A Novel Method for Objective Vision Testing in Canine Models of Inherited Retinal Disease

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PURPOSE. The use of canine models of retinal disease in the development of therapeutic strategies for inherited retinal disorders is a growing area of research. To evaluate accurately the success of potential vision-enhancing treatments, reliable methods for objectively assessing visual function in canine models is necessary.

METHODS. A simple vision-testing device was constructed that consisted of a junction box with four exit tunnels. Dogs were placed in the junction box and given one vision-based choice for exit. The first-choice tunnel and time to exit were recorded and analyzed. Two canine models of retinal disease with distinct molecular defects, a null mutation in the gene encoding the alpha subunit of rod cyclic GMP phosphodiesterase (PDE6A), and a null mutation in the gene encoding a retinal pigment epithelium-specific protein (RPE65) were tested and compared to those in unaffected dogs.

RESULTS. With the use of bright light versus dim red light, the test differentiated between unaffected dogs and dogs affected with either mutation with a high degree of certainty. The white-light intensity series showed a significantly different performance between the unaffected and affected dogs. A significant difference in performance was detected between the dogs with each mutation.

CONCLUSIONS. The results indicate that this novel canine vision-testing method is an accurate and sensitive means of distinguishing between unaffected dogs and dogs affected with two different forms of inherited retinal disease and should be useful as a means of assessing response to therapy in future studies. (Invest Ophthalmol Vis Sci. 2008;49:3568–3576) DOI: 10.1167/iovs.07-0625

Recently, the dog has become an important model in the study of inherited retinal degenerative diseases with several spontaneously occurring retinal diseases identified. Dog models have advantages over rodent models, including a larger eye that lends itself easily to surgical manipulation and a visual system that is adapted to vision in both day and night, which is more comparable to the human condition than rodent models. Ultimately, the goal of much research in this field is the development of therapeutic strategies for managing these conditions. Inroads toward successful gene therapy have already been made in the RPE65 mutant canine model, shown by electroretinography but with subjective visual function assessments. Any progress made toward therapeutic development must show, as an essential measure of success, a quantifiable improvement in a dog’s response to a visual cue. A quantifiable measure of improvement in visual performance in dog models is a current unmet need and represents a significant challenge to researchers in this field.

Currently, most researchers of canine retinal inherited diseases use subjective observation of a treated dog’s visual function when observed interacting with its environment or passing through an obstacle course compared with untreated dogs. Sometimes an attempt is made at a more objective assessment by having an observer blinded to the experimental conditions record the number of times a dog bumps into obstacles such as traffic cones, but the results are either recorded as the number of collisions or as pluses and minuses, neither of which yields satisfying statistical analyses. Jacobs et al. performed ocular motility recordings of the nystagmus associated with the RPE65 phenotype to examine phenotypic rescue after gene therapy, but were forced to correlate this measure with a subjective evaluation of visual performance in an obstacle course.

Obstacle course studies are limited by several factors. Individual dogs in a colony have significant differences in boldness and intelligence that affect behavior. Some dogs have no motivation to move through the obstacle course, and calling the animal excessively can arguably aid a vision-impaired dog in completing the task by relying on others. Sometimes even sighted dogs can be excitable and bump into obstacles, creating false or misleading results. In addition, in our experience, it is difficult even for an observer who is blinded to the experimental conditions to refrain from identifying with the dog and possibly interjecting observer bias.

Mice and rodent models are also used extensively in vision research and quantitative visual assessment has been developed and used in these species. Prusky et al. have developed a two-alternative, forced choice task that makes use of a water tank with a semisubmerged platform over which a visual cue is projected. The mice must swim and therefore learn to swim toward the visual cue and its platform. This forced-choice approach results in a test that is quantifiable and generates data that lends itself easily to statistical analysis. The dogs’ large size and behavioral differences from mice limit the ability to use such a forced-choice testing apparatus as a water maze. Optokinetic devices can be devised and used to measure visual function of small subjects such as rodents and chickens. Dogs will exhibit a postural change in response to optokinetic stimulation; however, these studies used small numbers of dogs that were painstakingly trained to stand still on a platform before testing could take place.

Training dogs and even larger animals such as horses to a task based on visual cues has been used to study visual behavior and pattern recognition. However, these studies either used harsh negative reinforcement or tended to use small numbers of animals and involve extensive positive reinforce-
ment training, with sometimes thousands of trials necessary to generate meaningful results. Similar visual-based testing has been used extensively to measure cognitive function in a canine model of aging in which some dogs learn a visual-based task more quickly than others. This type of training is also very time consuming, with dogs requiring daily training sessions for months in some studies. A larger number of dogs, usually with differing temperaments and abilities, are necessary for therapeutic trials than for visual behavior studies. Considering the need to minimize training time and avoid corporeal negative reinforcement, training for such visual tasks is not a practical option.

There is a clear need for a vision test that is simple, relatively rapid, and based on a behavior that is either a natural action or learned easily by most dogs. We observed that most of our dogs, when placed in a box or crate, naturally wanted to exit. We have used this natural behavior to create a four-alternative, choice-based visual testing system that offers a means of objectively quantifying visual performance under different lighting conditions in dogs with inherited retinal disease that is rapid, repeatable, quantifiable, and generates data that can be analyzed by statistical methods.

**METHODS**

**Animals**

Dogs used in the study were purpose-bred members of breeding colonies that were either homozygous for one of two mutations that result in retinal diseases (an RPE65 null mutation or a PDE6A null mutation), and unaffected normal or were nonphenotypically normal carrier animals used as control subjects. Table 1 contains a summary of dogs used in the study, their disease states, and which vision tests were performed. The PDE6A mutant dogs were of the Cardigan Welsh Corgi breed, and those with the RPE65 mutation were Beagle-Briard crosses. The dogs were all of a medium size, weighing between 20 and 40 pounds. The Corgi dogs were of shorter stature than the Beagle crosses, but generally weighed about the same. Ages of the 19 dogs used in the study ranged from 7 to 51 months. There were 15 females and 4 males. The dogs were housed in a vivarium under standard 12-hour light and dark cycles and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Test dogs** were placed in the junction box in the same orientation for each run, with the person placing the dog in the device remaining between two tunnels to observe the dog’s choices without being visible through an open tunnel. A second observer was in place between the opposite two tunnels so that the dog could not gain visual cues, and each observer monitored two tunnels. A stopwatch was started as the dog’s feet were placed on the floor inside the box, and the top was quickly placed on the box. The first tunnel chosen and time taken for the dog to exit the device were recorded.

The first-choice tunnel was defined as the first tunnel the dog entered. When a dog entered a tunnel, it caused the tunnel to rustle noticeably, which allowed us to detect the presence of the dog in the tunnel in all lighting conditions. The junction box was large enough for the dogs to turn in circles without rustling a tunnel, allowing us to distinguish easily between movement within the junction box and movement within the tunnels. Timing was stopped when the dog’s nose reached the open end of a tunnel. If the dog had not exited the device in 60 seconds, the run was stopped, and the time recorded as >60 seconds. If the dog remained in the junction box without entering a tunnel, the first choice was recorded as 0 and treated as an incorrect first choice in the analysis.

**Training**

Training was often unnecessary, as the dogs tended to exit the device naturally. The dogs were given praise as they exited the device. When dogs were introduced to the device, they were given several practice runs in full room light that were not timed or recorded to explore the tunnels. The tunnels were for exit only, and the dogs were never allowed to enter the device via the open tunnels, only by being picked up and placed in the junction box. During the practice runs, the dog’s name was used to call it from the device, but never from a location where the dog could look down the open tunnel and see the caller. Most dogs began exiting the device via the open tunnel, either immediately or within four to five runs. Dogs that did not feel motivated to exit the device during these training runs were encouraged to do so by the observer tapping on the outside of the box or tunnel, making noise, and creating mild negative reinforcement for staying inside, combined with praise on exit.

We observed certain behaviors to be indicative of loss of interest during testing for both affected and unaffected dogs. They included jumping upward after being placed in the junction box, scratching at
the sides of the device or lying down in a tunnel. If a dog exhibited any of these behaviors during testing, the box or tunnel would be tapped to give negative reinforcement, and that run was dropped from the analysis. If the dog's attention could not be regained within one or two runs, the dog was taken back to the kennel for a break. If a dog walked into an incorrect tunnel, simply stood still in the junction box, or turned circles without making a tunnel choice, it was not reprimanded with tapping on the outside of the device. Food rewards were not used in this study, partly to reduce the effect dropped crumbs may have on the dogs' performance and partly because this particular group of dogs seemed to be relatively more motivated by praise than food.

**Bright-Light versus Dim-Light Testing**

For the full-light condition, the overhead lights were on, and the dogs were run through the device at least seven times. Overhead lights were then turned off, and the test was run at least seven times in the dim red-light condition. The light detector of the photometer used in this study could not be used to measure the intensity of red light. Red lighting was set up in the identical way for each test dog. Seventeen dogs were tested under bright versus dim conditions, 11 unaffected normal control dogs, 3 affected dogs homozygous for the RPE65 mutation, and 3 affected dogs homozygous for the PDE6A mutation (Table 1).

**White-Light Intensity Series**

Light intensity at the opening of each tunnel was measured (IL 1700 Research Radiometer with SED033 silicon light detector; International Light, Inc, Peabody, MA). Under full room light conditions, all lamps were turned to the highest intensity and the overhead lights were kept on. The light level at each tunnel opening was recorded. We chose to measure light intensity at the tunnel opening rather than inside the device, because it could be rapidly and reproducibly measured without disturbing the device's position relative to all the lamps. The device was not light-tight, but the light level at the tunnel opening was at least 1 order of magnitude brighter than the light level inside the box or inside the tunnel for all conditions (data not shown). The different light intensities were created with the overhead lights off, using four lamps placed between tunnel openings with lights pointing upward. White light bulbs of various wattage and dimmer switches on each lamp were used to create the light intensity conditions. For a given lighting condition, the intensities at the tunnel openings were as close as possible to each other. The intensity reading at each opening was recorded and used individually in the analyses. Because the setup and method were being developed during the course of the study, the intensities used and the number of intensities were not the same for each dog. The number of intensities used in these series ranged from 12 to 6. Most dogs were tested with six intensity levels, with intensities falling into the broad ranges of 8 to 26, 1.5 to 4, 0.1 to 0.8, 0.04 to 0.09, 0.01 to 0.03, and 0.001 to 0.006 cd/m². For each intensity series experiment, no matter where in the broad range the intensity was, the light levels at the individual doors were made as close as possible to each other. They were typically within 1 to 3 cd/m² of each other for intensities between 1 to 4 cd/m², 0.1 to 0.3 cd/m² of each other for intensities between 0.9 to 1.0 cd/m², 0.01 to 0.03 cd/m² of each other for intensities between 0.09 to 0.1 cd/m², and so on. The greatest variability in intensities between tunnels was during the full-light condition due to the placement of overhead lights relative to tunnel openings. The intensity at each tunnel opening was used individually in all analyses. The normal dog (G) was subjected to four intensities, from 14 to 0.001 cd/m².

Seven dogs (dogs A–G) were tested in a light-intensity series. Three dogs with the RPE65 mutation (A–C) and three dogs with the PDE6A...
mutation (D-F) were tested. Individuals that were used for both bright versus dim testing, and the intensity series are indicated in Table 1.

Dog G in the intensity series testing was an unaffected dog and was also used in the bright and dim testing.

The dogs were run through the device at least seven times at each light intensity. Occasions when a dog was run through the device more than seven times included dogs that had apparent loss of attention during testing and were given a break (see the Training section), or cases in which in the same tunnel was open more than two times in a row, possibly leading to the subject’s choice being influenced by learning the location of the open tunnel. In these cases, seven additional runs were performed, and all test runs were included in the analysis. The intensity series testing was performed with each light intensity level in the series randomized rather than proceeding from brightest to dimmest or vice versa. The dogs were given from 5 to 10 minutes of adaptation time for each light condition before beginning runs through the device. Two to three runs at full light intensity separated each light condition.

**Data Analysis**

Two responses were recorded for each test: whether the first tunnel entered by the dog was the open exit (correct choice) and time to exit. The proportion of correct exit choice for full versus dim light were compared in a two-tailed test of proportions. Exit times at fixed light levels were approximately log-normally distributed. To assess a difference in performance between full- and dim-light conditions, a two-sample t-test was performed using the natural log of exit time. Exit times greater than 60 seconds were not recorded. In the case in which one or more of the dim-light exit times exceed 60 seconds those points were dropped from the time-to-exit analysis, and two-sample t-tests were performed for the reduced data sets. Because the only tests that were terminated occurred in the low-light conditions, the difference in performance estimated by the two-sample t-test with truncated data sets can only be smaller than the actual performance difference. In these cases, we quote the probabilities of the t-tests with the truncated data sets and note that the probabilities would have been even smaller if we had been able to include the longer exit times from the excluded tests. Note that had there been terminated tests in both the full-light and low-light conditions, we would not have been able to make this argument, and a more sophisticated analysis would have been necessary.

For full-intensity series, the exit time data were analyzed by using a linear regression of the natural log of exit time versus the natural log of light intensity. Correct exit choice as a function of natural log of light intensity was analyzed by binary logistic regression (Minitab ver. 14; Minitab, Inc. State College, PA).

**Electroretinogram (ERG)**

Animals were anesthetized under a dim red light. They were premedicated with acepromazine maleate (0.1–0.3 mg/kg) intramuscularly, induced with thiopental sodium (6–12 mg/kg IV) and maintained with isoflurane delivered in oxygen. A pulse-oximeter (Vet/Ox 4000; Heska Corp., Fort Collins, CO) was used to record pulse rate and oxygen saturation for the duration of the procedure. Body temperature was maintained with a heating pad. Pulse rate, oxygen saturation, and body temperature were recorded every 5 minutes during the ERG recording.

Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL). Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL). Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL). Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL). Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL). Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL). Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL). Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL). Both pupils were maximally dilated with 1% tropicamide (Mydriacyl; Alcon Laboratories, Honolulu, HI) and 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Buffalo Grove, IL).

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To assess performance in greater detail, a white-light intensity series was performed for one unaffected, three RPE65 mutant, and three PDE6A mutant dogs. Dogs A, B, and C were RPE65 mutation dogs; dogs D, E, and F were PDE6A mutation dogs, and dog G was an unaffected dog.

Figure 4 shows the probability of choosing the correct exit as a function of light intensity based on binary logistic regressions of light intensity versus first-choice tunnel. Because there are four exits from which to choose, a completely blind dog is expected to choose the correct exit tunnel randomly 25% of the time when using this test device. We verified this conjecture by testing an unaffected dog wearing opaque goggles in the device under full room light. The blinded dog chose the correct tunnel one time during seven runs through the device (data not shown). We were therefore interested in identifying the light intensity at which the affected dogs would begin randomly choosing tunnels. The unaffected dog (G) almost exclusively chose the correct tunnel first at all light intensities (Fig. 4, line G). We were unable to test a normal dog in the device in a condition dark enough for it to begin choosing randomly because we would have been unable to see well enough to determine tunnel choice or exit time. Therefore, the opaque goggles placed on a normal dog were used to show that it chose the open tunnel randomly in the absence of visual stimulation.

All six affected dogs, whether affected with the RPE65 mutation (dogs A–C) or the PDE6A mutation (dogs D–F), achieved or approached random-choice behavior as the light intensity decreased. There was a significant difference between the first-choice tunnel for each affected dog compared with the unaffected dog (Fig. 4, lines A–F).

Linear regressions of the resultant time to exit versus light intensity are shown in Figure 5. The unaffected dog (G) exited the device quickly at all light intensities used in the study, with no difference in performance as light intensity decreased. There was a significant difference between the first-choice tunnel for each affected dog compared with the unaffected dog (Fig. 5, lines A–F).

There was no significant effect of the age of any affected dog on performance in the test. In addition, there was no effect of run order on performance, indicating the dogs’ performance did not change with increasing experience in the device. The device was not impenetrable to light, but neither this nor spectral changes as the lights were dimmed affected the performance of the dogs (Gearhart P, et al., unpublished observations, 2004).

Distinguishing RPE65 and PDE6A Mutation Dogs

There was a significant difference in performance between the PDE6A (dogs D–F) and RPE65 (dogs A–C) mutant dogs in the light-intensity series, whether examining first-choice tunnel (Fig. 4) or time to exit (Fig. 5). The RPE65 mutant dogs began random selection of the correct open door at brighter light intensities than did the PDE6A dogs. The odds ratio for the disease state from binary logistic regression
Table 3 shows that the odds for the PDE6A mutant dogs selecting the correct tunnel was 2.48 times higher than that of the RPE65 mutation dogs. Similarly, the PDE6A mutation dogs were significantly faster to exit ($P < 0.0005$) than were the RPE65 mutant dogs (Table 4). There was no significant difference in performance for either time to exit or first-choice tunnel in the individual dogs with the same gene mutation.

**Table 2. Predictive Value of the Vision Test**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Full Light</th>
<th>Dim Light</th>
<th>Frequency of Correct Choice</th>
<th>Prediction of Disease Status</th>
<th>Actual Disease Status</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/7</td>
<td>4/7</td>
<td>U $P = 0.573$</td>
<td>U $P = 0.996$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6/7</td>
<td>5/7</td>
<td>U $P = 0.508$</td>
<td>U $P = 0.417$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4/7</td>
<td>5/7</td>
<td>U $P = 0.573$</td>
<td>U $P = 0.359$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6/7</td>
<td>7/7</td>
<td>U $P = 0.508$</td>
<td>U $P = 0.359$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6/7</td>
<td>7/7</td>
<td>U $P = 0.280$</td>
<td>U $P = 0.471$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9/10</td>
<td>6/10</td>
<td>U $P = 0.099$</td>
<td>U $P = 0.732$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5/8</td>
<td>6/8</td>
<td>U $P = 0.586$</td>
<td>U $P = 0.383$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6/7</td>
<td>4/7</td>
<td>U $P = 0.508$</td>
<td>U $P = 0.932$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>6/7</td>
<td>5/7</td>
<td>U $P = 0.508$</td>
<td>U $P = 0.850$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6/7</td>
<td>7/7</td>
<td>U $P = 0.280$</td>
<td>A $P = 0.017$</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>N/A</td>
<td>N/A</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>6/7</td>
<td>1/7</td>
<td>A $P &lt; 0.0005$</td>
<td>A $P &lt; 0.001$</td>
<td>A (RPE65)</td>
<td>Fearful dog; test aborted</td>
</tr>
<tr>
<td>13</td>
<td>12/13</td>
<td>6/11</td>
<td>A $P = 0.024$</td>
<td>A $P &lt; 0.0005$</td>
<td>A (RPE65)</td>
<td>Time truncated at 60 seconds</td>
</tr>
<tr>
<td>14</td>
<td>7/7</td>
<td>0/7</td>
<td>A $P &lt; 0.0005$</td>
<td>A $P = 0.001$</td>
<td>A (RPE65)</td>
<td>Time truncated at 60 seconds</td>
</tr>
<tr>
<td>15</td>
<td>6/7</td>
<td>1/7</td>
<td>A $P &lt; 0.0005$</td>
<td>A $P = 0.0005$</td>
<td>A (RPE65)</td>
<td>Dog D</td>
</tr>
<tr>
<td>16</td>
<td>7/7</td>
<td>4/7</td>
<td>A $P = 0.022$</td>
<td>A $P = 0.001$</td>
<td>A (PDE6A)</td>
<td>Dog F</td>
</tr>
<tr>
<td>17</td>
<td>6/7</td>
<td>3/9</td>
<td>A $P = 0.011$</td>
<td>A $P &lt; 0.0005$</td>
<td>A (PDE6A)</td>
<td>Dog D</td>
</tr>
</tbody>
</table>

The frequency of correct choices in full light and in dim light and the disease status as predicted by the test for correct choice and time to exit are shown, along with the actual disease status of the tested animals. Dogs that were also used in the intensity series are indicated in italics in the comments column. U, unaffected; A, affected; N/A, not applicable; RPE65, RPE65 mutant dog; PDE6A, PDE6A mutant dog.

**DISCUSSION**

We have successfully developed a four-choice vision-assessment system for use in dogs that is easy to perform and yields repeatable, quantitative results that can be analyzed by standard statistical methods. Until now, the best method available for characterizing vision in dogs was to set up obstacles such as traffic cones and observe the dogs among them. Our device is...
easy to build and the test is both simple and effective, detecting differences in performance to a high degree of statistical significance. The test is sensitive and can show differences in vision between the two different disease states tested to date. Finally, it is a task based on a natural reaction for most dogs.

The test is sensitive and can show differences in performance to a high degree of statistical significance. The test is sensitive and can show differences in vision between the two different disease states tested to date. Finally, it is a task based on a natural reaction for most dogs; therefore, very little training is necessary for successful vision testing.

Experience in the device seemed to have little effect on performance. At no time were run–order effects observed in the testing (data not shown), indicating the dogs’ performances neither improved with experience nor decreased with boredom or fatigue. In addition, when two light-intensity series were run using the same dog 3 months apart, there was good correlation between the regressions for each series (data not shown). Except for two individuals tested to date, variability in dog aptitude had little effect on the experimental results. The two dogs that had difficulties in the test (Table 2) were used in another study and therefore not retested. Any effects further training or experience with the test may have had on their performances is unknown.

There was a dramatic difference in performance between unaffected dogs and affected dogs of either mutation. The unaffected dogs rapidly exited the device with an average exit time in the light-intensity series of 2.28 seconds (Fig. 5). We also observed that affected dogs accurately exited the device within seconds when the room lights were turned on, even if the dog was unable to exit the device by 60 seconds under dim light (data not shown). The significant change in performance of all affected dogs with decreasing light level is consistent with the nature of the disease states in these dogs. Each diseased dog used in this study was affected with one of two retinal degenerations. The Cardigan Welsh Corgi dog, a model for autosomal recessive retinitis pigmentosa, has a null mutation in the gene encoding the α subunit of rod cyclic GMP phosphodiesterase (PDE6A)\textsuperscript{19} and the Briard-mix dog, a model for Leber congenital amaurosis, has a null mutation in the gene encoding the retinal pigment epithelium specific protein RPE65.\textsuperscript{18}

The PDE6A mutant dogs have a lack of pde6 activity, an absence of detectable rod-mediated ERG responses (Petersen-Jones SM, et al., unpublished findings, 2003) and undergo relatively rapid rod photoreceptor degeneration (Tuntivanich N, et al. IOVS 2005;46:ARVO E-Abstract 5238). The PDE6A mutation does not directly affect cone function, and the loss of rods is followed by a much slower secondary degeneration of cone photoreceptors that eventually leads to blindness. At the ages of PDE6A, mutant dogs used in this study there will have been loss of some, but not all cone photoreceptors. Because cones make up less than 5% of total photoreceptors in dogs, cone ERG waveforms are considerably lower in amplitude than are rod ERG waveforms. The dogs have no measurable rod function and the residual ERG responses originate from cones (Petersen-Jones SM, et al. IOVS 2003;44:ARVO E-Abstract 4537). They subjectively have vision under photopic conditions (not shown), yet very low to nonrecordable a- and b-wave ERG amplitudes (Fig. 1). In our test showed quantifiable visual function that declined in low lighting conditions (Figs. 4, 5).

The RPE65 mutant dogs have an absence of rpe65 protein in the retinal pigment epithelium.\textsuperscript{2} Extrapolation from studies in RPE65 mutant mice shows that it is very likely that in the RPE65 mutant dogs there is a lack of 11-cis retinal supply to the photoreceptors, resulting in very reduced function of both and rods and cones.\textsuperscript{20,21} The RPE65 mutant dog has a greatly elevated ERG threshold,\textsuperscript{2} which is illustrated in Figure 1. This finding is similar to that in RPE65 mutant mice, in which the predominant remaining ERG responses originate from rods with markedly reduced sensitivity.\textsuperscript{22} RPE65 mutant dogs have severely reduced scotopic and photopic vision,\textsuperscript{3,24} consistent with the known effect of lack of RPE65 function in other species.\textsuperscript{25,26} We were able to show that the RPE65 dogs had a more severe reduction in vision than did the PDE6A mutant dogs at the ages used in this study (Figs. 4, 5). However, they did have higher ERG amplitudes than did the PDE6A mutant dogs (Fig. 1). This apparent contradiction in which visual performance of the RPE65 dogs was worse than that of the PDE6A dogs and yet the ERG responses were of greater amplitude is readily explained when the effects of the two mutations are considered. Assuming that the RPE 65 mutant dogs are similar to the RPE65 mutant mouse, most of the recordable

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Fraction of correct first-choice tunnel versus light intensity. The fraction of correct first-choice tunnel was plotted against the light intensity on a log scale for the PDE6A mutation dogs (D–F), the RPE65 mutation dogs (A–C), and the unaffected dog (G). There was a significant correlation between log light intensity and correct first-choice tunnel (\(P < 0.005\)).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Regression of exit time versus light intensity. Regressions of the time to exit versus the light intensity on a log scale for the PDE6A mutation dogs (D–F), RPE65 mutation dogs (A–C), and the unaffected dog (G). Each line is the representative regression resulting from the plots of the individual time to exit and light intensity data points for each dog. Test runs truncated at 60 seconds were dropped from the analysis, making some regression lines shorter than others. There was a significant correlation between log light and exit time with \(P < 0.05\). \(R^2 = 26.2-41.5\).}
\end{figure}
ERGs in the RPE65 mutant dog should originate from rods, which make up more than 95% of the total photoreceptors. It is therefore not surprising that the ERG amplitudes from RPE65 mutant dogs are greater compared with the low-amplitude ERGs derived from the low number of cones in PDE6A mutant dogs, even though the RPE65 mutant dog rods have a marked loss of sensitivity and function. These results show that the test is sensitive enough to detect vision differences between animals with mutations causing different phenotypic diseases.

It is noteworthy that we observed variability in performance between individual affected dogs of the same species when comparing first-choice exit (Fig. 4) and time to exit (Fig. 5). For example, dog C (RPE65 mutation) showed a precipitous decline in the probability of correct tunnel choice, choosing randomly at the highest light intensity observed for any dog, yet was exiting the device much faster than the other RPE65 mutation dogs. Sometimes the opposite was observed (dog B). Similar variations occurred with the PDE6A mutation dogs.

Testing of a greater number of dogs is currently being performed. It is possible that these differences are the result of personality differences between the dogs. We have found various personality differences among the dogs in our colony that confound the subjective obstacle course testing, including slow dogs that refuse to walk through the cones, or energetic dogs that knock down all the cones surrounding them. Unlike the obstacle course, however, the results generated from the vision-testing device are interpretable even in the face of apparent behavioral differences between individual dogs.

It is possible that the differences in performance between dogs affected with the same mutation are the result of differences in the ages and therefore severity of disease. In this small sample, we found no significant difference in performance with age in dogs of either mutation. However, the ages of the PDE6A and RPE65 mutation dogs were 1.7, 3.3, and 3.4 years and 1.2, 1.7, and 2.2 years, respectively, making the age distribution too narrow to draw meaningful conclusions regarding age as a variable. Studies are currently under way to characterize further the possible effects of age on visual performance of dogs with either mutation.

In this investigation, we truncated the exit time data for dim-light conditions by terminating the tests if the dog had not exited by 60 seconds. For the specific case presented in this article, the dogs exited the device in full light quickly and consistently. Only in the dim-light conditions did the dogs take longer than a minute to exit the apparatus. Because the truncated data were restricted to dim-light conditions, we can state with confidence that the average exit time during dim-light conditions would have been longer had we not truncated the data and the average exit time under the full-light condition was not changed by the truncation. For this reason we were able to truncate the data and quoted an upper limit on the probability. However, for a general research procedure in which researchers must more accurately estimate visual performance, elimination of some points based on exit time will overestimate the visual performance. We find that the first-choice tunnel result is less ambiguous and lends itself to analysis because it eliminates this ambiguity.

The effect of dark adaptation time was not specifically addressed in the present study. Each light condition was tested in random order, rather than structuring the intensity series from brightest to dimmest light or vice versa. All dogs were given from 5 to 10 minutes of adaptation at each light intensity before the testing began. There was a lack of run-order effects within each lighting condition, indicating that the dogs’ performances were not improving as the number of runs (and therefore time) increased for a given light intensity. This suggests that increased dark adaptation time does not affect performance for the duration of our testing. However, a detailed analysis of the effect dark adaptation times may have on test performance for dogs with each mutation would be worthwhile.

Our test does not make an attempt to measure visual acuity in the dogs. In force-choice visual testing methods for mice, a grating can be projected over the submerged platform to which the mice must swim. After many trials, the mice learn to swim toward the grating to find the platform. By changing the projected grating, a measure of visual acuity can be made. Unlike the mouse test, the target to which our dogs were moving was large (the 28-inch diameter tunnel opening) without fine features or detail. The addition of an obstacle for the dog to avoid as it exited the tunnel was considered, but was not possible due to the size of the tunnel.
rejected on the basis that it would interfere with the same kind of observer bias experienced in the obstacle course testing, and a single obstacle would not be as beneficial to visual acuity assessment as the projected gratings used in the other species.

One goal in designing an objective test of visual function for dogs was its use in determining the response to therapeutic intervention. The test described herein objectively measures visual function and can distinguish not only between affected and unaffected, but between two different retinal disease processes. It therefore holds promise for use in therapeutic response trials in which light intensity series performed before and after treatment would be compared. In addition, the task is learned easily by most dogs, decreasing the need for extensive training of individual animals and making the system suitable for vision assessment of colony members or a study cohort.

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References