Activity of Vestibular Nuclei Neurons During Vestibular and Optokinetic Stimulation in the Alert Mouse

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Beraneck M, Cullen KE. Activity of vestibular nuclei neurons during vestibular and optokinetic stimulation in the alert mouse. *J Neurophysiol* 98: 1549–1565, 2007. First published July 11, 2007; doi:10.1152/jn.00590.2007. As a result of the availability of genetic mutant strains and development of noninvasive eye movements recording techniques, the mouse stands as a very interesting model for bridging the gap among behavioral responses, neuronal response dynamics studied in vivo, and cellular mechanisms investigated in vitro. Here we characterized the responses of individual neurons in the mouse vestibular nuclei during vestibular (horizontal whole body rotations) and full field visual stimulation. The majority of neurons (~2/3) were sensitive to vestibular stimulation but not to eye movements. During the vestibular-ocular reflex (VOR), these neurons discharged in a manner comparable to the "vestibular only" (VO) neurons that have been previously described in primates. The remaining neurons [eye-movement-sensitive (ES) neurons] encoded both head-velocity and eye-position information during the VOR. When vestibular and visual stimulation were applied so that there was sensory conflict, the behavioral gain of the VOR was reduced. In turn, the modulation of sensitivity of VO neurons remained unaffected, whereas that of ES neurons was reduced. ES neurons were also modulated in response to full field visual stimulation that evoked the optokinetic reflex (OKR). Mouse VO neurons, however, unlike their primate counterpart, were not modulated during OKR. Taken together, our results show that the integration of visual and vestibular information in the mouse vestibular nucleus is limited to a subpopulation of neurons which likely supports gaze stabilization for both VOR and OKR.

INTRODUCTION

The vestibuloocular reflex (VOR) and optokinetic reflex (OKR) have proven to be useful model motor systems for studying the neural correlates of behavioral plasticity (reviewed in Boyden et al. 2004; Broussard and Kassardjian 2004; Gittis and du Lac 2006). Much recent work has focused on the understanding of the mechanisms that mediate these reflexes (Katoh et al. 1998; Killian and Baker 2002; Stahl et al. 2000; van Alphen et al. 2001) and their adaptation during motor learning (Boyden and Raymond 2003; Iwashita et al. 2001; Katoh et al. 1998; van Alphen and De Zeeuw 2002) in mice. Developments in mouse genetic engineering, including the creation of transgenic and knockout mutant mice, provide the opportunity to study the links between genes and behavior. In addition, recent technical advances have made it possible to make accurate measurements of mouse eye movements without impeding normal behavior. Accordingly, a number of

studies have now demonstrated that mice generate robust eye-movement responses to stabilize their gaze relative to space. VOR and OKR responses driven by vestibular (head movement) and visual (retinal slip) inputs, respectively, stabilize gaze with respect to world during self-motion. In mice, as in primates, the optokinetic system is most effective at lower frequencies <1 Hz (Faulstich et al. 2004; Iwashita et al. 2001; Shutoh et al. 2002) such that it complements the frequency response of the VOR. As a result, the VOR and OKR together can effectively stabilize gaze over a wide range of head movements (Faulstich et al. 2006; Katoh et al. 2005; Kimpo et al. 2005; Stahl 2004; van Alphen et al. 2001).

A principal sensory input to vestibular nuclei (VN) is the angular head-velocity signal encoded by vestibular afferents that originate in the semicircular canals of the inner ears (Goldberg and Fernandez 1971). Neurons in the VN then integrate this information with other sensory- and motor-related information to stabilize gaze and posture during natural activities. Several groups have conducted in vitro studies in the mouse vestibular nuclei to establish the relationship between intrinsic membrane dynamics and spike discharges in normal animals (Camp et al. 2006; Dutia and Johnston 1998; Nelson et al. 2003; Sekirnjak and du Lac 2002, 2006). These studies emphasize that neuronal response dynamics of VN neurons are determined not only by the dynamics of their inputs but also by their intrinsic ion channel activity (Beraneck et al. 2007). For example, the subset of neurons that project to the extraocular motoneurons (i.e., neurons that mediate the VOR and/or OKR) is characterized by weak firing response adaptation during steady depolarization and little postinhibitory rebound firing after membrane hyperpolarization which allows the neurons to respond to head movements without substantially altering the dynamics of their inputs (Sekirnjak and du Lac 2006). In addition, experiments using mutant mice provided new insights into the cellular mechanisms that underlie motor learning in these reflex pathways (Kassardjian et al. 2005; Shutoh et al. 2006).

To date, in vivo studies have been only carried out in the unanesthetized paralyzed mouse (Baurle et al. 1997; Grusser-Cornehls et al. 1995). These prior investigations have shown that VN neurons are modulated in response to head-in-space velocity during passive whole-body rotations. However, this approach provides limited insight into the processing that occurs in this nucleus because the VN receive inputs from cortical, cerebellar, and other brain stem structures, in addition

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to inputs from the vestibular afferents (reviewed in Cullen and Roy 2004). These additional inputs relay somatosensory and visual inputs as well as signals related to eye movements and premotor head movement commands to the vestibular nuclei. As a result, in alert animals extra-vestibular inputs will modify the processing of vestibular information during behaviors including the VOR and OKR. Here we have characterized the signals that are encoded by the VN during the VOR and OKR by recording from individual vestibular neurons in alert mice while simultaneously recording eye movements.

The first objective of this study was to examine the response characteristics of mouse VN neurons during head and eye movements. Neurons in the rostral medial and the ventrolateral subdivisions of the vestibular nuclei receive direct inputs from horizontal semicircular afferents and so are responsive to rotation in the yaw axis. In primates, neurons in these regions of the VN can be largely grouped into three distinct classes based on their responses to en bloc rotation (VOR) and sensitivities to eye movements during saccades and steady fixation (Chubb et al. 1984; Fuchs and Kimm 1975; Keller and Daniels 1975; Lisberger and Miles 1980; Miles 1974; Tomlinson and Robinson 1984). Of these, two classes [i.e., position-vestibular-pause (PVP) neurons and eye/head (EH) neurons] encode horizontal head velocity during passive whole-body rotation and also encode eye movements in the absence of concurrent head motion. In contrast, the third cell class —vestibular-only (VO) neurons—is modulated by passive head rotation but not by eye movements. Here we establish that similar subpopulations of VN neurons can also be described in mouse.

Our second objective was to establish how vestibular and visual inputs are integrated at the level of single VN neurons in the alert mouse. The mouse, like other rodents (e.g., rat, gerbil, guinea pig) is a lateral-eyed animal, and its vision is inherently different from that of a frontal-eyed animal like the monkey. The mouse's retina lacks a fovea or an area centralis (Tauber and Atkin 1968), and as a result mice do not generate smooth pursuit eye movements and make few spontaneous saccades (Stahl 2004). In addition, although mice generate robust gaze stabilizing reflexes (i.e., VOR and OKR), there are noticeable differences in the response dynamics when compared with foveate animals. For example, OKR gains are maximal at lower velocities in mice (<10°/s) (Iwashita et al. 2001; Stahl 2004) and monocularly driven responses are restricted to stimuli moving from temporal to nasal direction in the visual field (rabbit: Collewijn 1969; rat: Hess et al. 1985; gerbil: Sontheimer and Hoffmann 1987; Tauber and Atkin 1968). Moreover, the build-up of eye velocity in response to optokinetic stimulation is smaller over time and has more variable dynamics in mice than primates (van Alphen et al. 2001). Thus we assessed whether the integration of visual and vestibular inputs at the level of the VN is different in mice compared with primates.

METHODS

Eight male C-57bl6 (30–35 g; Charles River Laboratories) adult mice were included in this study. All procedures were approved by the McGill University Animal Care Committee and were in strict compliance with the guidelines of the Canadian Council on Animal Care.

Head-post implantation and craniotomy

Mice were anesthetized with an intramuscular injection of a mixture of atropine (5×10^{-4} mg/g), ketamine (10^{-1} mg/g), acepromazine maleate (2×5.10^{-2} mg/g), xylazine (10^{-1} mg/g), and sterile saline. Animals were then secured in a stereotaxic frame. A 1 mm diam craniotomy was performed to provide access to the vestibular nuclei and a dental cement chamber (C&B Metabond) was constructed around the hole. A custom-built head holder was then cemented to the implant. After the surgery, animals were kept in isolated cages and closely monitored during the first 48 h. Buprenorphine (0.05 mg/kg) was utilized for postoperative analgesia, and xylocaine (2%, Astra Pharma, Ontario) was applied to the incision site. In addition, care was provided to avoid hypothermia and dehydration.

Recording sessions

During the experiment, animals were placed in a custom-built Plexiglas tube at the center of a turntable surrounded by a striped drum (vertical black and white stripes, visual angle width of 5°). The animal's head was fixed 35° nose down to align the horizontal semicircular canals with the horizontal plane (Calabrese and Hullar 2006; Vidal et al. 2004). Eye movements were recorded using the video-oculography method following the procedures described by Stahl et al. (2000). Extracellular single-unit activity was recorded using insulated tungsten microelectrodes (8–10 MΩ impedance, Frederic Haer). The methods for data acquisition were similar to those described for studies of the horizontal angular VOR in normal macaque monkeys (Huterer and Cullen 2002). Data acquisition was controlled by a QNX-based real-time data-acquisition system (REX) (Hayes et al. 1982). Eye- and head-position signals were low-pass filtered at 250 Hz by an 8 pole Bessel filter and sampled at 1 kHz. The sampled signals were digitally filtered at 125 Hz. Eye, head, table, and drum positions as well as unit activity were recorded on digital audio tape for later playback and off-line analysis.

Behavioral paradigms

Responses of central vestibular neurons were investigated in four different conditions, which were designed to evoke combined or dissociated head and eye movements (Fig. 1).

First, VOR: neuronal responses during the VOR were tested by rotating the turntable while the visual surround (drum) remained fixed relative to space. The search stimulus consisted in 0.5-Hz rotations at a velocity of 50°/s. When possible, supplementary frequencies were of 0.3, 1, and 2 Hz and velocities of 20 and 80°/s were also tested.

Second, static eye position (SEP): the turntable was slowly rotated through a series of steps to drive the eye at different eccentric positions in the orbit (range of ~20°). Only segments where both the head and eye were stable were used for the analysis (shaded area illustrated on Fig. 1 were excluded), as previously described for rabbit (Stahl and Simpson 1995).

Third, VVC: to dissociate responses to vestibular stimulation versus to the eye movements that were evoked via the VOR, neurons were tested during a condition in which eye-movement response were attenuated. Reduction of the normally evoked VOR was achieved by rotating the optokinetic drum and the turntable together at the same velocity and frequency. Stimulation parameters were set to cancel ~50% of the gain of the VOR in light (frequency of 0.3–0.5 Hz; velocities of 20–50°/s; see Fig. 7 and related text).

Fourth, OKR: neuronal responses during OKR were characterized using two different kinds of constant velocity full field visual stimulation: relatively slow velocity rotations (0–10°/s, 5-s duration) and higher velocity rotations (30°/s, 60-s duration). Responses to both ipsilaterally and contralaterally directed rotations (relative to the side of recordings) were tested.
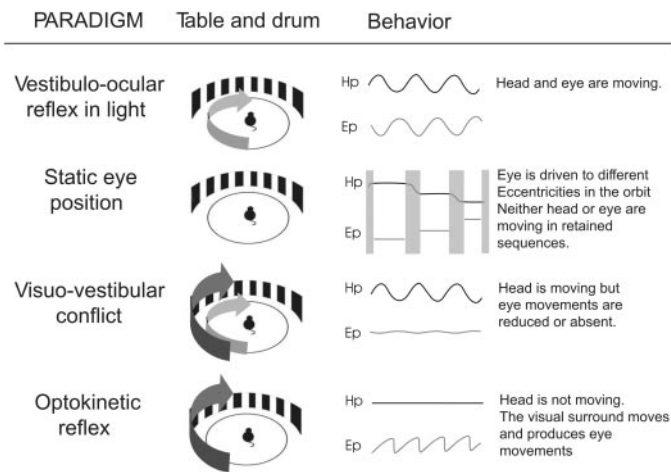
FIG. 1. Paradigms tested. Four different paradigms that evoked combined or dissociated head and eye movements were used: *Top*: neurons were identified by their response to horizontal head rotation during the vestibulo-ocular reflex (VOR) performed in the light (combined head and eye movements). Static eye position, the relation of the cell's discharge to the position of the eye in the orbit, was studied by slowly rotating the animal through a series of steps, which drove the eye to different eccentric positions in the orbit (range of ~20°). Only segments where neither the head nor the eye moved were used for the analysis. Cells were tested during a visuo-vestibular conflict produced by rotating en bloc the table and the drum surrounding the animal. The conflicting sensory information leads to an attenuation of the eye movements for rotation of limited frequency and velocity (see also Fig. 7). *Bottom*: influence of a pure visual stimulation was investigated with the optokinetic reflex, produced by rotating the striped drum around the animal without rotating the table. Hp, head position; Ep, eye position.

Testing with these four paradigms allowed us to describe the influence of eye movements versus vestibular and visual stimulation on neuronal discharges. Note that it was not possible to combine all these paradigms with a VOR performed in the dark condition because darkness induced a marked change in the pupils' size. Prior studies that monitored eye movements using a video-tracking system, have used drugs that induce mydriasis to keep the pupil size constant (e.g., physostigmine salicylate, Faulstich et al. 2004; pilocarpine hydrochlo-

ride, Iwashita et al. 2001). However, to ensure that the vision was normal in our experiments this approach was not used here.

Single-unit and field potential recordings

Of the more than 86 well-isolated units that were recorded, 52 were included in this study following off-line analysis. We included only those units that showed modulation ≥ 0.1 [(spike/s)(°/s)] during VOR and VVC. In addition, reliable eye-movement recordings were available for all of the tested stimulation protocols, such that each neuron's eye-position sensitivity (or lack of sensitivity) could be quantified.

Two animals were also implanted with vestibular stimulating electrodes. A lateral incision was made to expose the external auditory canal and the tympanic bulla. Bleeding was prevented using a portable cautery device (Allegiance Healthcare). The muscle covering the temporal bone and part of the tympanic bulla was dissected, and two Teflon-coated silver electrodes, denuded at their tips (ball electrodes), were placed over the round window of the middle ear cavity and in front of the semicircular ampullae, respectively. The vestibular nerve was electrically stimulated with brief current pulses (50–300 μA, 0.3 ms) delivered through the chronically implanted electrode using a stimulator and constant current—isolation units (model S88, SIU5, and CCU1; Grass Instruments). The maximum currents used to identify neurons that received a monosynaptic input from the nerve were twice the amplitude needed to elicit the maximum field potential. Neurons that were activated at latencies between 0.7 and 1.3 ms were considered to be second-order vestibular neurons (Fig. 2A).

Marking lesions

After completion of recording sessions, animals were killed. The recording electrode was then removed, and its location within the chamber was matched with the stainless steel electrode, which was then advanced to the same spot in depth. Electrolytic lesions were performed using a lesion producing device (Stoelting). Brains were then soaked overnight in a solution composed of 37% formaldehyde (30 ml), 99% ethyl alcohol (150 ml), glacial acetic acid (8 ml), potassium ferrocyanide (4 g), potassium ferricyanide (4 g), and distilled water (540 ml). During the following 48 h, brains were immersed in a solution of 30% sucrose formalin. They were then
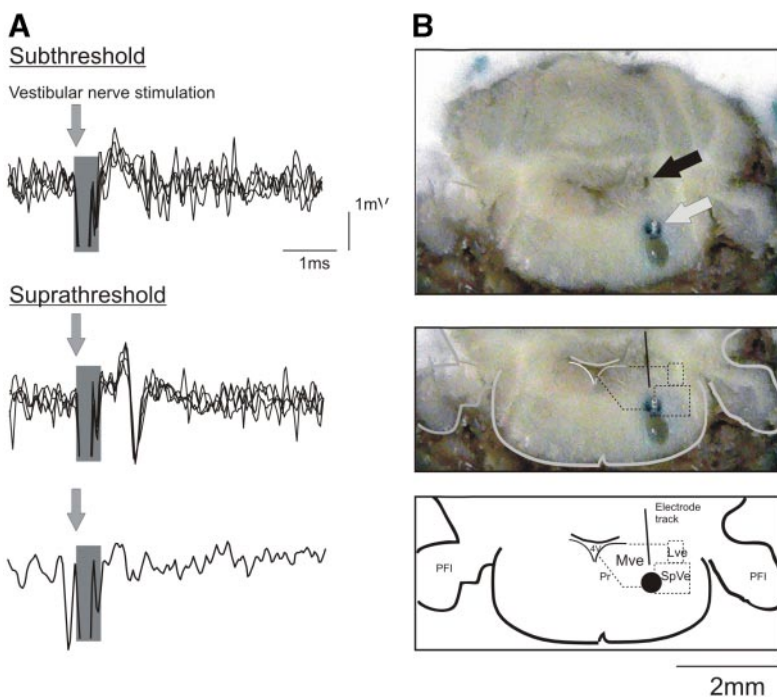

FIG. 2. Vestibular nerve stimulation and histology. A: vestibular nerve stimulation. After localization of the vestibular nuclei using field potential waves, neurons responsive for head rotation were tested with single shock (0.5 ms) electrical stimulation of the ipsilateral vestibular nerve. *Top*: subthreshold stimulations were applied and the intensity of the stimulation was increased until the threshold to evoke spikes was determined. *Middle*: suprathreshold stimulation of the same cell; this neuron was recruited with a monosynaptic latency of 1.2 ms. *Bottom*: cell failed to respond to suprathreshold stimulus because a spike was generated just before the shock. Arrows indicate the onset of stimulation. B: deep electrical lesions were produced with stainless steel electrodes to confirm the recording site. Brains were then processed with the blue spot marking method. *Top*: coronal section of a mouse brain. The black arrow indicates the electrode track. Gray arrow shows the blue spot marking. *Middle*: shapes of the 4th ventricle and paraflocculi were used as landmarks (superimposed in gray). Solid line illustrates the electrode track. Dotted lines indicate the boundaries of nuclei. This section is typical of the localization of our recording sites, ~6 mm caudal to bregma (in the rostral 1/3 of the vestibular nuclei). *Bottom*: scheme of the section showing the location of the electrode track and of the lesion relative to the vestibular nuclei. 4V, 4th ventricule; PFl, paraflocculus; Pr, prepositus hypoglossal nuclei; MVe, LVe, SpVe are medial, lateral, and spinal vestibular nuclei, respectively. Boundaries of nuclei are extracted from CMBL mouse brain atlas, section Bregma −5.88 mm.

embedded in OCT compound (Sakura) and frozen immediately by immersion in 2-methylbutane cooled in liquid nitrogen. Frozen coronal sections of 100 μm were cut with a cryostat and placed onto Probe-on Plus slides (Fisher Scientific, Houston, TX). The localization of the lesion (see Fig. 2*B*) was assessed using the shape of the fourth ventricle and of the paraflocculi as landmarks. The location of the lesions confirmed that our recordings were made in the rostral 1/3 of the vestibular nuclei.

Off-line analysis

All data were recorded on DAT tape for later playback. During play-back of the tape, the isolation of each unit was carefully evaluated. Action potentials were then discriminated using a windowing circuit (BAK) that was manually set to generate a pulse coincident with the rising phase of each action potential. Eye-, head-, table-, and drum-position signals were low-pass filtered at 250 Hz (8 poles Bessel filter) and sampled at 1,000 Hz. Recorded data were then imported into the Matlab (The MathWorks) programming environment for analysis. Horizontal eye- and head position data were digitally low-pass-filtered using a 51st-order finite-impulse-response (FIR) filter (cut-off frequency = 40 Hz) and differentiated to obtain velocity traces. A spike density function in which a Gaussian function was convolved with the spike train (SD of 10 ms) was utilized to represent the neuronal discharges (Cullen and Guitton 1997; Cullen et al. 1996; Sylvestre and Cullen 1999). Saccade and quick phase onsets and offsets were defined using a ±20°/s gaze velocity criterion. Segments of the data with saccades or quick phases were excluded from analysis of eye-position activity during SEP (Fig. 1) and slow phase responses during the VOR, VVC, and OKR (Sylvestre and Cullen 1999).

Neuronal responses to eye movement were first quantified in the absence of vestibular stimulation, by analyzing periods of steady fixation obtained during SEP paradigm to obtain an estimate of each neuron's resting discharge (bias, spike/s) and eye-position sensitivity [k, (spike/s)/°]. Next, a least-squared regression analysis was used to determine each neuron's phase shift relative to head velocity, resting discharge (bias, spike/s), and head-velocity sensitivity [g, (spike/s)/(°/s)] during VOR and VVC

$$Fr = bias + g*[Hv*(t-td)] \qquad (1)$$

where Fr is the firing rate; bias is the resting discharge; g is the sensitivity to head rotation; Hv is the head velocity; (t-td) represents the response phase reactive to head velocity. A regression model that included an eye position as well as head-movement term was also used to quantify responses of neurons that were sensitive to eye position during the SEP paradigm (Roy and Cullen 1998)

$$Fr = bias + g*[Hv*(t-td)] + [k*(Ep)] \qquad (2)$$

where Fr is the firing rate; bias is the resting discharge; g is the sensitivity to head rotation; Hv is the head velocity; (t-td) represents the response phase reactive to head velocity; and k is the eye-position sensitivity during static changes in eye position. Note that during vestibular stimulation, eye and head velocities are not independent. During the VOR, they are equal in amplitude and opposite in direction such that gaze remains stable in space, while during the VVC paradigm eye velocity is a scaled version of the head-velocity stimulus. Accordingly, in both conditions it is not possible to obtain coefficients for individual eye- and head-velocity terms. Instead, to establish whether changes in eye velocity were accompanied by changes in modulation during vestibular stimulation, we compare neuronal responses to VOR and VVC (see RESULTS). To determine whether neurons paused or burst during saccades, we assessed spike trains during the compensatory quick phases resulting from large amplitude rotations of the turntable (see Fig. 11).

Neuronal sensitivities to OKR were then quantified using a multiple regression analysis to estimate a neuron's resting discharge (bias, spike/s), eye-position sensitivity [k, (spike/s)/°], and eye-velocity sensitivity [r, (spike/s)/(°/s)]

$$Fr = bias + k*(Ep) + r*(Ev) \qquad (3)$$

where k and r represent the sensitivity to eye position and velocity, respectively. OKR responses were analyzed for intervals spanning from 300 ms after stimulus onset until end of stimulation (slow stimulation) or 3 s after stimulus onset until end of stimulation (faster stimulation). To quantify the ability of the linear regression analysis to model neuronal discharges in each condition, the variance-accounted-for (VAF) provided by each regression equation was determined (Roy and Cullen 1998). For these linear models, the VAF is the mathematical equivalent of the correlation coefficient R^2, such that a VAF value of 1 indicates a perfect fit to the data and a value of 0 indicates a fit that is equivalent to a mean value. Note that most VN neurons continued to fire through the entire stimulus cycles, and that only data for which the firing rate was >10 spike/s was included in the optimizations.

Statistical analysis

Statistical processing of all results was carried out using the Systat10.0 software (SPSS, Chicago, IL). For each parameter, normality of the distributions was assessed using one sample Kolmogorov-Smirnov tests, with significance set at $P = 0.05$. Statistical comparisons between numerical values were achieved through either parametric (if the distribution of the parameter was normal for all the samples involved and each sample included ≥15 values) or otherwise nonparametric tests, with the threshold for significance set at $P = 0.05$. Accordingly, two-by-two comparisons between cell groups were performed using Student's t-test or the nonparametric Mann-Whitney U test. All numbers in the results section are presented as means ± SE.

RESULTS

Neurons were categorized as either VO or eye-movement sensitive (ES) cells on the basis of their responses during the SEP and VOR paradigms. in the following text, we first illustrate an example VO neuron and ES neuron. This is then followed by a comparison of the resting discharge characteristics and head-velocity-related modulation of each population. We then consider the responses of each neuron class during a vestibular-visual conflict situation to address whether the altering visual input and/or the eye movement generation influences the modulation of VN neurons. Finally, we describe the influence of optokinetic stimulation on the discharge of each class of VN neurons as well as their quick phase related discharges.
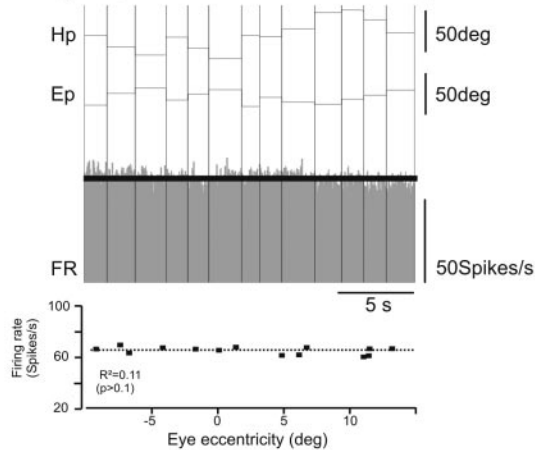
Characterization of VO neurons

In the present study, we found that a subset neurons with properties comparable to those of VO cells that have been previously described in monkeys (Cullen and McCrea 1993; McCrea et al. 1999; Scudder and Fuchs 1992) can be found in the VN of mice. The neuron illustrated in Fig. 3 is typical of our sample of VO neurons ($n = 37$). During passive whole-body rotation about an earth vertical axis (VOR paradigm: Fig. 1), the example neuron was strongly modulated in response to ipsilaterally directed head velocity [Fig. 3*A;* head-velocity sensitivity = 0.51 (spike/s)/°/s]. Depending on whether a neuron's firing rate increased during ipsilaterally (Fig. 3*A; n =*

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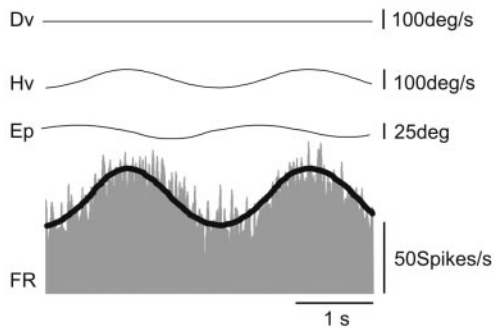
B Eye position sensitivity

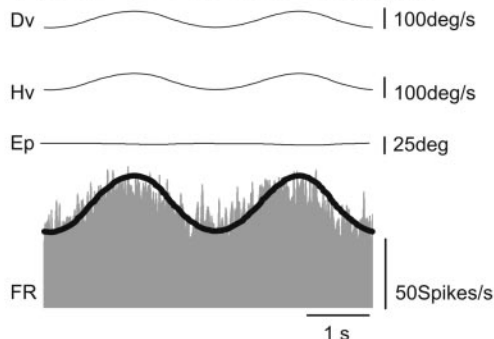## C Visuo-vestibular conflict

FIG. 3. Characterization of vestibular-only neurons. Activity of an example type I vestibular-only (VO, *unit 058c*) neuron during basic paradigms. *A*: VOR in light was used as a search stimulus (0.5 Hz; 50°/s) to identify cells responsive to horizontal head rotation. *B, top*: firing rate of the VO neuron while the eye is shifted to different positions in the orbit. Note that this figure and Fig. 4*B* display only time intervals during which the head was stable and so time is discontinuous between each vertical line. B*bottom*: mean firing rate of the neuron is not correlated with the horizontal eye position during the periods of steady fixation. Dotted line is the cell mean resting discharge. *C*: during VVC, the modulation of the cells discharge is not modified despite the absence of eye movements. A model (black lines; see *Eq. 1*) based on resting discharge and head-velocity sensitivity during VOR is superimposed to the firing rate traces. Dv, drum velocity; Hv, head velocity; FR, firing rate. In this and following figures, up is ipsilateral.

26) or contralaterally (*n* = 11) directed passive whole-body rotation, VO neurons were classified as type I or type II, respectively. The example type I neuron was representative of the VO cells in our sample in that its spontaneous discharge did

not vary as a function of the static orbital eye position (Fig. 3*B*). The lack of eye-position sensitivity (Fig. 3*B, inset, P >* 0.1) was quantified by comparing a neuron's mean firing rate at different static eye positions evoked during the SEP paradigm (see METHODS and Fig. 1*B*).

Because a compensatory eye velocity response was elicited during the VOR paradigm, neuronal responses were further characterized during the VVC (see METHODS and Fig. 1*C*) paradigm. The combined vestibular/visual stimulation resulted in a significant reduction in eye velocity (~75%, compare eye movement traces in Fig. 3, *A* and *C*) as compared with the VOR. However, as can be see for the example neuron, the head-velocity sensitivity of VO neurons was the same during the VVC and VOR paradigms [i.e., head-velocity sensitivity = 0.53 (spike/s)/(°/s)]. As a result, we found that a model based on the neuron's spontaneous discharge (bias) and head-velocity (Hv) sensitivity during the VOR (see *Eq. 1* in METHODS) could also be used to predict its firing rate during the VVC paradigm. For the example neuron, the same model provided an excellent description of responses during both the VOR and VVC condition (VAF$_{prediction}$ = 0.78 and 0.83, respectively).
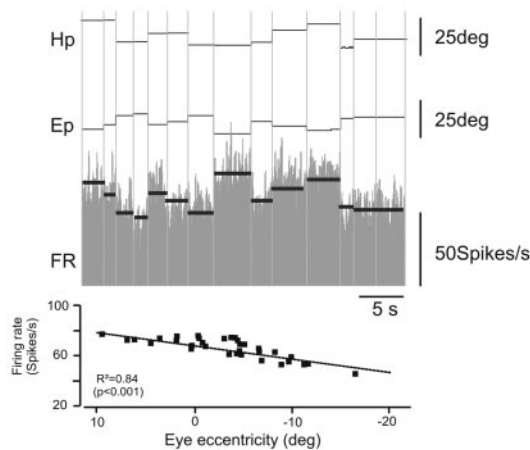
Characterization of ES neurons

We identified a second group of neurons, which were not only modulated in response to passive whole-body rotations but were also sensitive to the position of the eye relative to the orbit. Accordingly, we call this group of cells ES neurons. The response of a typical ES cell is shown in Fig. 4 during the VOR, SEP, and VVC paradigms. To quantify neuronal discharges during rotation, each neuron's resting discharge (bias), sensitivity to eye position [*k*, in (spike/s)/°] and sensitivity to passive head velocity [*g* in (spike/s)/(°/s)] were calculated using *Eq. 2* (see METHODS). The example neuron's firing rate increased during ipsilaterally directed whole-body rotation [*g* = 1.01 (spike/s)/(°/s); Fig. 4*A*] and thus was representative of most ES neurons in our sample (*n* = 11 type I vs. 4 type II). Moreover, in contrast to the VO neurons described in the preceding text, ES neuronal activity was correlated with orbital eye position during the SEP paradigm. For the example neuron, neuronal firing rate increased with increasing contralateral eye position [*k* = −0.84 (spike/s)/°; Fig. 4*B*]. Thus this neuron, like the vast majority (13/15) of ES was modulated in response to eye position and head velocity in opposite directions during the VOR and SEP paradigms. This response profile is a characteristic of the subclass of neurons in the monkey vestibular nuclei (i.e., position-vestibular-pause neurons) that are known to mediate the direct VOR pathway. We further consider the implications of this observation in the discussion.

As described in the preceding text for the VO neurons, we also used a VVC paradigm to further dissociate each ES neuron's sensitivity to vestibular stimuli from its eye-movement-related modulation. Although the activity of ES neurons was modulated in relation to rotation regardless of whether or not eye movements were substantially suppressed during rotation (compare Fig. 4, *A* and *C*), the example neuron was typical in that its head-velocity sensitivity was lower (~20%) when eye movements were suppressed during the VVC as compared with VOR paradigm [0.81 vs. 1.01 (spike/s)/(°/s)]. As a result, a model based on the neuron's head-velocity sensitivity during the VOR (*Eq. 2*) overpredicted the cell's discharge during

A Vestibulo-ocular reflex in light
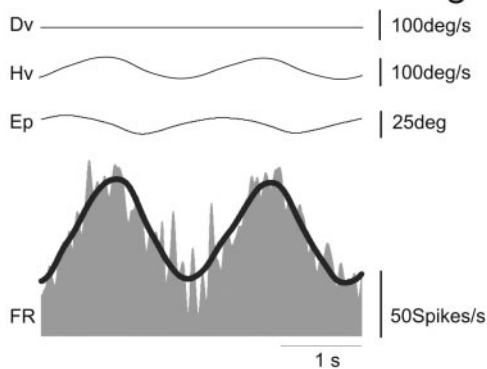
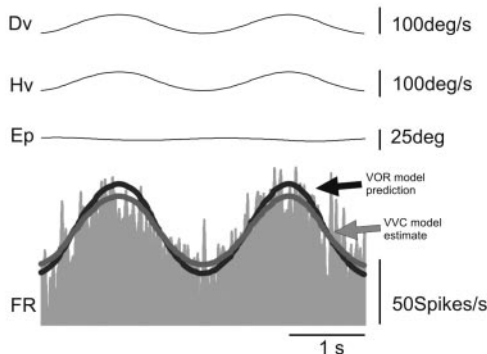## B Eye position sensitivity

C Visuo-vestibular conflict

FIG. 4. Characterization of eye-sensitive neurons. Activity of an example type I eye-sensitive (*unit 053c*) neuron during basic paradigms. *A*: modulation of the neuron's discharge during VOR in light. *B*: neuron's discharge increases when the position of the eye is shifted contralaterally and decreases when the eye is shifted ipsilaterally. Note that the figure only shows the segments in which the eye and head are not moving, as indicated by the vertical lines (discontinuous time). *C*: during a VVC, the modulation of the cell's discharge is decreased compared with VOR condition. Models (see *Eq. 2*) based on resting discharge, eye-position sensitivity during static eye position (SEP) and head-velocity sensitivity determined during VOR (black lines) or VVC (gray line) are superimposed to the firing rate traces.

VVC (Fig. 4*C;* compare model fits). This observation suggests that the modulation of ES neurons is attenuated during a visuo-vestibular conflict. We more explicitly address this possibility in relation to our presentation of the population responses of these neurons below (see Fig. 7 and associated text).

Comparison of resting discharge parameters, regularity, and rotational sensitivities of VO and ES neurons

In total, we recorded the single-unit activity of 52 rotationally sensitive neurons of which ~2/3 were classified as VO neurons and the remaining 1/3 as ES neurons (Fig. 5*A*). During horizontal head rotation at 0.5 Hz, the majority of neurons showed type I response, whereas the remaining neurons showed type II response. No type III activity was observed. The relative proportion of type I versus type II cells was similar for our subpopulations of VO and ES neurons (Table 1).

The resting discharge and coefficient of variation (CV) of the interspike interval were determined for each neuron, in the absence of vestibular or visual stimulation. For ES neurons, the resting discharge corresponded to the average discharge rate of the cell while the animal eyes were at their resting position (i.e., centered in the orbit). The distribution of the resting discharges of our entire population of VN neurons is presented on Fig. 5*B* (see also Table 1). On average, there was no difference in the resting discharge and regularity of the discharge (CV) between type I and type II units ($P = 0.40$ and $P = 0.41$, respectively). However, the discharges of VO neurons were more regular than those of ES neurons ($P = 0.026$; see Fig. 5*C* and Table 1). In contrast, although the resting discharges of VO neurons tended to be higher than those of ES neurons (Table 1), this difference was not statistically significant ($P = 0.098$; arrows in Fig. 5*B*).

Figure 5*D* illustrates the distribution of head sensitivities for our entire population of neurons during the VOR (0.5 Hz, $\pm 50°/s$; see table 1). The response sensitivities of type I units and type II units were comparable [0.46 ± 0.04 (spike/s)/(°/s) and 0.42 ± 0.06 (spike/s)/(°/s), respectively; $P = 0.59$]. While the modulation of ES neurons was greater than that of VO neurons ($P = 0.024$; table 1), there was no systematic relationship between head and eye sensitivities for the ES neurons when considered separately (see Fig. 5*D, inset; P = 0.280*). Finally, the response modulation of both the VO and ES neuron populations showed a similar phase lead relative to head velocity (7.1 $\pm$ 1.3° for VO; 7.6 $\pm$ 1.6° for ES; $P = 0.807$).

Dynamics of VN neurons discharge during the VOR

Most neurons ($n = 36$) were also tested at frequencies of 0.3, 1, and 2 Hz ($\pm 50°/s$), in addition to 0.5 Hz. Figure 6 compares the responses of an example VO and ES neuron during rotation at two different frequencies of 0.5 and 2 Hz (velocity of 50°/s). On average, the head-velocity sensitivity of VO neurons increased when rotational frequency increased from 0.3 to 2 Hz [■, Fig. 6*C;* 0.36 ± 0.05 to 0.45 ± 0.05 (spike/s)/(°/s), $P = 0.009$]. ES cells (○, Fig. 6*C*) demonstrated an even greater increase in response gain over this range; mean head-velocity sensitivity increased nearly 50% between stimulation at 0.3 and 1 Hz ($n = 8$, $P = 0.086$). Overall, the mean head sensitivity of ES was larger than that of VO neurons at 1 and 2 Hz, ($P = 0.014$ and 0.006, respectively) as well as at 0.5 Hz (Fig. 5*D, top*), indicating that the modulation of ES neurons was more robust not only for stimulation at 0.5 Hz (Fig. 5*D*) but also for higher frequencies.

The results shown in Fig. 6*C* (○) are computed by an analysis that accounted for the sensitivity of ES neurons to eye
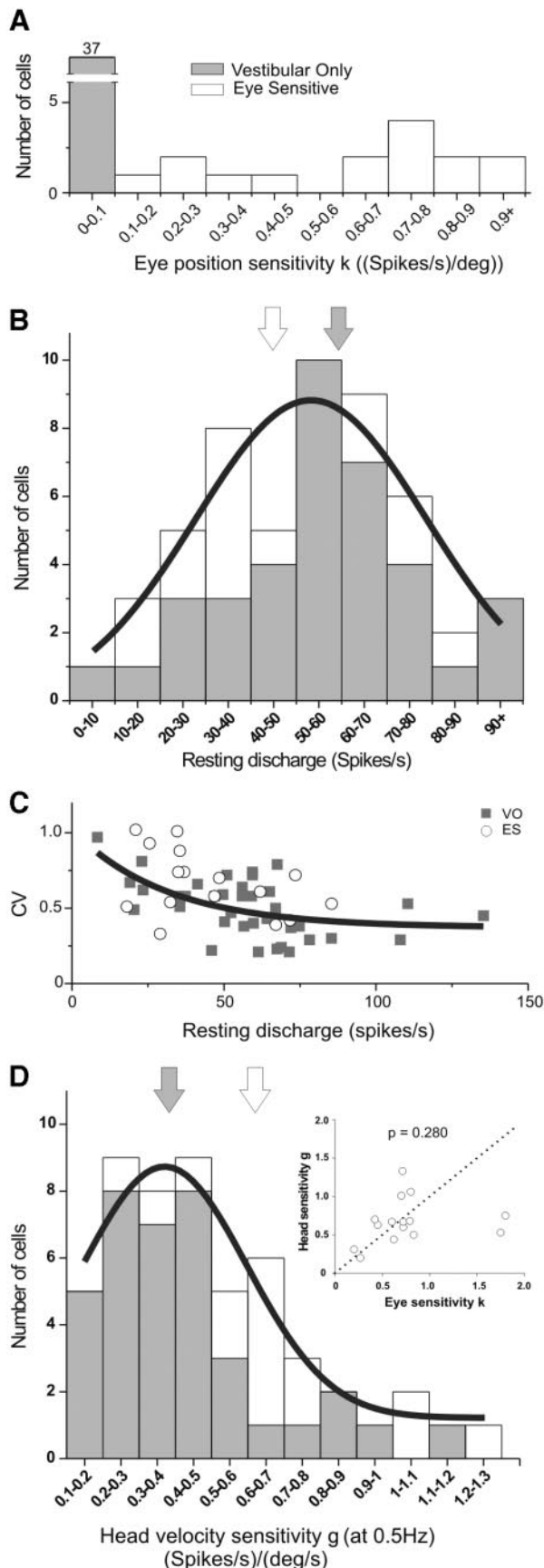
position to isolate that component of the response that could be attributed exclusively to head velocity. To understand how the actual modulation of the discharge of ES neuron was related to head rotation velocity, we also quantified each ES neuron's response without first correcting its sensitivity to eye position [i.e., using *Eq. 1* as was done for VO neurons, rather than *Eq. 2*]. Because the change in eye position will decrease with frequency [for a constant stimulation velocity (i.e., $\pm 50°/s$)], this analysis resulted in the largest differences at 0.3 Hz [0.15 (spike/s)/(°/s)] but only negligible differences at 2 Hz. Using this approach, we found that the average sensitivity of ES neurons remained relatively constant at ~ 0.6 (spike/s)/(°/s) over the full range of frequencies tested (◉, Fig. 6*C*; 0.56 at 0.3 vs. 0.68 at 2 Hz; $P > 0.6$).

Figure 6*D* illustrates the response phase for both subpopulations of neurons as a function of frequency. The response phase of VO neurons increased from a lead of $4.3 \pm 1.1°$ (0.3 Hz) to 10.6 ± 1.5 at 1 Hz ($P = 0.011$) and 17.9 ± 3.4 at 2 Hz ($P = 0.001$). In comparison, the response phase of the corrected ES neuron modulation (i.e., the head-velocity sensitivity estimated using *Eq. 2*) increased only up to 9.9 ± 1.7 at 2 Hz. There was no significant difference between VO and ES phase responses at all tested frequencies. When we did not correct the response of ES neurons for their sensitivity to eye position, the average response leads were reduced and were nearly zero for the highest frequency tested (○; Fig. 6*D*). This is expected because the position sensitive component of the response would by definition lag velocity.

Finally, the linearity of neuronal responses was tested for a subset of neurons by comparing modulations for rotations at $\pm 50°/s$ with modulations at $\pm 20°/s$ ($n = 21$; frequencies of 0.3 and 0.5 Hz) and $\pm 80°/s$ ($n = 27$, frequencies of 1 and 2 Hz). We found no significant differences for either VO or ES neurons when these data were compared with the results presented here for the corresponding frequency rotation and stimulation velocity of $\pm 50°/s$.

Comparison of neuronal responses during VOR and VVC

As described in the preceding text, because compensatory eye movements were elicited during the VOR paradigm, neuronal responses were further characterized during the VVC paradigm. As expected, eye velocity was significantly attenuated for lower frequency rotations during VVC because visually driven pathways contribute to gaze-stabilization in mice in this frequency range (see Stahl 2004). Figure 7*A* compares

FIG. 5. Distribution of eye-position sensitivity and basic discharge parameters of vestibular nuclei neurons. *A*: classification of vestibular neurons according to the sensitivity of their discharge to eye position (SEP paradigm). Cells with no eye-position sensitivity were classified as VO, whereas cells with an eye-position sensitivity were classified as eye-sensitive (ES) neurons. *B*: distribution of resting discharge for the entire population. ES neurons tend to have a lower resting discharge than do VO neurons. Gray and white arrows indicate the mean values for VO and ES, respectively. *C*: relationship between the coefficient of variation and the resting rate of vestibular VO and ES neurons. ES neurons discharge is more irregular than that of VO neurons. *D*: distribution of head-velocity sensitivities determined with the search stimulus during VOR in light paradigm. ES neurons have higher head sensitivities than do VO neurons. Gray and white arrows indicate the mean values for VO and ES neurons, respectively. *Inset*: there is no relation between the sensitivity of ES neurons to eye position and their sensitivity to head velocity. All curves are fits for the whole neuronal population.

TABLE 1. *Comparison of VO and ES neurons basic parameters*

	No. of Cells	Type I, %	Type II, %	Resting Discharge, spike/s	CV	Head-Velocity Sensitivity, spike/s/deg/s
Vestibular only	37	70.2	29.8	57.5 ± 4.2	0.51 ± 0.03	0.38 ± 0.04
Eye sensitive	15	73.3	26.7	45.7 ± 5.5	0.66 ± 0.006*	0.62 ± 0.06*
All	52	71	29	54.1 ± 3.4	0.55 ± 0.03	0.43 ± 0.03

*Statistical significance between VO and ES parameters ($P < 0.05$).

response gains during VOR in light and VVC paradigms ($n = 7$ animals) as a function of frequency (50°/s). For stimulation frequencies of 0.1–1 Hz, the gain of the VOR was relatively constant. In contrast, over the same range of frequencies,

average gains during VVC varied significantly (minimum: 0.06 ± 0.01 at 0.1 Hz vs. maximum: 0.77 ± 0.08 at 1 Hz). Neuronal responses were recorded during VVC paradigm at stimulating frequencies of 0.3 and 0.5 Hz for which ≥50% of

FIG. 6. Dynamics of vestibular neurons discharge during VOR performed in light. *A* and *B*: firing rate modulation during VOR in light paradigm of a VO neuron (*A, unit 057a*) and an ES neuron (*B, unit 053a*) tested at a velocity of 50°/s; *left*: frequency of 0.5 Hz; *right*: frequency of 2 Hz. Black lines, models based on *Eqs. 1* and *2* for the illustrated VO and ES neurons, respectively. *C* and *D*: bode plots of gain (*C*) and phase (*D*) for VO (▣) and ES (○) neurons. *, significant differences ($P < 0.05$) between ES and VO neurons. ◎, ES neuron responses when their eye sensitivity is not included in the fitting models. In this and following figures, right is up. Error bars indicate means ± SE.
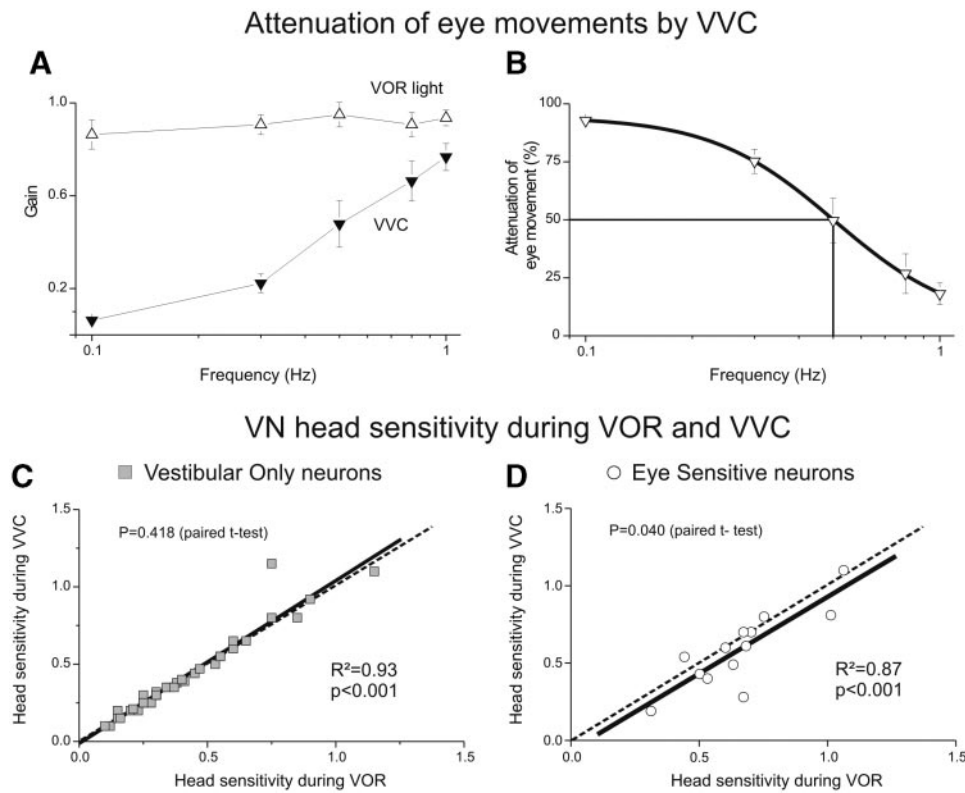
FIG. 7. Attenuation of eye movement amplitude and of vestibular nuclei (VN) neurons discharge modulation during VVC. *A* and *B*: comparison of the eye movements of 7 mice during VOR in light and VVC paradigms. *A*: VOR and VVC gain bode plots. *B*: relationship between the frequency of the stimulation and the amount of eye movement attenuation. Amplitude of the eye movement was reduced by ≥50% for frequencies <0.5 Hz. *C* and *D*: relationship between head sensitivities determined during VOR and VVC paradigms for VO (*C*) and ES neurons (*D*). Whereas VO neurons discharge is similar in the 2 conditions, ES neurons head-velocity sensitivity is significantly attenuated by the VVC.

the eye movement evoked by vestibular stimulation was suppressed by visual inputs (Fig. 7*B*).

As described for the example neuron in Fig. 3*C*, we established whether each VO neuron's response could be predicted based on its response during the VOR. Comparison of the VOR-based prediction and direct estimation of head-sensitivity parameter [i.e., the coefficient *g* in *Eq. 1*) provided similar results. First, both models provided comparable fits to the data (difference of VAF$_{prediction}$ vs. VAF$_{estimate}$ <5% for all VO neurons). Moreover the head-velocity sensitivities estimated during VVC were similar to those estimated for the VOR [*P* = 0.418; mean estimates: 0.37 ± 0.04 vs. 0.36 ± 0.04 (spike/s)/(°/s), respectively]. This is shown in Fig. 7*C*, which plots the head-velocity sensitivity estimates obtained during the VOR as a function of that obtained during the VVC for each VO neuron. Thus the head sensitivity of VO neurons was not modified during a VVC even though the amplitude of the compensatory eye movements was greatly reduced. This result is consistent with prior studies of VO in primates (see DISCUSSION).

Comparison of ES neuronal responses during VVC and the VOR showed that, unlike VO neurons, the modulation of this population of neurons was reduced by the suppression of eye movement. On average, comparison of the VOR-based prediction and direct estimation of head sensitivity parameter (i.e., the coefficient '*g*' in *Eq. 2,* see also Fig. 4C) revealed that there was an 11% reduction in the VAF provided by the VOR-based prediction. Furthermore, the head-velocity sensitivities estimated during VVC were significantly less than those estimated for the VOR [mean estimates: 0.56 ± 0.07 vs. 0.62 ± 0.06 (spike/s)/(°/s), respectively]. This result is shown in Fig. 7*D*, which plots the relationship between the head-velocity sensi-

tivity estimates obtained during the VOR and VVC for each ES neuron. Overall, however, response modulation of ES neurons was reduced by only ~10% (10.3 ± 1.1%) during VVC, whereas the gain of the eye velocity response reduced by more than half (0.32 ± 0.04) during the same single-unit recordings. Thus the reduction in neuronal response during VVC was small relative to the accompanying reduction in eye velocity response (i.e., 10 vs. 50%), consistent with the proposal that these neurons are primarily driven by vestibular input during these paradigms. This result is comparable to that previously described for PVP and eye-head neurons in the vestibular nuclei of monkey; nearly complete suppression of the VOR during a tracking task resulted in a ~25% reduction in neuronal modulation.

Neuronal responses during optokinetic stimulation in the optimal gain range

In monkey, the same neurons in the vestibular nuclei that respond to head rotation also respond to optokinetic stimuli (Boyle et al. 1985; Buettner and Buttner 1979; Reisine and Raphan 1992; Waespe and Henn 1977a,b). Here we addressed whether this is also the case in mice by recording the responses of VN neurons during full field visual stimulation. First, we measured the response gain of the optokinetic response (eye velocity/drum velocity) when mice viewed prolonged and constant velocity, full-field stimulation. Figure 8*A* shows the behavioral speed tuning curve for the eye-movement responses. Peak gains were associated with the lowest velocities of rotation (i.e., <5°/s), falling to gains <0.2 for velocities >10°/s. Similar trends were seen for drum movement directed either temporally or nasally relative to the recorded eye. This

A Tuning curve

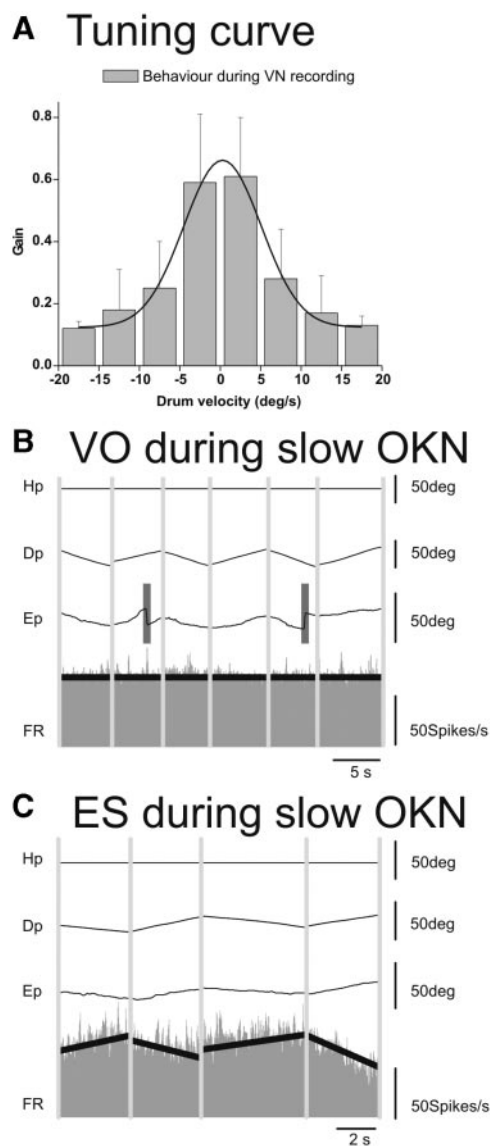
B VO during slow OKN

C ES during slow OKN

FIG. 8. Response of VN neurons during slow optokinetic reflex (OKR). *A*: tuning curve showing the relationship between the gain of the OKR and the velocity of the stimulus. Mouse OKR is maximal for velocity of 0–5°/s. *B*: response of a type I VO cell (*unit 056b*) during slow optokinetic stimulation. ▫, quick phases centering the eye back in the orbit. *C*: response of a type I ES neuron (*unit 054b*) during slow optokinetic stimulation. The firing rate of the cell increased for movement of the eye in the ON direction (slow phases directed contralaterally), and decreased in the OFF direction. Models (—) are based on eye position and eye velocity (*Eq. 3*). In this and following figures, right is up.

trend is comparable with that previously described in other behavioral studies of eye movements in mice (e.g., see Stahl 2004).

The responses of VO and ES neurons during stimulation were characterized in the optimal range of optokinetic responses (i.e., velocities <10°/s). In afoveate mammals, monocular responses are restricted to temporal to nasal stimulation (rabbit: Collewijn 1969; Erickson and Barmack 1980; rat: Hess et al. 1985). Binocular responses can be in either direction, optokinetic stimulations were therefore applied in both directions. The ON direction was defined as the optokinetic direction that was excitatory for a particular cell, whereas the OFF direction was inhibitory.

Each neuron's modulation during the slow phase portion of the optokinetic response was quantified using *Eq. 3* (see METHODS). The neuron illustrated in Fig. 8*B* is typical of the population of VO neurons (*n* = 15) recorded during optokinetic stimulation. Overall, the eye position and velocity terms in *Eq. 3* were negligible (mean values = −0.062 ± 0.05 and −0.008 ± 0.03, respectively), such that estimation of a simple bias term (e.g., resting discharge) provided equivalent fits to each neuron's discharge. For the population of VO neurons, the estimated bias during OKR was 54.8 ± 5.8 spike/s, which was comparable with the resting discharge of this same population of neurons (57.5 ± 4.2 spike/s). The lack of modulation was particularly striking, given that the mean maximal gain of the optokinetic response was 0.79 ± 0.06 for the analyzed sequences. The lack of modulation was observed for both type I and II VO neurons in both their ON and OFF directions. Taken together, these findings suggest that VO neurons neither are driven by constant velocity full-field visual stimulation nor contribute to the optokinetic reflex.

ES neurons were tested during the same visual stimulation paradigm (mean optokinetic response gain = 0.85 ± 0.03; *n* = 7, 5 type I, 2 type II), and their responses were quantified using *Eq. 3.* Optokinetic responses were significant in six of the seven cells tested. Figure 8*C* shows the response of a typical ES cell during optokinetic stimulation. For this type I cell the ON direction was for rotation toward the contralateral side. All the neurons tested had sensitivities to head rotation and to optokinetic rotation in opposite direction.

To illustrate the variability in the modulation of the discharge of ES neurons during slow optokinetic stimulation, we calculated the modulation of the firing rate of each neuron relative to its resting discharge after a 5 s long stimulation (drum velocity: 5°/s). As a mean, the discharge of ES neurons was increased by ~15% (range: 5–25) in response to stimulation in the ON direction, whereas it decreased by the same amount during stimulation in the OFF direction. The variation between the sensitivity of ES neurons to slow optokinetic stimulation largely reflects the variability of the sensitivity of their discharge to the position of the eye (see Figs. 5*A* and 6*B, inset*). Overall, each neuron's eye-position sensitivity (*k*) was comparable (paired *t*-test, *P* = 0.71) to its eye-position sensitivity during the SEP paradigm (see Fig. 1). Similarly, the estimated bias was 54.4 ± 9.0 spike/s, which was comparable with the resting discharge of this same population of neurons (53.2 ± 9.0 spike/s). In addition, we found that *Eq. 3* and a reduced version of *Eq. 3,* which did not include an eye velocity term provided comparable fits (mean VAF = 0.26 ± 0.04 vs. 0.23 ± 0.05, respectively). Thus neuronal responses could be well described by a constant bias and eye-position-dependent term. As is shown in the following text (see Fig. 9), the apparent lack of significant eye-velocity response was most likely the result of the minimal eye velocities that were evoked by this paradigm [mean 1.8 ± 1.7 (°/s)]; in response to these low-velocity stimuli, the eye-velocity-related component of any neuron's response would have made a relatively minor contribution to its net modulation in this paradigm.
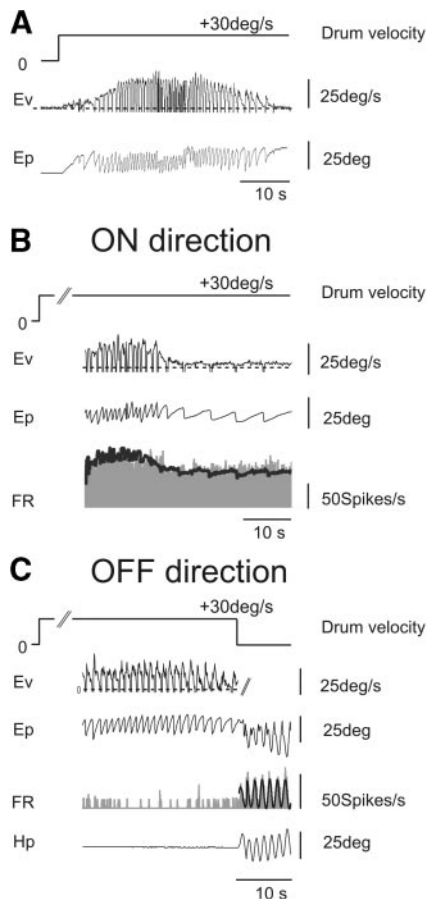
FIG. 9. Response of ES neurons during rapid long-lasting optokinetic stimulation. *A*: example of the behavioral response evoked by a sustained (~60 s), full field visual rotation at constant velocity (30°/s). Sudden illumination of the rotating drum led to an initial rise in the slow-phase eye velocity, which was followed by a steady state response. OKR then decayed while stimulation was still on. Both the gain of the response and the duration of the steady state varied from trial to trial. *B*: type II ES neuron (*unit 048d*) response during stimulation in the ON direction, the discharge of the cell was increased by ~50%. As shown by the fit of the discharge, the cell encodes both eye position and velocity (black line model is based on *Eq. 3*). *C*: type I ES neuron (*unit 025c*) response during stimulation in the OFF direction. The cells' firing decreased to reach complete silence. Note that at the end of the trace the turntable was rotated to verify that the cell was effectively not discharging. Model superimposed to the end of the trace is based on *Eq. 2*.

Neuronal responses during fast sustained optokinetic stimulation

As shown in Fig. 8*A*, the gain of the optokinetic response is maximal for low velocities and decreases substantially for higher velocity stimulation. However, as discussed in the following text, testing with lower velocity stimuli limits the estimation of neurons' sensitivities to eye velocity. To address whether VN neurons might encode eye-velocity signals in the absence of concurrent vestibular stimulation, we tested VN neurons during fast sustained optokinetic stimulation. For this purpose, mice were subjected to sustained (~60 s), constant velocity, full field visual rotation at relatively high velocities (30°/s). Figure 9*A* shows an example where sudden illumination of the rotating drum led to an initial rise (~5–10 s) in the slow-phase eye velocity followed by a robust steady-state response. The evoked behavioral response showed considerable variability in both the duration of the rising phase and

steady-state gain. Nevertheless regardless of the time course or magnitude of the behavioral response VO neurons were not modulated during either the initial buildup or sustained production of eye velocity.

Our quantitative analysis focused on the steady-state response because the eye and unit recordings collected during the first seconds of stimulation were frequently unstable; illumination of the cylinder was generally accompanied by blinking, marked contraction of the pupils, and strong behavioral responses. As for the analysis of slower optokinetic stimulation (Fig. 8*B*), a model based on both eye position and eye velocity (*Eq. 3*) was used to quantify each VO neuron's response. Again, both the eye-position and velocity coefficients were found to be negligible (mean $k = 0.1 \pm 0.1$; mean $r = 0.03 \pm 0.03$) and estimated mean biases were comparable ($P = 0.5$) with mean resting discharges (51.8 ± 8.0 vs. 54.5 ± 14.0 spike/s, respectively). Taken together, these results and those described in Fig. 8 show that VO cells are neither driven by full field visual stimulation nor involved in producing the optokinetic reflex in the alert mouse.

In contrast, ES neurons showed robust modulation in response to faster optokinetic stimulation. Figure 9*B* shows the response of an example type II ES neuron during stimulation in its ON direction. During this stimulation, slow phase velocity reached peak values of ~20–25°/s. The increase in slow phase eye velocity in a neuron's ON direction was accompanied by a concomitant increase in the cell discharge relative to resting discharge (~35 spike/s relative to the neuron's resting rate presented in Fig. 9*B*). The example neuron was typical in that all three terms in *Eq. 3* were required to describe its response to higher velocity optokinetic stimulation. In particular, an eye-velocity term [mean $= 0.55 \pm 0.20$ (spike/s)/(°/s)] was necessary to describe the cell's discharge in this condition ($\text{VAF}_{\text{velocity+position}} = 0.33$ vs. $\text{VAF}_{\text{position only}} = 0.02$). For the neurons tested ($n = 6$), removal of the eye-velocity term from *Eq. 3* resulted in a ~50% decrease in VAF provided by the model fit. During fast optokinetic stimulation in the OFF direction, neuronal responses were not only reduced but often driven to complete inhibition (Fig. 9*C*). These results and those described in the preceding text in Fig. 8 show that ES neurons are responsive to full field visual stimulation and are involved in generating the optokinetic reflex in the alert mouse.

Relation of the neuron's discharge to quick phases

In our initial characterizations of both VO and ES neurons, large-amplitude rotations of the turntable were applied to elicit many compensatory quick phases. VO cells ($n = 37$) did not demonstrate any quick phase-related activity; these neurons neither paused nor burst during VOR quick phases.

On the other hand, 40% of the ES neurons (6/15) showed discharge activity that was related to quick phase generation. Of these, half demonstrated a consistent pause during ipsilaterally directed quick phase. None paused during contralaterally directed quick phases. The other half showed a burst of activity during quick phases in either the ipsilateral or contralateral directions.

Figure 10, *A* and *B*, shows the quick phase related activity of an example type I and type II ES unit, respectively. The neuron shown in Fig. 10*A* paused ($\downarrow$) for ipsilaterally directed quick phases and was typical of our pausing neurons. Thus this cell
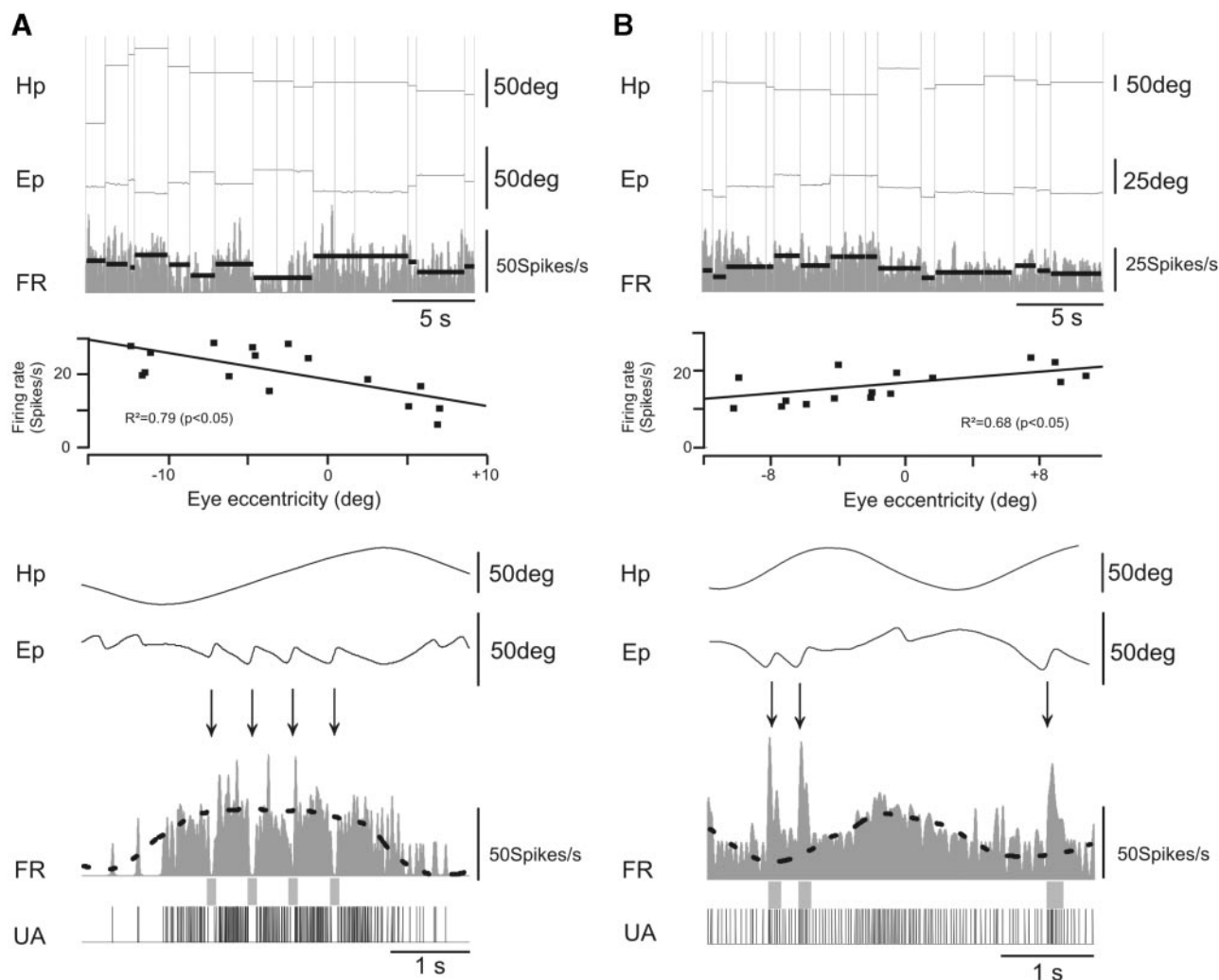
FIG. 10. ES neurons with quick phase related activity. *A*: example of a type I ES neuron (*unit 031b*) pausing during ipsilaterally directed quick phases. *B*: example of a type II ES neuron (*unit 025a*) bursting during ipsilaterally directed quick phases. *Top*: eye-position sensitivity of the cells determined during SEP paradigm, note that both cells have head and eye sensitivities in opposite directions (PVP like activities). *Bottom*: large-amplitude rotations performed in light were used to evoke quick phases. ↓, pauses and bursts in the discharge of these neurons, respectively. ⋯, model based on *Eq. 2* for both cells which illustrate the modulation of the discharge in response to head and eye movements; note that the models were based on desaccaded traces and not on the traces presented on *bottom panels*. UA, unit activity.

responded similarly to the type I position-vestibular-pause neurons, which have been previously described in monkeys and are known to mediate the direct VOR pathway. Figure 10*B* shows the typical bursting activity of a type II ES neuron. Note that both cells have head and eye sensitivities in opposite directions (*bottom panels*), which, as described in the preceding text, was the case for the majority of ES neurons (13 of 15).

In conclusion, all neurons that present quick phase related activity were ES neurons, which encode both eye-position and head-velocity signals comparable with PVP and EH neurons described in prior studies on primates.

DISCUSSION

During the past four decades, numerous studies have characterized the single-unit activity of neurons in the vestibular nuclei of the alert monkey. These studies have shown that neurons can be divided into distinct groups based on their responses during saccades, smooth pursuit, and passive whole-body rotations (reviewed in Cullen and Roy 2004). In the present study, we show that neurons in the vestibular nuclei of alert mouse can be similarly divided into two main groups (i.e., VO vs. ES neurons) based on their responses during eye movements and passive whole-body rotations. We further established that there are other marked differences between the response of VO and ES neurons. First, on average, eye-movement-sensitive neurons have more irregular spontaneous firing rates and higher sensitivities to rotation than do VO neurons. Second, VO cells do not demonstrate quick phase-related activity during vestibular or optokinetic nystagmus whereas about half of the eye-movement sensitive neurons produce a marked pause or burst for quick phases. Finally, the majority of ES cells, but none of the VO neurons, are activated by optokinetic stimulation.

Subpopulations of mouse VN neurons; comparison with other species

Comparison of our findings with those of previous studies that have characterized the responses of VN neurons in other

species of alert rodents (gerbil; Kaufman et al. 2000; guinea pig; Ris et al. 1995) suggests a number of similarities. First, in mice we found a comparable proportion of eye-movement-sensitive VN neurons as have been reported in prior studies of other rodents. In the present study, 1/3 of the neurons were sensitive to eye movements, which corresponds well to the proportions reported in alert gerbil and guinea pig [1/3 (Kaufman et al. 2000) and 1/5 (Ris et al. 1995), respectively]. Second, we found that in mouse ~50% of the eye-movement-sensitive neurons demonstrate quick phase-related activity comparable with proportion previously reported in guinea pig (Ris et al. 1995); data not available in gerbil (Kaufman et al. 2000). Third, eye-movement-sensitive neurons have generally higher sensitivities to head rotations than non-eye-movement-related neurons as previously described in gerbil (Kaufman et al. 2000); data not available in guinea pig (Ris et al. 1995). Finally, in mouse (this study, and see also Baurle et al. 1997 in anesthetized mouse) as in other rodents (Kaufman et al. 2000; Ris et al. 1995), the average head-velocity sensitivity of VN neurons is ~0.5–0.6 (spike/s)/(°/s) for rotation frequencies of 0.2–0.5 Hz. Moreover, previous studies have shown that response gain increases with stimulus frequency, whereas response phase remains close to zero relative to head velocity with increasing frequency. Our results are consistent with these findings and further show that observed changes in vestibular sensitivity are not dependent on the eye-position/movement sensitivities of the ES neurons (i.e., Fig. 6; compare neuronal responses in alert mice during the VOR and visual-vestibular conflict paradigms). Thus in summary, our findings show that the firing characteristics of the VN neurons in the alert mouse during vestibular stimulation are generally consistent with those of the VN neurons of other rodents.

Comparison of the results of single-unit studies in mouse and monkey also suggest similarities between the responses of VN neurons in these species. VO neurons, which are sensitive to vestibular stimulation during passive whole-body rotations and are insensitive to eye movements, are also frequently encountered in studies of head-restrained monkey (e.g., Cullen and McCrea 1993; Scudder and Fuchs 1992). These same studies of the monkey VN have described two additional classes of VN neurons [i.e., position-vestibular-pause (PVP) neurons and eye-head (EH) neurons], which are sensitive to eye movements as well to vestibular stimulation. In the present study, ~20% of eye-movement-sensitive neurons had PVP-like responses; they had oppositely directed eye- and head-movement sensitivities and paused for saccades made in the direction of their head-velocity sensitivity. In contrast, it is not possible to compare our neurons to EH cells because in monkey they are identified on the basis of their responses during smooth pursuit and voluntary cancellation of the VOR (Roy and Cullen 2003; Scudder and Fuchs 1992). In primates, each of the neuron classes supports different functions (reviewed in Cullen and Roy 2004). Although PVP and EH neurons generate the VOR and OKR via their direct projections to the extraocular motoneurons (Cullen and McCrea 1993; McCrea et al. 1987; Roy and Cullen 2002; Scudder and Fuchs 1992), the descending projections of VO neurons to the spinal cord underlie postural reflexes such as the vestibulocollic reflex (Boyle 1993; Boyle et al. 1996; Wilson et al. 1990). Although recent studies have shown that this reflex is also robust in mouse (Baker 2005; Takemura and King 2005), whether the

ES and VO neurons in mice subserve similar functions remains to be determined.

Implications of the lower eye- and head- movements sensitivities found in mouse VN neurons

Mouse VN neurons show relatively low sensitivities to both head velocity and eye position as compared with other species such as rabbit (Stahl and Simpson 1995), cat (Cheron et al. 1996; Escudero et al. 1992) or monkey (Cullen and McCrea 1993; Cullen et al. 1993a). The relatively low head-velocity sensitivities of mouse VN neurons are consistent with the recent finding that mouse afferents are on average three to four times less sensitive to head velocity in mouse relative to monkey (personal communication; see Lasker et al. 2004). This observation, taken together with our results suggests a co-adaptation of the firing properties of vestibular afferents and those of central vestibular neurons as shown for frog VN neurons (Beraneck et al. 2007).

What is the physiological basis for the reduced neuronal firing strategy in the pathways that process vestibular and/or gaze related information in mouse? One possibility is that the relatively lower discharges are matched to the specific mechanical constraints of the mouse oculomotor plant (e.g., Robinson 1964; Sklavos et al. 2005). The lower eye-movement sensitivities observed in mouse could reflect that fewer spikes are required for the motor and, by extension, premotor control of eye movements. For example, gaze control might have a "lower neural cost" in mice because the oculomotor plant has a lower inertia load than larger species. However, this idea is not supported by a preliminary report that suggests that the sensitivities of mouse extraocular motoneurons lie intermediate between those of the monkey and rabbit (personal communication; see Stahl et al. 2006). Nonetheless, further experiments aimed at characterizing the mouse eye plant (e.g., its inertia, plant elasticity and viscosity, and muscle physiology) could potentially provide insights into the neural coding strategy that is used in VN of mouse as compared with other species.

A second possible explanation for the relatively lower sensitivities of mouse VN neurons is that the mouse is far less dependent on gaze stabilization reflexes than are other species such as cat or monkey. Mice are afoveates with relatively poor visual acuity (Schmucker et al. 2005; Wong and Brown 2006), and so it follows that the low gain encoding of head and eye movement by central vestibular neurons might reflect less robust gaze stabilization reflexes in this species. However, this proposal is not consistent with the results of our study or those of prior investigations (Katoh et al. 2005; Stahl 2000), which have shown that the gain of the mouse VOR in light is nearly optimal and is in fact comparable to that in primate. A third and related possibility is then that the lower vestibular sensitivity in mice might help to extend the linear range over which head movement is encoded. Because the head of the mouse has less inertia load than that of larger species, it is likely the VOR would be required to compensate for higher velocity and frequency head movements than in primates. Further studies will be required to establish whether this proposal is correct.

A final possibility is that reduced sensitivity to eye and head movement in the mouse VN reflects a limited need for eye-

movement accuracy compared with foveate animals such as monkey. For example, monkeys make accurate orienting eye movements to scan their environments, whereas such movements are absent (i.e., pursuit) or infrequent (saccades) in mouse (Stahl 2004). Thus the specific firing behavior of mouse VN neurons might be tuned to match these less demanding requirements for the control of gaze. We have shown that compared with rhesus monkey (e.g., Roy and Cullen 2001, 2002), mouse VN neurons generally show lower and more irregular discharges as well as lower eye- and head-movement sensitivities. Together, these differences suggest that neurons in the mouse VN encode less information than the VN neurons of monkey (Borst and Haag 2001; Vinje and Gallant 2000). Recent work in the different fly species has shown that there is a cost for better performance in neural coding and suggests that evolutionary pressure reduces performance to the minimum required for adequate function and what constitutes "adequate function" can differ across species (Niven et al. 2007).

Most mouse VN neurons are not part of the pathway mediating OKR

The second specific objective of the present study was to establish how vestibular and visual inputs are integrated at the level of single VN neurons in the alert mouse. The vestibular nuclei are an important component of the pathways that processes optokinetic stimuli and generates OKR (goldfish; Dichgans et al. 1973; cat; Keller and Precht 1979; rat; Lannou et al. 1982; monkey; Waespe and Henn 1977a). Single-unit recording experiments in monkey had suggested that all classes of VN neurons (VO as well as ES neurons) can also be driven by optokinetic as well as vestibular stimulation (Boyle et al. 1985; Buettner and Buttner 1979; Reisine and Raphan 1992; Waespe and Henn 1977a,b). Moreover, these studies suggested that the modulation of both VO and ES neurons mirrors both the build-up of optokinetic slow phase eye velocity and exponential decay of optokinetic after nystagmus (OKAN). These findings have led to the proposal that VO neurons contributes to a "velocity storage" network, which uses visual information to supplementing the decaying signal from the vestibular afferents during sustained head movements to encode self-motion (Angelaki and Hess 1995; Cohen et al. 1983; Wearne et al. 1998).

Prior to our study, no previous report had described the responses of VN neurons in the alert rodent during OKR. We found that in alert mouse, optokinetic responses are present in the large majority of ES neurons, but not in VO neurons. Thus overall only ~1/3 of VN neurons appear to be involved in the generation of visually driven gaze stabilization. Similarly, other studies in afoveate mammals have found that only minority of the neurons that respond to head rotation are also responsive to visual stimulation [40% in rabbit during the OKR (Neverov et al. 1980) and 25% in the anesthetized guinea pig during nystagmus induced by optic nerve stimulation (Petrosini and Troiani 1978)]. There is some evidence to suggest that a larger proportion of VN neurons might be activated by optokinetic stimulation in a nonphysiological situation (paralyzed, alert rat) (Cazin et al. 1980) where, because the eyes cannot move in response to stimulation, the retinal slip velocity is equal to the velocity of the visual surround stimulus. However, it is possible that the neuronal modulation that was observed

during head rotation in the Cazin study encoded premotor/motor eye commands [as would be the case for "burst-tonic" neurons in the most medial zone of the medial VN and/or nucleus prepositus (Cullen et al. 1993b; McFarland and Fuchs 1992)] rather than a vestibularly derived signal, per se. Our population of recorded neurons did not include any cells that showed burst-tonic responses.

Taken together, our present results differ with those of previous studies of primate VO neurons. We found no evidence that, in mouse, VO neurons encode visually derived inputs to supplement the signal from vestibular afferents during sustained head movements. Instead, we found that the integration of visual-vestibular inputs in the VN is limited to a subpopulation of eye-movement-sensitive neurons that are primarily responsible for gaze stabilization during the optokinetic as well as VORs. Differences between how mice and monkeys control gaze, at least in part, may underlie this apparent discrepancy. First, as was discussed in the preceding text, mice are afoveate, lateral-eyed animals that produce a relatively limited repertoire of gaze behaviors, largely limited to gaze stabilizing reflexes (i.e., the VOR and OKR) (Stahl 2004). Second, OKR gains in mice are greatest in response to relatively low velocities of stimulation (<10°/s) (Iwashita et al. 2001; Stahl 2004) and the resultant build-up of eye velocity in response is less marked with more variable dynamics than in primates (van Alphen et al. 2001). Finally, monocular optokinetic responses are restricted to movements in the temporal-nasal direction in mice (Collewijn 1969; Hess et al. 1985; Sontheimer and Hoffmann 1987; Tauber and Atkin 1968). Further experiments will be needed to establish the relative contributions of processing in the VN versus in the upstream pathways that mediate sensory processing of visual motion shaping the specific dynamics of the OKR response in mice.

Bridging the gap: the relation between in vivo and in vitro properties of mouse VN neurons

Because the development of video-oculography techniques, it is now possible to record the eye movements of the mouse in a noninvasive manner (Iwashita et al. 2001; Stahl et al. 2000). The mouse therefore stands as a very interesting model to bridge the gap between gaze stabilization responses, neuronal response dynamics studied in vivo, and cellular mechanisms investigated in vitro. Thus the question arises whether the results of our in vivo studies can be related to previous in vitro characterizations of mouse VN neurons. In vitro studies have shown that VN neurons can be separated into two groups (type A and B) based on the shape of their action potentials and the subsequent after hyperpolarization (du Lac and Lisberger 1995; Johnston et al. 1994; Serafin et al. 1991). Although most of type A are GABAergic neurons, type B MVN neurons are either GABAergic, glutamatergic or glycinergic (Bagnall et al. 2007; Takazawa et al. 2004). Analysis of intrinsic membrane dynamics suggests that type B neurons function as active filters, which promote high-frequency responses, whereas type A neurons behave more like low-pass filters (Beraneck et al. 2003; Ris et al. 2001; Sekirnjak and du Lac 2002). Thus it has been proposed that type A and type B neurons can be considered channels for encoding low- and high-frequency signals, respectively (Av-Ron and Vidal 1999; Ris et al. 2001; Straka et al. 2005).

One interesting possibility is that the ES neurons recorded in our in vivo studies might correspond to type B neurons, which have been described in vitro. When recorded in the in vitro guinea pig whole brain preparation, type B cells have more irregular resting rates than do type A cells (Babalian et al. 1997). Similarly, we found that ES neurons had more irregular spontaneous firing rates than did VO neurons. Moreover, recent in vitro studies have further shown that type B neurons, presumably glutamatergic (Bagnall et al. 2007), send direct projections to the oculomotor nucleus (OMN) (Sekirnjak and du Lac 2006). These OMN projecting neurons have relatively higher excitabilities than nonprojecting neurons in the VN (Sekirnjak and du Lac 2006). Likewise, the ES neurons in our study had higher rotational sensitivities than did VO neurons. OMN-projecting neurons also exhibit relatively low rebound firing after an inhibitory hyperpolarizing step of current (Sekirnjak and du Lac 2006). This property could be useful for ensuring discharge recovery after the quick phase responses of ES neurons.

Prior work has further shown that neurons that receive inputs from the flocculus are type B neurons (Babalian and Vidal 2000; Sekirnjak et al. 2003). Thus at least a subset of type B cells that have been described in in vitro studies are likely to correspond to the floccular target/eye-head neurons that have previously been described in in vivo studies and have been shown to play an important role in visually induced VOR motor learning in primates (Blazquez et al. 2006; Lisberger et al. 1994). In alert guinea pig, most VN neurons exhibit a decrease in their head sensitivity in an acute VOR training paradigm (Serafin et al. 1999), and recent studies further suggest longer-term changes at the same level underlie memory consolidation required for chronic changes in VOR gain (Kassardjian et al. 2005; Shutoh et al. 2006). Here we have shown that the integration of visual and vestibular information in the mouse VN is restricted to ES neurons during the VOR and OKR. Nevertheless, it is possible that changes in both populations of VN neurons (i.e., VO as well as ES cells) accompany short- and long-term VOR motor learning. Future studies are required to establish the neural basis for motor learning in the mouse VN.

REFERENCES

Angelaki DE, Hess BJ. Inertial representation of angular motion in the vestibular system of rhesus monkeys. II. Otolith-controlled transformation that depends on an intact cerebellar nodulus. *J Neurophysiol* 73: 1729–1751, 1995.

Av-Ron E, Vidal PP. Intrinsic membrane properties and dynamics of medial vestibular neurons: a simulation. *Biol Cybern* 80: 383–392, 1999.

Babalian A, Vibert N, Assie G, Serafin M, Muhlethaler M, Vidal PP. Central vestibular networks in the guinea pig: functional characterization in the isolated whole brain in vitro. *Neuroscience* 81: 405–426, 1997.

Babalian AL, Vidal PP. Floccular modulation of vestibuloocular pathways and cerebellum-related plasticity: an in vitro whole brain study. *J Neurophysiol* 84: 2514–2528, 2000.

Bagnall MW, Stevens RJ, du Lac S. Transgenic mouse lines subdivide medial vestibular nucleus neurons into discrete, neurochemically distinct populations. *J Neurosci* 27: 2318–2330, 2007.

Baker JF. Dynamics and directionality of the vestibulo-collic reflex (VCR) in mice. *Exp Brain Res* 167: 108–113, 2005.

Baurle J, Helmchen C, Grusser-Cornehls U. Diverse effects of Purkinje cell loss on deep cerebellar and vestibular nuclei neurons in Purkinje cell degeneration mutant mice: a possible compensatory mechanism. *J Comp Neurol* 384: 580–596, 1997.

Beraneck M, Hachemaoui M, Idoux E, Ris L, Uno A, Godaux E, Vidal PP, Moore LE, Vibert N. Long-term plasticity of ipsilesional medial vestibular nucleus neurons after unilateral labyrinthectomy. *J Neurophysiol* 90: 184–203, 2003.

Beraneck M, Pfanzelt S, Vassias I, Rohregger M, Vibert N, Vidal PP, Moore LE, Straka H. Differential intrinsic response dynamics determine synaptic signal processing in frog vestibular neurons. *J Neurosci* 27: 4283–4296, 2007.

Blazquez PM, Hirata Y, Highstein SM. Chronic changes in inputs to dorsal Y neurons accompany VOR motor learning. *J Neurophysiol* 95: 1812–1825, 2006.

Borst A, Haag J. Effects of mean firing on neural information rate. *J Comput Neurosci* 10: 213–221, 2001.

Boyden ES, Katoh A, Raymond JL. Cerebellum-dependent learning: the role of multiple plasticity mechanisms. *Annu Rev Neurosci* 27: 581–609, 2004.

Boyden ES, Raymond JL. Active reversal of motor memories reveals rules governing memory encoding. *Neuron* 39: 1031–1042, 2003.

Boyle R. Activity of medial vestibulospinal tract cells during rotation and ocular movement in the alert squirrel monkey. *J Neurophysiol* 70: 2176–2180, 1993.

Boyle R, Belton T, McCrea RA. Responses of identified vestibulospinal neurons to voluntary eye and head movements in the squirrel monkey. *Ann NY Acad Sci* 781: 244–263, 1996.

Boyle R, Buttner U, Markert G. Vestibular nuclei activity and eye movements in the alert monkey during sinusoidal optokinetic stimulation. *Exp Brain Res* 57: 362–369, 1985.

Broussard DM, Kassardjian CD. Learning in a simple motor system. *Learn Mem* 11: 127–136, 2004.

Buettner UW, Buttner U. Vestibular nuclei activity in the alert monkey during suppression of vestibular and optokinetic nystagmus. *Exp Brain Res* 37: 581–593, 1979.

Calabrese DR, Hullar TE. Planar relationships of the semicircular canals in two strains of mice. *J Assoc Res Otolaryngol* 7: 151–159, 2006.

Camp AJ, Callister RJ, Brichta AM. Inhibitory synaptic transmission differs in mouse type A and B medial vestibular nucleus neurons in vitro. *J Neurophysiol* 95: 3208–3218, 2006.

Cazin L, Precht W, Lannou J. Optokinetic responses of vestibular nucleus neurons in the rat. *Pfluegers* 384: 31–38, 1980.

Cheron G, Escudero M, Godaux E. Discharge properties of brain stem neurons projecting to the flocculus in the alert cat. I. Medical vestibular nucleus. *J Neurophysiol* 76: 1759–1774, 1996.

Chubb MC, Fuchs AF, Scudder CA. Neuron activity in monkey vestibular nuclei during vertical vestibular stimulation and eye movements. *J Neurophysiol* 52: 724–742, 1984.

Cohen B, Suzuki JI, Raphan T. Role of the otolith organs in generation of horizontal nystagmus: effects of selective labyrinthine lesions. *Brain Res* 276: 159–164, 1983.

Collewijn H. Optokinetic eye movements in the rabbit: input-output relations. *Vision Res* 9: 117–132, 1969.

Cullen KE, Chen-Huang C, McCrea RA. Firing behavior of brain stem neurons during voluntary cancellation of the horizontal vestibuloocular reflex. II. Eye movement related neurons. *J Neurophysiol* 70: 844–856, 1993a.

Cullen KE, Guitton D. Analysis of primate IBN spike trains using system identification techniques. I. Relationship To eye movement dynamics during head-fixed saccades. *J Neurophysiol* 78: 3259–3282, 1997.

Cullen KE, Guitton D, Rey CG, Jiang W. Gaze-related activity of putative inhibitory burst neurons in the head-free cat. *J Neurophysiol* 70: 2678–2683, 1993b.

Cullen KE, McCrea RA. Firing behavior of brain stem neurons during voluntary cancellation of the horizontal vestibuloocular reflex. I. Secondary vestibular neurons. *J Neurophysiol* 70: 828–843, 1993.

Cullen KE, Rey CG, Guitton D, Galiana HL. The use of system identification techniques in the analysis of oculomotor burst neuron spike train dynamics. *J Comput Neurosci* 3: 347–368, 1996.

Cullen KE, Roy JE. Signal processing in the vestibular system during active versus passive head movements. *J Neurophysiol* 91: 1919–1933, 2004.

Dichgans J, Schmidt CL, Graf W. Visual input improves the speedometer function of the vestibular nuclei in the goldfish. *Exp Brain Res* 18: 319–322, 1973.

du Lac S, Lisberger SG. Membrane and firing properties of avian medial vestibular nucleus neurons in vitro. *J Comp Physiol [A]* 176: 641–651, 1995.

Dutia MB, Johnston AR. Development of action potentials and apamin-sensitive after-potentials in mouse vestibular nucleus neurones. *Exp Brain Res* 118: 148–154, 1998.

Erickson RG, Barmack NH. A comparison of the horizontal and vertical optokinetic reflexes of the rabbit. *Exp Brain Res* 40: 448–456, 1980.

Escudero M, de la Cruz RR, Delgado-Garcia JM. A physiological study of vestibular and prepositus hypoglossi neurones projecting to the abducens nucleus in the alert cat. *J Physiol* 458: 539–560, 1992.

Faulstich BM, Onori KA, du Lac S. Comparison of plasticity and development of mouse optokinetic and vestibulo-ocular reflexes suggests differential gain control mechanisms. *Vision Res* 44: 3419–3427, 2004.

Faulstich M, van Alphen AM, Luo C, du Lac S, De Zeeuw CI. Oculomotor plasticity during vestibular compensation does not depend on cerebellar LTD. *J Neurophysiol* 96: 1187–1195, 2006.

Fuchs AF, Kimm J. Unit activity in vestibular nucleus of the alert monkey during horizontal angular acceleration and eye movement. *J Neurophysiol* 38: 1140–1161, 1975.

Gittis AH, du Lac S. Intrinsic and synaptic plasticity in the vestibular system. *Curr Opin Neurobiol* 16: 385–390, 2006.

Goldberg JM, Fernandez C. Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. I. Resting discharge and response to constant angular accelerations. *J Neurophysiol* 34: 635–660, 1971.

Grusser-Cornehls U, Luy M, Baurle J. Electrophysiology and GABA-immunocytochemistry in the vestibular nuclei of normal (C57BL/6J) and Leaner mutant mice. *Brain Res* 703: 51–62, 1995.

Hayes AV, Richmond BJ, Optican LM. A UNIX-based multiple process system for real-time data acquisition and control. *WESCON Conf Proc* 2: 1–10, 1982.

Hess BJ, Precht W, Reber A, Cazin L. Horizontal optokinetic ocular nystagmus in the pigmented rat. *Neuroscience* 15: 97–107, 1985.

Huterer M, Cullen KE. Vestibuloocular reflex dynamics during high-frequency and high-acceleration rotations of the head on body in rhesus monkey. *J Neurophysiol* 88: 13–28, 2002.

Iwashita M, Kanai R, Funabiki K, Matsuda K, Hirano T. Dynamic properties, interactions and adaptive modifications of vestibulo-ocular reflex and optokinetic response in mice. *Neurosci Res* 39: 299–311, 2001.

Johnston AR, MacLeod NK, Dutia MB. Ionic conductances contributing to spike repolarization and after-potentials in rat medial vestibular nucleus neurones. *J Physiol* 481: 61–77, 1994.

Kassardjian CD, Tan YF, Chung JY, Heskin R, Peterson MJ, Broussard DM. The site of a motor memory shifts with consolidation. *J Neurosci* 25: 7979–7985, 2005.

Katoh A, Kitazawa H, Itohara S, Nagao S. Dynamic characteristics and adaptability of mouse vestibulo-ocular and optokinetic response eye movements and the role of the flocculo-olivary system revealed by chemical lesions. *Proc Natl Acad Sci USA* 95: 7705–7710, 1998.

Katoh A, Yoshida T, Himeshima Y, Mishina M, Hirano T. Defective control and adaptation of reflex eye movements in mutant mice deficient in either the glutamate receptor delta2 subunit or Purkinje cells. *Eur J Neurosci* 21: 1315–1326, 2005.

Kaufman GD, Shinder ME, Perachio AA. Convergent properties of vestibular-related brain stem neurons in the gerbil. *J Neurophysiol* 83: 1958–1971, 2000.

Keller EL, Daniels PD. Oculomotor related interaction of vestibular and visual stimulation in vestibular nucleus cells in alert monkey. *Exp Neurol* 46: 187–198, 1975.

Keller EL, Precht W. Visual-vestibular responses in vestibular nuclear neurons in the intact and cerebellectomized, alert cat. *Neuroscience* 4: 1599–1613, 1979.

Killian JE, Baker JF. Horizontal vestibuloocular reflex (VOR) head-velocity estimation in Purkinje cell degeneration (pcd/pcd) mutant mice. *J Neurophysiol* 87: 1159–1164, 2002.

Kimpo RR, Boyden ES, Katoh A, Ke MC, Raymond JL. Distinct patterns of stimulus generalization of increases and decreases in VOR gain. *J Neurophysiol* 94: 3092–3100, 2005.

Lannou J, Cazin L, Precht W, Toupet M. Optokinetic, vestibular, and optokinetic-vestibular responses in albino and pigmented rats. *Pfluegers* 393: 42–44, 1982.

Lasker D, Park H, and Minor L. Extracellular recordings from vestibular-nerve afferents in normal C57BL/6 mouse. *Midwinter Mtg Assoc for Research in Otolaryngology,* Daytona Beach, FL, #849. 2004.

Lisberger SG, Miles FA. Role of primate medial vestibular nucleus in long-term adaptive plasticity of vestibuloocular reflex. *J Neurophysiol* 43: 1725–1745, 1980.

Lisberger SG, Pavelko TA, Broussard DM. Neural basis for motor learning in the vestibuloocular reflex of primates. I. Changes in the responses of brain stem neurons. *J Neurophysiol* 72: 928–953, 1994.

McCrea RA, Gdowski GT, Boyle R, Belton T. Firing behavior of vestibular neurons during active and passive head movements: vestibulo-spinal and other non-eye-movement related neurons. *J Neurophysiol* 82: 416–428, 1999.

McCrea RA, Strassman A, May E, Highstein SM. Anatomical and physiological characteristics of vestibular neurons mediating the horizontal vestibulo-ocular reflex of the squirrel monkey. *J Comp Neurol* 264: 547–570, 1987.

McFarland JL, Fuchs AF. Discharge patterns in nucleus prepositus hypoglossi and adjacent medial vestibular nucleus during horizontal eye movement in behaving macaques. *J Neurophysiol* 68: 319–332, 1992.

Miles FA. Single unit firing patterns in the vestibular nuclei related to voluntary eye movements and passive body rotation in conscious monkeys. *Brain Res* 71: 215–224, 1974.

Nelson AB, Krispel CM, Sekirnjak C, du Lac S. Long-lasting increases in intrinsic excitability triggered by inhibition. *Neuron* 40: 609–620, 2003.

Neverov VP, Sterc J, Bures J. Electrophysiological correlates of the reversed postoptokinetic nystagmus in the rabbit: activity of vestibular and floccular neurons. *Brain Res* 189: 355–367, 1980.

Niven JE, Anderson JC, Laughlin SB. Fly photoreceptors demonstrate energy-information trade-offs in neural coding. *PLoS Biol* 5: e116, doi: 10.1371/journal. Pbio.0050116, 2007.

Petrosini L, Troiani D. Optic nystagmus and vestibular nuclei: unitary activity of vestibular neurons during nystagmus. *Exp Neurol* 60: 337–346, 1978.

Reisine H, Raphan T. Neural basis for eye velocity generation in the vestibular nuclei of alert monkeys during off-vertical axis rotation. *Exp Brain Res* 92: 209–226, 1992.

Ris L, de Waele C, Serafin M, Vidal PP, Godaux E. Neuronal activity in the ipsilateral vestibular nucleus following unilateral labyrinthectomy in the alert guinea pig. *J Neurophysiol* 74: 2087–2099, 1995.

Ris L, Hachemaoui M, Vibert N, Godaux E, Vidal PP, Moore LE. Resonance of spike discharge modulation in neurons of the guinea pig medial vestibular nucleus. *J Neurophysiol* 86: 703–716, 2001.

Robinson DA. The mechanics of human saccadic eye movement. *J Physiol* 174: 245–264, 1964.

Roy JE, Cullen KE. A neural correlate for vestibulo-ocular reflex suppression during voluntary eye-head gaze shifts. *Nat Neurosci* 1: 404–410, 1998.

Roy JE, Cullen KE. Selective processing of vestibular reafference during self-generated head motion. *J Neurosci* 21: 2131–2142, 2001.

Roy JE, Cullen KE. Vestibuloocular reflex signal modulation during voluntary and passive head movements. *J Neurophysiol* 87: 2337–2357, 2002.

Roy JE, Cullen KE. Brain stem pursuit pathways: dissociating visual, vestibular, and proprioceptive inputs during combined eye-head gaze tracking. *J Neurophysiol* 90: 271–290, 2003.

Schmucker C, Seeliger M, Humphries P, Biel M, Schaeffel F. Grating acuity at different luminances in wild-type mice and in mice lacking rod or cone function. *Invest Ophthalmol Vis Sci* 46: 398–407, 2005.

Scudder CA, Fuchs AF. Physiological and behavioral identification of vestibular nucleus neurons mediating the horizontal vestibuloocular reflex in trained rhesus monkeys. *J Neurophysiol* 68: 244–264, 1992.

Sekirnjak C, du Lac S. Intrinsic firing dynamics of vestibular nucleus neurons. *J Neurosci* 22: 2083–2095, 2002.

Sekirnjak C, du Lac S. Physiological and anatomical properties of mouse medial vestibular nucleus neurons projecting to the oculomotor nucleus. *J Neurophysiol* 95: 3012–3023, 2006.

Sekirnjak C, Vissel B, Bollinger J, Faulstich M, du Lac S. Purkinje cell synapses target physiologically unique brainstem neurons. *J Neurosci* 23: 6392–6398, 2003.

Serafin M, de Waele C, Khateb A, Vidal PP, Muhlethaler M. Medial vestibular nucleus in the guinea pig. I. Intrinsic membrane properties in brainstem slices. *Exp Brain Res* 84: 417–425, 1991.

Serafin M, Ris L, Bernard P, Muhlethaler M, Godaux E, Vidal PP. Neuronal correlates of vestibulo-ocular reflex adaptation in the alert guinea pig. *Eur J Neurosci* 11: 1827–1830, 1999.

Shutoh F, Katoh A, Kitazawa H, Aiba A, Itohara S, Nagao S. Loss of adaptability of horizontal optokinetic response eye movements in mGluR1 knockout mice. *Neurosci Res* 42: 141–145, 2002.

Shutoh F, Ohki M, Kitazawa H, Itohara S, Nagao S. Memory trace of motor learning shifts transsynaptically from cerebellar cortex to nuclei for consolidation. *Neuroscience* 139: 767–777, 2006.

Sklavos S, Porrill J, Kaneko CR, Dean P. Evidence for wide range of time scales in oculomotor plant dynamics: implications for models of eye-movement control. *Vision Res* 45: 1525–1542, 2005.

Sontheimer H, Hoffmann KP. Horizontal optokinetic reflex in light reared and dark reared Israelian gerbils (*Meriones tristrami*). *Exp Brain Res* 66: 440–444, 1987.

Stahl JS. Using eye movements to assess brain function in mice. *Vision Res* 44: 3401–3410, 2004.

Stahl JS, James RA, Oommen BS. Comparative dynamics of abducens nucleus neurons in awake mice, rabbits, and monkeys. *Soc Neurosci Abstr* 345.14, 2006.

Stahl JS, Simpson JI. Dynamics of abducens nucleus neurons in the awake rabbit. *J Neurophysiol* 73: 1383–1395, 1995.

Stahl JS, van Alphen AM, De Zeeuw CI. A comparison of video and magnetic search coil recordings of mouse eye movements. *J Neurosci Methods* 99: 101–110, 2000.

Straka H, Vibert N, Vidal PP, Moore LE, Dutia MB. Intrinsic membrane properties of vertebrate vestibular neurons: function, development and plasticity. *Prog Neurobiol* 76: 349–392, 2005.

Sylvestre PA, Cullen KE. Quantitative analysis of abducens neuron discharge dynamics during saccadic and slow eye movements. *J Neurophysiol* 82: 2612–2632, 1999.

Takazawa T, Saito Y, Tsuzuki K, Ozawa S. Membrane and firing properties of glutamatergic and GABAergic neurons in the rat medial vestibular nucleus. *J Neurophysiol* 92: 3106–3120, 2004.

Takemura K, King WM. Vestibulo-collic reflex (VCR) in mice. *Exp Brain Res* 167: 103–107, 2005.

Tauber ES, Atkin A. Optomotor responses to monocular stimulation: relation to visual system organization. *Science* 160: 1365–1367, 1968.

Tomlinson RD, Robinson DA. Signals in vestibular nucleus mediating vertical eye movements in the monkey. *J Neurophysiol* 51: 1121–1136, 1984.

van Alphen AM, Stahl JS, De Zeeuw CI. The dynamic characteristics of the mouse horizontal vestibulo-ocular and optokinetic response. *Brain Res* 890: 296–305, 2001.

van Alphen AM, De Zeeuw CI. Cerebellar LTD facilitates but is not essential for long-term adaptation of the vestibulo-ocular reflex. *Eur J Neurosci* 16: 486–490, 2002.

Vidal PP, Degallaix L, Josset P, Gasc JP, Cullen KE. Postural and loco-motor control in normal and vestibularly deficient mice. *J Physiol* 559: 625–638, 2004.

Vinje WE, Gallant JL. Sparse coding and decorrelation in primary visual cortex during natural vision. *Science* 287: 1273–1276, 2000.

Waespe W, Henn V. Neuronal activity in the vestibular nuclei of the alert monkey during vestibular and optokinetic stimulation. *Exp Brain Res* 27: 523–538, 1977a.

Waespe W, Henn V. Vestibular nuclei activity during optokinetic after-nystagmus (OKAN) in the alert monkey. *Exp Brain Res* 30: 323–330, 1977b.

Wearne S, Raphan T, Cohen B. Control of spatial orientation of the angular vestibuloocular reflex by the nodulus and uvula. *J Neurophysiol* 79: 2690–2715, 1998.

Wilson VJ, Yamagata Y, Yates BJ, Schor RH, Nonaka S. Response of vestibular neurons to head rotations in vertical planes. III. Response of vestibulocollic neurons to vestibular and neck stimulation. *J Neurophysiol* 64: 1695–1703, 1990.

Wong AA, Brown RE. Age-related changes in visual acuity, learning and memory in C57BL/6J and DBA/2J mice. *Neurobiol Aging* doi:10.106/j.neurobiolaging.2006.07.023.2006.