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# Application of zebrafish oculomotor behavior to model human disorders

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## Abstract

To ensure high acuity vision, eye movements have to be controlled with astonishing precision by the oculomotor system. Many human diseases can lead to abnormal eye movements, typically of the involuntary oscillatory eye movements type called nystagmus. Such nystagmus can be congenital (infantile) or acquired later in life. Although the resulting eye movements are well characterized, there is only little information about the underlying etiology. This is in part owing to the lack of appropriate animal models. In this review article, we describe how the zebrafish with its quick maturing visual system can be used to model oculomotor pathologies. We compare the characteristics and assessment of human and zebrafish eye movements. We describe the oculomotor properties of the zebrafish mutant *belladonna*, which has non-crossing optical fibers, and is a particularly informative model for human oculomotor deficits. This mutant displays a reverse optokinetic response, spontaneous oscillations that closely mimic human congenital nystagmus and abnormal motor behavior linked to circular vection.

**Keywords:** eye movements; human disorders; nystagmus; oculomotor behavior; zebrafish.

## Introduction

Eye movements are a common behavior present in all vertebrates and an integral part of high resolution vision. The main purpose of the oculomotor system is to either stabilize an image on the retina or to shift the eyes to an object of interest. The latter movement is common in foveated animals and is hence referred to as foveation.

To reach maximal acuity in vision, motion of the image on the retina must be kept at a minimum. Motion of the retina relative to the environment, either caused by self-motion or motion of the object of interest, results in blurred vision. To adjust for this relative movement of the image on the retina, referred to as retinal slip, fine balanced compensatory eye movements have evolved in all vertebrates and in some invertebrates (reviewed in Huang and Neuhauss, 2008).

The regulation of these oculomotor movements is also integrated in the postural control system as efference or re-afference output signals to ensure postural stability. Therefore, eye movements have an impact on both visual system performance and postural stability.

Pathological forms of eye movements such as infantile nystagmus entail inconvenient side effects such as reduced visual acuity (Dell'Osso, 1991) or decreased motion perception (Bedell, 1992) along with general decrease in quality of life (Pilling et al., 2005).

## Types of eye movements

Different types of eye movements exist to stabilize the image on the retina. They can be classified into two groups: gaze stabilizing eye movements and gaze shifting eye movements. Gaze stabilizing eye movements include the optokinetic response (OKR) and the vestibulo-ocular reflex (VOR), whereas gaze shifting mechanisms comprise the saccadic system, smooth pursuit movements and vergence movements. Gaze stabilizing eye movements are compensatory oculomotor reflexes aiming to reduce retinal slip, whereas gaze shifting eye movements are used to attend to a previously identified target. These systems involve different neural circuits, but they converge on the same efferent oculomotor system, which includes the six eye muscles moving the eyes.

### OKR (optokinetic response)

The optokinetic system uses visual information to activate the oculomotor musculature. Movement of the visual world triggers eye movements trying to minimize retinal slip. The OKR produces a nystagmus (optokinetic nystagmus) when a scene is continuously moving. In this case slow eye movements following the moving scene are interspersed by fast resetting phases, also referred to as saccades.

Pretectal neurons that receive input from the retina encode velocity and direction of the retinal slip, in that their firing

rate increases when the velocity of retinal slip increases. This velocity information is relayed via pontine and medullary neurons to vestibular nuclei.

#### VOR (vestibulo-ocular reflex)

Rotations of the head are detected by the semicircular canals in the vestibular organs. The VOR uses this vestibular information to counter-rotate the eyes in order to stabilize the line of sight. Vestibular information (afferent signal) is sent to the vestibular nuclei, where visual and vestibular information is integrated and an efference signal, the oculomotor command, is generated and sent to the oculomotor neurons that control the extraocular muscles. Adaptation of the VOR is regulated by the cerebellum. Here visual information is used to monitor current VOR performance compensating for head movements. Deviations are signaled to adjust the VOR.

The VOR is highly efficient at high frequencies but rather inaccurate at low frequencies, whereas the OKR is most efficient at low frequencies and its capacity drops when reaching a frequency of approximately 1 Hz. Hence, the combination of OKR and VOR can achieve a near perfect stabilization across a broad range of movement velocities.

#### Saccadic system

Saccades are rapid ballistic movements used to shift gaze. The saccadic system uses visual, somatosensory, and auditory information to identify a target and shift the gaze to it.

#### Smooth pursuit

The smooth pursuit system serves to identify a small moving target and shift the gaze such that it follows the moving stimulus. Smooth pursuit eye movements minimize the retinal slip of a visual target while producing an increased retinal slip for the rest of the world. Thus, when the visual stimulus involves the entire visual world, the optokinetic system is used, whereas when the visual stimulus is only a tiny portion of the visual world, the smooth pursuit system comes to the fore.

Although gaze stabilizing mechanisms can be found in essentially all vertebrates (Walls, 1962), and even in some invertebrates (Horridge, 1967; Horridge and Burrows, 1968; Frost, 1975; Neil, 1975), gaze shifting mechanisms have evolved in a subgroup of vertebrates that exhibit a fovea. The fovea is a highly specialized region in the central retina that is densely packed with photoreceptor cells enabling high resolution. This structure would be useless, unless it could be specifically targeted to areas of interest in the visual world. To make best use of this retinal specialization, foveated species have evolved gaze shifting mechanisms, which employ the extraocular muscles and redirect the fovea on the target. Neural components of the gaze shifting system are thought to have evolved from gaze stabilizing mechanisms. The smooth pursuit system is believed to be evolutionarily tightly linked to the optokinetic system, as both systems produce eye movements that limit the velocity with which a visual stimulus moves across the retina. Similarly, the saccadic system appears to have evolved from behavioral mechanisms shared by the OKR and VOR (for a textbook treatise on eye movements see Squire et al., 2008).

### Comparison of human and zebrafish oculomotor movements

Because zebrafish eyes do not have a fovea, their eye movements are confined to the gaze stabilizing mechanisms OKR and VOR, which they exhibit robustly (Clark, 1981; Brockerhoff, 2006; Fleisch and Neuhauss, 2006; Huang et al., 2010).

Similarly, lacking cortical (cerebral) structures, zebrafish do not display smooth pursuit eye movements. Therefore, zebrafish allow the study of the OKR without the complication of smooth pursuit as present in humans and other foveated vertebrates. Another important difference that distinguishes the visual system of humans and zebrafish is the position of their eyes. Humans have fronted eyes with a large binocular overlap, whereas zebrafish are lateral eyed animals with minimal binocular overlap. Thus, the anatomy of the optic nerve projections is different. In zebrafish, all optic fibers cross at the midline forming a complete optic chiasm, whereas in humans approximately half of the optic fibers project to the ipsilateral side of the tectum. In summary, the rather simple brain structure of zebrafish with its well developed visual system gives this teleost animal certain advantages for vision study including ocular motor research.

#### Recording human eye movements

Several techniques are available to measure eye movements in humans, each with its relative merits. The simplest, but least precise method is clinical observation (including ophthalmoscopy). It is non-invasive, very efficient in assessing fixation, and has a resolution of approximately 10 min of arc (Zee, 1978). Clinical observation is subjective and does not allow for quantitative analysis.

The magnetic search coil technique is the most reliable and flexible method for measuring human eye movements and is therefore used in a wide range of applications (Robinson, 1963; Judge et al., 1980; Collewijn et al., 1985b; Imai et al., 2005). In this technique, a search coil (coils embedded into a tightly fitting contact lens) is positioned onto the eye. When applying a magnetic field, the amplitude of the electric current generated through electromagnetic induction varies with direction and angular displacement of the eye, enabling the exact determination of eye position. The search coil technique enables the measurement of eye movements around all three axes with a high angular (~0.05°) and temporal resolution (<500 Hz) (Fetter and Haslwanter, 1999; Bergamin et al., 2004). This very precise method is fairly expensive and the placement of the search coil can irritate the eye or scratch the cornea.

Electro-oculography (EOG) is the most widely used method for measuring eye movements in the clinic. It is non-invasive, inexpensive, and fairly precise. In this method the

standing voltage between the front and the back of the eye is recorded by suitably placed electrodes on the skin near the eye. The measured voltage correlates well with eye movements. EOG enables the accurate recording of a large range of horizontal eye movements. Owing to artifacts introduced by eye lids, vertical eye movements cannot be reliably measured (Barry and Jones, 1965). EOG, with a maximal angular resolution of approximately 1°, is relatively insensitive owing to voltage changes caused by muscle activity and other sources. Furthermore, EOG has a baseline drift and limited bandwidth as a result of the filtering necessary for the removal of electrical background noise. Recently, improved computer algorithms have become available to correct for some of these shortcomings (Coughlin et al., 2004).

Video-based systems (either by tracking the pupil or the reflection of an image on the cornea) to record eye movements are also popular because they are non-invasive and well tolerated, even by children. As head movements cannot be distinguished from eye movements, either the head has to be fixed, or the recording device has to be attached to the head (DiScenna et al., 1995; Das et al., 1996). The temporal resolution of video-based systems, although not as high as that obtained with the magnetic search coil technique, has improved in recent years. Other methods for the measurement of eye movements in humans exist such as infrared differential limbus reflection technique, Purkinje image tracker, and ocular electromyography which will not be discussed further here (Eggert, 2007).

### Recording zebrafish eye movements

The OKR is the most widely used oculomotor behavior assessed in zebrafish. Various types of set-ups to measure the larval (Roeser and Baier, 2003; Beck et al., 2004a; Orger et al., 2004; Rinner et al., 2005; Brockerhoff, 2006; Huang and Neuhauss, 2008) and adult (Mueller and Neuhauss, 2010; Zou et al., 2010) zebrafish OKR have been described. Although there are slight differences in these methods, they all follow the same basic principle: a moving grating is presented to an immobilized larva, which elicits robust eye movements allowing further investigation of visual behavioral properties of the fish.

To record a clean OKR without the influence of the vestibular input signal the larva needs to be restrained. Typically the larva is immobilized by placing it into methylcellulose (3%), a viscous non-toxic medium that allows oxygen consumption through the skin of the larva and constrains swimming without significantly influencing eye movements. Alternatively, the body of the larva can be embedded in a block of low-melting agarose with head and gills exposed to water (Beck et al., 2004a).

In initial experiments, the embedded larva was placed inside a rotating drum fitted with stripes of various width and contrast, a method still widely used today. In this application, temporal aspects can be changed simply by manipulating the speed of the drum. The most convenient stimulus to measure zebrafish OKR is a computer-generated sine-wave grating that allows continuous variation of contrast, velocity, spatial/temporal frequency, color, and any other stimulus property. A digital projector is used to project the moving grating to the paper drum surrounding the larvae. Different projection modes are applied such as direct linear projection (Rinner et al., 2005) allowing monocular stimulation or the projection via a mirror placed below the larval 'movie theater' (Roeser and Baier, 2003; Mueller and Neuhauss, 2010) enabling binocular stimulation (Figure 1A).

Eye movement tracking can be done more simply by visual inspection (Brockerhoff et al., 1995; Easter and Nicola, 1996, 1997; Brockerhoff et al., 1998; Neuhauss et al., 1999; Muto et al., 2005), allowing for a fast assessment of presence, absence, or impairment of the OKR. Although this qualitative method is suitable for fast screening of vision mutants, a more precise and quantitative method of eye movement recordings is video imaging which allows computer-based tracking of eye position (Roeser and Baier, 2003; Beck et al., 2004b; Rinner et al., 2005; Mueller and Neuhauss, 2010). For this method image series are acquired by a CCD camera mounted onto a microscope. The larva is illuminated from below with an infrared light to avoid interference with the light stimulus. The larval eyes are tracked by software that extracts the shape of the eye from the image based on the darker pigmentation of the eye (Figure 1B and C).

As stimulus parameters and experimental paradigms are arbitrarily variable, a very diverse data set can be generated that allows for a detailed analysis of eye movement properties.

The most important variable of quantitative OKR analysis is velocity. Velocity is usually referred to as slow phase velocity (SPV), referring to eye speed in degrees/second during slow phases. Therefore, fast phases (saccades) and slow phases have to be separated first by algorithms or 'saccade filters' that identify the fast phases based on the high peak velocity and the large acceleration at onset (Beck et al., 2004b; Mensh et al., 2004). Another frequently used measure is the gain (SPV/stimulus velocity), which measures the input-output efficiency. A gain of 1.0 denotes that the eye rotates with exactly the same speed as the stimulus, thus leading to perfect stabilization of the image on the retina. A lesser gain indicates that the eye lags behind the visual stimulus, thus only partly compensating for retinal slip. A gain higher than 1.0 indicates overcompensation as the eye rotates faster than the visual stimulus. Moreover, saccade performance can be analyzed by calculating the ratio of peak saccade velocity and amplitude (Beck et al., 2004a). The peak saccadic velocity follows a linear relationship with the saccade amplitude (Beck et al., 2004a), meaning that the greater the amplitude (displacement of the eye) the more rapidly the saccade is performed. Therefore, saccade performance can be expressed as the peak saccadic velocity-amplitude ratio.

## Application of the zebrafish OKR

### Screens for visually impaired zebrafish strains

The zebrafish OKR is a stereotypic and robust visually mediated behavior that starts during development at around 73 h

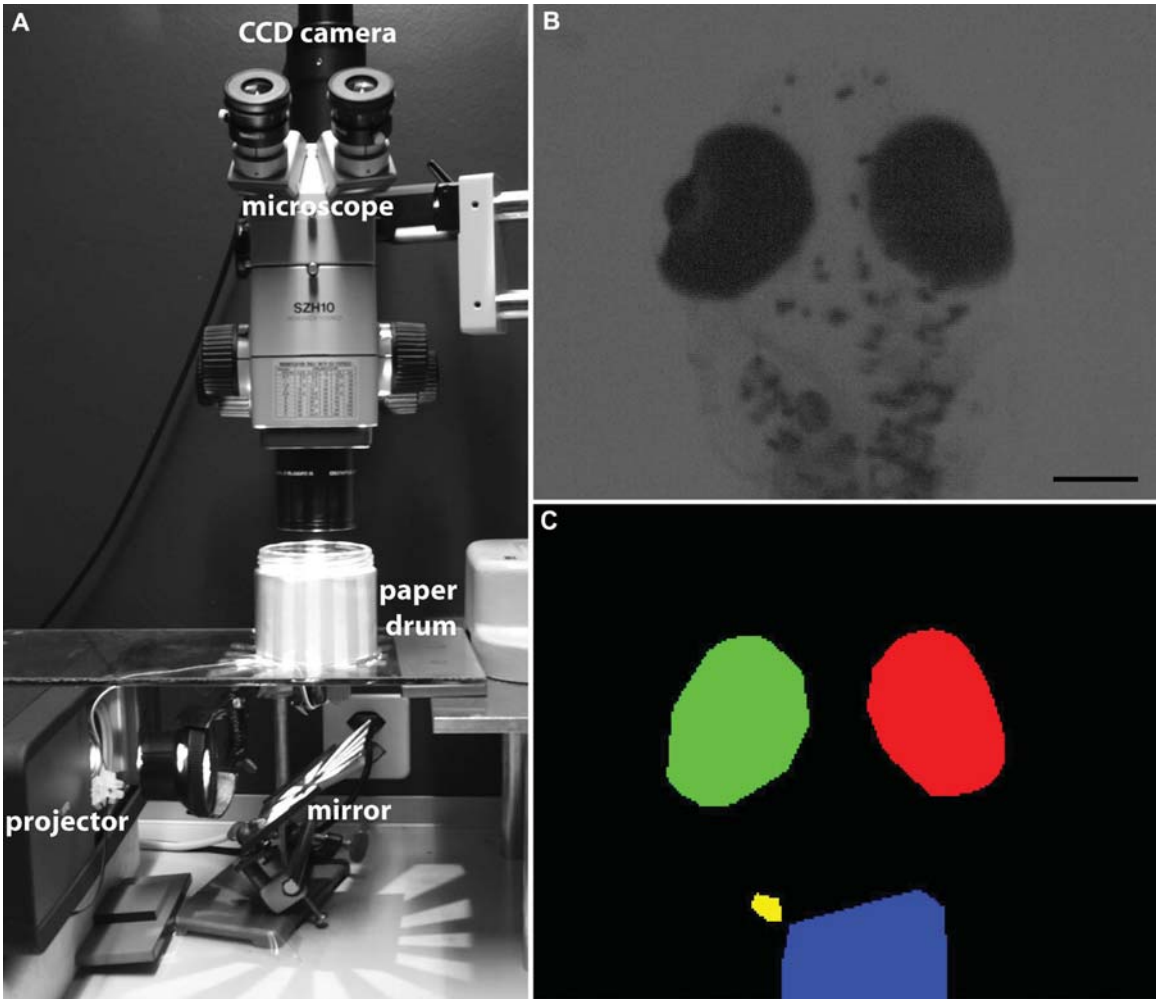


**Figure 1** Set-up to measure the zebrafish optokinetic response.
(A) Experimental apparatus to track the zebrafish OKR. A projector connected to a computer is used to generate the stimulus pattern which is projected to a mirror placed underneath the 'movie theater'. The stimulus pattern is reflected in the mirror and directed onto a paper drum surrounding the larva that is restrained in a dish in the center of the drum. A CCD camera on top of a binocular microscope is used to track eye movements of the larva. (B) High resolution image recorded by the CCD camera. (C) The software extracts the shapes of the eyes out of the image based on the pigmentation criteria. Scale bar is 100 μm.

post-fertilization (hpf) (Easter and Nicola, 1996, 1997) and reaches a steady adult-like level of performance at around 4 days post-fertilization (dpf). This is a reflexive behavior that needs no prior training to develop. This rapid development of the visual system function provides an excellent basis to study all types of functional aspects of vision. Zebrafish are not only diurnal (day active), hence having a cone dominant retina that supports tetrachromatic color vision, but are also well established as genetic models. The investigation of the zebrafish visual system was pioneered by Clark (1981) who was the first to suggest conducting mutagenesis screens for OKR defective mutants. Such a behavioral mutagenesis screen was later realized by John Dowling and colleagues who used the OKR to isolate several visually impaired zebrafish mutants (Brockerhoff et al., 1995). The success of this screen validated the concept of behavioral screening, which was subsequently continued by several other groups (Neuhauss et al., 1999; Neuhauss, 2003; Gross et al., 2005; Muto et al., 2005) and led to the isolation of a wealth of mutant strains. Genomic mapping of the mutated gene and the detailed analysis of the visual system by morphology, neural tracing, and electrophysiology revealed the underlying cause of visual deficiency of a growing number of mutants.

Visual behavior screening has so far focused on larval zebrafish, mainly owing to the lack of an adult OKR set-up. Only one small-scale adult vision screen for dominant mutants using the escape response has been conducted (Li and Dowling, 1997). Hence, a large number of adult visual mutants await isolation. Such mutants will be of particular medical interest, because many blinding diseases in humans are progressive leading to vision impairment in aged patients. The adult OKR is notoriously difficult to measure owing to the impossibility to immobilize the fish in methylcellulose, which would block the gills, the larger oxygen demand that cannot be sufficiently

met by diffusion through the skin and adult pigmentation, which complicates automated eye tracking. These difficulties have now been circumvented by the construction of a transparent flow-through chamber which restrains the fish and at the same time allows the supply of fresh water flushing the gills (Mueller and Neuhauss, 2010). The eye tracking software was adjusted by overlying a virtual white hourglass-shape in place of the pigmented body of the fish to extract the eye out of the image. This new set-up might not only allow screening for adult vision mutants but also for a more detailed quantitative analysis of vision, either in the wild-type, mutant, or pharmacologically treated fish. An alternative set-up for adult OKR has been described by Zou et al. (2010), who suggest immobilizing the adult fish with pins on a sponge. This method, however, is more time-consuming and relies on visual inspection of the eye movements and is, therefore, less suitable for a quantitative analysis of eye movements.

### Quantitative analysis of zebrafish visual performance

The zebrafish OKR has been successfully used to characterize visual performance (Easter and Nicola, 1996, 1997; Haug et al., 2010) to pinpoint defects in visual performance or visual pathway abnormalities (Rick et al., 2000; Huang et al., 2006) and to study how the retina decodes motion (Orger et al., 2000; Roeser and Baier, 2003).

Easter and Nicola (1996, 1997) undertook a detailed analysis of the development of the larval zebrafish oculomotor system. They have found that the earliest eye movements in response to a rotating drum can be evoked at 73 hpf. Being somewhat sluggish at this stage the accuracy steadily improves until adult-like performance is reached by 4 dpf. To deduce whether visual experience is needed to develop an OKR, they investigated the OKR of 5-day-old dark-reared fish and found an appropriate OKR response within minutes, indicating that no 'trial and error' phase of learning is required. Instead the polarity of the OKR is hard-wired. Moreover, the eye progresses from hyperopic (far-sighted) to emmetropic (in focus for objects at infinity) by approximately 72 hpf, coincident with the onset of OKR, without requiring visual experience. Therefore, the development of emmetropia does not contribute to the improvement of OKR between 72 hpf and 4 dpf. All six extraocular muscles were found to be present at 72 hpf supporting the onset of eye movements. The thickening of these muscles from 72 hpf to 4 dpf, however, might be at least partially responsible for the improvement of OKR. In summary, visual function is enabled by the simultaneous acquisition of retinal image formation, functional extraocular muscles, and projections from the retina to all retinofugal targets in the brain (Burrill and Easter, 1994) at 73 hpf. The improvement of OKR from 72 hpf to 4 dpf is probably attributable to the further maturation of the motor system rather than the sensory system (Easter and Nicola, 1996, 1997).

The OKR was recently used by Haug et al. (2010) to determine whether maximal visual acuity, as limited by photoreceptor spacing, can be fully translated into visual behavior. Visual acuity was behaviorally assessed by measuring the gain of the OKR evoked by a moving grating of varying spatial frequency. The visual acuity of a 5-day-old zebrafish larva was determined to be 0.16 cycles per degree (cpd) or 3.1°. This behavioral performance value was compared to the physical limitation of resolution imposed by photoreceptor spacing. The distance between red-green double cones, the relevant photoreceptors for motion detection (Schaerer and Neumeyer, 1996; Krauss and Neumeyer, 2003; Orger and Baier, 2005), was measured based on immunohistochemically stained tangential sections. The morphologically determined, theoretical maximal acuity value was found to be 0.24 cpd or 2.09°. Although small, the discrepancy between the behaviorally measured and the theoretical visual acuity indicates that the larval visual system cannot fully translate visual information into behavior.

Behavioral screening isolated a wide variety of visually impaired mutants with different defects. One mutant called *nrc* (no optokinetic response c) was isolated in such a screen owing to its defective OKR (Brockerhoff et al., 1995). The subsequent in-depth analysis of this mutant revealed that the ribbons in the cone photoreceptor synapse are unanchored (Allwardt et al., 2001) and that this was the underlying cause of defective visual transmission at the cone photoreceptor cell synapse revealed by electroretinography (Van Epps et al., 2001). The phenotype was linked to mutations in the synaptojanin 1 gene (Van Epps et al., 2004). Synaptojanin 1, a polyphosphoinositide phosphatase, was shown to be essential for proper cone photoreceptor ribbon synapse structure, function, and vesicle maintenance in the cone pedicle (Van Epps et al., 2004). Further visual behavior investigations showed that *nrc* mutants are still able to detect light decrements as they react with locomotion activity similar to wild-type larvae in response to light-off stimuli (Emran et al., 2008). The responses to light increments (light-on stimuli), by contrast, were sluggish and delayed compared with wild-type light-on responses. These visual motor response measurements and the investigation of the off-response by electroretinogram showed that the retinal OFF-pathway is not or only slightly affected by the loss of synaptojanin 1 function. Single-unit recordings of ganglion cells in the *nrc* retina confirmed that this mutant has a remaining OFF-pathway but has a defective ON-pathway, as ON-, but not OFF-responses of ganglion cells were found to be essentially absent in the *nrc* retina (Emran et al., 2008). These observations indicate that the retinal OFF-pathway in zebrafish cannot detect the motion required for initiating the OKR.

The *nrc* mutant is a prime example of how behavioral assessment can be used to isolate visual mutants, and how the combination of morphological, electrophysiological, and behavioral analysis leads to a more detailed understanding of synaptic function. Given that in this mutant only the ON-pathway is affected, it can be concluded that the OFF-pathway is not sufficient to drive the OKR.

## Modeling oculomotor defects

### Classification of waveform characteristics of nystagmus

To compare eye movement defects in the zebrafish to humans, it is necessary to accurately study the waveform characteristics

of the different forms of human nystagmus. Such a classification has been proposed by Dell'Osso and Daroff (1975), who grouped nystagmus waveforms into three main subgroups based on waveform characteristics: the pendular nystagmus, the jerk nystagmus, and the dual nystagmus (Figure 2).

The pendular nystagmus is a sinusoidal oscillation of the eyes biased such that the fovea rests on the target at one or the other peak of the waveform. The purity of the sinusoidal form is verified by the velocity waveform which is particularly sensitive to any saccadic components that can be present.

The Jerk nystagmus is initiated by a slow drift of the fovea off the target, followed by a fast phase (saccade) which both stops the drift and fully or partially corrects the eye position error (refoveating saccade). The waveform has a classical saw-tooth appearance and the velocity waveform shows spikes.

The dual jerk nystagmus consists of the simultaneous mixture of jerk and pendular nystagmus with the superimposition of a rapid small amplitude oscillation upon the larger amplitude jerk nystagmus.

### Types of human eye movement disorders

Nystagmus, involuntary oscillatory eye movements, can be induced in healthy subjects by self or world motion in an effort of the oculomotor system to stabilize the image of the visual world on the retina. The optokinetic as well as the vestibular system are involved in producing this involuntary, conjugate jerk nystagmus. However, nystagmus is mostly a pathology of the oculomotor system, resulting in several quality of life affecting issues, such as loss of visual acuity, decreased motion perception, and postural control problems (Dell'Osso, 1991; Bedell, 1992; Pilling et al., 2005).

There are several human diseases that result in nystagmus. Pathological nystagmus can be acquired or present at birth. The former can be caused by several neurological disorders, ranging from aniridia to tumors and stroke. Moreover, several drugs, toxins, and metabolic changes can lead to the development of nystagmus (Stahl et al., 2000).

Pathological forms of nystagmus that are present at birth, referred to as congenital or infantile nystagmus, can have a wide variety of poorly understood underlying causes. Therefore, a subclassification of congenital nystagmus (CN) forms based on clinical observation, as suggested by Gottlob (2000), is helpful. According to this classification scheme, CN can be classified as (i) idiopathic (of unknown cause), (ii) associated with albinism, (iii) latent/manifest, (iv) spasmus nutans (associated with torticollis and head nodding), and (v) sensory (associated with afferent visual system deficits).

A detailed review of human acquired and infantile nystagmus is beyond the scope of this article. Interested readers are referred to current textbooks (e.g., Brodsky, 2010).

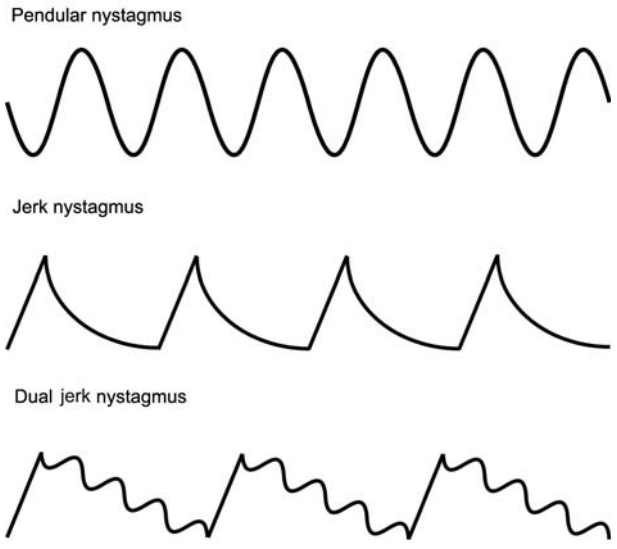


**Figure 2** Nystagmus waveforms.
Different forms of nystagmus can be grouped into three main groups based on their waveform characteristics (Dell'Osso and Daroff, 1975). Pendular nystagmus is a regular sinusoidal oscillation in which the target is foveated at each peak of the waveform. Jerk nystagmus is characterized by a slow drift off the target followed by a fast refoveating saccade. Depicted is a schematic waveform of a jerk nystagmus with decelerating velocity slow phase which is typical of cerebellar gaze-evoked nystagmus. However, slow phase velocity can also be constant (vestibular nystagmus) or accelerating (congenital nystagmus). Dual jerk nystagmus consists of a jerk nystagmus superimposed by a simultaneous pendular nystagmus of low amplitude.

### Modeling human eye movement disorders in zebrafish

Most zebrafish mutants isolated by using the OKR are characterized by the absence of visual stimulus inducible eye movements. Such mutants are very informative for outer retinal disorders, but less suitable for studying eye movement disorders that are not caused by loss of vision. One of the few mutants that proved to be informative for eye movement disorders is the *belladonna* mutant. This mutant is not blind, but has abnormal eye movements due to wiring defects of the optic nerve. It provides an excellent model for human eye movement disorders such as CN and non-decussating retinalfugal fiber syndrome.

Interestingly, none of the known hypopigmented zebrafish mutants show defects in optic nerve crossing, even those that are tyrosinase negative.

## The oculomotor mutant *belladonna*

The recessive *belladonna* mutant was isolated in a mutagenesis screen due to its aberrant retinotectal projections (Karlstrom et al., 1996). The pupils of homozygous *belladonna* larvae are enlarged due to a gap between retinal pigment epithelium and lens, which most probably results from a failure in posterior compartment formation (Seth et al., 2006). Thus, the mutant was named after a plant, *atropa belladonna*, whose toxin causes pupil dilation in humans.

*belladonna* carries a mutation in the gene coding for Lhx2, a Lim-homeodomain transcription factor. Disruption of this gene leads to defects in forebrain patterning and lack of midline crossing of retinal ganglion cell axons at the optic chiasm in the zebrafish (Seth et al., 2006). Consequently, in approximately half of homozygous mutant larvae, retinal ganglion cells project to the ipsilateral instead of the contralateral tectum, leading to achiasmatic larvae.

**OKR reversal** Analysis of the OKR in homozygous *belladonna* larvae, that were distinguishable by their eye phenotype, showed that 20–50% exhibited a curious reversal of the OKR. In these larvae, a clockwise moving stimulus resulted in counterclockwise pursuit movements (Neuhauss et al., 1999). This behavior was perfectly correlated to failure of the retinal ganglion cell axon to cross the midline (Neuhauss et al., 1999; Rick et al., 2000). This achiasmatic condition was termed *belladonna reversed* (*bel rev*), whereas the remaining homozygous mutants with normal crossing retinal ganglion cell projections were termed *belladonna forward* (*bel fwd*) (Huang et al., 2006). These *bel fwd* larvae serve as perfect controls because they have disrupted *lhx2* function but are not achiasmatic, helping to delineate defects that are attributable to the wiring defect and not to other defects caused by *lhx2* disruption.

The reversal of the optokinetic response in *bel rev* is readily explained by a simple qualitative model (Figure 3). In the chiasmatic wild type or *bel fwd* larva, visual information from a monocular stimulated eye crosses the midline and is received by a still unidentified pretectal midbrain nucleus X. In this parsimonious model, information is transmitted to the integrator nucleus, before it crosses the midline again to contact motor nuclei that drive eye muscles and thereby move the eye in the appropriate direction.

In achiasmatic *belladonna* mutants, information from the stimulated eye does not cross the midline. Hence, visual information is handled by the ipsilateral nucleus X. From there on the information path is the same as in unaffected larvae. Because visual information crosses the midline only once instead of twice, eye muscles of the unstimulated eye respond to the movement information from the stimulated eye. Consequently, the stimulated eye, due to conjugated eye movements, is moved in the reverse direction of the stimulus.

Therefore, the OKR which aims to minimize retinal slip leads to an increase in retinal slip in the *bel rev*. A detailed behavioral analysis of the OKR of *belladonna* showed that contrast sensitivity and peak saccadic velocity in *bel rev* mutants are similar to *bel fwd*. This implies that the oculomotor instabilities seen in *bel rev* can neither be explained by motor system impairments nor by the slightly compromised vision in *bel* mutants. Thus, neither information processing nor execution of commands can be linked to the *bel rev* behavioral phenotype, supporting the simple model.

Therefore, the reversed OKR found in *bel rev* mutants can be solely attributed to the ipsilateral projection of RGC axons. Further support for this interpretation stems from a quantitative mathematical model based on measured OKR parameters of wild-type larvae (Huang et al., 2006) (Figure 4A and B). This model can almost perfectly reproduce the waveform characteristics observed in *bel rev* mutants by solely inversing the sign of retinal slip error signal from 1 (*bel fwd* and wild type) to -1 (*bel rev*). Therefore, the quantitative model supports the hypothesis that axonal miswiring is the sole cause of the reversed OKR in *bel rev* mutants.

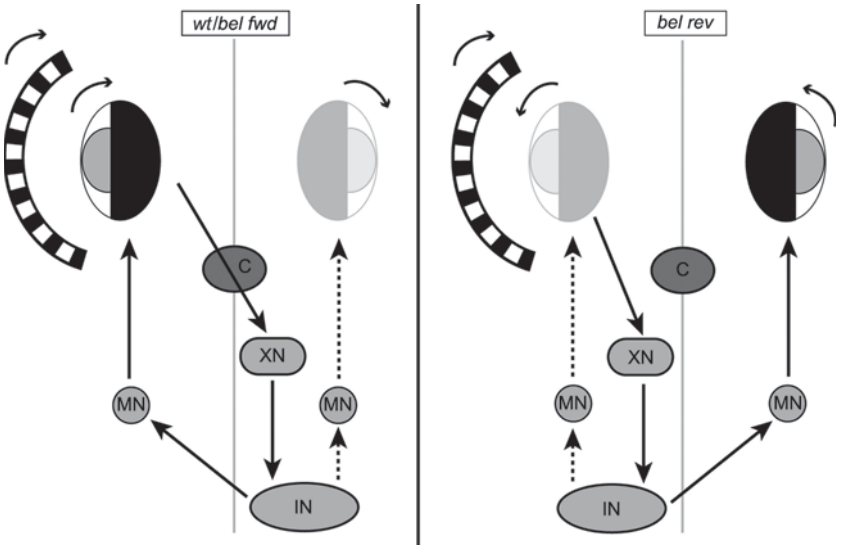


**Figure 3** Qualitative model of *belladonna* neuroanatomical properties.
In the *wt/bel fwd* case, the stimulated eye (darker) sends the retinal slip error signal to a yet unknown contralateral located nucleus X (XN) which forwards the information to the integrator nucleus (IN). From there, the signal again crosses the midline while it is passed to the motor nucleus (MN) of the stimulated eye, which leads to a compensatory eye movement in the direction of the stimulus. In *bel rev*, by contrast, the afferent signal from the stimulated eye is sent to the ipsilateral located nucleus X (XN), and from there processed as in *wt/bel fwd*. As a consequence, the stimulated eye is moved in the wrong direction leading to an increase of retinal slip.

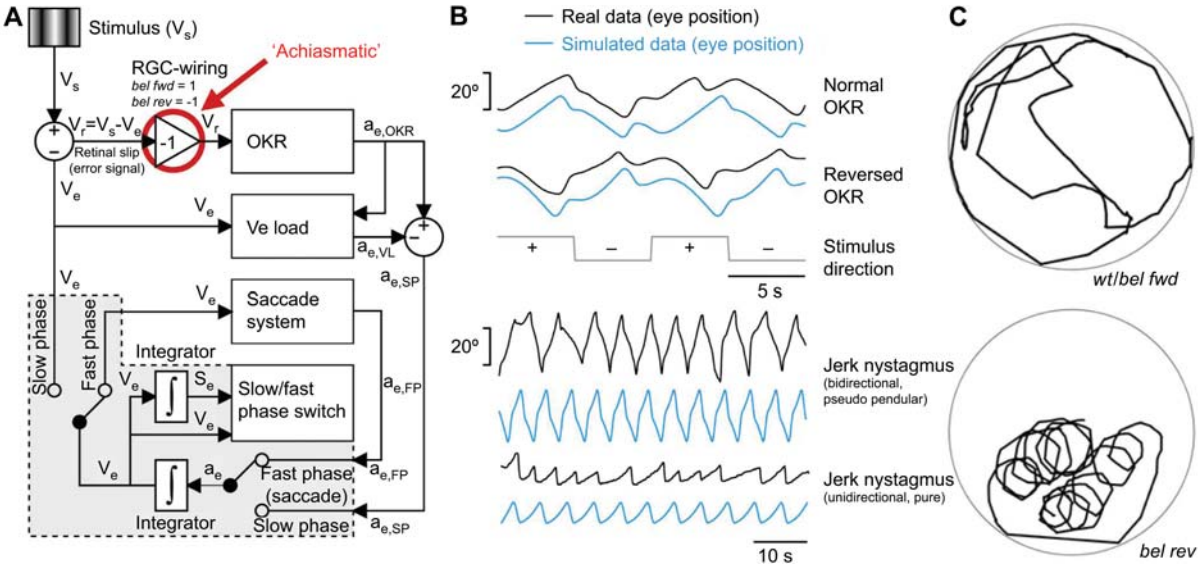


**Figure 4** The *belladonna* model and achiasma-related behaviors.
(A) A simplified version of the *belladonna* mathematical model is depicted. The input signal for the achiasmatic condition is changed from 1 to -1, which reproduces the waveforms recorded in *bel rev*. (B) Waveform traces of recorded data versus simulated data. The OKR is reversed in achiasmatic *belladonna* mutants; moreover, spontaneous oscillations (OSs) in these fish resemble the waveforms of CN patients exhibiting unidirectional and bidirectional jerk nystagmus. (C) Traces of locomotion tracking of a *wt/bel fwd* larva (upper panel) and a *bel rev* larva is shown. The *bel rev* larva circles around a virtual axis, a swimming behavior termed looping, whereas *wt/bel fwd* larva swim around randomly.

**Spontaneous oscillations** In addition to the reversed OKR, *bel rev* mutants show another intriguing oculomotor behavior, apparent as spontaneous oscillations (SOs), which closely resemble the involuntary eye movements of patients suffering from CN. SOs in *bel rev* mutants are triggered not only by an initial eye movement, which itself is induced by the moving pattern stimulus, but also by a prolonged presentation of a still grating leading to deferred initiation of SOs subsequent to a spontaneous saccade or body movement. In darkness, SOs cease in wild type, *bel fwd* as well as *bel rev* mutants, indicating that SOs are visual input-dependent. The additional observation that SOs are modulated by stimulus contrast in a similar way than the reversed OKR strongly implies that both oculomotor behaviors are caused by axonal miswiring. Again, the mathematical model helped to support this hypothesis, because also the SOs could be simulated by reversing the input sign in the model (Huang et al., 2006) (Figure 4B).

The mathematical model as well as *belladonna* waveform recordings are able to replicate transitions in waveforms similar to the periodic alternating nystagmus found in human patients with albinism (Abadi and Pascal, 1994; Shallo-Hoffmann et al., 1999). Periodic alternating nystagmus is characterized by regular epochs of 'active' and 'quiet' phases. The active phases of horizontal jerk oscillations (jerk nystagmus) with regular reversals in directions are separated by the quiet phases or transitional phases of low intensity eye movements. Unidirectional Jerk nystagmus simulation by the mathematical model is generated when pre- and postsaccadic eye velocity (Ve, exit) are equal in sign, whereas bidirectional jerk nystagmus is produced when pre- and postsaccadic velocities differ in sign. Random variation of the pre- and postsaccadic eye velocity results in periodic alternating nystagmus. This simulation shows that the SOs in the *bel rev* mutant can be compared to human CN waveforms and that the model even reproduces transitions in waveform characteristics.

**Postural control and looping** Eye movements serve to decrease the retinal slip in response to movements of the visual world. Retinal slip is not only perceived when the visual world moves but also during self-motion, such as head and body movements. Stabilization of an image on the retina can therefore be achieved by appropriate whole body movement. For instance, a fish floating with the current in a river can minimize retinal slip by swimming against the current. Sometimes world motion is perceived as illusionary self-motion (vection), which can lead to postural instability (Fushiki et al., 2005).

Accordingly, free swimming wild-type zebrafish larvae start to circle around a virtual axis outside of the body center when presented with a whole field 360° motion, a swimming behavior termed looping (Huang et al., 2009). Looping in wild-type larvae is a compensatory movement and can be triggered by the perception of illusionary self-motion (circular vection), in order to decrease retinal slip.

Circular vection is also perceived during involuntary eye movements. Similarly, reversed OKR and SOs, the two achiasma-related oculomotor instabilities in *bel rev* mutants, induce circular vection and trigger spontaneous looping behavior in *bel rev* mutants (Huang et al., 2009) (Figure 4C). As

expected, looping behavior tightly correlates with the achiasmatic condition. However, because higher brain centers in *bel rev* mutants receive a reversed afference signal due to the misprojection of RGCs the compensatory eye/body movements lead to an increased retinal slip and opens a negative feedback loop that reinforces looping behavior.

By carefully analyzing the looping behavior in *belladonna* mutants, Huang et al. (2009) could show that looping is visual input-dependent because it is decreased with lower contrast and absent in darkness. Moreover, based on the observation that eye movements are absent during looping behavior, they conclude that visual input (afferent signal) can directly influence postural control without requirements of eye movement related signals (efference copy/reafference signal) (Huang et al., 2009). This is in contrast to the 'efference/reafference-only' hypothesis of earlier studies that was made based on the observation that suppression of spontaneous nystagmus reduces postural sway and that while fixating a target in conditions of unstable stance the retinal slip is close to zero and therefore extraocular signals rather than visual signals are used for postural stability (Jahn et al., 2002; Strupp et al., 2003; Glasauer et al., 2005).

In summary, *belladonna rev* mutants display a set of behaviors that are functionally linked to an achiasmatic retinotectal projection.

Involuntary oscillatory eye movements observed in *bel rev* mutants have been shown to closely resemble the waveform characteristics of CN patients with probably similar underlying neuronal deficits (optic nerve projection defects) (Huang et al., 2006) suggesting that *belladonna* could serve as a disease model for axonal misrouting related CN in humans.

CN and reversed OKR in humans have been associated with albinism (St John et al., 1984; Collewijn et al., 1985a) as well as with hypochiasma (McCarty et al., 1992; Hertle et al., 2002). CN waveforms with exponentially shaped slow phases are a feature that is not only found in axonal misrouting related CN but might also be found in other forms of nystagmus. Therefore, the reversed OKR might be used as a particularly good diagnostic feature for CN linked to visual pathway abnormalities.

The mathematical model generated to simulate waveform characteristics of the *belladonna* eye movements helped to understand and pinpoint the underlying reason of oculomotor instabilities. Thus, whenever waveform characteristics of CN patients are similar to those of *bel rev*, namely exhibit an increasing exponential form and/or reversed OKR, visual pathway abnormalities might be the underlying pathology. Moreover, CN modeling in *bel rev* mutants extends the collection of CN waveform models (Optican and Zee, 1984; Broomhead et al., 2000; Jacobs and Dell'Osso, 2004) showing that mathematical modeling of human CN waveforms might help to decipher the underlying etiology in any form of nystagmus.

Postural instability is not among the main impairments of CN patients; however, it has been reported in connection with other more prominent symptoms of CN. Visually controlled postural imbalance and head tremor/nodding might, however, arise to compensate vection phenomena induced by oculomotor instabilities, in a similar way as looping behavior does in *bel rev* mutants (Gresty and Halmagyi, 1981; Gottlob et al., 1992). Therefore, looping can be compared with postural instability of human CN patients. Moreover, looping opens a door to study vection phenomena and the transition of world motion into self-motion perception, and neuronal input signals to postural control mechanisms.

### Genetic zebrafish models for oculomotor disorders

Apart from achiasmatic Belgian sheepdogs that have a spontaneously occurring mutation (Dell'Osso and Williams, 1995), zebrafish *belladonna* is the only genetic animal model for human oculomotor diseases. Strictly speaking, it is not a genetic model in the sense that the underlying genetic defect is not relevant for any human oculomotor disorder; however, the phenotype recapitulates several important behavioral and possibly anatomical features of human oculomotor diseases. Disrupting the mouse *Lhx2* gene leads to a much more severe phenotype, including anophthalmia, anemia, and cortex malformations (Porter et al., 1997). Human mutations in *LHX2* are likely to be not compatible with life and indeed have not been linked to any oculomotor disorders.

Recently, idiopathic congenital nystagmus (CIN) in humans has been linked to a mutation in the FERM-domain containing protein FRMD7 (Tarpey et al., 2006; Schorderet et al., 2007; Self et al., 2007; Shiels et al., 2007; Zhang et al., 2007a,b; He et al., 2008a,b; Kaplan et al., 2008; Li et al., 2008). The functional role of FRMD7 is under current investigation and might reveal important insights into the development and mechanisms of the oculomotor control system.

Several indications for FRMD7 function during neuronal development were recently revealed (Betts-Henderson et al., 2010). Expression of FRMD7 in the ventricular zone of the human forebrain during early developmental stages suggests a role for FRMD7 in asymmetrical cell division and radial migration of newborn neurons. In later stages, FRMD7 was found to be expressed in postmitotic cells within the developing subplate and cortical plate, suggesting an additional role in axonogenesis and dendritogenesis. Furthermore, *in vitro* studies showed that neuroblastoma cells that were pushed towards differentiation increase FRMD7 expression. Loss of FRMD7 function in these neuroblastoma cells leads a decreased length of neurites during differentiation, suggesting a role for FRMD7 in neuronal guidance and pathfinding. Indeed, the FRMD7 protein was found to localize to actin-rich regions in neurite processes as well as growth cones, indicating that FRMD7 might be involved in the regulation of neuronal cytoskeletal dynamics at the growth cone by transducing signals from membrane receptors to the cytoskeleton. Similar roles have been found for FARP1 and FARP2, two other FERM-domain containing proteins that are known to be modulators of neuronal cytoskeletal dynamics (Kubo et al., 2002; Toyofuku et al., 2005; Zhuang et al., 2009).

Currently, *FRMD7* is the only gene that has been implicated in infantile nystagmus in humans. However, ongoing genetic analyses of CN patients predict that there are more genes to be

linked with this disease (reviewed in Self and Lotery, 2007). The use of zebrafish to study such disease genes is advantageous and might provide valuable information on how they are involved in the pathogenesis of CIN. Excellent imaging techniques combined with the transparency of the zebrafish brain, as well established knockdown techniques, are features unique to the zebrafish and allow an extensive analysis of neuronal development. Moreover, behavioral consequences of loss-of-function can directly be analyzed by OKR testing.

## Outlook

The use of zebrafish to model oculomotor disorders, exemplified in the *belladonna* mutant, provides valuable insights to understand oculomotor diseases in humans such as CN. Moreover, the value of such zebrafish models is increasing because they can be used to screen for drugs or small compounds that potentially reduce the oculomotor instabilities.

Future research using the zebrafish as a model to study oculomotor behaviors, however, will probably focus on the behavior itself and on the identification and formation of the neural components underlying the zebrafish OKR, because little is known about the anatomical substrate of the OKR circuit in lower vertebrates. The emergence of new imaging techniques and optogenetic manipulations are ideally suited for zebrafish larvae with a translucent brain and a simple neuronal network.

Furthermore, the zebrafish has been successfully used in the past to study the expression and function of disease genes in reverse genetic approaches, by means of the morpholino knockdown technique (Penberthy et al., 2002; Ward and Lieschke, 2002). The identification of genes causing pathological forms of nystagmus when mutated in humans provides a basis for further oculomotor behavioral research in the zebrafish, because the tools to measure visual behavior abnormalities are readily available and are constantly being refined and improved. Such studies in the zebrafish will help to reveal the mechanisms causing enigmatic oculomotor deficits in humans.

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