Spontaneous Axial Myopia and Emmetropization in a Strain of Wild-Type Guinea Pig (Cavia porcellus)

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PURPOSE. To describe a wild-type guinea pig strain with an incidence of spontaneous axial myopia, minimal pupil responses, lack of accommodation, and apparently normal spatial vision. Such a strain is of interest because it may permit the exploration of defective emmetropization and mapping of the underlying quantitative trait loci.

METHODS. Twenty-eight guinea pigs were selected from 220 animals based on binocular myopia (exceeding −1.50 diopter [D]) or anisometropia (difference between both eyes exceeding 10 D) at 4 weeks of age. Refractive and pupil responses were measured with eccentric infrared photoretinoscopy, corneal curvature by modified conventional keratometer, and axial lengths by A-scan ultrasonography once a week. Twenty-one guinea pigs were raised under a normal 12-hour light/12-hour dark cycle. From a sample of 18 anisometropic guinea pigs, 11 were raised under normal light cycle and 7 were raised in the dark to determine the extent to which visual input guides emmetropization. Spatial vision was tested in an automated optomotor drum.

RESULTS. In 10 guinea pigs with myopia in both eyes, refractive errors ranged from −15.67 D to −1.50 D at 3 weeks with a high interocular correlation (R = 0.82); axial length and corneal curvature grew almost linearly over time. Strikingly, two patterns of recovery were observed in anisometropic guinea pigs: in 12 (67%) anisometropia persisted, and in 6 (33%) it declined over time. These ratios remained similar in dark-reared guinea pigs. Unlike published strains, all guinea pigs of this strain showed weak pupil responses and no signs of accommodation but up to 3 cyc/deg of spatial resolution.

CONCLUSIONS. This strain of guinea pigs has spontaneous axial refractive errors that may be genetically or epigenetically determined. Interestingly, it differs from other published strains that show no refractive errors, vivid accommodation, or pupil responses. (Invest Ophthalmol Vis Sci. 2009;50:1013–1019) DOI:10.1167/iovs.08-2463

Vertebrate eyes show generally scattered refractions at birth1–3 or after hatching.4 During development, initial refractive errors decline, and most eyes approach a near emmetropic state. Experiments in animal models have shown that this process of “emmetropization” is visually guided.5 The mechanism of emmetropization represents a research topic of considerable interest because of the high incidence of myopia in the industrialized world.6 In animal models, visual experience is experimentally changed by having animals wear diffusers or spectacle lenses. These manipulations induce changes in eye growth and refractive errors but they largely ignore potential genetic influences. In addition, in human populations, if visual experience differs severely among individuals, refractive errors are largely determined by environmental factors.7 On the other hand, genetic components of refractive development are most clearly visible when environmental factors are normalized,8 as in a homogeneous population in which everyone has similar visual experience. Given that near work can either cause or not cause myopia, some genetic factors must make the difference. Different chicken strains show different responses to normalized experimental manipulations,8 suggesting a genetic factor.

To determine genetic input into the development of refractive error in an animal model, it is necessary to study a population of animals that displays a large scatter of refractions despite similar visual experience. Unfortunately, the variability of refractive errors in animal models is usually small, and spontaneous myopia is rare.9 Nevertheless, Zhou and Williams10 and Zhou et al.11 crossed two recombinant inbred mouse strains with different average eye weights and were able to map two quantitative trait loci (QTL) that control eye size. Refractive states were not recorded, and it is unclear whether the mice with larger eyes also had more myopic refractions. Therefore, though this impressive study represents a promising attempt to find loci controlling eye size, there may be no association with myopia.

Some years ago, the guinea pig was introduced as a new animal model of myopia (McFadden SA, et al. IOVS 1995;36: ARVO Abstract 3504). Guinea pigs have a well-developed visual system—they open their eyes right after birth and reach a grating acuity of approximately 2.7 cyc/deg in adulthood12—perhaps even higher than the established model of the tree shrew.13 Furthermore, they are cooperative and easy to handle. In contrast to another new mammalian myopia model, the mouse, guinea pigs reliably develop refractive errors as ex-
perceived from imposed defocus (McFadden SA, et al. IOVS 1995; 36:ARVO Abstract 3504). In previous studies on guinea pigs, the scatter of spontaneous refractions was low at the age of 3 weeks (SD only 0.20 – 0.40 diopters [D] or 1.5–2.0 D). In these studies, guinea pigs were initially slightly hyperopic but approached emmetropia after approximately 3 weeks, when the small eye artifact was taken into account.

The population described in this study may offer new opportunities to study the heritability of refractive errors in guinea pigs by performing selective breeding, to study the mechanisms of natural emmetropization and the visual cues that might guide it by comparing refractive development in guinea pigs raised in light and dark, to measure visual performance and retinal function in guinea pigs with different spontaneous refractive errors, to explore how the optical properties of the ocular components (corneal power, lens power) determine refractive state are inherited, and, finally, to perform QTL mapping for all these variables, which may be facilitated by the fact that other guinea pig strains seem not to show these deficiencies.

METHODS
Animals
Two hundred twenty pigmented guinea pigs (Cavia porcellus [the “English short hair stock”], tricolor strain, crossed between strains 2 and 13) were refracted, and their axial eye lengths were measured at 3 weeks of age, when they were weaned. To evaluate the developmental changes in refraction, 134 eyes of 67 randomly selected animals from the same population were measured again at 9 weeks of age. Although there was some emmetropization since the SD declined from 3.89 to 3.49 D, some eyes remained highly myopic.

Twenty-eight guinea pigs were selected from the total sample based on high anisometropia exceeding 10 D (with one eye more hyperopic and the other more myopic than the baseline refraction of ±2.3 D) or binocular myopia exceeding −1.5 D in both eyes. Eleven anisometropic and 10 binocularly myopic guinea pigs were raised in the animal facilities, as previously described, with normal visual exposure to the cage environment. Two or three guinea pigs were raised together in one cage (25 × 26 × 35 cm) under a 12-hour light/12-hour dark cycle. Illuminance at the cage floor was approximately 450 lux. Seven other anisometropic guinea pigs were raised in complete darkness.

Refractions and axial lengths were measured every week until the age of 14 weeks in binocularly myopic animals and until the age of 8 weeks in the anisometropic guinea pigs reared under a 12-hour light/12-hour dark cycle. Guinea pigs reared in the dark from age 3 to 8 weeks were measured only at the beginning and the end of the dark-rearing period. Timetables, experimental protocols, and animal numbers are illustrated in Figure 1.

All guinea pigs had free access to standard food palettes and water, and fresh vegetables were provided twice a week. All treatments were in agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the animal research was approved by the Animal Care and Ethics Committee at Wenzhou Medical College, Wenzhou, China.

Measurement Techniques
Refractions. Refractive errors were measured in the vertical pupil meridian by eccentric infrared photoretinoscopy, as previously described for the mouse, with a camera distance of 60 cm. The software, written in Visual C++ by one of the authors (FS), continuously averaged 10 refractions that were recorded over 330 ms and that displayed the means and standard deviations on the left side of the display in seven eyes with lens powers ranging from −8 D to +8 D, tested in steps of 2 D. The offset was ignored since no correction was made for the small eye artifact. Alert guinea pigs are cooperative, and it was easy to align their heads by hand until the pupil was clearly visible in the video frame (see Fig. 7). Room light was dimmed to approximately 5 lux ambient illuminance during photorefraction; the only light source was the computer screen. Dim light is generally used in infrared photorefraction because large pupils improve the signal-to-

Figure 1. Flow chart showing the experimental protocols with timelines, number of guinea pigs and treatments, and measurements performed.
noise ratio of the measurements. In the present study, this would have been less important because the strain under consideration had only weak pupil responses (see Fig. 7).

Pupillography. During the continuous measurements of refraction and pupil size (see Refractions), a bright green LED attached to the photoretinoscope (5-mm diameter, 16 cd, peak emission 520 nm, radiation angle 30°; Conrad Electronics, Hirschau, Germany) could be flashed from the keyboard with the serial port of the computer, which was replicated through a USB to a serial adapter. Stimulation time was 80 ms. The continuous pupil trace was analyzed for pupil constriction amplitude and latency, as previously described for mouse measurements.18

Measurements of Ocular Dimensions. Corneal curvature was measured in alert guinea pigs with a keratometer (OM-4; Topcon, Tokyo, Japan) mounted with a +20 D aspherical lens, as described,19 immediately after the refraction measurement. A set of stainless steel ball bearings was used for calibration. Axial dimensions of the eyes were measured with A-scan ultrasound (AVISO Echograph Class I-Type Barco Mediphysics, Grenoble, France) at approximately 6 µs on the same days as the refractions. These measurements were also performed in alert guinea pigs, but the cornea was topically anesthetized with one drop of 0.5% proparacaine hydrochloride (Alcon, Belgium). Velocities of sound were assumed as described by Zhou et al.15: 1557.5 m/s for aqueous humor, 1723.3 m/s for the lens, and 1540 m/s for the vitreous humor. A stand-off rubber tube was attached to the probe tip, as described by Schaeffel and Howland.20 Each eye was measured eight times. Standard deviations from these repeated measurements ranged from 40 to 60 μm.

Optomotor Experiments. Eight-week-old guinea pigs, raised under 12-hour light/12-hour dark cycles, were individually placed in an optomotor drum with a diameter of 130 cm that rotated at an angular speed of 34.29°/s. The interior of the drum was covered with a pattern of vertical black and white stripes with exchangeable fundamental frequencies (0.05, 0.08, 0.15, 0.3, 0.6, 1.2, 1.8, 2.4, 3.0 cyc/deg). The guinea pigs tracked the drifting stripes by head nystagmus. Head movements were recorded by a video camera. Custom-made software, written in Visual C++, by one of the authors (FS), detected two white spots on a piece of black paper that was attached to the guinea pig’s head by a drop of glue. The movement of the white spots was automatically converted to angular head speed in the horizontal plane at a sampling rate of 30 Hz. The ratio of angular head speed and stripe speed (gain) was analyzed for different spatial frequencies of the stripes, as previously described.21

Statistical Analysis

There were significant correlations of the refractions and axial lengths between both eyes if the whole sample was evaluated. For statistical analysis both eyes were used.

Correlations between axial lengths and refractions were tested by linear regressions, and interocular correlations were tested by orthogonal regressions. Developmental trends in refractive states were also studied by linear regressions. To compare ocular parameters, t-tests were used; P < 0.05 was considered significant.

RESULTS

Distributions of Refractive Errors and Axial Lengths at 3 and 9 Weeks of Age

At the age of 3 weeks, the refractions of 220 guinea pigs (440 eyes) were widely scattered. The average refraction was +2.33D but the SD was 4.04D (Fig. 2A), much larger than in published studies in other strains of guinea pigs in which the standard deviation was only approximately 0.35 D.14,22 The large variability in refractions among different guinea pigs could not be attributed to measurement noise of the photorefractometer because repeated measurements at 2-day intervals in the same animals showed a correlation coefficient close to 1 (y = 1.0022x + 0.0048; R² = 0.9945; n = 10 animals). Furthermore, the measurement noise was very low, as can be seen on the refraction trace later in Figure 7.

At 9 weeks of age, a smaller sample of 67 guinea pigs (134 eyes) was refracted again. The distributions of the refractions of those 134 eyes at the age of 3 weeks are also shown in Figure 2A. It can be seen that the averages and standard deviations were similar in the subset and the full sample. Note that both eyes were used for the analyses.

Guinea pigs were randomly selected from the full sample shown in Figure 2. Some emmetropization had occurred in those guinea pigs in the range of moderate hyperopia (between +1 and +6 D) because there was a noticeable decline in the width of the distribution (Fig. 3A). However, a respectable number of more myopic guinea pigs did not undergo emmetropization at all. The distributions of their axial lengths at 3 and 9 weeks of age are shown in Figures 2B and 3B, respectively.

There were significant correlations between refractions and axial lengths, with longer eyes more myopic (Figs. 4A [3 weeks], 4B [9 weeks]). Correlation coefficients were 0.46 (P < 0.001) and 0.60 (P < 0.001) in 3-week-old and 9-week-old guinea pigs, respectively.

FIGURE 2. Distribution of refractive errors in the vertical pupil meridians (A) and axial lengths (B) in 440 eyes of 220 guinea pigs, measured at the age of 3 weeks. Note the wide scatter of the refractions, with a significant proportion of myopia, and the wide range of axial lengths. Distributions of the refractions (A) of the subset of the 134 eyes that were measured again at 9 weeks (see Fig. 3). The subset showed almost the same mean values and standard deviations.
Development of Refractive Errors, Corneal Curvatures, and Axial Lengths in Guinea Pigs with High Myopia: Individual Data

Binocular myopia (exceeding −1.5 D in both eyes) was observed in 10 of the 28 guinea pigs with myopic refractive errors. Refractions in the myopic guinea pigs were widely scattered at 3 weeks of age (Figs. 5A [right eyes], 5B [left eyes]). Corneal curvatures and axial lengths are also shown in Figure 5. Only two of them displayed some emmetropization, and one of them was the least myopic individual in the group (initially approximately −1.5 D of myopia in both eyes; uppermost curve with open circles in Fig. 5). The other guinea pig showing some recovery was initially highly myopic (−15 D) in the right and −6.25 D in the left eye; Fig 5, filled circles).

On average, the 10 guinea pigs with myopia in both eyes (20 eyes) grew approximately 60 μm per week in axial length with no signs of saturation during the first 14 weeks of life. Linear regression of axial length versus age had the equation:

\[ y = -7.50x + 62.60 \]

\[ R = 0.46 \]

Correlations between refractions in the right eyes (average refraction, −7.24 ± 4.70 D) and the left eyes (average refraction, −7.25 ± 5.52 D) were significant, at both the beginning and the end of the observation period. They are shown in Figure 6. Orthogonal regressions provided correlation coefficients of 0.68 \((P = 0.029)\) and 0.80 \((P = 0.006)\), respectively.

Development of Refractive Errors in Anisometropic Guinea Pigs

Eighteen guinea pigs were selected based on their anisometropia, exceeding 10 D at 3 weeks of age. The experimental protocol is shown in Figure 1, left side. In guinea pigs reared under 12-hour light/12-hour dark cycles, hyperopic eyes did not show any developmental change in refraction. Myopic eyes displayed two developmental patterns—recovery or no recovery—in the time frame from 3 to 8 weeks of age. Recovery was defined as a reduction of refractive error of >2 D. Four of the 11 guinea pigs (approximately 36%) showed recovery. At the end of the observation period, the mean interocular difference in the recovery group was −0.52 ± 3.11 D, and it was 10.90 ± 3.96 D if there was no recovery. Surprisingly, this pattern was not obviously different if the guinea pigs were reared in the dark. Two of the seven (approximately 30%) guinea pigs also showed recovery in the dark.

A further observation was that dark rearing generally reduced hyperopic refractive errors in the nonmyopic eyes (on average, from +6.95D to +3.02D in the guinea pigs with persistent anisometropia). As a result, the interocular differences in refractions declined from 13.5 D to 9.8 D. In guinea pigs that showed substantial recovery, hyperopia declined from +5.0 D to +2.9 D, and the interocular differences largely disappeared (from 9.48 D to −0.98 D).

Visual Functions: Pupil Responses, Accommodation, and Visual Acuity

An example of a continuous recording of pupil size (upper trace) and refraction (middle trace) in an alert guinea pig is...
shown in Figure 7. The peaks in the lowest trace denote the flashes of the green stimulation LED, attached to the photoretnoscope. Despite its very high stimulation intensity (see Methods), pupil size did not change much. Averaged over eight pupil responses from four guinea pigs, the pupil constriction was only approximately 7%, from 5.67 mm to 5.29 mm. A young human subject (23 years, −8.00 D in both eyes), exposed to the same stimulation, displayed a pupil constriction of 46%.

We also attempted to elicit accommodation in this guinea pig strain. However, no significant refraction changes could be observed on presentation of various fixation targets, such as a pencil or a pair of scissors, each of which elicited strong accommodation in chickens (data not shown).

Finally, because these observations may raise the suspicion that the guinea pigs were blind, their optomotor responses were studied in an optomotor drum. It became clear that their spatial vision was, in fact, good (Fig. 8) and was not different from that reported for wild-type guinea pigs (McFadden SA, et al. IOVS 1995;36:ARVO Abstract S758). Significant optomotor motor responses could be elicited for spatial frequencies of up to at least 3.0 cyc/deg. There was no significant difference in the optomotor gains at 1.2, 1.8, and 3.0 cyc/deg, and the gain at 3.0 cyc/deg was still significantly different from zero ($P < 0.001$). No differences were detected in the optomotor responses regardless of whether guinea pigs were myopic or hyperopic.
In contrast to the population studied by Howlett et al., we describe here an outbred population with a significant proportion of spontaneous myopia (ranging from $-15.67$ D to $-1.50$ D). Natural refractive error development and emmetropization were examined in animals not treated with lenses or diffusers. To select animals with refractive errors, screening for high myopia or anisometropia had taken place in a larger population of guinea pigs. Given that high myopia in humans typically develops without well-defined visual exposure, studies in a strain with spontaneous refractive errors may provide new insight into the mechanisms of high and nonenvironmental myopia in humans. This study showed a striking lack of light-induced pupil responses and no signs of accommodation, in contrast to published descriptions of other strains (McFadden SA, personal communication, 2008).

The most striking observation in this study was that the guinea pigs of this strain showed a large number of spontaneous refractive errors. Furthermore, unlike other strains (Howlett and McFadden14 and McFadden SA, personal communication, 2008), these guinea pigs had weak pupil responses, no detectable accommodation, but reasonable visual acuity in the optomotor drum. Interestingly, the same features were observed in guinea pig strains in Germany (University of Leipzig, Schaeffel F, Liu Q, Tornqist N, unpublished observations, 2007; and University of Tubingen, Schaeffel F, unpublished observations, 2008), suggesting that unpublished observations, there may be common mutation in tricolor wild-type guinea pig strains that reduces pupil responses and accommodation, consistent with an increased incidence of spontaneous refractive errors. Obviously, mapping the locus and identifying the underlying genes would be of great interest.

An unexpected observation was the absence of emmetropization in some myopic guinea pigs. Because of the lack of emmetropization, anisometropia often persisted over time. Apparently, it did not matter whether visual stimuli were available because there were no obvious differences in the recovery patterns from anisometropia when the guinea pigs were raised in the light or in the dark, though the small number of animals reared in the dark ($n = 7$, with 2 showing recovery) may limit the general validity of this conclusion.

At least in other animal models of myopia, emmetropization is believed to be guided by visual cues. Only a small fraction of emmetropization is “passive” and explained by “scaling” of the optical powers of cornea and lens. It is clear that this process alone cannot explain the reduction of large refractive errors in short time, as is usually seen in animal experiments. Nevertheless, two possibilities might explain the recovery from myopia in the dark. One is scaling, and the other is a hyperopic shift induced by exposure to the dark, as previously observed in monkeys. However, it is clear that more studies are necessary to determine the mechanisms for recovery from myopia in the dark.

Most of these observations suggested that the guinea pigs might have been blind, but optomotor experiments showed that this was not the case. In fact, even the highly myopic guinea pigs showed similar spatial vision though it remains to be studied in more detail how refractive errors affected their spatial resolution. No differences in pupil responses and accommodation were seen in myopic and normal guinea pigs.

Usually, guinea pigs reach adult-like refraction at approximately 3 to 4 weeks age.15,22 It is unexpected—but different from findings in guinea pigs, humans,1 rhesus monkeys,2 and chickens4—that emmetropization did not occur in several cases though vision was intact.

Perhaps a spontaneous mutation causing large anisometropia alone is enough to prevent emmetropization, irrespective of specific defects in pupil and accommodative responses. To test whether refractive errors in this sample of guinea pigs were genetically influenced, correlations between both eyes were studied. If no correlation existed despite identical genotypes in both eyes, it would have been questionable that genetic factors determined the refractions. The high correlations between both eyes in binocular myopic guinea pigs ($R = 0.82$) suggested a genetic component. It is more difficult to explain anisometropia. Of course, the interocular correlations were lower, ranging from $-0.44$ to $-0.79$ in guinea pigs that showed no recovery and $-0.98$ to 0.41 in those that did show recovery. Given that refractive errors in this strain may result partially from individual genetic differences and that this complete strain shows unusual differences in relation to other strains of guinea pigs, attempts of mapping the underlying QTLs may be promising.

**References**


