Individual Larvae of the Zebrafish Mutant belladonna Display Multiple Infantile Nystagmus-Like Waveforms that Are Influenced by Viewing Conditions

Sabina P. Huber-Reggi,1,2 Kaspar P. Mueller,1 Dominik Straumann,3,4 Melody Ying-Yu Huang,3,4 and Stephan C. F. Neuhauss1,3

1Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland
2Life Science Graduate School, Zurich, Switzerland
3Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland
4Department of Neurology, University Hospital Zurich, Zurich, Switzerland

Correspondence: Stephan C. F. Neuhauss, Institute of Molecular Life Sciences, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland; stephan.neuhauss@imls.uzh.ch.
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Purpose. Infantile nystagmus syndrome (INS) is characterized by involuntary eye oscillations that can assume different waveforms. Previous attempts to uncover reasons for the presence of several nystagmus waveforms have not led to a general consensus in the community. Recently, we characterized the belladonna (bel) zebrafish mutant strain, in which INS-like ocular motor abnormalities are caused by misprojection of a variable fraction of optic nerve fibers. Here we studied intrinsic and extrinsic factors influencing the occurrence of different waveforms in bel larvae.

Methods. Eye movements of bel larvae were recorded in the presence of a stationary grating pattern. Waveforms of spontaneous oscillations were grouped in three categories: “pendular,” “unidirectional jerk,” and “bidirectional jerk,” and the occurrences of each category were compared within and between individual larvae. Moreover, the effects of the characteristics of a preceding optokinetic response (OKR), of the field of view, and of the eye orbital position were analyzed.

Results. The different waveform categories co-occurred in most individuals. We found waveforms being influenced by the preceding OKR and by the field of view. Moreover, we found different kinds of relationships between orbital position and initiation of a specific waveform, including pendular nystagmus in a more eccentric orbital position, and differences among jerk oscillations regarding the beating direction of the first saccade or waveform amplitude.

Conclusions. Our data suggest that waveform categories in bel larvae do not reflect the severity of the morphological phenotype but rather are influenced by viewing conditions.

Keywords: infantile nystagmus, waveforms, zebrafish

Infantile nystagmus syndrome (INS), present at birth or shortly after, is a congenital human ocular motor disorder characterized by involuntary conjugate, predominantly horizontal, oscillations of the eyes,1,2 which can have an adverse impact on occupational and social functioning.3,4 The prevalence is approximately two cases per 1000 individuals.5 Eye oscillations can display pendular or jerk waveforms. Pendular nystagmus is a sinusoidal oscillation, whereas jerk nystagmus is characterized by accelerating slow drifts and fast resetting phases (saccades). Although INS can be idiopathic, it is often associated with visuosensory abnormalities such as congenital cataract, foveal hypoplasia, misprojecting optic nerve fibers, or aniridia.6 Attempts to classify INS according to the associated condition and the eye oscillation characteristics in patients have led to contradictory conclusions,7–11 due mainly to the variety of concomitant conditions. Mathematical models developed to generate common INS waveforms may be able to simulate recorded data of human eye movements, but they do not necessarily signify biological relevance. Therefore, a study of the occurrence and characteristics of nystagmus waveforms in an animal model with a well-defined underlying morphological phenotype is needed.

Recently, we introduced the zebrafish mutant strain belladonna (bel) as an animal model for INS.12,13 In homozygous bel larvae, a variable fraction of optic nerve fibers is misrouted in the optic chiasm and projects to the wrong brain hemisphere, a condition caused by mutations in Ibx2b, a Lim domain homeobox transcription factor.14–17 Depending on the number of misprojecting fibers, bel larvae display INS-like ocular motor instabilities such as a reversed optokinetic response (OKR) and, in the presence of a structured background, spontaneous eye oscillations with the same diagnostic waveforms reported in humans.12,18–20 Here, we investigated whether the occurrence of different waveforms varied among bel individuals, thus reflecting different morphological conditions (i.e., optic nerve projection phenotypes). Moreover, we investigated how viewing conditions affected waveform characteristics.
METHODS

All experiments were performed in accordance with the animal welfare guidelines of the Federal Veterinary Office of Switzerland. Experiments adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Fish Maintenance and Breeding

Fish were maintained and bred as previously described. Embryos were raised at 28°C in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.33 mM MgSO4) and staged according to development in days post fertilization (dpf). Homozygous bel (bel<sup>-/-</sup>) larvae were obtained from mating of identified heterozygous carriers. Homozygous larvae were selected according to the presence of an abnormal gap between lens and pigment epithelium. Larvae at 4 dpf were anesthetized with 200 mg/L 3-aminobenzoic acid ethyl ester (Sigma-Aldrich, Buchs, Switzerland) and methane sulfonate (Sigma-Aldrich, Buchs, Switzerland) and projected via a mirror to the internal walls of a paper drum 400 lux; spatial frequency, 0.56 cycles/degree) that was transformed, data were checked for normality with a test the influence of stimulus condition on the occurrence of each waveform, a two-way repeated-measures ANOVA was performed with stimulus condition and waveform as within-subject factors. Occurrence of eye oscillations under different stimulus conditions was compared with post hoc analysis for each waveform separately. Analysis was performed after transformation of percentage data, using the formula for transformation of proportional data sin^-1(√x), where x is the experimental data expressed as a percentage. Following this transformation, data were checked for normality with a Kolmogorov-Smirnov test.

RESULTS

Categorization of Nystagmus Waveforms

Nystagmus in bel mutants matches the diagnostic waveforms of INS described by Huang et al. and Dell Osso and Daroff, although eye movements are slower in bel larvae compared to humans. For quantification of waveform occurrences, we grouped waveforms into three main categories, pendular nystagmus, unidirectional jerk, and bidirectional jerk, depending on the presence and direction of intercalated saccades. Pendular nystagmus is a sinusoidal oscillation without saccades. The absence or presence of saccades was verified by examining the eye velocity trace. Unidirectional jerk consists of cycles of accelerating slow phases in one direction and braking saccades in the opposite direction. Bidirectional jerk consists of cycles in alternating directions of slow phases and saccades. Saccades were seen as spikes in the eye velocity trace, either always in the same direction (unidirectional jerk) or in alternating directions (bidirectional jerk) (Fig. 1A).

Co-Occurrence of Multiple Waveform Categories in a Single Larvae

To investigate whether individual larvae showed single or multiple waveform categories, we quantified the occurrence of waveform categories during eye movement recordings in individual larvae (n = 20). We tested our group of larvae for morphological heterogeneity (i.e., different numbers of misprojecting optic nerve fibers) by quantifying the slow-phase eye velocity during OKR stimulation with a rotating grating pattern (angular velocity, 7.5°/sec). We previously demonstrated...
ed that the slow-phase eye velocity during OKR exhibits a direct correlation with the number of misprojecting optic nerve fibers. Our sample consisted of individual larvae with a large range of slow-phase eye velocities (data not shown), which indicated a variable morphological phenotype among them. The presence of predominant waveforms in single larvae would suggest that different waveforms might reflect different morphological phenotypes.

To quantify occurrences of one or more waveforms in individual larvae, we selected the time point at which

**Figure 1.** Nystagmus waveforms. Movements of the right eye in the presence of a stationary grating pattern. A higher value on the y-axis indicates rotation into temporal direction. (A) Representative eye position traces (Se) are shown together with corresponding eye velocity traces (Ve). Arrows in the eye velocity trace indicate saccades. (B–E) Representative oscillations with waveform change are shown on eye position traces and their corresponding eye velocity traces. Arrows indicate the time point of waveform change. (B) A change from unidirectional jerk to bidirectional jerk is shown: after a saccade, the decelerating eye increases its velocity again before turning to the opposite direction. (C) A change from unidirectional jerk to pendular nystagmus is shown: a saccade is replaced by a slow eye movement. (D, E) A starting unidirectional jerk after a period without oscillations is shown. A period with nystagmus started with either an accelerating eye drift (D) or after a spontaneous saccade (E).

**Figure 2.** Co-occurring waveforms. A stationary grating pattern was presented to the full field of view of both eyes (binocular stimulation) for 5 minutes. (A) Representative segment of an eye position trace (Se) is shown together with the corresponding eye velocity trace (Ve). The three main waveform categories occurred without interruption of the oscillations in this larva. Pendular nystagmus (violet horizontal bar) is followed by unidirectional jerk (green horizontal bar) and by bidirectional jerk (blue horizontal bar). A higher eye position on the y-axis indicates rotation to a temporal direction. # indicates a body movement artifact. (B) Stacked bar graph shows the occurrence of spontaneous oscillation (SOs) waveforms in individual larvae. Asterisks indicate larvae that displayed all main waveforms within one recording (16 of 20 larvae). Five larvae displayed periods of unidirectional jerk with decelerating slow phases.
oscillations started or at which oscillations of one waveform changed to oscillations of another waveform. We frequently observed changes of waveforms without interruption of the ongoing oscillations (Figs. 1B, 1C). If oscillations stopped for a certain period, they spontaneously reappeared either with an accelerating slow drift of the eyes (Fig. 1D) or after a saccade (Fig. 1E).

Waveforms of different categories occurred over time within the same larva, and all three waveform categories co-occurred in 16 of 20 larvae (Fig. 2). Hence, classical waveform categories are not useful as predictors of specific morphological phenotypes.

In 5 of 20 larvae, some cycles of unidirectional jerk with decelerating slow phases were observed. Although this waveform is characteristic of fusion maldevelopment nystagmus (FMNS; formerly known as latent nystagmus), it has been reported to occur for short periods in INS patients as well.20

**Influence of Viewing Conditions on Nystagmus Waveforms**

The occurrence of different waveforms may be influenced by intrinsic and extrinsic factors. We previously showed that nystagmus in bel larvae depends on a sensory input.13 Here,
we asked how viewing conditions influenced nystagmus waveforms.

First, we asked whether the occurrence of waveform categories was influenced by a preceding optokinetic stimulation. Traces of eye movements during monocular presentation of a stationary grating pattern were analyzed in each larva (n = 16) after a period (1 minute) of a unidirectional or directionally alternating OKR, elicited by a rotating grating pattern (angular velocity, 7.5/s) (Fig. 3A). Unidirectional OKR, elicited by a unidirectional rotating grating pattern, is characterized by cycles of slow phases and resetting saccades. Directionally alternating OKR, elicited by a rotating grating pattern changing direction every 2 seconds, is characterized by short slow phases in alternating directions without saccades or with only a few saccades. The absence of saccades may be explained by the fact that slow phases would normally take longer than the short stimulation in one direction. We quantified the occurrence of spontaneous oscillations with each waveform and under each stimulus condition. We found the main effect of waveform (two-way repeated-measures ANOVA, F = 10.18, P = 0.002) but no significant main effect of OKR stimulus (F = 0.43, P = 0.524). There was a significant interaction effect of OKR stimulus × waveform (F = 8.49, P = 0.005). Post hoc analysis showed that unidirectional jerk occurred significantly more often following a unidirectional OKR than following a directionally alternating OKR (P = 0.01). In contrast, pendular nystagmus occurred significantly more often following a directionally alternating OKR than following a unidirectional OKR (P = 0.013). OKR properties did not have a significant effect on bidirectional jerk (P = 0.657) (Fig. 3B).

To ask whether the field of view influenced the occurrence of waveform categories, traces of eye movements were analyzed in each larva (n = 20) during monocular or binocular presentation of a stationary grating pattern (Fig. 3C). We quantified the occurrence of spontaneous oscillations for each waveform and under each stimulus condition. We found the main effect of waveform (F = 10.05, P < 0.001) but no significant main effect of field of view (F = 0.04, P = 0.838). Again, there was a significant interaction effect of field of view × waveform (F = 15.19, P < 0.001). Post hoc analysis showed that unidirectional jerk occurred significantly more often with a monocular field of view than with a binocular field of view (P = 0.001), whereas bidirectional jerk occurred significantly more often with a binocular than with a monocular field of view (P < 0.001). Field of view did not have a significant effect on the incidence of pendular nystagmus (P = 0.573) (Fig. 3D).
Influence of Orbital Position on Waveform Initiation

Because waveform changes are observed under the same stimulus conditions, there must be larval intrinsic factors that change over time and influence the nystagmus waveform. In human patients, waveforms and the intensity of eye movements are influenced by the eye position. To investigate a possible correlation between orbital position and initiation of specific waveforms in bel larvae, we recorded eye movements during monocular presentation of a stationary grating pattern, and we measured orbital position of the stimulated eye (Fig. 4A) when a period with a specific waveform started. The orbital position at the time point of waveform initiation varied widely among and within larvae. Nevertheless, some patterns were frequently observed. Often we could observe unidirectional jerk starting after a nasotemporal saccade and short periods of bidirectional jerk, typically one cycle, starting after a temporonasal saccade (Fig. 4B, pattern 1). Sometimes, pendular nystagmus occurred in a central position, whereas jerk nystagmus started after a centrifugal saccade or drift of the eye (Fig. 4C, pattern 2). The opposite was also observed with pendular nystagmus in an eccentric position and jerk nystagmus occurring after a resetting saccade to a more central position (Fig. 4D, pattern 3). Finally, bidirectional jerk was sometimes characterized by cycles of higher amplitude than those of unidirectional jerk or pendular nystagmus. Hence, the period of bidirectional jerk tended to start at a more peripheral eye position (Fig. 4E, pattern 4). Many recorded larvae showed a tendency toward one of the patterns described above, which was reflected by different mean orbital positions at the beginning of periods with different waveforms. In Supplementary Table S1, the mean orbital position at the beginning of each waveform and the predominant pattern are indicated for each larva (n = 27). The mean orbital position was only calculated if at least five periods of a specific waveform occurred.

Discussion

For a long time, research of the mechanisms underlying eye oscillations in INS has been based mainly on theoretical considerations and models due to the absence of suitable animal models. Recently, we introduced and characterized two new putative animal models displaying INS-like ocular motor abnormalities: albino mouse strains and the zebrafish bel damma (bel) mutant. We demonstrated that in the bel mutant, those abnormalities are caused by an aberrant decussation of retinofugal fibers at the optic chiasm, leading to the projection of variable numbers of optic nerve fibers to the wrong brain hemisphere. We could show that additional eye morphological defects do affect visual performance in bel larvae but are not related to ocular motor abnormalities. Thus, INS-like behavior in bel larvae is explained by a normally negative feedback loop that has become a positive loop, which increases retinal slip. In the present study, we investigated and described how nystagmus waveforms are influenced by intrinsic and extrinsic factors.

Occurrence of Nystagmus Waveforms in bel

In agreement with the concept of a destabilizing positive feedback loop, we observed periods of spontaneous eye oscillations following a reversed OKR, a spontaneous saccade or starting with a spontaneous eye drift of exponential velocity (Fig. 1).

We previously showed that only one mechanism, misrouting of optic nerve fibers, is at the origin of spontaneous eye oscillations in bel mutants. Nevertheless, INS-like oscillations displaying the shape of all classical INS waveforms are observed. Here, our main aim was to investigate whether waveform categories reflected specific morphological phenotypes, which in bel organisms would most likely be the extent of the underlying optic nerve projection phenotype. If this holds true, one would expect to see, depending on the underlying phenotype, a predominant waveform in each individual that could be used as a diagnostic tool. In contrast, we observed all main waveform categories co-occurring in the same individual in most cases (16 of 20 larvae), whereby waveform changes were often observed without interruption of the oscillations (Fig. 2). These data suggest that different waveforms in bel larvae are not linked to the severity of the underlying morphological phenotype.

Several factors may influence a sudden change in waveform or the relative occurrence of different waveforms. Here, we investigated the role of viewing conditions and observed a strong influence of both the properties of a preceding optokinetic stimulus and the field of view (Fig. 3). Pendular nystagmus was more often observed after a directionally alternating OKR than after a unidirectional OKR. In contrast, unidirectional jerk was more often observed after a unidirectional OKR than after a directionally alternating OKR. Our data suggest a possible biasing action of a preceding OKR on the spontaneous oscillation; that is, whereas both directionally alternating OKR and pendular nystagmus are characterized by a symmetric oscillation without or with only few saccades, both the unidirectional OKR and unidirectional jerk consist of cycles of slow phases and resetting saccades. The observed tendency toward spontaneous oscillations resembling a preceding OKR suggests that the ocular motor system might tend to keep the oscillation characteristics when real motion of the visual world is stopped but retinal slip is maintained by the positive feedback loop. The spontaneous oscillations following OKR described here should not be confused with “after-nystagmus” eye movements. After-nystagmus is characterized by jerk nystagmus with exponentially decreasing slow-phase eye velocity. Instead, the oscillations observed here that followed a period with OKR included features typical for INS (accelerating slow phases and characteristic waveforms).

We observed an effect of the visual field on the relative incidence of waveform categories. Unidirectional jerk occurred more frequently during monocular presentation of the stationary pattern (monocular field of view), whereas bidirectional jerk occurred more frequently during binocular presentation of the stationary pattern (binocular field of view). A possible explanation might lie in the preferential direction of nystagmus. With a monocular field of view, saccades of the stimulated eye were mostly nasotemporally directed, that is, a left-beating unidirectional jerk was more often observed if the left eye was stimulated with the stationary pattern and a right-beating unidirectional jerk was more often observed if the right eye was stimulated (data not shown). With a binocular field of view, both eyes were stimulated, and both eyes presented alternately nasotemporal saccades, giving rise to bidirectional jerk.

Despite the influence of environmental factors, waveform changes are often observed under the same stimulus conditions. Thus, changes in waveform must be triggered by intrinsic factors that change over time. A possible factor may be orbital position, shown to affect waveform changes in human INS patients. Here, we investigated a possible relationship between orbital position and waveform change in bel larvae. The effect of orbital position was unclear and highly variable. Although we described a tendency toward specific...
waveforms at specific orbital positions in a subset of larvae (Fig. 4, Supplementary Table S1), the kind of relationship varied considerably. A possible explanation may be that eye oscillations in bel larvae are of higher amplitude than in humans,26 often covering a large range of orbital positions within one cycle. Thus, the effect of gaze may be of less importance. This may be an interesting topic for future work, given the importance of eye position in nystagmus characteristics in humans.

Significance for INS Research

The mechanisms behind eye oscillations in INS are poorly understood. Eye movement analyses in human patients have led to contradictory conclusions.7–11 and mathematical models have led to different hypotheses. Some models only simulate some waveforms but not others,8,26 thus suggesting that different waveforms may reflect instabilities in different subsystems of the ocular motor system. Other models reproduce all waveforms starting from one mechanism,29,30 suggesting that waveforms may be a single entity and occur together in one individual or reflect a different severity of one pathological mechanism. Experimental data can help shed light on which ones of those different modeled mechanisms really occur in nature.

The data presented here demonstrate that in the zebrafish bel mutant, INS-like waveforms are a single entity caused by reversal of a velocity feedback loop. Such a feedback reversal has been previously suggested as the possible cause in human patients26 and is also supported by studies in goldfish and amphibians, in which ocular motor instabilities were induced by surgically produced achiasmia,31 or by rotation of the eye balls by 1808.32 Although INS in the zebrafish bel mutant is caused by optic nerve fiber misprojections,18 a reversed feedback loop could also be caused by other aberrant projections.26

Because all three main waveform categories co-occurred in most larvae, waveforms in bel larvae most likely do not reflect the severity of the morphological phenotype. Rather, they are influenced by viewing conditions, for example, as shown here, properties of the preceding optokinetic stimulus, field of view, and, partially, orbital position. Waveform changes under unaltered stimulus properties might depend on intrinsic factors such as eye position and eye velocity, regulated by the naturally fluctuating activity of a neural integrator network26 and/or by spontaneous saccades.29 Psychological factors, including stress, fatigue, and level of attention have been suggested as further influencing factors in humans.9,35

Some caveats must be considered when interpreting the results of our study. Here, we investigated only the occurrence of waveforms in the presence of a reversed feedback loop in zebrafish larvae. Thus, our data cannot be generalized to all INS patients, and we cannot exclude some differences in relative incidence of waveform categories among INS patients with different associated conditions. Such a difference has been previously described among patients with albinism and idiopathic INS.11 Moreover, we did not investigate the possibility that different mechanisms can lead to the same oscillations. Additionally, our data are specific for the zebrafish animal model and care should be taken when generalizing to human INS. In contrast to human INS, nystagmus in bel larvae is discontinued in darkness,13 as expected from a visually driven behavior. However, this difference could be explained by adaptive mechanisms in humans during development that are lacking in zebrafish at the larval stage. More importantly, although the shapes of nystagmus waveform are essentially the same in human INS and in bel larvae, nystagmus in bel larvae is characterized by cycles with much lower frequency and higher amplitude.12 The higher amplitude of nystagmus cycles in bel larvae might be an explanation for the weaker relationship between orbital eye position and nystagmus waveforms compared to human INS.

CONCLUSIONS

We have described an experimental model of INS in which 1 pathological mechanism leads to different waveform categories within 1 individual. Classical waveforms did not reflect the severity of the disorder, suggesting that they are unlikely to provide much diagnostic benefit for evaluation of the severity of the underlying condition in this model. Our findings of a strong influence of viewing conditions on nystagmus waveforms suggest that it may be difficult to compare different studies in which experimental conditions are different.

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References