Published in final edited form as:

J Comp Neurol. 2018 August 01; 526(11): 1749–1759. doi:10.1002/cne.24446.

A novel map of the mouse eye for orienting retinal topography in anatomical space

Maureen E. Stabio^{2,*}, Katelyn B. Sondereker¹, Sean D. Haghgou², Brittany L. Day¹, Berrien Chidsey², Shai Sabbah³, and Jordan M. Renna^{1,*}

¹Department of Biology, The University of Akron, Akron, Ohio, USA

²Department of Cell and Developmental Biology, University of Colorado School of Medicine, Aurora, Colorado, USA

³Department of Neuroscience, Brown University, Providence, RI, USA

Abstract

Functionally distinct retinal ganglion cells have density and size gradients across the mouse retina, and some degenerative eye diseases follow topographic-specific gradients of cell death. Hence, the anatomical orientation of the retina with respect to the orbit and head is important for understanding the functional anatomy of the retina in both health and disease. However, different research groups use different anatomical landmarks to determine retinal orientation (dorsal, ventral, temporal, nasal poles); variations in the accuracy and reliability in marking these landmarks during dissection may lead to discrepancies in the identification and reporting of retinal topography. The goal of this study was to compare the accuracy and reliability of the canthus, rectus muscle, and choroid fissure landmarks in reporting retinal orientation. The retinal relieving cut angle made from each landmark during dissection was calculated based on its relationship to the opsin transition zone, determined via a custom MATLAB script that aligns retinas from immunostained s-opsin. The choroid fissure and rectus muscle landmarks were the most accurate and reliable, while burn marks using the canthus as a reference were the least. These values were used to build an anatomical map that plots various ocular landmarks in relationship to one another, to the horizontal semicircular canals, to lambda-bregma, and to the earth's horizon. Surprisingly, during normal locomotion, the mouse's opsin gradient and the horizontal semicircular canals make equivalent 6° angles aligning the opsin transition zone near the earth's horizon, a feature which may enhance the mouse's ability to visually navigate through its environment.

*Corresponding Authors: Maureen Estevez Stabio, PhD, Department of Cell & Developmental Biology, University of Colorado School of Medicine, Mail Stop F435, 13001 E 17th Place, N5209B, Aurora, CO 80045, USA, Office: 303-724-7461, Fax: 303-724-3420, maureen.stabio@ucdenver.edu. Jordan M. Renna, PhD, Department of Biology, University of Akron, Akron, OH 44325-3908, Office: 330-972-6933, Fax: 330-972-8445, jrenna@uakron.edu.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest to report.

ROLE OF AUTHORS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: MES, JMR. Acquisition of data: KBS, BLD, SH, SS. Analysis and interpretation of data: MES, KBS, BLD, BC, SS, JMR. Drafting of the manuscript: MES, KBS, JMR. Critical revision of the manuscript for important intellectual content: MES, KBS, JMR. Statistical analysis: KBS. Obtained funding: JMR. Administrative, technical, and material support: MES, JMR. Study supervision: MES, JMR.

Graphical Abstract

In this study, we compared the accuracy and reliability of various anatomical landmarks used to orient the mouse retina during eye dissection. We built an anatomical map that plots four ocular landmarks in relationship to one another, to the horizontal semicircular canals, to lambda-bregma, and to the earth's horizon.

Keywords

retina; opsin; extraocular muscles; choroid fissure; topography; eye dissection; semicircular canals; lambda-bregma; mouse; RRID; AB_2158332

INTRODUCTION

The anatomical orientation of the mouse retina with respect to the orbit and head is important for visual neuroscience research, particularly studies that investigate directionally-selective retinal ganglion cells and the oculomotor system (Dhande et al., 2013; Huberman et al., 2009; Kim, Soto, & Kerschensteiner, 2015; Kretschmer, Tariq, Chatila, Wu, & Badea, 2017; Sabbah et al., 2017; Vaney, Sivyier, & Taylor, 2012; Weng, Sun, & He, 2005), topographic distribution of melanopsin retinal ganglion cells (Hughes, Watson, Foster, Peirson, & Hankins, 2013; Sondereker, Onyak, Islam, Ross, & Renna, 2017; Valiente-Soriano et al., 2014), and the topographic mapping of retinal anisotropies or “mini-foveas” in the mouse retina (Bleckert, Schwartz, Turner, Rieke, & Wong, 2014; Dhande & Huberman, 2014; Huberman & Niell, 2011; Ortin-Martinez et al., 2014; Sondereker et al., 2017; Sterratt, Lyngholm, Willshaw, & Thompson, 2013; Valiente-Soriano et al., 2014; J. Wang et al., 2017). Many of these anatomical studies report that functionally distinct retinal ganglion cell types have density and size gradients across the mouse retina. Similarly, studies investigating retinal degeneration have shown that some diseases follow topographic-specific gradients of cell death (de Lara et al., 2014; Hadj-Said et al., 2016; Maiorano & Hindges, 2013; Risner, Pasini, Cooper, Lambert, & Calkins, 2018; Soto et al., 2008; Tao et al., 2015; Ueki, Ramirez, Salcedo, Stabio, & Lefcort, 2016; Zhang et al., 2017). However, a review of the literature indicates that research groups use a variety of different anatomical landmarks during eye dissection to determine the topographic orientation (dorsal, ventral,

temporal, and nasal poles) of the mouse retina, and some groups do not report these aspects of dissection methodology at all. Currently, there is no study that compares these methods for accuracy or reliability or that relates these ocular landmarks to the mouse's skull or to other landmarks in the visual world through which the mouse navigates.

The canthus, an anatomical landmark where the upper and lower eyelids meet, is used in many studies to orient the mouse eye. The nasal canthus lies in the medial corner of each eye; the temporal canthus lies at the lateral margin. Typically, a burn mark, needle mark, suture mark, or dye mark is made on the cornea at the temporal or nasal canthus (Tao et al., 2015; Ueki et al., 2016). More commonly a mark is made at the dorsal or ventral cornea midway between the two canthi (Applebury et al., 2000; Dhande et al., 2013; Estevez et al., 2012; Hadj-Said et al., 2016; Kolesnikov & Kefalov, 2012; Lin, Wang, & Masland, 2004; Ortin-Martinez et al., 2014; Sondereker et al., 2017; J. Wang et al., 2017; Zhang et al., 2017). After the eye is removed from the orbit, a cut is made through the retinal eyecup at the corneal burn mark, from the limbus toward the optic disk. This relieving cut serves to flatten the retina and demarks on the retina the location of the corneal burn. According to this method, the dorsal relieving cut through the dorsal burn mark then serves as a marker for the “north pole” of the mouse eye.

Landmarks deep to the eye surface, including the choroid fissure and rectus muscles, are commonly used as dissection guides by other research groups (Bleckert et al., 2014; Sabbah et al., 2017; Sterratt et al., 2013; Valiente-Soriano et al., 2014; Wei, Elstrott, & Feller, 2010). The choroid fissure is a developmental remnant from the circumferential growth path of the eye cup. In the developing retina, it exists as a space between the two halves of the eye cup through which developing ganglion cell axons exit to form the optic nerve (Lamb, Collin, & Pugh, 2007). In the adult retina, the choroid fissure fuses, and all that remains is a faint line visible best under a light microscope on the back of the sclera, running from the nasal to temporal margins of the eye cup and faintly visible under infrared illumination through the retina anterior to the eye-cup (Wei et al., 2010). Many investigators use the choroid fissure as a guide on which to make temporal and nasal relieving cuts (Bleckert et al., 2014; Hilgen et al., 2017; Kim et al., 2015; Morrie & Feller, 2015; Nath & Schwartz, 2016; Osterhout, Stafford, Nguyen, Yoshihara, & Huberman, 2015; Shi et al., 2017; Stafford, Park, Wong, & Demb, 2014; Y. V. Wang, Weick, & Demb, 2011; Wei et al., 2010). Thus, in these cases, the nasal cut is labeled as 0° degrees on a polar plot, and a 90° superior rotation represents the “north pole” of the mouse eye.

The rectus muscles serve as another landmark for orientation of the mouse eye (Sabbah et al., 2017; Sterratt et al., 2013; Valiente-Soriano et al., 2014). In these cases, the insertion point of the superior rectus muscle at the limbus is bisected. A relieving cut through the eye cup at this point identifies the “dorsal pole” on a polar plot. Thus, in these cases, the insertion of the superior rectus muscle represents the “north pole” of the mouse eye.

Finally, in some cases the opsin transition zone (OTZ) is used as a landmark for orienting the retina in anatomical space (Hughes et al., 2013). The mouse retina contains 3% cones, which express either purely short wavelength (s-) opsin or a combination of s-opsin and mid-wavelength (m-) opsin (Carter-Dawson & LaVail, 1979). Cones that contain pure s-opsin are

distributed throughout the entire retina but represent only 3–5% of all mouse cones (Haverkamp et al., 2005). The majority of cones are m-cones, which co-express both m- and s-opsin in a gradient along the dorsoventral axis (Applebury et al., 2000). Additionally, s-opsin expression dominates the ventral retina whereas m-opsin expression dominates the dorsal retina (Applebury et al., 2000). Immunohistochemical staining for s-opsin reveals the OTZ (Chang, Breuninger, & Euler, 2013), which has been used as the dorsoventral axis for orienting the retina (Hughes et al., 2013). Thus, in these cases, a line drawn perpendicular to the OTZ represents the “north pole” of the mouse eye.

With the variety of anatomical landmarks used in the literature to orient the mouse retina, several uncertainties arise, particularly related to the accuracy and reliability of these methods. Which ocular landmark is the true “north pole” of the mouse eye (i.e. the most upward aspect in the normal posture of a mouse)? How do these various, commonly-used landmarks relate to each other, and what is the internal reliability of each landmark as an identifier of retinal orientation? How do these ocular landmarks relate to other important anatomical landmarks such as lambda-bregma or the semicircular canals? Most importantly, how do they relate to the earth’s horizon as the mouse ambulates through its visual world? Thus, the primary goal of this study was to compare the accuracy and reliability of the canthus, rectus muscle insertion, and choroid fissure landmarks in reporting retinal orientation in anatomical space. The second goal was to use this knowledge to build an accurate anatomical map of retinal landmarks that can be used as a reference for comparing past studies and for planning future studies. To accomplish this, we mapped and compared superficial ocular landmarks (the canthi) to deeper ocular landmarks (rectus muscles, choroid fissure, and OTZ) with skull landmarks (lambda-bregma and horizontal semicircular canals) and related these landmarks to the earth’s horizon as the mouse ambulates in its natural environment.

METHODS

Animals

All experiments were conducted according to NIH guidelines under protocols approved by the Institutional Animal Care and Use Committees of the University of Akron and Brown University. Thirty-seven wild-type male and female C57BL/6N adult mice between one and three months of age were used for all experiments. Mice were housed on a 12-hour light - dark cycle and with food and water ad libitum.

Antibodies

The polyclonal goat anti-s-opsin antibody (Santa Cruz Biotechnologies, Cat# sc14363, RRID: AB_2158332) raised against the 20 N-terminal amino acids of the human OPN1SW protein (EFYLFKNISSVGPWDGPQYH) was used. When pre-absorbed with its immunogen, this antibody exhibits lack of immunohistological reactivity (Schleich, Vielma, Glosmann, Palacios, & Peichl, 2010). Furthermore, this s-opsin antibody reliably labels s-opsin cone outer segments (Schiviz, Ruf, Kuebber-Heiss, Schubert, & Ahnelt, 2008).

Relieving cut dissections to identify retinal orientation

For all dissections, left and right eyes were segregated so as to be able to differentiate between nasal and temporal poles in the final stained tissue. For superficial landmarks, a burn was made prior to enucleation on either the dorsal or ventral cornea using a Bovie Low Temperature Cautery Ophthalmic Fine Tip cauterizer before enucleation (Figure 1a – left panel). For temporal burn cuts, a temporal burn was made on the cornea at the temporal canthus. Eyes were then enucleated and the globe was punctured at the location of the burn with a 20G (0.9 mm × 25 mm) needle. A cut was then made through the eyecup towards the optic disc (Figure 1a – middle panel) so the location of the burn was identifiable by a deep relieving cut in the dissected whole mount retina (Figure 1a – right panel).

Superior rectus muscle landmark—A burn mark was made prior to enucleation on the dorsal cornea using the cauterizer in order to be able to identify the superior rectus muscle after enucleation (Figure 1a – left panel). Curved tip forceps were used to enucleate the eye so that the extraocular muscles remained on the globe. The superior rectus muscle was identified as the extraocular muscle inserting closest to the dorsal corneal burn (Figure 1b – left panel). The cornea was punctured where the superior rectus muscle inserts onto the globe using a 20G (0.9 mm × 25 mm) needle (Figure 1b – left panel). A deep relieving cut bisecting the superior rectus muscle was made from the limbus towards the optic disc (Figure 1b – middle panel) so the location of the superior rectus was identifiable by a deep relieving cut in the dissected whole mount retina (Figure 1b – right panel).

Choroid fissure landmark—Eyes were enucleated and the choroid fissure was identified as the two dark lines on the sclera of the back of the eye that reach up from the optic nerve and terminate at the cornea-scleral border (Figure 1c – left panel). Cuts were made on either side of the choroid fissure down into the retina towards the optic disc (Figure 1c – middle panel) so the location of the choroid fissure was identifiable by two deep relieving cuts in the dissected whole mount retina (Figure 1c – right panel).

Immunohistochemistry

Criteria for dissection quality were applied before proceeding. Any retina that was appreciably torn in such a way as to mimic the appearance of a relieving cut, or any retina that exhibited significant tissue loss or damage of the central or mid-central retina was discarded. To observe the s-opsin gradient, dissected retinas (Figure 1d – left and middle panel) were mounted on nitrocellulose membrane paper and immunohistochemistry was conducted similarly to Sondereker et al. (2017). Retinas were fixed in 4% paraformaldehyde for 40 minutes and then rinsed (3 × 15 minutes) in 0.1M phosphate-buffered saline (PBS). Retinas were placed in blocking solution and incubated at 4° C overnight. Retinas were then incubated at 4° C in blocking solution for six days with primary antibody goat anti-s-opsin (Santa Cruz Biotechnologies, Cat# sc14363, RRID: AB_2158332). The retinas were washed (6 × 10 minutes) in 0.1M PBS and placed in blocking solution overnight at 4° C with secondary antibody donkey anti-goat Alexa Fluor 594 (Life Technologies, Cat# A11058, RRID: AB_2534105). Retinas were rinsed (6 × 10 minutes) in 0.1M PBS before being mounted on a glass slide with Aquamount and covered with a 1.5 µm thick coverslip.

Imaging, 3D Reconstruction, and Analysis

Whole mount retinas stained with s-opsin were viewed with an Olympus BX51 epifluorescence microscope and imaged at 4x with a camera in piecemeal. Multiple images of the same retina were stitched together in Adobe Photoshop CS6 (Figure 1d – right panel and Figure 2 left panels). Images of whole mounted retinas stained with s-opsin were then reconstructed to a 3D structure with Retistruct, an R plugin (Sterratt et al., 2013). Because s-opsin is expressed in highest concentration in the ventral retina (Applebury et al., 2000), retinas can be oriented according to this gradient.

To correctly identify the dorsal, ventral, temporal, and nasal poles of the retina, the 3D reconstructed retinas were analyzed with a custom MATLAB script so that the brightest half of the retina, the half with the highest s-opsin staining, was rotated to be positioned as the lower half of the circle. The 3D reconstructed retina was then divided into dorsal, ventral, nasal, and temporal poles based on the computer-aligned s-opsin gradient. Poles were assigned at 90° intervals on the reconstructed retina: 0° was considered nasal, 90° dorsal, 180° temporal, and 270° ventral. Because cuts are still visible as white lines on the reconstructed retina, the angle of the relieving cut was determined using the angle measurement tool in ImageJ. The three points forming the angle were calculated using the point of incision on the most distal edge of the cut, the optic disc, and the corresponding anatomical direction (dorsal, ventral, nasal, temporal) as set by the s-opsin gradient. The number of degrees that the cut deviated from the set degree value of either dorsal (90°), ventral (270°), temporal (180°), or nasal (0°) was also calculated (and labeled as the “relieving cut degree of error”).

Correlation with lambda-bregma

In the mouse skull, bregma is the anatomical intersection of the coronal and sagittal sutures where the frontal bone meets with the two parietal bones. Lambda is the point at which the two parietal bones meet the occipital bone and is used as a skull reference for stereotaxic studies. A line drawn between these two landmarks is called the lambda-bregma line. To examine the relationship between lambda-bregma, and the extraocular muscles, two corneal burns were made with a cauterizer when the mouse was anesthetized and the head stabilized in a stereotaxic apparatus. The head was positioned so that the lambda-bregma axis was parallel to the earth horizon and a stereotaxically mounted fine probe was used to make one burn on the rostral cornea (lambda) and another on the caudal cornea (bregma), both near the cornea-scleral border. These burns were made so as to mimic the lambda-bregma axis; if a line was to connect the two burns, it would represent the horizontal nature of the lambda-bregma line when the mouse is in standard stereotaxic position (Figures 4 and 5). The lambda-bregma line represented on the cornea between the two burn marks was compared to the insertion points of the lateral and medial extraocular rectus muscles. The angle between the lambda-bregma line and the globe insertion points of the extraocular muscles was then measured.

Statistics

Differences in the relieving cut degrees of error between anatomical landmark cuts were compared using a one-way ANOVA. A post-hoc Tukey-Kramer multiple comparisons test

was also used to analyze the differences in marking anatomical landmarks between experimenters. A post-hoc Bonferroni multiple comparisons test was used to analyze accuracy and reliability of each anatomical landmark for individual dissectors. Unless otherwise indicated, means are expressed as $\pm$ standard deviation (SD), and differences were significant at $p < 0.05$.

RESULTS

Using one of five ocular landmarks as a reference, adult mouse eyes were dissected with one relieving cut made from the limbus towards the optic disc. Reference landmarks were the ventral corneal burn (Figure 2a), dorsal corneal burn (Figure 2b), temporal corneal burn (Figure 2c), superior rectus insertion (Figure 2d), or choroid fissure at both nasal and temporal borders (Figure 2e). Flattened retinas were fixed, immuno-stained for s-opsin, reconstructed using Retistruct (Sterratt et al., 2013), and oriented along the s-opsin gradient (see Methods, Figure 2 middle panels). The relieving cuts for each trial were plotted on a polar plot in which the nasal opsin transition zone (OTZ) was set to 0° (Figure 2, right panel, dotted lines). Thus, 90° represents dorsal, 180° represents temporal, and 270° represents ventral in this figure, and the mean angle was also plotted (Figure 2, right panels, solid lines). Therefore, the s-opsin gradient served as the controlled variable for all dissection trials, the constant on which to compare each anatomical landmark cut as a method for identifying retinal orientation.

To assess the accuracy and reliability of each anatomical landmark as a method for identifying retinal orientation, we first determined the mean $\pm$ standard deviation angles for the ventral corneal burn ($250.2^\circ \pm 41.4^\circ$, $n = 7$), dorsal corneal burn ($65.4^\circ \pm 37.8^\circ$, $n = 6$), temporal corneal burn ($158.9^\circ \pm 32.9^\circ$, $n = 6$), superior rectus insertion ($96.9^\circ \pm 12.5^\circ$, $n = 6$), nasal choroid fissure ($357.5^\circ \pm 20.4^\circ$, $n = 4$), and temporal choroid fissure ($n = 178.9^\circ \pm 10.9^\circ$, $n = 4$; Figure 3a) and then compared these angles with the nasal point of the OTZ, which is arbitrarily set at 0° , as seen in Figure 2. From these data, it can be observed that the superficial landmark cuts (ventral, dorsal or temporal burns, average standard deviation of approximately 37°) had a standard deviation approximately 23° larger than that of internal ocular landmark cuts (rectus muscle insertion and choroid fissure, average standard deviation of approximately 14°). Thus, when compared to superior rectus muscle and choroid fissure cuts, superficial landmark cuts using corneal burns provide a less reliable mark for retinal orientation due to their high variability.

Second, the “relieving cut degrees of error” was calculated for all anatomical landmarks (Figure 3b). Relieving cut degrees of error was defined as the number of degrees a particular relieving cut deviated from the absolute 0° , 90° , 180° , or 270° , which were calculated on each retina according to the OTZ. Overall, the relieving cut degrees of error were significantly greater ($p < 0.05$) among the superficial landmark cuts that use corneal burns (ventral and dorsal burn cuts) compared to cuts using the rectus muscle insertion and choroid fissure landmarks. The rectus muscle and choroid fissure landmarks had the lowest relieving cut degrees of error ($12.1^\circ \pm 6.3^\circ$, $n = 6$, and $12.2^\circ \pm 8.0^\circ$, $n = 8$, respectively) and the dorsal and ventral corneal burn techniques the highest ($39.1^\circ \pm 24.8^\circ$, $n = 6$, and $39.5^\circ \pm 18.8^\circ$, $n = 7$, respectively; Figure 3b). Differences in relieving cut degrees of error for comparisons

between superior rectus versus choroid fissure and between dorsal versus ventral corneal burn were not statistically significant. The mean relieving cut degree of error for temporal burn cuts ($32.6^\circ \pm 18.5^\circ$, $n = 7$) was slightly lower than that of dorsal and ventral burn cuts, but was higher than that of superior rectus and choroid fissure cuts; however, these differences were not statistically significant. The experiments described in Figure 2 and 3 were repeated twice, each time by a different dissector (data not shown). These experiments yielded similar results suggesting that the dissector is not a confounding variable (no significant difference, $p > 0.05$). Taken in total, these data indicate that dissection methods that use deep structures of the eye, including rectus muscles and the choroid fissure, are more accurate and reliable than methods that use superficial anatomical landmarks (i.e. corneal burns) for orienting the retina in a test-retest protocol.

The average angle across trials from each anatomical landmark was plotted as a function of the s-opsin gradient (Figure 3c). From this polar plot, relationships between the cut angles and anatomical ocular landmarks can be inferred. While corneal burns cuts are not well aligned with either the OTZ or the rectus muscles, the choroid fissure cuts are aligned with the OTZ and are perpendicular to the superior rectus muscle cuts. But, how do OTZ, rectus muscle insertions, and choroid fissure relate to other important anatomical landmarks commonly used in the mouse, such as lambda-bregma?

To answer this question, a mouse's head was placed in a stereotaxic axis and tilted so that lambda-bregma fell along the horizontal plane (see Methods). The globe of the eye was then marked with two burns, one caudal and one rostral, so that a line connecting the burns would parallel the horizontal lambda-bregma axis (Figure 4a). Before isolation of the retina, cuts were made as to bisect the medial and lateral rectus muscle insertions (Figure 4b). The angle between the rectus muscle cuts and the lambda-bregma line was determined to be $35.1^\circ \pm 2.3^\circ$ ($n = 8$) with the medial rectus muscle located superior to the lateral rectus muscle (Figure 4c). Therefore, the medial rectus muscle is anatomically positioned at approximately 35° to lambda-bregma. Because the medial and lateral rectus muscle cuts also align with the nasal border of the OTZ (Figure 4b), their positioning can be applied to the position of the OTZ as well. All landmarks were mapped in relationship to each other in Figure 5, with all angles recalculated relative to the earth's horizon as the mouse ambulates normally (Oommen & Stahl, 2008). Note that the angles in Figures 2, 3 and 4 are calculated and plotted based off of the s-opsin gradient, where the nasal OTZ is set to equal 0° . In Figure 5, these angles have been recalculated and plotted so that 0° equals the earth's horizon.

DISCUSSION

The mouse is currently the most widely used model for investigating retinal structure, function, development, and disease. Methods to normalize data across research groups is important particularly to those studies that rely upon precise retinal orientation. A myriad of methods exist in the literature, but our study is the first to compare them and plot them all on a single anatomical map (Figure 5). We found that dissections relying on deep anatomical landmarks, such as the rectus muscles and choroid fissure, are more reliable compared to the use of superficial landmarks made by corneal burns. Superior rectus muscle and choroid fissure landmarks yielded the lowest standard deviation and lowest "relieving cut degree of

error” in a test-retest stability protocol. Furthermore, the superior rectus muscle cuts were perpendicular to the choroid fissure and OTZ, and all three of these anatomical landmark cuts were more precise compared to corneal burn cuts in orienting the mouse retina in anatomical space with respect to the earth’s horizon.

Previous work by Oommen and colleagues has demonstrated that, during normal ambulation, the mouse head is pitched inferiorly so that the lambda-bregma line on the skull is at a 29° downward angle to the earth’s horizon with the horizontal semicircular canals at a 6° downward angle to the earth’s horizon [Figure 5; (Oommen & Stahl, 2008)]. This causes optokinetic reflexes that position the eye into an “up and out” orientation, with a 22° superior rotation and 64° temporal rotation away from the midline during normal ambulation (Oommen & Stahl, 2008). If data from Oommen and colleagues is combined with our data showing that the medial and lateral rectus muscles are anatomically located at a 35° angle to the lambda-bregma line, it places both the medial and lateral rectus muscles and the OTZ at 6° upward from the earth’s horizon (Figure 5). Therefore, the mouse’s opsin gradient and the horizontal semicircular canals make equivalent 6° angles above and below the earth’s horizon, respectively. Together, these angles result in an ideal anatomical orientation that may allow the mouse to look for and avoid potential predators in visual areas above and around them while the head (and nose) is positioned downward for smelling and scavenging. Moreover, the equivalent and opposite angle positions of the OTZ and horizontal semicircular canals allow photons from the sky and grass to fall on the UV-sensitive ventral retina and green-sensitive dorsal retina, respectively.

It is important to note that the anatomical relationships of semicircular canals and OTZ to the earth’s horizon as described above are ambulatory phenomena that change with head position. Similarly, the position of the cornea with respect to the orbit also changes with eye position, which is optokinetically-influenced by head position through the position of the semicircular canals. This may contribute to the lower reliability of corneal burn dissection cuts: The “up and out” rest position of the mouse’s eye during normal ambulation would make a dorsal burn mark, which appears to be equidistant from the two canthi from the perspective of the dissector (Figure 1a, left panel), to actually result in a dorsal burn mark that is rotated significantly medially (nasally) in error with respect to the earth’s vertical as diagramed in Figure 5b. In contrast, the parallel alignments of the medial/lateral rectus muscle insertions to the OTZ and choroid fissure are independent of ambulation and head position: These anatomical relationships are always present, which may contribute in part to their higher reliability as dissection landmarks. Thus, the reported directional tuning curves of direction-selective retinal ganglion cells from laboratories that utilize the dorsal corneal burn approach could be up to 30° different from those generated from laboratories that utilize choroid fissure or rectus muscle landmarks.

Overall, this work emphasizes the utmost importance of reporting transparent and detailed descriptions of the retinal dissection methods used to orient the retina in anatomical space. Moreover, results related to retinal topography should be reported and interpreted with respect to the mid-sagittal and earth horizontal planes as in Figure 5, rather than as angles relative to an arbitrary zero. Finally, deep ocular landmarks, including the rectus muscles

and choroid fissure, provide more precise and reliable methodology to orient the retina when compared to superficial landmarks of the canthus and cornea.

Acknowledgments

We would like to thank Henry Astley, PhD, at University of Akron for assistance with MATLAB. We thank Nicole Shultz and Thomas Finger, PhD, at University of Colorado for technical assistance and advice. Acknowledgements of Support: NIH R15EY026255-01 and the Karl Kirchgeßner Foundation.

References

- Applebury ML, Antoch MP, Baxter LC, Chun LL, Falk JD, Farhangfar F, ... Robbins JT. The murine cone photoreceptor: a single cone type expresses both S and M opsins with retinal spatial patterning. *Neuron*. 2000; 27(3):513–523. [https://doi.org/10.1016/S0896-6273\(00\)00062-3](https://doi.org/10.1016/S0896-6273(00)00062-3). [PubMed: 11055434]
- Bleckert A, Schwartz GW, Turner MH, Rieke F, Wong RO. Visual space is represented by nonmatching topographies of distinct mouse retinal ganglion cell types. *Curr Biol*. 2014; 24(3):310–315. DOI: 10.1016/j.cub.2013.12.020 [PubMed: 24440397]
- Carter-Dawson LD, LaVail MM. Rods and cones in the mouse retina. I. Structural analysis using light and electron microscopy. *J Comp Neurol*. 1979; 188(2):245–262. DOI: 10.1002/cne.901880204 [PubMed: 500858]
- Chang L, Breuninger T, Euler T. Chromatic coding from cone-type unselective circuits in the mouse retina. *Neuron*. 2013; 77(3):559–571. DOI: 10.1016/j.neuron.2012.12.012 [PubMed: 23395380]
- de Lara MJP, Santano C, Guzman-Aranguez A, Valiente-Soriano FJ, Aviles-Trigueros M, Vidal-Sanz M, ... Pintor J. Assessment of inner retina dysfunction and progressive ganglion cell loss in a mouse model of glaucoma. *Experimental Eye Research*. 2014; 122:40–49. DOI: 10.1016/j.exer.2014.02.022 [PubMed: 24631335]
- Dhande OS, Estevez ME, Quattrochi LE, El-Danaf RN, Nguyen PL, Berson DM, Huberman AD. Genetic dissection of retinal inputs to brainstem nuclei controlling image stabilization. *J Neurosci*. 2013; 33(45):17797–17813. DOI: 10.1523/JNEUROSCI.2778-13.2013 [PubMed: 24198370]
- Dhande OS, Huberman AD. Visual circuits: mouse retina no longer a level playing field. *Curr Biol*. 2014; 24(4):R155–156. DOI: 10.1016/j.cub.2013.12.045 [PubMed: 24556437]
- Estevez ME, Fogerson PM, Ilardi MC, Borghuis BG, Chan E, Weng S, ... Berson DM. Form and function of the M4 cell, an intrinsically photosensitive retinal ganglion cell type contributing to geniculocortical vision. *J Neurosci*. 2012; 32(39):13608–13620. DOI: 10.1523/JNEUROSCI.1422-12.2012 [PubMed: 23015450]
- Hadj-Said W, Froger N, Ivkovic I, Jimenez-Lopez M, Dubus E, Degardin-Chicaud J, ... Garcia-Ayuso D. Quantitative and Topographical Analysis of the Losses of Cone Photoreceptors and Retinal Ganglion Cells Under Taurine Depletion. *Invest Ophthalmol Vis Sci*. 2016; 57(11):4692–4703. DOI: 10.1167/iovs.16-19535 [PubMed: 27607415]
- Haverkamp S, Wässle H, Dübels J, Künér T, Augustine GJ, Feng G, Euler T. The primordial, blue-cone color system of the mouse retina. *J Neurosci*. 2005; 25(22):5438–5445. DOI: 10.1523/JNEUROSCI.1117-05.2005 [PubMed: 15930394]
- Hilgen G, Pirmoradian S, Pamplona D, Kornprobst P, Cessac B, Hennig MH, Sernagor E. Pan-retinal characterisation of Light Responses from Ganglion Cells in the Developing Mouse Retina. *Sci Rep*. 2017; 7:42330.doi: 10.1038/srep42330 [PubMed: 28186129]
- Huberman AD, Niell CM. What can mice tell us about how vision works? *Trends Neurosci*. 2011; 34(9):464–473. DOI: 10.1016/j.tins.2011.07.002 [PubMed: 21840069]
- Huberman AD, Wei W, Elstrott J, Stafford BK, Feller MB, Barres BA. Genetic identification of an On-Off direction-selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. *Neuron*. 2009; 62(3):327–334. DOI: 10.1016/j.neuron.2009.04.014 [PubMed: 19447089]

- Hughes S, Watson TS, Foster RG, Peirson SN, Hankins MW. Nonuniform distribution and spectral tuning of photosensitive retinal ganglion cells of the mouse retina. *Curr Biol*. 2013; 23(17):1696–1701. DOI: 10.1016/j.cub.2013.07.010 [PubMed: 23954426]
- Kim T, Soto F, Kerschensteiner D. An excitatory amacrine cell detects object motion and provides feature-selective input to ganglion cells in the mouse retina. *Elife*. 2015; 4doi: 10.7554/eLife.08025
- Kolesnikov AV, Kefalov VJ. Transretinal ERG recordings from mouse retina: rod and cone photoresponses. *J Vis Exp*. 2012; (61)doi: 10.3791/3424
- Kretschmer F, Tariq M, Chatila W, Wu B, Badea TC. Comparison of optomotor and optokinetic reflexes in mice. *J Neurophysiol*. 2017; 118(1):300–316. DOI: 10.1152/jn.00055.2017 [PubMed: 28424291]
- Lamb TD, Collin SP, Pugh EN Jr. Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. *Nat Rev Neurosci*. 2007; 8(12):960–976. DOI: 10.1038/nrn2283 [PubMed: 18026166]
- Lin B, Wang SW, Masland RH. Retinal ganglion cell type, size, and spacing can be specified independent of homotypic dendritic contacts. *Neuron*. 2004; 43(4):475–485. DOI: 10.1016/j.neuron.2004.08.002 [PubMed: 15312647]
- Maiorano NA, Hindges R. Restricted perinatal retinal degeneration induces retina reshaping and correlated structural rearrangement of the retinotopic map. *Nat Commun*. 2013; 4:1938.doi: 10.1038/ncomms2926 [PubMed: 23733098]
- Morrie RD, Feller MB. An Asymmetric Increase in Inhibitory Synapse Number Underlies the Development of a Direction Selective Circuit in the Retina. *J Neurosci*. 2015; 35(25):9281–9286. DOI: 10.1523/JNEUROSCI.0670-15.2015 [PubMed: 26109653]
- Nath A, Schwartz GW. Cardinal Orientation Selectivity Is Represented by Two Distinct Ganglion Cell Types in Mouse Retina. *J Neurosci*. 2016; 36(11):3208–3221. DOI: 10.1523/JNEUROSCI.4554-15.2016 [PubMed: 26985031]
- Oommen BS, Stahl JS. Eye orientation during static tilts and its relationship to spontaneous head pitch in the laboratory mouse. *Brain Res*. 2008; 1193:57–66. DOI: 10.1016/j.brainres.2007.11.053 [PubMed: 18178173]
- Ortin-Martinez A, Nadal-Nicolas FM, Jimenez-Lopez M, Albuquerque-Bejar JJ, Nieto-Lopez L, Garcia-Ayuso D, ... Agudo-Barriuso M. Number and distribution of mouse retinal cone photoreceptors: differences between an albino (Swiss) and a pigmented (C57/BL6) strain. *PLoS One*. 2014; 9(7):e102392.doi: 10.1371/journal.pone.0102392 [PubMed: 25029531]
- Osterhout JA, Stafford BK, Nguyen PL, Yoshihara Y, Huberman AD. Contactin-4 mediates axon-target specificity and functional development of the accessory optic system. *Neuron*. 2015; 86(4):985–999. DOI: 10.1016/j.neuron.2015.04.005 [PubMed: 25959733]
- Risner ML, Pasini S, Cooper ML, Lambert WS, Calkins DJ. Axogenic mechanism enhances retinal ganglion cell excitability during early progression in glaucoma. *Proc Natl Acad Sci U S A*. 2018; doi: 10.1073/pnas.1714888115
- Sabbah S, Gemmer JA, Bhatia-Lin A, Manoff G, Castro G, Siegel JK, ... Berson DM. A retinal code for motion along the gravitational and body axes. *Nature*. 2017; 546(7659):492–497. DOI: 10.1038/nature22818 [PubMed: 28607486]
- Schiviz AN, Ruf T, Kuebber-Heiss A, Schubert C, Ahnelt PK. Retinal cone topography of artiodactyl mammals: influence of body height and habitat. *J Comp Neurol*. 2008; 507(3):1336–1350. DOI: 10.1002/cne.21626 [PubMed: 18189305]
- Schleich CE, Vielma A, Glosmann M, Palacios AG, Peichl L. Retinal photoreceptors of two subterranean tuco-tuco species (Rodentia, Ctenomys): morphology, topography, and spectral sensitivity. *J Comp Neurol*. 2010; 518(19):4001–4015. DOI: 10.1002/cne.22440 [PubMed: 20737597]
- Shi X, Barchini J, Ledesma HA, Koren D, Jin Y, Liu X, ... Cang J. Retinal origin of direction selectivity in the superior colliculus. *Nat Neurosci*. 2017; 20(4):550–558. DOI: 10.1038/nn.4498 [PubMed: 28192394]
- Sondereker KB, Onyak JR, Islam SW, Ross CL, Renna JM. Melanopsin ganglion cell outer retinal dendrites: Morphologically distinct and asymmetrically distributed in the mouse retina. *J Comp Neurol*. 2017; doi: 10.1002/cne.24293

- Soto I, Oglesby E, Buckingham BP, Son JL, Roberson ED, Steele MR, ... Marsh-Armstrong N. Retinal ganglion cells downregulate gene expression and lose their axons within the optic nerve head in a mouse glaucoma model. *J Neurosci*. 2008; 28(2):548–561. DOI: 10.1523/JNEUROSCI.3714-07.2008 [PubMed: 18184797]
- Stafford BK, Park SJ, Wong KY, Demb JB. Developmental changes in NMDA receptor subunit composition at ON and OFF bipolar cell synapses onto direction-selective retinal ganglion cells. *J Neurosci*. 2014; 34(5):1942–1948. DOI: 10.1523/JNEUROSCI.4461-13.2014 [PubMed: 24478373]
- Sterratt DC, Lyngholm D, Willshaw DJ, Thompson ID. Standard anatomical and visual space for the mouse retina: computational reconstruction and transformation of flattened retinæ with the RetiStruct package. *PLoS Comput Biol*. 2013; 9(2):e1002921.doi: 10.1371/journal.pcbi.1002921 [PubMed: 23468609]
- Tao Y, Chen T, Fang W, Peng G, Wang L, Qin L, ... Fei Huang Y. The temporal topography of the N-Methyl- N-nitrosourea induced photoreceptor degeneration in mouse retina. *Sci Rep*. 2015; 5:18612.doi: 10.1038/srep18612 [PubMed: 26685797]
- Ueki Y, Ramirez G, Salcedo E, Stabio ME, Lefcort F. Loss of Ikbkap Causes Slow, Progressive Retinal Degeneration in a Mouse Model of Familial Dysautonomia. *eNeuro*. 2016; 3(5)doi: 10.1523/ENEURO.0143-16.2016
- Valiente-Soriano FJ, Garcia-Ayuso D, Ortin-Martinez A, Jimenez-Lopez M, Galindo-Romero C, Villegas-Perez MP, ... Vidal-Sanz M. Distribution of melanopsin positive neurons in pigmented and albino mice: evidence for melanopsin interneurons in the mouse retina. *Front Neuroanat*. 2014; 8:131.doi: 10.3389/fnana.2014.00131 [PubMed: 25477787]
- Vaney DI, Sivyer B, Taylor WR. Direction selectivity in the retina: symmetry and asymmetry in structure and function. *Nat Rev Neurosci*. 2012; 13(3):194–208. DOI: 10.1038/nrn3165 [PubMed: 22314444]
- Wang J, O'Sullivan ML, Mukherjee D, Punal VM, Farsiu S, Kay JN. Anatomy and spatial organization of Muller glia in mouse retina. *J Comp Neurol*. 2017; 525(8):1759–1777. DOI: 10.1002/cne.24153 [PubMed: 27997986]
- Wang YV, Weick M, Demb JB. Spectral and temporal sensitivity of cone-mediated responses in mouse retinal ganglion cells. *J Neurosci*. 2011; 31(21):7670–7681. DOI: 10.1523/JNEUROSCI.0629-11.2011 [PubMed: 21613480]
- Wei W, Elstrott J, Feller MB. Two-photon targeted recording of GFP-expressing neurons for light responses and live-cell imaging in the mouse retina. *Nat Protoc*. 2010; 5(7):1347–1352. DOI: 10.1038/nprot.2010.106 [PubMed: 20595962]
- Weng S, Sun W, He S. Identification of ON-OFF direction-selective ganglion cells in the mouse retina. *J Physiol*. 2005; 562(Pt 3):915–923. DOI: 10.1113/jphysiol.2004.076695 [PubMed: 15564281]
- Zhang H, Li X, Dai X, Han J, Zhang Y, Qi Y, ... Pang JJ. The Degeneration and Apoptosis Patterns of Cone Photoreceptors in rd11 Mice. *J Ophthalmol*. 2017; 2017:9721362.doi: 10.1155/2017/9721362 [PubMed: 28168050]

Figure 1.

Schematic of the various ocular landmarks used to mark and orient the retina during mouse eye dissection. The anatomical landmarks on the wild type C57BL6/N mouse include the canthi (a), the extraocular muscles (b), the choroid fissure (shown from the posterior view of the globe, c), and the gradient of immunostained s-opsin (blue, d). In each case, the globe is enucleated and hemisected to create an eye cup (middle panels). Fine scissors are used to make a large relieving cut through the eye cup to demarcate a specific anatomical landmark (corneal burn, superior rectus or choroid fissure). Smaller, shallower cuts are made to facilitate flattening the retina for a whole mount preparation (right panels). Blue arrows indicate large relieving cuts. OTZ = opsin transition zone. D = dorsal, N = nasal, V = ventral, T = temporal. See also Methods.

Figure 2.

Comparison of different ocular landmarks for identifying retinal orientation. From left to right: whole mount retina with identifying cut, 3D image of dissected retinas reconstructed with Retistruct and rotated via MATLAB to the s-opsin gradient, polar plot with individual cuts as dashed lines and the averages as solid lines. Retinal orientation was determined by s-opsin immunohistochemistry (cyan) because s-opsin is asymmetrically expressed favoring the ventral retina. All angles were calculated based off the opsin gradient where the nasal OTZ was set to 0° (a) An example of a ventral burn cut dissection (n = 7). (b) An example of a dorsal burn cut dissection (n = 6). (c) An example of a temporal burn cut dissection (n = 6). (d) An example of a superior rectus muscle cut dissection (n = 6). (e) An example of choroid fissure dissection (n = 4). D = dorsal, N = nasal, V = ventral, T = temporal. Scale bar = 1 mm.

Figure 3.

Anatomical landmarks deep within the eye provide a more accurate and reliable marker for retinal orientation than superficial ocular landmarks. (a) scatter plot of cut angles from Figure 2 (right panels) with means (solid lines) plotted in relationship to raw data points (circles), where 0° represents the nasal OTZ. (b) Relieving cut error was greater for dorsal and ventral corneal burn cuts compared to superior rectus muscle and choroid fissure cuts when comparing cut location to a standardized retinal orientation determined by s-opsin immunohistochemistry ($p < 0.05$). Superficial burn cuts did not differ between each other in the reliability of identifying retinal orientation ($p > 0.05$). (c) Polar plot comparing all anatomical landmark cuts with the nasal opsin transition zone set to 0°. Ventral corneal burn cuts identified a ventral pole at $250.2^\circ \pm 41.4^\circ$ ($n = 7$). Dorsal corneal burn cuts identified the dorsal pole at $65.4^\circ \pm 37.8^\circ$ ($n = 6$). Temporal cuts identified the temporal pole at 158.9°

$\pm 32.9^\circ$ ($n = 6$). Superior rectus muscle cuts identified the dorsal pole at $96.9^\circ \pm 12.5^\circ$ ($n = 6$). Choroid fissure cuts identified the nasal pole at $357.5 \pm 20.4^\circ$ and the temporal pole at $178.9^\circ \pm 10.9^\circ$ ($n = 4$). Means reported as $\pm$ SD.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 4.

The s-opsin gradient border is demarcated by the lateral and medial rectus muscle insertions and these deep anatomical landmarks can be related to the stereotaxic markers lambda-bregma. (a) An example of an enucleated globe with two burn marks made on the cornea, one on the rostral limit and the other on the caudal limit. The line connecting these two burns replicates the horizontal position of the lambda-bregma line when the mouse head is in typical stereotaxic position. (b) The dissected retina stained with s-opsin immunohistochemistry (white puncta). After enucleation, two relieving cuts were made into the globe so as to bisect the lateral rectus muscle on the temporal pole and the medial rectus muscle on the nasal pole. The relieving cuts are indicated with arrows. (c) The 3D image of the retina reconstructed with Retistruct with the nasal OTZ set to equal 0°. The lateral and medial rectus muscle cuts are highlighted in red and blue, respectively. These data

demonstrate that the s-opsin transition zone is rotated counter-clockwise at a mean angle of $35.1^{\circ} \pm 2.3^{\circ}$ ($n = 8$). This has been superimposed onto a diagram to display the relationship of the stereotaxic marker lambda-bregma, the s-opsin gradient, and the lateral and medial rectus muscles insertions on the globe. Thus, anatomically, the line between the lateral and medial rectus muscle insertion points is located at a 35° angle to the horizontal lambda-bregma line. Scale bar in (b) = 1 mm.

Figure 5.

Anatomical map relating ocular and retinal landmarks to visual space. The ocular landmarks illustrated in Figure 1 were compared to the horizontal semicircular canals (a) and the lambda-bregma line (c) on the wild type C57BL6/N mouse. These anatomical landmarks were plotted with respect to the earth's horizon (which is set to 0° in (b) and (c)). Angle measurements of lambda-bregma to earth's horizon (29°) and horizontal canals to earth's horizon (6°) come from Oommen & Stahl, 2008; all other values come from original data. The angle between the opsin transition zone (OTZ) and earth's horizon is a 6°, equivalent and opposite to the angle between the horizontal semicircular canals and earth's horizon. Moreover, the “up and out” rest position of the mouse's eye during normal ambulation makes a dorsal burn mark, which appears to be equidistant from the two canthi from the perspective of the dissector (Figure 1a, left panel), to actually result in a dorsal burn mark that is rotated significantly medially (nasally) in error with respect to the earth's vertical (asterisks in b). D = dorsal, N = nasal, V = ventral, T = temporal.