A segregated cortical stream for retinal direction selectivity

Rune Rasmussen1,2, Akihiro Matsumoto1,2, Monica Dahlstrup Sietam1 & Keisuke Yonehara1

Visual features extracted by retinal circuits are streamed into higher visual areas (HVAs) after being processed along the visual hierarchy. However, how specialized neuronal representations of HVAs are built, based on retinal output channels, remained unclear. Here, we addressed this question by determining the effects of genetically disrupting retinal direction selectivity on motion-evoked responses in visual stages from the retina to HVAs in mice. Direction-selective (DS) cells in the rostrolateral (RL) area that prefer higher temporal frequencies, and that change direction tuning bias as the temporal frequency of a stimulus increases, are selectively reduced upon retinal manipulation. DS cells in the primary visual cortex projecting to area RL, but not to the posteromedial area, were similarly affected. Therefore, the specific connectivity of cortico-cortical projection neurons routes feedforward signaling originating from retinal DS cells preferentially to area RL. We thus identify a cortical processing stream for motion computed in the retina.
The mammalian visual system analyzes the external world through a set of distinct spatio-temporal channels. The mouse retina contains >40 distinct types of ganglion cells, each encoding a discrete set of visual features such as color, luminance, edges, and motion direction. The general consensus is that central visual areas combine signaling originating from different ganglion-cell types, and the output from each ganglion-cell type diverges into multiple central visual areas, embodying a feature-combinatorial system.

The mouse visual cortex includes up to 16 retinotopically organized higher visual areas (HVAs), varying in preferences for temporal and spatial frequency, motion speed, color, and visual-field coverage. In rodents, functionally segregated streams are already observed in the cortico-cortical projection neurons of the primary visual cortex (V1). However, it is not yet understood how signaling originating from individual retinal channels influences the activity of HVAs and, hence, how HVA-specific properties and distinct visual streams emerge.

One possibility is that each retinal channel influences all HVAs in a similar extent. Alternatively, in an extreme scenario, the impact of each retinal channel may be localized to a single HVA. If so, it will be critical to determine how V1 cortico-cortical projection neurons integrate inputs originating from individual retinal ganglion-cell types.

One fundamental task of the visual system is to detect the direction and speed of visual motion to analyze object motion or optic flow. The direction and speed of visual motion are first encoded by retinal direction-selective (DS) cells, preferentially responding to visual stimuli moving in a particular direction. Retinal ON-OFF DS cells include four subtypes: each prefers one of four cardinal directions, and genetic knockout of retinal horizontal direction selectivity reduces a posterior motion preference in V1 layer L2/3 neurons of control mice. In areas LM and AM, only posterior responses at 1.2 Hz were significantly decreased and increased, respectively, in Frmd7tm mice. These findings suggest that retinal horizontal direction selectivity contributes to motion responses in a subset of visual cortical areas. In particular, the higher-TF preference of areas V1 and RL for horizontal motion was dominantly impaired in mice with disrupted retinal horizontal DS signaling.

**Results**

**Mapping the sensitivity of cortical areas.** We mapped the visual cortex organization in anesthetized control and Frmd7tm mice using intrinsic signal optical imaging (ISOI; Fig. 1a). We generated visual-field sign maps (Fig. 1a) from horizontal and vertical retinotopic maps, and reliably identified six visual areas: V1, lateralmedial (LM), anterolateral (AL), RL, anteromedial (AM), and PM (Fig. 1a). It is worth noting that the RL area we identified may possibly include the anterior HVA (area A), which has been identified previously.

We found no differences in visual cortical organization or size proportions between control and Frmd7tm mice (Supplementary Fig. 1). To test the contribution of retinal direction selectivity to motion responses in visual areas, we measured evoked intrinsic signal activity levels in response to gratings drifting in the cardinal directions at a TF of 0.3, 0.75, 1.2, or 1.8 Hz with a fixed spatial frequency of 0.03 cycles/°. In areas V1 and RL, we found significantly decreased responses to horizontal motion in Frmd7tm mice at multiple TFs (Fig. 1b). In control mice, these areas responded strongly to horizontal motion moving at higher TFs (Fig. 1b). In areas LM and AM, only posterior responses at 1.2 Hz were significantly decreased and increased, respectively, in Frmd7tm mice (Fig. 1b). These findings suggest that retinal horizontal direction selectivity contributes to motion responses in a subset of visual cortical areas. In particular, the higher-TF preference of areas V1 and RL for horizontal motion was dominantly impaired in mice with disrupted retinal horizontal DS signaling.

**RL DS cells rely on retinal direction selectivity.** To elucidate the cellular underpinnings of the ISOI results, we used in-vivo two-photon calcium imaging from anesthetized mice (Fig. 1c and Supplementary Fig. 2). We focused on the areas RL and PM because RL was notably affected in Frmd7tm mice, whereas PM was unaffected (Fig. 1b). We imaged neurons in L2/3 using the virally transduced GCAMP6f, and stimuli consisted of gratings drifting in 12 directions at TFs of 0.3, 0.75, 1.2, or 1.8 Hz with a fixed spatial frequency of 0.03 cycles/° (Fig. 1d–f). RL DS cells (direction selectivity index [DSI] > 0.3) in control mice preferred higher TFs, particularly in the posterior direction (Fig. 1g, h), whereas PM DS cells preferred lower TFs at a similar level in all directions (Fig. 1g, h). In Frmd7tm mice, RL DS cells preferred lower TFs (Fig. 1g, h), whereas the preference of PM DS cells was unchanged (Fig. 1g, h). We found no differences in vertical motion responses in area RL or PM between control and Frmd7tm mice (Fig. 1h). In both control and Frmd7tm mice, RL DS cells developed a posterior bias in the distribution of preferred directions as the TF increased from 0.3 to 1.2 Hz (P ≥ 0.05 and P < 0.01, Rayleigh test; Fig. 1i): however, a notably smaller fraction of DS cells in RL of Frmd7tm mice preferred posterior motion (29.3%) compared to control mice (48.1%) at 1.2 Hz (Fig. 1j). Preferred directions of PM DS cells showed a posterior bias at 0.3 and 1.2 Hz (P < 0.001, Rayleigh test; Fig. 1j) in both control and Frmd7tm mice (Fig. 1i, j).

Anesthesia is known to influence, for example, cortical dynamics and synaptic excitation and inhibition. Thus, to validate our findings, we repeated the experiment in awake, quietly resting mice (Supplementary Fig. 3). Overall, these experiments confirmed that the key findings in anesthetized mice (Fig. 1f–j) were preserved in awake mice. RL DS cells in awake control mice preferred motion along the horizontal axis at higher TFs, while PM DS cells preferred motion at low TFs equally across all directions (Supplementary Fig. 3c, d). In contrast, RL DS cells in awake Frmd7tm mice preferred low TFs, whereas the preference of PM DS cells was unchanged (Supplementary Fig. 3c, d). Importantly, as the...
TF increased from 0.3 to 1.2 Hz, RL but not PM DS cells from awake control mice developed a strong bias for posterior motion, and this bias was significantly impaired in Frmd7tm mice (Supplementary Fig. 3e, f). These results suggest the following: First, increased response amplitude at higher TFs is correlated with a gain of bias towards posterior motion in RL DS cells. Second, these TF-dependent changes in response amplitude and directional preference in area RL depend on retinal horizontal direction selectivity. Third, the effect of disrupting retinal direction selectivity appears to be specific to certain HVAs.

**Fig. 1 Posterior motion preference of RL neurons at higher TFs depends on retinal horizontal direction selectivity.** a Upper: ISOI in control and Frmd7tm mice. Lower: Example vertical and horizontal retinotopic maps and the computed visual-field sign map. Scale bar, 1 mm. b Response strength as a function of motion direction (anterior [A], posterior [P], dorsal [D], and ventral [V]) and TF (Hz) and (five mice per genetic group). White and black asterisks: significantly decreased and increased responses, respectively, two-sided Mann-Whitney U-test. c Two-photon calcium imaging from L2/3 in areas RL and PM of control (1452 and 1098 DS cells, respectively; ten mice) and Frmd7tm mice (1387 and 1217 DS cells, respectively; 11 mice). d Example control and Frmd7tm RL and PM neurons expressing GCaMP6f (scale bar, 5 μm) and trial-averaged fluorescence (ΔF0/F0) time courses for the same neurons. Shading indicates SEM. e Tuning curves for neurons shown in d. Error bars are SEM; solid line is Gaussian fit. f Fraction of DS cells in RL and PM (two-sided χ² test with Yates correction). g Preferred TF for DS cells in RL (two-sided Mann-Whitney U-test) and PM (two-sided Mann-Whitney U-test). Triangles show medians. h Response amplitude as a function of motion direction and TF for RL and PM DS cells. White asterisks: significantly increased response amplitude in Frmd7tm mice, two-sided Mann–Whitney U-test. i Fractional distributions of preferred motion directions for RL and PM DS cells at 0.3 and 1.2 Hz; fractions are normalized to the largest fraction across genetic groups. j Distributions of preferred direction at 0.3 and 1.2 Hz in RL and PM (two-sided Kolmogorov-Smirnov test). **P < 0.01, ***P < 0.001, n.s., not significant, in f, g, and j. Source data are provided as a Source Data file.

RL DS cells are impaired by retinal starburst cell ablation. The effect of Frmd7 mutation on starburst cells is chronic from birth; potentially triggering plasticity-related changes in the downstream visual pathways. Thus, we next genetically ablated retinal starburst amacrine cells in adult mice to acutely abolish retinal direction selectivity. For this we used ChAT-Cre × LSL-DTR mice in which diphtheria toxin receptors are selectively expressed in starburst amacrine cells.25,29. Intravitreal injection of diphtheria toxin into these mice led to the selective ablation of starburst amacrine cells (Supplementary Fig. 4; referred to as ‘starburst-ablated mice’) and a loss of optomotor responses (OMR; Supplementary Fig. 4). We imaged neurons in L2/3 of areas RL and PM in awake, quietly resting mice (Supplementary Fig. 5). We found no differences in the fraction of DS cells between control and starburst-ablated mice in area RL or PM (Supplementary Fig. 5b). In starburst-ablated mice the distribution of TF preference was shifted toward slower
TFs for RL DS cells compared to control mice, whereas we found no difference for PM DS cells (Supplementary Fig. 5c). Similar to what we observed in Frmd7tm mice, RL DS cells from starburst-ablated mice showed a lack of horizontal motion response preference at higher TFs, in contrast to RL DS cells from control mice (Supplementary Fig. 5d). Notably, the posterior motion bias at 1.2 Hz was significantly impaired in RL DS cells from starburst-ablated mice (19.4% of DS cells) compared to control mice (29% of DS cells), whereas we found no difference for PM DS cells (Supplementary Fig. 5e). Thus, the key findings from Frmd7tm mice were largely supported by results from starburst-ablated mice.

Distinct RL neurons rely on retinal motion computation. To test whether there is a trend in affected response properties in Frmd7tm mice, we performed decomposition and segmentation on the datasets from areas RL and PM. First, we composed a TF-dependent response matrix for RL L2/3 DS cells, pooled from control and Frmd7tm mice (Fig. 2a). Next, we used principal component analysis (PCA) to decompose the response matrix into two dimensions (Fig. 2b). Noticeably, the PCA distribution showed a clear distribution trend depending on the TF preference: neurons sharing the same TF preference tended to be locally clustered (Fig. 2c). We then determined the fraction of RL neurons in local regions of the PCA distribution by superimposing 8 × 8 grids (Fig. 2d and Supplementary Fig. 6). Statistical comparisons of fractions between control and Frmd7tm mice revealed grids where the fraction of neurons was decreased, increased, or unchanged in Frmd7tm mice (Fig. 2e). Next, we probed the functional characteristics of these three groups. Notably, RL neurons that were decreased in Frmd7tm mice showed a prominent response amplitude increase as the TF increased ($P < 0.001$ for 0.3 versus 1.2 Hz for control and Frmd7tm mice, Wilcoxon signed-rank; Fig. 2f). In contrast, neurons that were increased or unchanged in Frmd7tm mice showed a TF-dependent decrease in response ($P < 0.05$ for 0.3 versus 1.2 Hz for control and Frmd7tm mice, Wilcoxon signed-rank; Fig. 2f). Neurons that were decreased in Frmd7tm mice showed a clear shift in direction tuning: at 0.3 Hz neurons uniformly encoded all directions while at 1.2 Hz they developed a posterior bias in DSI ($P < 0.001$ for 0.3 versus 1.2 Hz, respectively, Rayleigh test) with significantly less bias in Frmd7tm than in control mice at 1.2 Hz (Fig. 3g, h). PM-projecting DS cells from both control and Frmd7tm mice showed a posterior bias as the TF increased from 0.3 to 1.2 Hz ($P \geq 0.05$ and $P < 0.001$, Rayleigh test; Fig. 3g, h). RL-projecting DS cells from control mice developed a posterior bias ($P < 0.05$ and $P < 0.001$ for 0.3 and 1.2 Hz, respectively, Rayleigh test), while this did not occur in Frmd7tm mice (Fig. 3g, h). These data suggest that the distinct sensitivity of areas RL and PM to disruption of retinal horizontal direction selectivity is already found in V1 L2/3 DS cells that project to these areas.

Projection-specific impairment of V1 neurons. Previous work has shown that V1 neurons provide target-specific input to HVAs\(^{13,14}\). Here, we investigated whether the sensitivity to disruption of retinal horizontal direction selectivity is routed by V1 DS cells that project to areas RL and PM. Alternatively, such sensitivity may originate from inputs from other HVAs or higher-order thalamic areas. To probe this, we labeled RL- and PM-projecting V1 L2/3 neurons by injecting rAAV2-retro expressing GCaMP6m into either area PM or RL (Supplementary Fig. 7) and imaged these neurons and target-unspecific V1 L2/3 neurons in anaesthetized mice (Fig. 3a–c). The density of GCaMP6-labeled projection neurons did not differ significantly between genetic conditions (Supplementary Fig. 7e). Notably, the fraction of RL-projecting neurons that showed direction selectivity was reduced from 60% in control mice to 34% in Frmd7tm mice, whereas the fraction of DS cells among PM-projecting neurons was not altered in Frmd7tm mice (Fig. 3d). RL-projecting and target-unspecific DS cells from control mice preferred higher TFs, particularly in horizontal directions (Fig. 3e, f). In Frmd7tm mice, RL-projecting and target-unspecific DS cells preferred the lowest TF, showing lower responses than control mice to horizontal motion at 1.2 Hz (Fig. 3e, f). PM-projecting DS cells preferred low TFs for all directions in control mice, and this was the same in Frmd7tm mice (Fig. 3e, f). Consistent with previous work\(^{25}\), target-unspecific neurons developed a posterior bias in the distribution of preferred directions as the TF increased ($P < 0.05$ and $P < 0.05$ for 0.3 and 1.2 Hz, respectively, Rayleigh test) with significantly less bias in Frmd7tm than in control mice at 1.2 Hz (Fig. 3g, h). PM-projecting DS cells from both control and Frmd7tm mice showed a posterior bias as the TF increased from 0.3 to 1.2 Hz ($P \geq 0.05$ and $P < 0.001$, Rayleigh test; Fig. 3g, h). RL-projecting DS cells from control mice developed a posterior bias ($P < 0.05$ and $P < 0.001$ for 0.3 and 1.2 Hz, respectively, Rayleigh test), but this did not occur in Frmd7tm mice (Fig. 3g, h). These data suggest that the distinct sensitivity of areas RL and PM to disruption of retinal horizontal direction selectivity is already found in V1 L2/3 DS cells that project to these areas.

Distinct V1 neurons rely on retinal motion computation. Next, we correlated the response properties of V1 L2/3 DS cells and the differences between control and Frmd7tm by first decomposing the response matrix into two dimensions using PCA (Fig. 4a, b). Similar to in areas RL and PM, V1 L2/3 neurons sharing the same TF preference were locally clustered (Fig. 4c). We found grids where the fraction of neurons was decreased, increased or unchanged in Frmd7tm mice (Fig. 4e). Neurons that were decreased in Frmd7tm mice increased their response amplitude as the TF increased ($P < 0.001$ for 0.3 versus 1.2 Hz for control and Frmd7tm mice, Wilcoxon signed-rank; Fig. 4f). In contrast, neurons that were increased in Frmd7tm mice showed a TF-dependent decrease in responses ($P < 0.05$ for 0.3 versus 1.2 Hz for control and Frmd7tm mice, Wilcoxon signed-rank; Fig. 4f). We repeated the above analysis for PM L2/3 DS cells (Fig. 2h). Similar to in area RL, neurons from area PM showed a TF-dependent PCA distribution trend (Fig. 2i, j). We found no grids where the fraction of neurons was significantly different between control and Frmd7tm mice (Fig. 2k, l). PM neurons decreased their response amplitude as a function of increasing TF (Fig. 2m), with no clear change in direction tuning bias or DSI ($P \geq 0.05$ for 0.3 versus 1.2 Hz for control and Frmd7tm mice, Wilcoxon signed-rank; Fig. 2n). Together, these data indicate that the coupling of a high-TF motion preference and the development of posterior motion bias at high TFs is the major response feature that depends on retinal horizontal direction selectivity. Thus, the retinal contribution to motion processing in HVAs appears to be specific not only to area, but also to ensemble.
rank; Fig. 4g). Finally, we investigated the relationship between the effects of altered retinal horizontal direction selectivity and the target region of V1-projecting DS cells. We analyzed the fraction of PM- and RL-projecting neurons in each of the significantly affected grids. Importantly, this showed that the grids enriched in RL- and PM-projecting DS cells tend to be decreased and increased in *Frmd7tm* mice, respectively (Fig. 4h). These results suggest that the impact of disrupted retinal horizontal direction selectivity in L2/3 of V1 is biased to a subset of neurons that prefer high-TF motion and preferentially encode posterior motion at high TFs. Furthermore, this subset of V1 neurons appeared to preferentially project to area RL.
V1 L4 DS cells are insensitive to retinal manipulation. Signaling from the retina-geniculate pathway is conveyed to L2/3 of V1 mainly via L4 (ref. 32), and feature-tuned V1 L2/3 neurons receive L4 inputs33. Here, we investigated whether retina-originating DS signaling is routed to V1 L2/3 via L4, in addition to a previously suggested dLG N to V1 L1/2 shortcut pathway23. We imaged GCaMP6f-labeled neurons in L4 of V1 in anaesthetized mice (Supplementary Fig. 8), and identified DS cells from control and Frmd7tm mice (Fig. 5a). In contrast to L2/3, L4 DS cells from control and Frmd7tm mice were quantitatively very similar, both preferring 0.75 Hz TF, and showing no significant response amplitude differences (Fig. 5b, c). In control mice, the preferred directions of L4 DS cells showed a posterior bias at 0.3 and 1.2 Hz (P < 0.05, Rayleigh test; Fig. 5d), and this effect was not significantly different in Frmd7tm mice (Fig. 5d, e). The insensitivity of L4 DS cells to disruption of retinal horizontal direction selectivity at the population level was also supported by decomposition and segmentation analyses (Fig. 5f–h): none of the grids showed significantly different fractions between control and Frmd7tm mice (Fig. 5i, g). V1 L4 neurons showed no obvious change in direction tuning bias as a function of increasing TF, but neurons in both control and Frmd7tm mice increased their DSI (P < 0.01 for 0.3 versus 1.2 Hz for control and Frmd7tm mice, Wilcoxon signed-rank; Fig. 5i). These data show that DS cells in L4 of V1 are not noticeably sensitive to disruption of retinal horizontal direction selectivity, suggesting that retina-originating DS signaling reaches L2/3 of V1 via a pathway bypassing L4.

DS thalamic axons are affected by retinal manipulation. We examined whether the coupling of a high-TF preference and the TF-dependent development of posterior motion preference, which relies on retinal horizontal direction selectivity in areas V1 and RL, is already established in dLG N neurons. For this, we transected dLG N neurons with GCaMP6f and imaged axons in L1 and L2/3 of V1 of anaesthetized mice (Fig. 6a). During grating stimuli, fluorescence increased in individual micron-sized varicosities along the axonal arborizations (Fig. 6b, c), confirming they were putative presynaptic boutons13,23. The fraction of DS boutons preferring high and low TFs decreased and increased, respectively, in Frmd7tm mice (Fig. 6e). In control mice, DS boutons preferred higher TFs in response to horizontal motion (Fig. 6f). In contrast, in Frmd7tm mice, DS boutons preferred lower TFs in horizontal directions, showing higher responses than control mice at 0.3 Hz and markedly lower responses at 1.2 and 1.8 Hz (Fig. 6f). Responses to vertical motion were unaltered in control and Frmd7tm mice (Fig. 6f). In control mice, the fraction of horizontally tuned DS boutons was significantly higher than vertically tuned boutons at both 0.3 and 1.2 Hz (Fig. 6g), whereas the fraction of vertically tuned DS boutons in Frmd7tm mice was significantly higher at 1.2 Hz than horizontally tuned boutons (Fig. 6g). Thus, in control mice, the preferred directions of DS boutons showed a TF-invariant bias for posterior motion (P < 0.001 for 0.3 and 1.2 Hz, Rayleigh test; Fig. 6h), whereas they were biased to ventral at 1.2 Hz in Frmd7tm mice (P ≥ 0.05 and P < 0.01 for 0.3 and 1.2 Hz, respectively; Rayleigh test; Fig. 6h, i). In control mice, 36.9% of DS boutons increased response amplitudes as the TF increased from 0.3 to 1.2 Hz, whereas only 17.1% increased in Frmd7tm mice (Fig. 6i). Noticeably, posteriorly tuned DS boutons in control mice showed larger TF-dependent response increments than boutons preferring other directions (Fig. 6j, k). Conversely, DS boutons from Frmd7tm mice decreased their responses as the TF increased in all preferred directions (Fig. 6k), although posteriorly tuned boutons did show a smaller response decrement than anteriorly tuned boutons (Fig. 6k). These data suggest that two response characteristics of dLG N DS axons arriving in L1 and L2/3 of V1 were impaired in Frmd7tm mice: the preference for high-TF horizontal motion and the TF-invariant population bias for preferring posterior motion.

Altered TF-dependent responses in the retina of Frmd7tm mice. TF-dependent responses of retinal neurons in Frmd7tm mice have not previously been examined25,27. We sought to link the TF-dependent response modulation observed in thalamic axons and cortical neurons to that in retinal neurons. We performed two-photon calcium imaging from the ganglion-cell layer in isolated retinas by the virally transduced GCaMP6s (Fig. 7a–c). In control retinas, neurons preferred high-TF stimuli moving in posterior, anterior, and ventral directions (i.e., nasal, temporal, and superior on the retina, respectively), whereas we found no TF-dependent response modulation for dorsal motion (i.e., inferior on the retina; Fig. 7d). In Frmd7tm retinas, the high-TF preference of neurons was impaired for horizontal motion: their responses to higher TFs were significantly lower than control mice (Fig. 7d). In contrast, responses to vertical motion were unaltered, as has been shown previously27.

Next, we restricted our analyses to retinal neurons showing both ON-OFF responses and DS tuning (36.7% and 35.8% of all responsive neurons in control and Frmd7tm retinas, respectively; Supplementary Fig. 9). As previously reported27, Frmd7tm retinas showed significantly decreased ON-OFF DS responses to horizontal motion (Fig. 7e). In control mice, there was a population bias for posterior motion at 1.2 Hz (P < 0.05, Rayleigh test; Fig. 7f), whereas DS cells from Frmd7tm mice were biased towards vertical motion at both 0.3 and 1.2 Hz (P < 0.001 for 0.3 and 1.2 Hz, Rayleigh test; Fig. 7f, g). Interestingly, only posteriorly tuned ON-OFF DS cells showed larger TF-dependent response increments and a larger TF-dependent increase in DSI in control retinas (P < 0.001 and P ≥ 0.05 for all comparisons in control and Frmd7tm mice).
mice, respectively, Mann–Whitney U-test; Fig. 7h, i). These data suggest at least two mechanisms underlying the retinal over-representation of a preference for high-TF posterior motion: First, ON-OFF DS cells that prefer posterior motion are more abundant than other populations, which becomes more evident as the DSI of these cells increases at high TFs. Second, posteriorly tuned retinal DS cells show larger response increments than DS cells preferring other directions as TF increases. Importantly, both of these posterior biases are markedly impaired in Frmd7tm retinas.

Discussion

Our results provide three major insights into the functional organization of murine visual pathways for processing visual
**Fig. 3** V1 DS cells projecting to area RL or PM respond differently to disruption of retinal direction selectivity. 

a Target-unspecific V1 neurons were labeled by injecting AAV2/1-GCaMP6f into the V1. PM- (PM-p) and RL-projecting (RL-p) V1 neurons were labeled by injecting rAAV2-retro-GCaMP6m into PM and RL, respectively. 

b Two-photon calcium imaging from L2/3 in V1 of control (1087 target-unspecific, 109 PM-p, and 513 RL-p DS cells; 5 mice per group) and Frmd7tm mice (954 target-unspecific, 93 PM-p, and 235 RL-p DS cells; five mice per group). Left: example PM- and RL-projecting neurons expressing GCaMP6m (scale bar, 10 μm). Right: trial-averaged fluorescence (ΔF0/F0) time courses for the same neurons. Shading: SEM. c Tuning curves for neurons shown in b. Error bars: SEM. Solid line: Gaussian fit. d Fraction of DS cells in target-unspecific, PM-p, and RL-p V1 neuronal populations (two-sided χ² test with Yates correction). e Preferred TF for target-unspecific (two-sided Mann-Whitney U-test), PM-p (two-sided Mann-Whitney U-test), and RL-p (two-sided Mann-Whitney U-test) V1 DS cells. Triangles: Medians. f Response amplitude as a function of motion direction and TF for target-unspecific, PM-p, and RL-p V1 DS cells. White asterisks: significantly decreased in Frmd7tm mice, two-sided Mann–Whitney U-test. g Fractional distributions of preferred motion directions for target-unspecific, PM-p, and RL-p V1 DS cells at 0.3 and 1.2 Hz. The fractions are normalized to the largest fraction across genetic groups. h Distributions of preferred motion directions at 0.3 and 1.2 Hz for target-unspecific, PM-p, and RL-p V1 DS cells (two-sided Kolmogorov–Smirnov test). **P < 0.01, ***P < 0.001, n.s., not significant, in d, e, and h. Source data are provided as a Source Data file.

**Fig. 4** V1 L2/3 neurons with distinct functional characteristics are sensitive to disruption of retinal horizontal direction selectivity. 

a Response matrix composed of TF-dependent response amplitudes and DSI for all pooled V1 L2/3 DS cells (target-unspecific, PM-p, and RL-p) sorted by TF preference. 

b Two-dimensional (2D) visualization of the 1st and 2nd principal components for the response matrix shown in a. Each point represents one neuron. c TF preference of individual V1 neurons. d Fraction of neurons in 8 × 8 grids (gray lines) calculated from the PCA plot shown in a for control and Frmd7tm mice. e Fraction difference map between control and Frmd7tm mice. Black and white asterisks: significantly decreased and increased fractions in Frmd7tm mice, respectively, P < 0.05, two-sided χ² test with Yates correction. f Peak response amplitude as a function of TF for three groups (decreased, increased, or unchanged in Frmd7tm mice) in control (886, 845, and 591 DS cells, respectively) and Frmd7tm mice (169, 825, and 381 DS cells, respectively). Error bars are SEM. g TF-dependent tuning characteristics of individual V1 neurons from the three groups in control and Frmd7tm mice. Angular coordinate: preferred direction. Radial coordinate: DSI. Inner circle: DSI of 0.5. h Relationship between effect of Frmd7tm mutation and enrichment in PM-p or RL-p neurons. x-axis: Index comparing axonal projection pattern for the individual grids in e that were decreased and increased in Frmd7tm mice; groups with positive and negative index values are enriched in RL- and PM-projecting neurons, respectively. y-axis: Index comparing sensitivity to altered retinal direction selectivity for grids; grids with a positive and negative index value are decreased and increased in Frmd7tm mice, respectively. Source data are provided as a Source Data file.
Recent research has demonstrated that the connections between projection neurons in V1 that project to different targets are rare, regardless of response similarities. Thus, our results suggest that each of these segregated subnetworks potentially receives a unique combination of retina-geniculate inputs, enabling specialized responses of individual HVAs to emerge. Our findings invoke the intriguing question of how such specific multisynaptic connectivity may be established during development. One possibility is that downstream circuits of retinal DS cells are wired together based on a set of uniquely expressed molecules. Alternatively, or synergistically, mechanisms dependent on patterned spontaneous activity or visual experience may guide the synaptic connections.

By analyzing the PCA distributions of the imaged neurons, we identified that the coupling of response amplitude increments and the development of a posterior motion preference as the stimulus TF increases are the key response features in RL and V1 L2/3 DS cells that rely on retinal motion computations along the
Fig. 6 Directionally tuned thalamic boutons in the superficial V1 are sensitive to disruption of retinal horizontal direction selectivity. a Top: thalamocortical axons in V1 were labeled by injecting AAV2/1-GCaMP6f into the dLGN. Bottom: Example GCaMP6f-positive neurons in dLGN (scale bar, 100 µm; inset, 10 µm). b Left top: Example two-photon image of dLGN axons in L2/3. Left bottom: magnified axonal boutons expressing GCaMP6f (scale bar, 35 µm; inset, 10 µm). Right: trial-averaged fluorescence (ΔF/F₀) time courses for the boutons indicated. Shading: SEM. c Tuning curves for boutons shown in b. Error bars: SEM. Solid line: Gaussian fit. d Fraction of DS boutons (5,121 and 5,525 DS boutons from control and Fmrd7tm mice, respectively; five mice per group; two-sided χ² test with Yates correction). e Fraction of horizontally- and vertically tuned DS boutons at 0.3 and 1.2 Hz (two-sided Mann–Whitney U-test). g Fraction of horizontally- and vertically tuned DS boutons at 0.3 and 1.2 Hz (two-sided Mann–Whitney U-test). h Fractional distributions of preferred motion directions for DS boutons at 0.3 and 1.2 Hz, normalized to the largest fraction across genetic groups. i Distribution of preferred motion directions at 0.3 and 1.2 Hz in DS boutons (two-sided Kolmogorov–Smirnov test). j TF-dependent characteristics of DS boutons. Angular coordinate: Directional preference at 1.2 Hz. Radial coordinate: Ratio of response amplitudes at 1.2 and 0.3 Hz. Inner circle: Response ratio of 1. k Ratio of response amplitudes at 1.2 and 0.3 Hz as a function of motion direction preference of DS boutons (two-sided Kolmogorov–Smirnov test). Center line is median, box limits are 25th and 75th percentiles, and whiskers show minimum and maximum values. *P < 0.05, **P < 0.01, ***P < 0.001, n.s., not significant. Source data are provided as a Source Data file.

cannot exclude the possibility that signaling from retinal DS cells is also conveyed to area RL via extrageniculate pathways48–50, given the innervation of the superior colliculus by retinal OFF DS cells41, despite collicular outputs preferentially target the posttrinal cortex15.

What is the possible behavioral role of area RL and retinal ON-OFF DS cells? In mice, area RL is thought to belong to a dorsal-like stream5,11,12,38, and the anterior part of RL is considered to be part of the posterior parietal cortex42,43, which is important for visually guided navigation44,45. Furthermore, area RL is strongly interconnected with other areas such as area AL, the primary somatosensory cortex, and the secondary motor cortex46. Notably, >50% of RL L2/3 pyramidal neurons are multisensory, integrating both tactile and visual inputs47, and RL is also thought to be involved in visuo-motor integration48. Lastly, the receptive field location of RL neurons is biased to the anterior, lower visual-field48 and the neurons respond to visual stimuli very close to the mouse49. Altogether, these prior findings indicate that area RL is ideally adapted to sensori-motor coordination for the lower horizontal axis. How do such TF-dependent changes in responses and tuning rely on retinal motion computations? We propose that the retinal over-representation of posterior motion that develops with increasing TF, and which is conveyed via the dLGN, is amplified by the local circuitry within V1, shifting the balance of cortical DS responses to the posterior direction. The suppression of responses to non-posterior directions at higher TFs could result from cortical normalization37. The larger retinal population responses to posterior compared to anterior motion at higher TFs may be advantageous for distinguishing rotational and translational optic flow by enabling differences of summed output activity from left versus right retinas. The higher TF sensitivity of horizontal compared to vertical retinal DS cells may be explained by the two-dimensional nature of terrestrial navigation in mice. In contrast to RL and V1 L2/3 neurons, direction selectivity in V1 L4 and PM L2/3 neurons is most likely generated by non-retinal mechanisms26 (Figs. 1 and 5), indicating spatially segregated processing of direction selectivity computed by retina-dependent and -independent mechanisms. Lastly, it is worth noting that we
visuo-tactile space near the face of the mouse. Interestingly, the ventral intraparietal area of the human posterior parietal cortex, which is referred to as the dorsal stream of vision, also contains visual and tactile maps, and is focused on processing the face-centered sensory space. In macaque monkeys, this area contains many DS cells, which prefer high speeds, and some of these cells are sensitive to the trajectory of visual stimuli moving toward the face. Furthermore, this area jointly represents translation direction and rotation velocity during self-motion based on optic flow. Together with a recent finding that preferred directions of control DS and non-DS cells are different, this suggests a role for DS cells in the control of self-motion. The figure shows the response of DS cells to horizontal and vertical motion at different speeds and directions.
Fig. 7 Preference of retinal neurons to posterior motion at higher TFs is disrupted in Frmd7tm mice. a Two-photon calcium imaging was performed on retinas from control (1157; four mice) and Frmd7tm mice (953; four mice). b Left: example control and Frmd7tm retinal neurons expressing GCaMP6s (scale bar, 10 μm). Right: trial-averaged fluorescence (ΔF/F0) time courses for the same. Shading indicates SEM. c Tuning curves for neurons shown in b. Error bars are SEM. Solid line: Gaussian fit. d Response amplitude as a function of motion direction (nasal [N], temporal [T], inferior [I], and superior [S]) and TF for retinal white astersix: significantly decreased response in Frmd7tm mice (two-sided Mann–Whitney U-test). e Fraction of horizontally- and vertically-tuned retinal ON-OFF DS cells (425 and 342 ON-OFF DS cells in control and Frmd7tm mice, respectively) at 0.3 and 1.2 Hz (two-sided χ²-test with Yates correction). f Fractional distributions of preferred motion directions for ON-OFF DS cells at 0.3 and 1.2 Hz in ON-OFF DS cells (two-sided Kolmogorov–Smirnov test). g Distribution of preferred motion directions at 0.3 and 1.2 Hz in ON-OFF DS cells (two-sided Mann–Whitney U-test). h TF-dependent characteristics of ON-OFF DS cells. Angular coordinate: Directional preference at 1.2 Hz. Radial coordinate: Ratio of response amplitudes at 1.2 and 0.3 Hz. Inner circle: response ratio of 1. Ratio of response amplitudes at 1.2 and 0.3 Hz as a function of motion direction preference of cells that showed ON-OFF DS responses (two-sided Mann–Whitney U-test). Center line is median, box limits are 25th and 75th percentiles, and whiskers show minimum and maximum values. j Schematic diagram of proposed neural pathway linking retinal ON-OFF DS cells to RL DS cells. *P < 0.05, **P < 0.01, ***P < 0.001, n.s., not significant. Source data are provided as a Source Data file.

Methods

Experimental animals. Wild-type control mice (C57BL/6J) were obtained from Janvier Labs. Frmd7tm mice are homozygous female or hemizygous male Frmd7tm (KOMP/WSI) mice, which were obtained as Frmd7tm (KOMP/WSI) mice from the Knockout Mouse Project (KOMP) Repository.25,27: Exon 4 and neo cassette flanked by loxP sequences were removed by crossing with female Cre-deleter Edil2tm2Sel/sc32 (strain: B6.129X1-Mef2cCre/J, Jackson laboratory stock: 007900). A 5 mm glass coverslip sterilized by ozonolysis21 were removed, and a custom head-plate and cranial window implantation was mounted on the skull using cyanoacrylate-based glue (Super Glue Precision, Loctite) and black dental cement (Jet Denture Repair Powder) to allow optical access to the cortex, a 5 mm diameter craniotomy was performed. After removing the dura spontaneously detached with the skull and/or cement mixture dissolved in saline, and after recovering on a heating pad for one hour.

Retinal ON-OFF DS cells are aligned with translatory and rotatory optic flow fields21, our findings raise the intriguing hypotheses that the mouse area RL may be a functional counterpart of the primate ventral intraparietal area, and visual motion analyses in the primate posterior parietal cortex may rely on signaling from such retinal DS cells. However, it is still unknown if dLGN-projecting retinal DS cells exist in primates.

Visual stimuli for ISOI. Retinotopic maps were generated by sweeping a spatially corrected (Matlab code provided by Spencer Smith: https://labrigger.com/blog/2012/03/06/mouse-visual-stim/) full-field bar across the screen in both azimuth and elevation directions.37. The bar contained a flickering black-and-white checkerboard pattern on a black background.37. The width of the bar was 12.5° and the checkerboard square size was 25°. Each square alternated between black and white at 4 Hz. In each trial, the bar was drifted 10 times in each of the four cardinal directions (0°, 90°, 180°, and 270°), moving at 8–9°/s. Usually, two to three trials were sufficient to achieve well-defined retinotopic maps. For measuring and characterizing the evoked areal activity in V1 and HVAs we presented 100% contrast black and white sinusoidal gratings drifting in each of the four cardinal directions. We presented gratings with four different TFs: 0.3, 0.75, 1.2, and 1.8 Hz (0.03 cycles/°). Each stimulus was stationary for 10 s and in motion for 10 s, comprising a stimulus period of 20 s, which was repeated 5 times in each direction. All stimuli used for ISOI were produced and presented using Matlab and the Psychophysics Toolbox.38

Image analysis for ISOI. To generate functional visual cortex maps from the raw image data, we took the response time course for each pixel and computed the phase and magnitude of the Fourier transform at the stimulus frequencies (0.067 and 0.088 Hz, for azimuth and vertical, respectively).39 The bar was drifted in opposite directions in order to subtract the delay in the intrinsic signal relative to neuronal activity.35 The resulting phase maps were then converted into retinotopic coordinates (visual degrees) from the known geometry of our setup to retrieve absolute retinotopy. We used automated, publicly available code to identify visual area borders based on their visual-field sign maps37 (see also description below).

Intrinsic imaging. For ISOI mice were anesthetized with isoflurane (2–3% induction) and head-fixed in a custom holder. Chlorprothoxine was administered intraperitoneally (2.5 mg/kg body weight; Sigma) as a sedative,40 and isoflurane reduced to 0.5–1% and kept constant during visual stimulation. Core body temperature was maintained at 37–38 °C using a feedback-controlled heating pad (ATC2000, World Precision Instruments). The stimulated contralateral eye was kept wet by topical application of a thin layer of silicone oil and fluorescein (10,000, molecular weight). The experimental setup employed for ISOI was adapted from a similar system41 and made publicly available (https://snic.github.io/ISOI/). A 2 × air-objective (Olympus, 0.08 NA, 4 mm field of view) was mounted on our Scienta InVivoScope, which was equipped with a CMOS camera (HD1-D-D1312-160-CL12, PhotonFocus), a large-depth camera that offers high signal-to-noise measurements in bright light conditions. The camera was connected to a Matrox Solios (eCL/XCL-B) frame-grabber via Camera Link. The acquisition code for the Matrox board was written in Matlab using the Image Acquisition Toolbox.

For ISOI mice, the microscope was defocused to 2000 μm, where intrinsic signals were excited using a red LED (KLI300, Scit). Delivered via light guides through a 610 nm long-pass filter (Chroma). Reflective light was captured through a 700 ± 50 nm (mean ± SEM) band-pass filter (Chroma) positioned right in front of the camera at a rate of 6 frames per second (512 × 512 pixels). At 700 nm there is a large change in the absorption coefficient between oxy- and deoxyhemoglobin, contributing to the intrinsic signal measured in these experiments.41 The 47.65 × 26.87 cm (width × height) screen was angled 30° from the mouse’s midline and positioned so that the perpendicular bisector was 10 cm from the bottom of the screen, centered on the screen left to right (23.8 cm on each side), and 10 cm from the eye.31 This resulted in a visual-field coverage from −14.98° to 60.77° (total 102.75°) in elevation and from −67.23° to 67.23° (total 134.46°) in azimuth. Thus, the stimulus covered almost the entire known visual hemifield of the mouse, which is estimated to be at most 110° vertically and 140° horizontally. For the ISOI experiments presented in Fig. 1, each mouse was imaged on three separate days, and the data averaged to reduce the chance of day-to-day variations confounding group-level results.

Head-plate and cranial window implantation. Mice were anesthetized with an intraperitoneal injection of a fentanyl (0.05 mg/kg body weight; Hameln), midazolam (5.0 mg/kg body weight; Hameln) and medetomidine (0.5 mg/kg body weight; Domitor, Orion) mixture dissolved in saline. The depth of anesthesia was monitored by the pinch withdrawal reflex throughout the surgery. Core body temperature was monitored using a rectal probe and temperature maintained at 37–38 °C using a feedback-controlled heating pad (ATC2000, World Precision Instruments). Eyes were protected from dehydration during the surgery with eye ointment (Ocu loculate Augengel). The scalp overlaying the left visual cortex was removed, and a custom head-fixing imaging head-plate with a circular 8 mm diameter opening was mounted on the skull using cyanoacrylate-based glue (Super Glue Precision, Locite) and dental cement (Jet Denture Repair Powder) to allow for subsequent head fixation during imaging. The center of the head-plate was positioned above V1, 2.5 mm lateral and 1 mm anterior of lambda.55. To gain optical access to the cortex, a 5 mm diameter craniotomy was performed. After removing the skull flap, the cortical surface was left moist with a cortex buffer containing 125 mM NaCl, 5 mM KCl, 10 mM glucose, and 4 mM HEPS, 2 mM MgSO4, and 2 mM CaCl2. The dura was left intact (except in two animals in which the dura spontaneously detached with the skull flap) and any occasional bleedings were immediately stopped with Gelfoam (Pfizer). A 5 mm glass coverslip sterilized in ethanol (0.15 mm thickness, Warner Instruments) was placed onto the brain to gently compress the underlying cortex and dampen biological motion during subsequent imaging.36 The cranial window was hermetically sealed using a cyanoacrylate-based glue (Super Glue Precision, Locite) mixed with black dental cement (Jet Denture Repair Powder mixed with iron oxide powdered pigment) to prevent the entry of stray light from the screen through the skull and/or cement during imaging.36 Mice were returned to their home cage after anesthesia was reversed with an intraperitoneal injection of a flumazenil (0.5 mg/kg body weight; Hameln) and atipamezole (2.5 mg/kg body weight; Antisedan, Orion Pharma) mixture dissolved in saline, and after recovering on a heating pad for one hour.
and superimposed those borders with the anatomical blood-vessel images to accurately localize V1 and individual HVAs.

To evaluate and quantify the spatial properties of visual areas, we first computed and identified the borders of each visual area, using a dissociation algorithm and the Image Processing Toolbox in Matlab. The identification process constituted three conventional edge-detection steps. (1) Thresholding of the obtained visual-field sign map. For this we used a definition of 1 SD (1) where I equals the intensity of a pixel and denotes the mean intensity of neighboring pixels. (2) Isolation and segmentation of pixels. For this step we used the 8-neighbors criterion. If there were >4 non-zero pixels among the eight neighbors of one pixel, the pixel was retained and any gaps between pixels within the eight neighbors were filled with a non-zero value. If this criterion was not met, the pixel's value was set to 0. The value of all non-zero pixels was set to 1. (3) Edge detection. The isolated and segmented pixels were next binarized, and each edge was computed based on a Sobel method using the edge function in Matlab. After the isolated and segmented pixels were next binarized, and each edge was converted to mm²) and de

The thresholded image was smoothed using a median filter size, 3 × 3 neighborhoods. The isotropic and segmented pixels were next binnedarized, and each edge was

calculated as the peak power of the filter size, 3 × 3

equals the filter size, 3 × 3

and the response strength for a given area was averaged across the three experimental days before pooling data from all mice.

Local viral labeling. For local viral injections in the visual cortex or dLGN, mice were first anesthetized with an intraperitoneal injection of a fentanyl (0.05 mg/kg body weight; Hameln), midazolam (0.5 mg/kg body weight; Hameln) and medetomidine (0.5 mg/kg body weight; Domitor, Orion) mixture dissolved in saline. For injections yielding GCaMP6 expression in areas V1, RL and PM, three 0.4 mm diameter craniotomies were performed over the left visual cortex and 100 nl AAV2/1-Syn-GCaMP6f-WPRE (2.13 × 10¹³ vg/ml, Penn Vector Core #AV-1-PV2822) slowly injected at depths of 100–500 μm using a borosilicate glass micropipette (30 μm tip diameter) and a pressure injection system (Picospritzer III, Parker). For labeling geniculo-cortical axons projecting from the dLGN, 20–40 nl AAV2/1-Syn-GCaMP6f-WPRE (2.13 × 10¹³ vg/ml, Penn Vector Core #AV-1-PV2822) was slowly injected into the left dLGN using stereotoxic coordinates: 2.1 mm posterior of the Bregma; 2.2 mm lateral of the midline; 2.3 mm below the pial surface. To prevent backflow during withdrawal, the micropipette was kept in place for at least 5 min before it was slowly retracted. The skin was afterwards sutured shut. Mice were returned to their home cage after the anesthesia was reversed with an intraperitoneal injection of a flumazenil (0.5 mg/kg body weight; Hameln) and atipamezole (2.5 mg/kg body weight; Antisedan, Orion Pharma) mixture dissolved in saline. For imaging, the mouse was placed under the microscope 10 cm from the 47.65 × 26.87 cm (width × height) screen, with the screen subtending 134.46° in azimuth and 102.75° in elevation and angled 30° from the mouse’s midline. The visual area targeted for two-photon calcium imaging was localized based on superimposing the IS0I border map onto the cortical surface. Imaging was performed 50–100 μm (L1, 120–250 μm (L2)/3, and 350–500 μm (L4) below the dura using a scanning microscope (VivoScope, Scientifica) with a 7.9 kHz resonant scanner running ScScan version 1.3 with dispersion-compensated 940 nm excitation provided by a mode-locked Ti:Sapphire laser (MaiTai DeepSee, Spectra-Powers) through either a Nikon 16x (0.8 NA; soma imaging) or an Olympus 25x (0.15 NA; axonal bouton imaging) objective. Clear ultrason gel was used as an immersion medium (Aquaasonic, Parker Laboratories). To prevent light leak originating from the visualization or fixation, an imaging area was protected with a black O-ring and the objective shielded with black tape. Average excitation power after the exit pupil of the objective varied from 25 to 60 mW. Typical images had 512 × 512 pixels, at 0.3–0.5 μm per pixel for axons, and 0.92 μm per pixel for somata, and were acquired at 30.9 Hz using bidirectional scanning. By correcting for any slow drifts in neuron or axon location within the field of view using a reference image26, we could be able to record from the same population of neurons or axons over extended periods of time (∼40 min), allowing us to assess responses as a function of TF conditions. There was no evidence of GCaMP6 bleaching during experiments. Each mouse was imaged repeatedly over the course of 1–2 weeks.

Visual stimuli for cortical two-photon calcium imaging. Visual stimulation for cortical two-photon calcium imaging was generated and presented via Pybon, a Python-based custom-made software. Visual stimuli were presented in a binocularly and binocularly-opposed 180° contrast black and white sinusoidal drifting gratings. Drifting gratings were presented in six trials for 3 s at a time, with 3 s of gray screen between presentations, and were drifted in 12 different directions (0°, 30°, 60°, 90°, 120°, 150°, 180°, 210°, 240°, 270°, 300°, and 330°) in a pseudorandomized order, with a spatial frequency of 0.03 cycles/° and TFs of 0.3, 0.75, 1.2, and 1.8 Hz.

Image analysis for cortical two-photon calcium imaging. Imaging data were excluded from analysis if motion along the z-axis was detected. Raw images from soma imaging were corrected for in-plane motion via a correlation-based approach in Matlab25. Raw images from axonal bouton imaging were corrected for in-plane motion using a piecewise non-rigid motion correction algorithm26. Sinusoidal gratings were presented in ImageJ (https://github.com/Fislab/CorticalMap) and selected based on mean and maximum fluorescence imge26; somata ROIs were polygonal; axonal bouton ROIs were circular. The same
ROIs set was used for all imaging stacks acquired in a given field of view. Fluorescence time courses were computed as the mean of all pixels within ROIs and were extracted using MIJ [http://bigwww.epfl.ch/sage/software/mij/]. Baseline-normalized fluorescence time courses ($\Delta F/F_0$) were computed using a $60 \times 10$ percentile filter and 0.01 Hz low-pass Butterworth filter to define $F_0$ (ref. 56). For each of the twelve directions, the response amplitude in each trial was determined by sorting all $\Delta F/F_0$ values (down-sampled to 15.4 Hz) during the 3 s drift period, and taking the mean of the larger 30% of data points. Somata and axonal boutons were defined as visually responsive if $\Delta F/F_0$ in the preferred direction of motion exceeded 0.06 (refs. 78,98) in at least one of the four TFs. They were defined as DS if: (1) They were visually responsive; and (2) their DSI exceeded 0.3 (refs. 26,50) in at least one of the four TFs:

$$DSI = \frac{R_{pref} - \bar{R}_{opp}}{R_{pref} + \bar{R}_{opp}}$$

where $R_{pref}$ denotes the mean $\Delta F/F_0$ response to the preferred direction of motion and $\bar{R}_{opp}$ the mean $\Delta F/F_0$ response to the opposite direction. The preferred direction of motion for each cell was calculated as the angle, in polar coordinates, of the vector sum$^{27,55}$:

$$\theta = \tan^{-1} \left( \frac{\sum_{i=1}^{N} R_i \sin \theta_i}{\sum_{i=1}^{N} R_i \cos \theta_i} \right)$$

where $\theta_i$ denotes the direction of motion for cell $i$ and $R_i$ the mean $\Delta F/F_0$ response to direction $i$.

OSI was computed as:

$$OSI = \frac{R_{off} - \bar{R}_{on}}{R_{off} + \bar{R}_{on}}$$

where $R_{off}$ denotes the mean $\Delta F/F_0$ response to the preferred orientation and $\bar{R}_{on}$ the mean $\Delta F/F_0$ response to the orthogonal orientation. The preferred orientation was defined as the axis including the preferred direction and its opposite direction.

Data decomposition and segmentation. To correlate the TF-dependent response properties in individual cortical DS cells and the fractional changes of neurons between control and Frmd7tm mice, we performed decomposition and segmentation of datasets summarizing response features of the identified DS cells. First, we computed a response matrix for each visual area (e.g., area RL) using a total of eight parameters for each neuron: peak $\Delta F/F_0$, amplitudes and OSI values under each of the four TF conditions. The response matrix included datasets from both control and Frmd7tm mice. For the V1 L2/3 response matrix, we pooled the datasets from target-unspecific, PM-projecting, and RL-projecting DS cells. Next, the response matrix was decomposed into two dimensions by PCA. The resulting PCA distributions showed a distribution trend depending on the TF preference of the individual neurons, indicating that neurons sharing the same TF preference tended to be clustered in the local region of the PCA distribution. To quantify the localization of neurons, we segmented the PCA distribution by $8 \times 8$ grids. We then calculated the fraction of neurons located within each of the grids, and examined the fractional changes between control and Frmd7tm mice. Based on the fractional changes, we statistically classified neurons into three groups: (1) “Increased”, (2) “Decreased”, and (3) “Unchanged” in Frmd7tm mice, compared to control mice. We tested the number of grids for this segmentation, and confirmed that the results were not qualitatively changed by the size of the grids (Supplementary Fig. 6).

To investigate the relationship between the effects of altered retinal horizontal direction selectivity and the target region of V1-projecting DS cells, we analyzed the fraction of PM- and RL-projecting cells in each grid based on a projection target index (PTI): $PTI = \frac{\bar{F}_P - F_{Padj}}{F_{Padj} + \bar{F}_R}$

Where $F_P$ denotes the fraction of cells projecting to area $y$ in a grid $x$. A positive PTI indicates that the neurons within the grid are biased towards RL-projecting neurons, while a negative PTI indicates a bias towards PM-projecting neurons.

The effects of altered retinal horizontal direction selectivity was evaluated using a mutation index (MI):

$$MI = \frac{F_{On} - F_{Off}}{F_{On} + F_{Off}}$$

Where $F_y$ denotes the fraction of cells in population $y$ in grid $x$. A positive MI indicates that the fraction of neurons originating from Frmd7tm mice is decreased in the grid, while a negative MI indicates that the fraction is increased.

**Visual stimuli for retinal two-photon calcium imaging.** The visual stimulation was generated via custom-made software (Python and LabVIEW) developed by Zoltan Raics, projected by a DLP projector (LightCrafter Fiber F4050 MJK, EKB Technologies) coupled via a liquid light guide to an LED source (4-wavelength high-power LED Source, Thorlabs) with a 400 nm LED (LZU-08U-A00, LED Engin) through a band-pass optical filter (ET405/40x, Chroma) and focused onto the photoreceptor layer of the mounted retina through a condenser (W1-DICD, Olympus). The stimuli were exclusively presented during the fly-back period of the horizontal scanning mirror$^{68}$. To measure directional tuning and TF preference, we presented 100% contrast black and white sinusoidal drifting gratings (mean intensity, 0.058 mW/cm$^2$). Light intensity was measured using a power meter (PM200, Thorlabs) and a spectrometer (USB4000-XR1, Ocean Optics). Drifting gratings were presented in 3 trials for 3 s at a time, with 3 s of gray screen between presentations, and shown in 8 different directions ($0°$, $45°$, $90°$, $135°$, $180°$, $225°$, $270°$, and $315°$) in a pseudorandomized fashion, with a spatial frequency of 0.03 cycles° and TFs of 0.3, 0.75, 1.2, and 1.8 Hz. To measure ON and OFF responses, we presented static flash spots (2 s in duration, 50, 100, 200, 400, 800 µm in diameter). To classify retinal cells into ON-OFF and non-ON-OFF populations, we used an ON-OFF index (OOI)$^{57}$ (Supplementary Fig. 9):

$$OOI = \frac{R_{OFF} - R_{ON}}{R_{OFF} + R_{ON}}$$

Where $R_{ON}$ and $R_{OFF}$ denote peak calcium responses during the static spot illumination phase, and the phase after the illumination, respectively. If the mean OOI for 50–800 µm spots was <0.3, the cell was defined as an OFF-ON cell. We calculated the OSI for individual cells, and defined cells with OSI > 0.3 as DS cells, similar to experiments performed in the cortex.

**Image analysis for retinal two-photon calcium imaging.** The raw two-photon scanning imaging data acquired was initially loaded into Matlab and converted into accessible image files. The ROIs for cell bodies of retinal cells were drawn in Matlab by fitting polygons, and selected based on mean and maximum fluorescence intensities. Fluorescence time courses were computed as the mean of all pixels within the ROI at each time point and were extracted in Matlab. The raw GCaMP6 fluorescence signals for each ROI were normalized ($\Delta F/F_0$) using the mean fluorescence ($F_0$) in a 2 s window before visual stimulation, and then synchronized with visual stimulus information. The $\Delta F/F_0$ signals were resampled using the interpolation function in Matlab, and smoothed by a moving average filter (with two data points). To evaluate cell responsiveness, we determined a threshold for each cell as $mean_{F_0} + 2 SD_{F_0}$, and any cell with response amplitudes higher than their threshold was defined as visually responsive and included in further analysis.

**Histology and confocal imaging to validate the injection site in the dLGN.** Mice were anesthetized with an intraperitoneal injection of a fentanyl (0.05 mg/kg body weight; Hameln) and medetomidine (0.2 mg/kg body weight; Domitor, Orion) mixture dissolved in saline. We made a small hole at the border between the sclera and the cornea with a 30-gauge needle. Next, we loaded 2 µl of AAV1–CAG-ECFP-6a/VP2-FV333 into a pulled borosilicate glass micropipette, and the AAV was pressure-injected through the hole into the vitreous of the left eye using a Picospritzer III (Parker). Mice were returned to their home cage after anesthesia was reversed by an intraperitoneal injection of a flumazenil (0.5 mg/kg body weight; Hameln) and atipamezole (2.5 mg/kg body weight; Antisedan, Orion Pharma) mixture dissolved in saline.

**Retinal two-photon calcium imaging.** Retinal imaging was performed 3–4 weeks after virus injections. Mice were first dark-adapted for 1 h, and next the retina was prepared$^{68}$. The retina was isolated from the left eye, and mounted ganglion-cell side up on a small piece of filter paper (Millipore, MF-membrane), in which a $2 \times 2$ mm aperture window had previously been cut. During the procedure, the retina was illuminated by dim red light (KL1600 LED, Schott) filtered with a 650 nm high-pass optical filter (650/45x, Chroma) and bathed extracellular solution (in mM): NaCl 2.5 KCl 1 CaCl$_2$ 1.6 MgCl$_2$ 10 d-glucose, 22 NaHCO$_3$, bubbled with 5% CO$_2$, 95% O$_2$. The retina was kept at 35–36 °C and continuously superfused with oxygenated extracellular solution during recordings. For retinal two-photon calcium imaging we employed an equipment setup similar to that previously employed$^{68}$. The isolated retina was placed under a microscope (Si-Scence, Scientifica) equipped with a galvo-galvo scanning mirror system (8315 K, Cambridge Technologies), a mode-locked Ti:Sapphire laser tuned to 940 nm (MaiTai DeepSee, Spectra-Physics), and an Olympus 20x (1.0 NA) objective. The GCaMP6s signals emitted were passed through a set of optical filters (ET525/50m, Chroma, GG495, Schott) and collected using a GaAsP detector (Scientifica). Images were acquired at 6–10 Hz using custom-made software developed by Zoltan Raics (SELS Software).
using a confocal microscope (Zeiss LSM 780) with a 10× (0.45 NA) objective. To validate the specificity of starburst amacrine cell ablation in diphtheria toxin injected Cre-expressing DTR mice and PBS-injected littermates, we performed immunohistochemical analyses of the retinas27. A nasal mark was applied to the eyes and fixed for 20 min at room temperature (RT) in 4% PFA before dissection. Afterwards, eyes were rinsed in PBS, dissected, mounted on flatmount paper in 4% PFA for 30 min at RT and then washed with PBS overnight at 4 °C on a shaker. The next day, retinas were incubated in 30% sucrose in PBS for at least 3 h at RT. Afterwards, retinas were transferred in the sucrose buffer to microscope slides (SUPERFROST PLUS, Thermo Scientific) and frozen and thawed three times using dry ice to enhance antibody penetration. After washing with PBS, retinas were blocked for 3 h in blocking buffer (1% bovine serum albumin (BSA), 10% normal donkey serum (NDS), 0.5% Triton X 100, 0.02% sodium azide in PBS) at RT. Primary antibodies (rabbit anti-RPMS 1:500 [Milipore, ABN1362] and goat anti-ChAT 1:200 [Milipore, ABN1144P]) were incubated for 5 days in antibody reaction buffer (1% BSA, 3% NDS, 0.5% Triton X 100, 0.02% sodium azide in PBS) at 4 °C on a shaker. Secondary antibodies (donkey anti-rabbit IgG Alexa Fluor 568 1:200 [Invitrogen], donkey anti-goat IgG Alexa Fluor 488 1:200 [Life Technologies] together with DAPI 1:1000 [Thermofisher]) were incubated overnight at 4 °C in antibody reaction buffer. After a final wash in PBS, retinas were embedded in Fluoromount-G (eBioscience). For cell density analysis, z-stacks containing images of 1024×1024 pixels (1.38 μm per pixel) were acquired at an interval of 4 μm (total thickness of 75–80 μm) with a confocal microscope (Zeiss LSM 780) using a 10× (0.45 NA) objective, and cells were counted using ImageJ. For detailed confocal z-stacks, we used a 40× (1.4 NA) objective and acquired 1024×1024×1024 (0.35 μm per pixel) images at an interval of 0.3 μm (total thickness of ~75 μm).

Statistical analysis. Statistical tests were performed in Matlab and we used the following statistical tests: (1) where appropriate: Mann–Whitney U-test, Wilcoxon signed-rank, Kolmogorov–Smirnov, and χ² with Yates correction. Rayleigh’s test for non-uniformity of circular data was performed using the Circular Statistics Toolbox49. No testing was performed to check for normality or homogeneity of variance. Center and spread values are reported as mean ± SEM. We used no statistical methods to plan sample sizes, but used sample sizes similar to those reported for non-uniformity of circular data was performed using the Circular Statistics Toolbox69. No testing was performed to check for normality or homogeneity of variance. Center and spread values are reported as mean ± SEM. We used no statistical methods to plan sample sizes, but used sample sizes similar to those reported in the manuscript text or depicted in figure legends. We did not use any randomization; data collection and analysis were not performed blind to the conditions of the experiments. No collected data were excluded from analysis. P-values <0.05 were considered to be statistically significant. When a statistical test was used, the P-value is noted either in the manuscript text or depicted in figures and legends as: *P<0.05, **P<0.01, ***P<0.001, n.s., not significant, P ≥ 0.05.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All relevant data is provided in the Source Data file or is available from the corresponding author upon reasonable request.

Code availability
All relevant code is available from the corresponding author upon reasonable request.

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Author contributions
R.R., A.M. and K.Y. conceived and designed the experiments and analyses. R.R. performed the intrinsic signal optical imaging experiments; R.R. and A.M. analyzed the data. R.R. performed the in-vivo calcium imaging experiments; R.R. and A.M. analyzed the data. A.M. performed the in-vitro calcium imaging experiments; R.R. and A.M. analyzed the data. M.D.S. performed the optomotor reflex experiments; M.D.S. and R.R. analyzed the data. R.R., M.D.S. and K.Y. performed the immunohistochemistry experiments; R.R., M.D.S. and K.Y. analyzed the data. R.R., A.M. and K.Y. interpreted the data and wrote the paper.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to K.Y.

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