an identical manner to that of the ipsilateral sciatic pool and reached a peak at the same time, one day after the nerve lesion. Furthermore, because we prelabelled the motoneurones we were able to demonstrate a small but clear decline in the number of surviving contralateral sciatic motoneurones during the early postoperative period, which could not be established with the labelling methods used previously.

Like Koltzenburg *et al.*, we have considered both peripheral and central mechanisms to explain contralateral motoneurone susceptibility to nerve injury, and agree that a central neuronal mechanism is most likely. In view of the fact that our studies are carried out in neonates, two particular points might be pertinent. First, neuronal connections crossing the midline are thought to be more substantial in immature spinal cord. Second, there is evidence that increased activation alone can cause degeneration of immature motoneurones[4]. Thus, we suggested that death of contralateral motoneurones might be attributable to increased activation through a pathway crossing the midline.

The use of apoptotic techniques has also allowed us to observe the effect of a nerve lesion on spinal interneurones. Previously, we established that these cells underwent a discreet wave of naturally occurring cell death immediately after birth[5], but following sciatic nerve injury, interneurone death was significantly higher than the background level both ipsilaterally and contralaterally[2]. The increase on both sides occurred simultaneously but was delayed until three days after the peak of motoneurone death. Interneurone death was more obvious in the dorsal horns, suggesting that it was either consequent on altered sensory input, perhaps through increased activation, or secondary to loss of dorsal-root-ganglion cells.

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References

1 **Koltzenburg, M., Wall, P.D. and McMahon, S.B.** (1999) *Trends Neurosci.* 22, 122–127
2 **Lawson, S.J. and Lowrie, M.B.** (1998) *Neuroscience* 87, 337–348
3 **Lowrie, M.B. and Vrbová, G.** (1992) *Trends Neurosci.* 15, 80–84
4 **Sanusi, J. *et al.*** (1998) *Dev. Neurosci.* 20, 546–551
5 **Lawson, S.J. *et al.*** (1997) *Eur. J. Neurosci.* 9, 794–799

REVIEW

Rod pathways: the importance of seeing nothing

Lindsay T. Sharpe and Andrew Stockman

Anatomical and physiological studies of the mammalian retina have revealed two primary pathways available for the transmission of rod signals to the ganglion cells: one via ON rod bipolars, amacrine II cells, and ON and OFF cone bipolars, which is exquisitely designed for the transmission of single-photon absorption events; and a second via rod–cone gap junctions, and ON and OFF cone bipolars, which is designed for the transmission of multiple photon-absorption events at higher light levels. Psychophysical and electroretinographic (ERG) studies in normal observers and in two rare types of observer, who are devoid of either rod or cone function, support an analogous duality in the human visual system, the clearest signature of which is a loss of flicker visibility and ERG amplitude at frequencies near 15 Hz that results from destructive interference between sensitive 'slow' and insensitive 'fast' rod signals. The slow rod signal is most probably derived from the ON rod bipolar pathway and the fast signal from the rod–cone gap junction and cone pathways. Evidence has emerged recently for a third, insensitive rod pathway between rods and OFF cone bipolars, but it has so far only been observed clearly in rodents.

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THE MAMMALIAN RETINA contains two distinct types of photoreceptor: the rods and cones (see Fig. 1). Both hyperpolarize in response to light, reducing the synaptic release of their transmitter, glutamate. Cones subserve daylight or photopic vision, when photons are abundant. Together with specially evolved neural circuitry, they are responsible for the perception of fine temporal and spatial detail, and for colour vision. In contrast, rods mediate starlight–twilight or scotopic vision, when photons are few. Their design is optimized for the reliable transduction of single-photon absorptions. Aside from specialized bipolar cells and amacrine cells (see below), however, the retinal circuitry for rod signals appears to be superimposed upon pre-existing cone circuitry[3,4] in ways that afford multiple opportunities for signal transmission.

In this article, evidence for the routing of rod signals over more than one pathway is reviewed from: (1) non-human anatomy and physiology (see earlier reviews in Refs 1,5); and (2) human electrophysiology and psychophysics. The amalgamation of information from these disciplines yields insights into the behavioral and functional significance of having multiple rod pathways.

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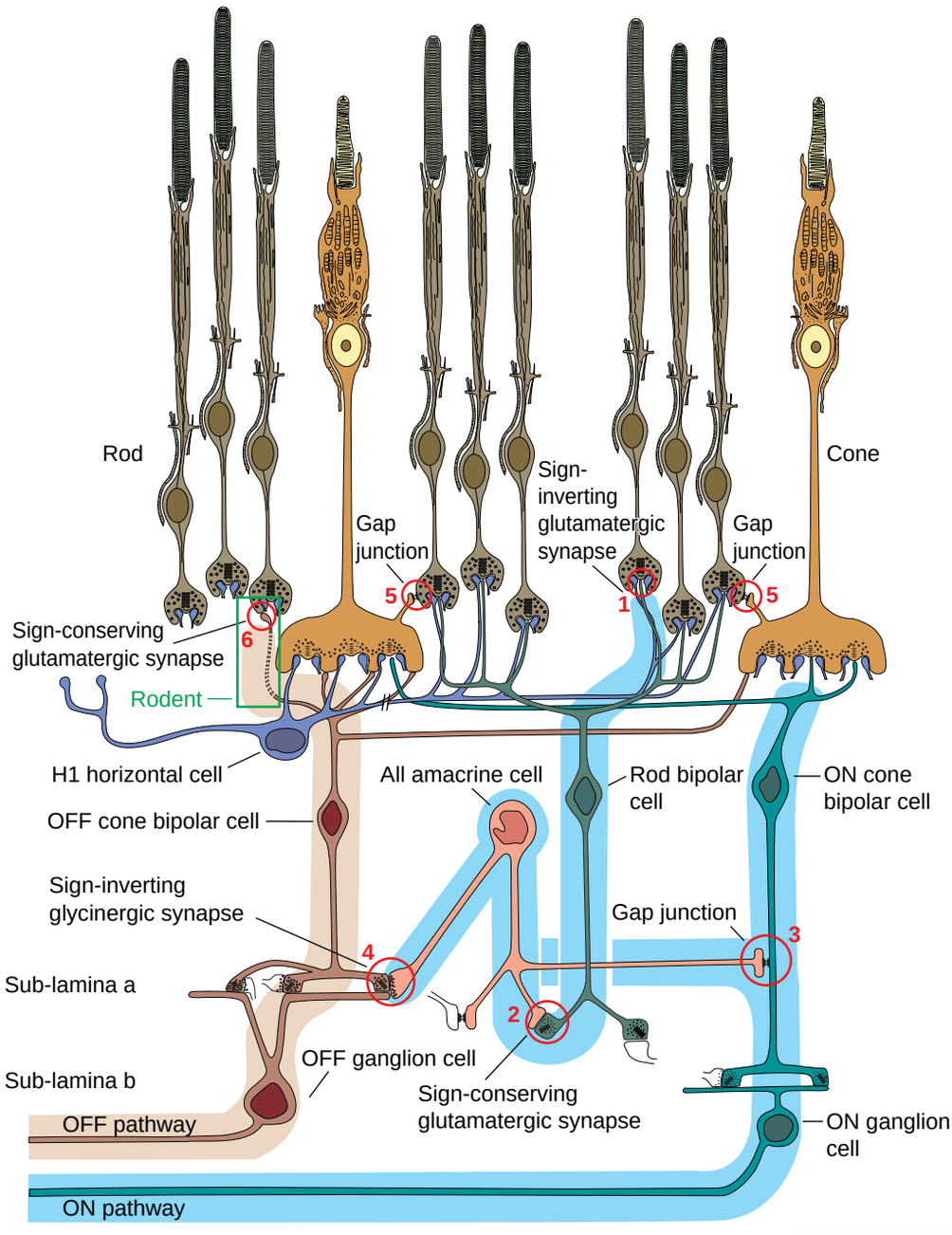

Fig. 1. ***Rod and cone pathways in the mammalian retina.*** *The retina is a complex neural tissue interweaving multiple circuits for transmitting photon signals from the light-sensitive rod and cone photoreceptors to the ON and OFF ganglion cells, the axons of which form the optic nerve. Integral to the circuits are bipolar, amacrine and horizontal cells, which maintain or enhance the linkage. The highly schematic retinal diagram depicted here (see also Refs 1,2) concentrates on the pathways available to the rods, all of which either infiltrate or superimpose upon the cone circuitry. The numbered circles highlight the six so-far identified or inferred regions of rod-signal transmission: (1) the rod–rod bipolar metabotropic (sign-inverting) glutamatergic synapse; (2) the rod bipolar–amacrine AII cell (sign-conserving) glutamatergic synapse; (3) the amacrine II–ON cone bipolar (sign-conserving) electrical gap junction; (4) the amacrine II–OFF cone bipolar (sign-inverting) glycinergic synapse; (5) the rod–cone (sign-conserving) electrical gap junction (shown twice, once each for the ON and OFF pathways); and (6) the inferred rod–OFF cone bipolar ionotropic (sign-conserving) glutamatergic synapse. Only the parasol ON (light green) and OFF (beige) pathways, which transmit the largest rod signals, are shown. The cone–cone gap junctions and H2 horizontal cells (the axons of which do not connect to rods) are not shown. The //, which cuts the axon of the H1 horizontal cell, indicates that the axon is much longer than depicted here.*

Anatomical and physiological evidence for multiple rod pathways

ON rod bipolar and AII amacrine pathway

The first route to be identified was the rod ON bipolar and AII amacrine pathway. Unlike cold-blooded vertebrate rods, mammalian rods are thought to synapse with a single type of bipolar cell[6–8], the so-called ON type, which depolarizes following light stimulation (but see below). The synapse is at the apex of the deeply clefted rod spherule (see Fig. 1). One synaptic ribbon, which is densely filled with vesicles containing glutamate, interacts with two or more invaginating rod ON bipolar dendrites that bear mainly the high-affinity sixth-subtype metabotropic glutamate receptor (mGluR6)[9–12], but that also contain some ionotropic glutamate receptor (iGluR) subunits[13]. The rod ON bipolar, in turn, contacts the amacrine AII cell at a sign-conserving glutamate synapse[3,14–17] (a synapse at which neurotransmitter release results in hyperpolarization of the postsynaptic membrane). Signals from the amacrine cell then infiltrate the main cone circuitry (see Box 1) by exciting ON (depolarizing) cone bipolar cells through sign-conserving electrical gap junctions and inhibiting OFF (hyperpolarizing) cone bipolar cells through glycinergic synapses (see Fig. 1)[3,17–20]. Thereafter, the signal separation is maintained: ON bipolars excite ON ganglion cells and OFF bipolars excite OFF ganglion cells.

Rod–cone gap-junction pathway

A second pathway infiltrates the ON and OFF cone bipolar circuitry at the earliest possible stage. Telodendria projecting from the sides and bases of neighbouring cone pedicles make minute gap-junction contacts with rod spherules (see Fig. 1), which allow electrical synaptic transmission[21–25]. Between three and five gap junctions occur on a single rod spherule with the majority originating from either red (long-wavelength-sensitive) or green (middle-wavelength-sensitive) cones[26]. Through the gap junctions, rod signals have access to ON and OFF cone bipolars and thence to ON and OFF ganglion cells[5,23,24,27].

The viability of the rod–cone gap junctions as a means of transmitting rod signals has been established in the cat by Nelson[23], and in the macaque monkey by Schneeweis and Schnapf[28], both of whom demonstrated the presence of rod signals in cones. In addition, the cell bodies of primate H1 horizontal cells have been shown to receive rod input[29,30], which is likely to arrive via the rod–cone gap junctions, cones and H1 dendrites. Although the H1 axonal tree contacts rods, the long thin axon isolates electrotonically the axonal tree from the cell body and dendritic tree that only contacts cones[31,32].

Box 1. Cone pathways

At least nine types of ON and OFF bipolar cell contact the cone pedicle[a]. Their response polarity is determined by the nature of their receptors. In ON bipolars, metabotropic glutamate receptors link glutamate to the closure of cation channels; whereas in OFF bipolars, ionotropic glutamate receptors link glutamate to Na^+ influx[b–e]. The ON bipolar axons terminate in the sublamina b of the inner plexiform layer (IPL), synapsing with ON-centre ganglion cells; whereas the OFF bipolar axons terminate in sublamina a, synapsing with OFF-centre ganglion cells[f].

Midget bipolars contact exclusively, at least out to retinal eccentricities of 50° of visual angle, single M- or L-cone pedicles. They are either of the invaginating (ON bipolar) or the semi-invaginating (OFF bipolar) type. In turn, they connect to single midget ganglion cells of the same sign (D.J. Calkins, PhD Thesis, University of Pennsylvania, 1994)[g–j], the projections of which, to the lateral geniculate nucleus, form the major substrate of the parvocellular pathway[h].

Diffuse bipolar cells pool information from several cones, contacting between 5 and 10 cones in central to mid-peripheral retina[a,i–k]. They are thought to connect to the parasol ganglion cells with which they form the main substrate of the early magnocellular pathway[a,l]. There are at least six varieties[a], which are identified according to whether they have predominantly invaginating or basal contacts with cone pedicles and according to whether they synapse in sublamina a or b of the IPL. Types DB1, DB2 and DB3, believed to be OFF types, have basal contacts and terminate in sublamina a layer of the IPL (Refs a,j,m); types DB4, DB5 and DB6, believed to be ON types, terminate in sublamina b (Refs a,j,n) and have predominantly 'invaginating' contacts (though types DB4 and DB6 have significant numbers of basal contacts[i,j,o]).

In primate retina, the AII amacrine cells of the primary rod circuit probably contact both midget and the diffuse bipolar cells[p,q], and so provide (along with the rod–cone gap junctions) rod input to both the parvocellular and magnocellular pathways. In general, however, rod signals appear to be more prevalent in magnocellular than in parvocellular projecting cells[r,s].

References

a **Boycott, B.B. and Wässle, H.** (1991) *Eur. J. Neurosci.* 3, 1069–1088
b **Slaughter, M.M. and Miller, R.F.** (1981) *Science* 211, 182–185
c **Nawy, S. and Copenhagen, D.R.** (1987) *Nature* 325, 56–58
d **Nawy, S. and Jahr, C.E.** (1990) *Nature* 346, 269–271
e **Shiells, R.A. and Falk, G.** (1990) *Proc. R. Soc. London B Biol. Sci.* 242, 91–94
f **Nelson, R., Famiglietti, E.V. and Kolb, H.** (1978) *J. Neurophysiol.* 41, 472–483
g **Dacey, D.M. and Lee, B.B.** (1999) in *Color Vision: From Genes to Perception* (Gegenfurtner, K. and Sharpe, L.T., eds), pp. 181–202, Cambridge University Press
h **Perry, V.H., Oehler, R. and Cowey, A.** (1984) *Neuroscience* 12, 1110–1123
i **Hopkins, J.M. and Boycott, B.B.** (1995) *J. Neurocytol.* 24, 680–694
j **Hopkins, J.M. and Boycott, B.B.** (1996) *J. Neurocytol.* 25, 381–390
k **Wässle, H.** (1999) in *Color Vision: From Genes to Perception* (Gegenfurtner, K. and Sharpe, L.T., eds), pp. 145–162, Cambridge University Press
l **Kolb, H. and Nelson, R.** (1995) in *Neurobiology and Clinical Aspects of the Outer Retina* (Djamgoz, M.B.A., Archer, S.N. and Vallerga, S., eds), pp. 273–296, Chapman & Hall
m **Grünert, U., Martin, P.R. and Wässle, H.** (1994) *J. Comp. Neurol.* 348, 607–627
n **Saito, T.** (1987) *Vis. Res.* 27, 135–142
o **Hopkins, J.M. and Boycott, B.B.** (1997) *J. Neurocytol.* 26, 313–325
p **Grünert, U. and Wässle, H.** (1996) *Visual Neurosci.* 13, 101–115
q **Grünert, U.** (1997) *Eur. J. Neurosci.* 9, 617–621
r **Purpura, K., Kaplan, E. and Shapley, R.M.** (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 4534–4537
s **Lee, B.B. *et al.*** (1997) *Vis. Res.* 37, 2813–2828

An OFF bipolar pathway?

Evidence for a third pathway, involving a direct rod to OFF bipolar connection (see Fig. 1), comes from recent experiments on transgenic mice that lack all long-wavelength-sensitive cones and more than 95% of short-wavelength sensitive cones[33]. In such mice, an OFF response at the ganglion cell survived the elimination of mGluRs of ON rod bipolar cells by the glutamate analog L-(+)-2-amino-4-phosphonobutyric acid (APB) and the elimination of glycinergic synapses of AII amacrine cells by strychnine, both of which should incapacitate the primary rod pathway (see Box 2). Given that the secondary pathway via gap junctions is also incapacitated by the lack of cones, the survival of an APB-resistant OFF response suggests the existence of a third pathway, perhaps connecting rod and OFF bipolar cells[33].

The presence of a third, direct OFF pathway accords with the observations of 'non-ribbon' contacts between OFF bipolars and rods[34], and of flat contacts between OFF bipolar cells and rods that possess iGluR receptors[35] in rat retina; with the observations of comparable contacts in gray squirrel[36]; and with observations that normal mouse retina contains OFF bipolar dendrites that contact rods (Y. Tsukamoto, pers. commun.). These findings argue against the possibility that direct OFF cone bipolar connections to rods exist in 'coneless' transgenic mice because of the absence of cones; in other words, they suggest that the connections arise through plasticity and reorganization in the developing retina, and are not present in the normal rodent retina. The possibility remains, however, that such connections are more numerous or more prominent, or both, in the coneless retina, owing to plasticity. Flat contacts between the rod spherules and putative OFF bipolar cells have not been found in cat or macaque monkey retina[6,11,37–40].

Direct OFF cone bipolar connections to rods could be a characteristic that is specific to the smaller eyes of rodents, but absent in the larger eyes of cats, monkeys and humans, and depends on rodent rods receiving, on average, more quanta than rods in larger eyes (R.G. Smith, pers. commun.). The substantial differences in quantum catch arise because the size of the rods remains roughly constant across these species, despite large changes in the size of the eye. Thus, rodent rods collect light from a larger visual angle than cat, primate or human rods. A second characteristic linked to the higher quantum catch might be the existence of rod–rod gap

Box 2. Receptor antagonists

Chemical antagonists can be used to reveal the flow of photoreceptor signal transmission in retinal networks by blocking transmission at a subset of synapses. The glutamate analog, APB, acts selectively on metabotropic glutamate receptors, blocking transmission from photoreceptors to ON bipolar cells[a–c], but not to the OFF bipolar cells[a,d]. Strychnine interferes with the glycinergic synapse of the AII amacrine cell; blocking transmission to the cone OFF bipolar cell[d].

References

a **Slaughter, M.M. and Miller, R.F.** (1981) *Science* 211, 182–185
b **Shiells, R.A. and Falk, G.** (1990) *Proc. R. Soc. London B Biol. Sci.* 242, 91–94
c **Nawy, S. and Jahr, C.E.** (1990) *Nature* 346, 269–271
d **Müller, F., Wässle, H. and Voight, T.** (1988) *J. Neurophysiol.* 59, 1657–1672

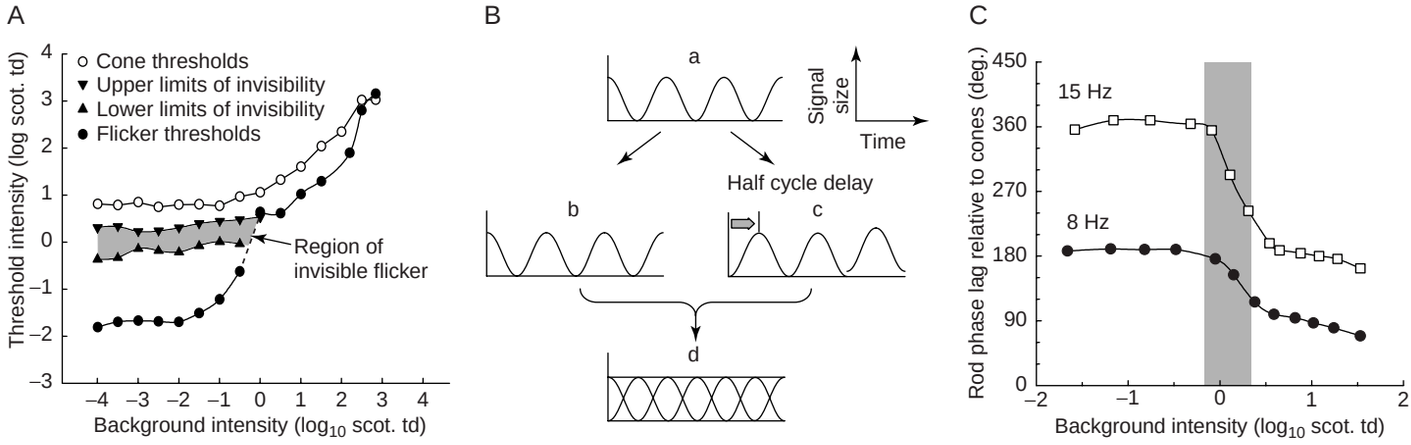

Fig. 2. ***Rod self-cancellation in human rod flicker data.*** **(A)** *15 Hz rod-flicker detectability data for a normal observer plotted as a function of background retinal illuminance [log_{10} scotopic troland (scot. td)]. The filled circles represent a rod-flicker threshold versus intensity (TVI) function (that is, they represent the lowest intensity at which rod flicker is just detectable plotted as a function of background intensity). The function is clearly double-branched. The upright and inverted triangles on the left of the TVI function represent, respectively, the lower and upper limits of the region within which 15 Hz flicker could not be seen (shaded gray). The disappearance of flicker occurs well below the cone-flicker thresholds measured before the rods have recovered from an intense bleaching light (open circles). The disappearance is also found in a rod monochromat, who lacks functioning cones*[49]. **(B)** *Illustration of how 15 Hz self-cancellation might occur. A single flickering 15 Hz rod stimulus (a) produces a fast signal (b), and a slow signal (c) that is delayed by a half cycle (33.3 ms) relative to the fast one. When recombined, the slow and fast signals destructively interfere (d) to produce a steady signal with no visible flicker.* **(C)** *Rod–cone phase lags measured as a function of scotopic intensity at flicker frequencies of 8 Hz (filled circles) and 15 Hz (open squares) in the same normal observer as in* (A). *The region of invisible 15 Hz flicker is shown in gray.* Data in (A) and (C) are replotted from Ref. 50.

junctions between rodent rods, which reduce the noise associated with multiple-photon signal transmission across the chemical synapse of the OFF bipolar cell in rodents[41]. Such junctions are not found in cats and primates[22,27,42].

Evidence from human psychophysics and electrophysiology for multiple rod pathways

Are the multiple rod pathways demonstrated in mouse, rat, cat, rabbit and macaque retinae also found in the human visual system? And, if so, what is the behavioral and functional significance of each of them? The answer to the second question is important because some 'pathways' could be involved in modulatory functions, such as sensitivity regulation, rather than the transmission of crucial details of the visual world.

First evidence

The earliest evidence for two rod pathways pre-dates the physiological and anatomical evidence. It came from psychophysical measurements of temporal resolution made more than 50 years ago in a rare, totally colour-blind human observer, a rod monochromat who lacked cone vision[43,44]. The measurements revealed two distinct stages in the function that relates critical flicker frequency (CFF, the frequency above which flicker can no longer be perceived) to intensity, separated by an inflection near 0 log_{10} scotopic troland (scot. td), a twilight-intensity region. Subsequent findings in other rod monochromats[45–47] and also in normal observers under conditions that isolate the rod response[48] show that the lower stage asymptotes at a CFF of approximately 15 Hz; whereas the higher stage attains a CFF as high as 28 Hz. Although a duality is expected in the normal eye at higher intensities, because detection passes from the sluggish rods to the much brisker cones (which can attain a CFF greater than 50 Hz), it was totally unexpected in the rod-only visual system.

Psychophysical evidence from normals

Other rod-mediated psychophysical functions also demonstrate conspicuous discontinuities in flicker sensitivity near a temporal frequency of 15 Hz and an intensity of 0 log_{10} scot. td. Figure 2A shows a typical example in the form of rod-flicker threshold data measured in a normal observer under conditions chosen to favour rod-flicker detection[50]. Conspicuously, the rod-determined part of the flicker threshold versus intensity (TVI) curve, like the CFF function, exhibits two stages[51], with a break occurring near a background intensity of 0 log_{10} scot. td. Crucially, alongside the break in the curve is a region within which 15 Hz flicker is invisible[50]. Thus, as the intensity of the flickering target is increased, the flicker becomes visible, but then disappears before reappearing again at higher intensities.

The disappearance of 15 Hz flicker is consistent with destructive interference between a fast rod signal and a slow one that is delayed by approximately 33.3 ms (that is, by half the 15 Hz period). As illustrated in Fig. 2B, a relative delay of 33.3 ms means that, when recombined, the two signals are in opposite phase and cancel each other. Measurements of the perceptual delay between rod and cone signals at intensities just above and just below the intensity region in which the flicker is invisible[50] confirm that these perceptual phenomena arise from destructive interference between two rod signals with different latencies. Results of such measurements are shown as a function of intensity in Fig. 2C for frequencies of 8 Hz and 15 Hz. The disappearance of flicker is associated with an abrupt change in phase delay from approximately 180° to 90° for 8 Hz flicker and from 360° to 180° for 15 Hz flicker, which are consistent with a reduction in time delay of approximately 33.3 ms (Ref. 50).

Electrophysiological evidence from normals

Further electrophysiological evidence for multiple rod signals that are both frequency- and intensity-dependent can be found in the human electroretinogram or ERG (Refs 49,52). Figure 3 shows ERG responses to 15 Hz Ganzfeld (full-field) flicker in the normal observer[52]. With increasing flicker intensity, the ERG amplitude grows slightly, but then falls to a minimum at an

intensity corresponding to the perceptual disappearance of the flicker (0.02–0.15 $\log_{10}$ scot. td) before growing once more. Consistent with destructive interference being the cause of the flicker loss, the phase of the response abruptly reverses by a half cycle as the minimum is crossed.

Special cases: evidence from human observers devoid of cone or rod function

A strategy in psychophysics that is often revealing is the use of observers with special visual deficits. Two types of observers have been used to study the underlying physiology and anatomy of the rod pathways: rod monochromats and individuals with congenital stationary night blindness (CSNB).

Rod monochromat

The classical signatures of the two rod signals, including the dual CFF and TVI functions, the flicker disappearance, and the minimum and phase reversal in the ERG, are all also found in a rod monochromat[47,50,52] whose vision has consistently been demonstrated to lack functioning cones. Molecular-genetic analysis has established that he has homologous mis-sense mutations in a gene (*CNGA3*) on chromosome 2 that encodes the α-subunit of the cGMP-gated cation channel in the cone photoreceptors, the presence of which renders them inexcitable[53].

Figure 4A,B highlights the similarity of the ERG estimates of phase delay between the slow and fast rod signals in the rod monochromat and normal subject[52]. Given that the cones in the rod monochromat are compromised, these similarities pose a challenge for the model in which fast rod signals travel by way of rod–cone gap junctions. The model can only be sustained if some functioning rod–cone gap junctions survive in the rod monochromat. Whilst the anatomical reports differ somewhat (see Ref. 54 for a review), all reports[55–58] do reveal morphologically intact cones, or cone-like structures in the rod-monochromat eye; although the three most-reliable and most-recent studies[56–58] find that the number of cones is much fewer than the number found in the normal retina (being perhaps 5–10% of normal numbers in the peripheral retina). Functioning rod–cone gap junctions would not be required if the faster rod pathway depended instead on a direct rod to cone OFF bipolar pathway.

Congenital stationary night blindness

Although CSNB observers of the complete Schubert–Bornschein type[59,60] have physiologically intact rod receptors, which exhibit normal photopigment bleaching and regeneration[61,62], and elicit normal rod signals in the flash ERG a-wave[63,64], they apparently have no post-receptoral rod function[63,64]. The deficit is believed to be in the rod to rod–bipolar synapse[61,65–67], which would abolish rod signals carried by direct bipolar cell contacts to the rod, but not signals routed through rod–cone gap junctions.

Flicker ERG recordings from two CSNB observers of the complete Schubert–Bornschein type, however, exhibit no clear evidence for a fast rod signal[52]. If the rod–cone gap junctions are viable in these observers, the absence of a fast signal poses another challenge for the gap-junction model. A small, residual rod response in these observers can be measured psychophysically at intensity levels near those at which the fast signal appears in normals, but, as shown in Fig. 4C, it is restricted to 0.5 and 1 Hz at 0 $\log_{10}$ scot. td (L.T. Sharpe, T. Wolfe and A. Stockman, unpublished observations).

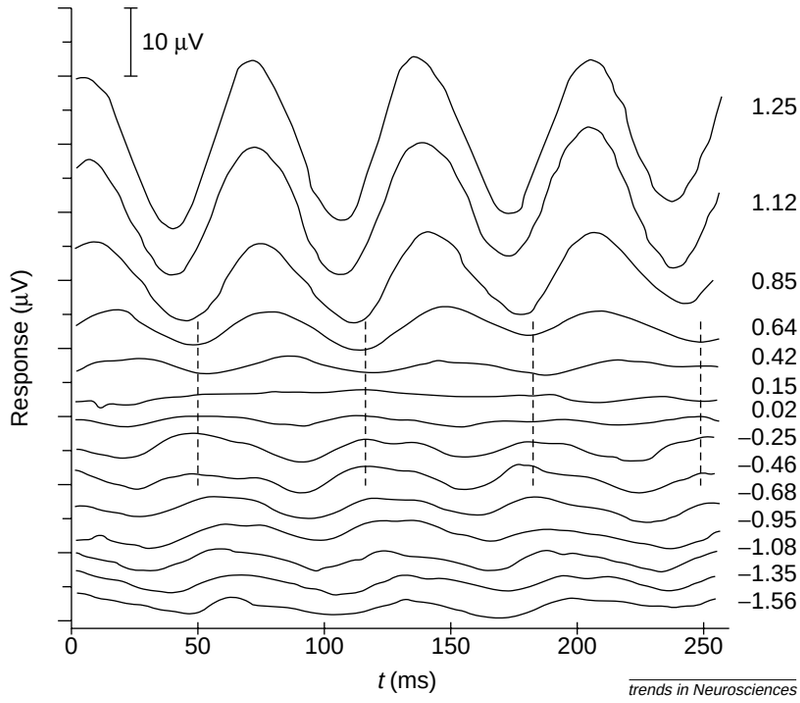

Fig. 3. ***Rod self-cancellation in human electrophysiogical data.*** *Ganzfeld electroretinogram (ERG) recordings at 15 Hz for the normal observer. The time-averaged flicker intensity increases upwards in steps of approximately 0.22 $\log_{10}$ unit ranging from −1.56 to 1.25 $\log_{10}$ scotopic troland (scot. td). The mean intensity in $\log_{10}$ scot. td is noted to the right of each record. The vertical broken lines highlight how the phase abruptly changes by 180° between the flicker intensities immediately above and below the region (~0.15 $\log_{10}$) in which rod flicker is invisible. The positions of the ERG responses with respect to the* y*-axis are arbitrary.* Data replotted from Ref. 52.

Conceivably, this sluggish, insensitive signal could be the one being conveyed by the rod–cone gap junctions, but it is not the same fast rod signal that cancels the slow rod signal psychophysically and electrophysiologically at 15 Hz. It should be noted, however, that the results from observers with congenital visual deficits might be unrepresentative. Through plasticity, those deficits could lead to the development or prominence of retinal connections that are normally missing or unimportant.

Bringing together the human and non-human evidence

The psychophysical evidence clearly indicates at least a duality of rod vision. A slow, sensitive rod signal is superceded by a fast, insensitive one as the light level increases from scotopic to mesopic levels. The remarkable consistency of the electrophysiological and psychophysical data suggests that the two rod signals are retinal in origin. Support for a retinal location also comes from one of the few physiological studies measured under relevant conditions that uses flicker: in the cat, Nelson *et al*.[68] reported phase delays between ganglion and AII amacrine cells at mesopic levels (levels at which both rods and cones are active) that were comparable to those found in humans. So how do the slow and fast rod-flicker signals correspond to the anatomically described retinal rod pathways? (For alternative possibilities and the problems associated with them, see Box 3.)

The slow rod signal

The sensitive, slow rod signal is most likely to be transmitted by the primary ON rod pathway routed by way of rod bipolars and AII amacrine cells. Several lines of reasoning support the assignment. First, the pathway

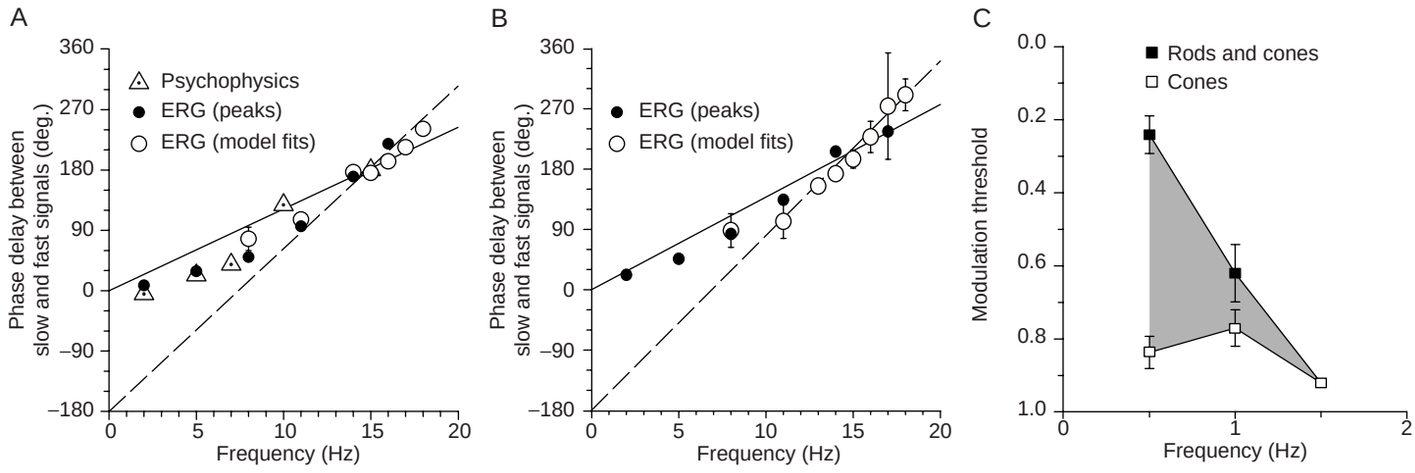

Fig. 4. ***Phase relation between the slow and fast rod signals in the normal and rod monochromat, and congenital stationary night blindness (CSNB) rod threshold data.*** **(A)** *Phase delays in degrees between the slow and fast signals for the normal observer, estimated from electroretinogram (ERG) measurements. Estimates are based on: (1) the relative positions of the peaks of the ERG flicker response above and below the null intensity (filled circles) from Ref. 50; (2) a Fourier analysis of the ERG flicker response above and below the null intensity (open circles) from Ref. 52; and (3) psychophysical estimates of the phase delays above and below the null intensity (dotted triangles) from Ref. 49. Also shown is the prediction of a simple time delay between slow and fast signals of the same sign (continuous line), and the prediction of a simple time delay between slow and fast signals of opposite sign (broken line).* **(B)** *Phase delays between the slow and fast signals for the rod monochromat estimated from ERG measurements. Details the same as in* (A)*, except no psychophysical rod–cone measurements are shown, as they are impossible.* **(C)** *Flicker modulation thresholds for a CSNB observer of the complete Schubert–Bornschein type obtained at a scotopic luminance of 0.0 $\log_{10}$ scotopic troland (scot. td) after complete dark adaptation (filled squares) and after the cones, but not rods, had recovered from an intense bleaching light (open squares). Detection within the shaded area from 0.5 to 1.5 Hz is mediated by rods.* (A) and (B) data replotted from Refs 50,52.

is well designed for high sensitivity. In cat central retina, signals from ~1500 rods converge onto a single ganglion cell, principally at the stages from rods to rod bipolar cells and from rod bipolar to AII amacrine cells[69]. With amplification at each stage, threshold nonlinearities to reduce transmitted noise at the rod to rod-bipolar synapse and noise averaging at the AII amacrine cell, the circuit is exquisitely designed[27,69–71] to enable the transmission of single photon events[72,73]. Second, the sensitive ON and OFF rod responses are eliminated at ganglion cells by blocking the ON rod bipolar cells with APB in cats and mice (see Box 2)[20,33]. Third, strychnine blocks the sensitive OFF rod response (see Box 2)[20]. Fourth, AII amacrine cells are active at scotopic but not high mesopic levels[74]. Fifth, the scotopic threshold response in cat, which is likely to be generated by rod-driven amacrine cells[75–77], has a similar response-intensity function (linear growth with $\log_{10}$ intensity, followed by saturation) to that of the slow rod signal in the human ERG; and both are very different from the function for the fast rod signal (linear growth with linear intensity)[52].

The fast rod signal

The origin of the fast rod signal observed in humans is less certain. What does seem clear, given that Nelson *et al.*[68] observed a clear rod response at mesopic levels in cat ganglion cells but none in AII cells, is that the fast signal bypasses the AII amacrine cells. The most-obvious route by which this can be effected is via rod–cone gap junctions. Evidence from cat and monkey shows that signal transmission by way of the rod–cone gap junction is indeed viable[23,28]; and calculations suggest that the gap-junction signal should become significant at the appropriate luminance levels[27].

Although the results obtained in the rod monochromat and CSNB observers of the complete Schubert–Bornschein type cast some doubts, most of the evidence supports the gap-junction model of the fast pathway, in which a multi-photon signal is 'piggybacked' onto the cone bipolar circuit. The fast signal might survive in rod monochromats because the few non-functioning cones that remain (see above) can provide enough rod–cone gap junctions to support a fast signal. In addition, CSNB patients of the complete Schubert–Bornschein type might fail to receive the fast rod signals because of possible post-receptoral abnormalities in both their rod and their cone bipolar pathways (see, for example, Refs 78–81). The finding of a transient as well as a sustained rod response in cones and in the cell bodies of the H1 cells[28,30] suggests that rod–cone gap junctions in the normal retina can transmit high as well as low temporal frequencies.

The phase data in Fig. 4A,B imply that the predominant fast rod signal at low frequencies must have the same sign as the slow signal, as they tend towards 0° at 0 Hz. However, as noted by Stockman *et al.*[52], at frequencies above 10 Hz the data, particularly those for the rod monochromat, might also be consistent with the fast rod signal being opposite in sign to the slow signal, as the higher frequency data extrapolate to −180° at 0 Hz. A speculative conclusion is that the 'fast' rod signal is actually composed of two signals: (1) a sluggish ON signal transmitted by rod–cone gap junctions that predominates at low frequencies, which can be seen most clearly in the CSNB data; and (2) a faster OFF signal transmitted by rod to OFF cone bipolar connections that predominates at frequencies above 10 Hz, which can be seen most clearly in the rod monochromat data. Overall, however, the simpler model seems more plausible.

Concluding remarks: multiple pathways

It might be unrealistic to impose a simple two- or even three-pathway scheme on the maze of largely unexplored post-receptoral circuitry in the mammalian retina. The rod signals that we identify as 'slow' and 'fast' might derive from several sources. The fast rod

Box 3. Multiple rod signals: alternative explanations

Psychophysical and electroretinographic evidence in man is consistent with slow and fast rod signals being conveyed through the retina by the rod to rod–bipolar pathway and by the rod to cone to cone–bipolar pathway, respectively. However, other possible anatomical or physiological substrates for the two types of signal exist.

Two types of rod receptor

Hecht *et al.* postulated that the duality found in rod-mediated human flicker functions corresponds to detection by two types of rod photoreceptor[a]. Although there is evidence for such a bimodality in skate[b] (which also have ON and OFF rod bipolars[c]) and perhaps in the ground squirrel[d], there is no evidence for this bimodality in the cat, the monkey or in humans.

Two signals from one rod

More than one signal could arise from within the same rod. Voltage recordings of the primate photoreceptor response reveal an early transient phase of the rod response that emerges at higher scotopic intensities[e]. The interaction between the early transient response and the more-sluggish response will lead to sensitivity losses at some frequencies. Fourier transforms of published photovoltage responses[e] indicate that the principal frequency at which such losses occur increases from 22 to 40 Hz with increasing flash strength, but is never as low as the frequency (15 Hz) at which flicker cancellation between the two signals occurs in humans.

Horizontal cells

A slow rod signal could derive from the axon-terminal system of the H1 horizontal cells feeding back onto rods. Such a scheme is improbable, however, as the effect of the H1 signal on rods is likely to be inhibitory and, therefore, of the wrong sign. Alternatively, horizontal cells could reduce the effective delay of the signals they transmit by increasing the transience of the signal[f,g]; raising the possibility that the H1 signal might be the source of the fast rod signal. However, once again, the effect of such a signal is likely to be inhibitory and therefore of the wrong sign.

References

a **Hecht, S. *et al.*** (1948) *J. Gen. Physiol.* 31, 459–472
b **Green, D.G. and Siegel, I.M.** (1975) *Science* 188, 1120–1122
c **Schlemermeyer, E. and Chappell, R.L.** (1996) *J. Neurocytol.* 25, 625–635
d **Jacobs, G.H.** (1990) *Visual Neurosci.* 5, 311–318
e **Schneeweis, D.M. and Schnapf, J.L.** (1995) *Science* 268, 1053–1056
f **Ashmore, J.F. and Falk, G.** (1979) *Vis. Res.* 19, 419–423
g **Ashmore, J.F. and Falk, G.** (1980) *J. Physiol.* 300, 115–150

signal, in particular, could be composed of any rod signals that avoid the primary rod pathway and its gain control, whether they are ON signals that travel by way of rod–cone gap junctions or OFF signals that travel directly from rods to OFF bipolar cells.

Even the assumption that the slow signal is unitary might be an oversimplification. In the cat, amacrine cells other than the AII cells, such as the rod-dominated A17 amacrine cells[82], might be involved in rod-signal transmission near absolute threshold[74]. There are, in addition, amacrine cells with mixed rod and cone inputs, including the A6, A8, A13 and A14 cells (Ref. 14), which have yet to be fully characterized physiologically and to be incorporated into functional models (for a recent description of some mammalian amacrine-cell properties, see Ref. 83).

Multiple rod circuits might be required because each circuit is only able to operate effectively over a limited range of intensities (see Ref. 27). Thus, the necessarily high sensitivity of the primary rod system might cause it to saturate at low mesopic intensities, after which less-sensitive pathways with shorter integration times take over rod-signal transmission. Alternatively, the use of multiple circuits might be a means of speeding up the rod response as more photons become available and as high sensitivity becomes less important.

Whatever the utility of the multiple circuits, the transition between them, which is presumably achieved in part by the modulation of intercellular coupling (for a recent review, see Ref. 84), is normally smooth and unremarkable. At 15 Hz, however, the harmony breaks down. Destructive interference between the circuits stills the flicker percept to nothingness and offers a unique insight into the inner workings of the retina.

Selected references

1 **Daw, N.W., Jensen, R.J. and Brunken, W.J.** (1990) *Trends Neurosci.* 13, 110–115
2 **Rodieck, R.W.** (1998) *The First Steps in Seeing*, Sinauer
3 **Kolb, H. and Famiglietti, E.V.** (1974) *Science* 186, 47–49
4 **Wässle, H.** (1999) in *Color Vision: From Genes to Perception* (Gegenfurtner, K. and Sharpe, L.T., eds), pp. 447–480, Cambridge University Press
5 **Sterling, P., Freed, M. and Smith, R.G.** (1986) *Trends Neurosci.* 9, 186–192
6 **Boycott, B.B. and Dowling, J.E.** (1969) *Philos. Trans. R. Soc. London Ser. B* 255, 109–184
7 **Dacheux, R.F. and Raviola, E.** (1986) *J. Neurosci.* 6, 331–345
8 **Wässle, H. and Boycott, B.B.** (1991) *Physiol. Rev.* 71, 447–480
9 **Missotten, L.** (1965) *The Ultrastructure of the Human Retina*, Arscia Uitgaven
10 **Dowling, J.E. and Boycott, B.B.** (1966) *Proc. R. Soc. London B Biol. Sci.* 166, 80–111
11 **Kolb, H.** (1970) *Philos. Trans. R. Soc. London Ser. B* 258, 261–268
12 **Rao-Mirotznik, R. *et al.*** (1995) *Neuron* 14, 561–569
13 **Morigiwa, K. and Vardi, N.** (1999) *J. Comp. Neurol.* 405, 173–184
14 **Kolb, H. and Nelson, R.** (1983) *Vis. Res.* 23, 301–302
15 **McGuire, B.A., Stevens, J.K. and Sterling, P.** (1984) *J. Neurosci.* 4, 2920–2938
16 **Chun, M-H. *et al.*** (1993) *J. Comp. Neurol.* 332, 421–432
17 **Wässle, H. *et al.*** (1995) *J. Comp. Neurol.* 361, 537–551
18 **Famiglietti, E.V. and Kolb, H.** (1975) *Brain Res.* 84, 293–300
19 **Pourcho, R.G. and Goebel, D.J.** (1985) *J. Comp. Neurol.* 233, 473–480
20 **Müller, F., Wässle, H. and Voight, T.** (1988) *J. Neurophysiol.* 59, 1657–1672
21 **Raviola, E. and Gilula, N.B.** (1975) *J. Cell Biol.* 65, 192–222
22 **Kolb, H.** (1977) *J. Neurocytol.* 6, 131–153
23 **Nelson, R.** (1977) *J. Comp. Neurol.* 172, 109–136
24 **Nelson, R. and Kolb, H.** (1983) *Vis. Res.* 23, 1183–1195
25 **Nelson, R. *et al.*** (1985) in *Neurocircuitry of the Retina: a Cajal Memorial* (Gallego, A. and Gouras, P., eds), pp. 109–121, Elsevier Science
26 **Ahnelt, P.K., Keri, C. and Kolb, H.** (1990) *J. Comp. Neurol.* 293, 39–53
27 **Smith, R.G., Freed, M.A. and Sterling, P.** (1986) *J. Neurosci.* 6, 3505–3517
28 **Schneeweis, D.M. and Schnapf, J.L.** (1995) *Science* 268, 1053–1056
29 **Verweij, J., Dacey, D.M. and Buck, S.L.** (1998) *Invest. Ophthalmol. Vis. Sci.* 39, S209
30 **Verweij, J. *et al.*** (1999) *Vis. Res.* 39, 3662–3672
31 **Nelson, R. *et al.*** (1975) *Science* 189, 137–139
32 **Rodieck, R.W.** (1988) in *Comparative Primate Biology: Neurosciences* (Vol. 4) (Steklis, H.D. and Erwin, J., eds), pp. 203–278, A.R. Liss
33 **Soucy, E. *et al.*** (1998) *Neuron* 21, 481–493
34 **Muller, J.F., Lukasiewicz, P.D. and Silverman, M.S.** (1993) *Invest. Ophthalmol. Vis. Sci.* 34, S984
35 **Brandstätter, J.H., Hack, I. and Peichl, L.** (1999) *Invest. Ophthalmol. Vis. Sci.* 40, S790

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36 West, R.W. (1978) *Vis. Res.* 18, 129–136
37 Boycott, B.B. and Wässle, H. (1991) *Eur. J. Neurosci.* 3, 1069–1088
38 Hopkins, J.M. and Boycott, B.B. (1995) *J. Neurocytol.* 24, 680–694
39 Hopkins, J.M. and Boycott, B.B. (1996) *J. Neurocytol.* 25, 381–390
40 Hopkins, J.M. and Boycott, B.B. (1997) *J. Neurocytol.* 26, 313–325
41 Sterling, P. (1998) *Neuron* 21, 643–644
42 Raviola, E. and Gilula, N.B. (1973) *Proc. Natl. Acad. Sci. U. S. A.* 70, 1677–1681
43 Hecht, S. *et al.* (1938) *Am. J. Physiol.* 123, 94–95
44 Hecht, S. *et al.* (1948) *J. Gen. Psychol.* 31, 459–472
45 Alpern, M., Falls, H.F. and Lee, G.B. (1960) *Am. J. Ophthalmol.* 50, 996–1012
46 Blakemore, C.B. and Rushton, W.A.H. (1965) *J. Physiol.* 181, 612–628
47 Hess, R.F. and Nordby, K. (1986) *J. Physiol.* 371, 365–385
48 Conner, J.D. and MacLeod, D.I.A. (1977) *Science* 195, 689–699
49 Stockman, A. *et al.* (1991) *J. Opt. Soc. Am.* 8, 1657–1665
50 Sharpe, L.T., Stockman, A. and MacLeod, D.I.A. (1989) *Vis. Res.* 29, 1539–1559
51 Conner, J.D. (1982) *J. Physiol.* 332, 139–155
52 Stockman, A. *et al.* (1995) *Visual Neurosci.* 12, 951–970
53 Kohl, S. *et al.* (1998) *Nat. Genet.* 19, 257–259
54 Sharpe, L.T. and Nordby, K. (1990) in *Night Vision: Basic, Clinical and Applied Aspects* (Hess, R., Sharpe, L.T. and Nordby, K., eds), pp. 335–389, Cambridge University Press
55 Larsen, H. (1921) *Klin. Monatsbl. Augenheilkunde* 67, 301–302
56 Harrison, D., Hoefnagel, D. and Hayward, J.N. (1960) *Arch. Ophthalmol.* 64, 685–692
57 Falls, H.F., Wolter, J.R. and Alpern, M. (1965) *Arch. Ophthalmol.* 74, 610–616
58 Glickstein, M. and Heath, G.G. (1975) *Vis. Res.* 15, 633–636
59 Schubert, G. and Bornschein, H. (1952) *Ophthalmologica* 123, 396–413
60 Miyake, Y. *et al.* (1986) *Arch. Ophthalmol.* 104, 1013–1020
61 Carr, R.E. *et al.* (1966) *Invest. Ophthalmol. Vis. Sci.* 5, 497–507
62 Alpern, M., Holland, M.G. and Ohba, N. (1972) *J. Physiol.* 225, 457–476
63 Goodman, G. and Bornschein, H. (1957) *Arch. Ophthalmol.* 58, 174–182
64 Brown, K.T. and Watanabe, K. (1962) *Nature* 193, 958–960
65 Carr, R.E. and Siegel, I.M. (1964) *Am. J. Ophthalmol.* 58, 95–107
66 Carr, R.E. *et al.* (1966) *Invest. Ophthalmol. Vis. Sci.* 5, 508–514
67 Ripps, H. (1982) *Invest. Ophthalmol. Vis. Sci.* 23, 588–609
68 Nelson, R., Kolb, H. and Freed, M.A. (1993) *J. Comp. Neurol.* 329, 68–84
69 Sterling, P., Freed, M. and Smith, R.G. (1988) *J. Neurosci.* 8, 623–642
70 Smith, R.G. and Vardi, N. (1995) *Visual Neurosci.* 12, 851–860
71 van Rossum, M.C.W. and Smith, R.G. (1998) *Visual Neurosci.* 15, 809–821
72 Barlow, H.B., Levick, W.R. and Yoon, M. (1971) *Vis. Res.* (Suppl.) 3, 87–101
73 Mastronarde, D.N. (1983) *J. Neurophysiol.* 49, 325–349
74 Nelson, R. (1982) *J. Neurophysiol.* 47, 928–947
75 Frishman, L.J. and Steinberg, R.H. (1989) *J. Neurophysiol.* 61, 1233–1243
76 Sieving, P.A. and Wakabayashi, K. (1991) *Clin. Vis. Sci.* 6, 171–179
77 Steinberg, R.H., Frishman, L.J. and Sieving, P.A. (1991) in *Progress in Retinal Research* (Vol. 10) (Osborne, N. and Chader, G., eds), pp. 121–160, Pergamon Press
78 Krill, A.E. and Martin, D. (1974) *Invest. Ophthalmol. Vis. Sci.* 10, 625–636
79 Hill, D.A., Arbel, K.F. and Berson, E.L. (1974) *Am. J. Ophthalmol.* 78, 127–136
80 Miyake, Y. *et al.* (1987) *Jpn. J. Ophthalmol.* 31, 81–87
81 Houchin, K.W., Purple, R.L. and Wirtschafter, J.D. (1991) *Invest. Ophthalmol. Vis. Sci.* (Suppl.) 32, 1229
82 Kolb, H. and Nelson, R. (1981) *Vis. Res.* 21, 1625–1633
83 MacNeil, M.A. and Masland, R.H. (1998) *Neuron* 20, 971–982
84 Baldridge, W.H., Vaney, D.I. and Weiler, R. (1998) *Semin. Cell Dev. Biol.* 9, 311–318

Chemokines in the CNS: plurifunctional mediators in diverse states

Valérie C. Asensio and Iain L. Campbell

The past decade has witnessed the remarkable ascendance of chemokines as pivotal regulatory molecules in cellular communication and trafficking. Evidence increasingly implicates chemokines and chemokine receptors as plurifunctional molecules that have a significant impact on the CNS. Initially, these molecules were found to be involved in the pathogenesis of many important neuroinflammatory diseases that range from multiple sclerosis and stroke to HIV encephalopathy. However, more-recent studies have fuelled the realization that, in addition to their role in pathological states, chemokines and their receptors have an important role in cellular communication in the developing and the normal adult CNS. For example, stromal-cell-derived factor 1, which is synthesized constitutively in the developing brain, has an obligate role in neurone migration during the formation of the granule-cell layer of the cerebellum. Many chemokines are capable of directly regulating signal-transduction pathways that are involved in a variety of cellular functions, which range from synaptic transmission to growth. Clearly, the potential use of chemokines and their receptors as targets for therapeutic intervention in CNS disease might now have to be considered in the context of the broader physiological functions of these molecules.

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THE TERM 'CHEMOKINE' was originally adopted to describe a family of chemoattractant cytokines that were, on the whole, smaller than the inflammatory cytokines and exhibited a characteristic N-terminal cysteine motif (see below). In general, chemokines are small proteins (8 to 10 kD) that induce chemotaxis, tissue extravasation and functional modulation of a wide variety of leukocytes during inflammation[1,2]. In view of these properties, intense research efforts have focused recently on the possible involvement of chemokines

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