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Network-level analysis of light adaptation in rod cells under normal and altered conditions

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Electronic Supplementary Information

Contents:

TABLE S1

Reaction number	Reaction equation	Reaction kinetics	Comments
1	$R \xrightarrow{stimulus} R_0$	$v_f = stimulus \frac{R}{R_{tot}}$	Photoactivation of unphospshorylated R
2	$R_n + RK \underset{kRK_2}{\overset{kRK1_n}{\rightleftharpoons}} R_n \cdot RK_{pre}$ (n = 0, …,6)	$v_f = kRK1_n \times R_n \times RK$ $v_r = kRK_2 \times R_n \cdot RK_{pre}$	Binding of R_n and RK. The association rate constant is assumed to decrease exponentially with increasing phosphorylations: $kRK1_n = kRK1_0 \exp(-\omega n)$
3	$R_n \cdot RK_{pre} \xrightarrow{kRK3_{ATP}} R_{n+1} \cdot RK_{post}$ (n = 0, …,5)	$v_f = kRK3_{ATP} \times R_n \cdot RK_{pre}$	Phosporylation of R_n to give R_{n+1}
4	$R_n \cdot RK_{post} \xrightarrow{kRK4} R_n + RK$ (n = 1, …,6)	$v_f = kRK4 \times R_n \cdot RK_{post}$	Dissociation of the R_n·RK complex
5	$R_n + Arr \underset{kA2}{\overset{kA1_n}{\rightleftharpoons}} R_n \cdot Arr$ (n = 1, …,6)	$v_f = kA1_n \times R_n \times Arr$ $v_r = kA2 \times R_n \cdot Arr$	Binding of R_n and Arr. The association rate constant is assumed to increase exponentially with increasing phosphorylations: $kA1_n = kArr \exp(\omega_{arr} n)$
6	$R_n \cdot Arr \xrightarrow{kA3} Ops + Arr$ (n = 1, …,6)	$v_f = kA3 \times R_n \cdot Arr$	Arr- mediated inactivation of R_n. Ops indicates the inactivated receptor with unbound all-trans retinal
7	$R_n \xrightarrow{ktherm} Ops$ (n = 0, …,6)	$v_f = ktherm \times R_n$	Thermal decay of catalytic active form of R_n to give Ops
8	$Ops + Gt \underset{kG2}{\overset{kOps}{\rightleftharpoons}} Ops \cdot Gt$	$v_f = kOps \times Ops \times Gt$ $v_r = kG2 \times Ops \cdot Gt$	Spontaneous Ops activity
9	$Ops \cdot Gt \underset{kG4_{GDP}}{\overset{kG3}{\rightleftharpoons}} Ops \cdot G$	$v_f = kG3 \times Ops \cdot Gt$ $v_r = kG4_{GDP} \times Ops \cdot G$	GDP dissociation from the Ops·Gt complex
10	$Ops \cdot G \xrightarrow{kG5_{GTP}} Ops \cdot G_{GTP}$	$v_f = kG5_{GTP} \times Ops \cdot G$	GTP binding to the Ops·Gt complex
11	$Ops \cdot G_{GTP} \xrightarrow{kG6} Ops + G_{GTP}$	$v_f = kG6 \times Ops \cdot G_{GTP}$	Dissociation of the Ops·G_{GTP} complex

12	$Ops \xrightarrow{kRrecyc} R$	$v_f = kRrecyc \times Ops$	Chromophore regeneration by 11-cis retinal binding to Ops
13	$R_n + Gt \underset{kG2}{\overset{kG1_n}{\rightleftharpoons}} R_n \cdot Gt$ (n = 0, …,6)	$v_f = kG1_n \times R \times Gt$ $v_r = kG2 \times R_n \cdot Gt$	Binding of R_n and Gt. The association rate constant is assumed to decrease exponentially with increasing phosphorylations: $kG1_n = kG1_0 \exp(-\omega n)$
14	$R_n \cdot Gt \underset{kG4_{GDP}}{\overset{kG3}{\rightleftharpoons}} R_n \cdot G$ (n = 0, …,6)	$v_f = kG3 \times R_n \cdot Gt$ $v_r = kG4_{GDP} \times R_n \cdot G$	GDP dissociation from the R_n·Gt complex
15	$R_n \cdot G \xrightarrow{kG5_{GTP}} R_n \cdot G_{GTP}$ (n = 0, …,6)	$v_f = kG5_{GTP} \times R_n \cdot G$	GTP binding to the R_n·Gt complex
16	$R_n \cdot G_{GTP} \xrightarrow{kG6} R_n + G_{GTP}$ (n = 0, …,6)	$v_f = kG6 \times R_n \cdot G_{GTP}$	Dissociation of the R_n·G_{GTP} complex
17	$G_{GTP} \xrightarrow{kG7} G\alpha_{GTP} + G\beta\gamma$	$v_f = kG7 \times G_{GTP}$	Dissociation of trimeric Gt into α and βγ subunits
18	$PDE + G\alpha_{GTP} \underset{kP1rev}{\overset{kP1}{\rightleftharpoons}} PDE \cdot G\alpha_{GTP}$	$v_f = kP1 \times PDE \times G\alpha_{GTP}$ $(v_r = kP1rev \times PDE \cdot G\alpha_{GTP})$	Binding of $G\alpha_{GTP}$ to one PDE inactive subunit
19	$PDE \cdot G\alpha_{GTP} \xrightarrow{kP2} PDE^* \cdot G\alpha_{GTP}$	$v_f = kP2 \times PDE \cdot G\alpha_{GTP}$	Activation of the PDE·$G\alpha_{GTP}$ complex
20	$PDE^* \cdot G\alpha_{GTP} + G\alpha_{GTP} \xrightarrow{kP3} G\alpha_{GTP} \cdot PDE^* \cdot G\alpha_{GTP}$	$v_f = kP3 \times PDE^* \cdot G\alpha_{GTP} \times G\alpha_{GTP}$	Binding of $G\alpha_{GTP}$ to one PDE active subunit
21	$G\alpha_{GTP} \cdot PDE^* \cdot G\alpha_{GTP} \xrightarrow{kP4} G\alpha_{GTP} \cdot {}^*PDE^* \cdot G\alpha_{GTP}$	$v_f = kP4 \times G\alpha_{GTP} \cdot PDE^* \cdot G\alpha_{GTP}$	Activation of both the $G\alpha_{GTP}$-bound PDE subunits
22	$RGS + G\alpha_{GTP} \cdot {}^*PDE^* \cdot G\alpha_{GTP} \xrightarrow{kRGS1} RGS \cdot G\alpha_{GTP} \cdot {}^*PDE^* \cdot G\alpha_{GTP}$	$v_f = kRGS1 \times RGS \times G\alpha_{GTP} \cdot {}^*PDE^* \cdot G\alpha_{GTP}$	Binding of RGS9 complex to the completely activated PDE tetramer
23	$RGS \cdot G\alpha_{GTP} \cdot {}^*PDE^* \cdot G\alpha_{GTP} \xrightarrow{kRGS2} PDE^* \cdot G\alpha_{GTP} + RGS + G\alpha_{GDP}$	$v_f = kRGS2 \times RGS \cdot G\alpha_{GTP} \cdot {}^*PDE^* \cdot G\alpha_{GTP}$	RGS9-mediated deactivation of one of the two PDE active subunits
24	$RGS + PDE^* \cdot G\alpha_{GTP} \xrightarrow{kRGS1} RGS \cdot PDE^* \cdot G\alpha_{GTP}$	$v_f = kRGS1 \times RGS \times PDE^* \cdot G\alpha_{GTP}$	Binding of RGS9 complex to a PDE tetramer with one active subunit

25	$RGS \cdot PDE^{*} \cdot G\alpha_{GTP} \xrightarrow{kRGS2} PDE + RGS + G\alpha_{GDP}$	$v_f = kRGS2 \times RGS \cdot PDE^{*} \cdot G\alpha_{GTP}$	RGS9-mediated deactivation of the PDE only active subunit
26	$PDE^{*} \cdot G\alpha_{GTP} \xrightarrow{kPDE_{shutoff}} PDE + G\alpha_{GDP}$	$v_f = kPDE_{shutoff} \times PDE^{*} \cdot G\alpha_{GTP}$	Inactivation of the PDE*·$G\alpha_{GTP}$ complex by $G\alpha_{GTP}$'s GTPase activity
27	$G\alpha_{GTP} \cdot {}^{*}PDE^{*} \cdot G\alpha_{GTP} \xrightarrow{kPDE_{shutoff}} PDE^{*} \cdot G\alpha_{GTP} + G\alpha_{GDP}$	$v_f = kPDE_{shutoff} \times G\alpha_{GTP} \cdot {}^{*}PDE^{*} \cdot G\alpha_{GTP}$	Inactivation of one of the two active PDE subunits by $G\alpha_{GTP}$'s GTPase activity
28	$G\alpha_{GTP} \xrightarrow{kGshutoff} G\alpha_{GDP}$	$v_f = kGshutoff \times G\alpha_{GTP}$	$G\alpha_{GTP}$ auto-catalytic GTPase activity
29	$G\alpha_{GDP} + G\beta\gamma \xrightarrow{kGrecyc} Gt$	$v_f = kGrecyc \times G\alpha_{GDP} \times G\beta\gamma$	Reconstitution of Gt heterotrimer from its inactive subunits
30	$RK \underset{kRec4}{\overset{kRec3}{\rightleftharpoons}} Rec \cdot wCa^{2+} \cdot RK$	$v_f = kRec3 \times Rec \cdot wCa^{2+} \times RK$ $v_r = kRec4 \times Rec \cdot wCa^{2+} \cdot RK$	Rec-mediated regulation of RK. Rec·wCa^{2+} is determined under quasy-steady state assumption
31	$Ca^{2+}_{free} \underset{k2}{\overset{k1}{\rightleftharpoons}} Ca^{2+}_{buff}$	$v_f = k1 \times (eT - Ca^{2+}_{buff}) \times Ca^{2+}_{free}$ $v_r = k2 \times Ca^{2+}_{buff}$	Ca^{2+} association and dissociation from intracellular buffers with total concentration eT
32	$Ca^{2+}_{free} \xrightarrow{\gamma Ca}$	$v_f = \gamma Ca \times (Ca^{2+}_{free} - Ca^{2+}_{0})$	Intracellular Ca^{2+}-efflux via the Na^{+}/Ca^{2+} - K^{+} exchanger
33	$\longrightarrow Ca^{2+}_{free}$	$v_f = \frac{10^{6}\ fCa \times J_{dark}}{2 + fCa \times F \times v_{cyto}} \times \left(\frac{cGMP}{cGMP_{dark}} \right)^{n_{cg}}$	Extracellular Ca^{2+}-influx via the cGMP-gated cation channels
34	$\longrightarrow cGMP$	$v_f = \frac{a_{max}}{1 + \left(\frac{Ca^{2+}_{free}}{Kc} \right)^{m}}$	cGMP synthesis by guanylate cyclase
35	$cGMP \longrightarrow$	$v_f = (\beta_{dark} + \beta_{sub} \times E) \times cGMP$	cGMP hydrolysis by PDE

TABLE S2

Parameter	Value	Reaction	Description	Comments	References
flashBG	0	1	# photons in the background flash	See the definition of stimulus	(Hamer *et al*, 2005)
flash0Dur	0.001 s	1	Duration of the pre-flash	See the definition of stimulus	(Hamer *et al*, 2005)
flash0Mag	1	1	# photons in the pre-flash	See the definition of stimulus	(Hamer *et al*, 2005)
flashDelay	0 s	1	time-delay between the pre-flash and the flash	See the definition of stimulus	(Hamer *et al*, 2005)
flashDur	0.001 s	1	Duration of the flash	See the definition of stimulus	(Hamer *et al*, 2005)
flashMag	0	1	Amount of photons in the flash	See the definition of stimulus	(Hamer *et al*, 2005)
otherstimulus	0	1	Additional stimulus to be able to by-pass the flash settings	See the definition of stimulus	-
R_{tot}	3.6e9	1	Total amount of R		(Pugh and Lamb, 2000)
PDE_{tot}	1.335e7	18,20	Total amount of PDE6 tetramers		(Pugh *et al*, 2000)
G_{tot}	3.6e8	13	Total amount of Gt		(Pugh *et al*, 2000)
Arr_{tot}	3.13e7	5	Total amount of Arr		(Hamer *et al*, 2005; Hamm and Bownds, 1986)
Rec_{tot}	35 μM	30	Total concentration of Rec		(Ames *et al*, 1997; Kawamura and Murakami, 1991)

RK_{tot}	7 μM	-	Total concentration of RK	Only used for quasi- steady state assumption (QSSA)	(Klenchin *et al*, 1995)
RGS_{tot}	3e6	22,24	Total amount of RGS9	Value estimated from data in (Pugh *et al*, 2000)	-
kRK1_0	7.643e-3 s^{-1}	2	Rate of binding of RK to unphosphorylated R in the dark	Optimized by parameter estimation	-
ω	0.6	2,13	Exponential rate of decay of Gt and RK affinity for R_n as a function of the number of phosphorylations	Value obtained from fitting to experimental data. See the reference fo details	(Gibson *et al*, 2000)
kRK2	250 s^{-1}	2	Rate constant of dissociation of R_n from R_n·RK prior to phosphorylation		(Hamer *et al*, 2005)
RK_{dark}	10838	2	Amount of unbound RK in the dark state	Obtained from initial equilibrium between RK and Rec (QSSA)	-
kRK3[ATP]	400 s^{-1}	3	Rate of binding ATP to R_n·RK	Value already multiplied for [ATP]	(Hamer *et al*, 2005)
kRK4	20 s^{-1}	4	Rate constant of dissociation of R_n from R_n·RK following phosphorylation		(Hamer *et al*, 2005)
ω_{arr}	0.8132	5	Exponential rate of increase of Arr affinity for Rn as a function of the number of phosphorylations	Optimized by parameter estimation	-
kArr	6.092e-10 s^{-1}	5	Basis of the exponential rate of increase of Arr affinity for Rn	Optimized by parameter estimation	-
KA2	3.232e-3 s^{-1}	5	Rate constant of dissociation of Rn from Arr·Rn, without Rn inactivation	Optimized by parameter estimation	-

KA3	4.4509e-2 s^{-1}	6	Rate constant of dissociation and inactivation of R_n from R_n·Arr to give Ops	Optimized by parameter estimation	-
kOps	6.117e-13 s^{-1}	8	Rate constant of association of Ops and Gt due to basal activity	Estimated according to experimental evidence	(Cornwall and Fain, 1994; Melia *et al*, 1997)
kRrecyc	7e-4 s^{-1}	12	Rate constant of association of Ops and 11-cis ret to reconstitute inactive R	Value already multiplied by [11-cis retinal]	(Firsov *et al*, 2005)
kG1_0	3.059e-5 s^{-1}	13	Rate of binding G·GDP to unphosphorylated R_0 in the dark	Optimized by parameter estimation	-
kG2	2250.34 s^{-1}	8,13	Rate constant of dissociation of R_n from R_n·Gt	Optimized by parameter estimation	-
kG3	2000 s^{-1}	9,14	Rate constant of dissociation of GDP from R_n·Gt		(Hamer *et al*, 2005)
kG4[GDP]	600 s^{-1}	9,14	Rate of binding GDP to R_n·Gt		(Hamer *et al*, 2005)
kG5[GTP]	750 s^{-1}	10,15	Rate of binding GTP to R_n·G		(Hamer *et al*, 2005)
kG6	2000 s^{-1}	11,16	Rate constant of dissociation of R_n from R_n·G_{GTP}		(Hamer *et al*, 2005)
kG7	200 s^{-1}	17	Rate constant of dissociation of G_{GTP} into $G\alpha_{GTP}$ and $G\beta\gamma$		(Hamer *et al*, 2005)
kGrecyc	2 s^{-1}	29	Rate constant of Gt reconstitution from $G\alpha_{GTP}$ and $G\beta\gamma$ subunits		(Felber *et al*, 1996)

kGshutoff	0.05 s^{-1}	28	Rate of spontaneous self-catalyzed hydrolysis of GTP to GDP by Gα		(Felber *et al*, 1996)
kP1	5.5e-2 s^{-1}	18	Rate of binding PDE to Gα·GTP	Optimized by parameter estimation	-
$kP1_{rev}$	0 s^{-1}	18	Rate constant of dissociation of PDE·Gα·GTP	Set equal to zero in this model	-
kP2	940.7 s^{-1}	19	Rate constant of removal of inhibition from the γ subunit of PDE*·$G\alpha_{GTP}$	Optimized by parameter estimation	-
kP3	1.498e-9 s^{-1}	20	Rate of binding $G\alpha_{GTP}$ to an active PDE*·$G\alpha_{GTP}$ complex	Optimized by parameter estimation	-
kP4	21.09 s^{-1}	21	Rate constant of activation of the second subunit in $G\alpha_{GTP}$ ·PDE*·$G\alpha_{GTP}$	Optimized by parameter estimation	-
$kPDE_{shutoff}$	0.033 s^{-1}	26,27	Rate constant of PDE-induced shutoff of PDE*·$G\alpha_{GTP}$	Manually tuned	-
kRGS1	1.57e-7 s^{-1}	22,24	Rate of binding RGS to a PDE·Gα·GTP complex with either active subunits	Manually tuned and optimized by parameter estimation	-
kRGS2	256.07 s^{-1}	23,25	Rate constant of dissociation of one PDE*·Gα·GTP and GTP hydrolysis	Optimized by parameter estimation	-
kRec3	9.6878 $\mu M^{-1}s^{-1}$	30	Rate constant of binding RK to Rec·wCa^{2+}	Optimized by parameter estimation	-
kRec4	0.610084 s^{-1}	30	Rate constant of dissociation of RK from RK·Rec·wCa^{2+}	Optimized by parameter estimation	-

Kp	0.425272 μM	30	[Ca^{2+}] causing half maximal inhibition of Rec	Involved in Rec equilibrium. Optimized by parameter estimation	-
w	2	30	Hill coefficient for the action of Ca^{2+} on Rec	Involved in Rec equilibrium	(Klenchin *et al*, 1995) (Chen *et al*, 1995)
Vcyto	1 pL	33	Cytoplasmic rod volume		(Pugh *et al*, 2000)
Kc	0.17 μM	34	[Ca^{2+}] at which synthesis of cGMP is half of α_{max}	Involved in the expression defining α_{max}	(Hamer, 2000)
m	2.5	34	Hill coefficient for the action of Ca^{2+} on cyclase rate	Involved in the expression defining α_{max}	(Gorczyca *et al*, 1994)
β_{dark}	1.2 s^{-1}	35	Dark rate of cGMP hydrolysis	Involved in the expression defining α_{max}	(Hamer, 2000)
β_{sub}	4.3e-4 s^{-1}	35	Rate constant for a catalytic PDE subunit in a well-stirred volume, V*ctyo*		(Leskov *et al*, 2000)
fCa	0.2	33	Fraction of circulating current carried by Ca^{2+}	Involved in the expression defining J	(Ohyama *et al*, 2000)
J_{dark}	29.7778 pA	33	Dark circulating current	Involved in the expression defining J	(Hamer *et al*, 2005)
F	96485.34 cm^{-1}	33	Faraday constant		
$cGMP_{dark}$	4 μM	33	[cGMP] in the dark	Involved in the expression defining α_{max} and J	(Nikonov *et al*, 2000; Pugh and Lamb, 1990; Pugh *et al*, 2000)
n_{cg}	3	33	Hill coefficient for opening the cGMP-gated channels	Involved in the expression defining J	(Hamer, 2000)

γCa	47.554 s^{-1}	32	Rate constant of Ca^{2+} extrusion by Na^{+}/Ca^{2+}-K^{+} exchanger		(Hamer *et al*, 2005)
$Ca2_{dark}$	0.6 μM	-	$[Ca^{2+}]$ in the dark	Involved in the expression defining α_{max} and J	(Hamer *et al*, 2005)
$Ca2_0$	0.01 μM	32	Minimum intracellular $[Ca^{2+}]$	Involved in the expression defining J	(Gray-Keller and Detwiler, 1994; McCarthy *et al*, 1994; Sampath *et al*, 1998)
k1	0.3815 $\mu M^{-1}s^{-1}$	31	Rate constant of Ca^{2+} binding to buffers	Optimized by parameter estimation	-
k2	1.909 s^{-1}	31	Rate constant of Ca^{2+} dissociation from buffers	Optimized by parameter estimation	-
eT	400 μM	31	Total concentration of Ca^{2+} buffers		(Forti *et al*, 1989; Hamer *et al*, 2005)
k_{therm}	0.0238 s^{-1}	7	Rate constant of thermal decay of active R_n		(Xu *et al*, 1997)

TABLE S3

Parameter	kRK1_0	kA2	kA3	kG1_0	kG2	kRec3	kRec4	Kp	k1	k2
(Hamer *et al*, 2005)	0.0092	0.0023	0.035	0.000028	2000	10.00	0.50	0.40	0.50	2.50
Current model	0.0076	0.0032	0.045	0.000031	2250	9.69	0.61	0.43	0.38	1.91

Note to Table S3: In this table the ten parameters whose values were changed with respect to those in the template model (Hamer *et al*, 2005) are shown. They all are within a factor of 2 with respect to previous ones. The remaining 44 parameters in the template model were kept fixed.

TABLE S4

# of photoactivated rhodopsins	Second PDE subunit activation		RGS-mediated shutoff of one PDE subunit in the completely activated tetramer		PDE shutoff by GαGTP GTPase activity		GαGTP auto-catalytic GTPase activity	Reconstitution of Gt heterotrimer
	reaction # 20	reaction # 21	reaction # 22	reaction # 23	reaction # 26	reaction # 27	reaction # 28	reaction # 29
10	5.00E-09	5.00E-09	1.80E-09	1.80E-09	55	1.30E-10	2.00E-04	8.50E+02
50	1.10E-07	1.10E-07	4.00E-08	4.00E-08	260	2.80E-09	1.00E-03	4.00E+03
100	4.50E-07	4.50E-07	1.50E-07	1.50E-07	500	1.20E-08	2.00E-03	7.50E+03
500	9.00E-06	9.00E-06	3.00E-06	3.00E-06	2300	2.10E-07	1.00E-02	3.50E+04
1000	3.50E-05	3.50E-05	1.20E-05	1.20E-05	4500	8.00E-07	2.00E-02	7.00E+04
2000	1.40E-04	1.40E-04	4.50E-05	4.50E-05	9.00E+03	3.20E-06	4.00E-02	1.40E+05
5000	9.00E-04	9.00E-04	2.90E-04	2.90E-04	2.20E+04	2.00E-05	1.00E-01	3.30E+05
10000	3.60E-03	3.60E-03	1.20E-03	1.20E-03	4.40E+04	8.44E-05	2.00E-01	6.70E+05
20000	1.60E-02	1.60E-02	5.00E-03	5.00E-03	9.00E+04	3.60E-04	4.00E-01	1.30E+06
50000	1.40E-01	1.40E-01	4.50E-02	4.50E-02	2.10E+05	3.50E-03	1.04E+00	3.30E+06
100000	1.00E+01	1.00E+01	1.90E+00	1.90E+00	4.30E+05	1.30E-01	2.60E+01	6.50E+06
101000	2.36E+01	2.20E+01	3.20E+00	3.20E+00	4.36E+05	2.30E-01	6.00E+01	6.60E+06
102000	6.76E+02	5.44E+02	3.68E+01	3.68E+01	4.40E+05	2.60E+00	1.69E+03	6.66E+06
105000	8.89E+03	8.89E+03	1.33E+03	1.33E+03	4.39E+05	9.40E+01	2.25E+04	6.70E+06
110000	2.37E+04	2.37E+04	5.33E+03	5.33E+03	4.40E+05	3.76E+02	6.00E+04	6.72E+06
118000	4.80E+04	4.80E+04	1.44E+04	1.44E+04	4.40E+05	1.02E+03	1.22E+05	6.80E+06
120000	5.50E+04	5.50E+04	1.70E+04	1.70E+04	4.40E+05	1.20E+03	1.40E+05	6.80E+06

Note to Table S4: The velocities of some of the slowest reactions observed in our dynamic model are reported (see Table S1 for the definition of each reaction). The reactions were followed over a broad range of light intensities (10 up to 120,000 photoactivations) and were all found to increase their velocity for increased bleaching.

TABLE S5

% rod saturation	WT rod		Rpe65 knockout rod		Δ recovery time (s)
	# photoactivated Rhodopsins	recovery time (s)	# photoactivated isoRhodopsins	recovery time	
15%	4.5	7.7	305	6.4	1.3
35%	14	10.2	850	7.9	2.3
50%	25.75	11.8	1400	8.8	3
70%	56	15.4	2675	10.1	5.3
85%	116	16.7	5100	12	4.7

Note to Table S5: After simulating the wt and *Rpe65*$^{-/-}$ rod photoresponses to flashes leading from 15% to 85% of rod saturation, we measured the corresponding time of recovery, that is the time needed for the photocurrent to get back to the initial value before illumination. The measured values are displayed in this table, together with the intensities (expressed as the number of photoactivated pigments) needed to reach the same level of saturation in rod photocurrent. In the rightmost column the difference between times of recovery of *Rpe65*$^{-/-}$ and wt rod is shown.

REFERENCES

Ames JB, Ishima R, Tanaka T, Gordon JI, Stryer L, Ikura M (1997) Molecular mechanics of calcium-myristoyl switches. *Nature* 389: 198-202.

Chen CK, Inglese J, Lefkowitz RJ, Hurley JB (1995) Ca(2+)-dependent interaction of recoverin with rhodopsin kinase. *J Biol Chem* 270: 18060-18066.

Cornwall MC, Fain GL (1994) Bleached pigment activates transduction in isolated rods of the salamander retina. *J Physiol* 480 (Pt 2): 261-279.

Felber S, Breuer HP, Petruccione F, Honerkamp J, Hofmann KP (1996) Stochastic simulation of the transducin GTPase cycle. *Biophys J* 71: 3051-3063.

Firsov ML, Kolesnikov AV, Golobokova EY, Govardovskii VI (2005) Two realms of dark adaptation. *Vision Res* 45: 147-151.

Forti S, Menini A, Rispoli G, Torre V (1989) Kinetics of phototransduction in retinal rods of the newt Triturus cristatus. *J Physiol* 419: 265-295.

Gibson SK, Parkes JH, Liebman PA (2000) Phosphorylation modulates the affinity of light-activated rhodopsin for G protein and arrestin. *Biochemistry* 39: 5738-5749.

Gorczyca WA, Gray-Keller MP, Detwiler PB, Palczewski K (1994) Purification and physiological evaluation of a guanylate cyclase activating protein from retinal rods. *Proc Natl Acad Sci U S A* 91: 4014-4018.

Gray-Keller MP, Detwiler PB (1994) The calcium feedback signal in the phototransduction cascade of vertebrate rods. *Neuron* 13: 849-861.

Hamer RD (2000) Analysis of Ca++-dependent gain changes in PDE activation in vertebrate rod phototransduction. *Mol Vis* 6: 265-286.

Hamer RD, Nicholas SC, Tranchina D, Lamb TD, Jarvinen JL (2005) Toward a unified model of vertebrate rod phototransduction. *Vis Neurosci* 22: 417-436.

Hamm HE, Bownds MD (1986) Protein complement of rod outer segments of frog retina. *Biochemistry* 25: 4512-4523.

Kawamura S, Murakami M (1991) Calcium-dependent regulation of cyclic GMP phosphodiesterase by a protein from frog retinal rods. *Nature* 349: 420-423.

Klenchin VA, Calvert PD, Bownds MD (1995) Inhibition of rhodopsin kinase by recoverin. Further evidence for a negative feedback system in phototransduction. *J Biol Chem* 270: 16147-16152.

Leskov IB, Klenchin VA, Handy JW, Whitlock GG, Govardovskii VI, Bownds MD, Lamb TD, Pugh EN, Jr., Arshavsky VY (2000) The gain of rod phototransduction: reconciliation of biochemical and electrophysiological measurements. *Neuron* 27: 525-537.

McCarthy ST, Younger JP, Owen WG (1994) Free calcium concentrations in bullfrog rods determined in the presence of multiple forms of Fura-2. *Biophys J* 67: 2076-2089.

Melia TJ, Jr., Cowan CW, Angleson JK, Wensel TG (1997) A comparison of the efficiency of G protein activation by ligand-free and light-activated forms of rhodopsin. *Biophys J* 73: 3182-3191.

Nikonov S, Lamb TD, Pugh EN, Jr. (2000) The role of steady phosphodiesterase activity in the kinetics and sensitivity of the light-adapted salamander rod photoresponse. *J Gen Physiol* 116: 795-824.

Ohyama T, Hackos DH, Frings S, Hagen V, Kaupp UB, Korenbrot JI (2000) Fraction of the dark current carried by Ca(2+) through cGMP-gated ion channels of intact rod and cone photoreceptors. *J Gen Physiol* 116: 735-754.

Pugh EN, Jr., Lamb TD (1990) Cyclic GMP and calcium: the internal messengers of excitation and adaptation in vertebrate photoreceptors. *Vision Res* 30: 1923-1948.

Pugh EN, Jr., Lamb TD (2000) *Phototransduction in Vertebrate Rods and Cones: Molecular Mechanisms of Amplification, Recovery and Light Adaptation*, Vol. 3: Elsevier Science B.V. .

Sampath AP, Matthews HR, Cornwall MC, Fain GL (1998) Bleached pigment produces a maintained decrease in outer segment Ca2+ in salamander rods. *J Gen Physiol* 111: 53-64.

Xu J, Dodd RL, Makino CL, Simon MI, Baylor DA, Chen J (1997) Prolonged photoresponses in transgenic mouse rods lacking arrestin. *Nature* 389: 505-509.