

Monocular and binocular opto-locomotor reflex biases for random dot motion in mice

Wenjun Zhang

Department of Biophysics, Donders Institute, Radboud University, Nijmegen, The Netherlands

Zhara Rezvani

Institute for Cognitive and Brain Sciences, Shahid Beheshti University G.C., Tehran, Iran

Richard J. A. van Wezel

Department of Biophysics, Donders Institute, Radboud University, Nijmegen, The Netherlands
Biomedical Signals and Systems, TechMed Centre, Twente University, Enschede, The Netherlands

Laurens A. M. H. Kirkels

Department of Biophysics, Donders Institute, Radboud University, Nijmegen, The Netherlands

We investigated the relationship between eyes receiving visual input of large field translating random dot motion and subsequent reflexive changes in running direction in mice. The animals were head-fixed running on a Styrofoam ball and the opto-locomotor reflex (OLR) was measured in response to 2 s of dots patterns moving horizontally to the left or right. We measured the OLR in conditions with both eyes open (binocular) and one eye closed (monocular). When we covered the right or left eye in the monocular condition, we found reflexive behavior to be delayed for a few hundred milliseconds to leftward or rightward motion, respectively. After this delay, the bias disappeared and reflexive behavior was similar to responses to motion under binocular conditions. These results might be explained by different contributions of subcortical and cortical visual motion processing pathways to the OLR. Furthermore, we found no evidence for nonlinear interactions between the two eyes, because the sum of the OLR of the two monocular conditions was equal in amplitude and temporal characteristics to the OLR under binocular conditions.

(Huberman & Niell, 2011; Niell & Stryker, 2008; Priebe & McGee, 2014; Wang, Gao, & Burkhalter, 2011). Furthermore, advances in mice visual behavior training, optogenetics, and neurophysiology opened up possibilities to profoundly study visual cortical networks and their link to behavior (Akemann, Mutoh, Perron, Rossier, & Knöpfel, 2010; Antic, Empson, & Knöpfel, 2016; T.-W. Chen et al., 2013; Fenno, Yizhar, & Deisseroth, 2011; Han et al., 2009; Knöpfel, 2012; Packer, Roska, & Häusser, 2013; Tian et al., 2009; Tsien et al., 1997; Zhang et al., 2007). A classical method for testing visual function in mice is measuring the optokinetic response of eyes or head that compensates for a large moving stimulus (Cahill, Nathans, Mettens, Nagao, & Mobraaten, 2008; Cowey & Franzini, 1979; Prusky, Alam, Beekman, & Douglas, 2004; Umino, Solessio, & Barlow, 2008). In those experiments, mice are typically placed in a drum or cage with vertically oriented bars that are translating leftward or rightward. The reflexive movement to motion onset of the eyes or the head is observed manually or automatically tracked using image analysis. In our lab, we recently introduced another approach to measure sensitivity to motion by recording the opto-locomotor response (OLR) of head-fixed mice while they are running freely on an air-floating Styrofoam ball (Kirkels et al., 2018). This system enables us to efficiently record voluntary running behavior of mice in response to a large moving random dot stimulus without training.

Previous rodent and rabbit literature has shown strong eye-dependent biases in optokinetic reflexes

Introduction

In recent years, many behavioral measures have been developed for elucidating the functioning of the visual system in rodents (Pinto & Enroth-Cugell, 2000; Siemann et al., 2015; Thompson, Philp, & Stone, 2008). A reason for this increased interest is the wider use of rodents in vision research and the resemblance of the visual system of rodents to that of higher mammals

Citation: Zhang, W., Rezvani, Z., van Wezel, R. J. A., & Kirkels, L. A. M. H. (2020). Monocular and binocular opto-locomotor reflex biases for random dot motion in mice. *Journal of Vision*, 20(2):6, 1–12, <https://doi.org/10.1167/jov.20.2.6>.



(OKR) (Douglas et al., 2005; Grüsser-Cornehls & Böhm, 1988; Harvey, De'Sperati, & Strata, 1997; Hobbelen & Collewijn, 1971; Thomas, Seiler, Satta, Coffey, & Aramant, 2004). When one eye is closed, temporonasal (rightward for the left eye and leftward for the right eye) motion evokes an eye- or head-tracking response. Most literature reports that there is a complete, or almost complete, lack of reflexes for nasotemporal motion directions. Such a strong monocular asymmetry in rodents and rabbits is not seen in adult higher mammals but has been described for OKRs in affected or young higher mammals, such as human adults with strabismus (Kiorpes, Walton, O'Keefe, Movshon, & Lisberger, 1996), human and monkey infants, and kittens (Atkinson, 1979). Asymmetric OKRs have been attributed to primitive midbrain (subcortical) structures causing reflexive behaviors, with each hemisphere mostly responding to only one motion direction (Braddick, 1996). The strong eye-dependent biases in mice could therefore indicate that they are more reliant on subcortical processing for reflexive visual behavior.

Moreover, studies of the OKR in all sorts of animals describe multiple phases of the response, an initial phase and later phases (Büttner & Kremmyda, 2007; Collewijn, 1991; Distler & Hoffmann, 2003; Stahl, 2008, 2004). The initial acceleration of the OKR consists of an immediate phase of rapid eye acceleration and a gradual phase of slower acceleration reaching a steady-state slow phase velocity (Harvey et al., 1997). In primates, the immediate phase of the OKR is linked to the ocular following response (OFR) (Miles, 1998), which is used as measure for visual computation of input and its transduction to smooth eye movements (K. J. Chen, Sheliga, Fitzgibbon, & Miles, 2005; Kawano, 1999; Kawato, 1999; Masson, 2004; Miles, 1998; Takemura & Kawano, 2002). A more recent study suggests that the initial part of the OKR in mice also consists of two components (Tabata, Shimizu, Wada, Miura, & Kawano, 2010). This prompted us to investigate whether the reflexive OLR we measure in mice also consists of multiple phases. To this end, we used visual motion stimuli under monocular and binocular conditions and measured OLR. Furthermore, we investigated whether there is any evidence for nonlinear interactions between the two eyes by comparing binocular conditions with the sum of the two monocular conditions.

Methods

Animals

We used five C57BL/6J male mice ranging in age from 6 to 8 weeks in this experiment. Because of their normal vision, mice of this strain are often used in

behavioral studies of mouse vision (Bussey, Saksida, & Rothblat, 2001; Prusky & Douglas, 2003; Prusky, West, & Douglas, 2000; Sinex, Burdette, & Pearlman, 1979; Wong & Brown, 2006). The mice were kept in a well-ventilated, temperature-controlled room ($21 \pm 2^\circ\text{C}$) on a 12-hr dark/12-hr light cycle, and all experiments were performed during the dark cycle. In a habituation period of one week, the animals were fed and handled by the experimenter each day. Starting weights of all animals were determined (22.79 ± 0.3 g), and by giving 2.2 g of food per day, we maintained a weight between 85% and 95% of their starting weights. All experiments were conducted in compliance with Dutch and European laws and regulations and were approved by the animal ethical committee of Radboud University Nijmegen. All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgery

Mice were anesthetized with isoflurane via a nose tube during surgery (4% isoflurane in oxygen at induction, 1–1.5% during surgery, 0.2 l/min). After fixating the heads of the anesthetized animals in a stereotactic holder, their eyes were covered in sterile ocular lube (Puralube; Dechra, s-Hertogenbosch, The Netherlands) to prevent dehydration. Next, after shaving the head, skin on top of the head was cut out using fine scissors, and the exposed periosteum was anesthetized locally (1 mg/ml lidocaine HCL with 0.25 mg/ml Bupivacaine Actavis, Melsungen, Germany) before the skull was cleaned with a bone scraper. A custom-made titanium head plate was attached to the head with dental cement (Superbond C&B; Sun Medical, Marseille, France), to allow for fixation.

Habituation

After one week of recovery from surgery, mice were handled for 10 mins every day until they were able to run freely and comfortably from one hand to the other. Next, the mice were transferred to the setup and fixed to the head holder. At the beginning of habituation, the mice ran on the ball for 10 min. Over the following four to five days, the duration increased to two sessions of 40 min per day. The experiments lasted three weeks in total per mouse, without any reward during the experiment.

Visual stimulation

Stimuli consisted of white dots (0.20 cd/m²) on a black background (0.09 cd/m²), giving rise to a Michelson contrast of 0.37. The motion was shown at a speed of 36 deg/s; dots had a radius of 0.9 degrees.

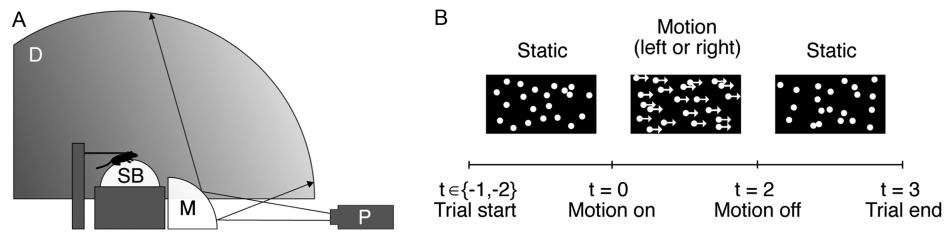


Figure 1. Experimental setup. (A) Schematic drawing of the setup. A projector (P) displayed patterns of randomly positioned dots via a mirror (M) onto the inside of a dome (D). Mice ran under head-fixed conditions on a Styrofoam ball (SB) floating on air. (B) Stimulus time course of one trial. Trials started with a static dot pattern. Motion onset ($t = 0$) occurred either 1 or 2 s after the start of the trial. The pattern of dots drifted either horizontally leftward or rightward for 2 s, producing optic flow consistent with leftward or rightward yaw of the mice, respectively. The trial ended with 1 s of static dots.

An OptomaX501 video projector (resolution: $1,920 \times 1,080 @ 60$ Hz) projected the stimuli onto the inside of a fiberglass-reinforced resin (Fibresports UK; Basildon, The United Kingdom) with a 112-cm inner diameter via a quarter spherical mirror (Figure 1A). At the center of the dome, a custom-made socket (University College London workshops) held a Styrofoam ball floating on air, which served as a treadmill for the mice to run on. Vertically, the visual stimuli covered from 10 degrees below to 80 degrees above the mouse; horizontally, 220 degrees of visual angle was covered (for further details, see Kirkels et al., 2018).

Each trial started with a newly generated random dot pattern that was static for 1 or 2 s, after which the dots started moving for 2 s. Following 2 s of motion, the dots stopped and remained 1 s static on the screen (Figure 1B). To reduce the animal's ability to anticipate motion onset, we interleaved the duration of the initial static phase (1 or 2 s) randomly. Motion (36 deg/s) was created by incrementing (or decrementing) the azimuths of the dots at each 17-ms video frame, resulting in a rotation around the vertical axis, or yaw.

Behavioral paradigm

The mice were running on the ball for two sessions, approximately 15 min each per day. In each session, one eye condition was pseudorandomly selected: binocular, monocular right, or monocular left open. In one session, the stationary random dot stimulus was repeated 80 times and the left and rightward moving stimulus 40 times each. This resulted in 160 trials in total for each session, and each eye condition was repeated in seven sessions, which resulted into 280 trials per stimulus/eye condition combination for the moving dot stimuli. We excluded those trials in which the mice were sitting still by requiring a mean forward speed of at least 1 cm/s over the course of the trial. Sessions with too few trials were subsequently excluded from further analysis. This led to removal of one session for one mouse.

Recording and data analysis

The spherical treadmill consisted of a 19.7-cm diameter Styrofoam ball, which was floating in a semispherical socket on pressurized air (Dombeck, Khabbaz, Collman, Adelman, & Tank, 2007), modified from insect studies (Dahmen, 1980; Mason, Oshinsky, & Hoy, 2001; Stevenson, 2005). The yaw of the ball was registered in deg/s by an optical computer mouse with a sampling rate of 60 Hz. Yaw is the axis of rotation of the visual stimulus and therefore used as a proxy for OLR. The yaw was smoothed with a 100-ms boxcar filtered. Next, the mean yaw during 500 ms before motion onset was determined per trial (baseline) and subtracted from the yaw time series. We statistically tested whether the mean OLR to either motion direction was different from the mean OLR to stationary patterns by comparing every 0.5-s bin between 0.5 and 2 s after motion onset using a Wilcoxon's rank test.

Results

When the mice got used to the setup, we measured OLRs to horizontally moving random dot patterns, either left- or rightward at a speed of 36 deg/s and for a duration of 2 s. In Figure 2, individual OLR traces of one mouse in response to stimulation of both eyes are shown for one session (Figure 2A) and for all seven sessions to leftward (Figure 2B) and rightward (Figure 2C) moving dot patterns. Corresponding to previous findings (Kirkels et al., 2018), mice responded to the motion by turning in the same direction as the dots. In Figure 2A, this is reflected by mostly positive OLRs to leftward motion (yellow lines) and negative OLRs to rightward motion (red lines). Furthermore, we show that OLRs in response to leftward (Figure 2B) or rightward (Figure 2C) motion do not change over the time course of the total experiment (seven sessions of approximately 40 trials per animal). The total trial

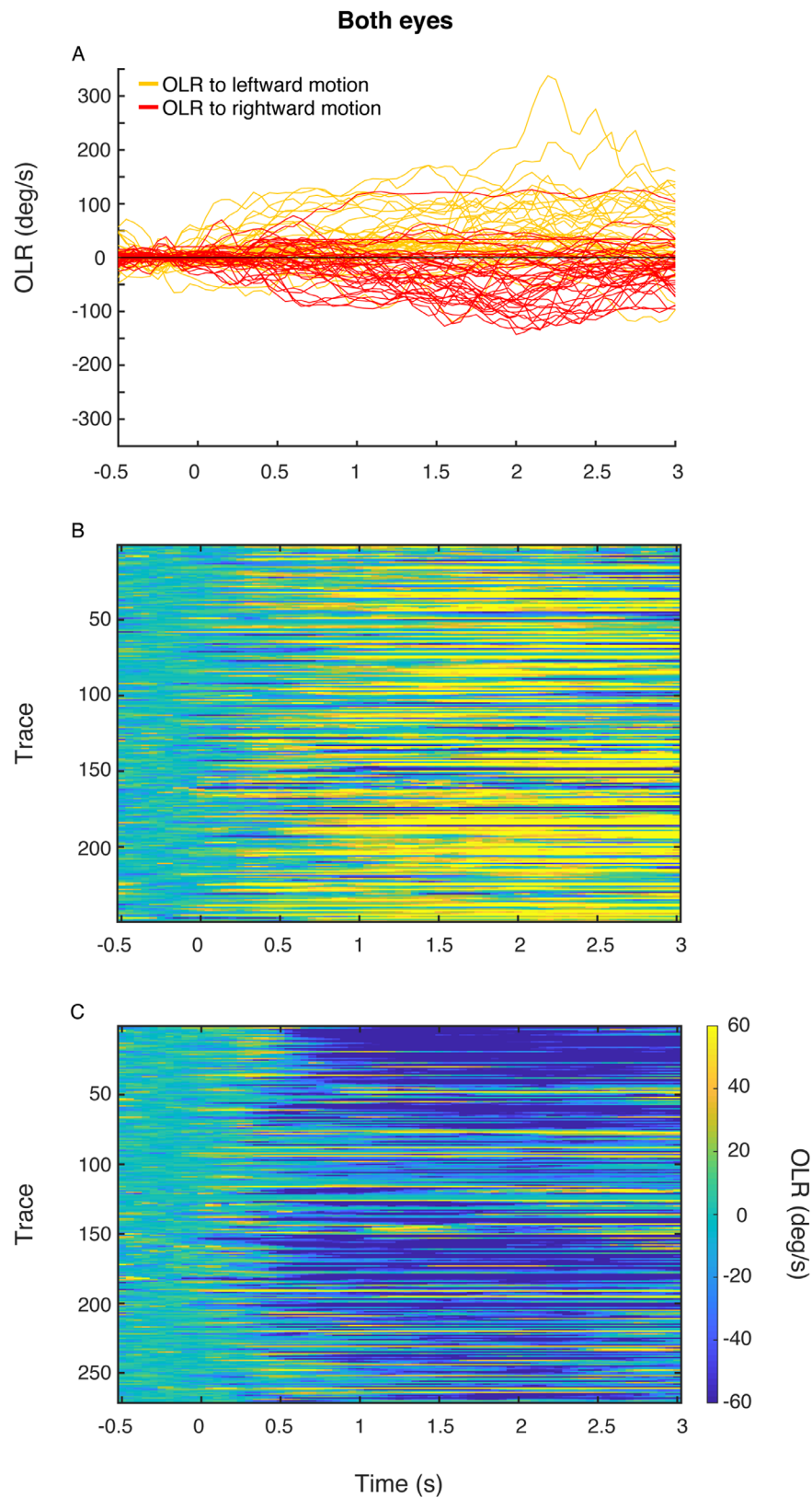


Figure 2. (A) Single OLR traces in response to random dot motion presented to both eyes against time after motion onset for one exemplary session (80 trials) in one exemplary mouse. Lines represent OLRs to a rightward (red line) or leftward (yellow line) moving random dot pattern. The mouse responds to motion by turning in the same direction as the dots. (B, C) Single OLR traces for all seven sessions in one exemplary mouse to dots moving left (B) or right (C). The color map indicates OLR speed of trials in degrees per second. The exemplary mouse in (A) is the same as in (B) and (C).

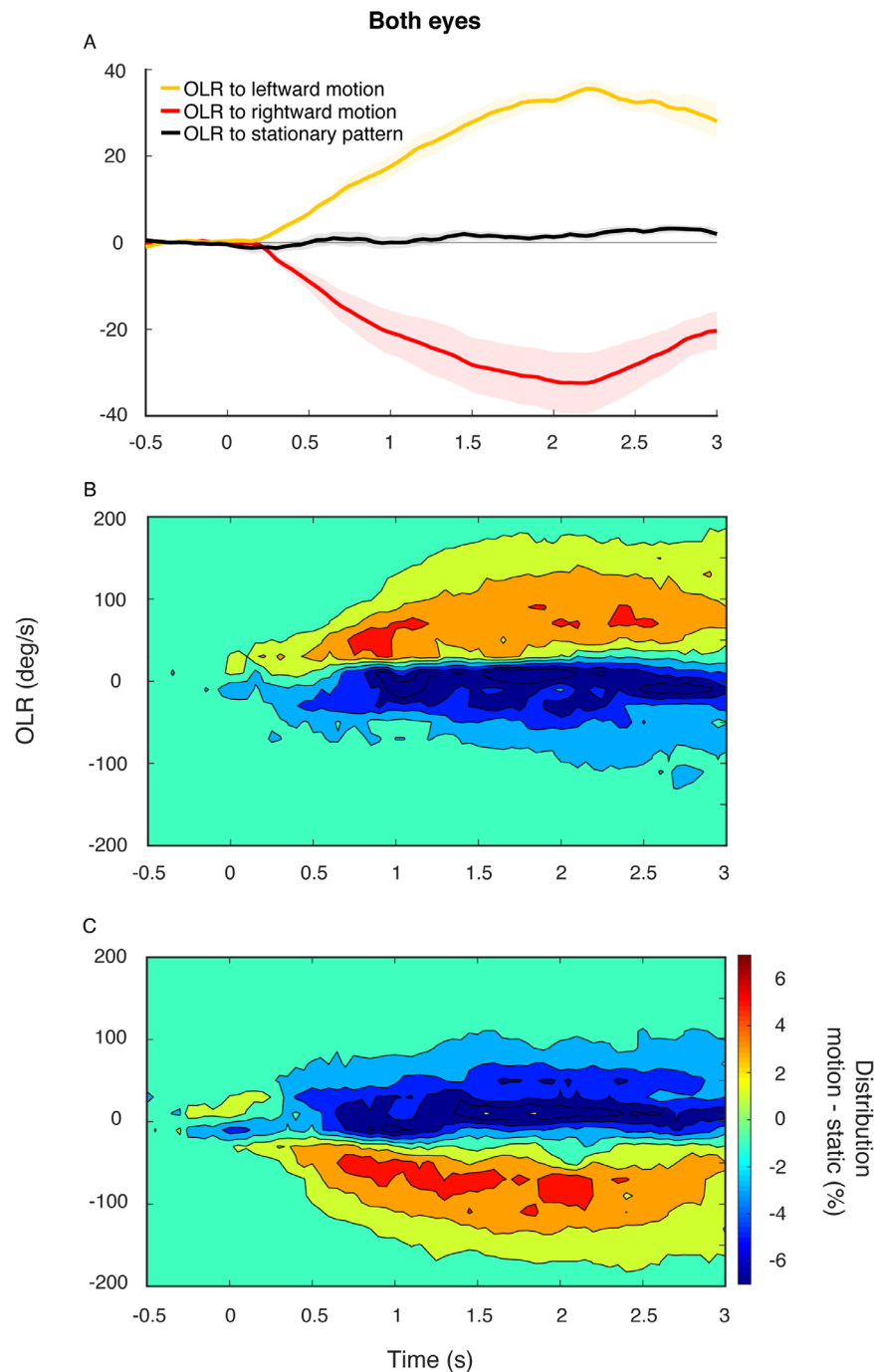


Figure 3. (A) Mean OLR of five mice for random dot motion presented to both eyes against time after motion onset. The lines represent OLR to a stationary (black line) or a rightward (red line) or leftward (yellow line) moving random dot pattern. The mice responded to the motion by turning in the same direction as the dots. Shaded bounds represent *SEM*. (B, C) The distribution of OLR values over time for all trials to the leftward and rightward binocularly presented moving patterns. In these contour plots, OLRs to stationary patterns were subtracted from the OLRs to dots moving left (B) or right (C). The color map shows the percentage of trials where the traces go through a certain point in the contour plot relative to the static condition.

number deviates from 280 because trials were discarded where the animal did not move enough.

In Figure 3A, the mean OLR of five animals to rightward motion (red), leftward motion (yellow), and stationary stimuli (black) is shown for mice with

uncovered eyes (binocular stimulation). OLRs to both motion directions start at about 250 ms after motion onset and show a peak response (both around 35 deg/s) around 2 s after motion onset. Additionally, the distribution of OLR values over time for all trials to the

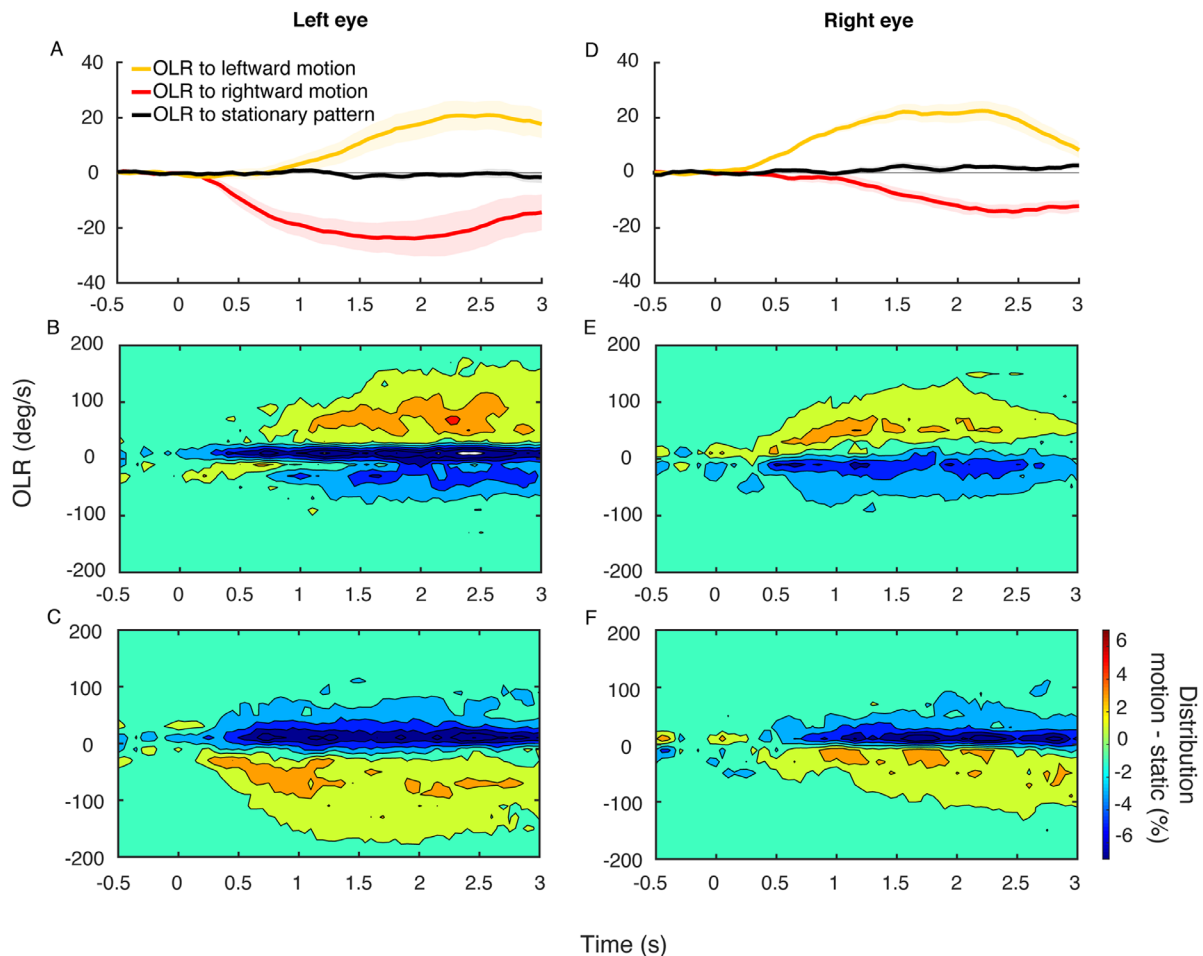


Figure 4. Mean ($n = 5$) OLR for random dot patterns presented only to one eye against time after motion onset for the left eye (A) and right eye (D). The patterns were stationary (black line), rightward (red line), or leftward (yellow line) moving. Shaded bounds represent *SEM*. (B, C, E, F) The distribution of OLR values over time for all trials to the leftward and rightward monocularly presented moving patterns. In these contour plots, OLRs to stationary patterns were subtracted from the OLRs to dots moving left (B, E) or right (C, F) for the left (B, C) and right (E, F) eye. The color map shows the percentage of trials where the traces go through a certain point in the contour plot relative to the static condition.

leftward and rightward moving patterns demonstrates that leftward motion predominantly results in positive OLR values when the baseline OLR to stationary motion is subtracted (Figure 3B) and vice versa for rightward motion (Figure 3C). When motion was presented to both eyes, the mean OLR to rightward and leftward motion was statistically different from the OLR to stationary patterns from 0.5 s after motion onset (Wilcoxon's rank test, $p < 0.01$ for all three 0.5 s bins from $t = 0.5$ –2 s).

Pseudorandomly interleaved with the binocular stimulation experiments, we used an eye cap to cover either the right or the left eye. When the left eye is open (Figure 4A), the OLR shows a delay of about 700 ms to leftward motion, compared to rightward motion. The same conclusion can be drawn from the contour plots for leftward (Figure 4B) and rightward (Figure 4C) motion. Responses for rightward motion start earlier

after motion onset. Results from the Wilcoxon's rank test demonstrated that the mean OLR to rightward motion differs significantly ($p < 0.01$ for all 0.5-s bins from $t = 0.5$ –2 s) from the mean OLR to stationary patterns, whereas the mean OLR to leftward motion only differs significantly from between 1.5 and 2 s after motion onset ($p = 0.04$). When the right eye is open, mice respond earlier and stronger to leftward motion compared to rightward motion (Figure 4D–F). The mean OLR to leftward motion differs significantly from the mean OLR to stationary patterns from 0.5 s to 2 s for all 0.5-s bins ($p < 0.05$). The mean OLR to rightward motion is only significantly different from the mean OLR to stationary patterns from 1.5 to 2 s after motion onset ($p < 0.01$).

We expect an interaction of visual input between the two eyes on the evoked OLR after the initial phase, where only one eye provides input. In order to

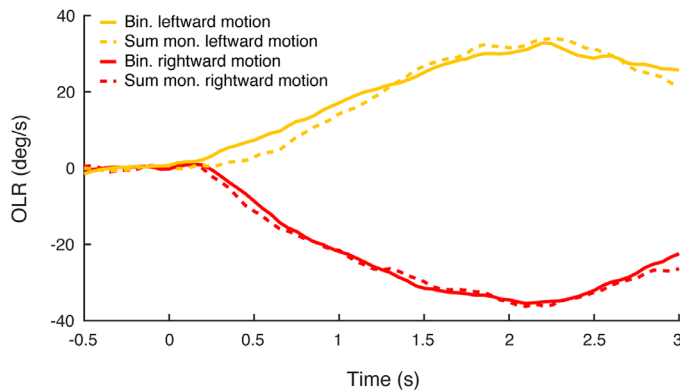


Figure 5. Comparison of the binocular OLR for leftward (red solid line) and rightward (yellow solid line) motion compared to the sum of the two related monocular OLRs (dashed lines).

determine whether there is interaction, we summed the OLRs to monocular stimulation and depicted them in the same figure as the OLRs to binocular stimulation. Figure 5 shows that the sum of the OLR to leftward motion monocularly presented in the left and right eye (dashed yellow line) is not significantly different from the OLR to leftward motion presented binocularly (solid yellow line) (Wilcoxon's rank test; $p > 0.4$ for all 0.5-s bins from 0.5–2 s). The same holds true for rightward motion (Wilcoxon's rank test; $p > 0.1$ for all 0.5-s bins from 0.5–2 s). These results show no evidence for averaging or nonlinear interactions between the eyes under these conditions.

Discussion

We measured OLRs of mice in response to random dot patterns moving horizontally left or right in binocular and monocular conditions. We found that in about the first 700 ms, there is a strong bias toward temporonasal stimulation, which is in accordance with a lot of previous animal literature on eye and head reflexes to large motion patterns (Douglas et al., 2005; Grüsser-Cornehls & Böhm, 1988; Harvey et al., 1997; Hobbelen & Collewijn, 1971; Thomas et al., 2004). However, this asymmetry largely resolves in the following second (Figure 4) and an OLR is also evoked, albeit somewhat smaller, by motion in the nasotemporal direction. Furthermore, when we summed the left and right monocular OLRs to leftward and rightward motion, the binocular OLRs to the same motion directions were similar, suggesting that in binocular vision, there are no strong nonlinear interactions between the two eyes (Figure 5).

Previous rodent and rabbit literature generally reports the (nearly) complete absence of OKR to nasotemporal motion in monocular stimulation

(Douglas et al., 2005; Grüsser-Cornehls & Böhm, 1988; Harvey et al., 1997; Hobbelen & Collewijn, 1971; Thomas et al., 2004). In contrast to this, we find that OLRs do not show this strong asymmetry at later stages of the response. We cannot rule out that this discrepancy can be explained by the characteristics of our visual stimulus (e.g., moving random dots in a whole field dome) compared to stimuli that are used in typical OKR experiments (moving vertical gratings on surrounding screens). Another factor that could play a role is our choice of animal model (C57BL/6J mice), but it has been shown that these mice also show strong asymmetries in OKR experiments (e.g., Tabata et al., 2010). The most probable reason for finding more symmetrical OLRs later in the response is that we measure running behavior instead of eye movements in OKR or head movements in OMR experiments (Kretschmer, Kretschmer, Kunze, & Kretzberg, 2013; Kretschmer, Sajgo, Kretschmer, & Badea, 2015). Furthermore, although OKRs occur in all vertebrates, their appearance differs from species to species. How gaze is stabilized differs per species. Birds, reptiles, and amphibians are more reliant on head and body movements, while mammals and fish depend on eye movements (Dieringer, Cochran, & Precht, 1983; Fritsches & Marshall, 2002; Straka & Dieringer, 2004). Consequently, these groups show discrepancies in velocity profiles and amplitude of eye movements (Dieringer, 1986; Huang & Neuhauss, 2008). From an evolutionary point of view, neuronal circuits underlying the OKR have changed in order to optimize the OKR. An obvious characteristic that emerged in the OKR is the asymmetry for motion in opposite directions when monocularly presented. In almost all species that display asymmetry, it is nasotemporal motion that results in no or much weaker responses compared to temporonasal motion (Bonaventure, Kim, & Jardon, 1992; Collewijn, 1975; Fite, Reiner, & Hunt, 1979; Fritsches & Marshall, 2002; Hess, Precht, Reber, & Cazin, 1985; Katte & Hoffmann, 1980; Wallman & Velez, 1985). This monocular asymmetry is predominantly seen in animals' laterally positioned eyes. A possible evolutionary advantage of the asymmetry in these animals is that it helps to suppress optokinetic drive due to nasotemporal optic flow on the retina during forward locomotion. This resulted in an OKR that is more sensitive to rotation, which helps them in gaze stabilization during head turns (Fritsches & Marshall, 2002).

Research into OKR symmetry has led to at least three theories that apply very well to mammals, as proposed by Masseck and Hoffmann (2009). First of all, retinal organization has been correlated with symmetry, dividing mammalian classes into foveates and afoveates. Possession of a fovea seems to be required for symmetry in the mammalian OKR (Tauber & Atkin, 1968). Another determining factor could be

the number of retinal fibers crossing in the optic chiasm. Supposedly, the more fibers that end ipsilaterally, the more symmetrical the OKR (Fukuda & Tokita, 1957). Furthermore, the size of the binocular visual field could be at the basis of OKR symmetry. Large binocular visual fields result in symmetrical OKRs (ter Braak, 1936). Whether one of these features or a combination of either of them is the underlying cause for OKR symmetry is hard to determine because of their intertwining; they are highly correlated and appear or are absent together. However, lesion and corticectomy studies of the visual cortex in cats and monkeys were reported to result in nystagmus or more asymmetrical OKRs, comparable to rats and rabbits (Flandrin, Courjon, Orban, & Sprague, 1992; Hamada, 1986; Montarolo, Precht, & Strata, 1981; Strong, Malach, Lee, & Van Sluyters, 1984; Tusa, Demer, & Herdman, 1989; Wood, Spear, & Braun, 1973; Zee, Tusa, Herdman, Butler, & Gucer, 1987). This clearly shows that the connection between visual cortex and subcortical structures is of importance for OKR symmetry. Specifically, neurons transferring the nasotemporal direction information to oculomotor areas are essential in this case. If these neurons do not exist or are not well developed, this leads to monocular OKR asymmetry (Masseck & Hoffmann, 2009). In line with this is the finding that lesions of visual cortex in rats or cerebral cortex in rabbits did not affect the OKR (Harvey et al., 1997; Hobbelen & Collewijn, 1971). Apparently, in these animals, nasotemporal direction information carrying neurons does not link to the oculomotor circuitry. However, more recent studies show that lesioning or silencing of the visual cortex does affect the OKR gain (Liu, Huberman, & Scanziani, 2016; Prusky, Alam, & Douglas, 2006; Prusky, Silver, Tschetter, Alam, & Douglas, 2008). These recent findings would indeed advocate for an involvement of the visual cortex besides subcortical structures in OKR plasticity. It would be very interesting to see what happens to the OLRs after visual cortex lesions in rodents.

The importance of connections between visual cortex and oculomotor areas for OKR symmetry is underlined by findings from OKR comparisons between infant and mature, as well as healthy and affected, higher mammals. Higher mammals such as cats and humans show an asymmetrical OKR at birth, and only over the course of weeks to months, respectively, when development takes place and cortical projections mature, the OKR becomes more symmetrical (Giolli, Blanks, & Lui, 2006). Moreover, disruption of cortical binocularity (e.g., in the case of strabismus or by monocular deprivation) leads to OKR asymmetry as well (Hoffmann, 1983; Tychsen & Lisberger, 1986). This all points to the idea that symmetry of reflexes and cortical control are inversely related. One might speculate that running behavior depends more on

cortical control than eye movements in mice, and because cortical pathways are symmetric, the OLR is more symmetric.

We show that when the stimulus is presented to one eye, the OLR starts off asymmetrically after about 250 ms after motion onset and resolves into a symmetrical response after about 700 ms. Compared to the OKR, the OLR is much slower, which could be explained by the involvement of different motor systems (e.g., fast responding eye and head movements versus slower changes in running behavior). Furthermore, it takes time for the mice to turn the Styrofoam ball into a different direction because of inertia. The OKR literature often describes an initial (first 500 ms from stimulus motion onset) and later parts of the response (Cohen, Matsuo, & Raphan, 1977). These two phases in the OKR resemble the initial and later part of the OLR of mice that we report. And although most OKR studies on signal processing in the mouse brain only focus on the first 500 ms (Tabata et al., 2010), at least for the OLR, later stages of the response also provide valuable information.

Adding monocular OLRs

Finally, we find that adding both individual monocular OLRs to either leftward or rightward motion matches the binocular OLR to that same motion direction. This result underlines the idea that binocular vision is the result of the sum of each eye's input and there is no averaging or winner-take-all mechanism at play (van Wezel & Britten, 2002). However, it is difficult to generalize this conclusion, because most other studies use eye or head movements and not running behavior. Despite this fact, our study delivered valuable information about monocular and binocular evoked opto-locomotor reflexes and (a)symmetry in response to monocular temporonasal and nasotemporal stimulation. Furthermore, it provides a platform to further investigate underlying mechanisms of normal binocular vision and visual abnormalities or diseases.

Conclusion

Here, we present evidence for a response not only to temporonasal but also to nasotemporal stimulation in monocular stimulation in mice. This is in stark contrast to what has been reported in the past, where head-tracking or OKR measurements show (almost) no response to nasotemporal stimulation in rodents and rabbits (Douglas et al., 2005; Grüsser-Cornehls & Böhm, 1988; Harvey et al., 1997; Hobbelen & Collewijn, 1971; Thomas et al., 2004). OKRs and OLRs manifest different temporal characteristics, possibly due

to different underlying connections between retina and oculomotor or motor areas, respectively. Although the actual origin of monocular asymmetry remains elusive, our results suggest that OLRs of mice have both a subcortical and cortical component. A delay in the response to monocular stimulation in the nasotemporal direction could be indicative of cortical processing time, whereas the response to temporonasal motion could reflect more instinctive reflexive behavior.

Keywords: opto-locomotor reflex, bias, nasotemporal, monocular, binocular

Acknowledgments

This work was funded by the China Scholarship Council 201406140123 and NWO/MaGW 404-10-405.

Commercial relationships: none.

Corresponding author: Richard van Wezel.

Email: r.vanwezel@donders.ru.nl.

Address: Department of Biophysics, Donders Institute, Radboud University, Nijmegen, The Netherlands.

References

- Akemann, W., Mutoh, H., Perron, A., Rossier, J., & Knöpfel, T. (2010). Imaging brain electric signals with /genetically targeted voltage-sensitive fluorescent proteins. *Nature Methods*, 7, 643–649. <https://doi.org/10.1038/nmeth.1479>.
- Antic, S. D., Empson, R. M., & Knöpfel, T. (2016). Voltage imaging to understand connections and functions of neuronal circuits. *Journal of Neurophysiology*, 116, 135–152. <https://doi.org/10.1152/jn.00226.2016>.
- Atkinson, J. (1979). Development of optokinetic nystagmus in the human infant and monkey infant: An analogue to development in kittens. In R. D. Freeman (Ed.) *Developmental Neurobiology of Vision* (pp. 277–287). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4684-3605-1_24.
- Bonaventure, N., Kim, M. S., & Jardon, B. (1992). Effects on the chicken monocular OKN of unilateral microinjections of GABAA antagonist into the mesencephalic structures responsible for OKN. *Experimental Brain Research*, 90, 63–71. <https://doi.org/10.1007/BF00229257>.
- Braddick, O. (1996). Motion processing: Where is the naso-temporal asymmetry? *Current Biology*, 6, 250–253. [https://doi.org/10.1016/S0960-9822\(02\)00470-0](https://doi.org/10.1016/S0960-9822(02)00470-0).
- Bussey, T. J., Saksida, L. M., & Rothblat, L. A. (2001). Discrimination of computer-graphic stimuli by mice: a method for the behavioral characterization of transgenic and gene-knockout models. *Behavioral Neuroscience*, 115, 957–960.
- Büttner, U., & Kremmyda, O. (2007). Smooth pursuit eye movements and optokinetic nystagmus. *Developments in Ophthalmology*, 40, 76–89. <https://doi.org/10.1159/000100350>.
- Cahill, H., Nathans, J., Mettens, P., Nagao, S., & Mobraaten, L. (2008). The optokinetic reflex as a tool for quantitative analyses of nervous system function in mice: Application to genetic and drug-induced variation. *PLoS ONE*, 3, e2055. <https://doi.org/10.1371/journal.pone.0002055>.
- Chen, K. J., Sheliga, B. M., Fitzgibbon, E. J., & Miles, F. A. (2005). Initial ocular following in humans depends critically on the Fourier components of the motion stimulus. *Annals of the New York Academy of Sciences*, 1039, 260–271. <https://doi.org/10.1196/annals.1325.025>.
- Chen, T.-W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., Schreiter, E. R., Kerr, R. A., Orger, M. B., Jayaraman, V., Looger, L. L., Svoboda, K., ... Kim, D. S. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature*, 499, 295–300. <https://doi.org/10.1038/nature12354>.
- Cohen, B., Matsuo, V., & Raphan, T. (1977). Quantitative analysis of the velocity characteristics of optokinetic nystagmus and optokinetic after-nystagmus. *Journal of Physiology*, 270, 321–344. <https://doi.org/10.1113/jphysiol.1977.sp011955>.
- Collewijn, H. (1975). Direction-selective units in the rabbit's nucleus of the optic tract. *Brain Research*, 100, 489–508. [https://doi.org/10.1016/0006-8993\(75\)90154-7](https://doi.org/10.1016/0006-8993(75)90154-7).
- Collewijn, H. (1991). The optokinetic contribution. In *Eye movements* (pp. 45–70). London: Macmillan.
- Cowey, A., & Franzini, C. (1979). The retinal origin of uncrossed optic nerve fibres in rats and their role in visual discrimination. *Experimental Brain Research*, 35, 443–455.
- Dahmen, H. J. (1980). A simple apparatus to investigate the orientation of walking insects. *Experientia*, 36, 685–687. <https://doi.org/10.1007/BF01970140>.
- Dieringer, N. (1986). Comparative neurobiology of the organization of gaze-stabilizing reflex systems in vertebrates. *Die Naturwissenschaften*, 73, 299–304.
- Dieringer, N., Cochran, S. L., & Precht, W. (1983). Differences in the central organization of gaze stabilizing reflexes between frog and turtle. *Journal of Comparative Physiology*, 153, 495–508. <https://doi.org/10.1007/BF00612604>.

- Distler, C., & Hoffmann, K.-P. (2003). Development of the optokinetic response in macaques. *Annals of the New York Academy of Sciences*, 1004, 10–18. <https://doi.org/10.1196/annals.1303.002>.
- Dombeck, D. A., Khabbaz, A. N., Collman, F., Adelman, T. L., & Tank, D. W. (2007). Imaging large-scale neural activity with cellular resolution in awake, mobile mice. *Neuron*, 56, 43–57. <https://doi.org/10.1016/j.neuron.2007.08.003>.
- Douglas, R. M., Alam, N. M., Silver, B. D., McGill, T. J., Tschetter, W. W., & Prusky, G. T. (2005). Independent visual threshold measurements in the two eyes of freely moving rats and mice using a virtual-reality optokinetic system. *Visual Neuroscience*, 22, 677–684. <https://doi.org/10.1017/S0952523805225166>.
- Fenko, L., Yizhar, O., & Deisseroth, K. (2011). The development and application of optogenetics. *Annual Review of Neuroscience*, 34, 389–412. <https://doi.org/10.1146/annurev-neuro-061010-113817>.
- Fite, K. V., Reiner, A., & Hunt, S. P. (1979). Optokinetic nystagmus and the accessory optic system of pigeon and turtle. *Brain, Behavior and Evolution*, 16, 192–202. <https://doi.org/10.1159/000121836>.
- Flandrin, J. M., Courjon, J. H., Orban, G. A., & Sprague, J. M. (1992). Longterm impairment of cat optokinetic nystagmus following visual cortical lesions. *Experimental Brain Research*, 88, 594–608. <https://doi.org/10.1007/BF00228188>.
- Fritsches, K. A., & Marshall, N. J. (2002). Independent and conjugate eye movements during optokinesis in teleost fish. *Journal of Experimental Biology*, 1252, 1241–1252.
- Fukuda, V. T., & Tokita, T. (1957). Relation of the direction of optical stimulation to the type of reflex of the oculomotor muscles. *Acta Oto-Laryngologica*, 48, 415–424. <https://doi.org/10.3109/00016485709126902>.
- Giolli, R. A., Blanks, R. H. I., & Lui, F. (2006). The accessory optic system: Basic organization with an update on connectivity, neurochemistry, and function. In T.-P. J. A. B., & Büttner-Ennever J. A. (Ed.), *Neuroanatomy of the oculomotor system* (Vol. 151, pp. 407–440). Munich, Germany: Elsevier. [https://doi.org/10.1016/S0079-6123\(05\)51013-6](https://doi.org/10.1016/S0079-6123(05)51013-6).
- Grüsser-Cornehls, U., & Böhm, P. (1988). Horizontal optokinetic ocular nystagmus in wildtype (B6CBA+/+) and weaver mutant mice. *Experimental Brain Research*, 72, 29–36. <https://doi.org/10.1007/BF00248497>.
- Hamada, T. (1986). Non-conjugate monocular optokinetic nystagmus in cats. *Vision Research*, 26, 1311–1314. [https://doi.org/10.1016/0042-6989\(86\)90114-8](https://doi.org/10.1016/0042-6989(86)90114-8).
- Han, X., Qian, X., Bernstein, J. G., Zhou, H.-H., Franzesi, G. T., Stern, P., ... Boyden, E. S. (2009). Millisecond-timescale optical control of neural dynamics in the nonhuman primate brain. *Neuron*, 62, 191–198. <https://doi.org/10.1016/j.neuron.2009.03.011>.
- Harvey, R. J., De'Sperati, C., & Strata, P. (1997). The early phase of horizontal optokinetic responses in the pigmented rat and the effects of lesions of the visual cortex. *Vision Research*, 37, 1615–1625. [https://doi.org/10.1016/S0042-6989\(96\)00292-1](https://doi.org/10.1016/S0042-6989(96)00292-1).
- Hess, B. J. M., Precht, W., Reber, A., & Cazin, L. (1985). Horizontal optokinetic ocular nystagmus in the pigmented rat. *Neuroscience*, 15, 97–107. [https://doi.org/10.1016/0306-4522\(85\)90126-5](https://doi.org/10.1016/0306-4522(85)90126-5).
- Hobbelen, J. F., & Collewijn, H. (1971). Effect of cerebro/cortical and collicular ablations upon the optokinetic reactions in the rabbit. *Documenta Ophthalmologica*, 30, 227–236. <https://doi.org/10.1007/BF00142521>.
- Hoffmann, K. P. (1983). Effects of early monocular deprivation on visual input to cat nucleus of the optic tract. *Experimental Brain Research*, 51, 236–246. <https://doi.org/10.1007/BF00237199>.
- Huang, Y.-Y., & Neuhauss, S. C. F. (2008). The optokinetic response in zebrafish and its applications. *Frontiers in Bioscience: A Journal and Virtual Library*, 13, 1899–1916.
- Huberman, A. D., & Niell, C. M. (2011). What can mice tell us about how vision works? *Trends in Neurosciences*, 34, 464–473. <https://doi.org/10.1016/j.tins.2011.07.002>.
- Katte, O., & Hoffmann, K.-P. (1980). Direction specific neurons in the pretectum of the frog (*Rana esculenta*). *Journal of Comparative Physiology*, 140, 53–57. <https://doi.org/10.1007/BF00613747>.
- Kawano, K. (1999). Ocular tracking: Behavior and neurophysiology. *Current Opinion in Neurobiology*, 9, 467–473. [https://doi.org/10.1016/S0959-4388\(99\)80070-1](https://doi.org/10.1016/S0959-4388(99)80070-1).
- Kawato, M. (1999). Internal models for motor control and trajectory planning. *Current Opinion in Neurobiology*, 9, 718–727. [https://doi.org/10.1016/S0959-4388\(99\)00028-8](https://doi.org/10.1016/S0959-4388(99)00028-8).
- Kiorpes, L., Walton, P. J., O'Keefe, L. P., Movshon, J. A., & Lisberger, S. G. (1996). Effects of early-onset artificial strabismus on pursuit eye movements and on neuronal responses in area MT of macaque monkeys. *Journal of Neuroscience*, 16, 6537–6553.
- Kirkels, L. A. M. H., Zhang, W., Havenith, M. N., Tiesinga, P., Glennon, J., Wezel, R. J. A. Van, ... Duijnhouwer, J. (2018). The opto-locomotor reflex as a tool to measure sensitivity to moving

- random dot patterns in mice. *Scientific Reports*, 8, . <https://doi.org/10.1038/s41598-018-25844-4>.
- Knöpfel, T. (2012). Genetically encoded optical indicators for the analysis of neuronal circuits. *Nature Reviews Neuroscience*, 13, 687–700. <https://doi.org/10.1038/nrn3293>.
- Kretschmer, F., Kretschmer, V., Kunze, V. P., & Kretzberg, J. (2013). OMR-arena: Automated measurement and stimulation system to determine mouse visual thresholds based on optomotor responses. *PLoS ONE*, 8, 1–12. <https://doi.org/10.1371/journal.pone.0078058>.
- Kretschmer, F., Sajgo, S., Kretschmer, V., & Badea, T. C. (2015). A system to measure the optokinetic and optomotor response in mice. *Journal of Neuroscience Methods*, 256, 91–105. <https://doi.org/10.1016/j.jneumeth.2015.08.007>.
- Liu, B.-H., Huberman, A. D., & Scanziani, M. (2016). Cortico-fugal output from visual cortex promotes plasticity of innate motor behaviour. *Nature*, 538, 383–387. <https://doi.org/10.1038/nature19818>.
- Mason, A. C., Oshinsky, M. L., & Hoy, R. R. (2001). Hyperacute directional hearing in a microscale auditory system. *Nature*, 410, 686–690. <https://doi.org/10.1038/35070564>.
- Masseck, O. A., & Hoffmann, K. P. (2009). Comparative neurobiology of the optokinetic reflex. *Annals of the New York Academy of Sciences*, 1164, 430–439. <https://doi.org/10.1111/j.1749-6632.2009.03854.x>.
- Masson, G. S. (2004). From 1D to 2D via 3D: Dynamics of surface motion segmentation for ocular tracking in primates. *Journal of Physiology*, 98, 35–52. <https://doi.org/10.1016/j.jphysparis.2004.03.017>.
- Miles, F. A. (1998). The neural processing of 3-D visual information: evidence from eye movements. *European Journal of Neuroscience*, 10, 811–822. <https://doi.org/10.1046/j.1460-9568.1998.00112.x>.
- Montarolo, P., Precht, W., & Strata, P. (1981). Functional organization of the mechanisms subserving the optokinetic nystagmus in the cat. *Neuroscience*, 6, 231–246. [https://doi.org/10.1016/0306-4522\(81\)90059-2](https://doi.org/10.1016/0306-4522(81)90059-2).
- Niell, C. M., & Stryker, M. P. (2008). Highly selective receptive fields in mouse visual cortex. *Journal of Neuroscience*, 28, 7520–7536. <https://doi.org/10.1523/JNEUROSCI.0623-08.2008>.
- Packer, A. M., Roska, B., & Häusser, M. (2013). Targeting neurons and photons for optogenetics. *Nature Neuroscience*, 16, 805–815. <https://doi.org/10.1038/nn.3427>.
- Pinto, L. H., & Enroth-Cugell, C. (2000). Tests of the mouse visual system. *Mammalian Genome*, 11, 531–536.
- Priebe, N. J., & McGee, A. W. (2014). Mouse vision as a gateway for understanding how experience shapes neural circuits. *Frontiers in Neural Circuits*, 8, 123. <https://doi.org/10.3389/fncir.2014.00123>.
- Prusky, G. T., Alam, N. M., & Douglas, R. M. (2006). Enhancement of vision by monocular deprivation in adult mice. *Journal of Neuroscience*, 26, 11554–11561. <https://doi.org/10.1523/JNEUROSCI.3396-06.2006>.
- Prusky, G. T., Alam, N. M., Beekman, S., & Douglas, R. M. (2004). Rapid quantification of adult and developing mouse spatial vision using a virtual optomotor system. *Investigative Ophthalmology & Visual Science*, 45, 4611. <https://doi.org/10.1167/iovs.04-0541>.
- Prusky, G. T., & Douglas, R. M. (2003). Developmental plasticity of mouse visual acuity. *European Journal of Neuroscience*, 17, 167–173. <https://doi.org/10.1046/j.1460-9568.2003.02420.x>.
- Prusky, G. T., Silver, B. D., Tschetter, W. W., Alam, N. M., & Douglas, R. M. (2008). Experience-dependent plasticity from eye opening enables lasting, visual cortex-dependent enhancement of motion vision. *Journal of Neuroscience*, 28, 9817–9827. <https://doi.org/10.1523/JNEUROSCI.1940-08.2008>.
- Prusky, G. T., West, P. W., & Douglas, R. M. (2000). Behavioral assessment of visual acuity in mice and rats. *Vision Research*, 40, 2201–2209.
- Siemann, J. K., Muller, C. L., Bamberger, G., Allison, J. D., Veenstra-VanderWeele, J., & Wallace, M. T. (2015). A novel behavioral paradigm to assess multisensory processing in mice. *Frontiers in Behavioral Neuroscience*, 8, 456. <https://doi.org/10.3389/fnbeh.2014.00456>.
- Sinex, D. G., Burdette, L. J., & Pearlman, A. L. (1979). A psychophysical investigation of spatial vision in the normal and reeler mutant mouse. *Vision Research*, 19, 853–857.
- Stahl, J. S. (2004). Using eye movements to assess brain function in mice. *Vision Research*, 44, 3401–3410. <https://doi.org/10.1016/j.visres.2004.09.011>.
- Stahl, J. S. (2008). Characteristics and applications of mouse eye movements. In L. M. Chalupa, & R. W. Williams (Ed.), *Eye, retina, and visual system of the mouse*. Boston, MA: The MIT Press.
- Stevenson, P. A. (2005). Octopamine and experience-dependent modulation of aggression in crickets. *Journal of Neuroscience*, 25, 1431–1441. <https://doi.org/10.1523/JNEUROSCI.4258-04.2005>.
- Straka, H., & Dieringer, N. (2004). Basic organization principles of the VOR: Lessons from frogs. *Progress in Neurobiology*, 73, 259–309. <https://doi.org/10.1016/j.pneurobio.2004.05.003>.

- Strong, N. P., Malach, R., Lee, P., & Van Sluyters, R. C. (1984). Horizontal optokinetic nystagmus in the cat: Recovery from cortical lesions. *Developmental Brain Research*, *13*, 179–192. [https://doi.org/10.1016/0165-3806\(84\)90153-6](https://doi.org/10.1016/0165-3806(84)90153-6).
- Tabata, H., Shimizu, N., Wada, Y., Miura, K., & Kawano, K. (2010). Initiation of the optokinetic response (OKR) in mice Hiromitsu Tabata. *Journal of Vision*, *10*, 1–17. <https://doi.org/10.1167/10.1.13.Introduction>.
- Takemura, A., & Kawano, K. (2002). Sensory-to-motor processing of the ocular-following response. *Neuroscience Research*, *43*, 201–206. [https://doi.org/10.1016/S0168-0102\(02\)00044-5](https://doi.org/10.1016/S0168-0102(02)00044-5).
- Tauber, E. S., & Atkin, A. (1968). Optomotor responses to monocular stimulation: Relation to visual system organization. *Science*, *160*, 1365 LP–1367. <https://doi.org/10.1126/science.160.3834.1365>.
- ter Braak, J. W. G. (1936). Studies on optokinetic nystagmus. *Archives Néerlandaises de Physiologie de l'Homme et des Animaux*, *21*, 309–376.
- Thomas, B. B., Seiler, M. J., Saddy, S. R., Coffey, P. J., & Aramant, R. B. (2004). Optokinetic test to evaluate visual acuity of each eye independently. *Journal of Neuroscience Methods*, *138*, 7–13. <https://doi.org/10.1016/j.jneumeth.2004.03.007>.
- Thompson, S., Philp, A. R., & Stone, E. M. (2008). Visual function testing: A quantifiable visually guided behavior in mice. *Vision Research*, *48*, 346–352. <https://doi.org/10.1016/j.visres.2007.07.020>.
- Tian, L., Hires, S. A., Mao, T., Huber, D., Chiappe, M. E., & Chalasani, S. H., & Looger, L. L. (2009). Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. *Nature Methods*, *6*, 875–881. <https://doi.org/10.1038/nmeth.1398>.
- Tsien, R. Y., Miyawaki, A., Llopis, J., Heim, R., McCaffery, J. M., Adams, J. A., . . . Ikura, M. (1997). Fluorescent indicators for Ca²⁺ based on green fluorescent proteins and calmodulin. *Nature*, *388*, 882–887. <https://doi.org/10.1038/42264>.
- Tusa, R. J., Demer, J. L., & Herdman, S. J. (1989). Cortical areas involved in OKN and VOR in cats: Cortical lesions. *Journal of Neuroscience*, *9*, 1163–1178.
- Tychsen, L., & Lisberger, S. (1986). Maldevelopment of visual motion processing in humans who had strabismus with onset in infancy. *Journal of Neuroscience*, *6*, 2495–2508. <https://doi.org/10.1523/jneurosci.06-09-02495.1986>.
- Umino, Y., Solessio, E., & Barlow, R. B. (2008). Speed, spatial, and temporal tuning of rod and cone vision in mouse. *Journal of Neuroscience*, *28*, 189–198. <https://doi.org/10.1523/JNEUROSCI.3551-07.2008>.
- van Wezel, R. J. A., & Britten, K. H. (2002). Multiple uses of visual motion: The case for stability in sensory cortex. *Neuroscience*, *111*, 739–759. [https://doi.org/10.1016/S0306-4522\(02\)00036-2](https://doi.org/10.1016/S0306-4522(02)00036-2).
- Wallman, J., & Velez, J. (1985). Directional developmental optic system asymmetries of optokinetic nystagmus: Changes and relation to the accessory and to the vestibular system. *Journal of Neuroscience*, *5*, 317.
- Wang, Q., Gao, E., & Burkhalter, A. (2011). Gateways of ventral and dorsal streams in mouse visual cortex. *Journal of Neuroscience*, *31*, 1905–1918. <https://doi.org/10.1523/JNEUROSCI.3488-10.2011>.
- Wong, A. A., & Brown, R. E. (2006). Visual detection, pattern discrimination and visual acuity in 14 strains of mice. *Genes, Brain and Behavior*, *5*, 389–403. <https://doi.org/10.1111/j.1601-183X.2005.00173.x>.
- Wood, C. C., Spear, P. D., & Braun, J. J. (1973). Direction-specific deficits in horizontal optokinetic nystagmus following removal of visual cortex in the cat. *Brain Research*, *60*, 231–237. [https://doi.org/10.1016/0006-8993\(73\)90862-7](https://doi.org/10.1016/0006-8993(73)90862-7).
- Zee, D. S., Tusa, R. J., Herdman, S. J., Butler, P. H., & Gucer, G. (1987). Effects of occipital lobectomy upon eye movements in primate. *Journal of Neurophysiology*, *58*, 883–907. <https://doi.org/10.1152/jn.1987.58.4.883>.
- Zhang, F., Wang, L.-P., Brauner, M., Liewald, J. F., Kay, K., & Watzke, N., & Deisseroth, K. (2007). Multimodal fast optical interrogation of neural circuitry. *Nature*, *446*, 633–639. <https://doi.org/10.1038/nature05744>.