Visual Influences on the Development and Recovery of the Vestibuloocular Reflex in the Chicken

CHRISTOPHER T. GOODE,[1,2] DONNA L. MANEY,[2] EDWIN W RUBEL,[1,2] AND ALBERT F. FUCHS[2,3]
[1]*Virginia Merrill Bloedel Hearing Research Center,* [2]*Program in Neurobiology and Behavior, and* [3]*Regional Primate Research Center, University of Washington, Seattle, Washington 98195*

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Goode, Christopher T., Donna L. Maney, Edwin W Rubel, and Albert F. Fuchs. Visual influences on the development and recovery of the vestibuloocular reflex in the chicken. *J Neurophysiol* 85: 1119–1128, 2001. Whenever the head turns, the vestibuloocular reflex (VOR) produces compensatory eye movements to help stabilize the image of the visual world on the retina. Uncompensated slip of the visual world across the retina results in a gradual change in VOR gain to minimize the image motion. VOR gain changes naturally during normal development and during recovery from neuronal damage. We ask here whether visual slip is necessary for the development of the chicken VOR (as in other species) and whether it is required for the recovery of the VOR after hair cell loss and regeneration. In the first experiment, chickens were reared under stroboscopic illumination, which eliminated visual slip. The horizontal and vertical VORs (h- and vVORs) were measured at different ages and compared with those of chickens reared in normal light. Strobe-rearing prevented the normal development of both h- and vVORs. After 8 wk of strobe-rearing, 3 days of exposure to normal light caused the VORs to recover partially but not to normal values. In the second experiment, 1-wk-old chicks were treated with streptomycin, which destroys most vestibular hair cells and reduces hVOR gain to zero. In birds, vestibular hair cells regenerate so that after 8 wk in normal illumination they appear normal and hVOR gain returns to values that are normal for birds of that age. The treated birds in this study recovered in either normal or stroboscopic illumination. Their hVOR and vVOR and vestibulocollic reflexes (VCR) were measured and compared with those of untreated, age-matched controls at 8 wk posthatch, when hair cell regeneration is known to be complete. As in previous studies, the gain of the VOR decreased immediately to zero after streptomycin treatment. After 8 wk of recovery under normal light, the hVOR was normal, but vVOR gain was less than normal. After 8 wk of recovery under stroboscopic illumination, hVOR gain was less than normal at all frequencies. VCR recovery was not affected by the strobe environment. When streptomycin-treated, strobe-recovered birds were then placed in normal light for 2 days, hVOR gain returned to normal. Taken together, the results of these experiments suggest that continuous visual feedback can adjust VOR gain. In the absence of appropriate visual stimuli, however, there is a default VOR gain and phase to which birds recover or revert, regardless of age. Thus an 8-wk-old chicken raised in a strobe environment from hatch would have the same gain as a streptomycin-treated chicken that recovers in a strobe environment.

INTRODUCTION

The vestibuloocular reflex (VOR) helps to maintain a stable image of the visual world on the retina during head movements by providing opposite movement of the eyes. The VOR is aided by the optokinetic response (OKR), which produces eye movements in the direction of the image motion that remains after the VOR. When these reflexes operating together do not produce a perfectly compensatory eye movement, visual images slip across the retina and cause the visual scene to blur. However, several lines of research indicate that the gain of the adult VOR changes in response to visual slip, thus minimizing the blur. This ability to respond to changing visual conditions produces an appropriate VOR, maintains it throughout life, and, if necessary, reestablishes the VOR after damage.

That visual slip helps maintain the VOR is well established. Adaptation to extreme visual environments has been demonstrated in numerous experiments in a variety of species by fitting subjects with magnifying, minimizing, or reversing goggles to create profound retinal slip. In response to these altered visual environments, the VOR gain (eye velocity/head velocity) will increase, decrease, or even reverse its sign (Gonshor and Melvill Jones 1971, 1976 ; Melvill Jones and Davies 1979; Miles and Eighmy 1980).

The importance of retinal slip during VOR development also has been confirmed in numerous species. The VOR gains of dark-reared cats, rabbits, fish, and tadpoles are lower than normal adult values (Collewijn 1977; Harris and Cynader 1981; Horn et al. 1996). Strobe-rearing, i.e., rearing an animal exclusively in a flashing visual environment to eliminate smoothly moving visual stimuli, and thus visual slip, also leads to decreased VOR gain in cats (Kennedy et al. 1982, but see Mandl et al. 1981). We wondered whether visual slip is necessary for the development of the avian VOR and, moreover, whether the slip must be experienced during a "critical period" for the VOR to be established at all.

We tested this by rearing chicks in a stroboscopic environment from hatch and measuring the VOR at several different ages. We compared the VOR of these birds to those of normal light-reared, age-matched controls. These data provided a "time line" of the effects of strobe-rearing that, to the best of our knowledge, is not available for this or any other species.

Visual slip also may play a role during recovery from injury. In birds, vestibular and auditory hair cells regenerate after they have been destroyed by ototoxic, aminoglycoside antibiotics (Cruz et al. 1987; Lippe et al. 1991; Weisleder and Rubel

Address for reprint requests: A. F. Fuchs, Regional Primate Research Center, Box 357330, University of Washington, Seattle, WA 98195-7330 (E-mail: fuchs@u.washington.edu).

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1993). Administration of streptomycin to birds causes most vestibular hair cells to degenerate. Within ~8–10 wk, however, the vestibular epithelia recover their normal morphological appearances (Weisleder and Rubel 1993). Regenerated vestibular hair cells apparently establish normal afferent connections because brain-stem-evoked potentials in response to linear acceleration resemble potentials recorded in normal controls (Jones and Nelson 1992).

We have shown elsewhere that horizontal VOR (hVOR) gain in the chicken diminishes essentially to zero in response to streptomycin-induced loss of hair cells but recovers as hair cells regenerate (Carey et al. 1996). The vestibulocollic reflex (VCR), which stabilizes the head in space during body rotations, also recovers with hair cell regeneration (Goode et al. 1999). In these two studies, chickens recovered in normal room light. It is possible that VOR and VCR recovery from hair cell loss did not require a visual error signal and that the requisite connections to reestablish these reflexes were guided by nonvisual mechanisms.

To test whether visual slip was necessary for VOR and VCR recovery, we treated hatchling chicks with streptomycin, allowed them to recover under stroboscopic conditions until hair cell regeneration was complete, and compared their VORs and VCRs with those of normal light-reared chickens (both streptomycin-treated and untreated, age-matched controls).

METHODS

General methods

EXPERIMENTAL ANIMALS. We used white leghorn chicks (*Gallus domesticus*) varying in age from 2 to 40 days posthatch (dph). Strobe-reared and -recovering chickens were housed in a room lit solely by two synchronized strobe lights (American DJ), flashing at 2.25 Hz, controlled by a Grass 1000 stimulator (Grass Instruments). The stimulator was controlled by a timer, which allowed 16 h of stroboscopic light and 8 h of darkness per day (16L:8D). Chickens housed under normal light were on the same light/dark schedule.

Chickens in both the normal light and strobe conditions had unrestricted access to food and water 24 h/d. All strobe-reared chickens ate and drank normally and weighed essentially the same as normal light-reared chickens. Animal care and experimental procedures conformed to the standards of the Institutional Animal Care and Use Committee at the University of Washington.

EYE COIL ATTACHMENT. The VOR was measured with the use of Robinson's (1963) magnetic search coil technique. Prefabricated eye coils, whose leads were twisted together and soldered to small, gold, female connector pins (Amphenol) that were imbedded in a small plug of dental acrylic, were implanted on the sclera (see Anastasio and Correia 1988; Quinn et al. 1998) of the left eye of each bird 48 h before testing (except for 2-day-old chicks, which received the eye coil 1 dph). Surgical procedures were performed with the birds under equithesin and ketamine anesthesia (1.5 ml/kg; 0.8 mg/kg, respectively). Strobe-reared chickens were anesthetized under strobe light and were returned to the stroboscopic environment for surgical recovery.

The coil was sewn to the sclera at three points, and its leads were directed under the skin to the top of the skull, where the plug exited the skin. The plug was sewn to the skull and the scalp was closed around the plug.

All animals recovered for ≥48 h before testing except the 2-day-old chicks, which underwent surgery 1 dph and recovered for 24 h. Strobe-reared chickens recovered from surgery in the strobe-illuminated environment.

VOR TESTING. Both the horizontal (yaw, about a vertical axis) and vertical (roll, about the anterior-posterior axis) VOR were measured in all chickens. All VOR testing was done in the dark. All strobe-reared chickens were prepared for testing in a darkened room.

Alert chickens were restrained in a supine plastic bottle with a hole cut out for the head. The heads of the birds were restrained by taping the beak firmly to a bone wax-lined beak holder, which was attached to the bottle, extending from the hole cut for the head. The bottle was then secured to a rotating turntable. The turntable was set to oscillate over a fixed angle of ±10° at frequencies of 0.1, 0.3, 0.5, and 0.8 Hz, i.e., at peak velocities of 6.28, 18.85, 31.42, and 50.2°/s, respectively. Two pairs of 14-in magnetic induction coils that produced alternating magnetic fields (35 kHz) at the subject's left eye rode on the turntable. Horizontal and vertical angular eye position signals were filtered at 500 Hz and recorded on video tape (Vetter 5000A PCM recorder, sample rate = 5 kHz/channel). Turntable position was measured with a potentiometer.

All signals were simultaneously digitized on-line with a Power Macintosh 7500/120 MHz and a MIO16 Digitizer (National Instruments). Digitizing software set the sampling rate at 600 samples for every cycle of oscillation, regardless of the frequency.

Search coils were calibrated at the beginning of each test session by suspending the animal in the center of the electromagnetic field and oscillating the turntable (and coils) at a frequency of 0.3 Hz around the animal, whose eyes remained essentially stationary in space.

VOR DATA ANALYSIS AND VECTOR AVERAGING. An interactive analysis program displayed single cycles of digitized sinusoidal horizontal or vertical turntable position, along with horizontal and vertical eye position, on a computer monitor. The program calculated digital derivatives of all position traces to produce velocities and fit each trace with a best-fit sine wave using a discrete Fourier transform (Fig. 1, *top 3 traces*). The rapid, nonperiodic saccades were easily identified by their rapid oscillations (see horizontal eye velocity) and were removed (Fig. 1, *bottom traces*). The program then calculated

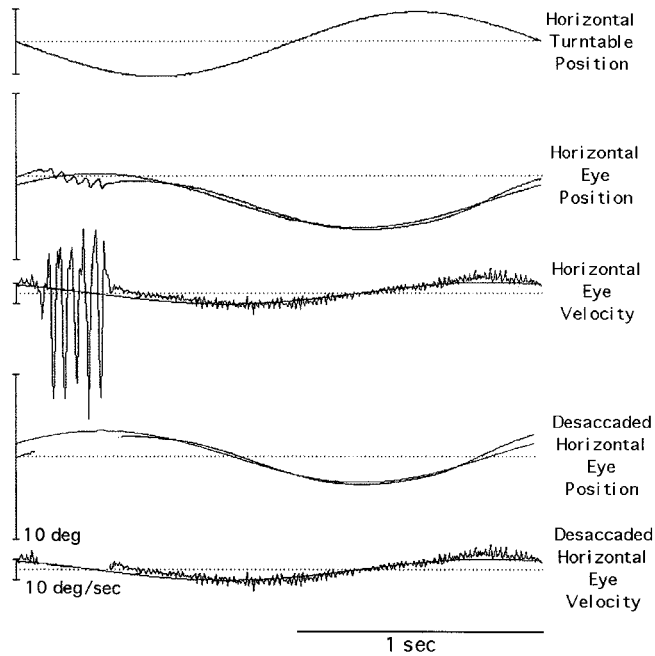

FIG. 1. Representative raw data showing a single cycle of the vestibulooocular reflex (VOR) of a strobe-reared chicken, 8 wk after streptomycin treatment and 48 h after exposure to normal light. *Top* to *bottom*: turntable (horizontal head) position, horizontal eye position, horizontal eye velocity, desaccaded horizontal eye position, and desaccaded eye velocity. The high-frequency oscillations produced at the beginning of the cycle are part of an avian saccade. "Desaccaded" eye position and velocity traces are what remain after the saccade has been removed. The smooth traces are the best-fit curves determined by the computer program. All bars on the traces are either 10° (position traces) or 10°/s (velocity traces).

VOR gain for each cycle of the saccade-free data as the ratio of eye to turntable velocity and the phase shift as the difference (in degrees) between the peak of the fitted turntable (and therefore head) velocity sinusoid and the peak of either the horizontal or vertical eye velocity sinusoid. By our convention, a perfectly compensatory VOR has a gain of 1.0 and a phase shift of 180°. Phase shifts between 0 and 180° indicate that eye velocity led head velocity.

Cycles were accepted for analysis on the basis of two criteria: after all saccades had been deleted, ≥60% of the data points in the cycle remained and ≥60% of the variance of eye velocity was sinusoidal (as calculated by the analysis program). For cycles with very low gains (<0.1) the second criterion was rarely met. Therefore for gains <0.1, which we show to demonstrate that strobe-rearing drove gains essentially to zero, phase values are not shown.

Both the gain and phase of the eye velocity were considered in the response averages, i.e., we performed vector averages. Each cycle response was represented by a vector whose size was equal to gain (eye velocity/head velocity) and whose direction was determined by the phase shift. Gain was decomposed into x (gain * cos ø) and y (gain * sin ø) vector components where ø is the phase in radians. The x and y components of the gain vector at each frequency were averaged separately and the magnitude of the gain vector was determined as $\sqrt{(x^2 + y^2)}$.

EXPERIMENT 1: EXPERIMENTAL DESIGN AND EXPERIMENTAL ANIMALS. The independent variables were age (4 levels) and lighting condition (stroboscopic and normal), yielding eight groups. Subjects were randomly assigned to these groups as follows. Of 56 chickens, 28 were placed in an incubator in the strobe environment 2 days before they hatched. After hatching, chicks were moved to brooders in the same stroboscopically illuminated room. Reptile heating pads (Cobra) on the floors of the brooders provided heat without light. The other 28 chicks were hatched in a normally illuminated (16L: 8D) incubator and served as age-matched controls. The horizontal and vertical VOR (hVOR and vVOR) were measured in strobe- and normal light-reared birds in four age groups: 2, 9, 25, and 40 dph ($n = 7$ at each age). Immediately after VOR testing, the strobe-reared, 40-dph chickens were moved to normal light conditions for 3 days and then tested again.

EXPERIMENT 2: EXPERIMENTAL DESIGN, EXPERIMENTAL ANIMALS AND STREPTOMYCIN TREATMENT. The independent variables were streptomycin treatment (treated and untreated) and lighting condition (stroboscopic and normal). Chickens were randomly assigned to one of the resulting four groups ($n = 4$ in each group) as follows. Of 24 white leghorn chicks, each 7 dph, 12 were injected with streptomycin sulfate ($1{,}200\ mg \cdot kg^{-1} \cdot d^{-1}$ im, for 5 days). This treatment produces profound damage of vestibular hair cells and reduces the gains of the hVOR and vestibulocollic reflex (VCR) to zero (Carey et al. 1996; Goode et al. 1999). The h- and vVOR of four streptomycin-treated birds were measured 2 days after the last injection and compared with those of four untreated, age-matched controls.

The day after the last injection, four of the remaining eight streptomycin-treated chicks were moved to the strobe environment and four remained under normal light conditions. Each group remained in its environment for 8 wk, long enough for complete hair cell regeneration and VOR recovery (Carey et al. 1996). Of the remaining eight untreated chicks, four were placed in the strobe environment at the same age as the streptomycin-treated chicks (12 dph) and four remained in normal light. Again, these conditions were maintained for 8 wk. Immediately after VOR testing at 8 wk, streptomycin-treated, strobe-reared chickens were placed in normal light for 2 days and then tested again.

STATISTICAL ANALYSES. Repeated-measures analyses of variance (ANOVA, Statview 4.5) were used to compare mean vector-averaged gain and phase data from strobe-reared and normal light-reared birds in both experiments. In *experiment 1*, between-subjects variables were age (4 levels) and lighting condition (stroboscopic vs. normal). In *experiment 2*, between-subjects variables were streptomycin treatment (treatment vs. no treatment) and lighting condition (stroboscopic vs. normal).

Scheffe post hoc tests (Statview 4.5) were used to compare means where significant main effects or interactions were detected by the ANOVA. Where ANOVA statistics are reported, the values of F and P (probability) are presented and between- and within-subjects degrees of freedom, respectively, are given in parentheses. Whenever individual means were compared, the probability value presented is the result of a Scheffe post hoc test. We consider probabilities <0.05 to be significant for both ANOVA and Scheffe statistics.

VCR TESTING. The VCR was measured in five additional chickens, of which three received the same dose of streptomycin as the 7-day-old chicks described in the previous section. All five chicks were placed in the stroboscopic environment the day after the streptomycin-treated chicks received their last injection. The VCR in these birds was measured after 21 days of recovery in the stroboscopic environment by which time VCR recovery under normal light is known to be complete (Goode et al. 1999). The head movements of the VCR were measured via a search coil attached to the side of the chicken's head (for measurement and analysis details, see Goode et al. 1999).

RESULTS

Experiment 1

VOR DEVELOPMENT IN NORMAL LIGHT-REARED CHICKENS. *Gain.* Average h- and vVOR gains were low (between 0.15 and 0.4 depending on frequency) when they were first measured at 2 dph (Fig. 2). Generally, both h- and vVOR gain increased with frequency [$F(3, 48) = 290.15$, $P < 0.0001$; $F(3, 48) = 155.68$,

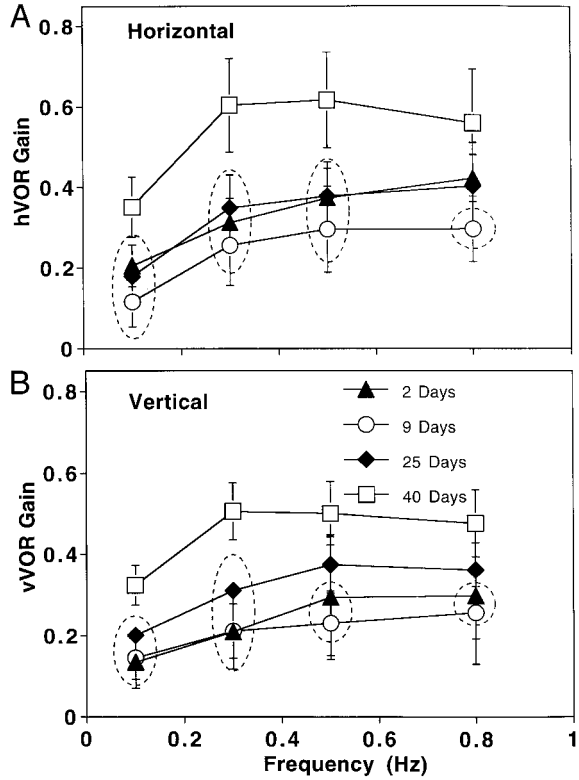

FIG. 2. Horizontal (*A*) and vertical (*B*) VOR gain (eye velocity/head velocity) as a function of oscillation frequency at 4 different ages after hatching. - - -, encloses symbols that are significantly lower than the 40-dph VOR gain at that frequency ($P < 0.05$, Scheffe) and not significantly different from each other. Each symbol represents mean gain at 1 frequency ($n = 7$). Lines connect symbols from the same 7 birds. Error bars in this an all subsequent figures represent ±1 SD.

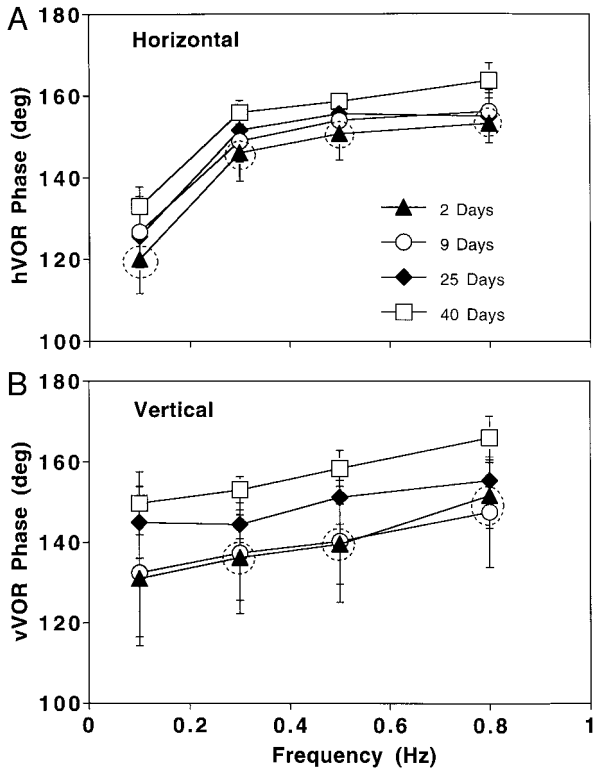

FIG. 3. Horizontal (*A*) and vertical (*B*) VOR phase shift (relative to head velocity) as a function of oscillation frequency at 4 different ages after hatching. - - -, encloses points that are significantly lower than the 40-dph VOR phase at that frequency ($P < 0.05$, Scheffe) and not significantly different from each other (note: - - - at 0.8 Hz in *A* encloses points for only 2 and 25 dph, not 9 dph). Each symbol represents mean phase shift at 1 frequency ($n = 7$). Lines connect symbols from the same 7 birds.

$P < 0.0001$, respectively] until 0.5 Hz, after which gain appeared to saturate (Fig. 2). Since the oscillation was of fixed amplitude, however, gain could have been related to velocity rather than frequency.

The relation between gain and frequency did not change significantly during the first 25 dph. By 40 dph, however, hVOR gain had increased at all frequencies and by >50% at 0.3 and 0.5 Hz (Fig. 2*A*). The vVOR gain showed a similar increase at 40 dph (Fig. 2*B*). The means that were significantly lower than those of 40-day-old chickens at each frequency ($P < 0.005$ for horizontal gain; $P < 0.05$ for vertical gain) are enclosed in Fig. 2 by - - -. At 0.8 Hz, the only significant difference in hVOR gain was between 9- and 40-dph birds.

Phase. If the VOR produced perfectly compensatory eye movements, VOR gain in the dark would equal 1.0 and eye velocity would be 180° out of phase with head velocity (phase shift would equal 180°). Figure 2 shows that the gain was not 1.0, and Fig. 3 shows that the phase shift was not 180°. For both the h- and vVOR, eye velocity led perfect compensation by less (phase shift moved closer to 180°) as frequency increased [Fig. 3; hVOR: $F(3, 48) = 99.25$, $P < 0.0001$; vVOR: $F(3, 48) = 24.81$, $P < 0.0001$]. For the hVOR, this frequency-dependent increase in phase shift appeared to saturate at ~0.3–0.5 Hz (Fig. 3*A*), similar to hVOR gain (Fig. 2*A*). However, vVOR phase shifts seemed to continue to increase gradually with frequency (Fig. 3*B*).

The VOR phase shifts increased more gradually with age than did the VOR gains. Between 2 and 40 dph, there generally were significant age-related differences in phase shift for the h- and vVOR at all frequencies. Means that were significantly lower than those at 40 dph ($P < 0.05$) are encircled (- - -). At 0.8 Hz, the mean hVOR phase shift at 9 dph was clustered near the means at 2 and 25 dph, but was not significantly different from the mean at 40 dph.

VOR DEVELOPMENT IN STROBE-REARED CHICKENS. In normal chickens, significant differences in VOR gain and phase shift occur between the lowest and highest stimulus frequencies (Carey et al. 1996). The lowest frequencies have the lower gains and the greater phase leads. We therefore compared the h- and vVOR of strobe-reared chickens with those of normal-light-reared chickens at the lowest and highest oscillation frequencies.

Gain. Strobe-rearing kept low-frequency VOR gains <0.1 through 40 dph. At 0.1 Hz, both hVOR gain (Fig. 4*A*) and vVOR gain (Fig. 4*B*) of strobe-reared chickens were lower than those of normal-light-reared chickens at all ages tested. Because both h- and vVOR gains in strobe-reared chickens were below our "noise floor" of 0.1 at every age, their associated phase shifts are omitted from Fig. 4. The suspect gain values from strobe-reared chickens are presented to show that they were <0.1 for the duration of the experiment.

Strobe-rearing had a similar but less dramatic effect on VOR gains at the highest frequency tested. At 0.8 Hz, hVOR gains of strobe-reared chickens were significantly lower than those of normal-light-reared chickens at 2, 9, and 40 dph (Fig. 5*A*; $P < 0.0001$, $P < 0.005$, $P < 0.005$, respectively). However, vVOR gains of strobe-reared chickens (Fig. 5*C*) were significantly

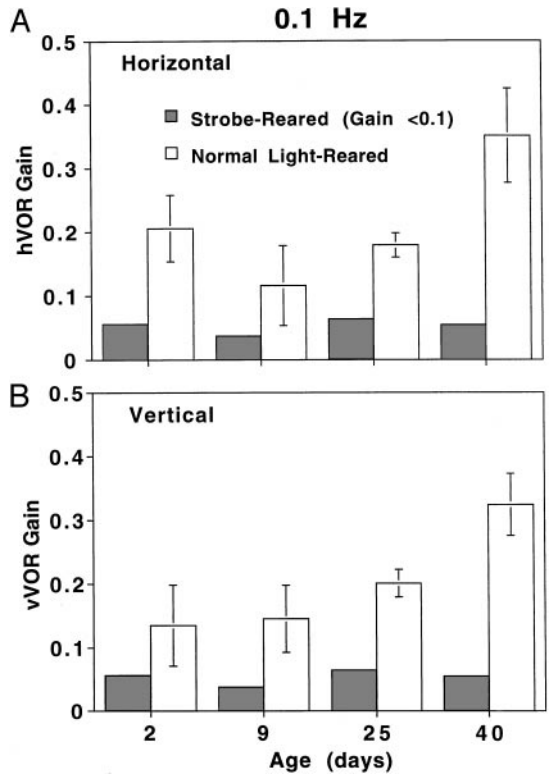

FIG. 4. Horizontal (*A*) and vertical (*B*) VOR gain at 0.1 Hz as a function of age. A comparison is made between strobe-reared (▣) and normal light-reared (□) chickens. Gains from strobe-reared chickens were below our noise floor and could not be measured reliably. Therefore they are presented as gray bars to distinguish them from reliable, higher-gain data, which appear as filled bars/symbols elsewhere. Each bar represents mean gain at 1 frequency ($n = 7$).

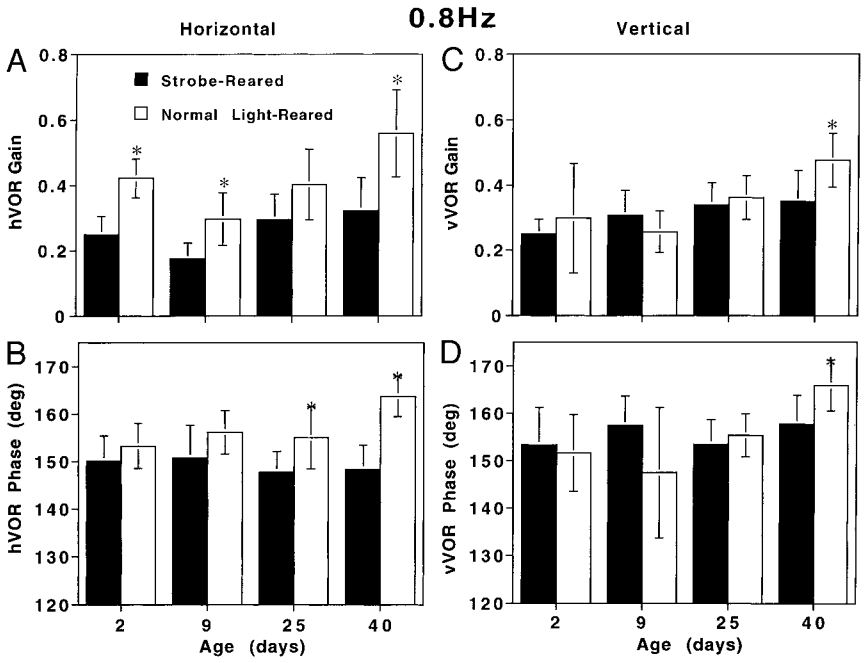

FIG. 5. Mean gain and phase shift of the hVOR (*A* and *B*) and vVOR (*C* and *D*) at 0.8 Hz as a function of age in strobe-reared (■) and normal-light-reared (□) chickens. *, normal-light-reared data that are significantly higher than strobe-reared data at the same age ($P < 0.01$, Scheffe). Each bar represents mean gain or phase shift at that frequency ($n = 7$).

lower than those of normal-light-reared chickens only at 40 dph ($P < 0.05$).

Phase shift. Although the hVOR phase shifts of strobe-reared chickens led perfect compensation more, on average, than those of normal-light-reared chickens of every age, strobe-rearing had no significant effect on both h- and vVOR phase shifts at 0.8 Hz until 40 dph (Fig. 5, *B* and *D*). At this age VOR phase shifts of strobe-reared chickens led perfect compensation (180°) by significantly more than did those of normal-light-reared chickens (Fig. 5*B*: hVOR: $P < 0.0001$; Fig. 5*D*: vVOR: $P < 0.05$).

EFFECTS OF PLACING STROBE-REARED CHICKENS IN NORMAL LIGHT. Immediately after strobe-reared chickens were tested 40 dph, they were moved to normal light conditions (16 h continuous light, 8 h dark). After 3 days of exposure to ambient room light, the hVOR gain in strobe-reared birds had increased at 0.1, 0.3, and 0.5 Hz (Fig. 6*A*). However, h- and vVOR gains in these chickens were still significantly lower overall than those of normal-light-reared chickens at all frequencies except for the vVOR at 0.8 Hz (Fig. 6, *A* and *C*; hVOR: 0.1 Hz, $P < 0.01$; 0.3 Hz, $P < 0.005$; 0.5 Hz, $P < 0.01$; 0.8 Hz, $P < 0.05$; Fig. 6*C*: vVOR: 0.1, 0.3, 0.5 Hz, $P < 0.05$; 0.8 Hz, $P > 0.5$).

Exposure to normal light essentially returned the phase lead of the hVOR of strobe-reared chickens to normal at all frequencies >0.1 Hz (Fig. 6*B*). There was no significant difference between the hVOR phase shift of strobe-reared birds that were then exposed to normal light and that of normal-light-reared chickens [Fig. 6*B*; $F(3, 6) = 0.42$, $P > 0.10$]. Strobe-rearing did not greatly affect vVOR phase shifts. The phase shifts of chickens that were strobe-reared and then exposed to

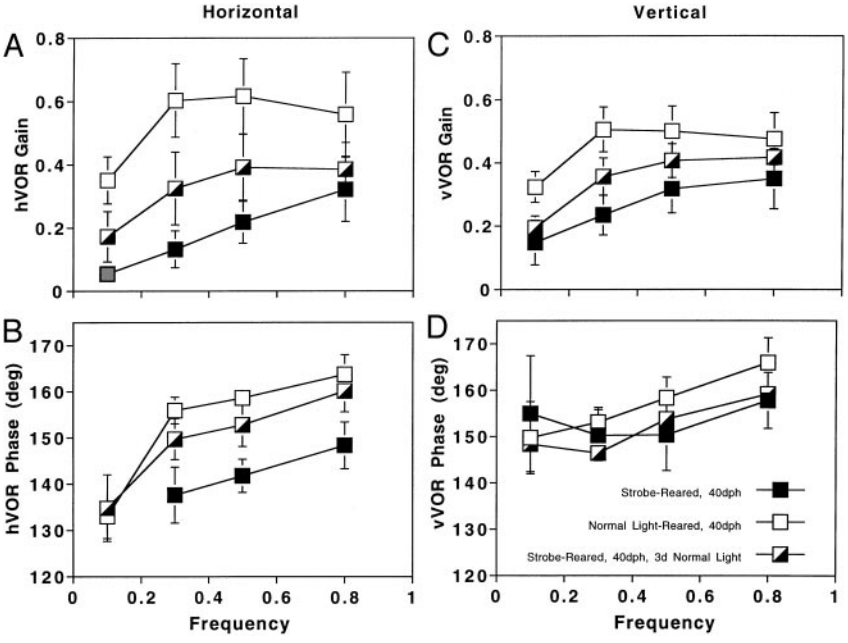

FIG. 6. Rescue of mean gain and phase shift of the hVOR (*A* and *B*) and vVOR (*C* and *D*) at 4 frequencies by normal light exposure in 40-dph strobe-reared chicks. Data are for chickens that experienced only strobe-rearing (■, $n = 7$), strobe-reared chickens that then experienced 3 days of normal light (◪, $n = 5$), and normal light-reared chickens of the same age (□, $n = 7$). Each symbol represents mean gain or phase shift at that frequency. Gray symbol indicates an average gain <0.1. Lines connect symbols representing data from the same birds.

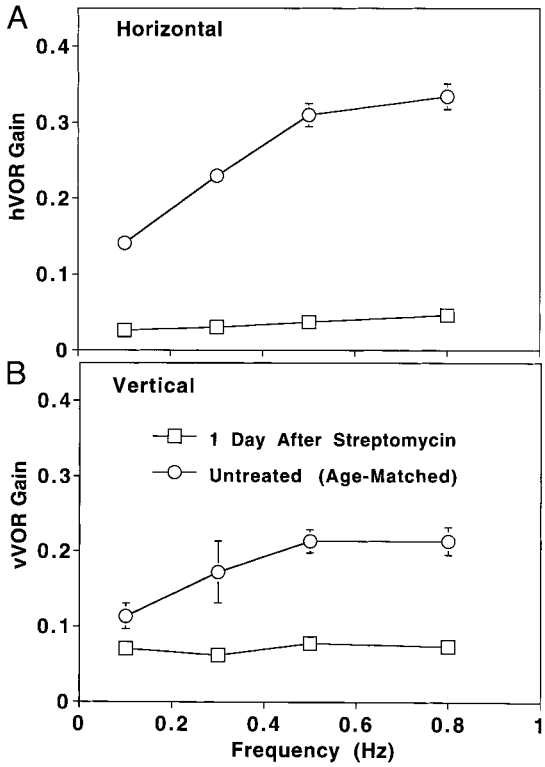

FIG. 7. Effects of streptomycin treatment on mean hVOR gain (*A*) and mean vVOR gain (*B*) as a function of the frequency of head oscillation. Data are for streptomycin-treated chicks in normal light 1 day after treatment and untreated age-matched controls. Each symbol represents mean gain at 1 frequency ($n = 4$). Lines connect symbols from the same 4 birds.

normal light did not differ significantly from those of normal-light-reared chickens [Fig. 6*D*; $F(3, 6) = 3.10$, $P > 0.10$].

Experiment 2

VOR RECOVERY OF STREPTOMYCIN-TREATED CHICKENS UNDER NORMAL LIGHT. One day after streptomycin treatment, both h- and vVOR gain were reduced essentially to zero at all frequencies (Fig. 7, *A* and *B*). The actual gains are shown in Fig. 7 to confirm that they all are <0.1, our inclusion threshold.

Streptomycin-treated chickens that were housed under normal light recovered their hVORs fully. Eight weeks after streptomycin treatment, hVOR gains and phase shifts of treated chickens were not significantly different from those of untreated chickens at all frequencies [Fig. 8, *A* and *B*; gain: $F(3, 6) = 0.001$, $P > 0.5$; phase: $F(3, 6) = 1.751$, $P > 0.1$]. The hVOR recovery in Fig. 8 is similar to that which we reported earlier (Carey et al. 1996), although the hVOR gains here were, on average, 10% lower.

In contrast, the vVOR gain had not fully recovered 8 wk after streptomycin treatment (Fig. 8*C*). The vVOR gain of streptomycin-treated, normal-light-reared chickens was significantly lower than that of untreated, normal-light-reared chickens at all frequencies [$F(3, 6) = 32.047$, $P < 0.05$]. Vertical VOR phase shifts in streptomycin-treated chickens were not significantly different from those of untreated chickens [Fig. 8*D*; $F(3, 6) = 1.4$, $P > 0.3$].

As in *experiment 1* (Fig. 2), we found the same frequency-dependent increase in VOR gain and a saturation between 0.3 and 0.5 Hz. Again, the saturation of the phase of the hVOR but not the vVOR seen in *experiment 1* (Fig. 3) was also found in the data of Fig. 8 (*B* and *D*). Clearly, then, there is a difference in the effect of streptomycin treatment on changes in the h- and vVORs.

VOR RECOVERY OF STREPTOMYCIN-TREATED CHICKENS IN A STROBOSCOPIC ENVIRONMENT. Allowing streptomycin-treated birds to recover in a stroboscopic environment prevented hVOR gain from recovering to normal values at all frequencies <0.8 Hz. Chickens that recovered in the stroboscopic environment had significantly lower hVOR gains at 0.1, 0.3, and 0.5 Hz than chickens that recovered in normal light ($P < 0.001$; $P < 0.001$, and $P < 0.01$, respectively). In fact, the hVOR gains of all strobe-recovered birds were lower than those of birds housed in normal light, whether they had been treated with streptomycin or not [Fig. 9*A*; $F(1, 12) = 23.05$, $P < 0.005$].

Recovery of streptomycin-treated chickens in a stroboscopic

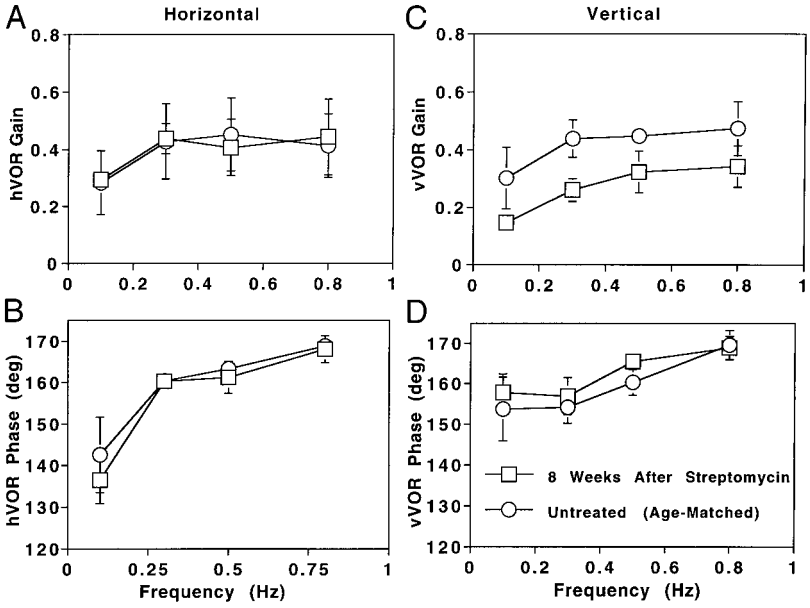

FIG. 8. Recovery in normal light of the mean gain and phase shift of hVOR (*A* and *B*) and vVOR (*C* and *D*) with frequency after treatment with streptomycin. Streptomycin-treated chicks 8 wk after treatment are compared with untreated age-matched controls. Each symbol represents mean gain or phase shift at 1 frequency ($n = 4$). Lines connect symbols from the same 4 birds.

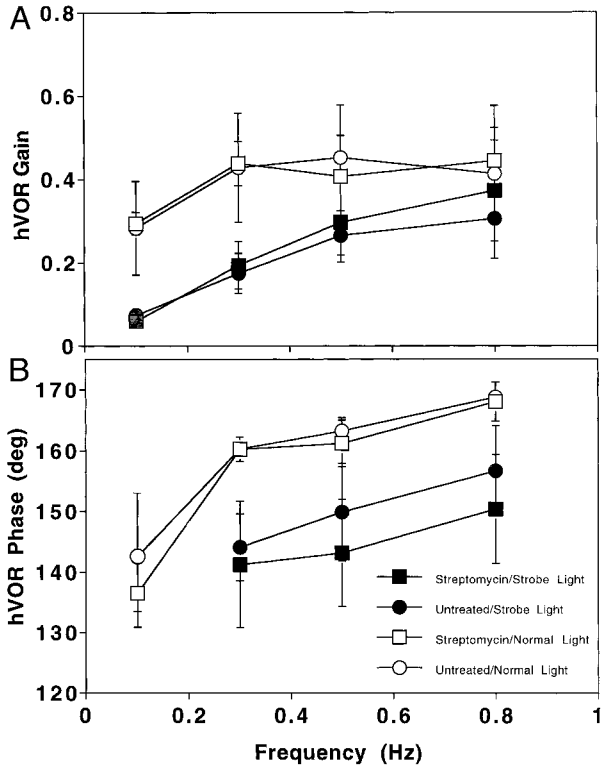

FIG. 9. Effects of stroboscopic illumination on recovery of the mean gain (*A*) and phase shift (*B*) of the hVOR with frequency after streptomycin treatment. Streptomycin-treated birds recovering in either normal or stroboscopic illumination are compared with untreated birds raised in either normal or stroboscopic illumination. Each symbol represents mean gain or phase shift at 1 frequency ($n = 4$). Gray symbols indicate average gains <0.1. Lines connect symbols from the same 4 birds.

environment also prevented the complete recovery of hVOR phase shifts (Fig. 9*B*). Again, whether treated with streptomycin or not, all strobe-reared birds had phase shifts that led perfect compensation (180°) by significantly more than did the phase shifts of birds that recovered in normal light [Fig. 9*B*; $F(1, 12) = 11.80$, $P < 0.005$]. This effect was driven by significantly lower mean hVOR phase shifts in strobe-recovered chickens at 0.3, 0.5, and 0.8 Hz ($P < 0.0005$; $P < 0.005$; $P < 0.005$, respectively).

Since the vVOR had not fully recovered when we measured it in normal-light-recovered chickens (Fig. 8*C*), it was impossible to determine what effect, if any, strobe-recovery may have had. Therefore we report only hVOR data from strobe-reared chickens here.

VOR IN STROBE-RECOVERED CHICKENS AFTER 48 H OF NORMAL LIGHT. Streptomycin-treated, strobe-recovered chickens were placed in normal light immediately after VOR testing 8 wk after streptomycin treatment. They were tested again ~48 h later at 0.1, 0.3, and 0.5 Hz. At this age, the chickens were quite large and boisterous, preventing measurement at the highest frequency of oscillation. Exposure to normal light brought the hVOR back to normal at all frequencies. Horizontal VOR gains in streptomycin-treated, strobe-recovered chickens that were exposed to normal light for 48 h were not significantly different from those of untreated chickens that were housed in normal light [Fig. 10*A*; $F(1, 6) = 0.11$, $P > 0.5$].

Horizontal VOR phase shifts, however, were still significantly lower in streptomycin-treated, strobe-recovered chickens that were briefly exposed to normal light than those of controls housed in normal light at 0.3 and 0.5 Hz (Fig. 10*B*; $P < 0.01$ for both).

VCR RECOVERY OF STREPTOMYCIN-TREATED CHICKENS IN A STROBOSCOPIC ENVIRONMENT. Three additional chicks were treated with the same dose of streptomycin (1,200 mg · kg $^{-1}$ · d^{-1} for 5 days) and allowed to recover in the stroboscopic environment along with two additional untreated chicks that were used as controls. After 3 wk, by which time the VCR recovers fully under normal light (Goode et al. 1999), the VCR was measured in all chickens and compared with that of untreated chickens housed in normal light in a previous experiment (Goode et al. 1999).

Both experimental and control birds had essentially normal VCRs at 3 wk posttreatment. Figure 11 shows that for frequencies ≥0.3 Hz, all birds raised in a stroboscopic environment had VCR gains that fell in the range (see shaded region) of birds raised in a normal environment. However, at the lowest frequency tested (0.1 Hz) streptomycin-treated, strobe-recovered chickens had lower VCR gains, on average, than untreated chickens that were housed in either strobe or normal light conditions.

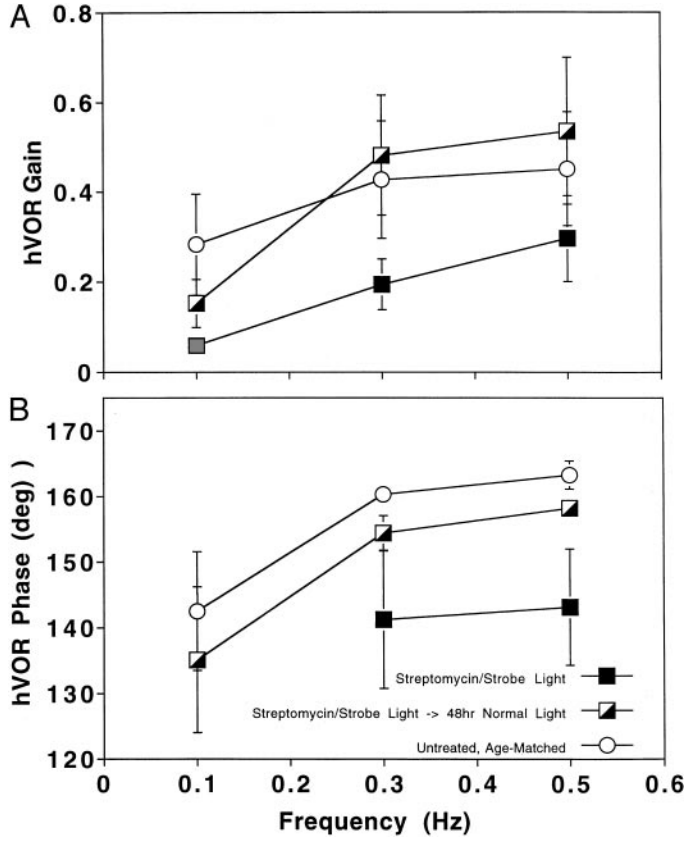

FIG. 10. Rescue of hVOR gain (*A*) and phase shift (*B*) by normal light exposure after birds treated with streptomycin had recovered in stroboscopic illumination. Data are for streptomycin-treated birds that recovered for 8 wk in a strobe environment, streptomycin-treated birds that had recovered for 8 wk in a strobe environment and then experienced 48 h of normal light, and untreated birds of the same age raised in normal light. Each symbol represents mean gain or phase shift at 1 frequency ($n = 4$). Gray symbol indicates an average gain <0.1. Lines connect symbols from the same 4 birds. Error bars represent ±1 SD.

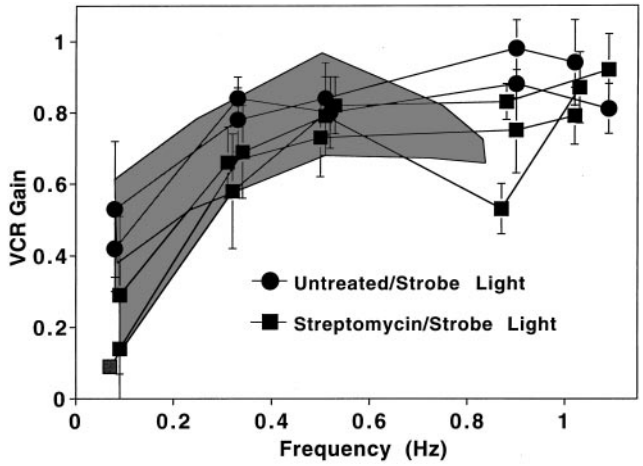

FIG. 11. VCR gain as a function of oscillation frequency. Streptomycin-treated, strobe-recovered chickens (■) are compared with untreated, strobe-recovered chickens (●). Each symbol represents mean VCR gain from 1 subject. Lines connect symbols from the same subject. The shaded area represents the range of VCR gains of untreated, normal light-recovered chickens (from Goode et al. 1999). Error bars represent ±1 SD.

DISCUSSION

Chickens that were reared from hatch in a stroboscopic environment, which eliminates visual slip, failed to develop a normal VOR. Both the gain and phase shifts of the VOR remained immature through 40 dph. Chicks that were treated with streptomycin at 7 dph and then placed in a strobe environment did not recover their VOR although streptomycin-treated chicks that recovered in normal light did. The VOR gain and phase shifts of streptomycin-treated, strobe-recovered chickens were similar to the gain and phase shifts of strobe-reared chickens of a similar age. Together, these experiments show that for a change in the VOR to occur, chickens must experience visual slip. This is true whether that change is over the course of development or during recovery from hair cell loss. In the absence of visual slip, the VOR has abnormal characteristics, which are the same in both streptomycin-treated and untreated birds. However, when slip is restored even after a long period of 40 days, the VOR still can improve.

Poor image stabilization early in development

Our results are consistent with other reports that the gain of the hVOR in newly hatched chickens is low, ranging from ~0.07 at 0.125 Hz to ~0.2 at 1.0 Hz (Carey et al. 1996; Wallman et al. 1982). By 4–6 wk of age in normal ambient light, hVOR gain increases to 0.35 at 0.125 Hz and to 0.6 at 1.0 Hz (Wallman et al. 1982). Our measurements of hVOR gain (Fig. 2) at 40 dph are nearly identical to those reported by Wallman et al. (1982) and Carey et al. (1996).

At low frequencies, both the gain and phase of the VOR, which is measured in the dark, would not provide adequate image stabilization. At higher frequencies and older ages, the gain and phase become more compensatory but far from ideal. If the adult chicken is oscillated in the light to activate the optokinetic reflex as well as the VOR, the gain of the compensatory eye movements is near 1.0 at all frequencies tested (0.125–1.0 Hz) (Wallman et al. 1982).

The time course of VOR development in chickens is much longer than the development of several other visual capabilities. For example, both depth perception (Shinkman 1963; Tallarico and Farrell 1964) and visual acuity (Over and Moore 1981) in chickens are fully developed by 2 dph. Thus developmental changes in VOR gain and phase shift are probably not secondary to, nor a consequence of, immature visual processes.

Different time courses of VOR gain and phase development

The gain of both the h- and the vVOR followed a different developmental time course than did their associated phase shifts. For both the vVOR and hVOR, little change in gain occurred between the 2nd and 25th dph. The greatest change occurred between the 25th and 40th day, when gain increased significantly (Fig. 2, *A* and *B*). In contrast, the phase shift of the hVOR showed very little change (<10° at each frequency) over the same 40-day period. Between 2 and 40 dph, the phase shift of the vVOR showed a slightly more substantial increase, on the order of 20° (Fig. 3, *A* and *B*).

Different time courses of the gain and phase changes also occur during VOR adaptation with optical devices. In cats whose VOR was adapted with reversing prism masks, hVOR gain decreased rapidly in the first 10 days, but hVOR phase shifts did not change noticeably until after 10 days. When the prisms were removed, the phase shift returned to preadapted levels within the first few days, but it was much longer before the gain approached its preadapted state (Melvill Jones and Davies 1979). VOR gain and phase, then, can be adapted at different rates when the visual environment is artificially controlled. Our study shows that VOR gain and phase shift develop at different rates as well, suggesting that gain and phase can be adjusted independently in a variety of situations.

Development of the VOR in a strobe environment

Chickens reared from hatch in the strobe environment did not develop a normal VOR. Strobe-rearing affected both the h- and vVOR at all frequencies of oscillation but especially at lower frequencies. The gain of the VOR at 0.1 Hz was near zero in strobe-reared chickens at each age tested (Fig. 4). These low gains prevented us from measuring phase shifts reliably. Generally, the effects of strobe-rearing were not as robust at 0.8 Hz, although VOR gains were still significantly lower in strobe-reared chickens on most test days (Fig. 5, *A* and *C*). At this frequency, h- and vVOR phase shifts did not develop, i.e., they remained unchanged for the entire 40 days of the experiment (Fig. 5, *B* and *D*).

The frequency dependence of the effects of strobe-rearing suggests a symbiosis with the frequency characteristics of the developing optokinetic response (OKR). At 0.1 Hz, the OKR has a higher gain than the VOR in both young and older chickens (Wallman et al. 1982) and thus contributes more to compensatory eye movements in a visual environment at this frequency. At 0.8 Hz, OKR gain is <0.1 in adult chickens (Wallman et al. 1982) and therefore contributes little to compensatory eye movements. Therefore depriving chickens of smoothly moving visual input affects the VOR more at frequencies where visual following generated by the OKR would normally aid gaze stabilization.

The effects of altered visual environments, either strobe- or dark-rearing, on both the h- and the vVOR have been examined

in several other species. Dark-rearing until either 3 or 7 mo of age in rabbits (Collewijn 1977; Favilla et al. 1984a,b) or for 11–15 mo of age in cats (Harris and Cynader 1981) reduced VOR gain by about half, on average, although phase shifts were close to normal. Similarly, strobe-reared cats had a significantly lower VOR gain after 14 mo of strobe-rearing (Kennedy et al. 1982). In congenitally blind adult humans, the VOR is completely absent (Kömpf and Piper 1987; Sherman and Keller 1986). Those studies showed that a normal visual environment is necessary for the development of the VOR. Here, we show that deprivation of visual slip has both immediate and late effects on VOR development. Horizontal VOR gain in strobe-reared chicks was significantly lower than that of normal-light-reared chicks after only 2 days in the strobe environment (Figs. 4*A* and 5*A*). On the other hand, the effects of strobe-rearing on the VOR phase shift were not apparent until 25–40 dph (Fig. 5, *B* and *D*).

Rescue of the strobe-reared VOR gain by brief exposure to continuous light

After strobe-rearing, exposure to normal light for 3 days drove both the gain and phase shift of the VOR toward normal values. However, the gains of both the h- and vVOR in these chickens were still significantly lower than in normal-light-reared chickens (Fig. 6, *A* and *C*) although the difference was less for the vVOR. In contrast, the phase shift of the v- and hVORs essentially recovered completely. These data provide additional support for our earlier suggestion that the gain and phase (direction) of the VOR can be adjusted somewhat independently. Because the hVOR phase of cats fitted with reversing prisms returns to normal more rapidly than does hVOR gain ($<$5 vs. $>$30 days) (Melvill Jones and Davies 1979), we may have missed subsequent VOR gain changes by leaving our strobe-reared birds in normal light for only 3 days.

Some recovery of VOR function by exposure to normal light after dark- or strobe-rearing has been demonstrated in mammals. Dark-reared rabbits recover some (Collewijn 1977) or all (Favilla et al. 1984a) VOR function after normal light exposure. However, dark-reared (Harris and Cynader 1981) and strobe-reared (Kennedy et al. 1982) cats fail to develop a normal VOR even after 5 mo in normal light. This suggests that cats have some critical period when visual slip is crucial for VOR development.

Our results suggest that if there is a critical period for normal VOR development in the chicken, there may be different critical periods for gain and phase. Since VOR phase recovered completely after 40-dph strobe-reared chickens were exposed to normal light for only a few days, the critical period for VOR phase must extend past 40 days, if one exists at all. Since VOR gain in these birds showed some improvement, but was still significantly lower than in normal-light-reared chickens, the critical period for VOR gain may be close to 40 days.

Recovery from streptomycin treatment

As shown in our previous studies, streptomycin effectively eliminates the hVOR immediately after treatment. We show here that the vVOR is similarly compromised. After 8 wk of recovery under normal light, the hVOR recovered completely (Fig. 8, *A* and *B*) but the vVOR gain was still significantly lower than that of untreated controls (Fig. 8, *C* and *D*).

What could account for the differential recovery of the h- and vVOR? One possibility is that there isn't sufficient vertical slip to drive vVOR adaptation. If, during recovery, a chicken experienced less slip in the vertical plane than in the horizontal, one might expect to see more recovery in the hVOR than the vVOR. However, chickens that were recovering in normal light made numerous head movements about the roll axis, apparently to direct gaze toward the floor of their cage where the feed was scattered. These head movements would create vertical slip when the VOR gain was recovering. Therefore the difference between h- and vVOR recovery cannot be explained by insufficient visual slip in the vertical plane.

A second possibility is that there is a late recovery of the type of hair cell that may be primarily responsible for the vVOR. After 8 wk of recovery, hair cell regeneration is largely complete, but the density of Type I hair cells still is lower in streptomycin-treated chickens than in age-matched controls (Carey et al. 1996; Goode et al. 1999). These differences are not significant, but it is possible that the vVOR is more dependent on Type I hair cells than is the hVOR. Although this explanation is much more likely than insufficient vertical visual slip, we do not have enough information to accept it conclusively. If it is true, future studies should find that the density of Type I hair cells is correlated more strongly with vVOR gain than with hVOR gain and that the vVOR should be fully recovered when the regeneration of Type I hair cells is complete.

Effects of strobe illumination on hVOR recovery

The major finding of *experiment 2* is that visual slip is necessary for the complete recovery of the hVOR after streptomycin treatment. Both the gain and phase shift of the hVOR were generally lower than normal in strobe-recovered chickens whether they were treated with streptomycin or not (Fig. 9). These data suggest that functional recovery from hair cell damage cannot be explained solely on the basis of neuronal factors such as axon guidance or the ratio of different hair cell types. In retrospect, this is perhaps not so surprising. Complete functional recovery after hair cell damage depends on several events. Hair cells must regenerate and differentiate into either Type I or II (Weisleder et al. 1995). The regenerated hair cells then must make afferent connections with the appropriate fibers of the 8th nerve. These connections must be very specific: Type I hair cells generally should connect with regularly firing afferents and Type II hair cells with irregularly firing afferents. In turn, these afferents must be connected to circuits involved in either the hVOR, vVOR, or VCR. We show here that this complicated process is facilitated by visual slip signals.

Rescue of hVOR gain by normal light

When our streptomycin-treated, strobe-recovered chickens were then exposed to normal light for only 48 h, the gain and phase of the hVOR recovered almost to normal (Fig. 10). This finding is in marked contrast to results in cats and rabbits. Strobe-reared (Kennedy et al. 1982) and dark-reared (Harris and Cynader 1981) cats failed to recover the VOR when exposed to normal light, and dark-reared rabbits had only partial recovery when subsequently exposed to normal light

(Collewijn 1977; Favilla et al. 1984a,b). Apparently, birds and mammals have different adaptation mechanisms. A VOR adaptation mechanism is functional early in the life of the bird. As soon as they hatch, chicks exhibit hVOR gain changes to retinal slip stimuli (Wallman et al. 1982). Our results suggest that this adaptation system remains ready to adjust hVOR gain even after 40 days of deprivation from its relevant error signal.

Effects of strobe illumination on VCR recovery

Although we tested only a small number of animals, our preliminary data indicate that retinal slip does not affect the VCR in the same way as it does the VOR. At frequencies >0.3 Hz, VCR gain in streptomycin-treated/strobe-recovered chickens was identical to that in untreated chickens housed in normal light (Fig. 11). Furthermore these data indicate that, as expected, the vestibular organs were not directly affected by strobe-rearing as they still were able to drive the VCR normally at all frequencies ≥ 0.3 Hz. In fact, at 0.1 Hz, we detected low VCR gains in only two streptomycin-treated, strobe-recovered chickens. Therefore the effects of stroboscopic illumination on the VOR must be strictly visual in nature. These data suggest that some other error signal must help guide recovery of the VCR. Perhaps there is sufficiently accurate information from the neck receptors to reestablish normal VCR gain after streptomycin damage.

General conclusions

The results of these two experiments support the hypothesis that the major factor that determines the status of an improving VOR, whether the improvement occurs during development from hatch or during recovery from hair cell loss, is the visual environment. However, some functional recovery occurs even in the stroboscopic environment. It is possible that during recovery from streptomycin intoxication, regenerating hair cells are soon contacted by the correct nearby afferent terminals, which have retained their central VOR connections. As more hair cells are produced and connect, hVOR gain increases even without proper visual feedback that the VOR is improving. The same argument could be used to explain the partial development of the VOR in the absence of visual slip.

Perhaps visual information *is* being used in our strobe environment but only at the fastest natural head velocities. It is possible (though unlikely) that very rapid head movements during the brief flash of the strobe create some retinal slip, which accounts for the relatively more normal hVOR behavior at high frequencies.

In any case, it appears that without any useful visual feedback, both the recovering and developing hVOR progress (or revert) to the same default gain and phase shift values regardless of age. When appropriate visual feedback is reintroduced, however, both the developing and recovering VOR still are capable of further improvement.

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Present address of C. T. Goode and D. L. Maney: Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218.

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