Responses of ventral posterior thalamus neurons to three-dimensional vestibular and optic flow stimulation

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Running Head: Vestibular and optic flow convergence in the macaque thalamus

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Abstract

Multisensory neurons tuned to both vestibular and visual motion (optic flow) signals are found in several cortical areas in the dorsal visual stream. Here we examine whether such convergence occurs subcortically in the macaque thalamus. We searched the ventral posterior nuclei, including the anterior pulvinar, as well as the ventro-lateral and ventral posterior lateral nuclei, areas that receive vestibular signals from brainstem and deep cerebellar nuclei. Approximately a quarter of cells responded to three-dimensional (3D) translational and/or rotational motion. More than half of the responsive cells were convergent, thus responded during both rotation and translation. The preferred axes of translation/rotation were distributed throughout 3D space. The majority of the neurons were excited, but some were inhibited, during rotation/translation in darkness. Only a couple of neurons were multisensory, being tuned to both vestibular and optic flow stimuli. We conclude that multisensory vestibular/optic flow neurons, which are commonly found in cortical visual and visuomotor areas, are rare in the ventral posterior thalamus.
Introduction

Unlike other sensory systems, the thalamo-cortical processing of vestibular information remains poorly understood. In monkeys, vestibular-responsive cells have been identified in and around the primary somatosensory cortex, specifically area 2v (Büttner and Buettner, 1978) and the arm/neck regions of area 3a (Guldin et al., 1992; Guldin and Grüsser, 1998; Odkvist et al., 1974). Grüsser and colleagues have subsequently identified the parieto-insular vestibular cortex (PIVC) as an additional cortical vestibular area (Grüsser et al., 1982; 1990a,b; Guldin et al., 1992). The PIVC in macaques lies in the retroinsular cortex, at and around the tip of the lateral sulcus and adjacent or overlapping with the secondary somatosensory cortex (Chen et al. 2010).

In recent years, vestibular signals have also been identified in areas of the dorsal visual stream and gaze-related, visuo-motor cortical areas. In particular, vestibular modulation has been described in the frontal eye fields (FEF), including the frontal pursuit area (Ebata et al. 2004; Fukushima et al. 2000), the ventral intraparietal area (VIP) (Bremmer et al., 2002; Chen et al. 2007; Klam and Graf, 2003; Schlack et al., 2002) and the dorsal medial superior temporal area (MSTd) (Duffy 1998; Page and Duffy 2003; Gu et al. 2006; 2007). Many neurons in FEF/MSTd/VIP are multimodal; they are spatially tuned during both motion in darkness and optic flow stimulation (Bremmer et al. 2002; Duffy 1998; Gu et al. 2006; 2007; 2008; Page and Duffy 2003; Takahashi et al. 2007). These visual and visuomotor cortical areas responsive to vestibular stimuli are also tuned to optic flow, that is the complex visual motion patterns that are experienced during self-motion, including contraction, expansion and spiral optic flow (Duffy and Wurtz 1995; Graziano et al. 1994).

The anatomical routes by which vestibular signals reach these extrastriate, visuo-motor and somatosensory-related cortical areas have not been well characterized. In contrast to other sensory and motor signals that are found in discrete areas of the thalamus, vestibular termination zones are patchy and less well organized (Büttner and Henn 1976; Büttner et al., 1977; Magnin and Fuchs 1977; Lang et al. 1979; Meng et al. 2001; 2007). Using both physiological and neuroanatomical techniques, recent studies in macaques and squirrel monkeys have identified the ventral posterior lateral (VPL)
and the ventral lateral (VL) nuclei, as well as the anterior pulvinar as the main routes for vestibular signals to the cortex (Meng et al. 2007; Marlinski and McCrea, 2008a,b). The core region where vestibular neurons were encountered on either side of the somatosensory VPL and somatomotor VL were shown to receive inputs from both vestibular (Asanuma et al., 1983; Lang et al., 1979; Meng et al. 2007) and deep cerebellar nuclei (Asanuma et al., 1983; Batton et al., 1977; Middleton and Strick 1997; Meng et al. 2007; Sakai et al., 1996). The rostral and dorsal VPL is thought to project to area 3a, while PIVC receives its main thalamic input from caudal VPL and from the anterior pulvinar (Akbarian et al., 1992). Projections from the VL could also transfer vestibular signals to premotor/motor cortex, as well as the FEF (Huerta et al., 1986).

How vestibular information reaches other cortical areas responsive to rotation and translation stimuli in darkness remains unknown. Optic flow signals in MSTd, VIP and FEF are thought to originate from earlier visual motion areas, in particular the medial temporal area (MT) (Baizer et al. 1991; Desimone and Ungerleider 1986; Grant and Hilgetag 2005). However, neurons in MT do not respond to vestibular stimulation (Chowdhury et al. 2009). Thus, it is possible that the convergence of vestibular and optic flow signals occurs first in MSTd/VIP/FEF. Alternatively, it is also possible that the visual/optic flow convergence seen in dorsal stream visual areas could have occurred already at the level of vestibular neurons in the brainstem and deep cerebellar nuclei. Thus, it could also represent a property of thalamic neurons carrying vestibular signals to the cortex.

In support of the latter hypothesis, convergence between vestibular and optokinetic signals is relatively common for neurons in the vestibular nuclei (Bryan and Angelaki 2009; Waespe and Henn 1977a,b), deep cerebellar nuclei (Bryan and Angelaki 2009; Büttner et al. 1991) and has also been reported in the ventral posterior nuclei of the thalamus (Büttner and Henn 1976; Büttner et al. 1977). However, the types of optic flow stimuli used to characterize visual/vestibular neurons in the dorsal visual stream (Duffy and Wurtz 1995; Graziano et al. 1994; Gu et al. 2006) are very different from the optokinetic stimuli used to characterize vestibular neurons in past studies. The traditional optokinetic stimuli consist of large white/black dots or bars on the inner surface of a sphere or ‘drum’ that rotates around the subject. Optokinetic stimuli are
optimized to generate reflexive eye movements, thus they typically are characterized by low spatial frequency, a factor that is critical for eliciting optokinetic nystagmus when the field of view is large (Schor and Narayan 1981). Importantly, only low temporal frequency stimuli drive vestibular neurons. For example, cells in the vestibular nuclei only modulate during low-frequency optokinetic stimulation; frequencies >= 0.5 Hz are ineffective (Boyle et al. 1985; Bryan and Angelaki 2009). In contrast, the visual stimuli traditionally used to characterize cortical neurons in the dorsal visual stream consist of the complex optic flow patterns experienced during egomotion, including expansion (simulating forward egomotion) and contraction (simulating backward egomotion). These responses are robust even with optic flow stimuli at 0.5-1 Hz (Gu et al. 2006). To date, brainstem and cerebellar neurons, known to respond to low frequency optokinetic stimulation, have never been tested with the optic flow stimuli used to characterize visual motion responses in the dorsal visual stream. Thus, it is presently unknown whether the vestibular/optic flow convergence seen in cortical areas is shared by subcortical neurons.

There were two goals for this study. The first goal was to record from vestibular-responsive neurons in the ventral posterior thalamus (VP) and investigate whether they are also tuned to optic flow. To achieve this goal, it was important that we use optic flow stimuli identical to those driving neurons in the dorsal visual stream (Chen et al. 2007; Gu et al. 2006). Because our goal was to examine vestibular/optic flow convergence, and not optic flow responsiveness alone, we first identified neurons that were responsive to vestibular stimulation and then tested those neurons with optic flow. The second goal was to characterize thalamic neuron responses using three-dimensional (3D) motion stimuli. Note that previous studies were limited to yaw rotation and/or horizontal plane translation (Büttner and Henn 1976; Büttner et al., 1977; Magnin and Fuchs 1977; Marlinski and McCrea, 2008a,b; Meng et al. 2007). Thus, it was not possible to investigate the percent of neurons responsive to vestibular stimulation. For example, one could not exclude that a cell unresponsive to yaw rotation and horizontal plane translation might prefer vertical translation. Thus, by measuring neural responses during yaw, pitch and roll rotation, as well as fore-aft, lateral and vertical translation, and by recording from all well-isolated neurons, we identify how common vestibular
modulation is in the ventral posterior thalamus. Results of this work have appeared in abstract form (Meng and Angelaki 2008).

Methods

Experiments were performed on 2 juvenile macaque monkeys (Macaca mulatta). The animals were chronically instrumented with a circular molded, lightweight plastic ring, which was implanted in the stereotaxic horizontal plane and anchored to the skull using titanium inverted T-bolts and dental acrylic. For single-unit recording from the thalamus, a delrin platform was stereotaxically secured to the skull and fitted inside the head ring. The platform had staggered rows of holes (spaced 0.8 mm apart) that extended from the midline to the area overlying the thalamus bilaterally. All monkeys were also implanted with scleral coils for measuring eye movements in a magnetic field (details can be found in previous work: Meng et al. 2007; Gu et al. 2006). All surgical and experimental procedures were performed under sterile conditions, in accordance with the Institutional Animal Care and Use Committee at Washington University and National Institutes of Health guidelines.

Experimental setup: Motion platform and visual stimuli

Sinusoidal 3D motion was delivered using a six degrees of freedom motion platform (MOOG 6DOF2000E; East Aurora, NY). In all experiments, the head was positioned such that the horizontal stereotaxic plane was earth-horizontal, with the axis of rotation always centered in the middle of the interaural axis of the head. Visual stimuli were generated by a 3-chip DLP projector (Christie Digital Mirage 2000, Christie Digital systems, Cypress, CA) that was mounted on top of the motion platform and rear-projected images onto a 60 × 60 cm tangent screen that was viewed by the monkey from a distance of 30 cm (thus subtending 90 × 90° of visual angle; see Gu et al. 2006 for details). This projector incorporates special circuitry such that image updating is precisely time-locked to the vertical refresh of the video input (with a one-frame delay). The tangent screen was mounted on the front of the field coil frame. The sides and top
of the coil frame were covered with black enclosures such that the monkey’s field of view was restricted to visual stimuli presented on the screen.

Visual stimuli depicted movement of the animal through a 3D cloud of ‘stars’ that occupied a virtual space 100 cm wide, 100 cm tall, and 50 cm deep. Roughly 1500 stars were visible at any time within the field of view of the screen. Stereoscopic images were generated using an OpenGL accelerator board (nVidia Quadro FX 3000G). Visual stimuli were plotted with sub-pixel accuracy using hardware anti-aliasing. Accurate rendering of the optic flow, motion parallax, and size cues that accompanied translation of the monkey were achieved by plotting the star field in a 3D virtual workspace and by moving the OpenGL ‘camera’ through this space along the exact trajectory followed by the monkey’s head. Image resolution was 1280x1024 pixels, 32-bit color depth and refresh rate was 60 Hz (same as the platform trajectory). Dot density was 0.01/cm³, with each dot rendered as a 0.15cm × 0.15cm triangle. Dots were fixed in the environment, such that they changed in size as appropriate for simulating egomotion.

The display screen was located in the center of the star field before stimulus onset and remained well within the depth of the star field throughout the motion trajectory. To avoid extremely large (near) stars from appearing in the display, a near clipping plane was imposed such that stimulus elements within 5cm of the eyes were not rendered. Stimuli were presented stereoscopically (simulated by two OpenGL cameras separated by the inter-pupillary distance) as red and green anaglyphs, viewed through Wratten filters (red no. 29, green no. 61, Kodak, Rochester, NY) that were mounted on custom made goggles. The stimuli contained a variety of depth cues, including horizontal disparity, motion parallax and size information.

**Experimental protocols**

Extracellular recordings were obtained using epoxy-coated, etched tungsten microelectrodes using standard electrophysiological techniques. In this study, we directed all penetrations into the VP nuclei, whose location was identified based on previous experience (one animal was the same as that used by Meng et al. 2007). Typically we first identified the oculomotor nuclei and the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) based on their characteristic firing patterns.
during fixation, pursuit and saccadic eye movements. These two structures were then used as landmarks for guiding electrode penetrations into the ventral posterior thalamus. The riMLF, a small area (< 2 mm across) whose cells burst during vertical and torsional saccadic eye movements, is located just ventromedial to the ventral posterior complex (Fig. 1), the anatomical location of the vestibulothalamic projection zone (Lang et al., 1979). As the electrode was lowered into VP, sinusoidal rotations about the roll, pitch and yaw axes, and sinusoidal translations along the left-right, up-down and fore-aft axes were used to drive potentially silent neurons. We recorded from every VP cell that was well isolated. The order of the experimental protocols was as follows:

(1) Sinusoidal stimuli (vestibular only): All well-isolated neurons were first tested during: (1) 0.5 Hz, ±7° yaw, pitch and roll rotation; and (2) 0.5 Hz, ±10cm left-right, up-down and fore-aft translation in complete darkness. For these sinusoidal stimuli, data acquisition was controlled by Spike2 scripts and the CED system (Cambridge Electronic Design, Cambridge UK). All neurons presented here did not have eye movement sensitivity, as evidenced by lack of modulation (based on a permutation test; see Data analysis) during horizontal/vertical smooth pursuit (0.5 Hz, ±10°).

(2) Transient stimuli (vestibular and visual): Neurons that modulated during sinusoidal motion in darkness were further tested using an identical protocol to that used previously by Gu et al. (2006), Takahashi et al. (2007) and Chen et al. (2007) to characterize the vestibular and visual (optic flow) tuning in visual cortical areas MSTd and VIP. This protocol consisted of 4 stimulus conditions:

(a) A “vestibular translation protocol” consisted of straight (translational) movements along 26 directions corresponding to all combinations of azimuth and elevation angles in increments of 45°. This included all combinations of movement vectors having 8 different azimuth angles (0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315°) and 3 different elevation angles: 0° (the horizontal plane) and ±45° (8 × 3 = 24 directions). In addition, elevation angles of 90° and -90° were included to generate upward and downward movement directions, respectively (for a total of 26 motion directions).

(b) A “vestibular rotation protocol” consisted of rotations about the same 26 directions, each of which now represents the corresponding axis of rotation according to
the right hand rule. For example, azimuths of 270° and 90° (elevation = 0°) correspond
to pitch-up and pitch-down rotations, respectively. Azimuths of 0° and 180° (elevation =
0°) correspond to roll rotation (right-ear-down and left-ear-down, respectively). Finally,
elevation angles of 90° or -90° correspond to leftward or rightward yaw rotation,
respectively.

During both vestibular translation and rotation stimuli, the screen was blank,
except for a head-fixed target (fixation windows spanned 2 × 2° of visual angle) that the
animals had to foveate during motion.

(c) A “visual translation protocol”, where the motion platform was stationary, while
optic flow simulating translational egomotion through a 3D cloud of stars was presented
on the screen (see Takahashi et al. 2007; Gu et al. 2008 for details).

(d) A “visual rotation protocol”, where the motion platform was stationary, while
optic flow simulating rotational egomotion through a 3D cloud of stars was presented on
the screen. During both visual translation and visual rotation stimuli, animals had to
fixate (2 × 2°) the same head-fixed target. Note that all stimulus directions were
referenced to body motion (real or simulated). Thus, a neuron with similar preferred
directions in the visual and vestibular conditions would be considered ‘congruent’.

For both visual and vestibular stimuli, the motion had a Gaussian velocity profile
and a corresponding biphasic acceleration profile (2s duration). The translation
amplitude was 13 cm (total displacement), with a peak acceleration of ~0.1 G
(~0.98m/s²) and a peak velocity of ~30cm/s. The rotation amplitude was 9° and peak
angular velocity was 23.5°/s. The animals were rewarded at the end of each trial for
maintaining fixation throughout the stimulus presentation. If fixation was broken at any
time during the stimulus, the trial was aborted and the data were discarded. For the
transient visual/vestibular protocol, behavioral tasks and data acquisition were
controlled by a commercially available software package (Reflective Computing, St.
Louis, MO).

The translation and rotation protocols were delivered in separate blocks of trials.
Within each block, ‘vestibular’ and ‘visual’ stimuli were randomly interleaved, each
typically having 5 repetitions, along with a (null) condition in which the motion platform
remained stationary and no star field was shown. Note that we refer to the motion
stimuli as the ‘vestibular’ condition for simplicity, even though other extra-retinal signal contributions (e.g., from body proprioception) cannot be excluded.

Following termination of single unit recordings, three electrolytic lesions were made within the region of vestibularly responsive cells in one of the animals to verify the penetration sites and allow more precise reconstruction of cell location.

Data analysis
All data were analyzed offline using Matlab (Mathworks Inc., Natick, MA). We describe separately the analyses used for sinusoidal and transient responses.

Sinusoidal analyses: To assign a statistical significance to the response modulation for each neuron, we computed average response histograms (40 bins per cycle) and a ‘Fourier Ratio (FR)’, defined as the ratio of the fundamental over the maximum of the first 20 harmonics. The statistical significance of FR was then based on a permutation analysis. Briefly, the 40 response bins were shuffled randomly, thus destroying the systematic modulation in the data but maintaining the inherent variability of the responses. The FR was then computed from those randomly permuted histograms, and the randomization process was repeated 1,000 times. If the FR for the original data exceeded that for 99% of the permuted data sets, we considered the temporal modulation to be statistically significant (p<0.01).

For responses that passed this criterion, instantaneous firing rate (IFR) was calculated as 1/interspike interval and assigned to the middle of the interval. IFRs from multiple stimulus cycles were then folded in time into a single cycle instantaneous frequency response for each stimulus condition. This procedure provides no averaging, as all spike occurrences are represented in time (see Meng et al. 2005 for details). To compute the neural gain and phase during translation/rotation, a function consisting of the 1st and 2nd harmonics (plus DC offset) was fitted to both response and stimulus using a nonlinear least-squares algorithm (Levenberg-Marquardt method). Neuronal gain for translation was expressed relative to linear acceleration (in units of spikes/s/G where G=9.81 m/s²). For rotations, neuronal gain was expressed relative to head velocity (in units of spikes/s/°/s).
The preferred direction in 3D was computed using a spatiotemporal model (Angelaki, 1991; 1992; Schor and Angelaki 1992). This was done by first computing the preferred direction in the horizontal plane. The polar angle of this vector defined the azimuth of the 3D preferred response. Then we computed its elevation by applying the spatiotemporal model in a vertical plane defined by the preferred direction in the horizontal plane and the vertical axis. Based on this analysis, the following parameters were computed for the rotational and/or translational response of each cell: (1) the 3D preferred direction was quantified in spherical coordinates, using two angles: azimuth (defined in the range of [-180, 180]) and elevation (range: [-90, 90]); (2) the gain and phase of the cell during stimulation along the preferred direction.

To determine whether a measured distribution was significantly different from uniform, we performed a re-sampling analysis (Takahashi et al. 2007). First, we calculated the sum squared error (across bins) between the measured distribution and an ideal uniform distribution containing the same number of observations. Next, we computed the sum squared error between the ideal uniform distribution and a random distribution generated by drawing the same number of data points from a uniform distribution. This second step was repeated 1000 times to generate a distribution of sum squared error values that represent random deviations from an ideal uniform distribution. If the sum squared error for the experimentally measured distribution laid outside the 95% confidence interval of values from the randomized distributions, then the measured distribution was considered to be significantly different from uniform (p < 0.05). For non-uniform distributions, a multi-modality test based on the kernel density estimate method was used to further assess the number of modes in a distribution (see Takahashi et al. 2007). The test gave a p-value (puni) for unimodality. If puni was > 0.05, the distribution was unimodal; If puni was < 0.05, unimodality was rejected (in which case, bimodality can be tested next; see Takahashi et al. 2007 for details).

**Transient response analyses:** To analyze neural responses to transient motion stimuli, we first constructed peristimulus time histograms (PSTHs) for each direction of translation and/or rotation. PSTHs for all 26 directions were computed using 25 ms time bins smoothed with a 400 ms boxcar filter. The following procedure was used to identify and classify PSTHs with significant temporal modulation. First, we computed the
distribution of spike counts (across repetitions) for each possible 400ms time window (in
25 ms steps) between 500-2000 ms after the onset of the stimulus. Each of these
distributions was then compared to a baseline response distribution, obtained from the
400ms time window from -100 to 300ms post-stimulus onset (an interval in which
stimulus velocity and acceleration has changed little), using the Wilcoxon signed rank
test (p<0.01). We then identified the 400ms time windows that passed the significance
test and contained the maximum (peak) and minimum (trough) spike counts. To avoid
false positives, we required at least 4 overlapping windows, spaced 25 ms apart, to be
significantly different from baseline.

This statistical test thus identified a significant peak and/or trough in the PSTH for
each stimulus condition/direction (if they existed). Accordingly, responses along each of
the 26 different directions were divided into inhibitory (only significant trough, not
significant peak) and excitatory (significant peak); otherwise the cell was considered
unresponsive for stimulation in that direction. For a neuron to be considered to have
significant temporal modulation in response to the transient stimuli, it had to show
significant excitatory or significant inhibitory responses to at least two nearby directions
(45 deg apart in azimuth or elevation). Neurons not meeting this criterion were
considered “unresponsive” (if no two nearby directions had significant modulation).

For cells that were considered responsive to 3D rotation or translation, the
statistical significance of their spatial tuning was assessed using one-way ANOVA. For
visualization of the data, mean responses were plotted as a function of azimuth and
elevation to create 3D tuning functions. To plot these spherical data onto Cartesian
axes, the data were transformed using the Lambert cylindrical equal-area projection
(see Gu et al. 2006 for details). This produced flattened representations where the
abscissa represents azimuth angle, and the ordinate corresponds to a sinusoidally
transformed version of the elevation angle.

Fig. 1 illustrates the histological reconstruction of vestibular cells from the animal
with the electrolytic lesions (filled up triangles/circles; open circles show cells not
responding to vestibular stimulation). Each cell location was reconstructed according to
the relationship between its recorded position (based on the predrilled recording grid
hole and micromanipulator depth reading) and the location of the borders of the riMLF.
and oculomotor nuclei, as well as the electrolytic lesions (two of the three are marked with arrows and asterisks in Fig. 1). Down triangles in Fig. 1 illustrate the cells recorded in a second animal. Because no electrolytic lesions were made in this case, reconstruction was based on stereotaxic coordinates and the borders of riMLF and oculomotor nuclei. In identifying the respective nuclei in the thalamus, we followed the procedure outlined by Meng et al. (2007).

Results

We recorded from all well-isolated neurons in the ventral posterior thalamus (abbreviated here as VP). Recordings were centered within the VL, VPL and anterior pulvinar. The anterior extent of the cells with vestibular responses reached the borders between VL, the intralaminar (centro-lateral, CL) nuclei and ventral anterior (VA) nuclei (Fig. 1). Vestibular cells extended posteriorly to the borders of anterior pulvinar with the posterior nuclei (PO) and the lateral pulvinar (lPul). One hundred and sixty two neurons in VP were tested during sinusoidal vestibular stimulation in darkness (Fig. 2A). Of these, 44 (27.2%) cells showed significant modulation (permutation test, p<0.01, see Methods) for at least one motion stimulus (yaw, pitch or roll rotation, as well as left-right, up-down or fore-aft translation). More than half (24; 55%) of the responsive cells were “convergent” neurons, i.e., they modulated during both rotation and translation (Fig. 2B). A smaller percentage of neurons only modulated during rotation (12; 27%) or translation (8; 18%) (Fig. 2B).

For cells with significant modulation to rotation about or translation along at least one axis in 3D space, we computed their preferred direction (see Methods). The distributions of response gains along the computed preferred direction are shown in Fig. 3A and 3B for translation and rotation, respectively. Gains averaged 203±219 (SD) spikes/s/G for translation and 0.77±0.71 spikes/s per º/s for rotation. The corresponding azimuth and elevation angles of the preferred directions have been plotted both as a scatter plot and as marginal distributions in Fig. 3C and 3D. Distributions of preferred directions were uniform (uniformity test, p>0.05), suggesting that all motion directions were equally represented in the population. The only exception was the distribution of
rotation elevation angles, which were not uniformly distributed (uniformity test, p=0.003); Instead, the distribution was unimodal (modality test, $p_{uni}=0.55$), with most cells preferring earth-horizontal axis rotations (e.g., pitch and roll, Fig. 3D).

There was no relationship between the preferred direction of translation in the horizontal plane and the preferred rotation axis in the horizontal plane, as illustrated graphically by the scatter plot in Fig. 2C. Instead, the distribution of the difference in preferred azimuth for the rotation and translation preferences of convergent cells was uniform (uniformity test, $p>0.05$; Fig. 2C, marginal distribution along the diagonal). That is, cells that preferred fore-aft motion did not necessarily prefer pitch rotations and cells that preferred left-right translation did not necessarily prefer roll rotations.

Neurons that modulated during sinusoidal rotation or translation were further tested during an interleaved visual (optic flow) and vestibular protocol, similar to that used to characterize multimodal MSTd neurons (Gu et al. 2006; Takahashi et al. 2007; see Methods). Fig. 4 illustrates responses to these transient vestibular/visual stimuli for an example neuron that was significantly tuned to both. The PSTHs in Fig. 4A and 4C show the vestibular and visual responses, respectively, and are plotted separately for each motion direction, as defined by the corresponding azimuth (abscissa) and elevation (ordinate). To quantify the spatial tuning in 3D, we computed mean firing rates for each stimulus direction during the 400 ms interval with the largest amplitude, as determined along the stimulus direction for which the cell fired maximally (Fig. 4A and 4C, red asterisks; the 400-ms interval for which mean firing rate was computed for all motion directions is also illustrated with vertical gray bars).

These mean firing rates were then used to compute 3D spatial maps, illustrated as contour plots of mean firing rate as a function of azimuth (abscissa) and elevation (ordinate) in Fig. 4B and 4D for vestibular and visual stimulation, respectively. During translation (Fig. 4A and 4B), the cell preferred leftward motion under the vestibular condition, but rightward motion during optic flow (Note that stimulus directions are referenced to the body’s motion, be it real or visually-simulated). During rotation (Fig. 4C and 4D), the cell preferred leftward yaw rotation in the vestibular stimulus condition and rightward yaw rotation during optic flow. Thus, visual and vestibular responses in this cell would be incongruent under natural stimulus conditions (Gu et al. 2006).
The visual tuning in Fig. 4C,D was not a typical finding in the ventral posterior thalamus. The majority of cells responding during vestibular stimulation were not responsive to optic flow (Fig. 5A, B). More than two thirds of the cells did not pass the criteria for temporal modulation, and this was true independently of whether the optic flow stimulus simulated translation or rotation. A smaller number of cells (5 for translation and 4 for rotation) were inhibited along most or all directions. Other than the cell of Fig. 4, one additional vestibular rotation-sensitive cell was significantly-tuned under both visual rotation and visual translation. A third vestibular rotation-sensitive cell was significantly tuned during visual translation. Thus, congruent and opposite multimodal neurons, which are abundant in cortical visual and gaze-related areas, appear rare in the VP thalamus.

By comparison, approximately half of vestibular translation responses (12/28) were excitatory and spatially-tuned. Another 9 cells were “inhibited” and 7 were “unresponsive”, i.e., they did not pass the criteria for temporal modulation. During rotation, 10/29 neurons were excitatory and had significant 3D tuning. Of the remaining, 14 cells were “unresponsive” (no temporal modulation), whereas 5 cells were “inhibited”. For the excitatory cells, the average vestibular PSTHs along the maximum response direction are illustrated in Fig. 6. For both translation (Fig. 6A) and rotation (Fig. 6B), each population response (thick lines) was computed by summing up each cell’s PSTH along the direction that produced the maximum response, after each 400 ms bin was normalized by the maximum bin (shown as thin lines). Note that some cells peaked at the time of peaked velocity, some much later, and some even had biphasic modulation, qualitatively resembling the biphasic profile of linear acceleration. Because of the small number of spatially-tuned excitatory cells, these properties have not been further quantified.

Discussion

There were two goals for this study. First, investigate whether vestibular neurons in the ventral posterior thalamus are tuned to optic flow. We found that few neurons were multisensory, being tuned to both vestibular and optic flow stimuli. Second,
characterize vestibular responses in thalamic neurons with three-dimensional (3D) stimuli. With the use of 3D stimuli and by recording from all well-isolated neurons, we found that about a quarter of cells in the ventral posterior thalamus modulate during vestibular stimulation.

Although some of the properties of these neurons have been recently characterized in both macaques (Meng et al. 2007) and squirrel monkeys (Marlinski and McCrea, 2008a,b), none of these studies used 3D stimuli. The percent of responsive neurons identified here using quantitative criteria (see Methods) is higher than that reported previously using qualitative criteria (e.g., audible modulation; Meng et al. 2007; Marlinski and McCrea, 2008a,b) for at least two reasons. First, unlike previous studies where a larger area in the thalamus was explored, here we focused exclusively on VP, areas that were previously identified to have vestibular responsiveness (Meng et al. 2007). Second, for the first time we have used 3D rotation/translation, in contrast to previous studies which were all limited in the stimuli used to test neurons.

A little more than half (55%, which represent only 24 of the total 162 neurons) of the responsive cells were convergent neurons, i.e., they modulated during both rotation and translation. The preferred directions of vestibular cells in the thalamus were distributed throughout 3D space. However, there was no systematic relationship between the preferred directions during translation and rotation for axes of motion in the horizontal plane. That is, cells that preferred fore-aft motion did not necessarily prefer pitch rotations and cells that preferred left-right translation did not necessarily prefer roll rotations. Such lack of tilt-aligned rotation/translation preferences in the thalamus is consistent with macaque vestibular and fastigial/interposed nuclei neurons (Dickman and Angelaki 2002; Bryan and Angelaki 2009; Shaikh et al. 2005) and differ from those in lateral-eyed species (Angelaki et al. 1993; Bush et al. 1993).

Responsive neurons were further tested with transient 3D translational and rotational stimuli, where the motion stimulus consisted of a smooth linear or angular displacement. Excitatory responses that are spatially tuned comprise almost half of the sinusoidally-modulated neurons. The other half of the cells judged to be significantly modulated during sinusoidal stimulation were either inhibited along most directions or did not even pass the temporal modulation criterion during transient movements (see
Methods). Notably, the transient motion profile has a dominant frequency of 0.5 Hz (Fetsch et al. 2008); thus, the difference cannot be explained simply by differences in frequency. It is possible that steady-state sinusoidal stimuli are more likely to activate extra-vestibular (e.g., somatosensory) signals than the smooth transient displacements.

The most important finding in these experiments was the lack of widespread convergence between vestibular and optic flow signals in the ventral posterior thalamus. The optic flow stimuli used to search for vestibular/optic flow convergence was identical to those previously used to characterize cortical neurons (Chen et al. 2007; Gu et al. 2008; Takahashi et al. 2007). Few cells responded to both vestibular and optic flow stimuli (Fig. 5). Such rare occurrence of visual/vestibular convergence in VP contrasts with the large percentage of multimodal neurons in extrastriate visual cortical areas MSTd and VIP (Bremmer et al., 2002; Klam and Graf, 2003; Schlack et al., 2002; Duffy 1998; Page and Duffy 2003; Gu et al. 2006; 2007; 2008). However, it is similar to the vestibular nuclei, where vestibular/optic flow convergence is rare for neurons that are not sensitive to eye movements (Sheng Liu, Ayanna Bryan and Dora Angelaki, unpublished observations).

Unlike our findings, neuronal modulation to low frequency optokinetic stimulation (which elicits optokinetic nystagmus and optokinetic afternystagmus) has been reported in the vestibular nuclei (Boyle et al. 1985; Waespe and Henn 1977a, b; 1978) and fastigial nuclei (Büttner et al. 1991). Optokinetic responses have also been reported in vestibular-responding neurons in the thalamus (Büttner and Henn 1976; Büttner et al. 1977) and PIVC (Grusser et al. 1990a,b). However, it is important to differentiate the stimuli used here (3D optic flow) from the low frequency or constant velocity optokinetic stimulation that has been traditionally used to study visual/vestibular convergence in the past. The 3D optic flow stimuli used here simulated self-translation and self-rotation through space (see Methods) and were not optimized to elicit optokinetic nystagmus. Importantly, the optokinetic stimuli used in the past to test vestibular neurons differed from the rotational optic flow used here in several aspects: First, the optokinetic stimuli in past studies were optimized to elicit reflexive eye movements, thus were characterized by very low spatial frequency (Schor and Narayan 1981). By contrast, our stimuli when presented to animals allowed to freely move their eyes elicit little
optokinetic nystagmus. Second, our stimuli had a dominant frequency of ~0.5-1 Hz, although optokinetic stimuli that evoked responses from vestibular neurons in the past were typically either low frequency sinusoids or constant velocity motion. Finally, we recorded neuronal responses while the animal fixated a head-fixed target, thereby controlling for a consistent visual motion stimulus to the retina. By contrast, previous optokinetic studies reported neuronal modulation while the animals were allowed to generate optokinetic nystagmus.

Collectively, the present results and previous findings suggest that visual/vestibular convergence might depend critically on the nature of the visual motion stimulus. In particular, optokinetic/vestibular convergence might occur in the thalamus and vestibular nuclei, although such convergence might only apply to low spatial frequency visual motion that generates reflexive eye movements and low temporal frequency stimulation that activates velocity storage (Boyle et al. 1985; Bryan and Angelaki 2009; Buettner and Büttner 1979; Henn et al. 1974; Waespe and Henn 1977a,b).

It is important to point out that there is no known direct connectivity between VP and MSTd/VIP. Ventral posterior nuclei (e.g., VL, VPL, anterior pulvinar) project to motor and somatosensory cortices, including vestibular areas 3a, 2v and PIVC (Akbarian et al. 1992), but not to extrastriate dorsal stream areas (Jones 1987; 2000; Steriade et al. 1997). Instead, thalamic inputs to visual motion areas like MT, MSTd and VIP arise from the medial inferior pulvinar (Adams et al. 2000; Bender 1982; Boussaoud et al. 1992; Kaas and Lyon 2007). However, there are no projections from the vestibular or deep cerebellar nuclei to the visual pulvinar, thus it appears unlikely that vestibular/optic flow convergence takes place within neurons of the medial inferior pulvinar. Indeed, preliminary experiments in one of the animals, where electrode penetrations extended into the visual pulvinar, revealed no vestibular modulation (unpublished observations). Although this negative finding needs to be further verified in future experiments, the present results suggest that there is little convergence between vestibular and optic flow signals related to egomotion and heading perception in the ventral posterior thalamus.
References


Henn V, Young LR, Finley C. (1974) Vestibular nucleus units in alert monkeys are also influenced by moving visual fields. Brain Res 71:144-149.


Figure Legends

**Fig. 1** Reconstructed locations of responsive (filled circles/triangles) and unresponsive (open circles) thalamic neurons in representative frontal brain sections. Consecutive sections are arranged in rostral to caudal order. The numbers represent distance from the interaural line. Cells recorded bilaterally have been superimposed in the left half of each section. Up and down triangles represent data from the two animals (see Methods). Filled circles are used for multisensory cells (responding to both visual and vestibular stimulation); up/down triangles are used for cells responding to vestibular stimulation only. Black arrows and asterisks point to the electrolytic lesions (section 8.4 and 6.8). aPul: anterior pulvinar nucleus; CL: centrolateral nucleus; CM: central medial nucleus; HB: habenula; III: oculomotor nucleus; LD: lateral dorsal nucleus; lPul: lateral pulvinar nucleus; MD: mediodorsal nucleus; MG: medial geniculate nucleus; PF: parafascicular nucleus; PO: posterior nucleus; riMLF: rostral interstitial nucleus of the medial longitudinal fasciculus; RT: reticular thalamic nucleus; SG: suprageniculate; ST: subthalamic nucleus; VA: ventral anterior nucleus; VL: ventral lateral nucleus; VPI: ventral posterior inferior nucleus; VPL: ventral posterior lateral nucleus; VPM: ventral posterior medial nucleus; ZI: zona incerta.

**Fig. 2** Summary of vestibular responses. (A), (B) Percentages of thalamic cells responsive to 3D vestibular translation and/or rotation (C) Relationship between the translation and rotation preferred directions in the horizontal plane (azimuth angle) for convergent neurons. Dashed lines illustrate the 0º and ±90º differences between the preferred azimuth for rotation and translation (±90º differences would correspond to an alignment between pitch and fore-aft translation or roll and lateral translation). Also shown is the marginal distribution of the difference in preferred direction azimuth between rotation and translation.

**Fig. 3** Summary of 3D sinusoidal responses. (A) and (B) show the distributions of response gains along the 3D preferred direction for translation and rotation, respectively. (C) and (D) plot the corresponding preferred direction in 3D. The data are illustrated
using spherical coordinates (i.e., azimuth and elevation) plotted on Cartesian axes that represent the Lambert cylindrical equal-area projection of the spherical stimulus space. Histograms on top and right sides of each scatter plot show the marginal distributions. Cartoon drawings define the azimuth and elevation angles used to express the preferred direction.

**Fig. 4** An example cell with significant tuning during both the visual (optic flow) and vestibular stimulus conditions. (A) and (C) illustrate response PSTHs for each of 26 directions during translation and rotation, respectively. Red asterisks mark the maximum response directions. The corresponding peak-times are marked by gray bars. (B) and (D) show the corresponding color contour tuning (at peak time) for each stimulus condition.

**Fig. 5** Percentages of thalamic cells responsive to 3D optic flow (visual stimulation) simulating (A) translation and (B) rotation.

**Fig. 6** Normalized vestibular population responses to (A) translation and (B) rotation. Thin gray lines illustrate normalized responses of individual cells along the maximum response direction. Thick lines show population averages. Stimulus velocity and acceleration have been superimposed for comparison (smooth solid and dashed black lines).
Fig 1
Fig. 2

A
- Responsive
- Not Responsive

118 (73%)

B
- Convergent
- Rotation only
- Translation only

44 (27%)
12 (27%)
8 (18%)

24 (55%)

C
Azimuth, Rotation (deg)
Azimuth, Translation (deg)

Backward Right Forward Left Backward
Fig. 3

A. Translation

Translation Rotation AB

Elevation (deg) Azimuth (deg)

-180 -90 0 90 180

Number of cells

Gain (sp/s/G)

0 200 400 600 800 1000 1200

B. Rotation

Number of cells

Gain (sp/s/deg/s)

0 1 2 3 4

C. Elevation (deg)

Down Up

-90 90 0 -90 90

Azimuth (deg)

-180 -90 0 90 180

Number of cells

Gain (sp/s/deg/s)

0 1 2 3 4

D. Azimuth (deg)

Roll Pitch Roll Pitch Roll

Azimuth (deg)

-180 -90 0 90 180
Fig. 4

Translation

A

Vestibular

Visual

Azimuth (deg)

Elevation (deg)

50 sp/s

Azimuth (deg)

Elevation (deg)

2 s

Visual

50 sp/s

Azimuth (deg)

Elevation (deg)

2 s

Visual

Azimuth (deg)

Elevation (deg)

Translation

B

Rotation

C

Rotation

D

Rotation
Fig. 5

<table>
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<th>Category</th>
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<th>Inhibited-tuned</th>
<th>Inhibited-not tuned</th>
<th>Unresponsive</th>
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<td><strong>Translation</strong></td>
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<td>2 (7%)</td>
<td>3 (11%)</td>
<td>20 (71%)</td>
</tr>
<tr>
<td><strong>Rotation</strong></td>
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<td>2 (7%)</td>
<td>2 (7%)</td>
<td>23 (79%)</td>
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</tbody>
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**Visual**

A  

B
Fig. 6