# Eye Movement Recordings as an Effectiveness Indicator of Gene Therapy in *RPE65*-Deficient Canines: Implications for the Ocular Motor System

*Jonathan B. Jacobs,*[1,2] *Louis F. Dell'Osso,*[1,2,3] *Richard W. Hertle,*[4,5] *Gregory M. Acland,*[6] *and Jean Bennett*[7]

**Purpose.** To perform ocular motility recordings of infantile nystagmus (IN) in *RPE65*-deficient canines and determine whether they can be used as a motor indicator of restored retinal function to investigate the effects of gene therapy.

**Methods.** Treated and untreated canines were comfortably suspended in a custom-built sling and encouraged to fixate on distant targets at gaze angles varying between ±15° horizontally and ±10° vertically. Ocular motility recordings were made, using two distinct methods—infrared reflection and high-speed video. The resultant recordings from three untreated, four treated, and three pre- and post-treatment dogs were analyzed for using the eXpanded Nystagmus Acuity Function (NAFX), which yields an objective assessment of best potential visual acuity, based on the duration and repeatable accuracy of foveation and centralisation.

**Results.** During fixation, the untreated dogs exhibited large-amplitude, classic IN waveforms, including pendular and jerk in both the horizontal and vertical planes, which prevented them from keeping the targets within the area centralis (the region of highest receptor density, spanning ±3° horizontally by ±1.5° vertically, analogous to the fovea). Some untreated dogs also had small-amplitude (0.5–1°), high-frequency (6–9 Hz) oscillations. Under the same conditions, successfully treated canines no longer exhibited clinically detectable IN. Their IN was converted to waveforms with very low amplitudes that yielded higher NAFX values and allowed target images to remain well within the area centralis. Of note, uniocular treatment appeared to damp the IN in both eyes. Behaviorally, the treated dogs were able to successfully navigate through obstacles more easily without inadvertent contact, a task beyond the untreated dogs' ability.

**Conclusions.** Gene therapy that successfully restored retinal function also reduced the accompanying IN to such a great extent that it was not clinically detectable ~90% of the time in many of the dogs. IN improvement, as quantified by the NAFX, is an objective motor indicator of visual improvement due to gene therapy. (*Invest Ophthalmol Vis Sci.* 2006;47:2865–2875) DOI:10.1167/iovs.05-1233

Leber congenital amaurosis (LCA) is the designation for a group of currently untreatable, autosomal-recessive retinal dystrophies that are the most common genetic cause of visual impairment in children. At birth, the retinas frequently appear normal, yet already have greatly reduced function. Usually, there is some vision initially, but it degenerates over the course of childhood, often leading to total blindness. This loss of vision is frequently accompanied by nystagmus, an involuntary oscillation of the eyes where slow-phase movements take the fovea off of the target, and fast-phase movements acting in opposition bring the fovea back toward the target. More than 45 different types of nystagmus have been characterized, arising from a great variety of etiologies, from benign to pathologic, appearing at (or near) birth, or being acquired later in life.[1] The qualitative progression of LCA in dogs is similar to that in humans with early and severe visual impairment but a more slowly progressive degeneration of retinal morphology.[2,3]

The molecular genetics of LCA are broad and quite complex, and can be traced to defects in any one of at least six different genes: *RPE65*, *CRX*, *GC1*, *AIPL1*, *RPGRIP1*, *TULP1*. This study concentrates on the *RPE65* gene-deficient variant, which accounts for 10% to 20% of LCA cases. There are two animal models for this variant—the Rpe65$^{-/-}$ mouse, and the Briard dog (discussed later), both the end products of years of careful breeding—and they serve as the basis for the ongoing development of a potential therapy consisting of the subretinal injection of approximately 150 to 255 μL of an adenoassociated virus (AAV) that has been modified to carry the DNA encoding RPE65 in vivo (AAV-*RPE65*).[4] This virus was selected because it targets retinal pigment epithelium (RPE) in a stable fashion and has not been associated with any human or animal disease.

The primary outcome measures used in the initial studies included electroretinograms (ERGs),[5,6] which must be performed on anesthetized dogs, and pupillometry, which is a less direct measure. We hypothesized that the application of the expanded nystagmus acuity function (NAFX— described later) would provide an objective estimate of the dogs' visual function before and after therapy. The NAFX provides an assessment of how much visual loss could be ascribed to ocular motor system instability alone. It is important to remember that it is a *predictor*, rather than a direct measure, of best *possible*

From the [1]Daroff-Dell'Osso Ocular Motility Laboratory, Veterans Affairs Medical Center; and the Departments of [2]Neurology and [3]Biomedical Engineering, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, Ohio; the Departments of [4]Pediatric Ophthalmology and [5]Ophthalmology, Children's Hospital of Pittsburgh and Pittsburgh Eye and Ear Institute, University of Pittsburgh Medical Center, Pittsburgh Pennsylvania; the [6]James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York; and the [7]F. M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, University of Pennsylvania, Philadelphia.

Supported in part by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs; National Eye Institute Grants EY06855, EY10820, U10UF01109, and Training Grant EY07157; Research to Prevent Blindness, Foundation Fighting Blindness; the F. M. Kirby Foundation; and the Paul and Evanina Mackall Foundation Trust.

Submitted for publication September 16, 2005; revised March 1 and 9, 2006; accepted May 17, 2006.

Disclosure: **J.B. Jacobs**, None; **L.F. Dell'Osso**, None; **R.W. Hertle**, None; **G.M. Acland**, None; **J. Bennett**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Louis F. Dell'Osso, Daroff-Dell'Osso Ocular Motility Laboratory, Louis Stokes Cleveland Veterans Affairs Medical Center, 10701 East Boulevard, Cleveland, OH 44106; lfd@case.edu.

visual acuity when there is no afferent defect. Because the lack of retinal function was the major acuity-limiting factor in the dogs, their NAFX-potential acuities would be higher than their actual acuities. Therefore, the NAFX provides a direct, objective measure of nystagmus improvement and an indirect measure of acuity improvement. In assessing the direct therapeutic efficacy of nystagmus surgery, the NAFX is the best direct measure of nystagmus improvement because, unlike prior measures (amplitude, frequency, slow-phase velocities), it is also an estimate of potential visual acuity improvement. In this study, the NAFX provides an indirect motor measure of the direct outcome of gene therapy (i.e., improved visual acuity).

Initial clinical examinations of the dogs' eye movements suggested a strong similarity to those in the human infantile nystagmus syndrome (INS)[7]—formerly known as congenital nystagmus (CN)— both clinically and on examination of the waveforms. As in most humans, the dogs' nystagmus was noted to appear shortly after birth (in the case of the dogs, shortly after eye opening at approximately 14 days after birth) and always occurred in the horizontal plane, although vertical and torsional components have been often noted. In addition, because the motor signals that cause the eyes to oscillate also drive efferents to the neck muscles,[8] it is very common to observe a small head "tremor" accompanying the eye movements. In humans, this head movement is generally not under conscious control, but can be stopped when brought to the subject's attention. In dogs, there is no such social pressure to suppress it when it occurs. We saw these movements occasionally, observing the dogs when they were relaxed and back in their kennels; however, when we were handling them, they were too active, making too many volitional head movements to allow reliable detection.

Acuity depends on *foveation periods*, which are the times when both eye position (i.e., target error) and velocity criteria (i.e., retinal slip, or how fast the target is moving across the retina) fall below a certain threshold (in humans, within one foveal radius, 0.5°, and <40°/sec). IN intensity (amplitude × frequency) is usually modulated by emotional state and attention. We could clearly observe several dogs' nystagmus increase as they got excited, such as during times when they were initially taken from the kennel to the examination room, or during "play time," the handling we performed to socialize them and get them used to us.

## METHODS

### Subjects

The *RPE65*$^{-}$ mutant strain of dog is an autosomal recessive model of LCA but displays no other abnormalities. Its disease originated as a naturally occurring disorder affecting the Briard breed of dog in several countries.[3,9] Although initially[9] termed a congenital stationary night blindness, in affected dogs, the disease exhibits variably severe abnormalities of cone-mediated vision as well, shows slow progression of symptoms with age and very slow developments of degenerative retinal morphologic changes.[10] The *RPE65*$^{-}$ mutant strain used in this study is a crossbred strain derived by breeding a single affected Briard dog to the research colony strain of normal beagle-crossbred dogs. The natural course of the disease in dogs means that there is an extremely long window of time in which therapeutic trials can be undertaken.

Over the course of 17 months, we performed ocular motility recordings on 11 dogs, divided into two groups. Group 1 included dogs BR33, BR47, and BR58, which were treated before ocular motility recordings could be made, and dogs BR108, BR118, BR138, and BR57 (littermate to BR58), which were recorded but not treated and were included for ocular motor comparison to the treated dogs. The group-2 dogs BR158, BR161, BR164, and BR174 were recorded before treatment, and at 3 and 10 months after treatment, with the latter data used for this study. The differing treatment status of the dogs in group 1 reflects the fact that we were invited to study the eye movements of these dogs after the gene-therapy studies had commenced. The dogs in group 1 were recorded by three of the authors (JBJ, LFD, RWH), who were not informed which dogs were treated and which were not. This procedure was used to assess the ability of ocular motor analysis to provide another measure of the effectiveness of gene therapy. All animal work followed the guidelines set forth in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Clinical Examination

Before any recordings, each dog's eye movements were examined for qualitative appearance with either an ophthalmoscope or a high-positive magnifying glass and penlight. Nystagmus, if present, was roughly characterized for amplitude, frequency, and plane(s). Ocular alignment was noted as well. Visual behavior was tested by allowing the dog to wander freely in the examination room (14 × 12 feet) and observing whether it reacted to movements made by the people in the room and how well it could avoid obstacles during its explorations. This behavior was videotaped. If the dog was able to navigate the room in normal light, the light level was then reduced to the lowest level that would still permit videotaping, and the dog's performance was again observed as an indicator of low-light visual function.

### Recording

Initial eye movement recordings were made using a uniplanar infrared reflection (IR) eye tracker (Applied Scientific Laboratories, Waltham, MA). In the horizontal plane the system was linear to ±20° and monotonic to ±25 ° to 30° with a sensitivity of 0.25°. The total system bandwidth (position and velocity) was 0 to 100 Hz. The data were digitized at 500 Hz with 16-bit resolution. The remaining data were recorded using a high-speed video system (EyeLink II; SR Research Ltd., Mississagua, Ontario, Canada) capable of measuring horizontal and vertical movement simultaneously at a sampling frequency of 500 Hz and a resolution of 16 bits. Although the EyeLink II system was designed for operation with human subjects, we were able to modify it for operation with dogs by repositioning the cameras more laterally to compensate for the greater interpupillary distance in dogs, whose eyes are not as forward-pointing as those of humans (Fig. 1). Because the EyeLink II cameras are mounted on a headband (specifically designed for human head-fixed operation) that does not fit a dog's head, we mounted the cameras to an adjustable and lockable earth-fixed frame. As shown in Figure 1, the cooperative dogs needed only the guidance of human hands to encourage them to keep their heads still during the short recording times. Any movement was reported on videotape.

Dogs with long hair that could potentially cover their eyes, or otherwise interfere with the sensors or cameras were shaved the day before recording. Their eyes were irrigated to remove any stray hairs, and they were examined with an ophthalmoscope for signs of irritation. They were then returned to their kennels after a brief session of socialization.

In previous work[11] we described the techniques necessary to record eye movements, using head-mounted IR from comfortably restrained, alert, relaxed, cooperating, but untrained dogs. For this study, the only modification needed was to assure that the dogs did not move their heads during recording, which—if not detected during experiments—would lead to motion artifacts. Firm but gentle hand pressure behind the dog's occiput accomplished this. Such contact served three purposes: In addition to restraint, it allowed the investigator to report on any movement so that corrupted data could be discarded. Furthermore, the constant contact helped to relax the dog. As an additional safeguard against poor data quality due to the dog's movement or inattention, sessions were video recorded, with a live video feed also present at the data acquisition operator's station.

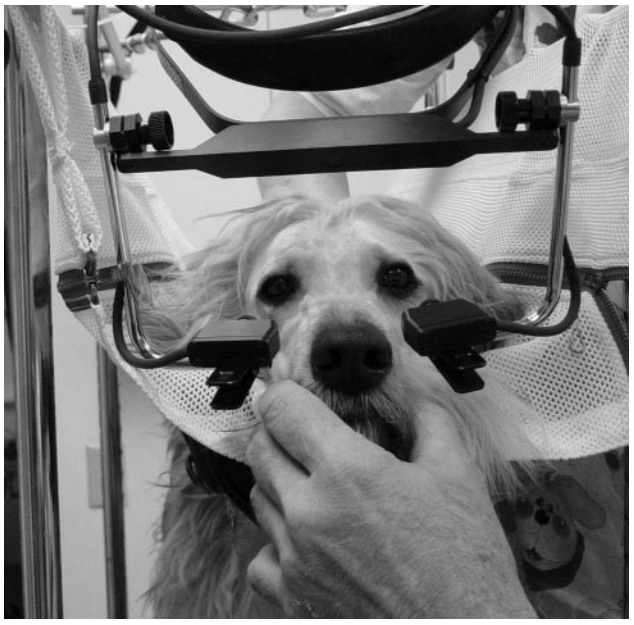

**FIGURE 1.** EyeLink II (SR Research Ltd., Mississagua, Ontario, Canada) camera setup. The dog's head is supported from below and stabilized from behind.

Before recording, the dogs' eye movements were calibrated against known targets in the horizontal and vertical planes. Special effort was made to increase accuracy during calibration. Each target was presented multiple times within the binocular field, to ensure sufficient accurate conjugate saccades to the target. These techniques allowed for a "ballpark" calibration of the EyeLink II system, by using its native routines (which are suitable for behaving subjects, such as humans or lower primates that have been trained to fixate a target reliably). A final, more accurate calibration was applied post hoc to the data using custom software developed (MATLAB; The MathWorks, Natick, MA) to examine where each dog looked during the repeated target presentations. Finally, to ensure accuracy over the course of an experiment, the calibration presentation was repeated during each trial.

## Protocol

All ocular motility recordings were performed in accordance with the Institutional Animal Use and Care Committees' (IACUC) guidelines regarding animal experimentation. A trained veterinarian was present, or could be summoned, at all times. An experiment consisted of between two and seven trials, determined by how cooperative the investigators felt the dog to be. If a dog refused to allow its head to be held still, or ceased to attend to targets, it was allowed to rest in the sling apparatus for several minutes before further attempts were made. If it still refused to cooperate or began to struggle against the restraints, it was immediately released for that session. No more than two sessions were attempted for any dog per day.

Each trial lasted from 30 to 90 seconds, again depending on the dog's ability and willingness to cooperate. During each trial, the dog was required to fix on an object that was determined to hold its interest, such as an electronic toy that made noise and had a flashing light. One examiner stood 57 in. from the dog's eyes and, holding the fixation target near his own eyes, moved repeatedly between the points of 0°, ±15° horizontally, and ±10° vertically, holding the target for approximately 5 seconds at each point. Because he was looking directly into the dog's eyes, the examiner was able to verify when the dog looked at each target and to report if the dog failed to do so. The data-acquisition operator also monitored the dog's performance using both a live feed from the video camera recording the session and the on-screen feedback provided by the EyeLink II. Recordings during which a dog ceased to pay attention to the target or moved its head (also monitored by the investigator stabilizing the dog's head) were marked accordingly.

## Analysis

Eye movement recordings by the EyeLink II system were exported using routines provided by SR Research into ASCII format that could be read into the computer program and analyzed, using custom-written MATLAB software. Only position data were measured directly. Velocity was calculated by means of a two-point central differentiator algorithm that also acted as a low-order, low-pass filter whose cutoff frequency decreased as the separation between the difference points increased. More information on this algorithm and its consequences may be found elsewhere.[12] Further filtering was performed, as needed, using a fourth-order low-pass filter with a cutoff frequency of 20 Hz, sufficient to reduce noise while retaining the eye-movement data.

Foveation quality and its potential effect (not accounting for afferent deficit) on human visual acuity were calculated using the NAFX, which returns a single value that ranges linearly between 0.0 and 1.0, corresponding to no vision at the low end, to a Snellen acuity of 20/15 (1.33) at the high end for young adults. It is important to note that these values represent human vision; most dog breeds have a lower maximum acuity, approximately 20/70 (e.g., several breeds of working dogs, especially hunting dogs), owing to the less developed nature of the canine area centralis (AC) compared with the fovea. The NAFX calculates this value based on the duration and repeatability (i.e., the standard deviations of fixation positions and velocities) of centralisation periods, defined as the data points that simultaneously satisfy particular position and velocity limits, defined as the *centralisation window.* Although details for application of the NAFX have been described previously,[13] it is instructive to summarize the most important rules: (1) ensure that the data are accurately calibrated to allow identification of the fixating eye; (2) select only segments of data where the subject is known to have been attending to the target, because without attention, foveation does not have a physical meaning; and (3) avoid long stretches of data recording (e.g., minutes), during which the subject (nystagmus subject, normal human, or canine) does not always maintain concentration or, in many cases, may switch which eye is fixing the target. Failure to adhere to these rules produces noisy data and precludes accurate measure of the quality of fixation, the nystagmus mechanisms, or pre- and post-therapy nystagmus.[14] Using the NAFX requires selecting the position and velocity criteria for the foveation window as small as necessary to suit the fixation ability of the subject being measured, *but no smaller than that.* In the case of the dogs, the centralisation window's lower boundaries were ±3.0° horizontally and ±1.5° vertically, reflecting the extent of the AC; the velocity limits were set between ±4°/s and ±10°/s, as in humans.[15] We used the same minimum velocity as for humans, because no psychophysical data exist for dogs, and the underlying physical constraints are equivalent. We limited our analyses to data segments that were no longer than 10 seconds and that showed no changes (or loss) in fixation during that time. Records where the dogs made head movements or failed to attend to the targets were not analyzed.

# RESULTS

## Nystagmus before and after Treatment

Of the 11 dogs in the study, 4 (BR108, BR118, BR138, BR57) had only ocular motility recordings made while untreated; 3 (BR33, BR47, BR58) had only ocular motility recordings made after treatment; and 4 (BR158, BR161, BR164 and BR174) had recordings made both before and after treatment. The nystagmus of the untreated dogs in group 1 was observed clinically

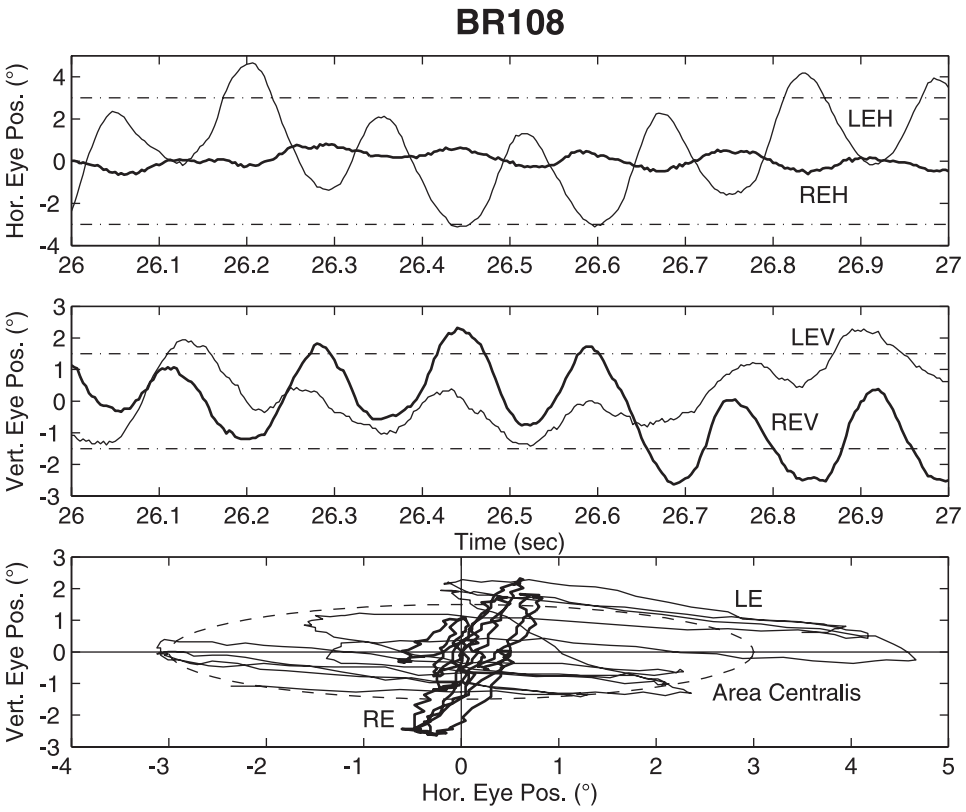


**FIGURE 2.** Horizontal (*top*) and vertical (*middle*) eye movements versus time and nystagmus scan paths (*bottom*) of both eyes of untreated dog BR108. *Heavy lines:* right eye; *dot-dashed* (time plots) or *dashed lines* (scan paths): the horizontal and vertical extents of the AC.

by two of the authors (GMA, JB) who also noted the clinical absence of nystagmus in the three treated dogs. Ocular motor recordings documented the nystagmus characteristics of both the untreated and treated dogs in group 1.

Movie 1 (all movies are online at http://www.iovs.org/cgi/content/full/47/7/2865/DC1) shows a video of untreated dog BR108. Figure 2 shows nystagmus in the horizontal (top) and vertical (middle) planes for BR108. The waveform was predominantly pendular with an average frequency of 6.5 Hz, which is toward the high end for IN. The (horizontal) peak-to-peak amplitude ranged from 1.0 ° to 2.8° when the right eye (RE) was viewing and from 0.7 ° to 2.0° for left eye (LE) viewing. These amplitudes were smaller than the extent of the AC (represented by the dot-dashed lines at ±3°). Similarly, the vertical oscillations were on the order of magnitude of the vertical extent of the AC (±1.5°); therefore, we would expect that visual acuity should not be badly compromised by the oscillation, as long as there were sufficient periods of low velocity during each cycle. It is important to note that though the overall peak-to-peak amplitude of the nystagmus would allow the target to remain in the AC, the dog did not have perfect control of its centralisation, and these turn-around points did not always line up from cycle to cycle. The resultant "drift" in the waveform meant that the target ended up outside the AC, thereby lowering the potential for visual acuity.

There were differences in the *phase relationship* between the LE versus RE in both the horizontal and vertical eye planes. These relationships were variable, so that the eyes appeared at times to mimic vergence nystagmus (Movies 2, 3) or see-saw nystagmus (Movies 4, 5). By plotting the vertical versus horizontal movement in a *nystagmus scan path*, it is easy to appreciate the oblique trajectory of the eyes due to the horizontal and vertical components. Note that the RE axis of travel is oriented more toward the vertical, whereas the LE is oriented more horizontally, due to the smaller relative contribution of the RE's horizontal component. Such unequal amplitudes are not uncommon in human IN.

We were unable to make quantitative recordings of the treated dogs in group 1 before their injections, although qualitative clinical examinations were performed. It was noted that the siblings BR57 and BR58 had nystagmus that could not be distinguished from one another by inspection, before BR58's treatment. Analysis of BR57's nystagmus revealed it to be strikingly similar to those of BR108, including the unequal magnitudes and variable phases between RE and LE and horizontal and vertical planes. Figure 3 and Movies 6,7 and 8 show BR57's nystagmus with its oppositely directed elliptical trajectories. The results of the nystagmus analyses are summarized in Table 1. In Group 2, one of the dogs did not have nystagmus. Because of the ranges of both frequencies and amplitudes within and between dogs, these gross nystagmus characteristics did not differentiate between the treated and untreated condition.

Clinically, BR58 and the other treated dogs displayed no nystagmus, and it was necessary to search thoroughly all the data taken for these dogs to find any intervals where nystagmus was present. Most of the time (~90%) there was no nystagmus in the treated dogs, and that corresponds to an NAFX of 1.0. From ERG studies, it was determined (by GMA) that the receptor function was restored to, on average, approximately 16% of normal function, and behaviorally it was clear that these dogs had greatly improved visual function that allowed them to navigate around obstacles that they previously could not avoid. This improvement was noted under the low-light conditions described earlier, as well as under normal room lighting levels. The post-treatment eye movements of BR58 (Movies 9, 10, 11), during a rare burst of oscillatory movements, are shown in Figure 4, and those of another treated dog, BR47 are shown in Figure 5. BR58's waveform remained pendular, and the frequency of the nystagmus was unchanged from that observed for its littermate (BR57), whereas the peak-to-peak amplitudes

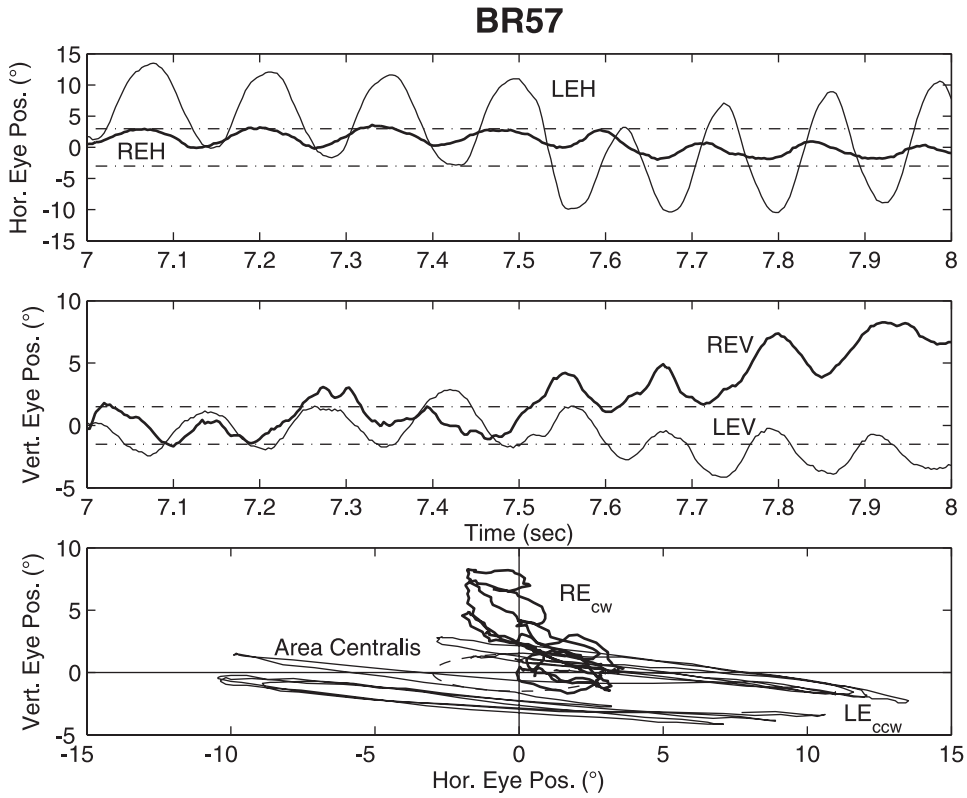


**FIGURE 3.** Horizontal (*top*) and vertical (*middle*) eye movements versus time and nystagmus scan paths (*bottom*) of both eyes of untreated dog BR57. RE motion is clockwise elliptical and LE motion is counterclockwise elliptical with both major axes in the second quadrant.

were so greatly reduced that the target remained within the AC at all times, unless carried off by nonnystagmus drifts or inattention, as shown in the bottom panels of both these figures and their related movies. Note that BR58 received injections in both eyes, whereas BR47 was treated in only the RE (as was dog BR33), yet this uniocular restoration of vision was sufficient to reduce the nystagmus in *both* eyes. That is, no untreated dog had an NAFX $>$ 0.512, yet both uniocularly treated dogs had NAFX's $>$ 0.7, a 40% increase. Also, all pretreated dogs had clinically observable nystagmus (in both eyes) whereas after treatment, the nystagmus was not clinically observable. Note that the drift of the untreated LE was much greater than that of the treated RE, allowing the target to go beyond the bounds of the AC.

It was not possible to examine changes in the phase relationships between eyes or planes, because there were not

**TABLE 1.** Ocular Motor Data

| Group 1 | Age at OMR (mo) | Tx Age (Eye) | IN Freq (HZ) | IN RE Fix (°pp) | IN LE Fix (°pp) |
|---|---|---|---|---|---|
| BR108 | 12 | NA | 6.5 | 1.0–2.8 | 0.7–2.0 |
| BR118 | 11 | NA | 3.5–6.0 | 3.0–6.3 | 1.5–5.0 |
| BR138 | 3 | NA | 3.5–6.0 | 1.8–10 | 1.4–10 |
| BR57* | 19 | NA | 7.0–9.0 | 0.5–2.8 | 1.0–5.2 |
| BR58* | 19 | 11 mo (BE) | 7.0–15† | 0.2–1.8† | 0.2–1.0† |
| BR47 | 26 | 3 mo (RE) | 5.0–10† | 0.3–1.0† | 0.5–3.8† |
| BR33 | 27 | 4 mo (RE) | 2.0–14† | 0.4–2.5† | 0.3–2.8† |

| Group 2 | Age at OMR (mo) | Tx Age (Eye) | IN Freq (HZ) (pre)‡ | IN BE Fix (°pp) (pre)‡ |
|---|---|---|---|---|
| BR158 | 16 | 6 mo (BE) | P:3.0–7.0§<br>J:<1.0–1.0 | P:1.0–6.0§<br>J:7.0–15.0 |
| BR161 | 15 | 5 mo (BE) | No IN | No IN |
| BR164 | 15 | 5 mo (BE) | 3.3 | 1.5–2.5 |
| BR174 | 14 | 4 mo (BE) | 7.5 | 1.0–2.0 |

OMR, ocular motility recording; P, pendular; J, Jerk; Tx, treatment RE/LE/BE Fix, right/left/both eye Fixation.

* Littermates.

† Only occasional cycles of nystagmus; 90% of the time, no nystagmus.

‡ Posttreatment characteristics unchanged (when nystagmus present).

§ Intermittent.

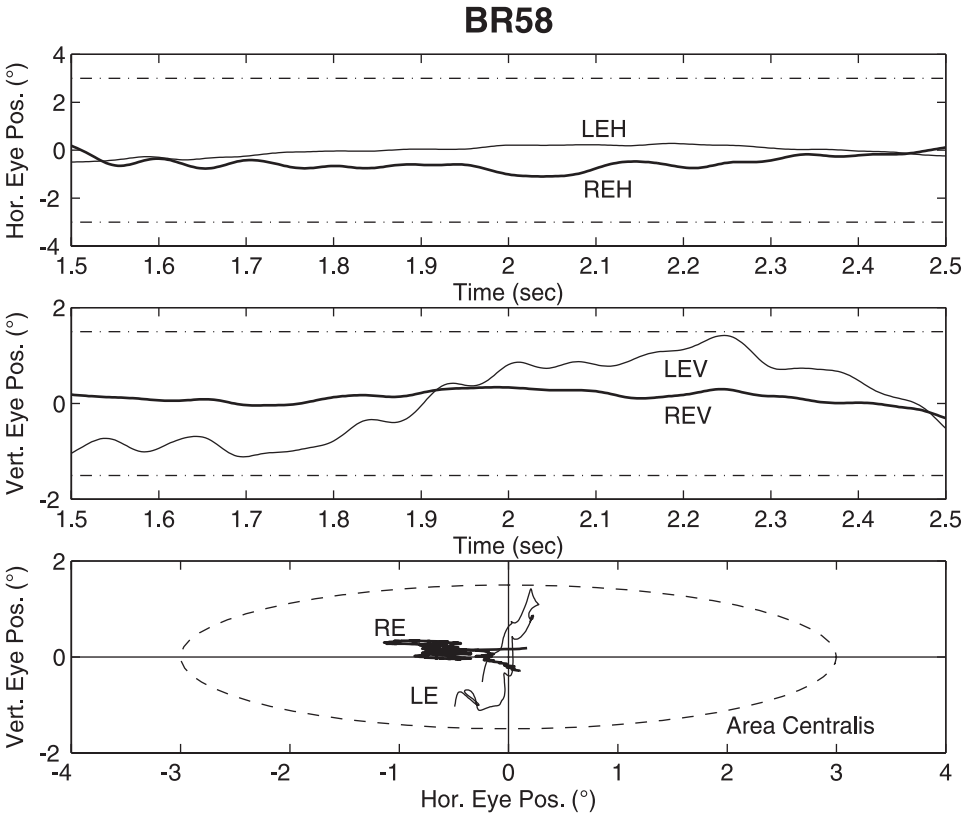


**FIGURE 4.** Horizontal (*top*) and vertical (*middle*) eye movements versus time and nystagmus scan paths (*bottom*) of both eyes of treated dog BR58. Note the absence of any sustained nystagmus in either plane, allowing the image of the target to remain within the AC.

enough times when the nystagmus existed in more than one eye or plane simultaneously.

The dogs in group 2 were recorded immediately before treatment and at 3 and 10 months after receiving binocular injections. Figure 6, top, shows output from the NAFX program and RE horizontal data from BR158 immediately before injection. The heavy points in Figures 6 and 7 represent the times during which the algorithm determined that the eyes

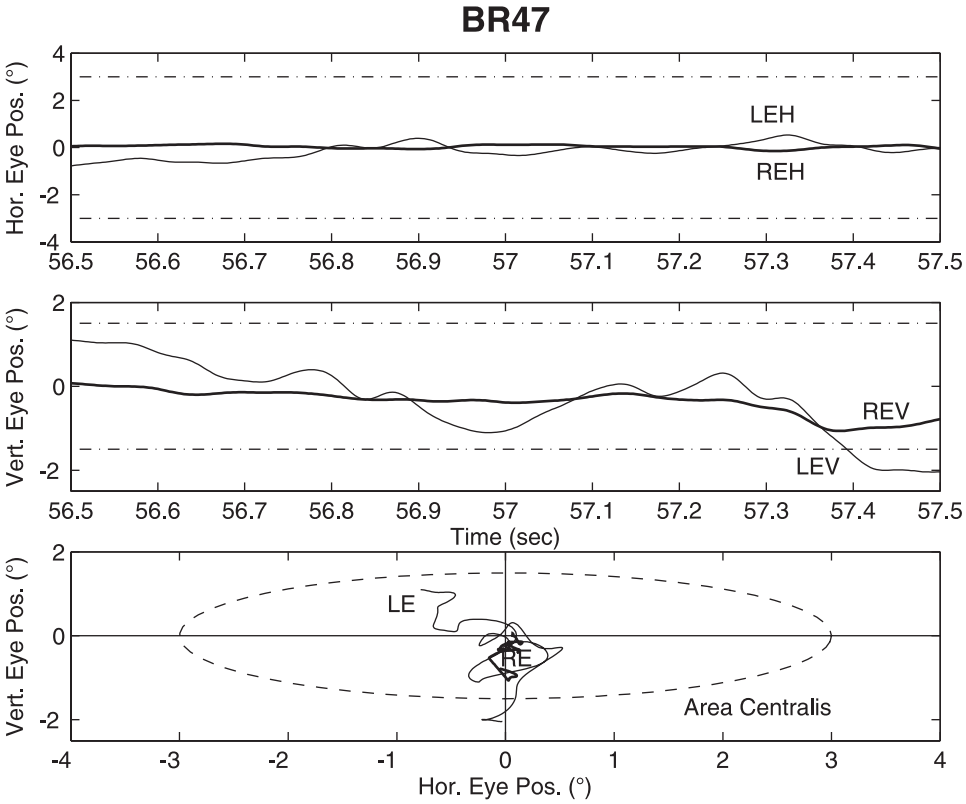


**FIGURE 5.** Horizontal (*top*) and vertical (*middle*) eye movements versus time and nystagmus scan paths (*bottom*) of both eyes of treated dog BR47. Note the absence of any sustained nystagmus in either plane, allowing the image of the target to remain within the AC.

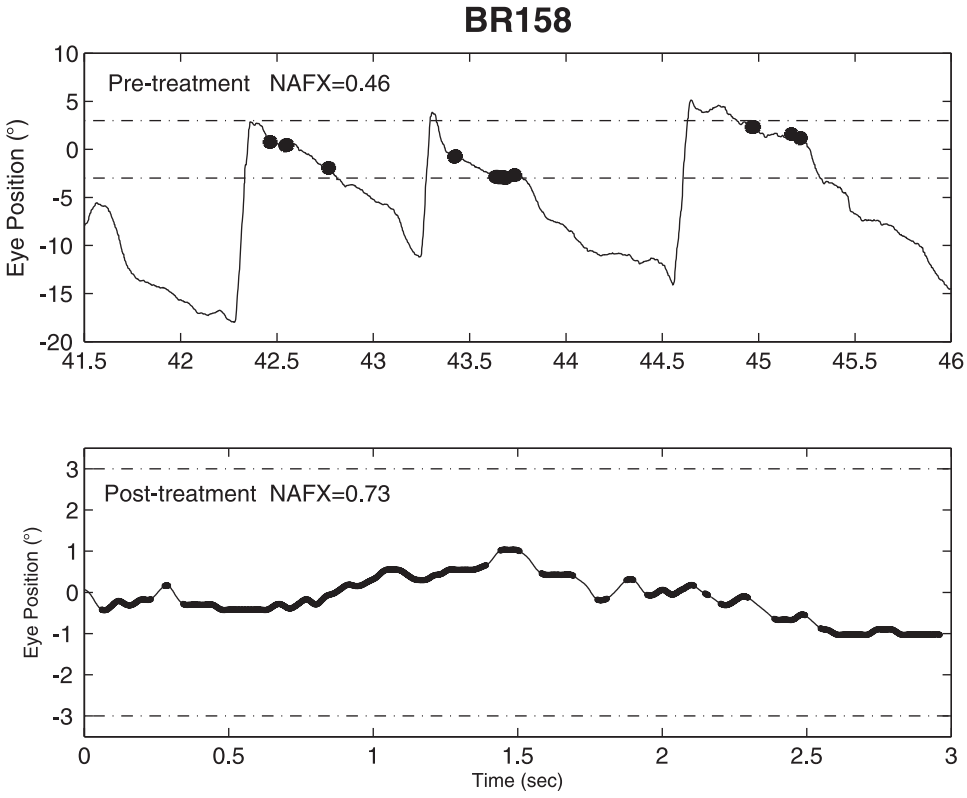


**FIGURE 6.** NAFX program output showing horizontal eye movements of BR158 before (*top*) and after (*bottom*) treatment. Pretreatment nystagmus is large-amplitude (~20° peak-to-peak), right-beating jerk with few foveation points. After treatment, the target remains reliably within the AC, and there are many points that satisfy velocity criterion. In this figure and in Figure 7, dark segments are data points simultaneously satisfying the imposed position and velocity criteria of the foveation window used to calculate the NAFX. They are automatically provided by the NAFX software.

simultaneously satisfied the position and velocity criteria necessary for good visual acuity (the centralisation window discussed in the Methods section). This waveform is a very large-amplitude, right-beating, dual-jerk ($DJ_R$, high-frequency pendular superimposed on a low-frequency jerk waveform)[16] with peak-to-peak amplitudes as great as 20°, and a frequency of ~1 Hz for the jerk component. The pendular component is much smaller and faster, averaging under 2° peak-to-peak, and up-

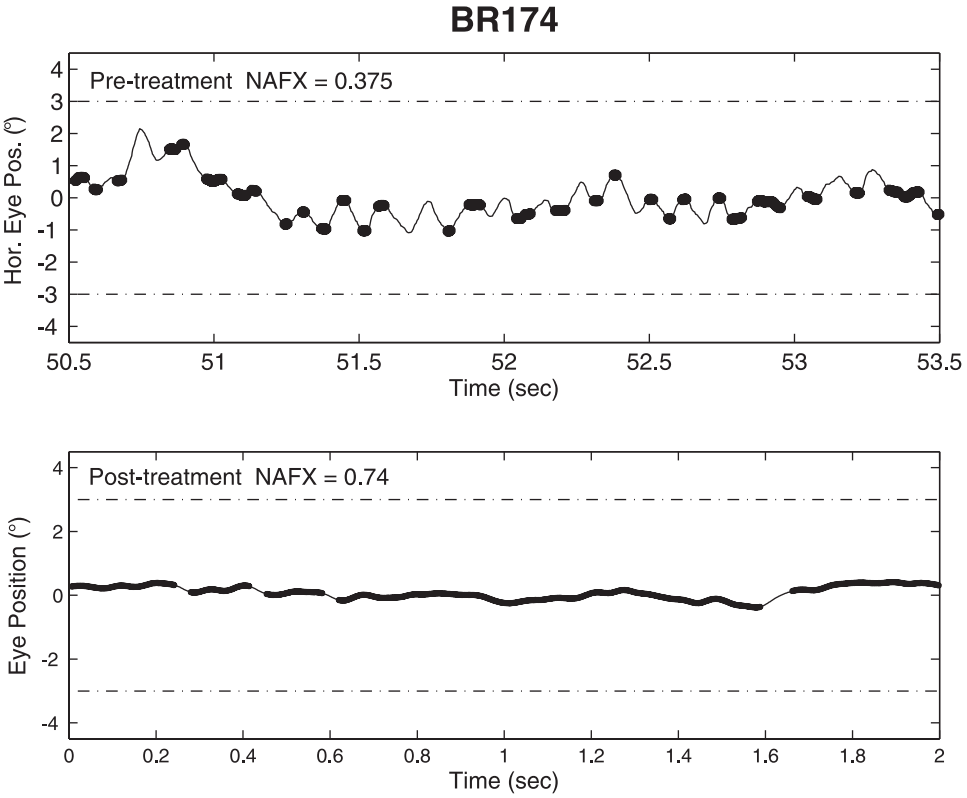


**FIGURE 7.** NAFX program output showing horizontal eye movements of BR174 before (*top*) and after (*bottom*) treatment. Contrast "before" case to that in Figure 5. Note that pretreatment amplitude is already small and many points fall below velocity criterion. After treatment, amplitude is further diminished and velocity is almost always slow.

**TABLE 2.** NAFX Data

| Group 1 | Age at OMR (mo) | Tx Age (Eye) | NAFX | | |
|---|---|---|---|---|---|
| | | | RE | LE | BE |
| BR108 | 12 | NA | 0.467 | 0.333 | 0.512 |
| BR118 | 11 | NA | 0.095 | 0.393 | 0.132 |
| BR138 | 3 | NA | 0.228 | 0.247 | 0.256 |
| BR57* | 19 | NA | 0.270 | 0.229 | 0.333 |
| BR58* | 19 | 11 mo (BE) | 0.626† | 0.557† | 0.711† |
| BR47 | 26 | 3 mo (RE) | 0.631† | 0.581† | 0.783† |
| BR33 | 27 | 4 mo (RE) | 0.639† | 0.609† | 0.711† |

| Group 2 | Age at OMR (mo)‡ | Tx Age (Eye) | ERG FA | | NAFX BE | |
|---|---|---|---|---|---|---|
| | | | LE | RE | (Pre) | (Post) |
| BR158 | 16 | 6 mo (BE) | 2.0 | 2.5 | 0.592 | 0.561 |
| BR161 | 15 | 5 mo (BE) | 2.0 | 2.0 | NA (No IN) | NA (No IN) |
| BR164 | 15 | 5 mo (BE) | 3.5 | 2.0 | 0.661 | 0.619 |
| BR174 | 14 | 4 mo (BE) | 3.0 | 3.0 | 0.375 | 0.542 |

NAFX values are average values from all trials. ERG FA, electro retinogram functional assessment. 0, No ERG; 1, Untreated ERG; 2, Small but significant treatment effect; 3, Large treatment effect; 4, Normal ERG. Abbreviations are as in Table 1.

* Littermates.

† Only occasional cycles of nystagmus; 90% of the time, no nystagmus, NAFX = 1.0.

‡ Age at post-treatment OMR; age at pretreatment OMR is same as age at treatment.

wards of 5 Hz, respectively. Clearly, there are few times when the eye is on target and moving slowly enough to allow target centralisation, and the resultant NAFX for this segment is 0.46, corresponding to a canine-adjusted Snellen fraction of ~20/150. Behaviorally, this dog had some difficulty navigating around objects, even in full light and, in low-light conditions, was effectively blind. Examples of this dog's eye movements before treatment are shown in Movies 12 and 13 and demonstrate the great difficulty this dog had keeping its AC near the target.

The output of the NAFX program presented in Figure 6, bottom, was performed on horizontal RE data recorded 10 months after treatment. The large jerk component of the nystagmus was eliminated, leaving only the smaller pendular oscillation, which is only occasionally present. The dog was now capable of keeping the target within the AC during fixation attempts, and eye velocity is slow enough for almost continuous centralisation. Accordingly, the NAFX for this record increased to 0.73, a canine Snellen fraction of 20/95. Behaviorally, the dog was able to move freely through the room without collisions and could react appropriately to movements made by the investigators—that is, without being startled or panicked, allowing for easier interaction with its handlers in general. Movies 14 and 15 show the much reduced (and sometimes totally damped) nystagmus, with peak-to-peak excursions now smaller than the extent of the AC. At other times, eye velocity was still great enough—due to the remaining high-frequency pendular component of the original dual-jerk waveform—that even though the target remained completely within the AC, the NAFX showed only a marginal increase over pretreatment values.

Output of the NAFX program for dog BR174 is presented in Figure 7. The top panel shows horizontal movements recorded from the right eye immediately before treatment. In contrast to the previously shown "before" data, this dog had much smaller peak-to-peak excursions, often remaining completely within the AC. However, as shown by the relative scarcity of heavy points, velocity was often beyond threshold, leading to diminished potential visual acuity, in this case a lowered NAFX of 0.375 that still offered room for improvement. The recording in the bottom panel was made 10 months after treatment of both eyes. When present, the pendular oscillation had a maximum peak-to-peak amplitude of 1 ° to 2°, and the velocity was so reduced that all samples satisfied the foveation criteria: The NAFX increased to 0.74. Behaviorally, the changes in this dog were not as great as those observed in the others, perhaps owing to its higher initial vision.

Table 2 shows the NAFX data for all dogs tested; BR161 had no nystagmus. Note that BR108 had a relatively high NAFX of 0.512 (both eyes viewing) corresponding to a Snellen acuity of 20/135, which is better than any of the untreated dogs but somewhat lower than any of the treated dogs. It is important to keep in mind that the NAFX values presented for the treated dogs represent the *worst case* vision because, most of the time, no nystagmus was present at all (i.e., NAFX = 1.0), and therefore visual acuity would be limited only by the afferent defects in the dogs' visual systems. In group 1, the NAFX for the treated dogs, during the infrequent times when they exhibited nystagmus, were much higher than for the untreated dogs. In group 2, the ERG functional assessments for each eye were determined 1 month after therapy. BR174 showed the largest increase in ERGs of both eyes and NAFX (44.5%) after treatment. BR158 and BR161 (who had no nystagmus) showed only minimal ERG increases, whereas BR164 showed only a large ERG increase in the LE.

## DISCUSSION

Before this study, we are aware of no evidence, other than anecdotal, that it is possible to reduce IN so thoroughly as effectively to abolish it. One published report showed the elimination of an *acquired* periodic alternating nystagmus (PAN) in a middle-aged man that arose after bilateral vitreous hemorrhages.[17] Vision was restored to this patient by performing vitrectomy in stages, and the investigators reported that after the restoration of vision to the right eye, the nystagmus was no longer clinically evident during right eye fixation and

reduced in amplitude during left eye fixation. After vitrectomy of the left eye, no nystagmus was clinically present, although recordings performed in the dark showed that the PAN was still present and maintained its previous pattern, although at lower amplitude.

Another report noted that the removal of bilateral congenital cataracts from infants younger than 1 year of age caused "a resolution of nystagmus or reduction to latent nystagmus" when the surgery was performed within 1 month of the onset of the nystagmus.[18] Unfortunately, the report made no attempts to quantify or classify the nystagmus. Without objective ocular motor recordings, it is impossible to rule out that the nystagmus in these cases was originally a manifest latent nystagmus that converted to a pure latent nystagmus. Both these reports illustrate the importance of the presence of visual input for initiating and maintaining the calibration of the ocular motor system, but neither demonstrably shows that it is possible to affect IN. Early treatment may be important for improving vision, by affecting cortical development. How the ocular motor system uses early improvement of the visual inputs to affect motor calibration is unknown, but possibly important.

Over the past 50-plus years a variety of techniques, ranging from pharmacological to surgical to optical and beyond have been developed to treat nystagmus, including IN,[19–23] acquired (Dell'Osso LF, et al. *IOVS* 2003;44:ARVO E-Abstract 2403),[24–34] and PAN.[24,26,31,35–39] Although these approaches have mostly been effective in partly damping the oscillations, it has been our experience that the amount of reduction usually cannot be determined by inspection but must be quantified by eye-movement recordings. Yet, as clearly demonstrated by our results, there are conditions under which it is possible, both reliably and repeatedly, to extinguish IN unambiguously in these canines. At first, our circumstances seem to represent an unusual case, for the nystagmus is the result of a severe afferent deficit, specially bred for, that can be substantially reversed with a highly specialized treatment. Although undoubtedly there are some factors unique to this present study (e.g., species or Leber mutation), it is not unreasonable to consider other circumstances that are at least superficially similar. In particular, as mentioned earlier, children born with congenital cataracts can have severely limited vision accompanied by IN, as the developing ocular motor system is deprived of the visual input it needs to properly calibrate itself. It is yet to be determined how little restoration of visual input is actually needed to allow the ocular motor system to begin its recalibration and to damp or eliminate IN. The role of this sensory loss in the pathogenesis of INS may also be elucidated. It is significant that, despite LCA, BR161 had no nystagmus, just as many patients with visual deficits may not have nystagmus. In canines, as in humans, visual sensory deficits may facilitate the development of nystagmus, but they are not its direct cause.

Full development of the ocular motor system takes years after birth in humans, yet even when it is mature, it must remain plastic to some degree, necessarily recalibrating throughout life as conditions change. This self-correcting property of homeostasis is quite valuable and necessary for the proper functioning of a dynamic system (such as a biological organism) over a large range of possible conditions. An example of this recalibration can be found in patients with a paretic eye, or with myasthenia gravis.[40] In these cases, as the effectiveness of the transmission of the signal to the muscles decreases, the ocular motor system must increase its gain to allow for the production of an appropriately sized saccade. Equally, this intrinsic recalibration can work *against* attempts at therapy. In the case of maximal recession surgery,[41,42] if the reduction of the moment arm of the muscles is too great, there is the potential that the central gain would simply end up having to increase long term, working against the intended effects of the surgery.

The results of our study suggest that late plasticity can be considerable, as even the dogs that were almost fully matured at the time of treatment and had poor-to-negligible vision and IN throughout their lives were able to recalibrate their ocular motor systems once sufficient visual input was available. These results lead us to question whether there is a critical phase for ocular motor system development similar to that in the development, or failure to develop, of stereoscopic vision. In that case, the human "window" appears to close some time within the second year of life, at approximately 18 to 24 months, as the ocular dominance columns in the visual cortex either develop having received visual information from both eyes or even if NO visual input reaches the target (e.g., animals reared in the dark)[43,44] or fail to develop if only one eye supplies a signal. This developmental window is so strong and well documented that it is now customary for pediatric ophthalmologists to perform strabismus and cataract surgery in infants during the first months of life, allowing sufficient time before the window's closing to provide the child with the best possible chance for binocular and central vision. Alignment or congenital cataract surgery performed later in life cannot restore these lost functions. We are unaware of early visual sensory deficits producing irreversible deficits in either the smooth pursuit, vestibuloocular reflex (VOR), or optokinetic nystagmus (OKN) responses in INS when properly studied with accurate eye-movement systems (clinical observations of "reversals" in these responses notwithstanding).

The fact that all dogs with this afferent defect did not also have nystagmus parallels humans, who may have afferent deficits, INS, or both. Afferent deficits may predispose a failure to calibrate key ocular motor subsystems properly, but do not directly cause INS, which also exists in the absence of such deficits. Similarly, the lack of improvement in NAFX of BR158 (and BR164; Table 2) may be due to the initial high value; this also agrees with our observations in humans. It also may be due to the small ERG improvement in the retina. It is also important to remember that the post-treatment values represent the *worst-case* waveforms—that is, times when the nystagmus could be detected. It is also possible to be misled by false expectations of a high NAFX, based solely on inspection of the position waveform only. There were many occasions when, even though the peak-to-peak amplitude of the nystagmus stayed entirely within the AC, the velocity of the eyes was so great as to make foveation impossible. BR164 had a large ERG increase in the left eye but not in the right eye. The pretreatment NAFX values were the largest in this group, meaning that although the retinal input was poor, the nystagmus waveform already exhibited well-developed foveation, a condition that presumably allowed the dog to use the improved retinal function in the LE and achieve better acuity post treatment. Humans with afferent deficits (e.g., those with albinism) may also exhibit high NAFXs due to well-developed foveation periods provided by their ocular motor system. It is critical to remember that, in such cases, the afferent deficit is most likely the limiting factor for visual acuity, and even if the nystagmus were to be completely eliminated, the improvement in vision could not match the improvement indicated by the NAFX. Furthermore, a patient with profound, long-term visual loss might also experience cortical changes that further limit restored acuity, regardless of enhanced ocular motor stability.

We were also struck by the degree of the yoking between the left and right eyes. Previously, we had observed dogs[11] and a human (Garbutt S, et al. *IOVS* 2003;44:ARVO E-Abstract 1968) making uniocular saccades, suggesting that the anatomic overlay has more variability than previously assumed. In this

study, we noted that the smooth pursuit system, too, may not always be tightly yoked. Even though some of the dogs received only uniocular treatment, the nystagmus showed binocular damping, which may reflect the combining of the visual signals from each eye in the LGN. However, the untreated eye tended to drift off target, and the nystagmus, when present, often varied in phase and amplitude, and even planes, with one eye moving in a mainly horizontal ellipse, whereas the other's trajectory was more vertically oriented.

In future studies, we hope to document the time course of nystagmus damping and to investigate the effects of varied dosages of injected AAV-*RPE65* to provide more insight into what is necessary to help the OMS recalibrate.

## *Acknowledgments*

The authors thank the following individuals who performed the core afferent–system studies in the RPE65 mutant dogs that were essential for the present study to be conducted: Gustavo D. Aguirre, who characterized the pathogenetic basis of the disease in the RPE65 mutant dogs and developed the colony of these animals; Samuel G. Jacobson, Artur V. Cideciyan, and Tomas S. Aleman, who performed the psychophysical and ERG studies evaluating effects of gene delivery on visual function; Nadine S. Dejneka, who cloned the cDNA encoding human RPE65 that was used to generate AAV; William W. Hauswirth, who generated and purified the AAV.RPE65 vector; Albert M. Maguire, who surgically delivered the AAV.RPE65 to the mutant dogs; and Andras Komaromy, who assisted with clinical/surgical procedures in the affected dogs; and the staff at the University of Pennsylvania's New Bolton animal facility: Amanda Nichols, Gerri Antonini, Shannon Edwards, Tracy Greiner, Alice Eidson, Siobhan Spears, and Jill Wells—for invaluable assistance working with the dogs to gain their trust and to collect the data.

## *References*

1. Dell'Osso LF, Daroff RB. Nystagmus and saccadic intrusions and oscillations. In: Glaser JS, ed. *Neuro-Ophthalmology.* 3rd ed. Philadelphia: Lippincott, Williams & Wilkins; 1999:369–401.
2. Van Hooser JP, Aleman TS, He YG, et al. Rapid restoration of visual pigment and function with oral retinoid in a mouse model of childhood blindness. *Proc Natl Acad Sci USA*. 2000;97:8623–8628.
3. Aguirre GD, Baldwin V, Pearce-Kelling S, Narfstrom K, Ray K, Acland GM. Congenital stationary night blindness in the dog: common mutation in the RPE65 gene indicates founder effect. *Mol Vis*. 1998;4:23.
4. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet*. 2001;28:92–95.
5. Acland GM, Aguirre GD, Bennett J, et al. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. *Mol Ther*. 2005;12:1072–1082.
6. Jacobson SG, Aleman TS, Cideciyan AV, et al. Identifying photoreceptors in blind eyes caused by RPE65 mutations: prerequisite for human gene therapy success. *Proc Natl Acad Sci USA*. 2005;102:6177–6182.
7. CEMAS Working Group. *A National Eye Institute Sponsored Workshop and Publication on The Classification of Eye Movement Abnormalities and Strabismus* (*CEMAS*). Bethesda, MD: National Institutes of Health, National Eye Institute: Bethesda, MD; 2000. Available at www.nei.nih.gov.
8. 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.
9. Narfstrom K, Wrigstad A, Nilsson SE. The Briard dog: a new animal model of congenital stationary night blindness. *Br J Ophthalmol*. 1989;73:750–756.
10. Wrigstad A, Nilsson SE, Narfstrom K. Ultrastructural changes of the retina and the retinal pigment epithelium in Briard dogs with hereditary congenital night blindness and partial day blindness. *Exp Eye Res*. 1992;55:805–818.
11. Dell'Osso LF, Williams RW, Jacobs JB, Erchul DM. The congenital and see-saw nystagmus in the prototypical achiasma of canines: comparison to the human achiasmatic prototype. *Vision Res*. 1998;38:1629–1641.
12. Jacobs JB, Dell'Osso LF, Leigh RJ. Characteristics of braking saccades in congenital nystagmus. *Doc Ophthalmol*. 2003;107:137–154.
13. 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.
14. Dell'Osso LF. Tenotomy and congenital nystagmus: a failure to answer the wrong question. *Vision Res*. 2004;44:3091–3094.
15. Dell'Osso LF, Williams RW. Ocular motor abnormalities in achiasmatic mutant Belgian sheepdogs: unyoked eye movements in a mammal. *Vision Res*. 1995;35:109–116.
16. Dell'Osso LF, Daroff RB. Congenital nystagmus waveforms and foveation strategy. *Doc Ophthalmol*. 1975;39:155–182.
17. Cross SA, Smith JL, Norton EWD. Periodic alternating nystagmus clearing after vitrectomy. *J Clin Neuro-ophthalmol*. 1982;2:5–11.
18. Yagasaki T, Sato M, Awaya S, Nakamura N. Changes in nystagmus after simultaneous surgery for bilateral congenital cataracts. *Jpn J Ophthalmol*. 1993;37:330–338.
19. Kestenbaum A. [New operation for nystagmus.]. *Bull Soc Ophtalmol Fr*. 1953;6:599–602.
20. von Noorden GK, Wong SY. Surgical results in nystagmus blockage syndrome. *Ophthalmology*. 1986;93:1028–1031.
21. Dell'Osso LF, Hertle RW, Williams RW, Jacobs JB. A new surgery for congenital nystagmus: effects of tenotomy on an achiasmatic canine and the role of extraocular proprioception. *J AAPOS*. 1999;3:166–182.
22. Dell'Osso LF. Development of new treatments for congenital nystagmus. *Ann NY Acad Sci.* 2002;956:361–379.
23. Blekher T, Yamada T, Yee RD, Abel LA. Effects of acupuncture on foveation characteristics in congenital nystagmus. *Br J Ophthalmol*. 1998;82:115–120.
24. Averbuch-Heller L. Acquired Nystagmus. *Curr Treat Options Neurol*. 1999;1:68–73.
25. Averbuch-Heller L, Leigh RJ. Medical treatments for abnormal eye movements: pharmacological, optical and immunological strategies. *Aust NZ J Ophthalmol*. 1997;25:7–13.
26. Averbuch-Heller L, Tusa RJ, Fuhry L, et al. A double-blind controlled study of gabapentin and baclofen as treatment for acquired nystagmus. *Ann Neurol*. 1997;41:818–825.
27. Dell'Osso LF, Tomsak RL, Rucker JC, Leigh RJ, Bienfang DC, Jacobs JB. Combined surgical and drug treatment of acquired pendular nystagmus and oscillopsia in MS. NANOS Poster 28 Abstr, 2005: www.nanosweb.org/meetings/nanos2005/syllabus.asp.
28. Depalo C, Hertle RW, Yang D. Eight muscle surgical treatment in a patient with acquired nystagmus and strabismus. *Binoc Vis Strabismus*. 2003;18:151–158.
29. Helveston EM, Pogrebniak AE. Treatment of acquired nystagmus with botulinum A toxin. *Am J Ophthalmol*. 1988;106:584–586.
30. Leigh RJ, Rushton DN, Thurston SE, Hertle RW. Optical treatment of oscillopsia due to acquired nystagmus. *Neurology*. 1986;36(suppl 1):252.
31. Stahl JS, Lehmkuhle M, Wu K, Burke B, Saghafi D, Pesh-Imam S. Prospects for treating acquired pendular nystagmus with servo-controlled optics. *Invest Ophthalmol Vis Sci*. 2000;41:1084–1090.
32. Stahl JS, Rottach KG, Averbuch-Heller L, Von Maydell RD, Collins SD, Leigh RJ. A pilot study of gabapentin as treatment for acquired nystagmus. *Neuro-ophthalmology*. 1996;16:107–113.
33. Tomsak RL, Remler BF, Averbuch-Heller L, Chandran M, Leigh RJ. Unsatisfactory treatment of acquired nystagmus with retrobulbar injection of botulinum toxin. *Am J Ophthalmol*. 1995;119:489–496.
34. Traccis S, Rosati G, Monaco MF, Aiello I, Agnetti V. Successful treatment of acquired pendular elliptical nystagmus in multiple sclerosis with isoniazid and base-out prisms. *Neurology*. 1990;40:492–494.

35. Halmagyi GM, Rudge P, Gresty MA, Leigh RJ, Zee DS. Treatment of periodic alternating nystagmus. *Ann Neurol*. 1980;8:609–611.
36. Hertle RW, Dell'Osso LF, FitzGibbon EJ, Thompson D, Yang D, Mellow SD. Horizontal rectus tenotomy in patients with congenital nystagmus: results in 10 adults. *Ophthalmology*. 2003;110:2097–2105.
37. Solomon D, Shepard N, Mishra A. Congenital periodic alternating nystagmus: response to baclofen. *Ann NY Acad Sci*. 2002;956:611–615.
38. Troost BT, Janton F, Weaver R. Periodic alternating oscillopsia: a symptom of alternating nystagmus abolished by baclofen. *J Clin Neuroophthalmol*. 1990;10:273–277.
39. Uemura T, Inoue H, Hirano T. The effects of baclofen on periodic alternating nystagmus and experimentally induced nystagmus. *Adv Otorhinolaryngol*. 1988;42:254–259.
40. Abel LA, Daroff RB, Schmidt D, Dell'Osso LF. Saccadic eye movements in myasthenia gravis. *Ann NY Acad Sci*. 1981;377:805.
41. Helveston EM, Ellis FD, Plager DA. Large recession of the horizontal recti for treatment of nystagmus. *Ophthalmology*. 1991;98:1302–1305.
42. von Noorden GK, Sprunger DT. Large rectus muscle recessions for the treatment of congenital nystagmus. *Arch Ophthalmol*. 1991;109:221–224.
43. Elliott T, Shadbolt NR. Dissociating ocular dominance column development and ocular dominance plasticity: a neurotrophic model. *Biol Cybern*. 2002;86:281–292.
44. Wiesel TN, Hubel DH. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *J Neurophysiol*. 1965;28(6):1029–1040.

## APPENDIX: MOVIES

Movies are available online at http://www.iovs.org/cgi/content/full/47/7/2865/DC1.