Rod Sensitivity of Neonatal Mouse and Rat

Dong-Gen Luo[1] and King-Wai Yau[1,2]

[1]Department of Neuroscience and [2]Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

We have measured the sensitivity of rod photoreceptors isolated from overnight dark–adapted mice of age P12 (neonate) through P45 (adult) with suction-pipette recording. During this age period, the dark current increased roughly in direct proportion to the length of the rod outer segment. In the same period, the flash sensitivity of rods (reciprocal of the half-saturating flash intensity) increased by ∼1.5-fold. This slight developmental change in sensitivity was not accentuated by dark adapting the animal for just 1 h or by increasing the ambient luminance by sixfold during the prior light exposure. The same small, age-dependent change in rod sensitivity was found with rat. After preincubation of the isolated retina with 9-cis-retinal, neonatal mouse rods showed the same sensitivity as adult rods, suggesting the presence of a small amount of free opsin being responsible for their lower sensitivity. The sensitivity of neonate rods could also be increased to the adult level by dark adapting the animal continuously for several days. By comparing the sensitivity of neonate rods in darkness to that of adult rods after light bleaches, we estimated that ∼1% of rod opsin in neonatal mouse was devoid of chromophore even after overnight dark adaptation. Overall, we were unable to confirm a previous report that a 50-fold difference in rod sensitivity existed between neonatal and adult rats.

INTRODUCTION

The b-wave of the dark-adapted electroretinogram increases 50–100-fold in photosensitivity (defined as reciprocal of the half-saturating flash intensity) from 12 d postnatal (P12) to 18 d postnatal (P18) in rat (Dowling and Sidman, 1962; Fulton and Graves, 1980). This developmental change in sensitivity may reside in the rod photoreceptors or in the transmission to bipolar cells. Subsequently, Ratto et al. (1991) reported that the sensitivity of rods isolated from neonatal rat retina was ∼50 times lower than that of adult rods, and that this low neonatal rod sensitivity could be restored to the adult level by exogenous chromophore. This work therefore suggested that the low sensitivity of the rod pathway in neonates resulted from a low sensitivity of the rods themselves, and that this low rod sensitivity arose from insufficient endogenous chromophore so that a substantial fraction of the rhodopsin existed as free opsin (i.e., devoid of chromophore) even under dark-adapted conditions.

Interestingly, Ratto et al. (1991) did not observe a smaller single-photon response (which reflects phototransduction gain) in neonatal rat rods, and attributed essentially all of the 50-fold lower rod sensitivity to a decrease in photon capture. These findings are surprising because they imply that 98% of all neonatal rod opsin had no chromophore, yet the presence of the free opsin did not reduce the phototransduction gain. In experiments by others, the phototransduction gain was strongly decreased by the presence of free opsin whether produced by bleaching light, a genetic deficiency in chromophore regeneration, or the removal of chromophore from pigment in darkness (Cornwall and Fain, 1994; Van Hooser et al., 2002; Fan et al., 2005; Kefalov et al., 2005).

Intrigued by the seemingly anomalous behavior of the neonatal rat rods, we have reexamined this question. Much to our surprise, we found only a 1.5-fold difference in flash sensitivity between neonatal and adult rods. The basis for the difference between our results and those of Ratto et al. (1991) is not clear.

MATERIALS AND METHODS

Preparation

Pigmented mice (C57BL/6) were used for most experiments, but some albino rats (Sprague Dawley) were also studied for comparison. The animals were housed in the animal quarters of the Johns Hopkins University School of Medicine under 14-h light/10-h dark cycles (7 a.m.–9 p.m. light and 9 p.m.–7 a.m. dark). The light was from regular ceiling fluorescent tubes, measured to have an average white light level of ∼11 lux (lumens m^{-2}) at the location of the animal cages. For most experiments, which involved dark adaptation for 14 h immediately before experimentation, an animal kept in the above light/dark cycle for at least 4–6 d was removed from the animal room and dark adapted overnight (6 p.m.–8 a.m.) in the experimental room, and then killed by CO_2 asphyxiation under dim red light, and the eyes removed. For experiments involving only 1 h dark adaptation before experimentation, the animals were photoentrained to an 8 a.m.–6 p.m. dark/6 p.m.–8 a.m. light cycle for 4–6 d in the experimental room, and then dark adapted for just 1 h (8 a.m.–9 a.m.) before experiments.

Under infrared light, the eyes were hemisected, and the retinae removed, cut into several small pieces, and stored in dark-

ness up to 4–6 h in L-15 medium (GIBCO BRL) supplemented with 10 mM glucose and 0.1 mg/ml BSA (Sigma-Aldrich) on ice. When needed, a retinal piece was chopped with a razor blade under chilled L-15 medium on a Sylgard-coated surface. The retinal fragments were transferred to the recording chamber, allowed to settle, and perfused with bicarbonate-buffered Locke's solution: 112.5 mM NaCl, 3.6 mM KCl, 2.4 mM $MgCl_2$, 1.2 mM $CaCl_2$, 10 mM HEPES (pH 7.4), 0.02 mM EDTA, 20 mM $NaHCO_3$, 3 mM Na_2-succinate, 0.5 mM Na-glutamate, 10 mM glucose, 0.1% vitamins (Sigma-Aldrich), and 0.1% amino-acid supplement (Sigma-Aldrich), bubbled with 95% O_2/5% CO_2. The perfusion solution was heated to 37–38°C with a resistor heater (Reisert and Matthews, 2001). The temperature of the solution was monitored continuously with a tele-thermometer situated within 200 μm from the recorded cell.

Electrical Recordings and Light Stimulation

A rod outer segment protruding from a retinal fragment was drawn into a snug-fitting glass suction electrode containing 140 mM NaCl, 3.6 mM KCl, 2.4 mM $MgCl_2$, 1.2 mM $CaCl_2$, 3 mM HEPES (pH 7.4), 0.02 mM EDTA, and 10 mM glucose. Single-cell recordings were done as previously described (Yang et al., 1999). Membrane current was measured with a current-to-voltage amplifier (Axopatch 200B; Axon Instruments). All signals were low-pass filtered at 20 Hz (8-pole Bessel) and sampled at 500 Hz.

Brief flashes (10 ms) of 500-nm light were delivered at 8-s intervals. The effective collecting area, A_e, of an outer segment for incident light approximately perpendicular to the longitudinal axis of the outer segment is given by $A_e = 2.303\pi d^2 lQ\alpha f/4$ (Baylor et al., 1979), where d and l are the diameter and length of the rod outer segment, respectively, Q is the quantum efficiency of isomerization, α is the transverse specific optical density of the outer segment, and f is a factor that depends on the polarization of the incident light. For unpolarized light, f is 0.5 (Baylor et al., 1979). We have adopted $\alpha = 0.016$ μm^{-1} at λ_{max} and Q = 0.67. An outer segment diameter of 1.4 μm was adopted throughout (Carter-Dawson and LaVail, 1979; see also Ratto et al., 1991). With mouse rods being so tiny, it was difficult to measure l reliably during the experiment. Accordingly, we simply used the age-dependent measurements published by LaVail (1973).

9-cis-retinal Application

A stock solution was prepared by adding just-sufficient ethanol to dissolve a small amount (microgram-range) of the 9-cis-retinal crystals. The chromophore concentration in this stock solution was determined by spectrophotometry after a 2,000-fold dilution in ethanol, using a molar extinction coefficient for 9-cis-retinal of 36,100 M^{-1} cm^{-1} at λ_{max} (Morton, 1972). 25-μl aliquots of the solution were placed in individual vials, dried under a gentle stream of nitrogen, capped, and stored in darkness at −80°C to be used within 1–2 wk. When needed, an aliquot was dissolved in minimal ethanol and diluted with normal Ringer to give a final chromophore concentration of 35 μM (final ethanol concentration was <0.1% vol/vol) (Cornwall et al., 2000). A piece of retina was incubated for ~10 min in this solution in darkness, after which it was transferred into 0.4 ml of normal Ringer and finely chopped as previously described.

Data Analysis

The relation between the peak amplitude of the flash response and flash intensity was fit with the exponential saturation function, $r/r_{max} = 1 - \exp(-\gamma I_F)$, where r is peak response amplitude, r_{max} is maximum peak response amplitude, γ is a constant proportional to the flash sensitivity of the cell, and I_F is the flash strength. We take the half-saturating flash intensity ($\sigma_F = ln2/\gamma$)

as the indicator of rod sensitivity, a parameter inversely proportional to σ_F.

The single-photon response was calculated in two ways (Baylor et al., 1979). The first was to use the response ensemble variance-to-mean ratio (σ^2/μ) obtained from a series of 60 identical dim flashes delivered to the cell. The second way was to divide the mean response amplitude, μ, to a dim flash by the mean number of photoisomerizations, ϕ. ϕ was calculated by multiplying the flash intensity with the effective collecting area, A_e, of the outer segment at a given age (see above).

In the bleaching experiment, with a bleaching light step of intensity I_S (photons μm^{-2} s^{-1} at 500 nm) and duration T seconds, the fractional bleach of the rod outer segment is given by $1 - \exp(-2.303Q\alpha f I_S T/\rho N_{av})$, where ρ is pigment density in the rod outer segment and N_{av} is Avogadro's number. With a ρ of ~3.5 mM (Harosi, 1975) and the above Q, α, and f values, the fractional bleach is $1 - \exp(-5.9 \times 10^{-9}I_S T)$. For small bleaches (<15%), this reduces to $5.9 \times 10^{-9}I_S T$.

The total change in sensitivity at a given fractional level of free opsin, P, due to bleaching can be calculated from the following equation (Jones et al., 1996; Xiong and Yau, 2002):

$$\frac{S_F}{S_F^D} = \frac{(1-P)}{(1+kP)}, \tag{1}$$

where S_F is the sensitivity for a given P, S_F^D is the sensitivity for $P = 0$, and k is a constant. In this model, the desensitization due to a light bleach is caused by a combination of reduction in photon catch (numerator) and an additional factor that increases linearly with free opsin (denominator), the latter arising from a weak ability of free opsin to activate phototransduction (Cornwall and Fain, 1994; Jones et al., 1996; Xiong and Yau, 2002).

RESULTS

Physical Dimensions, Dark Current, and Sensitivity of Mouse Rods during Development

While the mouse rod outer segment changes little in diameter during development, it elongates at an almost linear rate from P11 to P17, reaching adult length by P19–25 (LaVail, 1973). Thus, the surface area of the outer segment simply increases in direct proportion to its length. P12 was before eye opening, but we found P12 rods to be light sensitive, although the dark current was rather small (~5 pA or less; Fig. 1). Thereafter, the dark current increased steadily until reaching a maximum at around P20. As shown in Fig. 1, the increase in dark current with age coincided quite well with the growth of the outer segment length (but see Ratto et al., 1991). Thus, it appeared that the density of cyclic GMP-gated channels per unit area of the plasma membrane remained constant through the developmental stages.
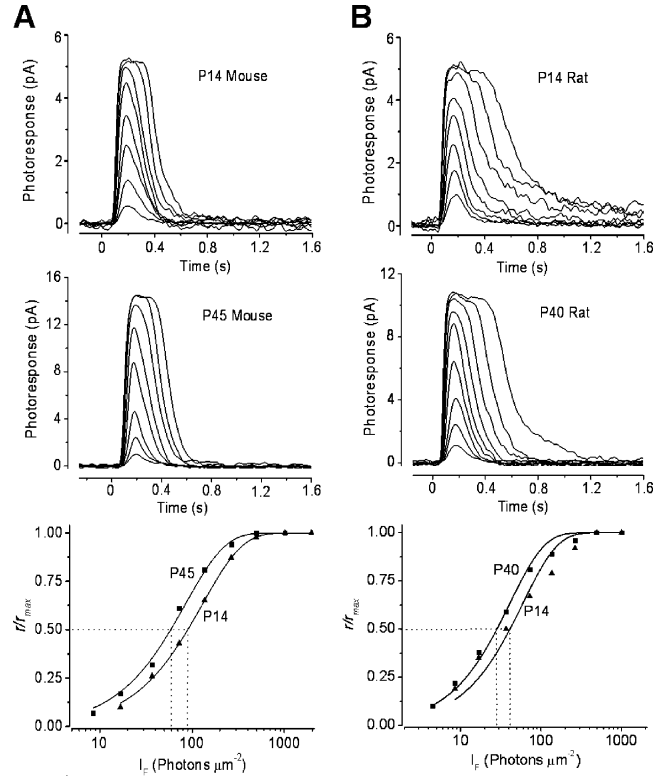
To measure the sensitivity of rods in darkness, we used mice that had been kept in 14/10 h light/dark cycles for 4–6 d before being dark adapted for 14 h before experiment (see MATERIALS AND METHODS). In contrast to the dramatic increase in rod sensitivity associated with rat development as previously reported by others (Ratto et al., 1991), we found only a small in-
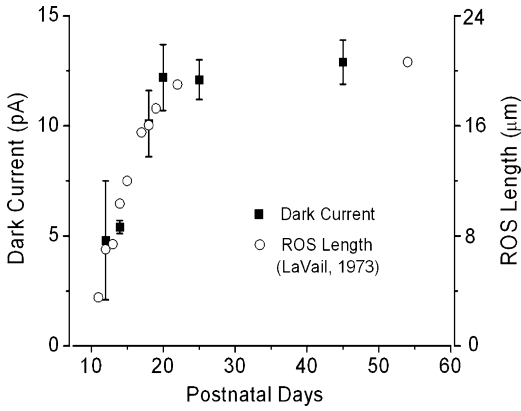
Figure 1. Dark-current amplitude and length of rod outer segment (ROS) at different developmental stages of mouse. The error bars indicate SEM. For the measurements on dark current (equivalent to the maximum photoresponse), the numbers of rods studied at each stage were: 4(P12), 6(P14), 8(P18), 5(P20), 5(P25), and 14(P45). The ROS length data are from LaVail (1973).


Figure 2. Comparison between neonatal and adult rod flash sensitivities. A, mouse; B, rat. (A, top and middle panels) Flash response families from P14 and P45 mouse rods. Rods were overnight dark adapted (14 h). Flash delivered at time 0, with intensities of 16.8, 36.8, 72.6, 136.6, 266.2, 493.9, 1010.8, 1956.6 photons μm^{-2}, respectively, for the P14 rod, and 8.5, 16.8, 36.8, 72.6, 136.6, 266.2, 493.9, 1010.8 photons μm^{-2}, respectively, for the P45 rod. Bottom panel shows the intensity–response relations, with the fitted solid curves drawn from a saturating exponential function (see MATERIALS AND METHODS) with a half-saturating intensity, σ_F, of 89.8 photons μm^{-2} (P14) and 58.7 photons μm^{-2} (P45), respectively. (B, top and middle panels) Flash response families from P14 and P40 rat rods. Same dark-adaptation conditions as in A. Flash intensities of 8.5, 16.8, 36.8, 72.6, 136.6, 266.2, 493.9, 1010.8 photons μm^{-2}, respectively for both cells, plus 4.5 photons μm^{-2} for the P40 rod. The solid curves in bottom panel are drawn with σ_F of 41.1 photons μm^{-2} (P14) and 28.3 photons μm^{-2} (P40), respectively.

crease in sensitivity from P12 to P45. In Fig. 2 A, flash response families derived from P14 and P45 mouse rods were compared. In both cases, the intensity–response relation fit well to a saturating exponential curve (see MATERIALS AND METHODS), with a half-saturating flash intensity (σ_F) of 89.8 photons μm^{-2} for the P14 rod and 58.7 photons μm^{-2} for the P45 rod. Collected results gave σ_F values of 77.3 ± 14.2 (mean ± SEM, $n = 6$) and 52.7 ± 3.4 photons μm^{-2} ($n = 14$) for P14 and adult rods, respectively. Thus, the flash sensitivity increased by only 1.5-fold. The complete data for σ_F at different ages are shown in Fig. 3 A. In Fig. 3 B, the single-photon response amplitudes at different ages are plotted, calculated from either the response ensemble variance-to-mean ratio (σ^2/μ) or the mean dim-flash response amplitude divided by the number of photoisomerizations (μ/ϕ). The two calculated values broadly overlapped. The perhaps smaller value derived with the second method may reflect the fact that the rod outer segment was not necessarily entirely inside the suction pipette during recording (so not all of the dark current was recorded), but the effective collecting area, A_e, was calculated from the entire rod outer segment (See MATERIALS AND METHODS). Either way, the single-photon response showed the trend of the rod as revealed by σ_F, namely, a small increase in sensitivity with age, by about twofold from P14 to P45.

The kinetics of the dim-flash response changed very little during development: from 206 ± 8 ms at P14 to 217 ± 11 ms at P45 for the time-to-peak, and from 282 ± 40 ms at P14 to 335 ± 40 ms at P45 for the integration time ($n = 6$ and 14, respectively). Interestingly, the kinetics was slowest at P12, with a flash response time-to-peak of 273 ± 14 ms ($n = 4$, unpublished data).

Complete data on the response kinetics from P14 to P45 at different mouse ages are shown in Fig. 3 C.

Lack of Effect of Decreasing the Dark-adaptation Period or Increasing the Light Intensity during the Light Period

In their experiments, Ratto et al. (1991) dark adapted the animals for at least 1 h. Accordingly, we repeated our experiments with mice dark adapted for just 1 h (see MATERIALS AND METHODS for exact timings). Nonetheless, this much shorter dark-adaptation period did not further reduce the sensitivity of P14 rods ($\sigma_F = 74.7 \pm 14.0$ photons μm^{-2}, $n = 7$). To check for any effect of the luminance level during prior light exposure, we also increased the ambient light level in
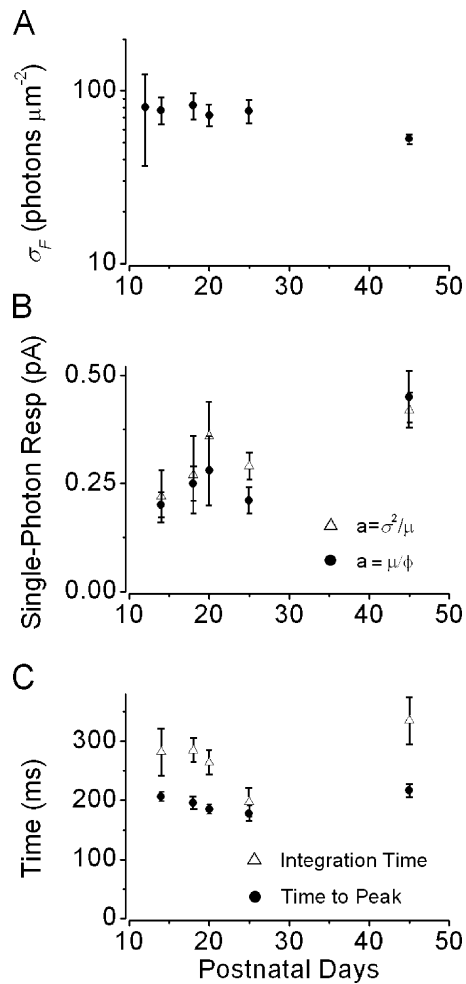
Figure 3. Changes in sensitivity, single-photon response, and response kinetics of mouse rods during development. Data are averaged, with SEM shown. (A) Half-saturating flash intensity (reciprocally related to sensitivity) at different developmental stages. (B) Change in the single-photon response amplitude, a, during development. See MATERIALS AND METHODS for calculations. (C) Changes in the kinetics of the dim-flash response during development. The integration time is defined as $\int f(t)\,dt/f_p$, where $f(t)$ is the dim-flash response waveform and f_p is the amplitude of $f(t)$ at time-to-peak. The number of recorded rods at each stage was 6(P14), 8(P18), 5(P20), 5(P25), 14(P45), respectively, in A, B, and C, plus 4(P12) in A.

which the animals were kept during the light period from 11 lux (for all of the above experiments) to 64 lux by positioning the animal cages much closer to the ceiling fluorescence lights for at least 4–6 d. This manipulation, when coupled to just 1-h dark adaptation, decreased sensitivity only slightly ($\sigma_F = 88.5 \pm 8.4$ photons μm^{-2}, $n = 9$). Incidentally, Ratto et al. (1991) reported keeping their animals in a luminance of only 7 lux. Thus, the discrepancy between our findings and theirs could not have resulted from a difference in the duration of dark adaptation or in the luminance level during the light period.

Experiments with Rats

The large discrepancy between our findings and those reported by Ratto et al. (1991) could reflect a species difference because the previous work was on rat. Accordingly, we also compared the sensitivities of rods from neonatal (P14) and adult (P40) rats after 1 h dark adaptation, with the same experimental procedures as for mice (11 lux). Again, we found only a small increase in rod sensitivity from neonate to adult (Fig. 2 B). Overall, from P14 to P40, the dark current increased from 5.6 ± 0.7 pA to 9.4 ± 0.6 pA, σ_F decreased from 41.2 ± 4.6 photons μm^{-2} to 33.8 ± 2.5 photons μm^{-2} (1.2-fold change), the single-photon response increased from 0.16 ± 0.05 pA to 0.33 ± 0.03 pA, the time-to-peak of the dim-flash response increased from 211 ± 8 ms to 252 ± 13 ms, and the response integration time from 273 ± 8 ms to 306 ± 21 ms ($n = 7$ and 9, respectively). Thus, the findings on mouse and rat were similar.

Effect of Exogenous Chromophore

Based on what Ratto et al. (1991) reported and also what we found in *Xenopus* tadpoles (Xiong and Yau, 2002), we asked whether the slightly lower sensitivity of neonatal rodent rods was due to the presence of free opsin. Accordingly, we preincubated the isolated mouse retina with 9-cis-retinal (used instead of the native 11-cis-retinal because it is inexpensive and readily available) in darkness before recording from single rods (see MATERIALS AND METHODS). Fig. 4 shows that preincubation with 9-cis-retinal increased the flash sensitivity of P14 mouse rods to the level of adult rods, confirming the presence of free opsin. In contrast, the same preincubation with 9-cis-retinal did not affect the sensitivity of adult rods. 9-cis-rhodopsin has a λ_{max} of $\sim$480 nm (Fukada et al., 1990; Kefalov et al., 2005), versus 500 nm for 11-cis-rhodopsin. The two pigments also have different extinction coefficients and quantum efficiencies of photoisomerization (Hubbard and Kropf, 1958). However, these differences should not have affected our measurements because the amount of free opsin was so minimal ($\sim$1%, see below).

Effect of Prolonged Dark Adaptation

In *Xenopus*, we found that the low sensitivity of rods during tadpole development could be alleviated by prolonged dark adaptation (Xiong and Yau, 2002). We found the same with mouse rods. Thus, dark adapting neonatal mice continuously for 6 d (from P8 to P14) before recordings decreased the value of σ_F from 77.3 ± 14.2 photons μm^{-2} ($n = 7$) to 47.0 ± 5.0 photons μm^{-2} ($n = 7$). The σ_F of adult rods was hardly affected by this procedure (57.9 ± 5.8 photons μm^{-2}, $n = 8$) (Fig. 4). Thus, the free opsin in neonatal animals appeared to result from a very slow regeneration of chromophore.
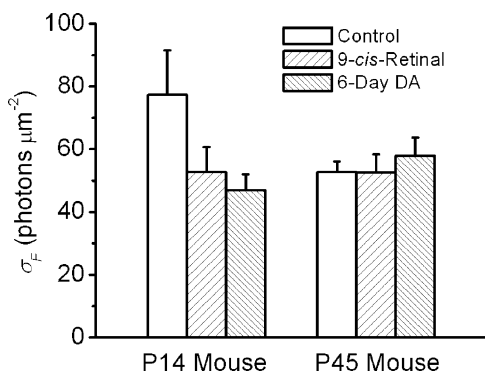
Figure 4. Effect of exogenous 9-cis-retinal or prolonged (6-d) dark adaptation (DA) on rod sensitivity of P14 and P45 mice. Average ± SEM. The σ_F values for P14 rods with control, 9-cis-retinal, and 6-d DA were, respectively, 77.3 ± 14.2 ($n = 6$), 52.7 ± 8.0 (7), and 47.0 ± 5.0 (7) photons μm^{-2}. For P45 rods, the σ_F values were, respectively, 52.7 ± 3.4 ($n = 14$), 52.6 ± 5.8 (8), and 57.9 ± 3.6 (8) photons μm^{-2}. The time-to-peak of the dim flash response was 220 ± 11 ms (9-cis), 233 ± 11 ms (6-day DA) for P14 mice, and 211 ± 13 ms (9-cis), 224 ± 9 ms (6-day DA) for P45 mice. Control time-to-peak was 206 ± 8 ms (P14) and 217 ± 11 ms (P45) (see first section in RESULTS).

Percentage of Free Opsin in Neonatal Mouse

How much free opsin was present in neonatal mouse rods? We estimated this percentage in P14 mouse rods by comparing their lower sensitivity to the desensitization produced by bleaching light in adult rods. We bleached different fractions of the pigment in adult rods and measured the change in flash sensitivity after the dark current reached steady state. Fig. 5 shows the data from seven rods (with each cell giving a single measurement). The two smooth curves bracketing the data are drawn from Eq. 1 with $k = 22$ and 40, respectively. Taking these data as a standard, a sensitivity decrease of 1.5-fold would correspond to ∼1% free opsin. Thus, we estimated that 99% of rhodopsin had chromophore in the neonatal mouse.

DISCUSSION

Our findings differ from those of Ratto et al. (1991), who found that rod sensitivity was 50-fold lower in neonatal rat than in adult. Instead, we found the difference to be only 1.5-fold (in terms of the reciprocal of the half-saturating flash intensity) in mouse, and even less in rat. The slightly lower sensitivity in neonatal mouse rods could be restored to adult level by exogenously applied chromophore or by dark adaptation of the animal for a prolonged period (several days). Thus, neonates appeared to be less efficient than adults in regenerating their rhodopsin, perhaps because of an immature retinoid turnover system in the retinal pigment epithelium. The percentage of free opsin, i.e., without chromophore, was nonetheless quite small,

only ∼1%, with its constitutive activity leading to a 1.5-fold desensitization of the rods. Dodge et al. (1996) also failed to detect any significant free opsin in neonatal rat rods, based on comparing light absorption by extracted rhodopsin in the absence and presence of exogenous chromophore. The same group also reported detecting a ≥2-fold lower density of rhodopsin in immature rods (see their Fig. 1). We have not measured this parameter directly, but we found that the single-photon response amplitudes derived separately from variance analysis and from the effective collecting area matched each other moderately well at each stage in development (see Fig. 3 B). Thus, there did not appear to be any significant change in α (the specific optical density of the outer segment), reflecting little change in rhodopsin density during development.

The large difference between our results and those of Ratto et al. (1991) is puzzling. We failed to duplicate their findings with mouse or rat, pigmented or albino animals (according to Ratto et al., the neonates of pigmented and albino rats showed similar, substantial desensitization), and short (1-h) or long (14-h) dark-adaptation periods. Even with a 10-fold higher luminance during the prior light period (up to 64 lux in our experiments versus 7 lux used by Ratto et al.) in a deliberate attempt to increase rhodopsin bleaching, we were unable to substantially accentuate the sensitivity difference between neonate and adult rods. One experimental condition incompletely specified by Ratto et al. is the period of dark adaptation, which they described only as "1 h or longer". Nonetheless, the fact that we found the same results with 1-h and 14-h dark adaptations should rule out this uncertainty as a factor. We are left with two remaining possibilities: the genetic line of the experimental animals and the rodent diet. The rats used by Ratto et al. (1991) were albino CHFB and pigmented Lister Hooded strains. As for diet, it is conceivable that vitamin A, the precursor of 11-cis-retinal, was for some reason deficient in the neonates used by Ratto et al. (1991), although these authors have specified the amount of vitamin A in the diet.

An additional unexplained point in the results of Ratto et al. (1991) is that the single-photon response retained an essentially normal amplitude despite the presence of a large amount of free opsin. Normally, free opsin reduces the amplification of phototransduction predominantly owing to its constitutive activity instead of by simply lowering the probability of photon capture (Cornwall and Fain, 1994; Van Hooser et al., 2002; Fan et al., 2005; Kefalov et al., 2005). This point can be appreciated from Fig. 5 inset, which shows the extrapolations of the curves in Fig. 5 to higher bleaches. For the present purpose, it is unimportant whether these extrapolations are precise or not (see Jones et al., 1996). Rather, the key point is that, even at
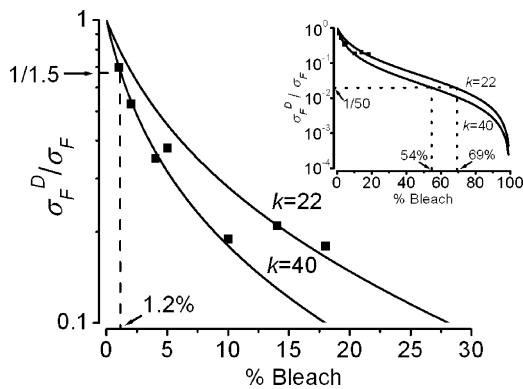
Figure 5. Estimation of percentage free opsin in neonatal mouse rods. The ratio between post-bleach and pre-bleach flash sensitivities in adult (P45 or older) mouse, represented by σ_F^D/σ_F, is plotted against the percentage of bleached pigment. Seven rods, each providing a single measurement (i.e., flash sensitivity after a single bleach). Continuous curves are Eq. 1 with $k = 22$ and $k = 40$, respectively, which bracket the scatter in the data. The ratio of 1/1.5, representing the "equivalent bleach" situation in neonates, is used for estimating the percentage of free opsin in P14 mouse rods, giving 1.2%. (Inset) The bleaching curves extended to the entire bleaching range. A 50-fold decrease in rod sensitivity requires only 54% ($k = 40$) to 69% ($k = 22$) of rhodopsin bleach.

just 50% bleach (corresponding to a reduction in the probability of photon capture by only half), the overall decrease in sensitivity is already 50-fold, due largely to a decrease in the phototransduction amplification mentioned above.

Previously, Fulton and Graves (1980) have shown that the a-wave of the electroretinogram (a reflection of rod response) from dark-adapted rats (24 h dark-adaptation, albino animals) showed little change in sensitivity (defined as reciprocal of the half-saturating flash intensity) from P12 to adulthood. This result is therefore in rough agreement with what we report here. Fulton and Graves found that the b-wave of the dark-adapted electroretinogram, a reflection of the light response of rod-bipolar cells, shows a 50–100-fold increase in sensitivity with age under the same experimental conditions. Thus, most of this increase in sensitivity during development appears to come from changes downstream of the rod photoreceptor, such as in synaptic maturation (Fisher, 1979; Feller, 2003). During the same period, the maximum amplitude of the b-wave increases by about fourfold (see Fig. 3 A in Fulton and Graves, 1980). At least part of this increase should arise from a doubling of the rod's dark current as a result of the increase in its outer segment length (Fig. 1). This increase in dark current enhances the dark release of glutamate and therefore, presumably, the maximum depolarizing response from the rod-bipolar cell triggered by light.

In human, there is likewise a large increase (50-fold) in dark-adapted visual sensitivity from 1 mo old on,

based on psychological testing (Powers et al., 1981). Suggestions for the underlying reasons in this developmental change have ranged from optical factors in the eye to elements postsynaptic to the photoreceptors (Hamer and Schneck, 1984; Brown, 1986, 1990; Banks and Bennett, 1988). However, only a small part of this change (about threefold) appears to originate from developmental changes in the photoreceptor themselves, such as photon capture and phototransduction (Nusinowitz et al., 1998). Thus, the overall picture may be qualitatively similar between rodents and primates.

REFERENCES

Baylor, D.A., T.D. Lamb, and K.-W. Yau. 1979. Responses of retinal rods to single photons. *J. Physiol.* 288:613–634.

Banks, M.S., and P.J. Bennett. 1988. Optical and photoreceptor immaturities limit the spatial and chromatic vision of human neonates. *J. Opt. Soc. Am. A.* 5:2059–2079.

Brown, A.M. 1986. Scotopic sensitivity of the two-month-old human infant. *Vision Res.* 26:707–710.

Brown, A.M. 1990. Development of visual sensitivity to light and color vision in human infants: a critical review. *Vision Res.* 30:1159–1188.

Carter-Dawson, L.D., and M.M. LaVail. 1979. Rods and cones in the mouse retina. I. Structural analysis using light and electron microscopy. *J. Comp. Neurol.* 188:245–262.

Cornwall, M.C., and G.L. Fain. 1994. Bleached pigment activates transduction in isolated rods of the salamander retina. *J. Physiol.* 480:261–279.

Cornwall, M.C., G.J. Jones, V.J. Kefalov, G.L. Fain, and H.R. Matthews. 2000. Electrophysiological methods for measurement of activation of phototransduction by bleached visual pigment in salamander photoreceptors. *Methods Enzymol.* 316:224–252.

Dodge, J., A.B. Fulton, C. Parker, R.M. Hansen, and T.P. Williams. 1996. Rhodopsin in immature rod outer segments. *Invest. Ophthalmol. Vis. Sci.* 37:1951–1956.

Dowling, J.E., and R.L. Sidman. 1962. Inherited retinal dystrophy in the rat. *J. Cell Biol.* 14:73–109.

Fan, J., M.L. Woodruff, M.C. Cilluffo, R.K. Crouch, and G.L. Fain. 2005. Opsin activation of transduction in the rods of dark-reared RPE65 knockout mice. *J. Physiol.* 10.1113/jphysiol.2005.091942.

Feller, M.B. 2003. Visual system plasticity begins in the retina. *Neuron.* 39:3–4.

Fisher, L.J. 1979. Development of synaptic arrays in the inner plexiform layer of neonatal mouse retina. *J. Comp. Neurol.* 187:359–372.

Fukada, Y., T. Okano, Y. Shichida, T. Yoshizawa, A. Trehan, D. Mead, M. Denny, A.E. Asato, and R.S.H. Liu. 1990. Comparative study on the chromophore binding sites of rod and red-sensitive cone visual pigments by use of synthetic retinal isomers and analogues. *Biochemistry.* 29:3133–3140.

Fulton, A.B., and A.L. Graves. 1980. Background adaptation in developing rat retina: an electroretinographic study. *Vision Res.* 20:

819–826.

Hamer, R.D., and M.E. Schneck. 1984. Spatial summation in dark-adapted human infants. *Vision Res.* 24:77–85.

Harosi, F.I. 1975. Absorption spectra and linear dichroism of some amphibian photoreceptors. *J. Gen. Physiol.* 66:357–382.

Hubbard, R., and A. Kropf. 1958. The action of light on rhodopsin. *Proc. Natl. Acad. Sci. USA.* 44:130–139.

Jones, G.J., M.C. Cornwall, and G.L. Fain. 1996. Equivalence of background and bleaching desensitization in isolated rod photoreceptors of the larval tiger salamander. *J. Gen. Physiol.* 108:333–340.

Kefalov, V.J., M. Estevez, M. Kono, P. Goletz, R.K. Crouch, M.C. Cornwall, and K.-W. Yau. 2005. Breaking the covalent bond – a pigment property that contributes to desensitization in cones. *Neuron.* 46:879–890.

LaVail, M.M. 1973. Kinetics of rod outer segment renewal in the developing mouse retina. *J. Cell Biol.* 58:650–661.

Morton, R.A. 1972. The chemistry of the visual pigments. *In* Photochemistry of Vision. H.J.A. Dartnall, editor. Springer-Verlag, Berlin. 33–68.

Nusinowitz, S., D.G. Birch, and E.E. Birch. 1998. Rod photore-sponses in 6-week and 4-month-old human infants. *Vision Res.* 38:627–635.

Powers, M.K., M. Schneck, and D.Y. Teller. 1981. Spectral sensitivity of human infants at absolute visual threshold. *Vision Res.* 21:1005–1016.

Ratto, G.M., D.W. Robinson, B. Yan, and P.A. McNaughton. 1991. Development of the light response in neonatal mammalian rods. *Nature.* 351:654–657.

Reisert, J., and H.R. Matthews. 2001. Response properties of isolated mouse olfactory receptor cells. *J. Physiol.* 530:113–122.

Van Hooser, J.P., Y. Liang, T. Maeda, V. Kuksa, G.-F. Jang, Y.-G. He, F. Rieke, H.K.W. Fong, P.B. Detwiler, and K. Palczewski. 2002. Recovery of visual functions in a mouse model of Leber Congenital Amaurosis. *J. Biol. Chem.* 277:19173–19182.

Xiong, W.-H., and K.-W. Yau. 2002. Rod sensitivity during *Xenopus* development. *J. Gen. Physiol.* 120:817–827.

Yang, R.-B., S.W. Robinson, W.-H. Xiong, K.-W. Yau, D.G. Birch, and D.L. Garbers. 1999. Disruption of a retinal guanylyl cyclase gene leads to cone-specific dystrophy and paradoxical rod behavior. *J. Neurosci.* 19:5889–5897.