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Anatomical Origins of Ocular Dominance in Mouse Primary Visual Cortex

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Abstract

Ocular dominance (OD) plasticity is a classic paradigm for studying the effect of experience and deprivation on cortical development, and is manifested as shifts in the relative strength of binocular inputs to primary visual cortex (V1). The mouse has become an increasingly popular model for mechanistic studies of OD plasticity and, consequently, it is important that we understand how binocularity is constructed in this species. One puzzling feature of the mouse visual system is the gross disparity between the physiological strength of each eye in V1 and their anatomical representation in the projection from retina to the dorsal lateral geniculate nucleus (dLGN). While the contralateral-to-ipsilateral (C/I) ratio of visually evoked responses in binocular V1 is ∼2:1, the ipsilateral retinal projection is weakly represented in terms of retinal ganglion cell (RGC) density where the C/I ratio is ∼9:1. The structural basis for this relative amplification of ipsilateral eye responses between retina and V1 is not known. Here we employed neuroanatomical tracing and morphometric techniques to quantify the relative magnitude of each eye’s input to and output from the binocular segment of dLGN. Our data are consistent with the previous suggestion that a point in space viewed by both eyes will activate 9X as many RGCs in the contralateral retina as in the ipsilateral retina. Nonetheless, the volume of the dLGN binocular segment occupied by contralateral retinogeniculate inputs is only 2.4X larger than the volume occupied by ipsilateral retinogeniculate inputs and recipient relay cells are evenly distributed among the input layers. The results from our morphometric analyses show that this reduction in input volume can be accounted for by a 3-to-1 convergence of contralateral eye RGC inputs to dLGN neurons. Together, our findings establish that the relative density of feed-forward dLGN inputs determines the C/I response ratio of mouse binocular V1.

Keywords

lateral geniculate nucleus; retinotopy; retinogeniculate; anatomy; ocular dominance plasticity; binocular

In mice and other mammals each eye transmits visual information along the retinogeniculate projection to relay cells located in eye-specific layers of the dorsal lateral geniculate nucleus.
(dLGN). This eye-specific information is in turn relayed to primary visual cortex (V1) with retinotopic precision via the geniculocortical pathway. In mammals with frontally positioned eyes, contralaterally and ipsilaterally projecting retinofugal pathways are roughly equal in magnitude (Fukuda and Stone, 1974, Stone and Fukuda, 1974) and, on average, cortical neurons in binocular visual cortex respond equally to stimulation of either eye (Wiesel and Hubel, 1963). However, in species such as mice that possess more laterally placed eyes, there is no obvious correlation between the anatomical and functional representation of the visual field from retina to V1 (Dräger, 1978, Dräger and Olsen, 1980). Less than 3% of the entire retinofugal pathway projects ipsilaterally (Dräger and Olsen, 1980), and the number of retinal ganglion cells (RGCs) viewing a point in binocular space is 9X greater for the contralateral retina than for the ipsilateral retina (Dräger and Olsen, 1980). Nonetheless, the relative drive on binocular neurons by the contralateral eye is, on average, only 2–3X greater than that for the ipsilateral eye (Dräger, 1978, Gordon and Stryker, 1996, Porciatti et al., 1999, Sawtell et al., 2003, Frenkel and Bear, 2004). Thus, it remains unknown whether ocular dominance (OD) in mouse V1 simply reflects the influence of feed-forward geniculocortical input or is constructed within the cortex. Knowledge of the anatomical basis of cortical OD is essential for the interpretation of OD plasticity following manipulations of visual experience in mice.

The aim of the current study was to understand the structure of geniculate inputs to binocular V1 in the mouse. Specifically, we wanted to determine whether it is reasonable to assume that the density of feed-forward geniculocortical inputs forms the basis for OD as measured physiologically in cortex. Because the mouse dLGN lacks cytoarchitectonic features that readily distinguish its binocular segment, we first employed neuroanatomical tracing and morphometric techniques to delineate this portion of dLGN. Using this information, we then determined how each eye is represented in terms of RGC inputs to, and geniculate relay cell outputs from, binocular dLGN. We demonstrate that the relative volume occupied by eye-specific retinal afferents in binocular dLGN is in accord with physiological measurements of OD in V1. In addition, results from morphometric analyses suggest that the relative magnitude of the contralateral retinogeniculate projection is reduced between retina and cortex by a convergence of its inputs at the level of dLGN. Our findings suggest that the OD of cortical neurons in mouse V1 results from feed-forward geniculocortical input in a manner similar to mammals with frontally placed eyes.

EXPERIMENTAL PROCEDURES

Animals

Adult wild-type C57/BL6J mice (postnatal day 60–90) were obtained from Jackson Laboratories (Bar Harbor, ME) and housed at MIT on a 12 h light, 12h dark cycle. All animals were handled according to the rules and guidelines set forth by the MIT Animal Care Committee.

Tracer Injections

Ocular—For ocular injections, mice were anesthetized by inhalation of isoflurane (1%–2%) and placed under a surgical microscope. The temporal portion of the conjuctiva was exposed. To access the vitreal chamber, the temporal portion of the globe was reflected anteriorly by gently pulling a 7-0 silk suture that was threaded through the conjuctiva and secured to the operating surface. The conjuctiva was then trimmed using fine angled scissors to expose the sclera. A small puncture was made into the vitreous chamber with a fine needle posterior to the corneoscleral junction. A glass micropipette with a tip diameter of ~30 µm was attached to a manually driven microinjection apparatus (MMP, World Precision Instruments, Sarasota, FL) and inserted into the vitreous chamber at a depth of approximately 2 mm. Both eyes were injected with approximately 1.0 µl of cholera toxin-B (CTB) subunit conjugated to Alexa-488,
Alexa-555 or Alexa-647 (Invitrogen, Carlsbad, CA) (0.5% in 0.01 M phosphate buffered saline and 2.0% DMSO). Different Alexa dyes were injected into each eye so each eye’s RGC axons could be delineated in one hemisphere. Following injection, the eye was rinsed with sterile eye drops and coated with an antibiotic ointment to prevent infection.

Visual cortex and dLGN—Mice were anesthetized with a mixture of ketamine and xylazine and prepared for stereotaxic injections as previously described (Frenkel et al., 2006). The following stereotaxic coordinates were used for each region: V1 monocular segment – 1.0 mm anterior to lambda and 2.5 mm lateral from the midline, 0.45 mm down from the dural surface; V1 binocular segment – 3.2 mm lateral from lambda; dLGN – 2.10 mm posterior to bregma and 2.19 mm lateral from the midline, 2.55 mm down from the dural surface. A small burr hole (~1.0 mm in diameter) was made at each coordinate and a small bone flap was removed to expose the underlying dura and cortex. Dyes (prepared as described above) were injected using a Nanoject II injection system (Drummond Scientific, Broomall, PA). Tip diameter for injection pipettes was approximately 20 µm and 5 × 4.6 nl or 10 × 13.2 nl (pausing 10–15 sec between injections) was injected into cortex and dLGN, respectively. The pipette was left in place for 2–5 min. Upon removal of the pipette, the exposed skull was covered with dental cement and the animals were allowed to recover for 2–3 days prior to perfusion.

Tissue preparation and immunohistochemistry

Animals were euthanized by an overdose of pentobarbital and transcardially perfused with ~50 ml of 4% paraformaldehyde in 0.1 M phosphate buffer. Eyes and brains were removed and postfixed for 1 day at room temperature or 2–5 days at 4°C. For tracer-labeled brain tissue, 100 µm-thick sections were cut in the coronal plane using a vibratome. Serial sections were mounted onto glass slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA), allowed to adhere and then counterstained with NeuroTrace-640 fluorescent Nissl stain (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. The addition of DMSO to the CTB solutions facilitated the spread of label in retina and brain tissue. Retinal flat-mounts were prepared and examined to ensure thorough labeling throughout the retinas. Samples were included for analysis if CTB tracer was present though the entire extent of medial dLGN or if the overlying optic tract was thoroughly labeled. All sections and flat-mounts were covered with an aqueous-based mounting medium (Biomeda GelMount, Foster City, CA) and coverslipped, allowed to cure at room temperature for 2–18 h and then sealed with clear nail polish to prevent drying.

Conofocal imaging

All images (1024 × 1024 pixels unless otherwise noted) were acquired with an Olympus (Melville, NY) FluoView 300 laser-scanning confocal microscope and FluoView 500 acquisition software using appropriate filter sets and excitation lasers. An Olympus 4x UPLanFl objective (NA = 0.13) was used to acquire low-magnification images of visual cortex (as in Figure 1A), with the confocal aperture (CA) set to 3 to maximize sensitivity. When acquiring images for morphometric analyses, we used Olympus 10x UPLanFl (NA = 0.30) and 20x UPLanFl (NA = 0.50) air objectives for the dLGN and retina, respectively. For these images, the CA was set to reduce noise and minimize collection of signal outside the excitation plane (CA = 1 for 10x; CA = 2 for 20x). For retinal regions, the image dimension was clipped to 512 × 512 pixels (0.123 mm²). In order to obtain adequate signal for delineating dLGN regions containing contralateral- or ipsilateral-eye afferents, 4 to 5 optical sections (1.0 µm step size) were acquired by scanning ± 2–2.5 µm from the brightest focal plane. For the Nissl-stain channel, the acquisition settings were tuned as described above and one optical section (approximately 3 µm thick in the Z-dimension) was acquired for each physical section so that each image was separated by approximately 100 µm (physical section thickness; pixel scale = 1.57 × 1.57 µm). Some images were pseudo-colored for presentation using the acquisition

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software. Contrast and brightness were adjusted using Adobe Photoshop software (Adobe Systems, Inc., San Jose, CA) to reduce background noise and improve the visibility of images for presentation purposes.

**Morphometric analyses**

**Retina**—Areas for regions of binocular correspondence were defined as previously described (Dräger and Olsen, 1980). The vertical meridian (which corresponds to the line of decussation, see Figure 1A) was easily identifiable because the ventrotemporal crescent (VTC) (i.e. the ipsilaterally projecting domain) was labeled in all retinas. Images (0.4 × 0.4 mm) of each RGC population were acquired about the vertical meridian as indicated in Figure 1. Maximum-intensity Z-projections (5–6 µm) were produced from the images acquired at each retinal region and imported into Adobe Photoshop. A new layer was made for each image and RGCs were manually identified and marked with a dot. The new layer was then exported to ImageJ (NIH Image) for analysis. The “analyze particles” function was used to count marked cells. When warranted, the effective area for each image was taken into account by subtracting damaged areas where cells could not be accurately counted (<0.5% of the total area analyzed). RGC density for each region was expressed as the number of RGCs per mm$^2$. Retinas were also imaged under low-magnification to obtain area measurements for calculating total RGC numbers. The vertical meridian was drawn along the border of the VTC (Dräger and Olsen, 1980). There are no anatomical landmarks demarcating the dorsonasal border of the contralaterally projecting binocular region of retina. Therefore, this border had to be estimated by drawing a line that connected from each tip of the VTC and that was positioned just ventral and temporal to the optic disk. As expected, areal measurements for contra- and ipsilaterally projecting regions were similar since each region should have equal spatial coverage of one binocular hemifield (see Results for details). In order to control for slight variations in RGC density throughout each binocularity corresponding region, we divided each retina into 4 areas (about the 4 sample regions) by drawing lines that extended radially from the optic disk (retinal center) to the periphery (Fig. 1C). To obtain visuotopically-localized estimates of cell number, the area for each division was multiplied by its corresponding RGC density. These numbers were then summed and averaged to obtain a total number for each binocularly corresponding region. As noted in the Results section, our calculations of VTC area and RGC number in the VTC were well matched with those previously reported in the literature (Dräger and Olsen, 1980, Rice et al., 1995).

**dLGN**—Analyses of the dLGN were performed using MetaMorph (version 6.1, Universal Imaging Corporation, Downingtown, PA) unless noted otherwise. The extreme rostral pole of dLGN and the last section beyond that containing terminating axons from the ipsilateral eye were included for analysis (resulting in 6 to 7 100 µm-thick sections for each hemisphere). Single-channel, maximum-intensity Z-projections were used for tracing the perimeters of RGC termination zones. The lateral edge of the binocular segment was demarcated by drawing a line along the lateral-most edge of ipsilateral laminae and parallel to the trajectory of anatomical projection lines as viewed through a series of coronal sections (see Results section for details on determining this trajectory). The line drawn along the edge of the binocular segment was then continued to encircle the rest of the labeled contralateral area medial to the border. A second bounding region was drawn around the ipsilateral “core” in the channel containing ipsilateral-eye label. These two regions were then transferred to the channel containing the Nissl-stained cells using the “transfer regions” functions in MetaMorph and the cells were counted as follows. Each image containing one optical section was smoothed using a nearest-neighbors deconvolution filter and a threshold was applied to ensure that Nissl-stained cell bodies with visible nuclei were included for counting. In most cases, the same threshold could be applied to an entire set of images from one hemisphere. Using the MetaMorph cell-counting module, the average cell size (standard area) was determined by sampling at least 10 cells from
each section that were representative of single-cell sizes. Cell standard areas varied slightly from image to image, ranging from 41 to 63 pixels. This setting allows the program to estimate cell number by pixel area so that if two or more pixel clusters (cell bodies) are connected (preventing them from being recognized as separate objects), each standard area (cell body) within the joined clusters will be recognized as an individual object to be counted. In addition, this method was specific for counting Nissl-stained neuronal cell bodies, which were readily distinguishable from glial cells using established criteria (see Williams and Rakic, 1988 for example). Counted cells had a large nucleus marked by smooth nucleoplasm and averaged 15 µm in diameter whereas glial-like cells or profiles that averaged <8 µm in diameter were excluded.

Total volumetric cell numbers for each region were estimated using stereological methods. Because we were able to efficiently count all cells within a section and the depth of Nissl staining was limited (thereby limiting our ability to obtain high-resolution, serial optical sections), we used the size-frequency method to calculate volumetric densities from each optical section \( N_V = N_A/d \), where \( N_V \) is the volumetric density, \( N_A \) is the number of objects per sampled area and \( d \) is the mean length of the test object). This method has been shown to be a robust alternative to dissector-based methods when counting disc-shaped objects in a 2-dimensional plane (Colonnier and Beaulieu, 1985, DeFelipe et al., 1999, Benes and Lange, 2001). The value for \( d \) was determined by computing the average caliper diameter (test object) for a subset of thresholded Nissl-stained cells in an area that measured 0.026 mm\(^2\) from each section using the integrated morphometry function in MetaMorph. The subset included cells that could be counted as individual objects with standard pixel areas of 41–63. The caliper diameter calculated from all sections averaged 15.0 ±0.18 µm (\( n = 173 \) cells), which is approximately 5X greater than the estimated thickness of each optical section (<3.0 µm). Areal measurements \( A \) for each region were obtained using MetaMorph for all sections of dLGN that contained ipsilateral-eye label. Reference volumes \( V_{\text{REF}} \) for each lamina of the binocular segment were calculated using \( A \) from each section and section thickness (\( t; 100 \) µm) and the following formula: \( V_{\text{REF}} = \frac{1}{2} A t \). Total cell number \( N \) within a given region was then calculated using the following formula: \( N = (N_V V_{\text{REF}}) + (N_V V_{\text{REF}})^2 + \ldots (N_V V_{\text{REF}})^n \). Note that the volume measurements presented here should be taken as underestimates of real brain volume as there was likely undetermined amounts of tissue shrinkage that occurred during tissue processing. However, this would not impact laminar comparisons of volume and cell number.

**Methodological considerations**—It is important to note that the introduction of small, systematic errors in measurements or cell number estimates would not be expected to significantly impact our conclusions. For example, we found that small variations in the angle of the border used to demarcate monocular and binocular dLGN did not significantly impact cell number and volume estimations, which varied by <5% when using angles that ranged 20 to 30 degrees from the vertical midline (see Results section for details). Furthermore, as reported in the Results section, our measurements and cell number estimates are consistent with those reported in previous studies and suggest that the rigor of our analyses is more than adequate to support our conclusions (Seecharan et al., 2003).

**RESULTS**

**Distribution of RGCs contributing to binocular vision**

RGCs in each retina were retrogradely labeled by injecting CTB-488 and CTB-555 directly into the left and right dLGN, respectively (see example injection in Figure 1B). Ipsilateral to the injections, the majority (>90%) of labeled RGCs were restricted to the VTC as previously described (Dräger and Olsen, 1980). Contralateral to the injections, we observed large numbers
of labeled cells throughout the retina. Our estimates of RGC numbers for both populations were nearly identical to previous reports, suggesting that we were able to achieve thorough labeling in binocular retina (Dräger and Olsen, 1980). We observed that contralaterally projecting RGCs were intermingled with ipsilaterally projecting RGCs in the VTC as has been noted previously in the rat; these cells do not play a role in binocular vision (Reese, 1986, 1988).

Figure 1C shows an example of retinas ipsi- and contralateral to one injected dLGN that were used for analysis. The VTC and contralaterally projecting binocularly corresponding regions measured $3.06 \pm 0.25 \text{ mm}^2$ and $4.05 \pm 0.18 \text{ mm}^2$, respectively (mean ± SEM; $n = 4$ retinas from 3 animals). The remaining area corresponding to the monocular segment measured $10.28 \pm 0.95 \text{ mm}^2$. In agreement with previous studies, our estimates show that total retinal area is $17.38 \pm 1.30 \text{ mm}^2$ and that the VTC represents 18% of total retinal area (Dräger and Olsen, 1980, Rice et al., 1995). These observations, taken together, confirmed that we were able to adequately label each population of RGCs via retrograde tracing with CTB.

Next, we determined RGC density and C/I density ratios at 4 visuotopically corresponding regions that lie about the vertical meridian (Fig. 1C). The results are summarized in Figure 2. There was no significant variation of RGC density within contralaterally or ipsilaterally projecting regions ($p > 0.05$, one-way ANOVA; $n = 4$ retinas). The range of average densities calculated for the contralaterally projecting region was 2620 ± 252 to 3201 ± 214 cells/mm$^2$, similar to previous estimates (Dräger and Olsen, 1980). In contrast, our estimates of RGC density within ipsilaterally projecting regions were slightly higher than those reported by Dräger and Olsen (Dräger and Olsen, 1980) (443 ± 56 to 555 ± 40 cells/mm$^2$, $n = 4$ retinas), a discrepancy that we attribute to differences in methodology. Due to these higher densities, the ratios of contra-to-ipsilaterally projecting RGC densities were slightly lower than previously reported (6–8:1 versus 9–10:1) (Dräger and Olsen, 1980). There was no statistically significant variation in RGC density between visuotopic locations. Together, our results confirm that, regardless of visuotopic representation, there is an inconsistency between anatomy and function with respect to the retinal projections to dLGN and the relative eye-specific responses evoked in binocular V1.

Relative magnitudes of inputs and outputs in the binocular segment of dLGN

Anatomical definition of binocular dLGN in mouse—Next, the C/I ratio of the geniculocortical projection that originates from the binocular segment of dLGN was determined. The rodent dLGN lacks overt cytoarchitectonic features that allow for easy identification of its binocular segment. Therefore, we first had to establish a method for demarcating the monocular and binocular portions of mouse dLGN and for determining the trajectory of projection lines. The trajectory of projection lines can be used to plot the location of cells representing conjugate points in binocular visual space (and with similar receptive fields) through the rostrocaudal extent of dLGN and can therefore be used to define the shape of its binocular segment (Bishop et al., 1962). In the ferret for example, projection lines run perpendicular to contralateral and ipsilateral laminae within dLGN, making it rather straightforward to discern the binocular-monocular border in coronal sections with a simple histological counterstain (Zahs and Stryker, 1985). In contrast, the trajectory of anatomical and physiological projection lines in rodent dLGN course at some unknown, oblique angle relative to the ipsilateral-eye termination zone (Reese and Cowey, 1983, Reese, 1988).

The first labeling experiment was performed to visualize the mediolateral positioning of binocular and monocular dLGN in the mouse. Geniculate relay cells projecting to binocular and monocular V1 and ipsilaterally projecting RGC termination zones were visualized in the same tissue by direct injection of differently colored tracers into V1 and one eye, respectively (Fig. 3A). As shown in Figure 3A, geniculate relay cells serving the binocular hemifield are
positioned more medially than those serving the monocular hemifield and overlap with both contralateral and ipsilateral retinal input.

A second set of labeling experiments was performed to visualize the retinotopic organization of relay cells in mouse dLGN so that the outer border of the dLGN binocular segment could be properly oriented. Figure 3B shows that retrogradely labeled clusters of relay cells serving the binocular visual field lie along “bundles” of projection lines, the center of which is estimated by the color-coded dot shown in each section. The angle of the trajectory of projection lines relative to the vertical midline of each section was then determined as shown in Figure 3B–D. The alignment of serial sections with labeled projection lines revealed that the trajectory of projection lines courses ventromedially and dorsolaterally along the rostrocaudal axis of dLGN in a manner similar to rat (Reese and Cowey, 1983). By aligning coronal sections containing the extreme poles of labeled projection lines, we determined that the angle of the trajectory of these lines as measured from the vertical midline in a single coronal section was approximately 22 degrees (Fig. 3D). Thus, a line drawn at this angle and along the lateral-most edge of the ipsilateral RGC termination zone defines the shape of binocular dLGN in coronal sections.

Volume of retinal termination zones and cell counts in binocular dLGN—Having anatomically defined monocular and binocular dLGN, we next wanted to know the volume of retinal synaptic territory occupied by each pathway in the binocular segment and the cell-packing density in eye-specific layers within each section. Eye-specific terminal fields within dLGN were labeled by injecting the left and right eyes of each animal with CTB-488 (green channel) and CTB-555 (red channel), respectively (Fig. 4A). CTB-labeled sections were counterstained with fluorescent Nissl stain, so that all three labels could be visualized in serial sections through the entire dLGN as shown in Figure 4A–D. Next, the binocular segment of dLGN was demarcated as indicated in Figure 3 and the ipsilateral termination area was also outlined (Fig. 4C). To verify the fidelity of the settings for automated counting of Nissl-stained cells within these termination zones (Fig. 4D–E), we performed manual and computer-assisted counts in several regions (0.025 mm$^2$) from different sections. The regions were sampled from at least one section obtained from each hemisphere used in the analysis. As shown in Figure 4F, manual and computer-assisted counts were well correlated ($r^2 = 0.87$), thereby confirming that the cell-counting protocol used in this study was capable of faithfully reporting cell number using the parameters described in the Methods section.

Figure 5A shows the shape and position of binocular dLGN in a series of coronal sections from one animal. The volume occupied by each eye’s retinal termination zone in binocular dLGN is summarized in Figure 5B. We found that the ipsilateral terminal field occupies 9–12% of the entire dLGN volume, which is consistent with previous estimates (Dräger and Olsen, 1980). Interestingly, using previously determined estimates of total dLGN volume in the mouse (0.2–0.3 mm$^3$) (Rice et al., 1995), the total volume of the binocular segment (summed laminar volumes) represents approximately one-third of the entire dLGN volume, which closely matches the cortical territory occupied by binocular V1 in occipital cortex (Dräger, 1975, Gordon and Stryker, 1996). Surprisingly however, despite the massive decussation that has been reported for retinal projections in mouse, our calculations showed that contralateral-eye input only occupies 2.4 times more volume than ipsilateral-eye input in the binocular segment ($n = 4$, contralateral laminae = .060 ± 0.002 mm$^3$ and ipsilateral lamina = .026 ± 0.003 mm$^3$). This, if one assumes that there is an equal distribution of synaptic input within each eye-specific layer then one might expect to see a higher density of target cells in contralateral laminae to accommodate its larger retinal input. To explore this possibility, we calculated the areal density of Nissl-stained cells in each lamina in serial sections that contained ipsilateral-eye label. As summarized in Figure 5C, areal cell densities in contralateral and ipsilateral laminae were nearly identical in each section and there was no significant variation between

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layers along the rostrocaudal axis ($P > 0.4$, one-way ANOVA; $n = 4$; contralateral laminae = 1446 ± 64 cells/µm$^2$; ipsilateral lamina = 1550 ± 66 cells/µm$^2$). Together, these data show that the relative magnitude (and influence) of each eye’s retinal input is altered at the level of dLGN and suggest that these inputs are somehow transformed as their information is relayed to binocular V1 via the geniculocortical projection.

**Numerical relationship between RGCs and geniculate relay cells**

Since there is only a 2.4-fold difference in the volume of each eye’s RGC input in dLGN, either ipsilaterally projecting RGCs diverge to amplify their input or contralaterally projecting RGCs converge to condense their input. As a first step towards addressing this issue, we investigated whether the degree of divergence or convergence based on numerical matching of inputs (RGC numbers) with outputs (geniculate relay cell numbers) differs between each pathway. This type of analysis has previously been used to show the degree of divergence and/or convergence in the visual pathways of other mammals (see (Alonso et al., 2006) and references therein).

First, we estimated the total number of Nissl-stained cells in each laminar volume of dLGN. These calculations revealed that contralateral and ipsilateral laminae contained 5794 ± 393 and 2594 ± 127 Nissl-stained cells, respectively. If we account for the fact that approximately 20% of these cells are interneurons (Arcelli et al., 1997), which are evenly distributed throughout the rodent dLGN (Gabbott et al., 1985 and unpublished observation), the estimated numbers of relay cells located in contralateral and ipsilateral laminae were 4635 ± 315 and 2075 ± 102, respectively. These data are summarized in Figure 6A. Based on these estimates, the ratio of the number of cells located in contralateral and ipsilateral terminal fields is 2.2, which is consistent with the average C/I volume ratio of 2.4 (Fig. 5B).

Second, we determined the number of RGCs serving each corresponding binocular visual hemifield. Binocularly corresponding retinal areas were subdivided into 4 regions by drawing lines that extended radially from the optic disk to the periphery (Fig. 1C). Total RGC numbers were calculated by multiplying local retinal areas by their corresponding cell densities and summing the resultant products. These calculations showed that 12,375 ± 745 RGCs project contralaterally and 1501 ± 140 RGCs project ipsilaterally to the binocular segment of the dLGN ($n = 4$ retinas) (Fig. 6B). Previous reports of contralaterally projecting RGC numbers always include those cells located in the VTC and in the monocular segment of retina, so a direct comparison with our data is not possible. However, our estimates of ipsilaterally projecting RGCs are in good agreement with previous estimates (Dräger and Olsen, 1980, Rice et al., 1995).

Finally, the potential for divergence or convergence in the retinogeniculate pathway was determined by examining the numerical relationship between RGCs and relay cells in the binocular segment. The data showed that there is an approximate 1-to-1 correspondence along the ipsilateral pathway and a 3-to-1 correspondence along the contralateral pathway. A straightforward interpretation of these results suggests that 1 ipsilaterally projecting RGC serves 1 corresponding relay cell whereas 3 contralaterally projecting RGCs converge onto 1 relay cell. Together, these data show that, based on synaptic resources in dLGN, convergence (or relative condensation) of contralateral retinal inputs is more likely to occur than divergence (or relative amplification) of ipsilateral retinal inputs.

**DISCUSSION**

In this study, we established a link between structure and function in the mouse binocular visual pathway by determining the relative proportion of each eye’s geniculocortical input to binocular V1. Our results show that C/I ratio of geniculate projections is 2.2, which directly corresponds to the relative amplitudes of visually evoked responses and eye dominance in V1.
Dräger and Olsen, 1980, Porciatti et al., 1999, Frenkel and Bear, 2004). While the C/I ratio of inputs to binocular dLGN averaged 6:1, there is a relative condensation of contralateral RGC input that effectively reduces the C/I ratio of its output from dLGN to ~2:1. These findings are summarized in the schematic shown in Figure 6C. As discussed below, these observations are consistent with the suggestion that OD in mouse V1 is primarily influenced by excitatory geniculocortical input from each eye. In addition, these results reinforce the point that despite the lack of any overt laminar organization characteristic of primates and carnivores, the mouse dLGN possesses a highly ordered laminar arrangement of topographically aligned binocular inputs.

Contralateral bias in the retinogeniculate projection

The results from our first set of experiments helped to rule out the possibility that the C/I density ratio for RGCs serving binocular vision in the mouse retina was overestimated in earlier studies (Fig. 1). In agreement with previous work (Dräger and Olsen, 1980), we found that the C/I ratio of RGC densities (~6:1) was too great to account for the C/I ratio of visually evoked responses in binocular V1 (~2:1) if they are primarily driven by geniculocortical input. Another possible explanation for the reduced magnitude of contralateral-eye response bias in cortex lies in the nature of the retinofugal projection. While 95–97% of RGCs project contralaterally, approximately half of these do not project to dLGN at all whereas nearly all ipsilaterally projecting RGCs terminate in dLGN (LaVail et al., 1978, Dräger and Olsen, 1980, Pak et al., 2004). Thus, bulk labeling of the optic tract or dLGN (where axons of passage can be labeled) could lead to an overestimation of the number of RGCs that actually target dLGN and the C/I ratio of their projections. However, RGC densities in the binocular portion of retina are similar whether determined after restricted dLGN injections (in which passing axons are avoided) or bulk labeling of the optic tract (Martin, 1986). In contrast, RGC densities in monocular retina are much lower when determined by restricted injections. Additionally, contralaterally projecting RGC density varies across mouse retina with eccentricity such that the areas of highest densities are located in the monocular portion and close to the optic disk, an increase that could reflect an additional RGC population that bypasses dLGN (Dräger and Olsen, 1980, 1981). Thus, it seems likely that C/I ratio of RGC density in binocular retina is indeed reflected by the retinogeniculate projection.

Lamination and retinotopy in mouse dLGN

One interesting observation from our RGC labeling experiments is that upon demarcating the monocular and binocular segments, a laminar structure emerges in the medial portion receiving binocular input. The lamination follows a contra-ipsi-contralateral pattern, reminiscent of the laminar pattern in other species with cytoarchitectonic lamination (Sherman and Guillery, 2006). While there is a disruption of this lamination by a narrow portion of the contralateral shell alongside the medial border of dLGN, much of this region receives a condensed projection from contralaterally projecting RGCs located in the VTC of rat retina and is not likely to play a role in binocular vision (Reese, 1988). Additionally, Figure 3 demonstrates that relay neurons within the mouse dLGN exhibit an arrangement nearly identical to the columnar projection lines found in rat, which course obliquely relative to the ipsilateral RGC terminal field (Reese and Cowey, 1983, Reese and Jeffery, 1983). Thus, the binocular segment of mouse dLGN is shaped by the visuotopic representation of these projection lines and by the presence of the ipsilateral eye’s projection field.

Functional implications

An important finding in this study is that the relative magnitude of eye-specific visual output from dLGN directly correlates with response magnitude in binocular V1. In previous studies, it had been posited that the contralateral response bias may be influenced by callosal projections.
in mouse (Dräger, 1975, 1978) or that OD shifts might be directly influenced by intracortical circuitry serving the monocular visual field (Gordon and Stryker, 1996). However, our findings clearly demonstrate that the C/I ratio of visual responses as measured using VEP (Porciatti et al., 1999, Sawtell et al., 2003) or single-unit recordings (Dräger, 1978) in V1 can be solely accounted for by direct relay of visual information along the retinogeniculocortical axis. Thus, these observations suggest that methods such as VEPs can be used to reliably report the relative activity of eye-specific geniculocortical synapses.

A simplified wiring diagram based on our current findings is shown in Figure 6C. Contrary to the suggestion that ipsilateral-eye responses are somehow amplified in dLGN relative to the contralateral-eye (see for example Dräger and Olsen, 1980 and Porciatti et al., 1999), the results from our final set of experiments suggest that contralateral retinal input to dLGN is actually condensed by an average 3-to-1 convergence of these inputs onto single geniculate relay cells. Evidence obtained from electrophysiological studies in rodent dLGN support this arrangement, having shown that retinogeniculate inputs exhibit varying degrees of convergence. During development, initially exuberant retinal axons are pruned to provide and average of 1 to 3 inputs to each geniculate relay cell in mice (Chen and Regehr, 2000, Jaubert-Miazza et al., 2005). Studies of retinogeniculate connections in slices of immature rat dLGN, which exhibit similar arrangements through development, have shown that relay cells receive an average of 3 contralateral inputs and 1 ipsilateral input (Ziburkus and Guido, 2006). Thus, our data provide an anatomical correlate of this relationship in mouse and show that the contralateral pathway is more tuned to promote convergence than the ipsilateral pathway. How or whether the degree of convergence varies among contralateral RGCs remains to be determined. It is possible that the degree of convergence may vary with eccentricity as in other species or with geniculate input layer (Yeh et al., 2009). Future experiments using physiological measures combined with restricted labeling of RGCs are needed to address these issues.

In summary, we demonstrate a clear anatomical correlation between the relative density of feed-forward inputs from binocular dLGN to V1 and the C/I ratio of VEPs and OD distribution of neurons in V1. The alternate wiring pattern between contra- and ipsilateral pathways from retina to dLGN implies that each pathway processes information from the same binocular hemifield differently. Similar differences between populations of RGCs exist in other species and although they are not between eye-specific pathways, they may be indicative of evolutionary vestiges or transitions (Arnason and Janke, 2002, Murphy et al., 2004). For example, a recent study in cat by Yeh et al. (2009) suggests that different degrees of convergence among Y projections to specific layers of dLGN are likely to result in the different spatiotemporal structures of geniculate cell receptive fields (Yeh et al., 2009). Grubb and Thompson (Grubb and Thompson, 2003) reported that the response properties of mouse dLGN neurons are rather homogeneous and exhibit X-like linear response properties, but they could not completely rule out the possibility that neurons with other distinct properties exist and did not take eye-specificity into account. Further physiological and morphological comparisons between eye-specific pathways are likely to provide key insights into visual processing in the mouse and will allow for the development of more architecturally detailed theoretical models of mouse cortical plasticity.

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Abbreviations

OD, ocular dominance; V1, primary visual cortex; dLGN, dorsal lateral geniculate nucleus; C/I, contralateral-to-ipsilateral; RGC, retinal ganglion cell; CTB, cholera toxin-B; CA, confocal aperture; VTC, ventrotemporal crescent.

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FIGURE 1. Distribution of RGCs in binocularly corresponding regions of retina

(A and B) (A) Schematic of the mouse visual field and its representation in retina (adapted from Dräger and Olsen, 1980). The upper panel shows the projection of the mouse visual field onto a flattened sphere. The mouse is to be imagined behind the page facing the intersection of the horizontal and vertical meridians. The binocular visual field is highlighted with two shades of green. The darker shade is the region viewed by the binocularly corresponding retinal regions highlighted by the darker hues in the lower panel. The numbers along the vertical meridian correspond to those in binocular retinal regions and show the location of the regions used for sampling RGC density (see panel C). Yellow and blue indicate the location of ipsilaterally and contralaterally projecting RGCs, respectively. The solid line located between...
the colored areas indicates the vertical meridian. The dashed line indicates the approximate location of the horizontal meridian. Ipsilaterally projecting RGCs that are located in the ventrottemporal crescent view the contralateral binocular visual hemifield. In the other retina, contralaterally projecting RGCs that view the same visual hemifield are located in another crescent that is shifted dorsonasally towards the optic disk (black circle). Note that this seemingly peculiar mapping is a result of the protruding, laterally placed eyes of the mouse. (B) Example confocal image showing the central location of the injection site in the left dLGN from animal CTB2 (green = CTB-488, blue = Nissl stain). (C) Outlines of left and right retinas from animal CTB2, which were ipsilateral and contralateral, respectively, to the injection of CTB-488. The gray areas indicate the binocularly corresponding regions of retina (boxes) that were used for analysis. Confocal images of retrogradely labeled RGC cell bodies in retinal regions used for analysis are shown adjacent to the region indicated in the retina. The numbers correspond to the retinal positions shown in A. 
D = dorsal; N = nasal; V = ventral; Scale bars: B = 200 µm; C = 1.0 mm (black) and 0.2 mm (white)
FIGURE 2. RGC density in visuotopically corresponding regions of ipsi- and contralateral retinas

**Left panel** - Summary of RGC density as a function of retinal location. There was no significant variation in RGC density across locations within ipsilateral or contralateral regions ($n = 4$ retinas from 2 animals; $P > 0.4$, one-way ANOVA).

**Right panel** - Summary of RGC density ratios plotted as a function of retinal location. The dashed line indicates the average density ratio of all regions.
FIGURE 3. Anatomical demarcation of mouse binocular dLGN
The panels contain example images of retrograde and anterograde tracing experiments with CTB that were used to anatomically define the location (A) and shape (B) of binocular dLGN in the mouse. (A) The mediolateral positioning of monocular and binocular dLGN was verified by retrograde labeling of geniculate relay cells that project to monocular (green, monoc) and binocular (red, binoc) V1. Two serial sections separated by 100 µm are shown for each brain region. The V1 panel shows DIC images merged with fluorescent CTB labels. The dLGN panel shows the termination zone of ipsilaterally projecting RGC axons, which were anterogradely labeled by ocular injection of CTB-647 (blue). Note that the relay cells projecting to binocular V1 (red) are located in more medial regions of dLGN that receive input from the ipsilateral (blue, ipsi) and contralateral (unlabeled region) retinas, whereas relay cells projecting to monocular V1 are located more laterally and never overlap with the ipsi termination zone. The arrowheads indicate the rough location of the border between monocular and binocular dLGN. (B) Confocal images of three serial sections through a portion of dLGN from another animal that received an injection of CTB-555 into binocular V1. Retinotopically aligned clusters of retrogradely labeled relay cells (red) are visible along “bundles” of projection lines, which course obliquely relative to the ipsi RGC terminal field in a ventromedial to dorsolateral direction along the rostrocaudal axis of dLGN. Each dLGN section and the estimated center of each labeled cell cluster are color-coded for presentation in panels C and D. (C) Three-dimensional illustration of the trajectory of a single projection line (dashed arrow) through aligned and rotated serial sections from B. (D) The angle of the trajectory of a projection line.
as viewed in a coronal section of dLGN. The projection line was viewed in a flattened and aligned series of coronal sections and its path traced. The angle of this path was then measured from the vertical midline, which measured 22 degrees in this example.

D = dorsal; M = medial; R = rostral; C = caudal; LPN = lateral posterior nucleus; IGL = intergeniculate leaflet; vLGN = ventral lateral geniculate nucleus; VPM = ventral posteromedial thalamic nucleus; Scale bars = 200 µm
FIGURE 4. Labeling of eye-specific laminae in dLGN and morphometric analyses

(A–C) Confocal images of RGC axon termination zones from contralateral (contra) and ipsilateral (ipsi) retinas labeled by intraocular injection of CTB-488 (green) and CTB-555 (red), respectively. (B) The lateral border of the binocular segment was drawn at the edge of the ipsi termination zone at an angle of 22 degrees from the vertical midline (see Figure 3D). (C) Example showing the bounding regions used for areal measurements and cell counting. Each input layer was outlined as shown for areal measurements and volume estimates. (D) A single optical section showing Nissl stain (blue) and bounding regions transferred from C. (E) Illustrative example of how the MetaMorph cell counting function used standard pixel areas to count thresholded cell profiles. The bounding regions represent the border of single cells or clusters of cells. The number of cells counted in each example is indicated by the adjacent numeral. The arrows point to profiles that fell below the threshold and were not counted. (F) Summary of manual versus automated cell counts ($r^2 = 0.87$). Scale bar = 400 µm.
FIGURE 5. Distribution of retinal terminal fields and geniculate relay cells in the binocular segment of dLGN

(A) Raster of confocal images through the entire dLGN of the right hemisphere from one animal with labeled contralateral (contra) and ipsilateral (ipsi) eye terminal fields (section number increases in the caudodorsal direction where s0 denotes the most caudal section). Dashed lines represent the lateral border used to separate monocular (lateral portion) and binocular (medial portion) segments in each section. (B) Histogram showing the average volume occupied by contra and ipsi terminal fields in the binocular segment (n = 4 hemispheres). (C) Summary of the C/I ratios of neuronal cell density in serial sections (each color corresponds to one hemisphere). The dashed line indicates the average C/I ratio for all sections.

D = dorsal; L = lateral; Scale bar = 250 µm

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FIGURE 6. Numerical relationship between topographically matched RGCs and geniculate relay cells

(A) Summary of estimated relay cell numbers within contra- and ipsilateral laminae of dLGN binocular segment as calculated using the size-frequency method (see Methods for details; \( n = 4 \) hemispheres). C-layer = Contralateral RGC input layer; I-layer = Ipsilateral RGC input layer. (B) Summary of RGC cell counts in binocularly corresponding regions of retina (\( n = 4 \)). (C) Schematic of the binocular visual pathway in mouse. The widths of the triangles and lines in the cartoon reflect the relative magnitude of the contralateral (blue) and ipsilateral (yellow) retinogeniculate and geniculocortical projections, respectively. The number of RGCs and relay cells estimated in this study are also indicated. The dLGN afferents serving each eye converge onto a subset of binocular cortical neurons in binocular V1 (V1-B) (green). (D) Schematic showing how the contralateral bias might be represented at different stages in the mouse binocular visual pathway. Based on the numerical relationship between RGCs and relay cells, a single ipsilateral RGC is likely to project to a single corresponding relay cell whereas an average of 3 contralateral RGCs project to converge onto a single corresponding relay cell (corresponding receptive fields are represented by lighter hues).