Gravity orientation tuning in macaque anterior thalamus

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Gravity may provide a ubiquitous allocentric reference to the brain’s spatial orientation circuits. Here we describe neurons in the macaque anterior thalamus tuned to pitch and roll orientation relative to gravity, independently of visual landmarks. We show that individual cells exhibit two-dimensional tuning curves, with peak firing rates at a preferred vertical orientation. These results identify a thalamic pathway for gravity cues to influence perception, action and spatial cognition.

Our brain has adapted to living on earth, and gravity likely sculpts how we interact with the world. Gravity is sensed by the otolith organs of the inner ear, but gravity and inertial accelerations must be differentiated, and this is achieved by a distributed brainstem and cerebellar circuit1–4 using an internal model of the physical laws of nature5–7. We have hypothesized that gravity provides a global allocentric reference for spatial orientation7. Head direction (HD) cells form a ‘neuronal compass’ encoding head orientation in the horizontal plane8, as well as head orientation relative to vertical, as shown recently in the dorsal presubiculum of bats5. Here we searched for gravity-tuned cells in the macaque anterior thalamus, where HD cells are found in rodents8.

We recorded from 95 neurons (Supplementary Fig. 1a,b) while head-fixed macaques were passively rotated inside an independently movable spherical enclosure (Supplementary Fig. 1c and Supplementary Video 1). First we rotated animals in a horizontal plane with them oriented upright relative to gravity. Most neurons, including the example cell in Figure 1, were unmodulated in this condition (Fig. 1a and Supplementary Fig. 2). Next we tilted the rotation axis and the visual environment together 30° away from vertical, right-ear down (Fig. 1b). Despite identical visual surround, the cell now exhibited strong directional tuning peaking at −90° orientation in the visual reference frame, corresponding to nose-down orientation in the gravity reference frame. Note that clockwise (CW) and counterclockwise (CCW) responses were similar for this neuron.

We tested whether the tuning in Figure 1b was anchored to gravity. First we tilted the setup to the opposite direction (left-ear down) by −30° (Fig. 1c). This reversed the head orientation relative to gravity while the visual surround remained unchanged. The cell’s tuning shifted relative to the visual reference frame by 180° (now peaking at 90°), but maintained the nose-down tuning in the gravity reference frame8.

Figure 1 Neuronal responses during yaw rotations around upright and tilted axes. (a–d) Example neuron responses in light during yaw rotation around an earth-vertical axis, with the animal upright (a) and yaw rotations around axes tilted ±30° in light (b,c) and darkness (d). NU, nose up; ND, nose down; LED, left ear down; RED, right ear down. Top: the stimuli. Middle: neuronal responses as a function of head orientation relative to the visual environment (a–d, top axis, black) or relative to gravity (b–d, bottom axis, green). Bottom: head position in space. Raster plots and traces show individual spikes and average response during multiple cycles of CCW (red) and CW (blue) rotation. Neuron recorded in animal L, right hemisphere. Forty-eight of 95 neurons were significantly modulated. (e–h) Population responses (n = 48). (e,g) Resultant (R) vector length. (e) Comparison between +30° and −30° tilt. (g) Comparison between rotations in light and darkness. (f,h) Distributions of the absolute difference in preferred direction (PD) relative to gravity between +30° and −30° tilt (f) and rotations in light and darkness (h). Each cell appears twice in red and blue plots, for CCW and CW rotations, respectively.

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Received 21 March; accepted 23 September; published online 24 October 2016; doi:10.1038/nn.4423
reference frame. Next we found that the cell’s tuning was maintained during rotation in complete darkness (Fig. 1d), further demonstrating that it was independent of visual cues. Third, we tested the cell during ±30° pitch and roll rotations (Supplementary Fig. 3a,b). The firing rate increased in the nose-down condition (forward pitch), consistent with results in the tilted yaw protocols. Thus, the cell exhibited a consistent tuning to orientation relative to gravity, regardless of visual cues and of the particular rotation plane being used to bring the animal into that orientation.

We analyzed 48 cells that were significantly tuned during tilted rotations. The population exhibited similar tuning strengths during +30° and −30° tilted yaw rotations (paired Wilcoxon rank test, P = 0.2; Fig. 1e) and aligned preferred direction (PD; |APD| < 45° in 83% of the cells; Fig. 1f). Both tuning strength and PD were identical when measured in darkness and light (Fig. 1g,h, P = 0.5). Furthermore, tuning strength and PD during vertical (pitch or roll) plane rotations were similar to those during tilted yaw rotation (Supplementary Fig. 3c–f).

Neuronal responses were also identical when only the rotation axis, and not the visual environment, was tilted (Supplementary Fig. 4). Collectively, these results demonstrate that neuronal responses were anchored to gravity and persisted in complete darkness.

Figure 1 demonstrates a prominent cell type that was tuned to gravity. In this cell type, CCW and CW responses were similar. Other cells exhibited comparable modulation but distinct preferred directions for CCW and CW rotations (Fig. 2a,b and Supplementary Fig. 5), indicating that they also encoded gravity velocity (Online Methods). We separated gravity (G) and gravity velocity (dG) responses (Supplementary Fig. 6) and classified cells into G-tuned (n = 26, Fig. 2), dG-tuned (n = 14) and intermediate (G+dG cells, n = 8) groups. The G tuning preferred mostly the pitch plane (that is, nose up and nose down; Fig. 2d; 24 of 34 cells; χ² = 14.2, d.f. = 3, P = 0.003), whereas the dG components appeared uniformly distributed (Fig. 2d, χ² = 6, d.f. = 3, P = 0.1).

In a subset of cells (n = 31), neuronal responses were measured at tilt angles ranging from 5° to 45°. In many neurons, tuning strength peaked at an intermediate tilt angle (for example, ±15°; Fig. 3a). In contrast, the tuning strength of afferent signals from the otolith gravity sensors would increase with tilt (Supplementary Fig. 7).

The reconstructed two-dimensional tuning curve of this example cell (Fig. 3b; see also Supplementary Fig. 8) formed a hill of activity, with the firing rate peaking at a preferred orientation and tilt angle. The directions (nose up, nose down, ipsilateral or contralateral) of peak firing for G-tuned cells (Fig. 3c) were distributed broadly (circular R test, n = 18, P = 0.14) and G-tuned cells had preferred tilt angles uniformly distributed in this plane (Kolmogorov-Smirnov test, P > 0.2; Fig. 3d). That is, a larger fraction of neurons preferred 30–40° tilt, compared to 0–10° tilt, since the area of the former sector is larger than that of the latter. Too few dG-tuned neurons were included in this sample (Fig. 3c) to justify firm conclusions.

In summary, we have shown that neurons in the macaque anterior thalamus carry a gravity-anchored orientation signal that is independent of visual landmarks. These neurons fire maximally at a particular head orientation relative to gravity, in a way analogous to rodent azimuth-tuned HD cells, whose preferred direction is anchored to visual landmarks.

The gravity-tuned cells did not appear to be tuned to head azimuth; however, the tuning of HD cells is frequently suppressed during head-fixed rotation in rodents. It is therefore unknown whether, but possible that, some of these gravity-tuned cells are traditional azimuth-tuned HD cells.

We found that gravity orientation tuning is two-dimensional, and accordingly individual gravity-tuned neurons exhibited two-dimensional hill-shaped tuning curves. A quarter of spatially modulated cells also exhibited gravity-derivative (velocity) tuning, implying a distributed representation of the integration process. The most remarkable property of gravity tuning is that it is independent of the rotation plane (in egocentric coordinates) that changes head orientation: we found similar tuning relative to gravity for yaw rotations, on one hand, and pitch and roll rotations, on the other hand. Thus, the underlying neuronal circuits should integrate rotation velocity signals around all three egocentric (yaw, pitch, roll) axes simultaneously. Indeed, the circuity that produces an internal model of gravity in the cerebellum has this exact property.

On the basis of these findings, we propose that gravity-tuned cells may comprise the two vertical degrees of freedom of a three-dimensional orientation compass in the macaque thalamus. This hypothesis could also be consistent with the finding of vertically tuned HD cells in bats. Both species show a small dominance of pitch-tuned over roll-tuned cells. Since roll does not change the direction the head is facing, and neither macaques nor bats perform frequent roll head movements, it is possible that there is a selective adaptation for spatial orientation to be tuned mostly in the direction the head faces. Yet, although less frequent, roll-tuned cells are found in both species. The gravity signals identified here may also govern the updating of the reference frame of azimuth-tuned HD cells during three-dimensional movements.

![Figure 2](image-url) **Figure 2** Gravity (G) and gravity-derivative (velocity, dG) responses. Cells are classified as G-tuned (green), dG-tuned (cyan) and G+dG-tuned (gray) (see Supplementary Fig. 6 for details). (a,b) Comparison of resultant vector length, |R|, and PD between CW and CCW rotations. Cells showed similar modulation strength during CW (|R| = 0.36 ± 0.2 s.d.) and CCW (|R| = 0.35 ± 0.2 s.d.) rotation (paired Wilcoxon rank test, P = 0.93), but often differed in PD. Identical PDs are characteristic of G-tuned whereas opposite PDs are characteristic of dG-tuned cells. (c) |R| of the G and dG components. G-tuned cells: |R(G)| = 0.37 ± 0.2 s.d.; |R(dG)| = 0.11 ± 0.1 s.d.; dG-tuned cells: |R(G)| = 0.18 ± 0.1 s.d.; |R(dG)| = 0.33 ± 0.1 s.d. (d) Distribution of the preferred direction of the G component (upper panel, G-tuned and G+dG-tuned cells) and dG component (lower panel, dG-tuned and G+dG-tuned cells). Nose down (ND), contralateral (Cont.), nose up (NU) and ipsilateral (Ipsi.) refer to the direction of tilt.

**G brief communications**
Future experiments should investigate whether gravity tuning is found in physiologically identified azimuth-tuned HD cells.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

This work was supported by NIH grant R01 EY012814 (D.E.A.), NIH grant R01DC004260 (D.E.A.) and NIH grant R01 DC014686 (J.D.D.).

AUTHOR CONTRIBUTIONS

J.L. analyzed the data and wrote the manuscript. B.K. performed the experiments. J.D.D. supervised the experiments and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Angelaki, D.E., Shaikh, A.G., Green, A.M. & Dickman, J.D. Nature 430, 560–564 (2004).
2. Laurens, J., Meng, H. & Angelaki, D.E. Nat. Neurosci. 16, 1701–1708 (2013a).
3. Laurens, J., Meng, H. & Angelaki, D.E. Neuron 80, 1508–1518 (2013b).
4. Zhou, W., Tang, B.F., Newlands, S.D. & King, W.M. J. Neurophysiol. 96, 2915–2930 (2006).
5. Merfeld, D.M., Zupan, L. & Peterka, R.J. Nature 398, 615–618 (1999).
6. Laurens, J. & Angelaki, D.E. Exp. Brain Res. 210, 407–422 (2011).
7. Yakusheva, T.A. et al. Neuron 54, 973–985 (2007).
8. Taube, J.S. Annu. Rev. Neurosci. 30, 181–207 (2007).
9. Yoder, R.M., Clark, B.J. & Taube, J.S. Trends Neurosci. 34, 561–571 (2011).
10. Shinder, M.E. & Taube, J.S. J. Neurophysiol. 111, 2479–2492 (2014).
11. Finkelstein, A. et al. Nature 517, 159–164 (2015).
12. Wilson, J.J., Page, H. & Jeffery, K. Preprint at bioRxiv http://dx.doi.org/10.1101/043711 (2016).
ONLINE METHODS

Animals. We recorded from the anterior thalamus of two naive male rhesus macaques (V and L, both 5 years old, pair-housed with normal light/dark cycle) implanted with a circular Delrin ring to immobilize the head, scleral search coils to measure eye movements, and a Delrin platform for neural recordings. Neuronal recordings in the anterior thalamus were performed in animal V before this study. Experimental procedures were conducted in accordance with US National Institutes of Health guidelines and approved by the Animal Studies and Use Committee at Baylor College of Medicine.

Electrodes were positioned daily using a Delrin platform that was implanted stereotaxically. The anterior thalamus was localized by matching stereotoxic MRI scans (Supplementary Fig. 1a,b) onto a vectorized brain atlas (15). Stereotoxic MRI scans were performed using MRI-compatible ear bars and additional MRI-compatible markers placed stereotaxically on the recording platform, allowing reconstruction of stereotactic coordinates directly from MRI images. Recording locations were reconstructed by superimposing MRI and recording platform coordinates. Positioning accuracy was verified in initial experiments, where two landmark regions (the oculomotor nuclei and midbrain burst neurons, identified physiologically by means of their characteristic firing patterns during vertical pursuit and saccadic eye movements) were recorded using the same positioning system. During experiments, the anterior thalamus was reached by lowering the electrode until it entered the lateral ventricle, which could be clearly recognized by the absence of neural activity. The electrode was lowered further and we searched for rotation-tuned cells as soon as neural activity resumed.

Experimental apparatus. During experiments the animal was comfortably seated in a primate chair secured inside a vestibular stimulator composed of a motorized three-axis rotator surrounded by a light-tight sphere (1.8 m diameter) (Acutronics Inc., Switzerland). A fourth rotation axis allowed tilting of the rotator and the sphere together as a single unit (Supplementary Fig. 1c). The inside of the sphere was painted in white and black dots of different sizes. Animals were positioned such that all four rotation axes were aligned with the center of the head and the stereotoxic-horizontal plane was earth-horizontal when the animal was at rest.

Experimental protocols. As illustrated in Supplementary Video 1, all yaw rotation protocols were delivered using the rotator’s innermost axis (axis I, Supplementary Fig. 1c). Animals were passively rotated alternately in counter-clockwise (CCW) and clockwise (CW) directions, at a constant velocity of 28.6°/s, thus pitching the animal alternatively forward and backward. A total of 24 movements (12 in each direction) were performed. Stimulus protocols were delivered as follows:

(1) Yaw rotation with the animal upright (no change in head orientation relative to gravity).
(2) Yaw rotation around a head and visual tilted axis (gravity/vison conflict). This was achieved using axis IV to tilt the whole system (3D rotator and spherical enclosure) either +30° or −30°. This condition generated a sensory conflict with visual cues identical to those observed during upright yaw rotation (condition 1), but with gravity cues signaling a different orientation relative to gravity.
(3) Yaw rotation around a head tilted axis only (non-conflict condition). In this case, the ±30° tilt was introduced using axis II (head/body), whereas axis IV (spherical enclosure) remained upright. In this condition, vestibular/somatosensory cues were congruent with visual cues (which indicated a tilted orientation within the upright visual surround).
(4) In addition, neurons were also tested with pitch rotations, where axis I was positioned such that axis II was aligned with the animal’s interaural axis. Axis II was then rotated back and forth to ±30°, at a constant velocity of 28.6°/s, thus pitching the animal alternatively forward and backward. A total of 24 movements (12 in each direction) were performed. We also performed roll rotations, where axis I was positioned such that axis II was behind the animal and aligned with the naso-occipital axis. Axis II was then rotated back and forth to ±30°, at a constant velocity of 28.6°/s, thus rolling the animal alternatively rightward and leftward.
(5) If cell isolation was maintained, the tilted yaw rotation protocol (3) was also delivered for tilt angles other than ±30°, ±15°, and ±45°.

All isolated neurons were first recorded during protocols (1) and (3), and the rest of the stimuli were used only for responsive neurons. Protocols were performed in the following order: (1), (3), (2), (4) and (5). Protocols (1) to (4) were performed first in light and then repeated in complete darkness.

Neural recordings. Extracellular recordings from isolated single neurons were obtained with epoxy-coated tungsten microelectrodes (1–2 MΩ impedance; FHC, Bowdoinham, ME). Each electrode reached the midbrain and thalamus through a 26-gauge cannula and was manipulated with a remote-controlled microdrive (FHC, Bowdoinham, ME). Neural activity was amplified and filtered (300 Hz–6 kHz). Neuronal data were also acquired at 33 kHz using an analog channel of a Power-1401 data acquisition interface (Cambridge Electronic Design Ltd) and analyzed offline using custom Matlab (MathWorks) scripts to extract spike timing from the raw neuronal data on the basis of spike statistics (amplitude, peak latency) and principal component analysis.

Statistics. No statistical methods were used to predetermine sample size, but our sample sizes are typical of those used in the field. Depth of modulation was compared across tasks using Wilcoxon rank-sum tests. All statistical tests were two-sided. Data collection and analysis were not performed blind to the conditions of the experiments. A Supplementary Methods Checklist is available.

Modulation during yaw rotation. We used Rayleigh’s mean vector R as a measure of modulation depth during yaw rotations around upright or tilted axes. Head orientation α, in the plane of rotation (i.e. the position of the yaw rotation axis), which ranged from 0° to 360°, was divided in 100 bins of width δα = 3.6°. We computed the mean firing rate, FR(α), within each bin. The mean vector R (as defined in ref. 16) is the complex number

\[
R = \frac{1}{N} \sum_{i=1}^{N} FR(\alpha_i) e^{\alpha_i/180} + \sum_{i=1}^{N} FR(\alpha_i) \cdot (c + (\delta\alpha/2)(180/\pi) \sin(\delta\alpha/2))
\]

The length of the mean vector, |R|, represents the strength of the neuron’s modulation and ranges from 0 (when spikes are distributed uniformly) to 1 (when all spikes occur at the same head orientation α). The phase of R represents the preferred direction of the neuron. Note that R was always computed independently for CCW and CW rotations because some neurons (dG-tuned, see below) exhibited out of phase responses during CCW and CW rotations. The significance of |R| was assessed by bootstrap analysis. Each bootstrap sample was constructed by splitting the data set into individual rotation cycles. Within each cycle, the head orientation variable (α) was shifted circularly by a random number ranging from 0° to 360°. A value of |R| was computed on the basis of this shuffled data set; and the procedure was repeated 1,000 times. Cells were considered significantly modulated when |R| was higher than in 99% of the bootstrap samples (i.e. |R| was significant at P = 1%) in both CCW and CW directions.

Reconstructing gravity and gravity-derivative tuning curve. We separated gravity (G) and gravity-derivative (dG) responses on the basis of the difference between the experimentally measured response curves (FRCW(α) and FRCW(α)) during CCW and CW rotations. Parameter α is expressed in a gravity reference frame, and data from protocols (2) and (3) in both light and in darkness were pooled for this analysis. Mathematically, FRCW(α) and FRCW(α) would be expected to be identical for a cell that responds to orientation (position) relative to gravity (as in Fig. 1; see also Supplementary Fig. 6). In contrast, FRCW(α) and FRCW(α) would be expected to be 180° out of phase for a cell that responds to dG (as in Supplementary Figs. 5 and 6). On the basis of these properties, we extracted a G tuning curve, T_G(α), and a dG tuning curve, T_dG(α), from the response curves FRGCW(α) and FRCW(α). This was performed by building a system of equations, as follows. During CCW rotation, we assumed that the firing rate was the sum of the G and dG tuning curves with an average firing rate, FR0, i.e.

\[
FR_{CCW}(\alpha) = FR_0 + T_G(\alpha) + T_dG(\alpha)
\]

(1)

During CW rotation, we assumed that the response to G remained identical but that the dG response would shift by 180°, i.e.

\[
FR_{CW}(\alpha) = FR_0 + T_G(\alpha) + T_dG(\alpha + 180°)
\]

(2)
Next we replaced $\alpha$ by $\alpha + 180^\circ$ in equations (1) and (2) and created two new equations:

$$FR_{CCW}(\alpha + 180^\circ) = FR_0 + T_C(\alpha + 180^\circ) + T_DG(\alpha + 180^\circ)$$

(3)

$$FR_{CW}(\alpha + 180^\circ) = FR_0 + T_C(\alpha + 180^\circ) + T_DG(\alpha)$$

(4)

Note that $T_DG(\alpha + 360^\circ)$ is replaced by $T_DG(\alpha)$ in equation (4) because $\alpha + 360^\circ$ and $\alpha$ are the same point. It can also be shown that the system has a rank of 3 and is therefore overdetermined. The unknown variables $T_C(\alpha)$ and $T_DG(\alpha)$, which represent the variations of the cell's firing rate around the average rate $FR_0$, can be reconstructed from these equations at each point $\alpha$.

**Cell classification.** We assessed how much G and dG contribute to a cell's firing rate by computing partial correlation coefficients\(^1\). Cells were classified as either G-tuned or dG-tuned on the basis of neuronal responses during yaw rotation at $\theta = 0^\circ$, $5^\circ$, $15^\circ$, $30^\circ$ and $45^\circ$ tilt angles (protocol 5). Because data were collected in this restricted range of tilt angle, we did not attempt to fit Gaussian curves to the tuning (topology of tilt orientation).

We assumed that the variations of $FR_0(\theta)$ are attributable to G and dG tuning in G- and dG-tuned cells respectively. On the basis of this assumption, the reconstructed 2D tuning curves of G- and dG-tuned cells were $T_C(\theta, \alpha) + FR_0(\theta)$ and $T_DG(\theta, \alpha) + FR_0(\theta)$, respectively. However, we could not make a similar assumption for G+dG cells, and therefore we did not reconstruct the 2D tuning of G+dG cells (Fig. 3c).

**Comparison of the responses to yaw and pitch/roll rotations.** To compare responses to yaw rotations around a tilted axis (protocols (2) and (3)) and to pitch/roll tilt (protocol (4)), a linear model ($FR = k_xG_x + k_yG_y + k_0$) was fitted to neuronal firing rate. On the basis of this model, the gravity gain and PD are $(k_x^2 + k_y^2)^{1/2}$ and atan$(k_y/k_x)$. The model was fitted independently to neuronal responses during yaw and pitch/roll and the resulting response gains and PD were compared (Supplementary Fig. 3c,d). The same approach but where $G_x$ and $G_y$ were replaced by $dG_x$ and $dG_y$ was used to compare the dG tuning during yaw and pitch/roll rotations (Supplementary Fig. 3e,f).

Although neuronal responses generally formed a hill of activity in the $G_x$-$G_y$ or $dG_x$-$dG_y$ plane, the linear model was used only as an approximation of the response at a given tilt angle. Because the same tilt angle ($30^\circ$) was used in protocols (2), (3) and (4), the projection of the gravity vector in the $x$-$y$ plane peaked at the same value of 0.5 G. This made the linear model well suited to compare G tuning in both protocols (Supplementary Fig. 3c,d). In contrast, the derivate of gravity had a magnitude of 0.25 G/s during protocols (2) and (3) and 0.5 G/s during protocol (4). Therefore, the comparison of the dG tuning was less accurate (Supplementary Fig. 3e,f).

**Topology of tilt orientation.** Whereas, for small tilt angles, we have approximated the 2D space of tilts using a polar plot (Fig. 3b,c and Supplementary Figs. 1d and 8), in general it adopts a spherical geometry. Intuitively, this is so because $180^\circ$ tilt in any direction (NU, ND, etc.) brings the head into the same orientation (upside-down). In other words, all points at $180^\circ$ tilt must be the same, and this is achieved by folding the plot into a sphere.

**Data and code availability.** The data that support the findings of this study and the analysis scripts are available from the corresponding author upon request.

13. Meng, H., May, P.J., Dickman, J.D. & Angelaki, D.E. J. Neurosci. 27, 13590–13602 (2007).
14. Meng, H., Green, A.M., Dickman, J.D. & Angelaki, D.E. J. Neurophysiol. 93, 3418–3433 (2005).
15. National Primate Research Center, University of Washington. BrainInfo http://www.braininfo.org/ (1991–present).
16. Zar, J.H. Biostatistical Analysis 4th edn. 592–615 (Prentice Hall, 1998).