Video Article

VisioTracker, an Innovative Automated Approach to Oculomotor Analysis

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Abstract

Investigations into the visual system development and function necessitate quantifiable behavioral models of visual performance that are easy to elicit, robust, and simple to manipulate. A suitable model has been found in the optokinetic response (OKR), a reflexive behavior present in all vertebrates due to its high selection value. The OKR involves slow stimulus-following movements of eyes alternated with rapid resetting saccades. The measurement of this behavior is easily carried out in zebrafish larvae, due to its early and stable onset (fully developed after 96 hours post fertilization (hpf)), and benefitting from the thorough knowledge about zebrafish genetics, for decades one of the favored model organisms in this field. Meanwhile the analysis of similar mechanisms in adult fish has gained importance, particularly for pharmacological and toxicological applications.

Here we describe VisioTracker, a fully automated, high-throughput system for quantitative analysis of visual performance. The system is based on research carried out in the group of Prof. Stephan Neuhauss and was re-designed by TSE Systems. It consists of an immobilizing device for small fish monitored by a high-quality video camera equipped with a high-resolution zoom lens. The fish container is surrounded by a drum screen, upon which computer-generated stimulus patterns can be projected. Eye movements are recorded and automatically analyzed by the VisioTracker software package in real time.

Data analysis enables immediate recognition of parameters such as slow and fast phase duration, movement cycle frequency, slow-phase gain, visual acuity, and contrast sensitivity.

Typical results allow for example the rapid identification of visual system mutants that show no apparent alteration in wild type morphology, or the determination of quantitative effects of pharmacological or toxic and mutagenic agents on visual system performance.

Video Link

The video component of this article can be found at https://www.jove.com/video/3556/

Protocol

1. Breeding of fish

Embryos were kept and raised under standard conditions (Brand 2002) and staged according to development in days post fertilization (dpf). Adults and larvae at 5 dpf were utilized for measurements.

2. Experimental procedure

1. Preparation of instrument

Larvae: Fish larvae were embedded in 3% pre-warmed (28°C) methylcellulose to prevent body movements. The embryos were placed dorsal side up in the VisioTracker, facing the projecting screen. Adult fish: Fish were briefly anesthetized in 300 mg/l MS-222, fitted into the immobilizing device and placed in the VisioTracker. Before measurements were initiated, they were left to recover for 1-2 min.

2. Generation of stimulus patterns

Stimulus patterns consisting of vertical black-and-white sine-wave gratings rotating around the fish were created using the proprietary software package. They could be modulated through the software package according to wave form, contrast, intensity, angular speed and spatial frequency. Patterns were projected onto the screen using a digital light projector contained within the VisioTracker. The approximate distance between the fish's eye and the screen was 4.5 cm, and projection size on-screen was 360 deg horizontally and 55 deg vertically. For larval fish,

direction of stimulation was altered with a frequency of 0.33 Hz to reduce saccade frequency. Adult fish were stimulated unidirectionally and only the eye stimulated in temporal-to-nasal direction was considered, since nasal-to-temporal eye velocity in general is significantly lower and less constant (refer to Mueller and Neuhauss, 2010).

3. Recording of eye movements

A bright-field image of the fish's head was fed to an infrared video camera. Infrared illumination of fish was effected from below. The camera recorded images at a rate of 5 frames/second (larvae) or 12.5 frames/second (adults), respectively. Images are automatically processed, corrected and smoothed for eye shape. Eye orientation in relation to the horizontal axis was then determined automatically and eye velocity was calculated by the proprietary software package. Small movements of the fish were automatically corrected for by the software. All recording and analysis was achieved in real time.

3. Post-experimental data processing

1. Raw measurements of eye velocities were filtered for saccades in order to extract slow-phase velocity.
2. Saccade-filtered eye velocity curves were smoothed by a running average with a sliding window of 7 frames.
3. Eye velocity was averaged over frames with identical stimulus conditions.
4. For larval fish, eye velocity was averaged over both eyes.

4. Representative results:

In order to assess the capabilities of the VisioTracker for larval and adult fish, experiments were performed with zebrafish larvae at 5 dpf, and adult zebrafish.

For larval Zebrafish, the *bumper* mutant was chosen. In this mutant, lens epithelial cells hyperproliferate, leading to reduced lens size and ectopic location of the lens. These morphological alterations are reflected by a significant reduction of contrast sensitivity and visual acuity (Schonthaler *et al.*, 2010). Figure 1 demonstrates the difference in contrast sensitivity of bumper mutants versus wild-type siblings. *bumper* mutants increasingly fail to adjust eye velocity as the stimulus contrast decreases. By analogy, when the stimulus spatial frequency is increased, i.e. the stimulus stripe width is decreased, *bumper* mutants likewise demonstrate reduced visual acuity (Fig. 2)

The dependence of adult Zebrafish visual performance on environmental conditions was investigated by subjecting the fish to varying concentrations of alcohol in their tank water for 30 minutes and subsequently measuring the optokinetic response under varying stimulus conditions. Adult Zebrafish show a marked reduction in contrast sensitivity when maintained in increasing alcohol concentrations (Fig. 3). A similar dose-dependent reduction of overall eye velocity over a wide range of spatial frequencies could be observed when the fish were treated with increasing alcohol concentrations (Fig. 4). Alcohol treatment furthermore dose-dependently reduces oculomotor performance at more demanding tasks as exemplified by increased stimulus speeds (Fig. 5).

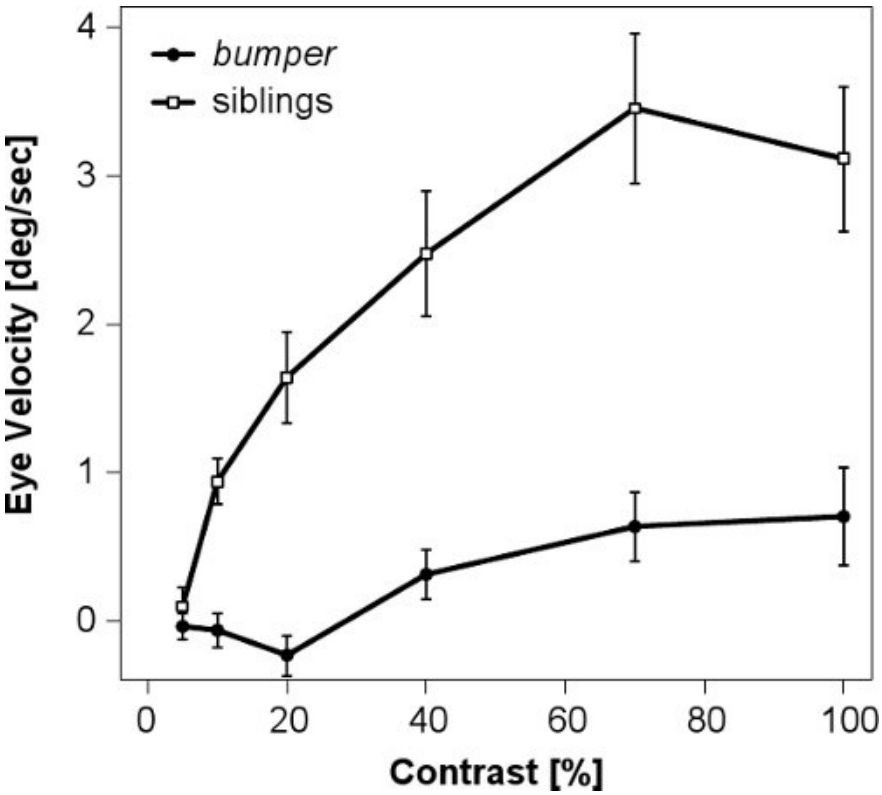

Figure 1. Zebrafish larval eye velocity is dependent on stimulus contrast. 10 *bumper* mutants and 10 wild-type siblings were analyzed at 5 dpf under varying stimulus stripe contrast conditions. Graph shows average eye velocity ± 1 SEM.

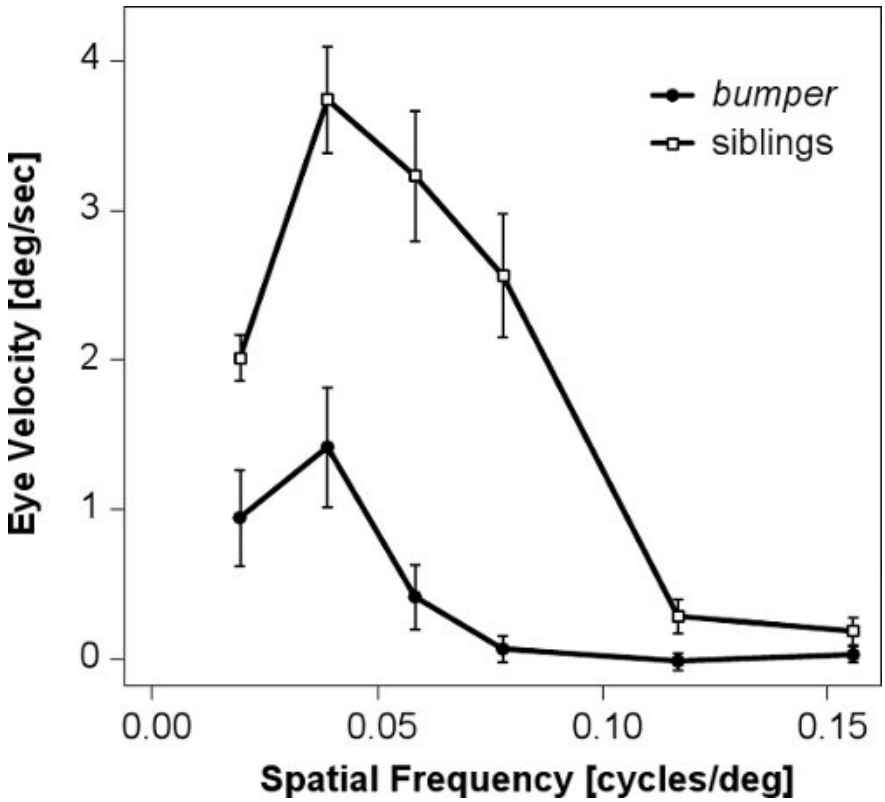

Figure 2. Zebrafish larval eye velocity is dependent on spatial frequency. 10 *bumper* mutants and 10 wild-type siblings were subjected to different stimulus stripe widths at 5 dpf and analyzed as described. Graph shows average eye velocity ± 1 SEM.

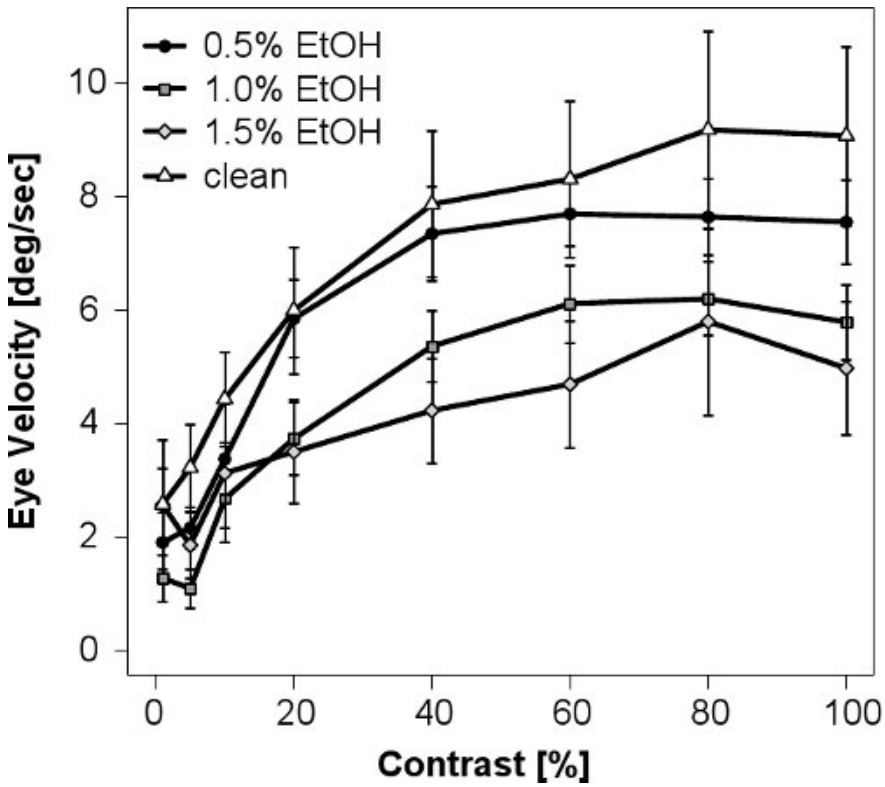

Figure 3. Adult Zebrafish show alcohol concentration-dependent reduction in contrast sensitivity. Adult Zebrafish were maintained in varying alcohol concentrations as indicated for 30 minutes and analyzed under varying stimulus stripe contrast conditions. Graph shows the average temporal-to-nasal eye velocity ± 1 SEM of 9 fish per group (except control group: n=11).

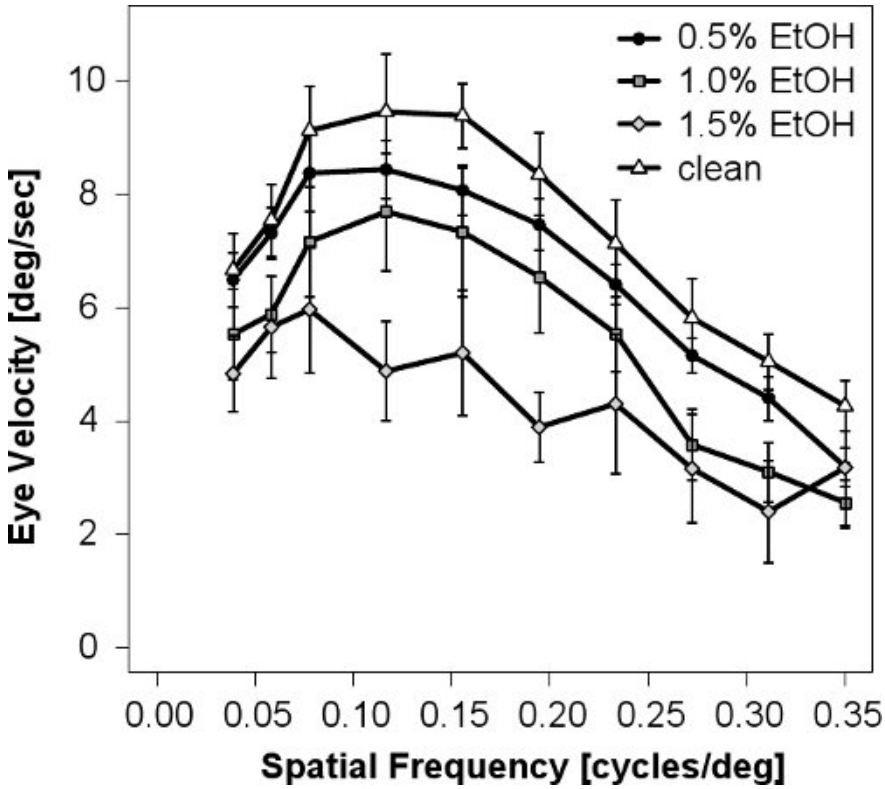

Figure 4. Adult Zebrafish show alcohol concentration-dependent reduction in overall eye movement over a wide range of stimulus stripe width. Adult Zebrafish were maintained in varying alcohol concentrations as indicated for 30 minutes and analyzed under varying stimulus stripe width conditions. Graph shows the average temporal-to-nasal eye velocity ± 1 SEM of 9 fish per group (except control group: n=11).

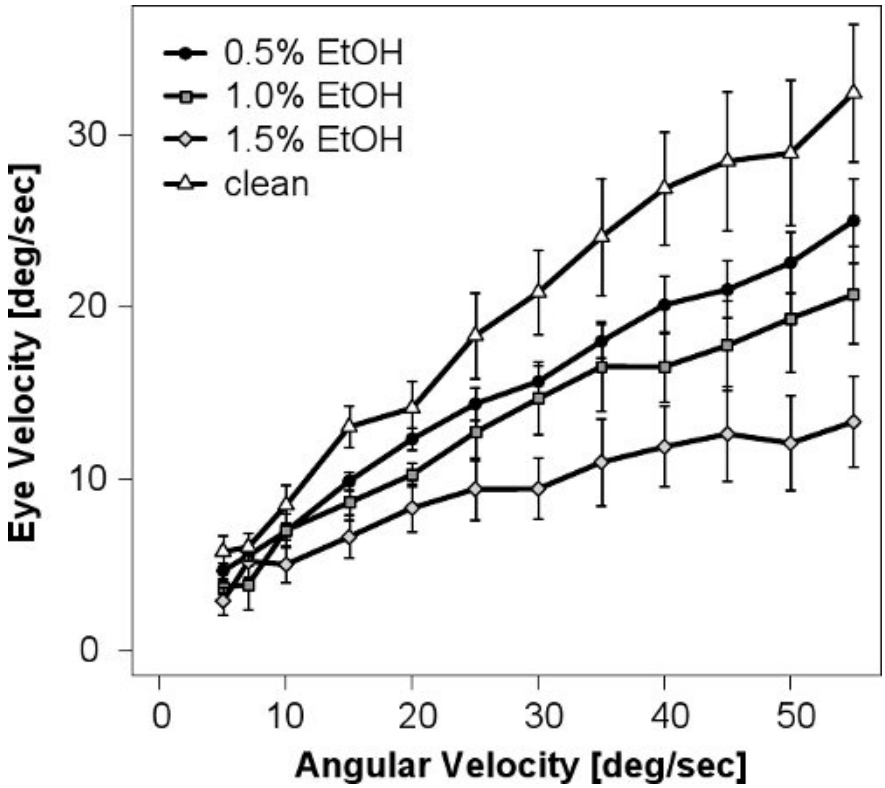

Figure 5. Adult Zebrafish show alcohol concentration-dependent reduction in overall eye movement over a wide range of stimulus speeds. Adult Zebrafish were maintained in varying alcohol concentrations as indicated for 30 minutes and analyzed under varying stimulus speed conditions. Graph shows the average temporal-to-nasal eye velocity ± 1 SEM of 9 fish per group (except control group: n=11).

Discussion

The importance of the OKR for the study of visual function has been recognized in the scientific community for a long time (Easter & Nicola 1996, 1997), and attempts to truly quantify the paradigm have started well over a decade ago. Easter and Nicola (1996) developed a system with motorized rotating striped drums, where the video recording of eye movement was analyzed manually. This system suffered from the lack of immobilization of the fish embryo, which required frequent manual repositioning, and could detect the tracking movements of eyes only with great difficulty. A step forward was the use of a video-projected striped drum to allow for more variable computer generated stimulus presentation (Roeser & Baier, 2003; Rinner *et al.*, 2005a).

The mostly manual, frame-by-frame analysis of videotaped recordings has proven to be extremely laborious, and to a certain degree hampered by observer bias (Beck *et al.*, 2004). Automated analysis in real-time was suggested to allow the use of behavioral feedback learning mechanisms (Major *et al.*, 2004). The use of infrared illumination and frequency-controlled rotating stimuli has been pioneered by Beck *et al.* (2004). However, the system described there has only been used for larvae, and analysis was carried out off-line. Furthermore, the VisioTracker allows complete control over stimuli, including changing the stimulus during the experiment, thereby allowing greater flexibility and spontaneous influence on the course of the experiment. Also, the digital stimulus creation used by the VisioTracker overcame problems mentioned earlier with acceleration of the inert mass of a striped stimulus drum (Beck *et al.*, 2004).

Larvae restraint by methylcellulose does not significantly interfere with eye movement and does not have any long-term effects on zebrafish well-being. Fish larvae have been successfully maintained embedded in methylcellulose for several days, until oxygen supply through the skin becomes insufficient for the demand with increasing age (Qian *et al.*, 2005).

The adult fish restraining method is equally easy on the animal. The short duration of the experiment, coupled with the option of rapidly exchanging the test animal for a different one, further adds to the positive animal welfare aspects of the system. Since the gills are continuously flushed by water, it is convenient to spike the water with any chemical of choice to study its effect on eye movements and visual performance. Similarly a wash-out experiment can be added without the need to handle the animal between the experiments.

Pixel noise in the video picture was minimized by smoothing algorithms of the proprietary VisioTracker software, enabling highly precise measurements of eye position and angular velocity. Furthermore, to facilitate statistical analysis, the software filtered out saccadic movements which occur at fixed velocity and do not contribute to the experimental statement. An averaging of velocity curves over 7 video frames facilitated later analysis.

The VisioTracker opens a new dimension for many varied research areas. The system and its predecessors have already been used successfully to quantify visual performance in zebrafish larvae, using parameters such as visual acuity, contrast sensitivity and light adaptation (Rinner *et al.*, 2005a, Schonthaler *et al.*, 2010), for functional analysis of cone photoreceptors following manipulation of members of the visual transduction cascade (e.g. Rinner *et al.*, 2005b, Renninger *et al.*, 2011), or the analysis of visual defects in mutant zebrafish larvae (e.g. Schonthaler *et al.*, 2005, 2008; Bahadori *et al.*, 2006). The interdependence of morphological and functional maturation of the visual system has been studied by OKR measurements to show that visual acuity is mainly but not completely limited by photoreceptor spacing at larval stages (Haug *et al.*, 2010).

The VisioTracker is equally suitable to analyze visual function in adult zebrafish and other similar sized fish species (Mueller and Neuhauss (2010), this report).

It is also conceivable to utilize the system in research areas such as toxicology or pharmacology whereby substances to be investigated might be added to the water flow surrounding the adult fish gills. Furthermore, the versatility of VisioTracker enables more thorough analyses for example of ontogenetics of visual function, neural circuit function and development, or sensorimotor control (see review in Huang & Neuhauss, 2008).

Disclosures

Oliver D.R. Schnaedelbach and Holger D. Russig are employees of TSE Systems GmbH that produces the visual performance tracking system used in this article. Production of this article was sponsored by TSE Systems GmbH. Stephan C.F. Neuhuass is an employee of the University of Zurich which receives remuneration by TSE Systems for each system sold.

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