

Differential topographic organization and retinal inheritance of direction and orientation selectivity in the visual thalamus

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This is a strong study that makes several important contributions to the field of visual neuroscience. Since the Berson lab demonstrated that retinal direction selectivity (DS) is organized along axes of optic flow (Sabbah et al 2017), there has been a need to understand whether this organization is also present in retinorecipient areas (like the thalamus and superior colliculus) and whether this organization is inherited from the retina or distinct. In this study, the authors perform in-vivo electrophysiological recordings of lateral geniculate nucleus (LGN) neurons in anesthetized mice and collect a large dataset of DS responses. The study first demonstrates that multiple subregions of the LGN exhibit direction selective (DS) responses, except for the dorsal LGN (dLGN) core (figure 1), which is consistent with previous neuroanatomical studies. The study then reports that DS responses show topographic organization in the dLGN and align most closely to axes of optic flow (figure 2), which coincides with reports that retinal DS also aligns to these axes. The study next demonstrates that most DS responses in the dLGN depend on retinal DS (figure 3). Another interesting observation made in this part of the paper is that very few DS responses are found in the center of the visual field.

Next the study shifts focus to the orientation/axial selective responses in the performed recordings. Orientation and axial selectivity are often interchangeable terms, though there is a detailed history about these terms and the authors do a great job of describing the distinction. Specifically, the authors demonstrate results that reject the hypothesis that LGN OS/AS responses arise from the inputs of two antiparallel DS retinal cells (Figure 6). Figures 4 and 5 demonstrate OS responses across the LGN and demonstrate that OS responses are highly susceptible to edge effects, consistent with previous reports. I think this is a strong paper with a lot of great findings, sound experiments, and an impressive amount of analysis techniques (e.g.: I found the use of the brain reconstruction package to be an impressive way to ensure confidence in fig 2 results). Many of the findings in this paper are important to the field of visual neuroscience and help us better understand how sensory processing is relayed from periphery to brain. I have a few major comments but all are aimed at making the paper stronger. I also note that I read the reporting summary and found it satisfactory.

Major comments

1. The title of the paper needs to be changed. The abstract and intro do a good job of setting up both the DS and OS parts of the story, but the title does not. The title really only refers to figure 2 but does not prime the reader for retina->LGN transfer of info (fig 3) or any of the OS results (figs 4-6).
2. The results in the dLGN shell presented in fig 1 and 2 are intriguing. Specifically, the study reports that "DS units in the dLGN-shell displayed a gradual shift in their preferred direction along the elevation axis". The authors interpret this result as consistent with the Sabbah et al 2017 study but this is only half of the story. The Sabbah et al paper argues that there are two axes of optic flow (translation and gravitational). What is intriguing is that the LGN recordings shown here might argue that only one of those axes is transferred to the dLGN shell (translation). This certainly could be due to anatomical reasons, as the authors reference studies showing that ventral-preferring cells (superior-preferring) project to the dLGN core. Would we expect the gravitational axis representation to be in the dLGN core then? The loss of transfer of the DS responses aligning to the gravitational axis should be addressed in the discussion.
3. After getting rid of edge effects (figure 5), would it be possible to perform an OS mapping analysis as was done for DS cells in figure 2? As mentioned by the authors, there are recent reports that OS retinal responses also change as a function of retinal location, so assessing this is important and fits within the scope of the paper. At the very least, see if the local OS map within a small region of the visual field changes as a function of visual field location.
4. Related to the above point, how much of the retina is activated by the setup displayed in Fig 1a? I assume based on

methods that the screen's coverage of the visual field is mostly sampling from temporal retina? I ask because it would be interesting to see if the organization shown in 5D changes depending on whether the visual stimulus samples nasal vs temporal retina. The second reason I mention this is that this should be addressed as a limitation of the study in the discussion. Although unlikely, it is possible that transfer of DS → LGN may be different across retinotopic space.

5. The analysis in Figure 2k-m is important. Unfortunately, it is unclear from the text and methods how the transformation was calculated. Specifically, the authors claim to use 36.7 degrees to estimate optic axis position (and specifically elevation) but it is unclear how this was determined. More detail is needed, and when referencing the methods in the results section, please reference the section in the method for ease of reading.

Reviewer #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)

In this manuscript, Yaakov, Heukamp et al, examine the topographic organization of direction selective (DS) and orientation selective (OS/AS) neurons in the mouse dorsal lateral geniculate nucleus (dLGN) using high density in vivo neuropixel recordings in 20 mice. The authors find that dLGN DS neurons are concentrated in the shell whereas OS neurons are found throughout the nucleus. Specific direction preferences of DS neurons follow translatory optic flow dynamics, consistent with those of DSGCs (on-off DSGCs) reported previously. Using genetic tools to disrupt DS in the retina (Chat-cre;IDTR), the authors find a loss of dLGN neurons responsive to DS but not to OS. Therefore, the authors conclude that DS neurons in the dLGN inherit their properties from the retina.

Most of the findings of this study support or confirm the conclusions of previously published studies in the literature. The potential novelty of the study comes from the authors conclusions that: 1) DS dLGN neurons are not present in regions of the nucleus representing central vision, and 2) OS/AS responses are inherited from the retina and not computed in the dLGN as previously suggested by other studies. However, there are a number of technical concerns that need to be addressed to make these claims more convincing. They include;

Major Concerns

1. It is really difficult to interpret the 3D-video of the recording tracks in dLGN (as well as Fig 2e). The color code of shell and core do not seem to encompass the entire nucleus in the video. The core seems to be floating in the middle of the dLGN with shell on all sides. There is a gradient of greys, not all are labeled in the legend. There is also no indication of where the ipsilateral patch (more about that later). According to this video, the electrode tracks only encompass part of the dLGN. There are no recordings from the anterior-medial portions of shell and core. The claim that there is little DS representation in regions of the dLGN that covers central vision is not convincing without recording from all of these areas. According to Piscopo et al, 2013 central vision representation includes several areas of the dLGN where the authors do not record from.

2. A simple explanation for the authors finding little DS representation in the areas of central vision is that their recordings are contaminated by units from the ipsilateral patch. The authors do not mention occluding the ipsilateral eye. Previous studies have already demonstrated that DSCG exclusively project contralaterally and that the ipsi patch is enriched in non-DSGC inputs (Johnson et al, Neuron 2021). It is important to indicate which electrode leads pass through the ipsilateral patch and whether there might be contamination of these units when calculating DS percentages.

3. Do the authors fully rely on anatomical registrations of electrode tracts to determine whether units were in dLGN shell, dLGN core, IGL, vLGN? It appears that this analysis is dependent on being able to precisely determine where the dye signal ends. Even if this is off by 50-100um, it could change classification of unit locations in an area as small as the LGN. The authors use elevation as a means to validate dLGN boundaries, but how can authors be sure about the borders between dLGN, IGL, vLGN? Can the authors use parameters such as RF size (which is known to be different across the different areas) to more precisely determine the boundaries?

4. AS vs. OS: The authors state: "The term axis selectivity became popular with respect to mouse LGN units, partly because of the hypothesis that two DSRGCs with opposing preferred directions converge onto one LGN neuron, which would consequently encode motion along a specific axis 34,35." This reviewer looked at both references cited for this statement. There is no mention that the term AS is used to define dLGN units that compute AS from the combination of DS inputs (as stated by the authors). AS is classically used as a conservative description of an axially selective neuron stimulated with moving stimuli. Without stationary bars, one cannot definitely call a response OS.

5. Related to #4, the authors interpret the results of the Diptheria toxin experiments as definitive evidence that dLGN neurons are OS inherited from the retina and not computed in the dLGN. However, they do not directly test whether the units they record from respond to stationary bars as has already been shown by Zhao et al. 2013. Zhao et al directly tested dLGN neuron feature selectivity by showing stationary bars and also demonstrated the presence of AS/OS RGCs in with MEA recordings in the retina. The authors should correct their citations and statements regarding measurements of OS:
a. Page 3: "This wiring scheme predicts the presence of DS in the dLGN, as well as the emergence of axis-selectivity^{35,36}". The Zhao et al., 2013 paper referenced here (36) actually states that dLGN responses are OS and present stationary bars to

show they are OS.

b. Page 3: "studies have not really distinguished them from OS responses". This is not a fair assessment based on Zhao et al. 2013. This reviewer would argue that the authors of this current paper do not really distinguish AS responses from OS responses.

6. The authors should address:

- a. Are there AS cells in the retina (that are not OS) that could be mediating AS/OS in the dLGN? Related to point #4-5 above, this reviewer would argue that the data in Fig 6a-d show that AS is not affected in the retina, not OS.
- b. Are there other types of DS RGCs that do not depend on SACs that could contribute to dLGN AS/OS?
- c. The authors should discuss whether there is the possibility that there are AS cells that are computed from DSGC inputs but they are of low frequency in dLGN.

7. The authors argue that dLGN DS neurons inherit their properties from DSGC in the retina. Are other response properties like their luminance polarity also preserved in DS dLGN neurons (i.e. are they also on-off)?

8. The authors use two parameters to determine whether a unit is visual: 1) a response 2 SDs above baseline or 2) an RQI value of 0.25 or greater. However, according to Baden et al. 2016, which the authors cite, the RQI for completely random responses is expected to be proportional to $1/(\# \text{ of trials})$, which in this case equals 0.2 (1/5 trials). In this case, a RQI cutoff of 0.25 is not particularly stringent for assessing response quality. To address this, the authors should present the distribution of RQI values in their recordings and demonstrate that their conclusions remain unchanged when using a more stringent RQI threshold.

9. The authors describe a 34 degree shift in elevation in aligning the preferred directions of DS to the predicted optic flow vectors. This is confusing to the reader. The authors should explain this in more detail and give an explanation for why there is a shift.

10. Fig 4c, 6h: the authors should also show the cumulative distributions of OSI/gOSI

Reviewer #4

(Remarks to the Author)

The paper of Yaakov et al addresses a fundamental set of questions about the organization of direction and orientation selectivity in the lateral geniculate nucleus. The authors show that while orientation selective neurons in LGN span the whole visual field while direction selective neurons are largely absent in the central visual field and are topographically aligned to translatory optic flow. Using transgenic mice lacking retinal direction selectivity, Yaakov et al demonstrate that LGN direction selectivity relies on retinal directional selectivity, suggesting that LGN DS is inherited from the retina. Moreover, the authors show that LGN orientation selectivity is not affected by the ablation of directional selective neurons in the retina, demonstrating that LGN orientation selectivity is not generated via the integration of direction selective retinal ganglion cells.

I think the manuscript is well written and shows strong convincing evidence for all the claims made in the paper.

(Minor) main point

-Throughout the manuscript the authors speak about orientation and direction selective neurons as two clearly separate populations of neurons. Is there an overlap in the populations selective for direction and orientation? Or are they clearly separate populations? . The selection of threshold criteria seems kinda arbitrary. I don't necessarily object to that since it's quite common in the field. However it would be good to show some plots in the supplements that undermine your choices of the criteria. The 6) criteria for assigning a neuron to one of these groups makes sure that a neuron is assigned to only one of the groups. However I am wondering if that is a fair criteria because a neuron that fulfills all other 5 criteria for orientation selectivity and direction selectivity would end as orientation selective if it has only slightly higher gOSI than gDSI. I think it could be useful to e.g. show a scatter plot in the SI where you plot direction vs orientation selectivity of all cells to see that this clear distinction is fair and not the result of tweaking the classification criteria

Minor points:

-Most abbreviations are introduced somewhere in the beginning of the paper but I failed to find a definition of AS "This wiring scheme predicts the presence of DS in the dLGN, as well as the emergence of axis-selectivity (AS) 35,36."

-How do you measure the optic axis in anesthetized mice? It's not clear to me. Doesn't this depend on the head position/orientation?

-In the 3 results Section "SAC ablation in the retina eliminates DS responses in the LGN" the authors write "The lack of DS representation in the central visual field as well as the topographic organization of DS preferred directions in the dLGN are different from DS in the retina." I think it would be good to add a sentence elaborating on the difference in the topographic organization.

-In the 4 result section the authors write: ". The LGN OS population exhibited general orientation tuning biases towards

horizontally, vertically and diagonally oriented gratings (mostly postero-superior to antero-inferior) (Fig. 4d).” What do the authors make out of this bias for diagonal/vertical/horizontal tuning? Maybe speculate about it in the discussion section

- Why do most DS units don't display a clear RF? Is it due to experimental constraints? Do you think they actually have clear RFs? I think it would be good to discuss it a bit

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors thoroughly addressed my feedback. From my assessment, I also think that authors adequately addressed other reviewers' feedback. I continue to think that this is a strong study that significantly contributes to 1) our understanding of how retinal computations are transferred to the visual thalamus and 2) how DS and OS are organized in the visual thalamus. The paper is even stronger post-revision, and I have no further feedback to give. Excellent study.

Reviewer #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)

In this revision, the authors have addressed some of the issues raised previously.

This reviewer appreciates the explanation of AS/OS and additional analyses the authors performed. However, there were several responses that require further clarification in order to strengthen the authors arguments.

Comments:

1. While the inclusion of color maps of the LGN in Figure S5 and Reviewer Figure 5 is helpful, it is still difficult to interpret the precise locations of probe insertions. The 3D representations (e.g., Figure 1c, 2e, and the supplementary video) are not accessible to most readers, including those familiar with the mouse visual system. The authors should also include the same color coding in the supplementary video for consistency and clarity.

2. With regards to the authors response to concerns about the limited coverage of the recordings, this reviewer is still not convinced. Based on the color-coded maps, the authors' reviewer figure 6 and the supplemental video, the electrode tracks and data miss regions of anterior as well as middle and posterior dLGN which have DS signals according to Piscopo et al. that may represent central vision (see the dark blue regions depicted in Reviewer Figure 6e, f and the green regions in 6g). The authors should more explicitly address the extent of their dLGN coverage in the main text and the possibility that some DS regions representing central vision may be missing. After rebutting this issue in the previous version of the paper, the authors state "Having said all the above, we agree that we cannot know if DS responses exist in regions that we did not record from, or if the center visual field has additional representation in the anterior dLGN." Yet, despite this statement in the rebuttal, the sentence "LGN DS responses are absent in the central visual field" is in the abstract and emphasized throughout the text.

Because the manuscript makes this negative claim (i.e., the absence of DS in central visual space), readers must be able to evaluate the spatial coverage of the recordings. The authors should include 2D coronal sections at various anterior-posterior positions with overlaid probe trajectories (very much as they did for reviewer figure 6c-f).

3. The author's response to Major concerns #2 reveals that they did not cover the ipsilateral eye during recordings of the binocular visual field (part of the central visual field). This is also apparent from data in Reviewer Figure 9 showing the ipsilateral patch is responsive to visual stimulation. This issue would influence the interpretation of the dLGN core analysis (Fig 2h). While this reviewer is in complete agreement that the ipsilateral patch is part of the "core", if recordings of the central visual field primarily come from the ipsilateral patch, it would not be surprising that there are no DS responses given the projections of DS RGCs. This reviewer was under the impression that the novelty of the study was that DS from the central retina is not transmitted to the dLGN (regardless of shell or core). Therefore, contamination from the ipsilateral patch could confound interpretation of results. The authors should show a separate graph of just the ipsi patch results (as in Figure 2g and 2h) and another with just responses from the contralateral eye. They should also report DS percentages in the core excluding the units in ipsi patch. This is reasonable given the Allen CCF data also distinguishes core and ipsi patch as in Reviewer Figure 9. Showing receptive field locations of ipsi patch units alone would also help corroborate that anatomical reconstructions were precise. Reviewer Figure 9 is not very convincing in showing that the authors can confidently distinguish between the different compartments of the LGN (dLGN, vLGN, IGL).

4. Authors should include ON-OFF data in the manuscript. While there are caveats to the data, as the authors nicely explain,

the data do potentially suggest that there is room for non-simple inheritance. Readers will benefit from seeing the data directly, along with a discussion of their limitations, as was articulated in the rebuttal.

5. The authors state that they do not argue OS is inherited from the retina, but the current manuscript title “Topographic organization and retinal inheritance of direction and orientation selectivity in the visual thalamus” implies otherwise. Most readers will interpret this title as indicating that both DS and OS are inherited from the retina. To avoid misinterpretation, the title should be revised to better reflect the data and the authors' intended message.

6. The authors acknowledge that the response quality index (RQI) cutoff they use is not very stringent and many of the units are not substantially distinguishable from random noise. The reviewer appreciates the analysis that the authors performed in Reviewer Figure 11. However, the figure only shows that units preferring different directions have similar average RQI values (Reviewer Figure 11a-c) and that the results from the DT experiments are “robust to stricter inclusion criteria,” as stated in the figure legend. The authors need to substantiate this claim further by re-generating other key results, such as the topographic maps in Figure 2i–m and the OS edge effects in Figure 5, using a more stringent RQI cutoff (e.g. 0.4). Although this will reduce the number of analyzable units, it is critical to assess whether the conclusions are robust with stricter inclusion criteria. RQI data should be included in the manuscript, not only referenced in the Methods section.

Reviewer #4

(Remarks to the Author)

The authors have carefully addressed all my queries and revised the manuscript accordingly.

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)

In this second revision of the manuscript, the authors have adequately addressed comments #1-5. In particular, further data analysis of the ipsi vs. contra region reveal that the frequency of encountering DS cells in the ipsi and contra core dLGN regions do not differ and overall occur at about 20% of that found in shell. This reviewer appreciates the authors' efforts to enhance transparency regarding recording tract locations, as demonstrated in Supplemental Figures 3 and 7. Additionally, the documentation that units recorded in the ipsilateral patch can exhibit receptive fields outside the central visual field and display direction selectivity (Supplemental Figure 8) provides valuable information for readers about the limitations of the methods used.

With regards to the response to comment #6, the authors reveal for the first time that the criteria used to identify units was not limited to the response quality index (RQI) cutoff of > 0.25 . The authors now clarify that there were three possible ways that a unit could be included: 1) $RQI > 0.25$, OR 2) responses exceeding 2 standard deviations above baseline, OR 3) the presence of clear receptive fields. The RQI histogram in Supplemental Figure 4a suggests that with a cutoff of $RQI > 0.25$, units shown in green are included while those below the threshold are excluded. However, when the authors raise the cutoff of $RQI > 0.4$, only 4 direction-selective units are excluded. If this reviewer understands what the authors are saying, this would mean that the majority of the DS units were included based on criteria #2 and 3.

Although the examples in Supplemental Figure 4b appear to support these criteria, this reviewer finds it challenging to assess how many cells were included based on single versus multiple criteria. To enhance transparency, the authors should consider presenting a scatter plot displaying response z-scores relative to baseline versus RQI, with color-coding to indicate included units. This visualization would improve reader comprehension of the dataset composition given these unconventional inclusion criteria that do not incorporate frequency-modulation response analysis.

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We thank the reviewers and the editor for their constructive feedback on our manuscript. We are grateful for the time and effort dedicated to reviewing our work and for the insightful suggestions that helped us improve the clarity, rigor, and overall quality of the paper. We have carefully considered each comment and made the corresponding changes in the revised manuscript. We added nine supplementary figures to support the analyses, and a new section to the discussion *Limitations of the study*. We also shortened some of the figure captions according to the journal requirements.

Please find our point-by-point responses (in blue) to the reviewers' comments (in black) below. All significant changes are highlighted in blue in the revised manuscript.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This is a strong study that makes several important contributions to the field of visual neuroscience. Since the Berson lab demonstrated that retinal direction selectivity (DS) is organized along axes of optic flow (Sabbah et al 2017), there has been a need to understand whether this organization is also present in retinorecipient areas (like the thalamus and superior colliculus) and whether this organization is inherited from the retina or distinct. In this study, the authors perform in-vivo electrophysiological recordings of lateral geniculate nucleus (LGN) neurons in anesthetized mice and collect a large dataset of DS responses. The study first demonstrates that multiple subregions of the LGN exhibit direction selective (DS) responses, except for the dorsal LGN (dLGN) core (figure 1), which is consistent with previous neuroanatomical studies. The study then reports that DS responses show topographic organization in the dLGN and align most closely to axes of optic flow (figure 2), which coincides with reports that retinal DS also aligns to these axes. The study next demonstrates that most DS responses in the dLGN depend on retinal DS (figure 3). Another interesting observation made in this part of the paper is that very few DS responses are found in the center of the visual field.

Next the study shifts focus to the orientation/axial selective responses in the performed recordings. Orientation and axial selectivity are often interchangeable terms, though there is a detailed history about these terms and the authors do a great job of describing the distinction. Specifically, the authors demonstrate results that reject the hypothesis that LGN OS/AS responses arise from the inputs of two antiparallel DS retinal cells (Figure 6). Figures 4 and 5 demonstrate OS responses across the LGN and demonstrate that OS responses are highly susceptible to edge effects, consistent with previous reports. I think this is a strong paper with a lot of great findings, sound experiments, and an impressive amount of analysis techniques (e.g.: I found the use of the brain reconstruction package to be an impressive way to ensure confidence in fig 2 results). Many of the findings in this paper are important to the field of visual neuroscience and help us better understand how sensory processing is relayed from periphery to brain. I have a few major comments but all are aimed at making the paper stronger. I also note that I read the reporting summary and found it satisfactory.

Major comments

1. The title of the paper needs to be changed. The abstract and intro do a good job of setting up both the DS and OS parts of the story, but the title does not. The title really only refers to figure 2 but does not prime the reader for retina->LGN transfer of info (fig 3) or any of the OS results (figs 4-6).

We have changed the title of the paper to: "Topographic organization and retinal inheritance of direction and orientation selectivity in the visual thalamus".

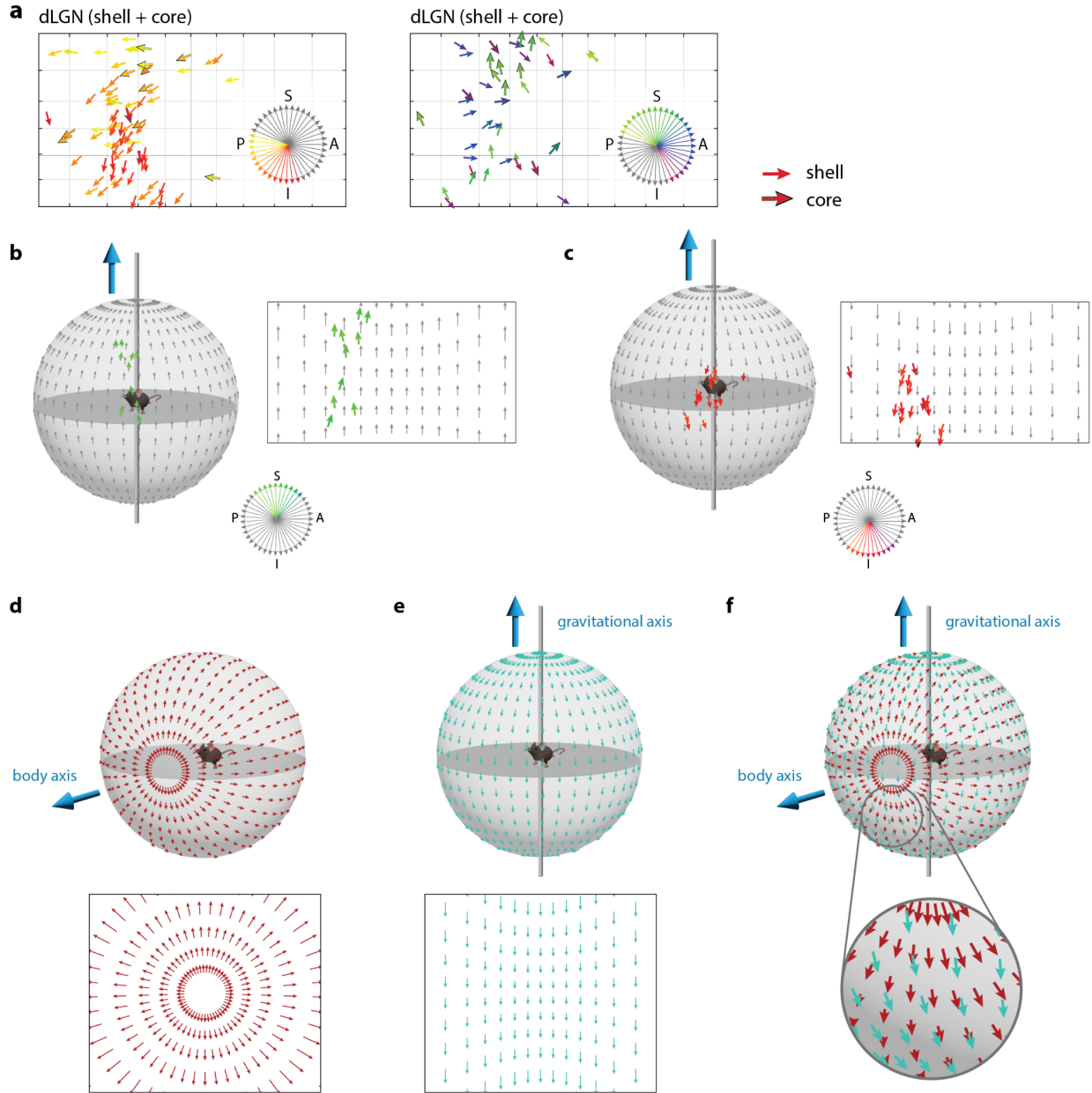
2. The results in the dLGN shell presented in fig 1 and 2 are intriguing. Specifically, the study reports that “DS units in the dLGN-shell displayed a gradual shift in their preferred direction along the elevation axis”. The authors interpret this result as consistent with the Sabbah et al 2017 study but this is only half of the story. The Sabbah et al paper argues that there are two axes of optic flow (translation and gravitational). What is intriguing is that the LGN recordings shown here might argue that only one of those axes is transferred to the dLGN shell (translation). This certainly could be due to anatomical reasons, as the authors reference studies showing that ventral-preferring cells (superior-preferring) project to the dLGN core. Would we expect the gravitational axis representation to be in the dLGN core then? The loss of transfer of the DS responses aligning to the gravitational axis should be addressed in the discussion. This is an interesting point. According to Sabbah et al., nasal and temporal DSRGCs are organized by translatory self-motion along the body axis (moving forward and backward, respectively), and ventral and dorsal DSRGCs are organized by self-motion along the gravitational axis (moving up and down, respectively). We find that in the dLGN there is an overrepresentation of preferred directions matching optic flow generated during forward motion. This bias may be partly inherited from the retina, as nasal DSRGCs outnumber all of the other DSRGC subtypes (Sabbah et al., Fig. 1i, j). Yet, we agree with the reviewer that the bias towards posterior-inferior directions is enhanced in our LGN data compared to the retinal data in Sabbah’s paper.

To answer the reviewer’s question, we now show all other DS units (the non inferior-posterior preferring cells), combined from shell and core (Reviewer Fig. 1a, right). We also calculated the optic flow generated during motion along the gravitational axis, either up or down (Reviewer Fig. 1b, c). To analyse how preferred directions of DS units align to this optic flow, we show either superior or inferior preferring DS units. In both cases, we could find units that are aligned with the optic flow’s direction. However, the number of these units is small, and they do not cover the entire hemifield as expected by the optic flow pattern. Optic flow along the gravitational axis is rarely generated naturally in an animal’s life (emerges when the animal is lifted up or down but the gaze is horizontal, for example, in a transparent elevator), and this could be the reason why its representation is not fully transferred to the LGN.

While the question of why the gravitational axis is represented in the retina and where it is transferred remains open, we would like to note that the optic flow generated by forward motion and by gravitational motion overlap in specific areas in the visual field (Reviewer Fig. 1d-f), and could combine in specific regions to generate the representation we find in the LGN.

We now refer to these points in the discussion and added panel a and panels d-f as Supplementary Figure 6 to the manuscript:

“In fact, the topographic organization of the preferred directions of DS dLGN cells may be partly inherited from the retina according to the tilt of preferred directions that match the translatory optic flow **along the body axis**¹⁵. **Other DSRGCs were shown to match the gravitational axis, which we find to be underrepresented in the dLGN. However, the optic flow generated along the body axis and along the gravitational axis overlap in specific areas in the visual field (Supp. Fig. 5b-d), and we therefore cannot rule out the possibility that both representations are transferred to the dLGN, at least for these specific areas. Indeed, given the known topographic variation in the mouse retina¹² it is possible that retina-dLGN projection rules are also topography-dependent**”.



Reviewer Figure 1. Topographic organization of all dLGN DS units. **a.** Left: Preferred directions of dLGN shell and core units on their receptive field locations, including units with preferred directions of the postero-inferior quadrant (like Fig. 2). Right: Same, but including the remaining DS units. **b.** Superior preferring DS units from dLGN plotted on the spherical world and projected onto the screen, with optic flow contours along the gravitational axis shown in gray. **c.** Like **b** for inferior-preferring DS units. **d.** Illustration of optic flow directions generated by translational movement along the body axis (top) and the same directions projected onto a flat surface (bottom). **e.** Like **d**, for optic flow along the gravitational axis. **f.** Overlay of **d** and **e** (top), and a close up of an area in the inferior visual field, where optic flow directions of both axes match.

3. After getting rid of edge effects (figure 5), would it be possible to perform an OS mapping analysis as was done for DS cells in figure 2? As mentioned by the authors, there are recent reports that OS retinal responses also change as a function of retinal location, so assessing this is important and fits within the scope of the paper. At the very least, see if the local OS map within a small region of the visual field changes as a function of visual field location.

We performed an analysis similar to that for the DS units shown in Fig. 2. Reviewer Fig. 2a-c shows the results for all dLGN OS units (after removing OS units near screen edges), revealing no clear topographic bias across the visual field.

On a finer scale, the local organization of OS units is indeed an interesting question. We attempted a similar analysis to that of Laniado et al. 2025, but were unsuccessful for several reasons:

- 1) We did not achieve 'clean' clustering into On/Off subtypes of OSRGCs based on the full-field spot stimulus that we used (Laniado et al. used a moving bar for the clustering), and our dataset of OS units is much smaller (280 units compared to 2100 RGCs used by Laniado et al, 2025).
- 2) We only have a small coverage of the visual field (for the corresponding retinal region see Reviewer Fig. 3), compared to the large coverage of the retinal region that was done in Laniado et al. Variations of preferred orientations that match specific latitude/longitude lines may become more apparent when considering the entire field of view.

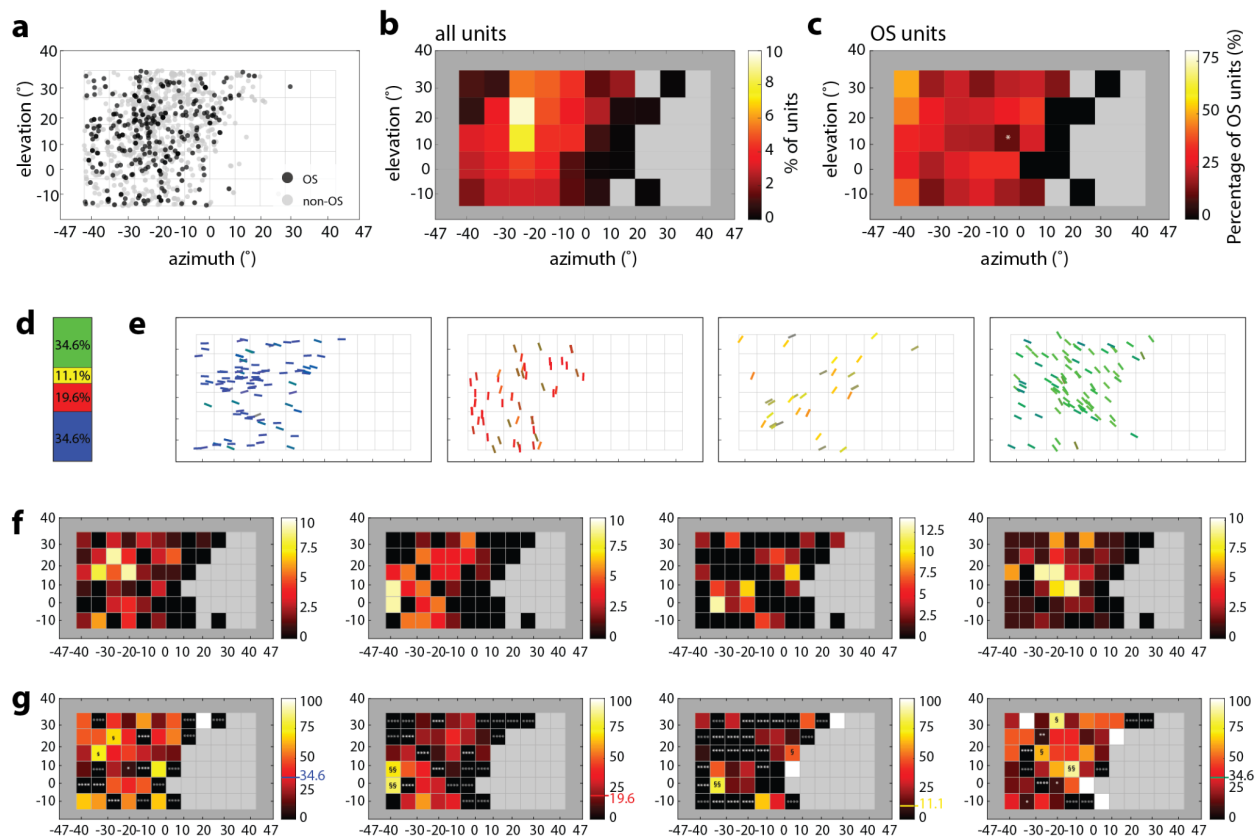
Therefore, we chose a slightly different analysis. We grouped OS units into four main groups based on their preferred orientation (horizontal, vertical and the two diagonals) and analyzed their distribution across visual space (Reviewer Fig. 2d-g). We did notice several subtle trends, for example, a seemingly higher density of horizontal preferring OS units at around 30 deg elevation (Reviewer Fig. 2c-e, left). Interestingly, this altitude corresponds with the horizon based on optic axis calculations (see below). We also found a streak of higher density of diagonal preferring OS units (Reviewer Fig. 2e-g, right).

We already referred to the arrangement of all OS units in the paper (page 13), but now added Reviewer Fig. 2 as Supplementary Fig. 11.

We made the following change in the manuscript:

"After excluding the edge units from the population, the orientation preference of LGN OS units fell along the diagonal and horizontal axes (Fig. 5d), **and we observed no area in the visual field having over- or underrepresentation of OS units (Supp. Fig. 11a-c). When grouping the OS units based on preferred orientation, we did notice some subtle trends of spatial organization along the horizon and around the center visual field (Supp. Fig. 11d-g)**".

For the new analysis we introduced a threshold where a spatial bin needs to have ≥ 5 units for the shuffling analysis. We also updated the DS analysis of Fig. 2 accordingly. Asterisks in bins with < 5 units are now shown in gray.



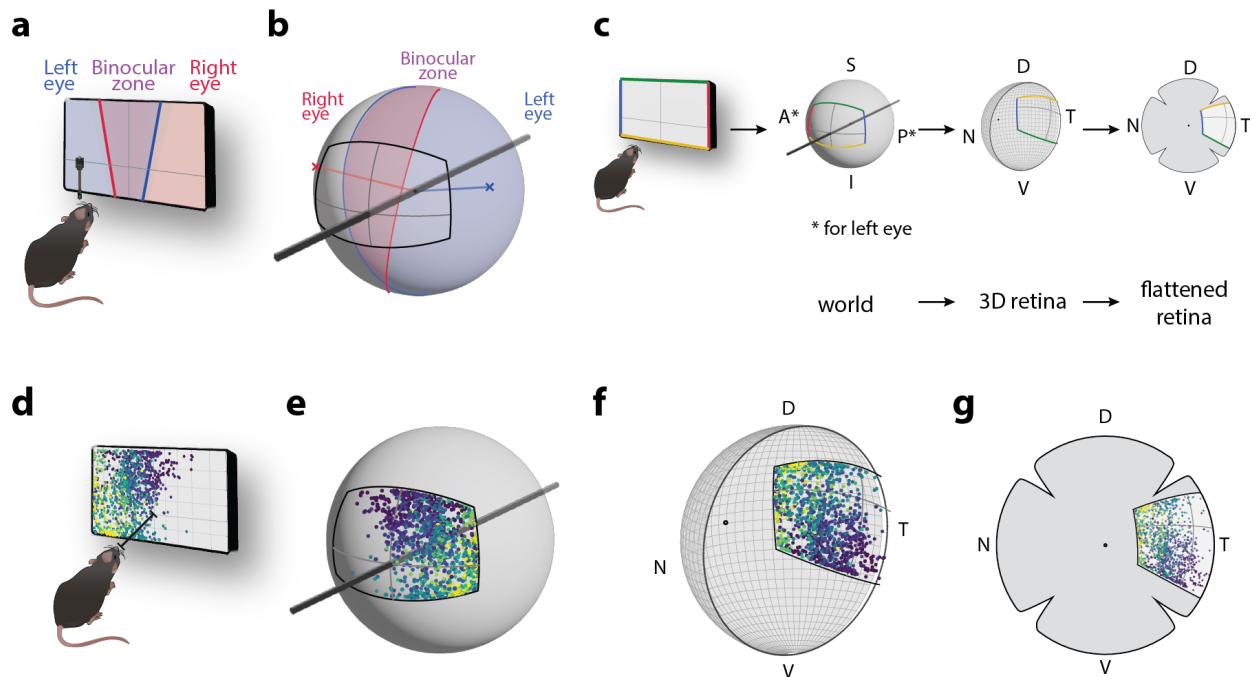
Reviewer Figure 2. Spatial organization of OS units. **a.** Receptive field of dLGN units. OS units are shown in black, non-OS units in gray. Units within 5° of the screen's edge were excluded. **b.** Proportion of recorded units across screen. **c.** Proportion of OS units out of all units. Significance was tested against shuffled data (see Methods). **d.** Percentages of OS units in the four subgroups based on their preferred orientation (horizontal, vertical or one of the diagonals) shown in e-g. **e.** OS units of four subgroups plotted on their receptive field location. **f.** Proportion of OS units within each group (based on e) to illustrate regions of higher density. Colorbar shows percentage out of all OS units in a subgroup. **g.** Like c, showing the percentage of OS units of each group out of the entire OS population. Values expected for uniform distribution correspond to percentages of OS units in each group (from d) and are shown on the colorbar for each subgroup. Statistical test as in c. For c and g, * and § represent lower and higher OS proportion than expected by chance, respectively. */§: $p < 0.05$, **/§§: $p < 0.01$, ***/§§§: $p < 0.001$, ****/§§§§: $p < 0.0001$. Gray asterisks in g indicate bins with < 5 OS units.

4. Related to the above point, how much of the retina is activated by the setup displayed in Fig 1a? I assume based on methods that the screen's coverage of the visual field is mostly sampling from temporal retina? I ask because it would be interesting to see if the organization shown in 5D changes depending on whether the visual stimulus samples nasal vs temporal retina. The second reason I mention this is that this should be addressed as a limitation of the study in the discussion. Although unlikely, it is possible that transfer of DS $\rightarrow$ LGN may be different across retinotopic space.

Indeed, the screen covers only a portion of the retina in the temporal region. We added a supplementary figure to illustrate the extent of the visual field that is stimulated by the screen, as well as a visualisation of how our recorded receptive fields can be mapped onto the retina (Supplementary Fig. 3 in the

manuscript, also shown below as Reviewer Fig. 3). We added a section to the Methods describing the calculations and transformations (*Transformation to retinal coordinates*).

We have added this aspect as a limitation to the discussion (new section *Limitations of the study*): “Our study was limited to the central visual field (Supp. Fig. 3), and wider investigation of the complete visual field and the entire dLGN would further reveal the topography of DSRGC-dLGN projection rules and additional representation patterns of DS in the periphery of the visual field”.



Reviewer Figure 3. Schematic of the mouse’s field of view and experimental setup. **a.** Schematic of the setup with borders of each eye’s field of view labelled on the screen. **b.** 3D representation of the left eye’s visual field (blue) and binocular zone (red). The mouse is placed at the center of the spherical coordinate system. The optic axes are shown as blue and red lines and were determined as described in the Methods (elevation: 36.7°, azimuth: 73.6°; section *Optic axis measurements*). **c.** Schematic of transformation between ‘world’ coordinates, a projection onto a 3D retina, and a flattened retina. Note that world coordinates (anterior (A) and posterior (P)) are shown for the left eye and will be opposite from the perspective of the right eye. **d.** Recorded receptive fields colored by depth (as in Fig. 2f) and added to the image of the setup. **e.** 3D representation of recorded receptive fields in the spherical ‘world’ coordinate system. **f.** Screen and receptive fields from e projected onto a 3D retina. **g.** Like f, on a flattened retina.

5. The analysis in Figure 2k-m is important. Unfortunately, it is unclear from the text and methods how the transformation was calculated. Specifically, the authors claim to use 36.7 degrees to estimate optic axis position (and specifically elevation) but it is unclear how this was determined. More detail is needed, and when referencing the methods in the results section, please reference the section in the method for ease of reading.

We realize this part was not clear in the text and apologize.

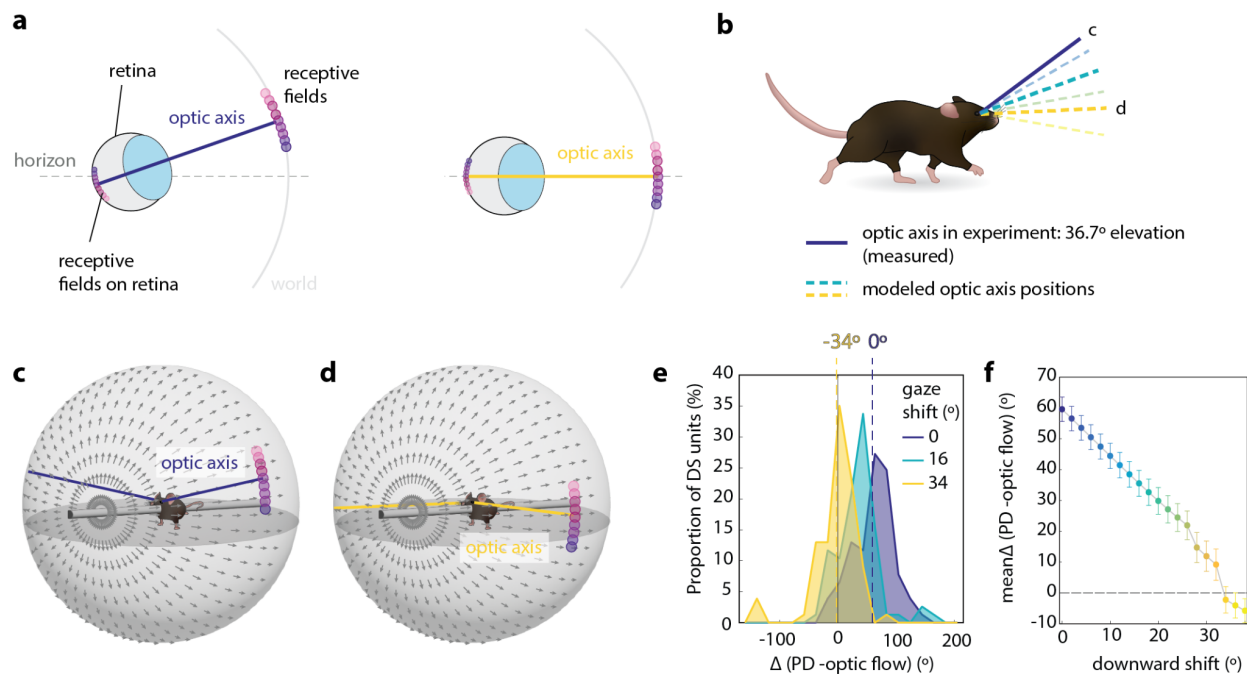
The 36.7 degree elevation was measured experimentally, and Reviewer Fig. 14 below (added as Supp. Fig. 8) illustrates the method of measuring the optic axis (see second Minor Point of Reviewer 4).

In the analysis of Fig. 2k-m we were asking what happens when the optic axis position changes. This happens in awake mice by moving the eyes (or both eyes and the head together). As a result, the animal’s

viewpoint would change, and neuronal receptive fields would now view different locations in the visual world (examples illustrated in Reviewer Fig. 4a-d). However, the optic flow generated by translatory motion (gray arrows in Reviewer Fig. 4c, d) are fixed when the mouse moves forward, regardless of its optic axis. As a result, the local optic flow direction can exactly match the preferred direction of a specific DS unit for one optic axis, but diverge from it for another optic axis. As we could not change the eye position of the anesthetized mice in our experiments, we calculated where the receptive fields of DS units would fall in the visual world for different optic axes, and resulting from this the difference between optic flow motion and the preferred directions. Specifically, we rotated the optic axis to change its elevation, resulting in a rotation of the receptive fields in the visual world (Reviewer Fig. 4b-d). For each optic axis rotation we quantified the difference between DS units' preferred direction and the optic flow experienced in their receptive field (Reviewer Fig. 4e, f). In the main Figure of the manuscript we only show two optic axis positions, here we now show multiple.

We have added Reviewer Figure 4 as a new supplementary figure in the revised manuscript (Supplementary Fig. 7), and added clarifications in the manuscript's main text and Methods, as well as references to specific Methods sections.

Additionally, we modified Fig. 2 and added an additional panel showing the same schematic as in Reviewer Fig. 4b to illustrate the optic axis, and to clarify the concepts explained above to the reader, as well as point to the new supplementary figures for more details.



Reviewer Figure 4. Illustration of optic axis and calculations performed for Figure 2. **a.** The optic axis is defined as a vector/light ray that passes through the center of the eye. Rotating the eye will change the optic axis position. Example receptive fields (pink/purple) will fall on different locations in the visual world. **b.** Schematic of optic axis position during experiment, obtained through measurements (solid dark blue line, elevation: 36.7°; see Methods), and schematics of optic axis positions after changing the optic axis elevation in a calculation (dashed lines). **c.** Schematic of optic axis position during the experiment with schematic receptive fields (pink/purple circles). Optic flow vectors from Fig 2 are shown in grey. **d.** Like c, for a lowered optic axis position. **e.** Distribution of differences between DS units' preferred directions and translational optic flow in their receptive fields for three optic axis positions (illustrated in b with matching

colors). f. Mean $\pm$ SD of differences between DS units' preferred directions and optic flow (as in e) for all calculated optic axis shifts.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3 (Remarks to the Author):

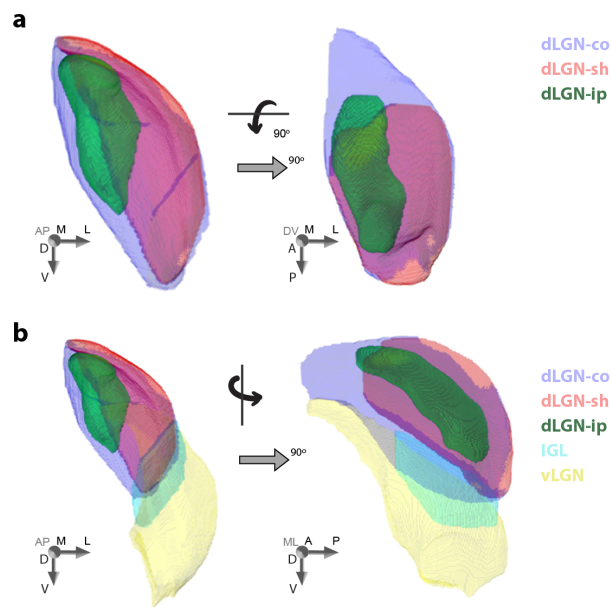
In this manuscript, Yaakov, Heukamp et al, examine the topographic organization of direction selective (DS) and orientation selective (OS/AS) neurons in the mouse dorsal lateral geniculate nucleus (dLGN) using high density in vivo neuropixel recordings in 20 mice. The authors find that dLGN DS neurons are concentrated in the shell whereas OS neurons are found throughout the nucleus. Specific direction preferences of DS neurons follow translatory optic flow dynamics, consistent with those of DSGCs (on-off DSGCs) reported previously. Using genetic tools to disrupt DS in the retina (Chat-cre;iDTR), the authors find a loss of dLGN neurons responsive to DS but not to OS. Therefore, the authors conclude that DS neurons in the dLGN inherit their properties from the retina.

Most of the findings of this study support or confirm the conclusions of previously published studies in the literature. The potential novelty of the study comes from the authors conclusions that: 1) DS dLGN neurons are not present in regions of the nucleus representing central vision, and 2) OS/AS responses are inherited from the retina and not computed in the dLGN as previously suggested by other studies. However, there are a number of technical concerns that need to be addressed to make these claims more convincing. They include;

Major Concerns

1. It is really difficult to interpret the 3D-video of the recording tracks in dLGN (as well as Fig 2e). The color code of shell and core do not seem to encompass the entire nucleus in the video. The core seems to be floating in the middle of the dLGN with shell on all sides. There is a gradient of greys, not all are labeled in the legend. There is also no indication of where the ipsilateral patch (more about that later). According to this video, the electrode tracks only encompass part of the dLGN. There are no recordings from the anterior-medial portions of shell and core. The claim that there is little DS representation in regions of the dLGN that covers central vision is not convincing without recording from all of these areas. According to Piscopo et al, 2013 central vision representation includes several areas of the dLGN where the authors do not record from.

We have added a supplementary figure (Supp. Fig. 5) with a different colormap of the LGN model to improve distinction of its subregions including the ipsilateral patch (see Reviewer Fig. 5).



Reviewer Figure 5. Subregions of the LGN. a. 3D model (adopted from Allen Mouse CCF) of the dLGN. Left: the nucleus is viewed from posterior. Right: viewed from above. b. The LGN including IGL and vLGN.

With regards to the limited coverage of our recordings: DSRGCs project mainly to the dLGN shell, and our probes' trajectories cover most of this region. The anterior part of the dLGN lacks the shell layer and accordingly has almost no DS projections. At the mid ranges of the dLGN, the shell is a thin layer that covers the lateral-dorsal surface of the core. Towards the posterior end of the dLGN, the shell takes over most space, and so does DS distribution in the dLGN. In fact, this is shown in Piscopo et al. 2013 (Figure 7C, the figure is shown here for convenience as Reviewer Fig. 6g). This is also evident in anatomical tracings of DSRGC projections (e.g. Jiang et al 2022, Fig 2). Importantly, our results include many responsive shell units with RFs in the central visual field, but DS was a rare feature amongst these units (Fig 2g).

We interpret the reviewer as specifically referring to Fig. 7 of Piscopo (retinotopic organisation, part a and b in their figure). We chose our most anterior insertion and compared it with the retinotopic organization shown in Piscopo et al. . Reviewer Fig. 6a, b shows the receptive field locations and histology of the probe, revealing that the receptive fields cover the entire elevation range of the screen and are located in the most peripheral azimuth of our screen (-47°). We then overlaid the probe trajectory onto the schematic from Piscopo et al. (Reviewer Figure 6c, e). We find our retinotopic organization to nicely match Piscopo's retinotopic trend. Please note that Piscopo's visual field mapping is limited and does not cover the full visual field of the mouse (e.g., the highest elevation is 40°). We believe that Piscopo's plot is representing a general trend of LGN retinotopy and that the more anterior we go the more peripheral the visual field is (in both azimuth and elevation). These regions are outside of our screen (please also see our answer to point 4 of Reviewer 1 and Reviewer Fig. 3). We therefore cannot evoke coherent light responses in these regions.

Having said all the above, we agree that we cannot know if DS responses exist in regions that we did not record from, or if the center visual field has additional representation in the anterior dLGN. We added a section *Limitations of our study* to the discussion, which directly refers to that.

[FIGURE REDACTED]

Reviewer Figure 6. Spatial organization of LGN functional responses. **a.** Receptive field location of our most anterior recording (AP=-2.1mm from bregma). **b.** Histology of probe trajectory through dLGN. Dashed red line is added for clarity. **c.** [REDACTED] **d.** Schematic of Piscopo's elevation colormap on the screen used in our setup. **e.** [REDACTED] **f.** Like d, for azimuth. **g.** [REDACTED]
Panels c and e and their captions are modified from Piscopo et al., Figure 7. Panel g and its caption are taken from Piscopo et al. Figure 7.

2. A simple explanation for the authors finding little DS representation in the areas of central vision is that their recordings are contaminated by units from the ipsilateral patch. The authors do not mention occluding the ipsilateral eye. Previous studies have already demonstrated that DSCG exclusively project contralaterally and that the ipsi patch is enriched in non-DSGC inputs (Johnson et al, Neuron 2021). It is important to indicate which electrode leads pass through the ipsilateral patch and whether there might be contamination of these units when calculating DS percentages.

Indeed, the dLGN contains a region which is specialized for inputs coming from the ipsilateral eye. However, this region is located in the dLGN **core**. The dLGN **shell** does *not* include projections from the ipsilateral eye (see Reviewer Fig. 7 adapted from Nakamoto et al., 2019, showing that the shell - superficial (dorsal) layer of the dLGN - has no/little ipsilateral projections.). Our analysis that shows little representation of DS in the central visual field is based only on units recorded in the shell (Figure 2g), and therefore does not involve the ipsilateral region. Also, see our answer to the reviewer's comment #3 below.

[REDACTED]

[FIGURE REDACTED]

The figure and its caption are adapted from Nakamoto et al., 2019.

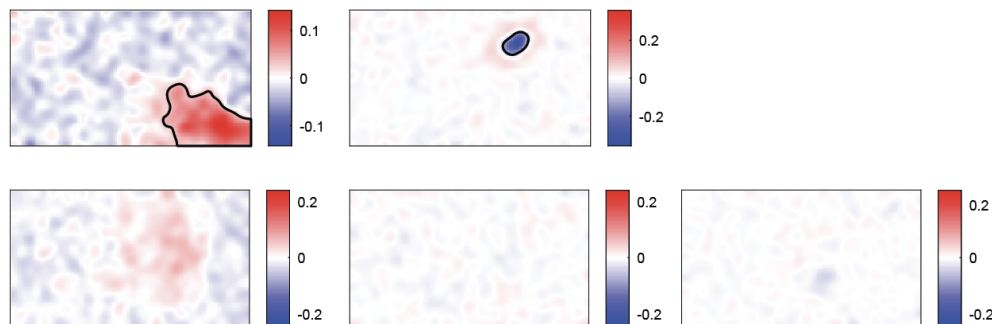
3. Do the authors fully rely on anatomical registrations of electrode tracts to determine whether units were in dLGN shell, dLGN core, IGL, vLGN? It appears that this analysis is dependent on being able to precisely determine where the dye signal ends. Even if this is off by 50-100um, it could change classification of unit locations in an area as small as the LGN. The authors use elevation as a means to validate dLGN boundaries, but how can authors be sure about the borders between dLGN, IGL, vLGN? Can the authors use parameters such as RF size (which is known to be different across the different areas) to more precisely determine the boundaries?

As mentioned in the methods section, anatomical registrations were based on histological sections, which revealed the probe's trajectory based on the Dil trace. In addition, we manually curated and corrected probe tip depth according to electrophysiological traces of the recorded units. The Neuropixels' probe

channel pitch of 20 μm allows for a high resolution in estimating the first visually responsive units as the probe traverses the hippocampal-LGN boundary. This method for mapping recorded units to their anatomical origin is consistent with current accepted practices, and like any other method – has its limitations.

It was previously shown that receptive fields in the vLGN (and IGL) are larger than those of the dLGN (Ciftcioglu et al., 2020), and this parameter could potentially serve as an additional measure for checking the probe alignment. While we found vLGN units that display large receptive fields, most vLGN (and IGL) units in our dataset did not display any clear receptive fields (~88% of units had no receptive field; for example receptive fields in the vLGN see Reviewer Fig. 8). This is probably due to the white noise stimulus that we used – we used a fixed pixel size of $\sim 4^\circ$, which worked nicely for reconstructing dLGN units' receptive fields. However, since vLGN units have larger receptive fields, they probably require a white noise stimulus with larger pixels. In Ciftcioglu et al., the authors use a range of white noise pixel sizes (2-20 $^\circ$), and it is possible that the larger pixels are more useful for reconstruction of the larger receptive fields (see for example Fig. 1 in Ciftcioglu et al.).

We added this information to the Results section: **“As opposed to the dLGN, IGL and vLGN units rarely displayed a classic compact receptive field, in line with Ciftcioglu 2020”.**



Reviewer Figure 8. Example receptive fields of vLGN units. Receptive fields of vLGN units recorded in response to a white noise stimulus ($\sim 4^\circ$ pixel size). Most vLGN units did not display clear receptive fields.

Since receptive fields unfortunately do not provide us a clear answer about the probe placement and borders between brain regions, we utilized a different approach to validate units' anatomical registration and resorted to light responses. If the probe alignment was accurate, we would expect to see light responses in visual brain areas, and no light responses in non-visual areas.

We considered all recorded units along the entire probe (containing also multi-units), and analysed the response quality to a full-field spot stimulus. We found significantly higher response quality values for LGN subregions compared to adjacent non-LGN regions, supporting our probe alignment (reviewer Fig. 9).

We added Reviewer Fig. 9 as Supplementary Fig. 2 to the manuscript and refer to it as follows in the methods section (Brain histology and probe alignment):

“We validated the assigned brain areas by quantifying light responsiveness and found that, as expected, visual areas displayed light responses while adjacent, non-visual areas, did not (Supp. Fig. 2).”

4. AS vs. OS: The authors state: “The term axis selectivity became popular with respect to mouse LGN units, partly because of the hypothesis that two DSRGCs with opposing preferred directions converge onto one LGN neuron, which would consequently encode motion along a specific axis 34,35.” This reviewer looked at both references cited for this statement. There is no mention that the term AS is used to define dLGN units that compute AS from the combination of DS inputs (as stated by the authors). AS is classically used as a conservative description of an axially selective neuron stimulated with moving stimuli. Without stationary bars, one cannot definitely call a response OS.

We agree that the statement cited by the reviewer reflects our interpretation of the papers. Yet, this interpretation is a direct outcome of the statements made in the papers, as both clearly suggest that two DSRGCs with opposing preferred directions may converge in the LGN and generate an axis selective neuron.

Marshall et al. 2012: “These results suggest that dLGN maintains and sharpens retinal direction selectivity and integrates opposing DSRGC subtypes in a functional-anatomical region, perhaps forming a feature representation for horizontal-axis motion, contrary to dLGN being a simple relay”.

Liang et al. 2018: “We observed significant finescale functional clustering of pairs with near opposite direction preferences (Figures 4 H, 4I, blue, and S4 E). Thus, a judicious arrangement exists whereby neighboring DS boutons demonstrate functional clustering for similar or opposite direction preferences, possibly contributing to direction tuning or axis tuning of target dLGN neurons, respectively”.

From our survey, most papers use the term ‘orientation selectivity’ despite using moving rather than static gratings. For example, Piscopo et al., 2013: “We refer to units selective to both directions of motion of a single orientation as “orientation selective” to follow recent nomenclature describing cortical tuning to moving gratings (Ringach et al., 2002; Kerlin et al., 2010; Rochefort et al., 2011). However, a more explicit description might be “bidirectional” selectivity because they respond to two opposing directions, or “axial” selectivity because they respond to motion about a single axis”.

Following the reviewer’s comment, and to avoid making statements in the name of the authors, we changed the sentence:

“The term axis selectivity became popular with respect to mouse LGN units. **We think that this is** partly because of the hypothesis that two DSRGCs with opposing preferred directions converge onto one LGN neuron, which would consequently encode motion along a specific axis 34,35.”

Of note, although it is common to use drifting gratings to define cells’ orientation/axial preference, neither definition is final based on drifting gratings alone. As the reviewer says - a cell cannot be defined as orientation selective based on the drifting gratings because it may respond to the motion direction (aka axis selective) rather than the orientation. At the same time, a cell cannot be defined as axis selective based on the drifting gratings because it may respond to the orientation (aka orientation selective) rather than the motion direction. For example, a true axis selective cell would respond to other moving stimuli that contain no orientation pattern, which would be a better stimulus to define those.

Please note that in the introduction we start with the term AS/OS, but then, for simplicity and consistency, we state we will use the term OS:

“Our results demonstrate that LGN AS/OS is not generated via the integration of two oppositely-tuned DSRGCs and we therefore refer to them simply as OS neurons, aligning with the original terminology used by Hubel and Wiesel⁴⁰ and that used today for both the retina and visual cortex”.

Following the reviewer’s comment, we have also added the following to the discussion:

“Since our recordings from control and SAC-ablated mice dispute this hypothesis by identifying similar percentages of AS/OS units in the LGN of the two groups, we chose to refer to these units as OS rather than AS. **However, only with additional stimuli – such as static orientations or non-oriented motion patterns – can the true nature of these cells, as either axis- or orientation-selective, be determined.**”

5. Related to #4, the authors interpret the results of the Diptheria toxin experiments as definitive evidence that dLGN neurons are OS inherited from the retina and not computed in the dLGN. However, they do not directly test whether the units they record from respond to stationary bars as has already been shown by Zhao et al. 2013. Zhao et al directly tested dLGN neuron feature selectivity by showing stationary bars and also demonstrated the presence of AS/OS RGCs in with MEA recordings in the retina. The authors should correct their citations and statements regarding measurements of OS:

a. Page 3: “This wiring scheme predicts the presence of DS in the dLGN, as well as the emergence of axis-selectivity^{35,36}”. The Zhao et al., 2013 paper referenced here (36) actually states that dLGN responses are OS and present stationary bars to show they are OS.

b. Page 3: “studies have not really distinguished them from OS responses”. This is not a fair assessment based on Zhao et al. 2013. This reviewer would argue that the authors of this current paper do not really distinguish AS responses from OS responses.

Please note that we did not claim that OS responses in the dLGN are inherited from the retina. As we do not have any mouse lines to specifically abolish OSRGCs, we currently have no way of testing this hypothesis. We only claim here that OS responses in the dLGN are *not dependent on retinal DS* (which we could directly test using the DT experiments that abolish retinal DS).

Zhao et al. demonstrated that most LGN cells that are orientation selective to drifting gratings tend to be tuned also to a flashed bar (their Figure 7). Nevertheless, in their figure 9F Zhao et al. suggest a “possible emergence of orientation selectivity in the dLGN by converging two direction-selective RGCs that have asymmetric excitation/inhibition in their RFs. The dLGN cell could be orientation selective to flashed bars due to its elongated inhibitory RF” (copied from their figure caption). For this reason we cited them in page 3 (point a). Although we believe the citation does support our statement, we agree this was not the major take home message of Zhao and removed the citation as the reviewer asked.

Regarding point b, we see the reviewer’s point and correct it to “**most** studies have not really distinguished them from OS responses”.

6. The authors should address:

a. Are there AS cells in the retina (that are not OS) that could be mediating AS/OS in the dLGN? Related to point #4-5 above, this reviewer would argue that the data in Fig 6a-d show that AS is not affected in the retina, not OS.

b. Are there other types of DS RGCs that do not depend on SACs that could contribute to dLGN AS/OS?

c. The authors should discuss whether there is the possibility that there are AS cells that are computed from DSGC inputs but they are of low frequency in dLGN.

Related to a: We currently have no indication of true axis-selective RGCs. Studies that characterized OSRGC first used static bars centered on an RGC and found tuned responses to the bar’s orientation (Nath et al., 2016; Nath et al., 2017). The authors also show that these RGCs are tuned to the same oriented bar moving through their receptive field. However, since these cells are already tuned to static bars they are OSRGCs (Nath et al., 2016; Nath et al., 2017). In retinal studies that use population recordings (e.g. Ca-imaging or MEA recordings) it is more difficult to center the stimuli directly on each cell, and therefore often only *moving* bars or gratings are used to find OSRGCs (e.g. Baden et al., 2016, Laniado et al., 2025, Vita et al., 2024). The advantage of moving stimuli is that each cell will see each position of the gratings or bar at some point, so there will be no bias of a specific location of a bar/stripe on a cell’s receptive field. However, this will of course complicate the distinction between orientation- and axis-selectivity since the stimulus is moving.

Most importantly, since moving grating is an oriented pattern, it would drive tuned responses for both AS and OS neurons. Thus, the results shown in Figure 6 cannot distinguish between the two, and

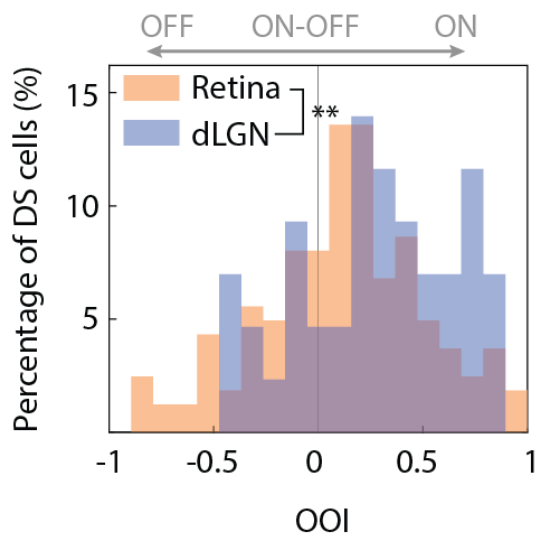
indicate that AS/OS units are unaffected. As we mentioned in our response to the reviewer's point #4, we refer to AS/OS cells as OS cells for simplicity.

Related to point b and c: Indeed, non-canonical DSRGCs whose DS is independent of SACs were shown to project to the LGN. However, these RGCs are usually tuned to vertical direction (Kim et al., 2008, Rousso et al., 2016) and tend to exhibit DS responses only under specific moving stimuli (Cooler & Schwartz 2021; Nath & Schwartz 2017; Joesch & Meister 2016). As our MEA recordings from SAC-ablated mice show that all DS responses to the drifting gratings were lost, we believe that non-canonical DSRGCs were not directionally tuned to the grating stimulus we used. As a result, the DS information was not transferred to the dLGN via the non-canonical DSRGCs, and could not mediate AS/OS responses (note that we used the same gratings parameters for retina and LGN recordings). Nevertheless, we agree with the reviewer that population extracellular recordings are generally biased towards specific cell types and sizes. Therefore, we may have undersampled some RGCs subtypes, including non-canonical DSRGCs. Similarly, we may have missed specific types of LGN neurons in our Neuropixels recordings. We have added the following sentence to the discussion to refer to this point (*Limitations of the study*): **"In this study we optimized the stimulus for canonical DSRGCs. Additional types of non-canonical DSRGCs are thought to be independent of SACs and to project to the LGN^{13,71} and their contribution to DS and AS/OS in the LGN was not tested here. Lastly, as for any recording technique for extracellular population recordings, our results may be biased towards specific cell types and sizes, and we may have missed cell types of very low frequencies."**

7. The authors argue that dLGN DS neurons inherit their properties from DSGC in the retina. Are other response properties like their luminance polarity also preserved in DS dLGN neurons (i.e. are they also on-off)?

This is an interesting point. We looked into the responses to a full-field white stimulus and quantified the On-Off-index (OOI) as the normalised difference between the On and Off responses. We only include cells with a response quality index > 0.25 and a mean firing rate > 1Hz. The OOI can reach values between -1 and 1, with -1 corresponding to Off responses, 1 to On responses, and OOI values around 0 to both On- and Off-responses. In Reviewer Fig. 10 we show the OOI distribution of DSRGCs (orange) and DS units of the dLGN (blue). As expected, DSRGCs are On-Off RGCs, and therefore their OOIs tend to cluster around 0. However, we found that the OOI distribution of DS units of the dLGN is shifted to the right, indicating they tend to have stronger On- than Off responses, with some cells being entirely On-cells. This difference was unexpected, as the DSRGCs that target the LGN have an On-Off preference.

There are several potential sources for the difference in OOI between DSRGCs and DS neurons in the LGN. Since we focussed on the transfer of direction-selectivity, we did not match the On-Off stimulus between the MEA and the *in vivo* setups. The stimuli between retina and LGN differed in their duration (2 sec On in the retina vs 4 sec in the LGN). We also did not match the exact light levels. The polarity preference of RGCs can be dynamic and change, for example with ambient light level (Pearson et al., 2015, Tikidji-Hamburyan et al., 2015). In addition, the contribution of receptive field surround depends on light levels, and it is possible that On and Off surround of DSRGCs are not affected similarly (Ankri et al., 2024). For example, if Off, but not On surround is stronger in our *in vivo* recording conditions, we might expect to see diminished Off responses to the full-field stimulus offset, but no change in the On response. We therefore feel that it is difficult to conclude from our data how dLGN DS units inherit their polarity preference. They likely inherit parts of it from DSRGCs, but whether the differences that we observe are due to light levels, stimulus duration, or additional inputs of non-DS ON-RGC to DS-LGN units, or from LGN interneurons, cannot be determined at this point.



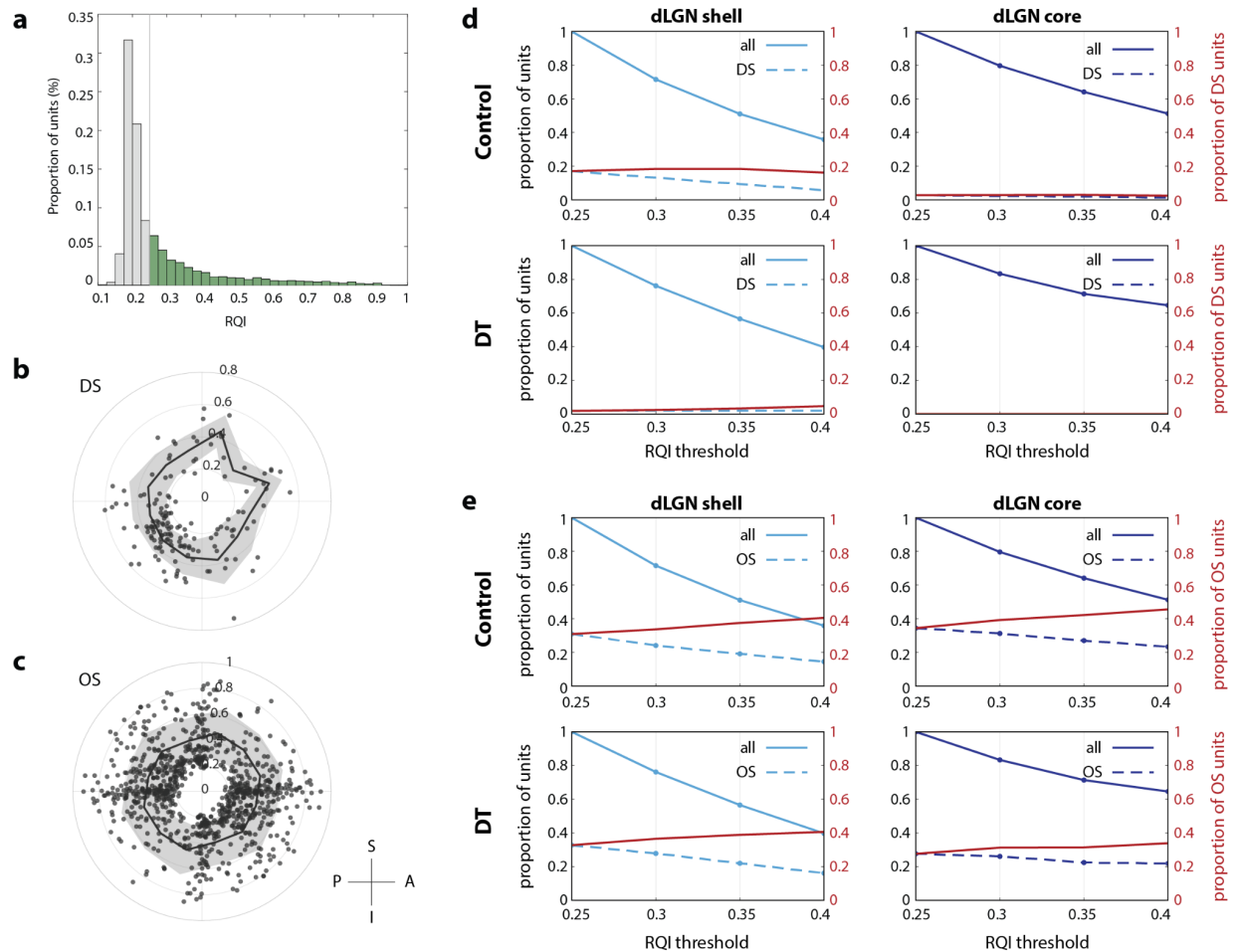
Reviewer Figure 10. On-Off index of DS units. OOI is calculated as the normalised difference between On and Off response and can reach values between -1 (Off cell), and +1 (On cell). On-Off neurons with balanced On and Off responses would present an OOI of 0.

8. The authors use two parameters to determine whether a unit is visual: 1) a response 2 SDs above baseline or 2) an RQI value of 0.25 or greater. However, according to Baden et al. 2016, which the authors cite, the RQI for completely random responses is expected to be proportional to $1/(\# \text{ of trials})$, which in this case equals 0.2 (1/5 trials). In this case, a RQI cutoff of 0.25 is not particularly stringent for assessing response quality. To address this, the authors should present the distribution of RQI values in their recordings and demonstrate that their conclusions remain unchanged when using a more stringent RQI threshold.

We agree. We provide a figure below showing the distribution of response quality index across the entire LGN, and show units with $RQI > 0.25$ in green (Reviewer Fig. 11a). It is true that for non-responsive units (random responses) the RQI will be 1/5 (see peak in the distribution at 0.2).

To check if the results are robust for stricter thresholds, we plotted the RQI of DS units as a function of their preferred direction (Reviewer Fig. 11b), showing that RQIs are uniformly distributed across preferred directions. Increasing the RQI inclusion threshold would therefore equally affect DS units of all preferred directions. The same is true for OS units (Reviewer Fig. 11c). Lastly, we plotted the percentage of included units out of all units for different RQI thresholds, where we define all units included at $RQI > 0.25$ as 100%. With increasing RQI threshold, the percentage of all included units decreases (solid lines). Similarly, both the percentage of DS and OS units decrease (dashed lines, Reviewer Fig. 11d and e, respectively). Nevertheless, the percentages of DS and OS units among all responsive units (red lines, right y-axis) remain relatively stable across RQI thresholds (Reviewer Fig. 11d,e). We conclude that the results are robust and independent of the RQI threshold.

We added the information to the Methods section, *Light-responsive units*.



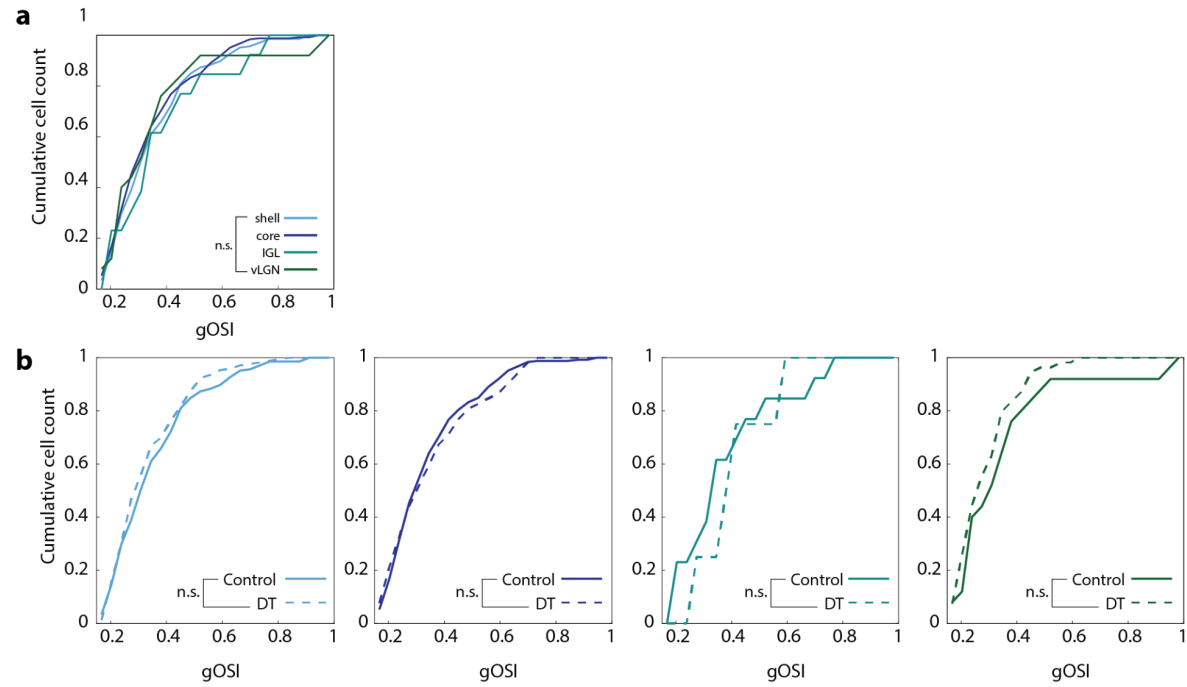
Reviewer Figure 11. Results are robust to stricter inclusion criteria. **a.** Distribution of response quality index of all LGN units with units of RQI>0.25 shown in green. **b.** RQI as a function of preferred direction for all LGN DS units. Mean $\pm$ SD across 30° bins are shown as gray line and shaded area. **c.** Like b for OS units and their preferred orientations. **d.** Proportion of units still included when increasing RQI threshold (compared to 100% of units included at RQI threshold of 0.25; solid blue line, left y-axis), and proportion of DS units (dashed blue line, left y-axis). Resulting proportion of DS units is shown in red (right y-axis). **e.** Like d for OS units.

9. The authors describe a 34 degree shift in elevation in aligning the preferred directions of DS to the predicted optic flow vectors. This is confusing to the reader. The authors should explain this in more detail and give an explanation for why there is a shift.

We understand this point was not clear and apologize. Please see our answer to Point 5 of Reviewer 1. We added clarifications in the text, as well as Supplementary Figure 7.

10. Fig 4c, 6h: the authors should also show the cumulative distributions of OSI/gOSI. We provide the cumulative distributions of the gOSI below in Reviewer Fig. 12. The tuning width and gOSI are related measures - both express tuning sharpness/tuning width. In the manuscript we decided to plot the tuning width in degrees because we felt like this would be a more intuitive measure than a gOSI. Importantly, our conclusion remains based on either measure (gOSI or tuning width): there is no significant difference between LGN subregions (Reviewer Fig. 12a) or between control and DT (Reviewer

Fig. 12b). We agree that the gOSI is an accepted measure, and added it as a supplementary figure (Supplementary Fig. 11).



Reviewer Figure 12. Cumulative distributions of gOSI. **a.** Cumulative distributions of gOSI values for OS units across LGN subregions (like tuning width in Fig. 4c). **b.** Cumulative distributions of gOSI for all OS units in control vs. DT condition, across LGN subregions (like tuning width in Fig. 6h).

Reviewer #4 (Remarks to the Author):

The paper of Yaakov et al addresses a fundamental set of questions about the organization of direction and orientation selectivity in the lateral geniculate nucleus. The authors show that while orientation selective neurons in LGN span the whole visual field while direction selective neurons are largely absent in the central visual field and are topographically aligned to translatory optic flow. Using transgenic mice lacking retinal direction selectivity, Yaakov et al demonstrate that LGN direction selectivity relies on retinal directional selectivity, suggesting that LGN DS is inherited from the retina. Moreover, the authors show that LGN orientation selectivity is not affected by the ablation of directional selective neurons in the retina, demonstrating that LGN orientation selectivity is not generated via the integration of direction selective retinal ganglion cells.

I think the manuscript is well written and shows strong convincing evidence for all the claims made in the paper.

(Minor) main point

-Throughout the manuscript the authors speak about orientation and direction selective neurons as two clearly separate populations of neurons. Is there an overlap in the populations selective for direction and orientation? Or are they clearly separate populations? . The selection of threshold criteria seems kinda arbitrary. I don't necessarily object to that since it's quite common in the field. However it would be good to show some plots in the supplements that undermine your choices of the criteria. The 6) criteria for assigning a neuron to one of these groups makes sure that a neuron is assigned to only one of the groups. However I am wondering if that is a fair criteria because a neuron that fulfills all other 5 criteria for orientation selectivity and direction selectivity would end as orientation selective if it has only slightly higher gOSI than gDSI. I think it could be useful to e.g. show a scatter plot in the SI where you plot direction vs orientation selectivity of all cells to see that this clear distinction is fair and not the result of tweaking the classification criteria

We agree that – although commonly used in the field – the thresholds for (g)DSI and (g)OSI are somewhat arbitrary. Because of how the gOSI (normalized vector sum) is calculated, DS cells can also have relatively high gOSI values. We illustrate this in Reviewer Fig. 13a, b for several made-up responses: some of these responses reach gOSI values above a certain threshold, but should still be classified as direction-selective. Therefore, our criterion 6 (where $gDSI > gOSI$ for DS units) is a valid criterion to use to correctly classify these units as DS (and not both).

However, there are cases in which distinguishing between DS and OS cells becomes more challenging, particularly, when the gOSI and gDSI are relatively similar. These cells could be interpreted as (imperfect) DS cells with some response in their null direction, as AS cells with an asymmetric response along their preferred axis, or as cells that are both DS and AS. (Indeed, DSRGC may uncover a 'null response' under certain conditions (Rivlin-Etzion et al., 2012)).

Reviewer Fig. 13c shows all dLGN shell units, with units either labelled as DS (pink) or AS/OS (teal). Notably, the plot indicates that the DS and OS units are largely distinct, as only a minority of the units lay close to the unity line. We provide some example tunings of units, including units with relatively similar gDSI and gOSI (Reviewer Fig. 13d-f). Currently, these ambiguous cells are assigned as DS or OS depending on whether they lie below or above the unity line. If we were to remove our last criteria (6: $gDSI > gOSI$ for DS cells) it would allow some ambiguous cells that were formerly classified as AS/OS to now be classified as DS (e.g. example cells 5 and 7 from Reviewer Fig. 13c, f).

While the number of these ambiguous cells that may pass as DS cells is relatively low ($n=12$ cells in the dLGN shell compared to 114 DS cells and 215 OS cells), we checked whether adding these cells to the general DS population would change our conclusions. We found that the bias of dLGN shell DS units

towards postero-inferior motion remains (Reviewer Fig. 13g), as well as the topographic organization (Reviewer Fig. 13h, i).

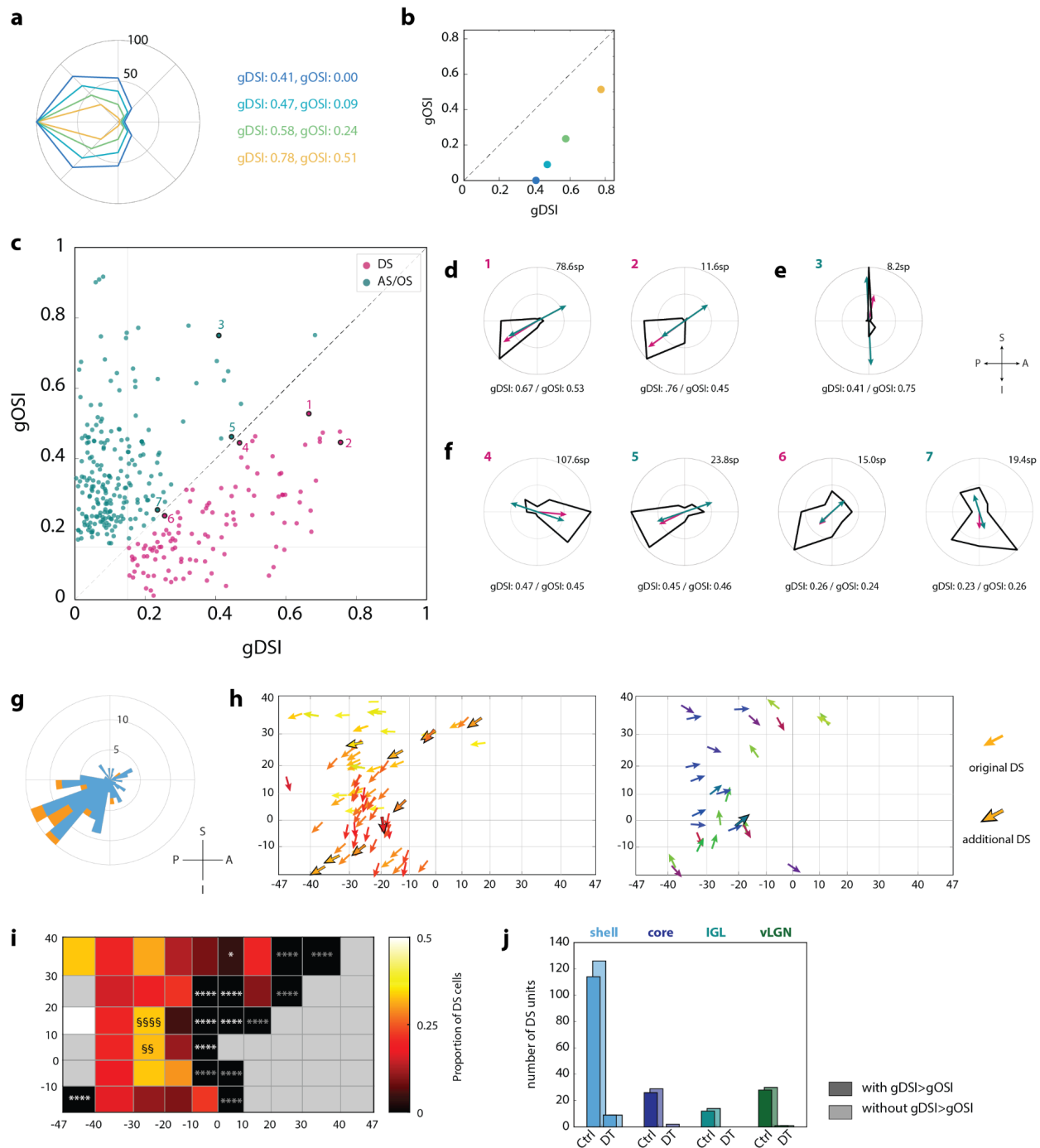
We also found that adding these ambiguous cells to the DS neuronal population does not affect the findings of Figure 3 (reduced DS in DT mice; Reviewer Fig. 13j). It is therefore possible that these cells are actually DS cells. However, they also fit the properties of the AS/OS population (diagonal preference).

As the classification of these cells remains somewhat ambiguous, and since the conclusions of the paper will not be affected by how these cells are classified, we did not change their label (i.e., these units are still considered as AS/OS).

We added panels c-f as a Supplementary Figure 1 and refer to the thresholding and classification in the main text now as follows:

“Animals were centered in front of a screen and presented with a binary noise stimulus for receptive field estimation and moving gratings in 8 directions, based on which we assessed the direction and orientation tuning of the units using accepted parameters (e.g., global direction- and orientation-selective index, gDSI and gOSI, respectively (Methods section *Direction, axis- and orientation selectivity*)). **The gOSI and gDSI populations formed two relatively distinct populations and allowed us to determine direction and orientation tuning of recorded LGN units (Supp. Fig. 1).**”.

We also added in the Methods (*Direction, axis and orientation selectivity*): “When removing criterion #6, the results of the study did not change.”



Reviewer Figure 13. Criteria for defining direction- and orientation-selectivity. **a.** Example made-up DS responses with their corresponding gDSI and gOSI values. **b.** Raster plot of gDSI and gOSI of example tunings shown in **a**. **c.** Scatter plot of gDSI and gOSI of all dLGN shell units. Thresholds of 0.15 are shown as gray lines. Dashed line is the unity line. Units are colored according to their classification as DS (pink) or AS/OS (teal). **d.** Example units from **c** with high gDSI and high gOSI classified as DS (example units 1 and 2). **e.** Example unit from **c** with high gDSI and high gOSI value classified as OS (example unit 3). **f.** Example units with similar gDSI and gOSI values that can be considered 'ambiguous'. **g.** Preferred directions of dLGN shell DS units of original classification (blue, like Fig. 1f), and of the DS population with the additional units classified as DS after removing criterion 6 (gDSI>gOSI for DS units). **h.** Preferred directions of original

DS units and newly classified DS units (black outline) plotted on their receptive field center (notation as in Fig. 2i). i. Proportion of DS units out of all units from dLGN shell. j. Number of DS units in all LGN subregions in control and DT condition. Dark bars represent original numbers, transparent bars represent numbers when removing criterion 6. For d-f, both the preferred direction (pink) and preferred motion axis (teal) are plotted. Please note that for easier comparison we plot here the axis of motion preference rather than the orientation axis.

Minor points:

-Most abbreviations are introduced somewhere in the beginning of the paper but I failed to find a definition of AS "This wiring scheme predicts the presence of DS in the dLGN, as well as the emergence of axis-selectivity (AS) 35,36."

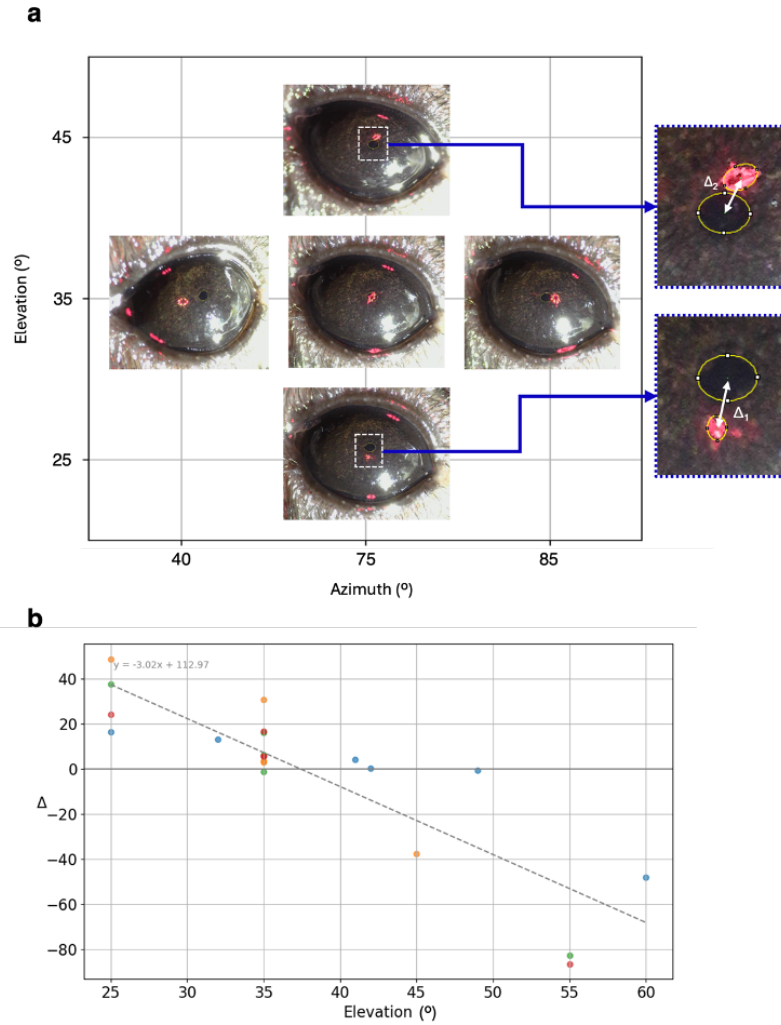
Thanks for noticing this. Done.

-How do you measure the optic axis in anesthetized mice? It's not clear to me. Doesn't this depend on the head position/orientation?

The method of optic axis extraction is shortly mentioned in the methods section along with the relevant reference (Stahl et al. 2000), which we based our measurement on and where the process is described in detail. We provide here Reviewer Fig. 14 to depict the methodology for clarity: a LED-girdled camera (Dino-Lite Edge) is mounted on a two axis gimbal, allowing us to precisely rotate the camera along the azimuth or elevation. The rotation center is manually adjusted to the center of the pupil as shown in the figure. This process already provides a pretty precise estimation of the optic axis. We then continue with further calibrations based on the distance between the reflection of the LED and the pupil in other positions of the camera with fixed small shifts in azimuth and elevation, as described in Stahl et al. (Reviewer Fig. 15).

We have added Reviewer Fig. 14 as a Supplementary Fig. 8 to clarify the methodology.

Regarding the second question in the comment – in our case the optic axis is fixed. First, the vestibulo-ocular reflex (VOR), which is responsible for complementary eye movements according to head shifts, is subdued in anesthetized animals, so the eyes do not move. Second, our experiments were done in head fixed mice, all of which were fixed at the same head-orientation (bregma-lambda aligns with horizon).



Reviewer Figure 14. Optic axis measurement. **a.** Images of an anesthetized mouse eye acquired from different camera elevation and azimuth perspectives. The camera's focal point was manually calibrated to minimize pupil deviations across camera angles (Methods section *Optic axis measurements*). Extracted Δ s (the distance between the camera's corneal reflection and the pupil's centers) were used to calculate and verify reasonable R_p values according to Stahl's previous works (see Figure 3 from Stahl et al., 2000). **b.** Scatterplot of all elevations and their corresponding Δ values (pixels). Linear regression (dashed gray line) predicts a similar elevation value to that derived using the geometric calibration process. Different colors represent values obtained from separate mice ($n=4$). Intersection of linear regression with $\Delta=0$ is at elevation 36.7° .

[REDACTED]

The figure and its caption are taken from Stahl et al., 2000 (Fig. 3).

-In the 3 results Section “SAC ablation in the retina eliminates DS responses in the LGN” the authors write “The lack of DS representation in the central visual field as well as the topographic organization of DS preferred directions in the dLGN are different from DS in the retina.” I think it would be good to add a sentence elaborating on the difference in the topographic organization.

We refined the sentence: “The lack of DS representation in the central visual field is unexpected to derive from the retina, as DSRGCs are thought to be distributed across the entire retinal surface, including the temporal region which represents the center visual field (Heukamp et al., 2020; Sabbah et al., 2017; Tiriach et al., 2022; El-Danaf et al., 2019)”.

-In the 4 result section the authors write: “. The LGN OS population exhibited general orientation tuning biases towards horizontally, vertically and diagonally oriented gratings (mostly postero-superior to antero-inferior) (Fig. 4d).” What do the authors make out of this bias for diagonal/vertical/horizontal tuning? Maybe speculate about it in the discussion section

We now refer to the arrangement of all OS units in the paper, and added a supplementary figure showing how the different OS units are organized across the screen (see our answer to comment #3 of reviewer 1, along with Reviewer Fig. 2/Supp. Fig. 11).

We find that horizontal OS cells are somewhat denser around 30 deg elevation (Reviewer Fig. 2e-g, left) - the altitude corresponding with the horizon based on our optic axis calculations. These cells may therefore contribute to orienting during navigation. We also found a streak of higher density of diagonal preferring OS units, tuned in supero-posterior to infero-anterior orientation, but not in the opposite diagonal (Reviewer Fig. 2e-g, right). In this case, we could not identify a simple explanation and believe

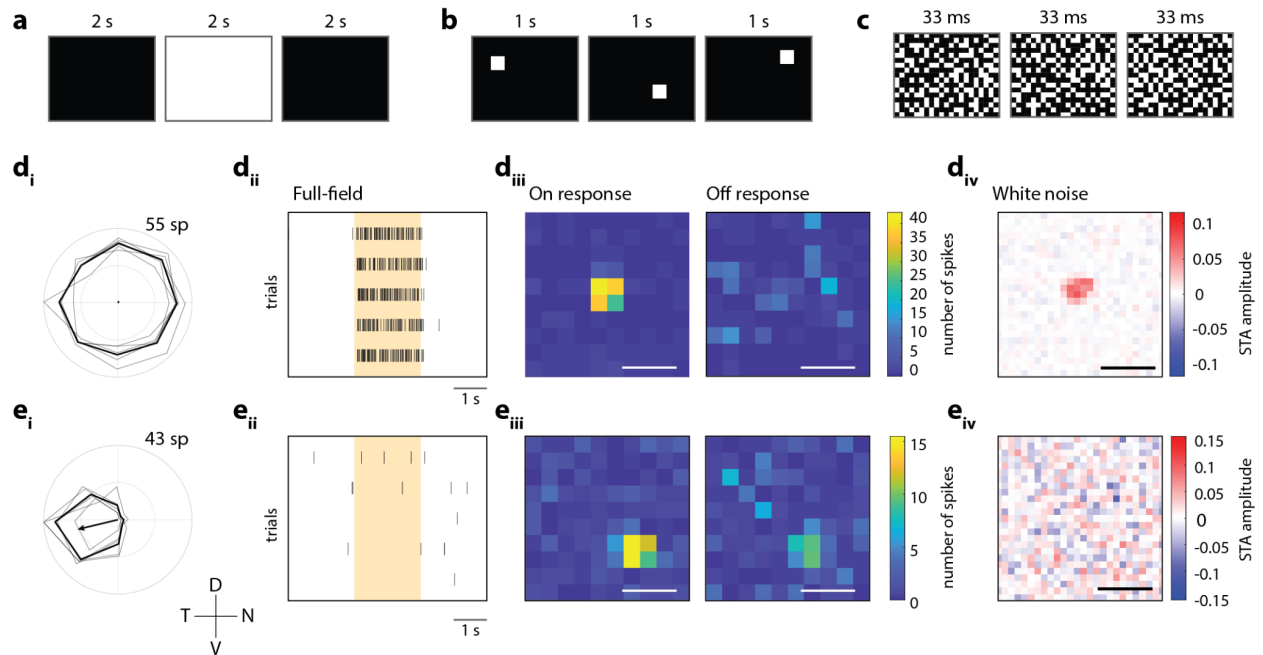
this pattern warrants a thorough investigation of the mouse's natural environment, behavioral repertoire, and locomotion patterns. We have added the following to the discussion (section *Role of direction and orientation selectivity in visually-guided behaviors*):

“We also found that horizontal OS cells are somewhat denser around 30 deg elevation - the altitude corresponding to the horizon. These cells may therefore contribute to orienting during navigation. We also found a streak of higher density of diagonal preferring OS units, the functional significance of which remains unclear and requires further investigation”.

- Why do most DS units don't display a clear RF? Is it due to experimental constraints? Do you think they actually have clear RFs? I think it would be good to discuss it a bit

From the retina, we know that DSRGCs's receptive fields display strong surround antagonism (Chan & Chiao 2008, Ankri et al., 2024). We believe that the antagonistic surround is recruited during the white noise stimulus and inhibits the DSRGC, thereby masking its center receptive field response. In our MEA recordings from the retina, we do find receptive fields of ~59% of DSRGCs to a white noise stimulus using the STA analysis, but they are often not as good as those of non-DSRGCs (e.g., they respond with lower firing rates and have lower STA amplitudes). Note however that our white noise stimulus for the retina differs from the LGN experiments, so we cannot directly compare the results (MEA recordings: squares of 2° size, temporal frequency of 30 Hz; Neuropixel recordings: squares of ~4° size, temporal frequency of 15 Hz). Moreover, we obtain better receptive fields by using a stimulus consisting of a single square flashed at different locations (sometimes called 'sparse noise') where we can ensure that only the center, but not the surround, is activated (see Reviewer Fig. 16 for an example for a full-field-response, sparse noise and white noise response for a non-DSRGC and a DSRGC). This is likely the case for LGN DS units as well, but we unfortunately did not use the sparse noise in our recordings.

We now refer to this point in the Results section *Direction-selective responses in the dLGN are topography-dependent*: “Some dLGN units showed a clear response to the moving gratings but poor responses to the white noise. Hence, we could not directly calculate their receptive field (34.8% of non-DS dLGN units, 62.9% of DS units). **The lack of clear receptive fields may occur when a cell with strong surround antagonism is presented with a white noise stimulation. Such strong surround was reported for DSRGCs^{45,46}, probably masking responses to stimuli in the center receptive field.**”



Reviewer Figure 16. Example receptive fields of RGCs to different stimuli. **a.** Schematic of a full-field spot stimulus. **b.** Square stimulus (150 μ m size; or 'sparse noise') used for receptive field mapping. **c.** White noise used for receptive field mapping (60 μ m pixel size, 30Hz). **d.** Example responses of a non-DSRGC to various stimuli: Moving gratings (d_i); full-field spot (d_{ii}, from a); square (or 'sparse noise', d_{iii}, from b), separated into onset (left) and offset (right) responses; White noise (d_{iv}, from c). **e.** Like d for a DSRGC. Scale bar in d_{iii}-d_{iv} and e_{iii}-e_{iv}: 500 μ m.

Reviewer #1 (Remarks to the Author):

The authors thoroughly addressed my feedback. From my assessment, I also think that authors adequately addressed other reviewers' feedback. I continue to think that this is a strong study that significantly contributes to 1) our understanding of how retinal computations are transferred to the visual thalamus and 2) how DS and OS are organized in the visual thalamus. The paper is even stronger post-revision, and I have no further feedback to give. Excellent study.

We thank the reviewer for the thoughtful comments and encouraging assessments. We are pleased that the revisions have strengthened the manuscript and that the reviewer finds our study to be a valuable contribution to understanding the transfer of retinal computations and the organization of direction and orientation selectivity in the visual thalamus.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We thank the reviewer and their co-reviewer for their joint evaluation. We appreciate the Nature Communications initiative supporting Early Career Researchers and are grateful for the time and expertise both reviewers dedicated to improving our manuscript.

Reviewer #3 (Remarks to the Author):

In this revision, the authors have addressed some of the issues raised previously. This reviewer appreciates the explanation of AS/OS and additional analyses the authors performed. However, there were several responses that require further clarification in order to strengthen the authors arguments.

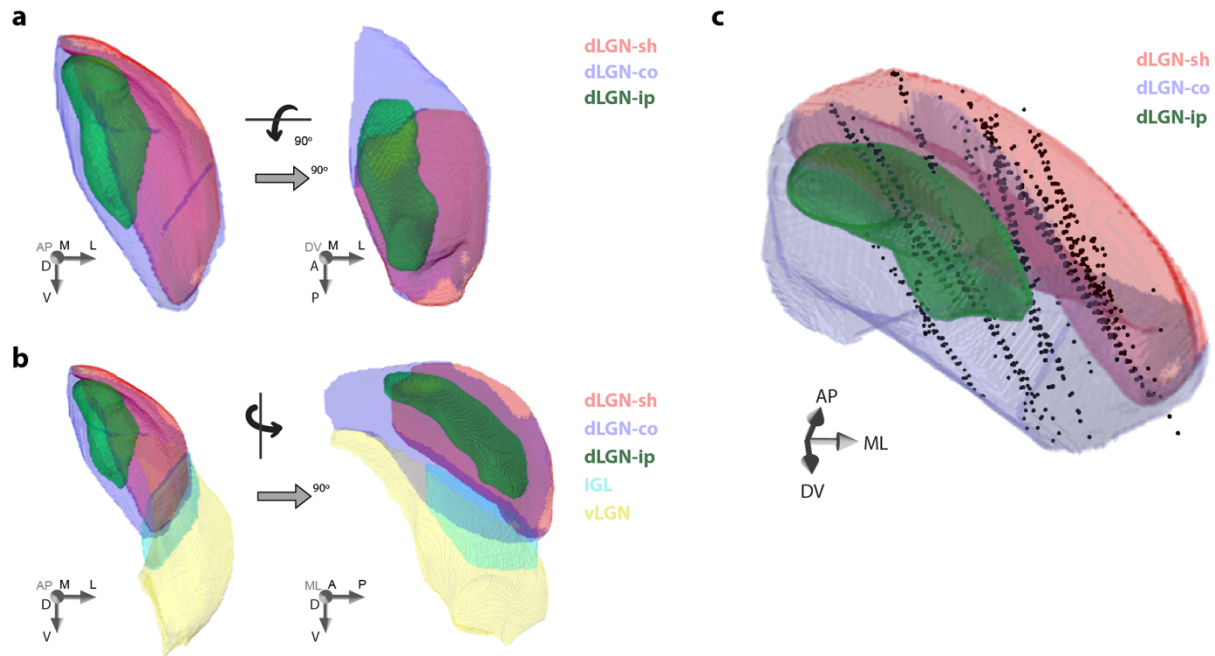
We thank the reviewer for acknowledging the improvements made in the revised manuscript and appreciate the reviewer's careful assessment. Below, we provide detailed responses to the points that were identified as needing further clarification. We hope these additional explanations and revisions adequately address the reviewer's concerns.

Comments:

1. While the inclusion of color maps of the LGN in Figure S5 and Reviewer Figure 5 is helpful, it is still difficult to interpret the precise locations of probe insertions. The 3D representations (e.g., Figure 1c, 2e, and the supplementary video) are not accessible to most readers, including those familiar with the mouse visual system. The authors should also include the same color coding in the supplementary video for consistency and clarity.

In Fig. 2e we are aiming to highlight the organization of units across the dLGN and their receptive field location on the screen. We therefore chose light colors for the dLGN subregions to allow the reader to focus on the colormap used for the recorded units. (We did try to combine the colormap of the units with the new color map of the dLGN, but this impaired discriminability.)

After the reviewer's suggestion, we now added an additional supplementary video using the same colourmap to highlight the LGN subregions (and we therefore plot all units in the same color; Supp. Video 2). We additionally added a new panel to the supplemental figure (Reviewer Fig. 1 below ; new Supp. Fig. 7) showing the same data as in Fig. 2e, but using the new color map that highlights the different LGN subregions.



Reviewer Figure 1. Subregions of the LGN. a. 3D model (adopted from Allen Mouse CCF) of the dLGN. Left: the nucleus is viewed from posterior. Right: viewed from above. **b.** Same as a, including IGL and vLGN. **c.** Same as Fig. 2e, highlighting the dLGN subregions as in a-b. Recorded units are shown in black. dLGN-sh: dLGN shell, dLGN-co: contralateral patch of the dLGN core; dLGN-ip: ipsilateral patch of the dLGN.

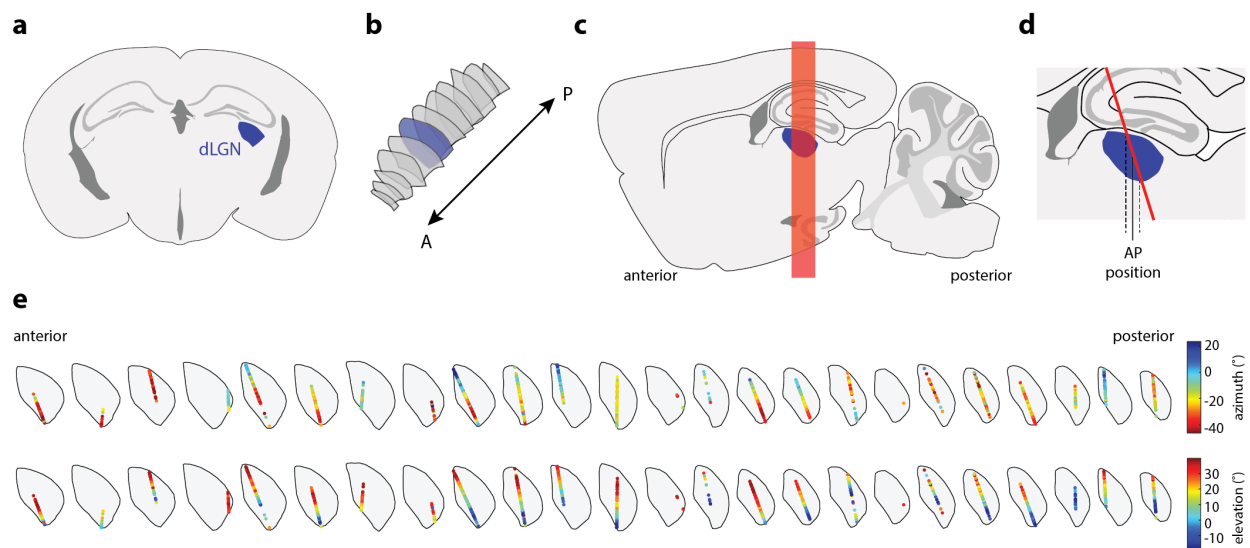
2. With regards to the authors response to concerns about the limited coverage of the recordings, this reviewer is still not convinced. Based on the color-coded maps, the authors' reviewer figure 6 and the supplemental video, the electrode tracks and data miss regions of anterior as well as middle and posterior dLGN which have DS signals according to Piscopo et al. that may represent central vision (see the dark blue regions depicted in Reviewer Figure 6e, f and the green regions in 6g). The authors should more explicitly address the extent of their dLGN coverage in the main text and the possibility that some DS regions representing central vision may be missing. After rebutting this issue in the previous version of the paper, the authors state "Having said all the above, we agree that we cannot know if DS responses exist in regions that we did not record from, or if the center visual field has additional representation in the anterior dLGN." Yet, despite this statement in the rebuttal, the sentence "LGN DS responses are absent in the central visual field" is in the abstract and emphasized throughout the text.

Because the manuscript makes this negative claim (i.e., the absence of DS in central visual space), readers must be able to evaluate the spatial coverage of the recordings. The authors should include 2D coronal sections at various anterior-posterior positions with overlaid probe trajectories (very much as they did for reviewer figure 6c-f).

We have made the following changes based on the points made in comment #2:

1. We have modified our statements regarding DS in the center visual field throughout the text (for example, in the abstract we changed “LGN DS responses are absent in the central visual field” to “LGN DS responses are **scarce** in the central visual field”).
2. We have added the following statement to the Discussion (section *Limitations of the study*): “Therefore, we cannot know if DS responses exist in brain regions we did not record from, or if the central visual field has additional representation in dLGN regions which we did not target”.
3. We have added a supplementary figure depicting the coverage of our dLGN recordings as well as slices of all insertions with units plotted on their location in the slice, colored by azimuth (top) and elevation (bottom) of their receptive field location (Reviewer Figure 2; new Supp. Fig. 3). Please note the probe was often tilted in order to maximize dLGN coverage, and therefore the slices presented in Reviewer figure 2e are arranged by their mean AP coordinate (see Reviewer Figure 2d).

While Piscopo et al. provide a foundational framework for understanding dLGN response properties and organization, their data have several limitations. First, recordings in their study contain a relatively low number of units per penetration (3-16 single units, 9 on average), whereas our Neuropixels recordings allow for a much denser coverage (on average 98 units in the dLGN). The total number of units in Piscopo is 257 units across the entire dLGN (this is under the assumption that *all* units in the Piscopo study were recorded in the dLGN, although they did not specify it in the study, and simply refer to the LGN throughout their paper); our study includes 1883 units in the dLGN. Second, Piscopo et al. found 11% (28 of 257) units that were *either* DS or OS; only 7 of them were DS (2.75% of all recorded units). Moreover, Piscopo’s histological dLGN organization figure (their Fig. 7) combines both OS and DS units, so DS organization alone cannot be inferred from the figure. Lastly, for the organization of azimuth, elevation, or the prevalence of DS and OS shown in Fig. 7 in Piscopo et al., only three averaged coronal slices are shown but without labelling their exact location. While this study certainly informs us of trends and general rules of the organization of LGN units, it required a high level of interpolation and smoothing of the data to achieve the color maps shown in their figure.



Reviewer Figure 2. Anterior-posterior organization of the dLGN and recorded locations. **a.** Exemplary coronal section showing the dLGN (modified from Franklin & Paxinos Atlas). **b.** dLGN area extracted from coronal slices at different AP positions. **c.** Sagittal section of a brain (modified from Franklin & Paxinos Atlas), showing the dLGN (blue) and the AP region covered by our recordings (red). **d.** Schematic of estimated AP positions of coronal slices shown in e. The AP position was estimated as the average between the dorsal and ventral AP positions of the probe intersecting with the dLGN border (probe trajectory was often tilted to maximize dLGN coverage). **e.** dLGN extracted from coronal sections of our recordings, showing recorded units that had receptive fields, colored by azimuth (top) and elevation (bottom). Slices are arranged according to AP coordinates, estimated as shown in d. Note that exact AP coordinates are not given to avoid confusion, since they vary across different atlases.

3. The author's response to Major concerns #2 reveals that they did not cover the ipsilateral eye during recordings of the binocular visual field (part of the central visual field). This is also apparent from data in Reviewer Figure 9 showing the ipsilateral patch is responsive to visual stimulation. This issue would influence the interpretation of the dLGN core analysis (Fig 2h). While this reviewer is in complete agreement that the ipsilateral patch is part of the "core", if recordings of the central visual field primarily come from the ipsilateral patch, it would not be surprising that there are no DS responses given the projections of DS RGCs. This reviewer was under the impression that the novelty of the study was that DS from the central retina is not transmitted to the dLGN (regardless of shell or core). Therefore, contamination from the ipsilateral patch could confound interpretation of results. The authors should show a separate graph of just the ipsi patch results (as in Figure 2g and 2h) and another with just responses from the contralateral eye. They should also report DS percentages in the core excluding the units in ipsi patch. This is reasonable given the Allen CCF data also distinguishes core and ipsi patch as in Reviewer Figure 9. Showing receptive field locations of ipsi patch units alone would also help corroborate that anatomical reconstructions were precise. Reviewer Figure 9 is not very convincing in showing that the authors can confidently distinguish between the different compartments of the LGN (dLGN, vLGN, IGL).

The reviewer raises valid concerns regarding the methods for assigning units to small subparcellations of the LGN. First and foremost, we want to reiterate that our central conclusions do not depend on the exact localization of units within small anatomical compartments. Therefore, the overall interpretation of our results remains robust and well-supported:

- (1) DS units in the dLGN are organized to represent optic flow during forward motion;
- (2) DS responses in the LGN are eliminated when SACs are eliminated;
- (3) Edge-induced OS responses are present in the LGN;
- (4) OS responses in the LGN do not depend on retinal SACs.

We have made several clarifications and added new analysis to address the issues raised by the reviewer:

1. Correct, we did not cover one eye. We now explicitly state that the mouse viewed the screen with both eyes to avoid confusion (added to Methods, section *Visual stimuli for in vivo recordings*)
2. We have modified the text to emphasize our finding that DS is scarce in the *central visual field*, which corresponds to the temporal retina, see Supp. Fig. 5. Note this does not correspond to central retina.
3. After the reviewer's suggestion, we added a new supplementary figure showing receptive fields of dLGN core units from the contra- and ipsilateral patch in separate panels as requested (Reviewer Fig. 3; new Supp. Fig. 8). We then calculated the DS percentages for units annotated as ipsilateral patch and units annotated as contralateral. The percentage of DS units remains low in both groups, with 2.61% (6 out of 230 units) in the ipsilateral and 2.98% (23 out of 773 units) in

the contralateral core territories, showing that our finding that DS responses are scarce in the dLGN core does not depend on the separation between ipsi- and contralateral patch.

We have added the following to the Results, section *Direction-selective responses in the dLGN are topography-dependent*:

“The low percentages of DS units in the core could originate from units in the ipsilateral patch, which tends to lack DSRGC inputs^{24,26,28}. We therefore separated the core units into ipsilateral and contralateral regions according to their assignment and found that DS units in each region comprised less than 3% of the cells (Supp. Fig. 8)”.

As the reviewer wrote, showing receptive field locations of ipsi patch units alone can also help corroborate our anatomical reconstructions. Most units we classified as belonging to the ipsilateral patch (75%) had RFs in the binocular zone, consistent with expectations. However, a quarter of these units had RFs in the monocular zone (Reviewer Fig. 3b, right).

This observation probably reveals a limitation of anatomical localization in small subregions like the ipsilateral patch, yet other sources may also contribute. First, despite the traditional division of the dLGN into distinct eye-specific territories, *in vivo* recordings from the dLGN revealed that a significant number of neurons collect retinal inputs from both eyes (Howarth et al., 2014). These binocular cells were found at highest density around zones of anatomically defined ipsilateral retinal projections, suggesting that some dLGN neurons in the ipsilateral patch collect inputs from the surrounding contralateral region (Hooks and Chen 2020). Indeed, it was shown that neurons in the ipsilateral patch extend dendrites into the contralateral patch of the dLGN core (Krahe et al., 2011). As a result, their RFs may deviate from the pure binocular region. Second, some of the units may represent interneurons, which comprise ~10% of the dLGN cells (Jaubert-Miazza et al. 2005). These interneurons are particularly large and may innervate functionally distinct regions (Zhu et al., 1999; Morgan & Lichtman 2020; Müllner & Roska 2024). Specifically, their processes frequently cross eye-specific borders (Seabrook et al., 2013). At this stage, we cannot determine if the units assigned to the ipsilateral patch are mislabeled, span their dendrites to the contralateral core, or are large interneurons. We assume that the population is composed of all three categories.

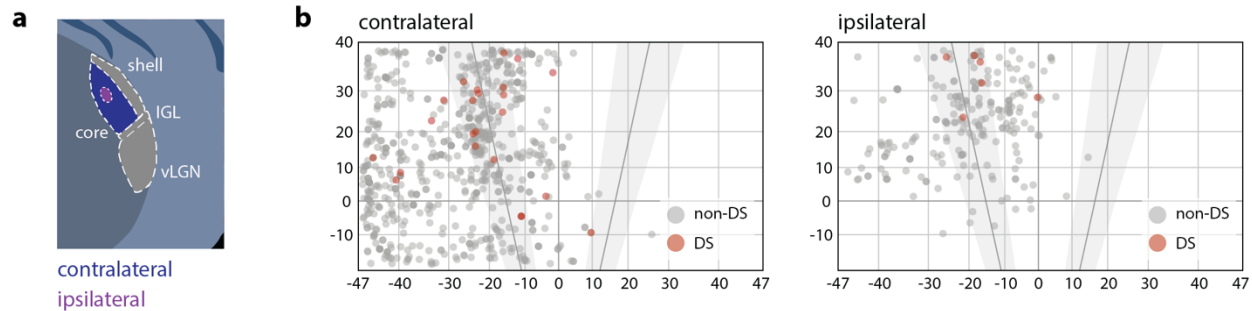
While mislabeling is plausible, our finding that the shell is abundant with DS cells and the core includes only a small portion of them is well supported by anatomical studies (Huberman et al., 2009, Jiang et al., 2022, Rivlin-Etzion et al., 2011, Kay et al., 2011) and further supports our approach for unit classification. Even though this approach is well-accepted in the field, we agree it has its limitations, particularly when assigning units in small brain regions.

4. We have added a reference to the RF location of units from the ipsilateral patch to the Results, section *Direction-selective responses in the dLGN are topography-dependent*:

“While most (75%) of the units assigned to the ipsilateral patch had receptive fields in the binocular zone, a quarter had receptive fields in the monocular zone. It was shown that dendrites of dLGN neurons may cross eye-specific borders and integrate inputs from both eyes^{33–35,49–52}, which may explain the peripheral receptive fields. Yet, these units may also reflect the limitation of our approach to assign units belonging to particularly small brain regions such as the ipsilateral patch”.

In addition, we have emphasized the limitations with respect to brain regions assignment (in Discussion section *Limitations of the study*):

“We also note that small errors in anatomical localization may occur, particularly in compact regions such as the ipsilateral patch or the IGL; however, none of our key conclusions depend on precise unit assignment within these compartments”.



Reviewer Fig. 3. Contra- and ipsilateral patch of the dLGN core. **a.** Schematic of the dLGN core showing the two regions receiving contralateral (blue) or ipsilateral (purple) inputs. **b.** Receptive fields of all units recorded in Control experiments in the dLGN core, showing the location of DS units, separated into the contra- (left) and ipsilateral (right) patch of the dLGN core.

4. Authors should include ON-OFF data in the manuscript. While there are caveats to the data, as the authors nicely explain, the data do potentially suggest that there is room for non-simple inheritance. Readers will benefit from seeing the data directly, along with a discussion of their limitations, as was articulated in the rebuttal.

We have modified the manuscript as follows:

1. We have added the Reviewer Figure to the paper (Supp. Fig. 12) and refer to it in the text (section *SAC ablation in the retina eliminates DS responses in the LGN*):
 "...while DSRGCs projecting to the LGN are reported to be On-Off RGCs that often exhibit balanced On and Off responses, we found that DS units of the dLGN tend to display stronger On- than Off responses, with some cells responding exclusively to On stimuli (Supp. Fig. 12)".
2. We have also added a discussion of the inheritance of polarity preference in dLGN DS neurons to the Discussion (section *Limitations of the study*):
 "Our study demonstrates that DS in the dLGN fully depends on retinal DS, but it remains unclear whether other properties, such as polarity preference, are inherited from DSRGCs. Several factors may underlie the observed differences in polarity preference (Supp. Fig. 12), including differences in On-Off stimulus parameters between MEA and in vivo recordings, such as light level, which was shown to influence RGC polarity^{77,78}. Light level-dependent surround effects may also differentially impact On and Off responses⁴⁸. Thus, while dLGN DS units likely inherit some of their polarity preference from DSRGCs, contributions from non-DS ON-RGCs or LGN interneurons cannot be excluded".

5. The authors state that they do not argue OS is inherited from the retina, but the current manuscript title "Topographic organization and retinal inheritance of direction and orientation selectivity in the visual thalamus" implies otherwise. Most readers will interpret this title as indicating that both DS and OS are inherited from the retina. To avoid misinterpretation, the title should be revised to better reflect the data and the authors' intended message.

We have changed the title to: "**Differential** Topographic organization and retinal inheritance of direction and orientation selectivity in the visual thalamus" to avoid confusion.

6. The authors acknowledge that the response quality index (RQI) cutoff they use is not very stringent and many of the units are not substantially distinguishable from random noise. The reviewer appreciates the analysis that the authors performed in Reviewer Figure 11. However, the figure only shows that units preferring different directions have similar average RQI values (Reviewer Figure 11a-c) and that the results from the DT experiments are “robust to stricter inclusion criteria,” as stated in the figure legend. The authors need to substantiate this claim further by re-generating other key results, such as the topographic maps in Figure 2i–m and the OS edge effects in Figure 5, using a more stringent RQI cutoff (e.g. 0.4). Although this will reduce the number of analyzable units, it is critical to assess whether the conclusions are robust with stricter inclusion criteria. RQI data should be included in the manuscript, not only referenced in the Methods section.

We have regenerated the key results (see Reviewer Fig. 4; new Supp. Fig. 4) and added the RQI data in the manuscript as the reviewer suggested.

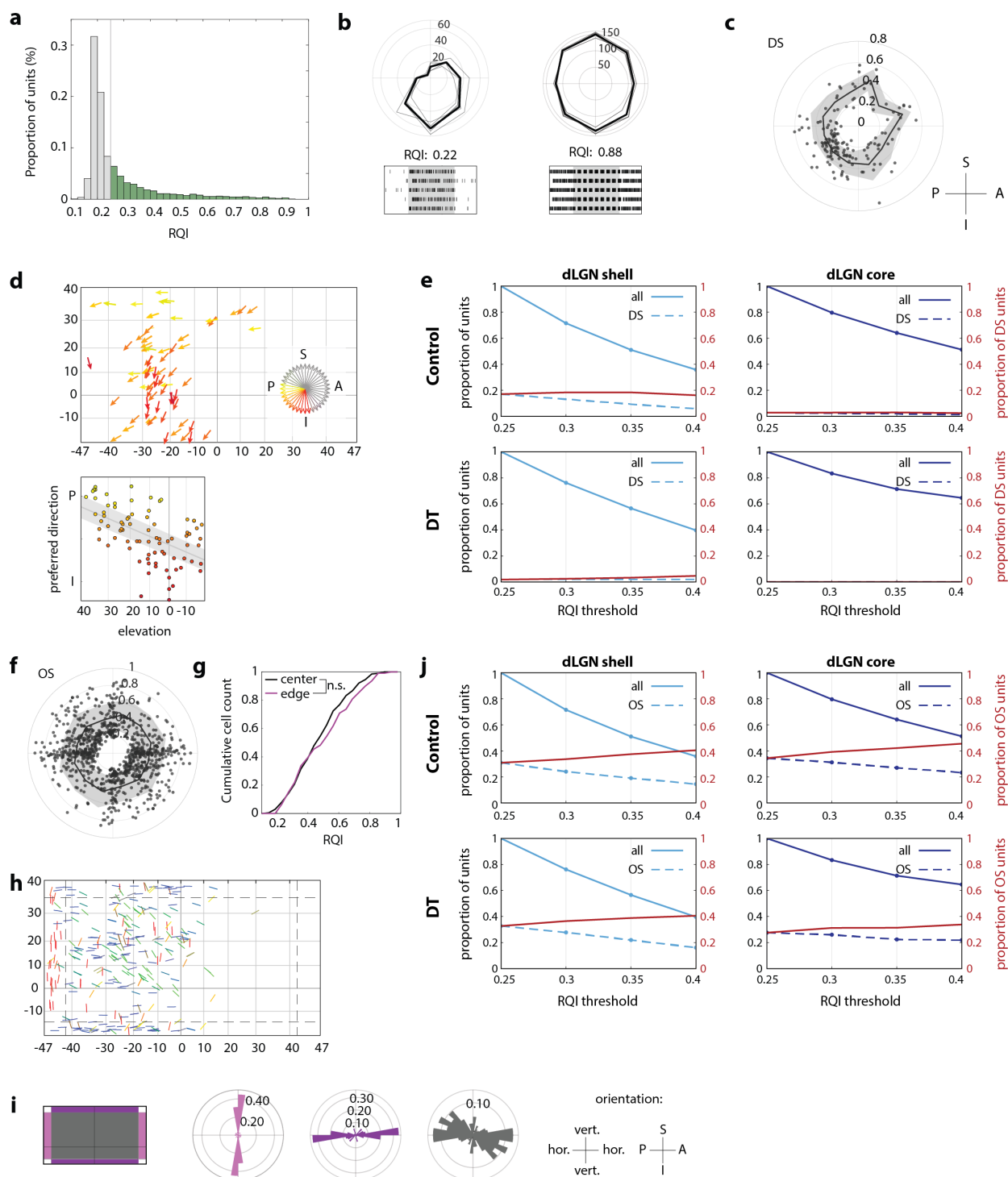
We would like to note that we use two criteria for responsiveness to gratings: (1) $RQI > 0.25$ or (2) $>2SD$ increase in firing rate. Each criterion is checked in the direction with the highest firing rate (please note that the original version did not include this last clarification. We apologize and updated the Methods accordingly (under *Analysis of Neuropixels recordings; Light-responsive units*)).

We illustrate the reason for choosing both these criteria via two example responses in Reviewer Fig. 4b, with (1) an example DS unit with a clear response to the gratings but a low RQI, and (2) a non-DS unit with almost no change in the number of spikes between baseline and grating response, but a clear time-locked response to the moving gratings (represented by a high RQI). The rationale for focusing on the direction with the highest firing rate is because DS and OS cells have reduced firing rate in non-preferred directions/orientations, and may be erroneously considered as unresponsive based on these. On the other hand, if a cell shows a high RQI or a significant increase even in one direction it is a responsive cell.

As the reviewer suggested, we now show:

1. Increasing the RQI threshold to 0.4 had little effect on the inclusion of DS units (excluding only 4 units from Fig. 2i (now shown in Reviewer Fig. 4d)).
2. Percentages of DS units among all responsive units remain relatively stable across RQI thresholds (Reviewer Fig. 4e; for this analysis we ignored the criterion of 2SD increase and units are included merely based on RQI values).
3. We additionally reproduced the finding of the edge-induced OS responses and found that these results are robust to increased RQI threshold, and that OS units from the edge and center of the screen do not significantly differ in their RQI (Reviewer Fig. 4f-i).
4. Finally, percentages of OS units among all responsive units remain relatively stable across RQI thresholds (here again, units are included merely based on RQI values).

We added Reviewer Figure 4 to the manuscript as Supp. Fig. 4 and now refer to it in throughout the text.



Reviewer Figure 4. Results are robust to stricter inclusion criteria. **a.** Distribution of response quality index of all LGN units with units of RQI > 0.25 shown in green. **b.** Two example units, with raster plot of example response to one motion direction (the direction with maximal firing rate). **c.** RQI as a function of preferred direction for all LGN DS units. Mean $\pm$ SD across 30° bins are shown as gray line and shaded area. **d.** Organization of preferred directions, including only DS units with RQI > 0.4. Annotations as in Fig. 2i. **e.** Proportion of units still included when using only RQI threshold criterion and increasing RQI threshold

(compared to 100% of units included at RQI threshold of 0.25; solid blue line, left y-axis), and proportion of DS units (dashed blue line, left y-axis). Resulting proportion of DS units is shown in red (right y-axis). Top row shows control data; bottom row DT data. **f.** Like c for OS units and their preferred orientations. **g.** Cumulative distribution of RQI of OS units of the center screen vs. OS units located along the edges. **h.** Organization of preferred orientations, including OS units with $RQI > 0.4$. Annotation as in Fig. 5b. **i.** Preferred orientations of OS units located along the vertical edge (pink), horizontal edge (purple) and in the center of the screen (gray) with $RQI > 0.4$. **j.** Like e for OS units.

Reviewer #4 (Remarks to the Author):

The authors have carefully addressed all my queries and revised the manuscript accordingly.

We thank the reviewer for the positive assessment and are pleased that our revisions have fully addressed the queries raised. We greatly appreciate the reviewer's thoughtful feedback, which helped improve the clarity and quality of the manuscript.

Reviewer #3 (Remarks to the Author):

In this second revision of the manuscript, the authors have adequately addressed comments #1-5. In particular, further data analysis of the ipsi vs. contra region reveal that the frequency of encountering DS cells in the ipsi and contra core dLGN regions do not differ and overall occur at about 20% of that found in shell. This reviewer appreciates the authors' efforts to enhance transparency regarding recording tract locations, as demonstrated in Supplemental Figures 3 and 7. Additionally, the documentation that units recorded in the ipsilateral patch can exhibit receptive fields outside the central visual field and display direction selectivity (Supplemental Figure 8) provides valuable information for readers about the limitations of the methods used.

With regards to the response to comment #6, the authors reveal for the first time that the criteria used to identify units was not limited to the response quality index (RQI) cutoff of > 0.25 . The authors now clarify that there were three possible ways that a unit could be included: 1) $RQI > 0.25$, OR 2) responses exceeding 2 standard deviations above baseline, OR 3) the presence of clear receptive fields. The RQI histogram in Supplemental Figure 4a suggests that with a cutoff of $RQI > 0.25$, units shown in green are included while those below the threshold are excluded. However, when the authors raise the cutoff of $RQI > 0.4$, only 4 direction-selective units are excluded. If this reviewer understands what the authors are saying, this would mean that the majority of the DS units were included based on criteria #2 and 3.

Although the examples in Supplemental Figure 4b appear to support these criteria, this reviewer finds it challenging to assess how many cells were included based on single versus multiple criteria. To enhance transparency, the authors should consider presenting a scatter plot displaying response z-scores relative to baseline versus RQI, with color-coding to indicate included units. This visualization would improve reader comprehension of the dataset composition given these unconventional inclusion criteria that do not incorporate frequency-modulation response analysis.

We appreciate the reviewer's evaluation of the additional analyses that we performed, and thank the reviewer for the constructive comments.

Regarding the criteria to include responsive units: It is true that many DS units are included based on criteria #2 and #3. To clarify the inclusion criteria, we now added additional panels to Supp. Fig. 4. First, as the reviewer suggested, we added a scatter plot of the z-scored response increase relative to baseline vs. RQI for all included cells (Supp. Fig. 4c). We show that most DS units have SD changes > 2 and would therefore not be affected by stricter RQI thresholds (also see example raster plot in Supp. Fig. 4b, left). Second, we also added pie charts showing what percentages of all units (DS or non-DS) were included based on the individual criteria or a combination of multiple (Supp. Fig. 4d). Out of all responsive cells, the majority of DS units are responsive to > 1 criterion (78%), and similar percentages are considered responsive because they either pass the SD or the RQI criteria ($\sim 10\%$).