Supplementary Figure 1

Afferents and efferents of LP.

(a) Injection of a retrograde tracer into LP. Coronal sections (100 μm) showing several planes with retrogradely labelled neurons along the antero-posterior axis. Schematic on the left depicts the injection site. An image of the injection site is shown in Fig. 1a. V1: primary visual cortex; VisM: medial higher visual areas; VisL: lateral higher visual areas; TEa: Temporal association area; PPC: posterior parietal cortex. (b) Co-injection of a retrograde and anterograde tracer reveals the organisation of input-output connectivity of LP. (b1) Schematic and image showing the injection site for the retrograde tracer CTB647 (red) and for the anterograde tracer AAV2.1-Ef1a-eGFP (green). Note the yellow area where both tracers co-localize. (b2-b5) Coronal sections showing retrogradely labelled somata and anterogradely labelled axons in the posterior parietal cortex (PPC, b2), in the temporal association area (TEa, b3), in the cingulate cortex (b4, ventral anterior cingulate cortex, ACAv, dorsal anterior cingulate cortex, ACAd, primary motor area, MO1, secondary motor area MO2), and in visual cortical areas (b5). Note the absence of LP axons in layer 4 of V1 compared to higher visual areas (VisL: lateral higher visual area; VisM: medial higher visual area). (b6) Close-up of the dashed rectangle over V1 in b5. (b7) Close-up of the lateral higher visual area highlighted in b5. (b8-b9) Confocal images of the caudoputamen (CP, b8) and the amygdala (b9, LA: lateral amygdalar nucleus; BLA: basolateral amygdalar nucleus; CEA: central amygdalar nucleus, CP: caudoputamen). Arrows represent the orientation of the coronal sections, similar in all images (M: Medial; D: Dorsal). (c) Summary table of telencephalic areas and layers projecting to LP, and receiving projections from LP.
Coarse topographic organization of LP inputs into V1.

(a-d) Organization of LP neurons projecting along the azimuth axis of V1. A subset of the data is shown in Fig. 1b. (a) Intrinsic imaging was used to determine the borders of V1 and to guide injections. Top: retinotopic phase map showing the phase thresholded by the normalized power of the Fourier component at the frequency of a vertical bar drifting leftward and rightward. White circles represent the locations of the three retrograde tracer injections. Arrows represent the orientation of the cortical surface (M: Medial; R: Rostral). Bottom: locations of the injections on the cortical blood vessel pattern. The outline of V1 is superimposed from the retinotopic map. (b) Confocal image of V1 showing the locations and extent of the injections (green: CTB488; red: CTB647; yellow: CTB555). (c) Stained cell bodies are found in dLGN and these are arranged in clear topographic order. (d) Coronal sections with stained cell bodies in LP. Arrow-heads show subdivisions with topographic organization. Arrows represent the orientation of the coronal section (M: Medial; D: Dorsal) (e-h) Same as a-d for injections along the V1 axis for elevation in the nasal visual field. (i-l) Same as a-d for injections along the V1 axis for azimuth in the lower visual field. The retinotopic map was obtained by using a horizontal bar drifting upward and downward. Stars indicate cells that are probably labelled due to leakage of the retrograde tracer CTB488 into a higher visual area in this animal.
Supplementary Figure 3

Injection sites and visually responsive boutons.

The thalamus of adult mice was injected with an adeno-associated virus carrying a genetically encoded calcium indicator (GCaMP5 or 6). Injections were made either into dLGN or LP. This was followed by two-photon calcium imaging of thalamocortical axons in the primary visual cortex for the characterization of visual response properties. (a,b) Coronal sections showing GCaMP-labelled neurons in the dLGN (a) and LP (b). Arrows represent the orientation of the coronal sections (M: Medial; D: Dorsal). (c,d) Two-photon image of dLGN (c) and LP projections (d) in layer 1 of V1. (e,f) ROI masks corresponding to the images in c,d. ROIs for which a visual receptive field could be obtained are shown in yellow, others in white.
Supplementary Figure 4

Additional receptive field (RF) properties.

(a) Receptive field examples of dLGN boutons, V1 somata and LP boutons with a single subfield. Scale bars, 10 deg. (b) Distributions of minor and major axis lengths of subfields from dLGN boutons, V1 somata and LP boutons. (c) Distributions of the major axis orientations of receptive fields. The subfields of LP receptive fields were more likely to be horizontally oriented, while the subfields of dLGN boutons were biased for vertical orientations. Subfields of V1 neurons were less orientation-biased. dLGN: n = 2317, LP: n = 1825, V1: n = 356 RF subfields. *, P < 0.05; ***, P < 10⁻¹⁰, Χ²-test for uniformity. (d) Examples of dLGN, LP and V1 receptive fields with significant ON and OFF subfields. About one third of LP and dLGN boutons and V1 neurons exhibited subfields of both signs (LP: 471 of 1354; dLGN: 590 of 1727; V1: 89 of 267). Scale bar, 10 deg. (e) Distributions of distances between the centroids of ON and OFF subfields for boutons or neurons with a significant ON and OFF subfield. The subfields of dLGN boutons were often arranged in a center-surround configuration, such that the distances between ON and OFF subfield centroids were relatively small. LP boutons showed a larger subfield separation while values for V1 neurons lay in between LP and dLGN. dLGN: n = 590, LP: n = 471, V1: n = 89 receptive fields. **, P < 0.0001; ***, P < 10⁻¹⁰, Wilcoxon rank-sum test. These differences in distance cannot be fully accounted for by differences in RF size. Calculating the RF subfield distance normalized to the average axis length of the subfields resulted in median distances between ON and OFF subfield centroids of 18% (dLGN), 22% (V1) and 25% (LP) of the average axis length. dLGN: 7 mice, LP: 13 mice, V1: 4 mice.
Supplementary Figure 5

Single-unit recordings in LP and dLGN.

(a) Coronal slice showing tracks of the four electrode shanks (2 tetrodes per shank, see Methods) stained with Dil. Hip: hippocampus; LPl: lateral posterior nucleus, lateral section; LPrm: lateral posterior nucleus, rostro-medial section. Scale bar, 600 µm. (b) Action potential waveform, autocorrelrogram (bin size = 2 ms) and spike raster plot in response to flashed 8-degree squares (bin size = 5 ms; stimulus onset at 0 ms) for example single units from LPl and dLGN. (c) Mean spontaneous firing rate measured during 30 s without visual stimulation. LPrm: n = 18; LPl: n = 34; dLGN: n = 14 units. (d) Mean visually-evoked firing rate to a flashed 8-degree square at the preferred position, from 50 ms after stimulus onset until 50 ms after stimulus offset. LPrm: n = 18; LPl: n = 34; dLGN: n = 14. (e) Average PSTH of all units, normalized to the maximum response. Stimulus onset at 0 ms. (f) Mean latency of visually-evoked responses of all units; LPrm: n = 18; LPl: n = 34; dLGN: n = 12. (g) Example receptive fields. (h) Receptive field size. LPrm: n = 16; LPl: n = 10; dLGN: n = 12. Error bars are s.e.m in all panels. **, P < 0.005; ***, P < 10^{-5}. Kruskal-Wallis test followed by Wilcoxon rank sum test with Bonferroni correction. n = 8 mice.
Supplementary Figure 6

Estimation of the number of boutons from different neurons.

(a,b) Matrices of pair-wise correlation coefficients from all boutons of an example imaged region containing dLGN axons (a) or LP axons (b). Correlation coefficients were obtained by correlating calcium traces from receptive field mapping data only. (c) Cumulative distribution functions of correlation coefficients of all pairs of visually responsive thalamocortical boutons (dLGN and LP data combined). The average correlation coefficient is close to zero (gray line (‘All pairs’), median = 0.037, n = 187590 pairs). Some pairs of boutons belonging to the same axon could be identified by visual inspection of averaged images. These exhibited, on average, much larger correlation coefficients (median = 0.476, n = 539). (d) In order to estimate how many boutons from different neurons were present in an imaged region we built clusters of correlated boutons that putatively belonged to the same neuron. We seeded the first cluster with the bouton exhibiting the strongest calcium response and assigned all boutons it was correlated with to the same cluster, then seeded the next cluster with the most strongly responding unassigned bouton, and so on. The graph shows the mean estimated number of visually-responsive boutons from distinct neurons per imaging region for different correlation coefficient cut-off values used for assigning boutons to the same cluster (0.18 is equal to P = 0.05 on the same-axon pair-wise correlation coefficient distribution in a). The column without cut-off shows the mean total number of responsive boutons per imaged area. Error bars represent s.e.m.
Supplementary Figure 7

Fine-scale retinotopic organization.

(a,b,c) Dots indicate cortical x-y positions of dLGN boutons (a), LP boutons (b) or V1 somata (c) within example imaged regions. In addition they are grayscale coded for receptive field position within the visual field with respect to relative elevation (top) or azimuth (bottom). Arrows indicate axes of cortical space that correlate best with changes in receptive field elevation (top) or azimuth (bottom).

(d,e,f) Top, cortical axes for elevation (left) and azimuth (right) for all regions containing V1 cells, or dLGN or LP axons with at least 35 boutons. Bottom, distributions of separation angles between the two axes for elevation and azimuth for dLGN bouton populations (d), for LP bouton populations (e), and for V1 layer 2/3 neurons (f). For regions containing dLGN boutons, the directions of the axes were similar to those of V1 layer 2/3 neurons and the angles that separated the azimuth and elevation axes were close to 90° for all imaged sites (dLGN and V1: P-values < 0.05, X²-test for uniformity), showing a consistent fine-scale retinotopic organization. In contrast, the axes directions and separation angles varied considerably in regions containing LP boutons (P = 0.52, X²-test for uniformity), suggesting that LP input is not as closely aligned with the retinotopic map of V1 as dLGN input. M: medial, R: rostral, L: lateral, C: caudal. dLGN: n = 11 regions, 5 mice, LP: n = 13 regions, 7 mice, V1: n = 8 regions, 4 mice.
**Supplementary Figure 8**

**Saccades in the dark.**

Top three rows: camera images of the contralateral eye showing saccades and blinks. Note that the pupil is dilated in the dark but nonetheless, eye movements are tractable. Top trace: horizontal pupil position showing eye movements and blink artefacts. The dotted lines represent saccades detected by our algorithm. Note that none of the blinks were classified as eye movements. Middle trace: Fluorescence trace of an example LP bouton responding selectively to medial-to-lateral saccades. Bottom trace: Inferred firing rate (arbitrary units) of the same bouton.
Supplementary Figure 9

Relationships between bouton activity and visual flow or running speed.

(a) Proportions of dLGN and LP boutons with significant visual flow tuning curves of different shapes obtained in the open-loop condition (significance test excluding stationary periods of running speed $< 3 \text{ cm s}^{-1}$, see Methods). Boutons either increased (incr.) or decreased (decr.) their activity with increasing visual flow speed, or they exhibited band-pass tuning curves (BP). Shown are mean proportions and s.e.m over sessions. A higher fraction of dLGN than LP boutons preferred high visual flow speed ($P = 0.0054$), while more LP boutons preferred low visual flow speed ($P = 0.0001$). (b) Proportions of dLGN and LP boutons with significant running speed tuning curves of different shapes obtained in the open-loop condition. No differences between dLGN and LP were observed ($P$-values $> 0.05$). (c) Left: examples of running speed tuning curves in the dark. Right: proportions of significant running speed tuning curves with different shapes as in b, obtained in the dark. No differences between dLGN and LP were observed ($P$-values $> 0.05$). (d) Proportions of boutons significantly informative about running speed in the dark (random forests decoder, PP > 0.16, see Methods) were not different between dLGN and LP ($P = 0.10$). (e) Examples of RS–VF tuning curves of dLGN and LP boutons in the open-loop condition of dLGN and LP boutons (left) and proportions of dLGN and LP boutons with significant RS–VF tuning curves of different shapes (right) obtained in the open-loop condition. Open-loop: dLGN, $n = 18$ sess.; LP, $n = 31$ sess.; dark: dLGN, $n = 18$ sess.; LP, $n = 29$ sess.; dLGN = 8 mice, LP = 10 mice. **, $P < 0.01$; ***, $P < 0.001$, Wilcoxon rank-sum test. Error bars indicate s.e.m.
Supplementary Figure 10

Comparison between linear correlations and nonlinear decoding; effect of PP threshold

(a) Relationship between the Pearson’s correlation coefficient R between inferred spike rate and running or visual flow speed, and the prediction power (PP) for speed traces obtained with the non-linear regression decoder for individual boutons. Black: boutons above the threshold of significant prediction power PP > 0.16. (b) Relationship between Pearson’s correlation coefficient R for running speed and visual flow speed for all LP (left) and dLGN boutons (right). Magenta (LP) and green (dLGN) boutons have a decoding |PP| > 0.16. Equivalent to Fig. 6d. (c) Circular histogram depicting the distribution of linear interaction angles $\theta_{\text{lin}}$ for running and visual flow speed computed from corr. coeff. of running and visual flow speed, for boutons with decoding |PP| > 0.16. Equivalent to Fig. 6e. Proportions of $\pm (\text{RS} - \text{VF})$ boutons (including angles of up to 22.5 deg off the diagonal): dLGN 19%, LP 39%; P < 10$^{-10}$, Z-test. Proportions of $\pm (\text{RS} + \text{VF})$ boutons: dLGN 36%, LP 25%; P < 10$^{-10}$, Z-test. dLGN: n = 2159 boutons; LP: n = 1617 boutons. (d) Scatter plots of signed decoder PP for running speed versus visual flow speed for all LP and dLGN boutons. Similar to Fig. 6d, colors indicate for which variable (running speed (RS), visual flow (VF), the difference between RS and VF (RS−VF) or the sum of RS and VF (RS+VF) the boutons had the highest prediction power. Grey: PP < 0.16 for all variables. LP: 10 mice, dLGN: 8 mice. (e) Circular interaction histograms showing the distribution of visual flow and running speed interaction angles computed from signed PPs, similar to Fig. 6e for boutons above varying PP thresholds. Proportions of $\pm (\text{RS} - \text{VF})$ and $\pm (\text{RS} + \text{VF})$ boutons were significantly different between dLGN and LP for all PP thresholds (P-values < 10$^{-10}$, Z-test).