Effect of Gabapentin/Memantine on the Infantile Nystagmus Syndrome in the Zebrafish Model: Implications for the Therapy of Ocular Motor Diseases

Journal Article

Author(s):
Bögli, Stefan Y.; Afthinos, Maresa; Huang, Melody Ying-Yu

Publication date:
2017-06

Permanent link:
https://doi.org/10.3929/ethz-b-000190818

Originally published in:
Investigative Ophthalmology & Visual Science 58(7), https://doi.org/10.1167/iovs.16-21308

Effect of Gabapentin/Memantine on the Infantile Nystagmus Syndrome in the Zebrafish Model: Implications for the Therapy of Ocular Motor Diseases

Stefan Yu Bögli,[1] Maresa Afthinos,[1] and Melody Ying-Yu Huang[1–3]

[1]Department of Neurology, University Hospital Zurich, University of Zurich, Switzerland
[2]Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Switzerland
[3]Neuroscience Center Zurich (ZNZ), University of Zurich and ETH Zurich, Switzerland

Correspondence: Melody Ying-Yu Huang, Department of Neurology, University Hospital Zurich, University of Zurich, Frauenklinikstrasse 26, CH-8091, Zürich, Switzerland; Ying-yu.Huang@usz.ch.

Submitted: December 15, 2016
Accepted: May 22, 2017

Citation: Bögli SY, Afthinos M, Huang MY-Y. Effect of gabapentin/memantine on the infantile nystagmus syndrome in the zebrafish model: implications for the therapy of ocular motor diseases. *Invest Ophthalmol Vis Sci*. 2017;58:3149–3157. DOI:10.1167/iovs.16-21308

Purpose. Infantile nystagmus syndrome (INS) is a disorder characterized by typical horizontal eye oscillations. Due to the uncertain etiology of INS, developing specific treatments remains difficult. Single reports demonstrated, on limited measures, alleviating effects of gabapentin and memantine. In the current study, we employed the zebrafish INS model *belladonna* (*bel*) to conduct an in-depth study of how gabapentin and memantine interventions alleviate INS signs, which may further restore visual conditions in affected subjects. Moreover, we described the influence of both medications on ocular motor functions in healthy zebrafish, evaluating possible iatrogenic effects.

Methods. Ocular motor function and INS characteristics were assessed by eliciting optokinetic response, spontaneous nystagmus, and spontaneous saccades in light and in dark, in 5- to 6-day postfertilization *bel* larvae and heterozygous siblings. Single larvae were recorded before and after a 1-hour drug treatment (200 mM gabapentin/0.2 mM memantine).

Results. Both interventions significantly reduced nystagmus intensity (gabapentin: 59.98%, memantine: 39.59%). However, while the application of gabapentin affected all tested ocular motor functions, memantine specifically reduced nystagmus amplitude and intensity, and thus left controls completely unaffected. Finally, both drug treatments resulted in specific changes in nystagmus waveform and velocity.

Conclusions. Our study provides deeper insight into gabapentin and memantine treatment effect in the zebrafish INS model. Moreover, this study should establish zebrafish as a pharmacologic animal model for treating nystagmus and ocular motor disease, serving as a basis for future large-scale drug screenings.

Keywords: infantile nystagmus syndrome, gabapentin, memantine, translational medicine, zebrafish

Infantile nystagmus syndrome (INS) is a congenital ocular motor disease characterized by specific, periodically reoccurring involuntary eye movements.[1] At least 12 typical INS waveforms have been documented in patients.[2] Infantile nystagmus syndrome has a prevalence of 1 to 1.5‰[3] and manifests 2 to 3 months after birth.[1] It can be idiopathic, but is often associated with anterior visual pathway anomalies (e.g., achiasma and cataract) or optic nerve disorders (e.g., hypoplasia and atrophy).[4] Infantile nystagmus syndrome is also associated with substantial visual impairments such as low visual acuity,[5] which adversely affects occupational capabilities and social functioning.[6] The psychological stress may be further exacerbated by the cosmetic appearance.[7]

Infantile nystagmus syndrome is treated by suppressing the nystagmus. This can be achieved by botulinum toxin injections or eye muscle operations.[8] Pharmacologically, the application of gabapentin or memantine has shown to suppress nystagmus.[9] However, to the best of our knowledge, no study has provided a comprehensive description of their effects on general ocular motor functions. Since most ocular motor functions are unaffected by INS,[10–12] altering these due to medication could potentially lead to additional iatrogenic symptoms. Possible side effects include convergence insufficiency, or oscillopsia during head movements caused by an insufficient vestibulo-ocular reflex.[8]

The mechanism of action of gabapentin and memantine in INS is unknown. Gabapentin is widely used to treat epilepsy and neuralgia.[13] It inhibits voltage-gated calcium channels and reduces the release of many excitatory neurotransmitters including glutamate.[14] Memantine, on the other hand, is a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist[15] used for the treatment of Alzheimer disease.[16] Both medications have been used to treat a various types of nystagmus.[8] In particular, gabapentin is the most commonly prescribed medication for acquired nystagmus.[17] Both gabapentin and memantine have been used off-label and in high dosages to treat INS. Although generally well tolerated, both treatments have occasionally led to side effects such as dizziness, headache, tiredness, and nausea.[9]

Zebrafish is a widely used animal model in ophthalmologic research.[18] Its benefits lie within the large amount of offspring, larval translucency, and the rapid development of their visual

system.[19] Visual behaviors, such as optokinetic response, reach adult-like behavior already at 4 days postfertilization.[20–23] Therefore, the larval zebrafish has been widely appreciated and employed as a valuable animal model for ophthalmic research.[18,24–27] A line of zebrafish *belladonna* (*bel*) strain is a particularly important INS animal model: Homozygous *bel* embryos carry a recessive mutation of *lhx2*, encoding a Lim domain homeobox protein Lhx2, while heterozygous embryos display normal visual functions.[28] It portrays all major nystagmus waveforms comparable to INS patients.[29] These include pure pendular nystagmus, pendular nystagmus with foveating saccades, pure unidirectional jerk nystagmus, pseudocycloid unidirectional jerk nystagmus, pseudopendular bidirectional jerk nystagmus, and triangular bidirectional jerk nystagmus.[29] As in humans, these waveforms are susceptible to many factors, including stress and viewing conditions, and can change at any given time.[29–31] Furthermore, it also displays a reversed optokinetic response that has been attributed to its underlying optic nerve misrouting.[28,32]

The treatment of INS remains a challenge for clinicians due to its diverse associations and manifestations. The aim of this study was to provide further insight into the mechanisms of action of memantine and gabapentin, as it is essential for treating physicians to choose the optimal medication on a case-by-case basis. Therefore, we described their effects not only specifically on alleviating nystagmus, but generally on other ocular motor functions as well. Moreover, we investigated waveform changes after treatments and discussed possible mechanisms, which could help explain the rapid improvement of vision in patients. Lastly, this study should establish zebrafish larvae as a pharmacologic animal model for the treatment of nystagmus and other ocular motor diseases.

Materials and Methods

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

Animal Maintenance and Breeding

The *belladonna* (*bel^{tv42}*) *bel danio rerio* zebrafish line was bred and maintained as previously described.[33] Identified carriers were crossed. Their embryos were raised under a 14-hour light, 10-hour dark cycle in 28°C E3 medium (in mM: 5 NaCl, 0.17 KCl, 0.33 $CaCl_2$, and 0.33 $MgSO_4$; Sigma-Aldrich Corp., St. Louis, MO, USA)[34] and staged according to developmental stage in days post fertilization (dpf). At 4 dpf, larvae were anesthetized with 0.882 mM 3-aminobenzonoic ethyl esther methanesulfonate (MESAB; Sigma-Aldrich Corp.) and sorted into homozygous (*bel* mutants)/heterozygous (*bel* sib, siblings) according to their eye pigmentation phenotype.[28] Furthermore, *bel* mutants were sorted based on their optokinetic response behavior (OKR), into reverse (rev) and forward (fwd) groups as described in the previous study.[28]

Recording of Eye/Body Movement

Single larvae were embedded in a transparent 21-mm diameter plastic tube filled with 3.5% methylcellulose. The tube was placed inside a glass cylinder covered with a translucent screen, and was illuminated and heated from below by infrared (IR) emitting diodes. Eye movements were recorded by an IR-sensitive charge-coupled device camera with a sampling rate of 40 frames/second. Binocular visual stimulation and recording were carried out throughout all experiments. Frames were processed by a custom-made eye recognition software (LabVIEW; National Instruments, Austin, Texas, USA).[35]

General Experimental Procedure

In each recording session 3 *bel* rev and 3 *bel* sib larvae at 5 to 6 dpf, randomly chosen from a single clutch, were individually recorded within a timeframe of 4 hours. During the same session, all tested larvae were recorded twice, once before the treatment and once after the treatment of either 200 mM gabapentin (GBP) in 1% dimethyl sulfoxide (DMSO), 0.2 mM memantine (MEM) in 1% DMSO, or only 1% DMSO (Sigma-Aldrich Corp.). All drug solutions were prepared in E3 medium, stored according to the instructions, and used within 1 week after preparation. In the same recording session, each of the three treatments was applied to a pair of larvae from both groups: one *bel* rev and one *bel* sib larva. During the experiment, single larvae were exposed to different computer-generated stimulus patterns (described below) projected by four beamers (SP-H03 Pico Projector; Samsung Group, Seoul, South Korea). Stimulus properties such as grating velocity, grating contrast and spatial frequency of the gratings were controlled by a custom-made program (National Instruments).[35]

Optokinetic Response

Optokinetic response is a reflexive eye movement evoked by whole-field motion of the visual scene. The response consists of slow eye movements (slow phases) that follow the moving scene and saccades (fast phases) that are directed in the opposite direction for resetting eye positions. The optokinetic response stimuli were composed of horizontally moving black/white vertical gratings. For data analysis, slow phase eye velocity was calculated by first removing all saccades from the eye movement traces followed by using a moving window for calculating velocities within each time-period. The absolute mean values of these measurements were presented in the paper as slow-phase velocities.[36]

Experimental Procedure

For each experiment, the same three consecutive visual stimuli (OKR, stationary pattern in light, and dark) were presented to the whole visual field of the fish. Larval eye movements were recorded binocularly in real time. General stimulus properties applied were a spatial frequency of 0.056 cyc/deg, 100% contrast, and a maximum illumination of 30.1 lux. Specifically, OKR stimulation was composed of 10-second alternating cycles of clockwise (cw) and counterclockwise (ccw) horizontally moving black/white gratings; there were a total of 10 cycles (i.e., five cw and five ccw cycles). The angular velocity of the stimulus was $\pm$10 degree per second (dps; $\pm$ for ccw/cw direction). There was a 10-second dark phase in-between each OKR cycle. After the OKR stimulation, subject's eye movements were recorded for 180 seconds in light under viewing condition of stationary vertical black/white gratings. This period was designed to record spontaneous nystagmus and eye movements of *bel* rev or spontaneous eye movements of *bel* sib. Nystagmus waveforms were analyzed and categorized into unidirectional and bidirectional types as previously described.[29] Alongside analyzing frequency and amplitude of nystagmus, we also calculated its intensity (product of amplitude and frequency), since nystagmus intensity is more commonly used in describing the treatment effect in patients.[9] The last stimulus condition was 180 seconds in the dark, when mostly spontaneous saccades were recorded. After the experiment was finished, the larva was removed from the

Investigative Ophthalmology & Visual Science

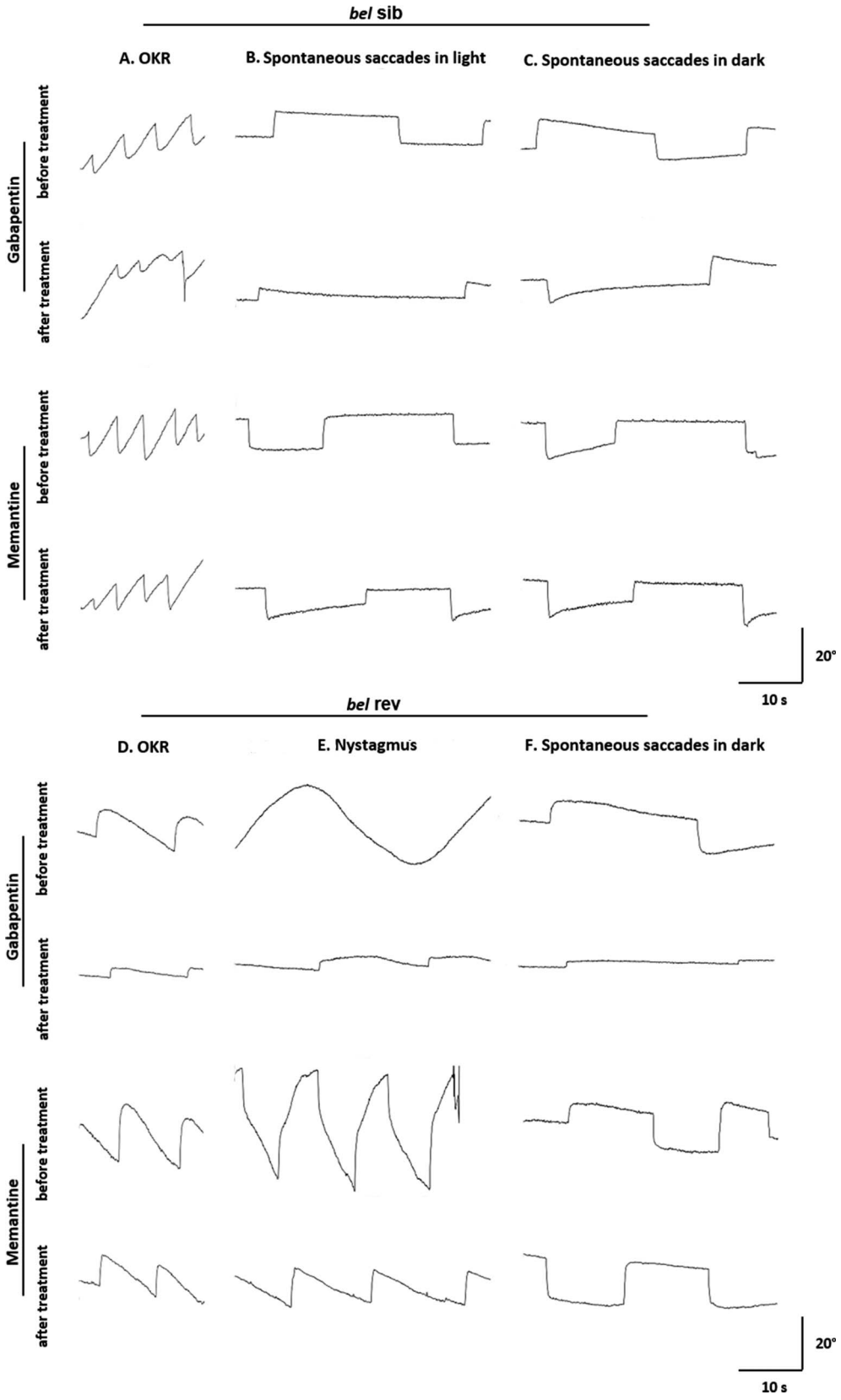

Figure 1. Representative eye movement traces. Typical eye movement traces before and after treatment using gabapentin (rows 1–2/5–6) and memantine (rows 3–4/7–8) in *bel* sib (rows 1–4) and *bel* rev (rows 5–8). Each pair of rows consists of a single zebrafish depicting excerpts of the whole recording. (**A**) OKR, (**B**) spontaneous saccades in light, and (**C**) spontaneous saccades in dark are depicted for *bel* sib, while reverse (**D**) OKR, (**E**) spontaneous nystagmus, and (**F**) spontaneous saccades in dark are depicted for the *bel* rev. (**A**, **D**) were recorded during stimulation using 10 dps ccw moving vertical black and white gratings; (**B**, **E**) under the same, however, stationary gratings; and (**C**, **F**) in complete darkness.

tube using a pipette and transferred into a dish filled with 28° E3 medium. The remaining gel was removed from the animal by gentle rinsing. Next, the larva was transferred into a microplate well containing the medication. After a 1-hour treatment, the larva was again embedded in methylcellulose and underwent the same experiment again.

Statistical Analysis

Analysis was executed using statistical software (SPSS; IBM Corp., Armonk, NY, USA). All values are shown as mean ± standard deviation. Values of *P* are indicated separately for each statistical test. In case of multiple dependent variables being compared (frequency and amplitude), a multivariate (M)ANOVA was performed. In case of significant results, an ANOVA with Bonferroni correction as alpha correction was applied as the follow-up test.

Results

In this study, we qualitatively and quantitatively described the effect of GBP and MEM on both naturally occurring eye movements and the characteristics of INS. Hereby, OKR, spontaneous nystagmus/spontaneous saccades in the light, and spontaneous saccades in the dark were investigated. Homozygous zebrafish *bel* mutants with *bel* rev, and *bel* sib were tested. Figure 1 shows eye movement traces recorded before and after GBP or MEM treatment in *bel* sib and *bel* rev. The treatment duration was 1 hour. For each treatment group, a total of 20 larvae were tested. Traces from all three parts of the experiment (i.e., OKR, spontaneous saccades in the light/nystagmus, spontaneous saccades in the dark) are presented. Figures 1A through 1C show eye movement traces of healthy zebrafish. Figure 1D shows typical reversed OKR trace of *bel* rev. Figure 1E shows several nystagmus waveforms of *bel* rev. Eye movement pattern changes as the consequences of drug treatments demonstrated in Figure 1 are representative of the following data.

In the first part of the experiment, OKR was elicited by horizontally moving gratings. The slow phase velocity was calculated (Fig. 2). After treatment using GBP, the slow-phase velocity decreased significantly from 8.493 ± 0.865 dps to 7.634 ± 0.746 dps in *bel* sib (paired *t*-test, $P = 0.002$, Fig. 2A), and from 7.579 ± 3.415 dps to 5.379 ± 2.427 dps in *bel* rev (paired *t*-test, $P = 0.006$, Fig. 2B). Treatment with MEM, on the other hand, showed no significant influence on OKR slow-phase velocity, both in *bel* sib (8.389 ± 1.391 dps to 8.314 ± 1.357 dps, paired *t*-test, $P = 0.779$, Fig. 2C) and in *bel* rev (6.440 ± 1.618 dps to 6.329 ± 2.170 dps, paired *t*-test, $P = 0.842$, Fig. 2D). In the control groups, results showed no significant change of OKR slow-phase velocity (8.939 ± 0.862 dps before and 8.361 ± 2.262 dps after treatment in *bel* sib (paired *t*-test, $P = 0.307$), and 8.035 ± 2.624 dps before and 7.355 ± 2.102 dps after treatment in *bel* rev (paired *t*-test, $P = 0.335$ Figs. 2E, 2F).

In the second part of the experiment, under stationary gratings, spontaneous saccades in *bel* sib and spontaneous nystagmus in *bel* rev were evoked. For spontaneous saccades, we measured both the frequency and amplitude; and for spontaneous nystagmus, we calculated the nystagmus intensity (i.e., product of frequency and amplitude) in addition to nystagmus frequency and amplitude (Fig. 3). In *bel* sib, saccade characteristics changed significantly after GBP treatment (MANOVA, $P = 0.006$). Both the frequency (from 0.115 ± 0.034 Hz to 0.077 ± 0.047 Hz, ANOVA, $P = 0.005$, Fig. 3A) and the amplitude (from 10.542 ± 4.190° to 7.131 ± 3.870°, ANOVA, $P = 0.011$, Fig. 3B) were significantly reduced.

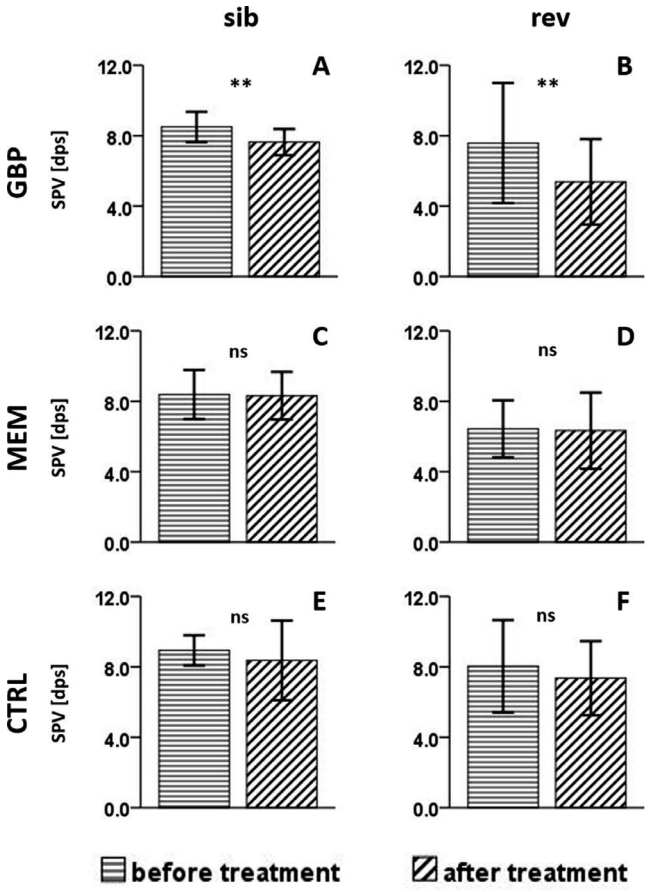

Figure 2. Optokinetic response. Mean OKR slow-phase velocity during stimulation using 10 dps moving black and white gratings before and after application of gabapentin: (**A**) *bel* sib; (**B**) *bel* rev; memantine: (**C**) *bel* sib, (**D**) *bel* rev; or DMSO: control (**E**) *bel* sib, (**F**) *bel* rev. Each group consists of 20 larvae. Ns, not significant; SPV, slow-phase velocity. ** Highly significant ($P < 0.01$).

Similarly, in *bel* rev, GBP significantly reduced nystagmus characteristics (MANOVA, $P = 0.001$). Again, both the frequency (from 0.179 ± 0.051 Hz to 0.125 ± 0.059 Hz, ANOVA, $P = 0.004$, Fig. 3C) and the amplitude (from 22.726 ± 10.264° to 11.463 ± 7.534°, ANOVA, $P < 0.001$, Fig. 3D) were reduced. This resulted in a highly significant drop of nystagmus intensity (from 4.163 ± 2.467 dps to 1.666 ± 1.358 dps [paired *t*-test, $P < 0.001$, Fig. 3E]). MEM did not have a significant influence on spontaneous saccades parameters (MANOVA, $P = 0.834$) in *bel* sib. The frequency was 0.099 ± 0.046 Hz before, and 0.095 ± 0.048 Hz after treatment (Fig. 3F), while the amplitude was 10.709 ± 3.537° before and 9.970 ± 4.081° after treatment (Fig. 3G). In *bel* rev, MEM had a significant effect on nystagmus (MANOVA, $P = 0.028$). While the frequency was not changed significantly (0.164 ± 0.042 Hz to 0.127 ± 0.074 Hz, ANOVA, $P = 0.058$, Fig. 3H), the amplitude was (19.325 ± 9.438° to 11.892 ± 9.321°, ANOVA, $P = 0.017$, Fig. 3I). The intensity of nystagmus was changed highly significant (3.089 ± 1.603 dps to 1.866 ± 1.569 dps, $P = 0.005$, Fig. 3J). In *bel* sib spontaneous saccades characteristics were unchanged in the control group (MANOVA, $P = 0.766$). The frequency was 0.116 ± 0.037 Hz before and 0.107 ± 0.045 Hz after treatment (Fig. 3K), while the amplitude was 11.208 ± 4.113° before and 10.916 ± 4.981° after treatment (Fig. 3L). In *bel* rev, spontaneous nystagmus characteristics were also unchanged in the control group (MANOVA, $P = 0.661$). The frequency was 0.178 ± 0.041 Hz before and 0.192

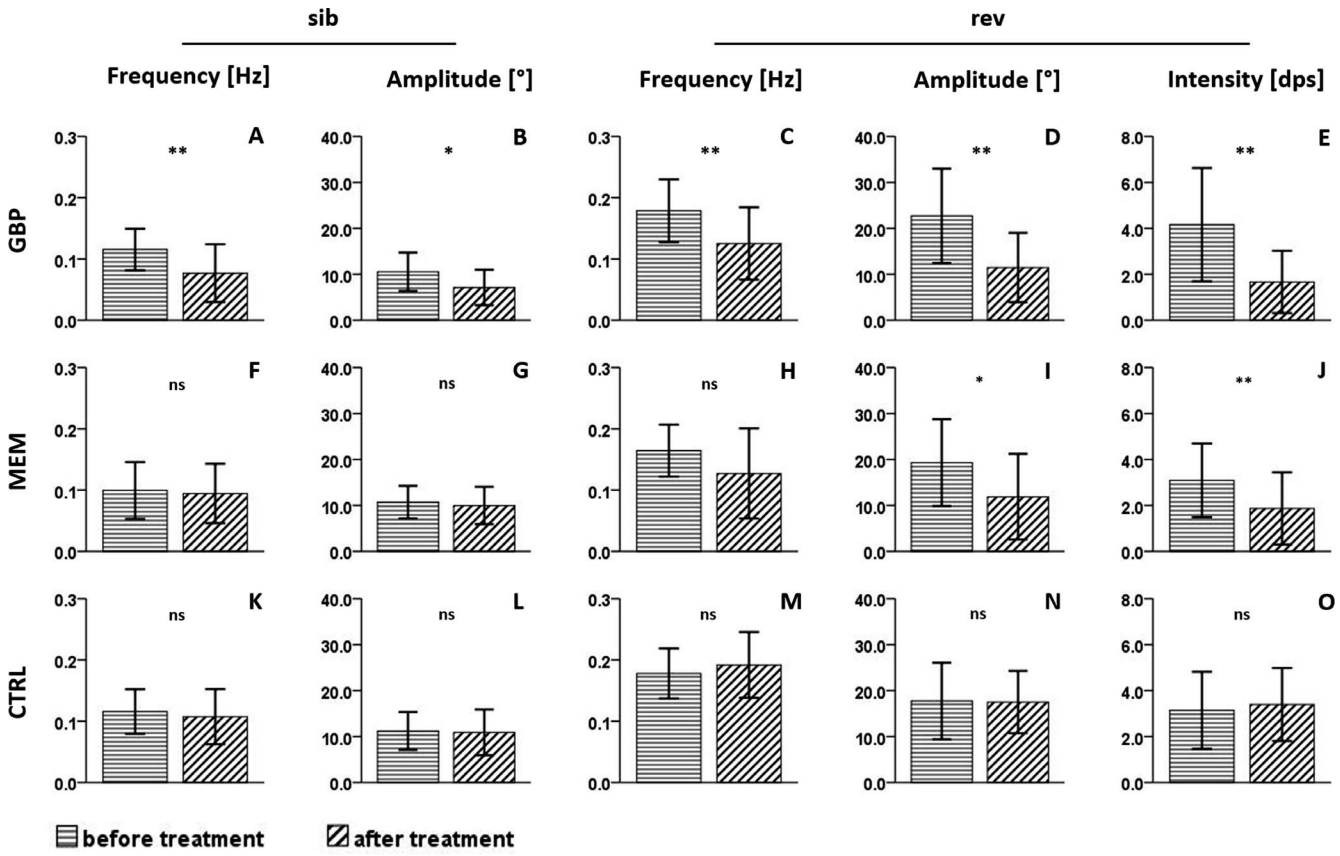

FIGURE 3. Nystagmus and spontaneous saccades in the light. Frequency (*column 1*) and amplitude (*column 2*) of spontaneous saccades in the light of *bel* sib; and frequency (*column 3*), amplitude (*column 4*), and intensity (*column 5*) of spontaneous nystagmus in light of *bel* rev before and after application of (**A–E**) gabapentin, (**F–J**) memantine, and (**K–O**) DMSO (control group). Each group consists of 20 larvae. Ns, not significant. ** Highly significant ($P < 0.01$). * Significant ($P < 0.05$).

± 0.053 Hz after treatment (Fig. 3M), while the amplitude was 17.729 ± 8.314° before and 17.493 ± 6.785° after treatment (Fig. 3N). The intensity of spontaneous nystagmus was 3.146 ± 1.675 dps before and 3.393 ± 1.589 dps after treatment (paired *t*-test, $P = 0.530$, Fig. 3O).

In the last part of the experiment, in complete darkness, spontaneous saccades were elicited in both *bel* sib and *bel* rev. Again, the frequency and amplitude thereof were analyzed (Fig. 4). After GBP treatment, spontaneous saccade characteristics were significantly changed in *bel* sib (MANOVA, $P = 0.027$). The frequency changed from 0.112 ± 0.040 Hz to 0.080 ± 0.042 Hz (ANOVA, $P = 0.019$, Fig. 4A), while the amplitude changed from 8.371 ± 3.045° to 6.465 ± 0.532° (ANOVA, $P = 0.033$, Fig. 4B). After GBP, spontaneous saccade characteristics were also changed significantly in *bel* rev (MANOVA, $P = 0.007$). The frequency was changed from 0.091 ± 0.039 Hz to 0.050 ± 0.037 Hz (ANOVA, $P = 0.002$, Fig. 4C) in *bel* rev; while the amplitude was changed from 7.085 ± 3.302° to 5.500 ± 3.904° (ANOVA, $P = 0.174$, Fig. 4D) in *bel* rev. In contrast, MEM treatment had no significant influence on spontaneous saccades in *bel* sib (MANOVA, $P = 0.169$). The frequency was 0.094 ± 0.009 Hz before, and 0.099 ± 0.041 Hz after treatment (Fig. 4E), while the amplitude was 7.775 ± 2.672° before, and 9.612 ± 3.602° after treatment (Fig. 4F). Memantine also had no significant influence on spontaneous saccades in *bel* rev (MANOVA, $P = 0.492$). The frequency was 0.106 ± 0.035 Hz before, and 0.100 ± 0.046 Hz after treatment (Fig. 4G), while the amplitude was 8.487 ± 3.352° before, and 9.620 ± 5.157° after treatment (Fig. 4H). Again, in *bel* sib, there were no changes in spontaneous saccade characteristics in complete darkness in the control group (MANOVA, $P = 0.809$). Saccade frequency was 0.117 ± 0.037 Hz before and 0.108 ± 0.48 Hz after treatment (Fig. 4I), while spontaneous saccade amplitude was 8.391 ± 3.427° before and 8.198 ± 3.578° after treatment (Fig. 4J). In *bel* rev, there were also no changes in spontaneous saccade characteristics in the control group (MANOVA, $P = 0.837$). The frequency was 0.120 ± 0.038 Hz before and 0.113 ± 0.044 Hz after treatment (Fig. 4K), and the spontaneous saccade amplitude was 8.921 ± 4.285° before and 9.101 ± 3.622° after treatment (Fig. 4L).

Furthermore, we used unpaired *t*-tests to directly compare the treatment efficacy of both drugs on nystagmus in *bel* rev. The mean Δ frequency was –0.053 ± 0.081 Hz after GBP treatment and –0.037 ± 0.080 Hz after MEM treatment ($P = 0.531$). The mean Δ amplitude was –11.263 ± 10.262° after GBP treatment and –7.434 ± 11.138° after MEM treatment ($P = 0.265$). The mean Δ intensity was –2.496 ± 2.473 dps after GBP treatment and –1.223 ± 1.701 dps after MEM treatment ($P = 0.065$). Thus, both medications showed no statistical difference in reducing nystagmus frequency, amplitude, and intensity.

To test if drug treatments changed nystagmus waveform characteristics, we analyzed spontaneous nystagmus waveforms before and after both MEM and GBP treatments. We resorted all tested larvae into two subgroups based on their major spontaneous nystagmus waveforms: In the first group, larvae displayed mainly nystagmus with bidirectional waveforms, and in the second group mainly unidirectional waveforms (Fig. 1E). Before MEM treatment, out of a total of

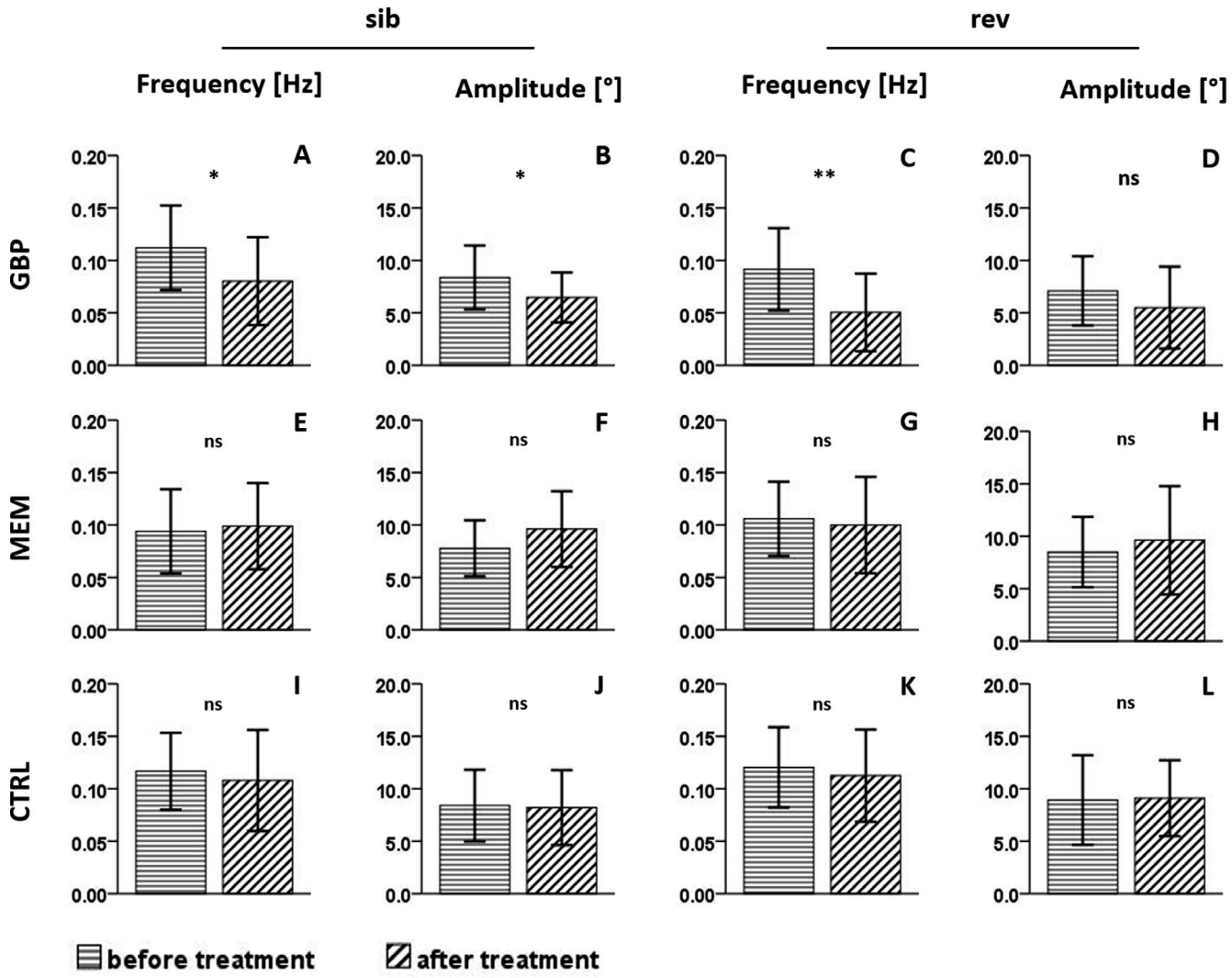

Figure 4. Spontaneous saccades in the dark. Frequency (*column 1*) and amplitude (*column 2*) of spontaneous saccades in dark of *bel* sib, and frequency (*column 3*), amplitude (*column 4*) of spontaneous nystagmus in dark of *bel* rev before and after application of (**A–D**) gabapentin, (**E–H**) memantine, and (**I–L**) DMSO (control group). Each group consists of 20 larvae. Ns, not significant. ** Highly significant ($P < 0.01$). * Significant ($P < 0.05$).

20 larvae 15 (75%) displayed bidirectional nystagmus while only 5 (25%) displayed unidirectional nystagmus. After MEM treatment, 6 (30%) larvae displayed bidirectional nystagmus while 10 (50%) larvae displayed unidirectional nystagmus, 4 (20%) displayed no nystagmus after the treatment. After treatment, nystagmus either disappeared or changed from bidirectional to unidirectional types in most larvae. One larva changed from the unidirectional to the bidirectional group. Similarly in the GBP treatment group, 18 out of a total of 20 larvae (90%) displayed bidirectional nystagmus, while only 2 (10%) displayed unidirectional nystagmus before drug treatment. After GBP treatment, 10 larvae (50%) displayed bidirectional nystagmus and 8 (40%) with unidirectional nystagmus, 2 (10%) larvae had no nystagmus after the treatment. As with MEM, one larva changed from the unidirectional to the bidirectional group after treatment. While we found no significant correlation between waveform category and treatment in the control group (Fisher's exact test, $P = 0.527$), waveform categories showed a significant dependence on treatment in both GBP (Fisher's exact test, $P = 0.027$) and MEM (Fisher's exact test, $P = 0.041$). Lastly, we calculated velocities of all spontaneous nystagmus. Gabapentin significantly lowered the slow-phase velocity of nystagmus (5.185 ± 2.250 dps before and 3.064 ± 1.631 dps after treatment, paired *t*-test, $P < 0.001$, Figs. 5A, 5B). Memantine also lowered the slow-phase velocity of nystagmus significantly (4.269 ± 1.637 dps before and 3.560 ± 1.644 dps after treatment, paired *t*-test, $P = 0.046$, Figs. 5C, 5D). We found no significant change of slow phase velocity within the control group (4.621 ± 2.220 dps before and 4.488 ± 1.649 dps after treatment, paired *t*-test, $P = 0.761$, Figs. 5E, 5F). We found bidirectional nystagmus to have significantly higher slow-phase velocity than unidirectional nystagmus (5.153 ± 1.824 dps for bidirectional and 3.008 ± 1.200 dps for unidirectional nystagmus, unpaired *t*-test, $P \leq 0.001$).

Discussion

In this study, we employed an INS model, the zebrafish mutant *bel*, to investigate the treatment effect of GBP and MEM. We assessed general ocular motor functions and INS characteristics in rev *bel* and sib before and after treatment using either GBP

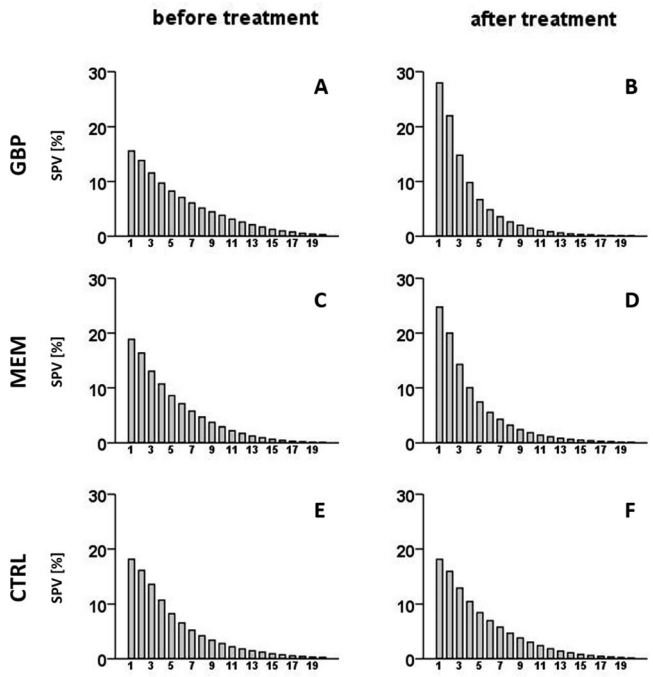

FIGURE 5. Distribution of velocity within nystagmus. Velocity histograms illustrating the percentage distribution of absolute velocities found during nystagmus before and after treatments using (**A**, **B**) gabapentin, (**C**, **D**) memantine, or (**E**, **F**) DMSO in *bel* rev. Both gabapentin and memantine groups show an increase of low velocities after treatment (**B**, **D**).

or MEM by measuring OKR, spontaneous nystagmus, and spontaneous saccades in light and dark.

Both GBP and MEM were highly and similarly effective at decreasing the intensity of spontaneous nystagmus in *bel* rev. In comparison, efficacy of GBP and MEM was also found to be similar in treating INS patients.[9] In humans, both GBP and MEM successfully decreased nystagmus intensity and increased visual acuity. Both medications exhibited equal efficacy and tolerability. Furthermore, only minor adverse effects such as dizziness and tiredness occurred during the treatment.[9,37] However, other ocular motor functions were not tested in relation to the medications. However, impairments of these functions might lead to further complications. Beside reducing the efficacy of MEM and GBP treatments, iatrogenic oscillopsia and/or diplopia could possibly occur.[8] In our study, using the INS zebrafish model, MEM decreased the amplitude and intensity of nystagmus, while other measured values including slow phase velocity of OKR remained within the normal ranges in *bel* rev. In *bel* sib, all measured factors were completely unaffected by MEM. Gabapentin, on the other hand, impaired almost all tested functions in both *bel* rev and *bel* sib. Although not directly applicable to humans, these observations in the animal model make certain ocular motor dysfunctions and/or adverse events caused by GBP treatment foreseeable in patients.

Aside from INS, both GBP and MEM are used to treat many other types of nystagmus including acquired nystagmus.[8] Acquired nystagmus is commonly associated with multiple sclerosis and stroke.[3] A study investigating the efficacy of GBP and MEM in dampening acquired nystagmus in multiple sclerosis found both treatments to be effective. However, MEM had the upper hand at decreasing nystagmus intensity.[38] Both GBP and MEM reached their highest efficacy when applied long term. Moreover, visual acuity improvement by GBP and MEM faded after cessation of the medication.[9] Gabapentin was found to have a high abuse potential with a prevalence of 40% to 65% among patients with prescriptions.[39] This is highly worrisome, not only due to the high prevalence of nystagmus,[3] but also due to GBP being one of the most commonly prescribed medications in acquired nystagmus.[17] Abuse led to an opioid- or benzodiazepine-like experience such as euphoria, increased energy, increased relaxation, and calmness.[39] Occasionally, GBP even led to severe addiction and subsequent withdrawal symptoms after cessation of the medication.[40] Furthermore, different types of nystagmus have all shown to cause high psychological stress,[7] which may increase the chance of self-medication and abuse. In contrast, MEM possesses no abuse potential[41]; moreover, it has even shown to be beneficial in terms of aiding restoration of functions after stroke.[42]

The common denominator of mechanism of action of GBP and MEM lies within the antiglutamatergic effect.[9] Memantine acts as an noncompetitive NMDA receptor antagonist.[15] Gabapentin, on the other hand, reduces release of excitatory neurotransmitters including glutamate, norepinephrine, serotonin, and dopamine.[43] As a specific glutamate antagonist, MEM apparently solely reduces nystagmus of INS in the zebrafish model; the reduction of several neurotransmitters by GBP, however, could be the reason behind the iatrogenic suppression of other ocular motor functions found in our study. Memantine singularly reduces excessive NMDA receptor excitation,[44] leaving normal levels of receptor activation unaffected. Besides explaining the unaffected ocular motor functions, increased release or sensitivity to glutamate could potentially be one of the underlying causes for idiopathic INS.

In this study, we also investigated the waveform characteristics of spontaneous nystagmus with respect to the drug treatments. Similar to the previous reports of INS patients and fish, we found a multitude of waveforms within single subjects and a high intersubject variability as well.[29–31] Both GBP and MEM led to consistent waveform changes from bidirectional to unidirectional types. In humans, both medications increased visual acuity subjectively and objectively.[9,37] Within such short timeframes of the treatment,[9,38] retinal changes/corrections on a cellular level are not expected. Nystagmus intensity reduction alone cannot explain the improvement of visual acuity, since it is poorly correlated to visual acuity.[45] Foveation time during nystagmus, on the other hand, highly correlates to the visual function.[46] It has been reported in INS that unidirectional waveforms generally possess longer foveation periods.[2] Based on our data, it could be attributed to the lower slow-phase velocities of the unidirectional nystagmus or the waveform type itself. A previous study of treatment effect of GBP and MEM on acquired nystagmus suggested the latter to be the cause of the visual acuity improvement; moreover, they found only a low correlation between the eye velocity and visual acuity.[47] Our results revealed a general trend of nystagmus waveform change toward the unidirectional type after INS drug treatments. This suggests the visual acuity improvement in INS patients after receiving the same medications could well be due to a similar change of waveform types.

In conclusion, our study provides a more comprehensive overview of both GBP and MEM's treatment effects on the nystagmus characteristics and on general ocular motor functions as well. Both medications were highly effective at reducing the nystagmus intensity. However, MEM more specifically affected/dampened the nystagmus, while GBP suppressed many additional ocular motor functions including the OKR. We also showed for the first time that both medications led to distinct changes of spontaneous nystagmus waveform characteristics, which could possibly explain the

visual acuity improvement in INS patients. Results of this study shall bring new insights for neuro-ophthalmologists who may provide better patient-based treatments; moreover, they also serve as the basis of utilizing zebrafish models for developing novel drug therapies for ocular motor diseases.

Acknowledgments

The authors thank Dominik Straumann for the discussions and for providing research infrastructure support, Marco Penner for his technical assistance, and Giovanni Bertolini for the data analysis software.

Supported by the Swiss National Science Foundation grants #139754 (Marie Heim-Vögtlin Programme) and #149521; the Betty and David Koetser Foundation for Brain Research; and the Dr. Dabbous Foundation.

Disclosure: **S.Y. Bögli**, None; **M. Afthinos**, None; **M.Y.-Y. Huang**, None

References

1. Richards MD, Wong A. Infantile nystagmus syndrome: clinical characteristics, current theories of pathogenesis, diagnosis, and management. *Can J Ophthalmol*. 2015;50:400–408.
2. Dell'Osso LF, Daroff RB. Congenital nystagmus waveforms and foveation strategy. *Doc Ophthalmol*. 1975;39:155–182.
3. Sarvananthan N, Surendran M, Roberts EO, et al. The prevalence of nystagmus: the leicestershire nystagmus survey. *Invest Ophthalmol Vis Sci*. 2009;50:5201–5206.
4. Gottlob I, Proudlock FA. Aetiology of infantile nystagmus. *Curr Opin Neurol*. 2014;27:83–91.
5. Fu VLN, Bilonick RA, Felius J, Hertle RW, Birch EE. Visual acuity development of children with infantile nystagmus syndrome. *Invest Ophthalmol Vis Sci*. 2011;52:1404–1411.
6. Pilling R, Thompson J, Gottlob I. Social and visual function in nystagmus. *Br J Ophthalmol*. 2005;89:1278–1281.
7. McLean RJ, Windridge KC, Gottlob I. Living with nystagmus: a qualitative study. *Br J Ophthalmol*. 2012;96:981–986.
8. Thurtell MJ, Leigh RJ. Therapy for nystagmus. *J Neuroophthalmol*. 2010;30:361–371.
9. McLean R, Proudlock F, Thomas S, Degg C, Gottlob I. Congenital nystagmus: randomized, controlled, double-masked trial of memantine/gabapentin. *Ann Neurol*. 2007; 61:130–138.
10. Dell'Osso LF, van der Steen J, Steinman RM, Collewijn H. Foveation dynamics in congenital nystagmus. III: Vestibulo-ocular reflex. *Doc Ophthalmol*. 1992;79:51–70.
11. Dell'Osso LF, van der Steen J, Steinman RM, Collewijn H. Foveation dynamics in congenital nystagmus. II: smooth pursuit. *Doc Ophthalmol*. 1992;79:25–49.
12. Dell'Osso LF, van der Steen J, Steinman RM, Collewijn H. Foveation dynamics in congenital nystagmus. I: fixation. *Doc Ophthalmol*. 1992;79:1–23.
13. Dougherty JA, Rhoney DH. Gabapentin: a unique antiepileptic agent. *Neurol Res*. 2001;23:821–829.
14. Sills GJ. The mechanisms of action of gabapentin and pregabalin. *Curr Opin Pharmacol*. 2006;6:108–113.
15. Johnson JW, Kotermanski SE. Mechanism of action of memantine. *Curr Opin Pharmacol*. 2006;6:61–67.
16. Wilkinson D. A review of the effects of memantine on clinical progression in Alzheimer's disease. *Int J Geriatr Psychiatry*. 2012;27:769–776.
17. Choudhuri I, Sarvananthan N, Gottlob I. Survey of management of acquired nystagmus in the United Kingdom. *Eye (Lond)*. 2007;21:1194–1197.
18. Maurer CM, Huang YY, Neuhauss SC. Application of zebrafish oculomotor behavior to model human disorders. *Rev Neurosci*. 2011;22:5–16.
19. Bilotta J, Saszik S. The zebrafish as a model visual system. *Int J Dev Neurosci*. 2001;19:621–629.
20. Beck JC, Gilland E, Tank DW, Baker R. Quantifying the ontogeny of optokinetic and vestibuloocular behaviors in zebrafish, medaka, and goldfish. *J Neurophysiol*. 2004;92: 3546–3561.
21. Easter SS, Nicola GN. The development of vision in the zebrafish (Danio rerio). *Dev Biol*. 1996;180:646–663.
22. Easter SS, Nicola GN. The development of eye movements in the zebrafish (Danio rerio). *Dev Psychobiol*. 1997;31:267–276.
23. Huang Y-Y, Neuhauss SCF. The optokinetic response in zebrafish and its applications. *Front Biosci*. 2008;13:1899–1916.
24. Baier H. Zebrafish on the move: towards a behavior-genetic analysis of vertebrate vision. *Curr Opin Neurobiol*. 2000;10: 451–455.
25. Gestri G, Link BA, Neuhauss SCF. The visual system of zebrafish and its use to model human ocular Diseases. *Dev Neurobiol*. 2012;72:302–327.
26. Gross JV, Perkins BD. Zebrafish mutants as models for congenital ocular disorders in humans. *Mol Reprod Dev*. 2008;75:547–555.
27. Fadool JM, Dowling JE. Zebrafish: a model system for the study of eye genetics. *Prog Retin Eye Res*. 2008;27:89–110.
28. Huang Y-Y, Rinner O, Hedinger P, Liu S-C, Neuhauss SCF. Oculomotor instabilities in zebrafish mutant belladonna: a behavioral model for congenital nystagmus caused by axonal misrouting. *J Neurosci*. 2006;26:9873–9880.
29. Huang MY, Chen CC, Huber-Reggi SP, Neuhauss SC, Straumann D. Comparison of infantile nystagmus syndrome in achiasmatic zebrafish and humans. *Ann N Y Acad Sci*. 2011; 1233:285–291.
30. Abadi RV, Dickinson CM. Waveform characteristics in congenital nystagmus. *Doc Ophthalmol*. 1986;64:153–167.
31. Yee RD, Wong EK, Baloh RW, Honrubia V. A study of congenital nystagmus: waveforms. *Neurology*. 1976;26:326–333.
32. Huber-Reggi SP, Chen C-C, Grimm L, Straumann D, Neuhauss SC, Huang MY. Severity of infantile nystagmus syndrome-like ocular motor phenotype is linked to the extent of the underlying optic nerve projection defect in zebrafish belladonna mutant. *J Neurosci*. 2012;32:18079–18086.
33. Mullins MC, Hammerschmidt M, Haffter P, Nüsslein-Volhard C. Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. *Curr Biol*. 1994;4: 189–202.
34. Haffter P, Granato M, Brand M, et al. The identification of genes with unique and essential functions in the development of the zebrafish, Danio rerio. *Development*. 1996;123:1–36.
35. Chen CC, Bockisch CJ, Bertolini G, et al. Velocity storage mechanism in zebrafish larvae. *J Physiol*. 2014;592:203–214.
36. Bogli SY, Afthinos M, Bertolini G, Straumann D, Huang MY. Unravelling stimulus direction dependency of visual acuity in larval zebrafish by consistent eye displacements upon optokinetic stimulation. *Invest Ophthalmol Vis Sci*. 2016; 57:1721–1727.
37. Shery T, Proudlock FA, Sarvananthan N, McLean RJ, Gottlob I. The effects of gabapentin and memantine in acquired and congenital nystagmus: a retrospective study. *Br J Ophthalmol*. 2006;90:839–843.
38. Starck M, Albrecht H, Pollmann W, Dieterich M, Straube A. Acquired pendular nystagmus in multiple sclerosis: an examiner-blind cross-over treatment study of memantine and gabapentin. *J Neurol*. 2010;257:322–327.

39. Smith RV, Havens JR, Walsh SL. Gabapentin misuse, abuse and diversion: a systematic review. *Addiction*. 2016;111:1160–1174.
40. Norton JW. Gabapentin withdrawal syndrome. *Clin Neuropharmacol*. 2001;24:245–246.
41. Johnson JW, Glasgow NG, Povysheva NV. Recent insights into the mode of action of memantine and ketamine. *Curr Opin Pharmacol*. 2015;20:54–63.
42. Lopez-Valdes HE, Clarkson AN, Ao Y, et al. Memantine enhances recovery from stroke. *Stroke*. 2014;45:2093–2100.
43. Taylor CP, Gee NS, Su TZ, et al. A summary of mechanistic hypotheses of gabapentin pharmacology. *Epilepsy Res*. 1998; 29:233–249.
44. Lipton SA. The molecular basis of memantine action in Alzheimer's disease and other neurologic disorders: low-affinity, uncompetitive antagonism. *Curr Alzheimer Res*. 2005;2:155–165.
45. Dell'Osso LF, Jacobs JB. An expanded nystagmus acuity function: intra- and intersubject prediction of best-corrected visual acuity. *Doc Ophthalmol*. 2002;104:249–276.
46. Sheth N, Dell'Osso L, Leigh R, Van Doren C, Peckman H. The effects of afferent stimulation on congenital nystagmus foveation periods. *Vision Res*. 1995;35:2371–2382.
47. Thurtell MJ, Joshi AC, Leone AC, et al. Crossover trial of gabapentin and memantine as treatment for acquired nystagmus. *Ann Neurol*. 2010;67:676–680.

Investigative Ophthalmology & Visual Science