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The Circadian Clock in the Retina Controls Rod-Cone Coupling

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SUMMARY

Although rod and cone photoreceptor cells in the vertebrate retina are anatomically connected or coupled by gap junctions, rod-cone coupling is thought to be weak. By using a combination of tracer labeling and electrical recording in the goldfish retina and tracer labeling in the mouse retina, we show that the retinal circadian clock, a local endogenous process, and not the retinal response to the visual environment, controls the extent and strength of rod-cone coupling by activating dopamine D_2-like receptors in the day, so that rod-cone coupling is weak during the day but remarkably robust at night. The results demonstrate that circadian control of rod-cone coupling serves as a synaptic switch between the rod and cone pathways, so that cones and neurons postsynaptic to cones receive very dim light signals from rods at night, but not in the day. The increase in the strength and extent of rod-cone coupling at night may enhance the reliability of the rod light response and facilitate the detection of large dim objects.

INTRODUCTION

Vision begins in the retina when rod and cone photoreceptor cells detect visual images and transduce them into neural signals. Rods and cones primarily function under different lighting conditions in that rods mediate dim light (scotopic) vision at night and cones mediate bright light (photopic) vision during the day (Dowling, 1987), enabling the retina to operate over the ~10 billion-fold change in ambient light intensity that occurs daily on a sunny day compared to a moonless night. Although ganglion cells, the output neurons of the retina that signal more central brain areas, indirectly receive both rod and cone input, the synaptic mechanisms and neural circuits that mediate the switch between rod pathway function at night and cone pathway function in the day remain largely unknown. Rod signals can reach ganglion cells via at least two separate pathways in all vertebrate species that have both rods and cones (Bloomfield and Dacheux, 2001; Copenhagen, 2004). Rods signal bipolar cells at chemical synapses. In addition, in both mammalian and non-mammalian retinas, rods and cones are anatomically connected or coupled by gap junctions (Raviola and Gilula, 1973; Dowling, 1987; Bloomfield and Dacheux, 2001; Copenhagen, 2004), a type of electrical synapse (Bennett and Zukin, 2004; Connors and Long, 2004) at which rod input

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can enter the cone circuit and thereby reach ganglion cells. However, evidence to date suggests that rod-cone coupling is relatively weak (Yang and Wu, 1989; Krizaj et al., 1998; Hornstein et al., 2005).

The circadian (24-hr) clock in the retina (Green and Besharse, 2004; Iuvone et al., 2005) regulates rod and cone pathways by activating dopamine D_2-like receptors in the day (Wang and Mangel, 1996; Barlow, 2001; Ribelayga et al., 2002, 2004). For example, by increasing dopamine release and activating D_2-like receptors in the day, the retinal clock regulates rod and cone input to fish cone horizontal cells (Wang and Mangel, 1996; Ribelayga et al., 2002, 2004), second order cells that receive synaptic contact from cones, but not from rods (Stell and Lightfoot, 1975). Due to the action of the clock, cone input to cone horizontal cells dominates during the day and rod input dominates at night (Wang and Mangel, 1996; Ribelayga et al., 2002, 2004). Because in most vertebrate species, including fish and mammals 1) rods and cones are connected by gap junctions (Raviola and Gilula, 1973; Bloomfield and Dacheux, 2001; Copenhagen, 2004), 2) D_2-like receptors are expressed by rods and cones, but not by horizontal cells (Cohen et al., 1992; Yazulla and Lin, 1995; Witkovsky, 2004), and 3) the retina contains a circadian clock (Green and Besharse, 2004; Iuvone et al., 2005), we directly tested the hypothesis that rod input reaches cones and then cone horizontal cells at night due to an increase in rod-cone electrical coupling.

RESULTS

Tracer coupling between rods and cones

We examined the extent of rod-cone tracer coupling during the subjective day (Circadian Time (CT) 2–10) and subjective night (CT 14–22) of a circadian cycle (i.e. constant darkness and temperature) and following 1 hr of dark adaptation during the day (Zeitgeber Time (ZT) 2–10) and night (ZT 14–22) of a regular 12-hr light /12-hr dark cycle (see Experimental Procedures). Under these dark-adapted conditions, when biocytin tracer was iontophoresed into individual cones, the tracer was restricted on average to a few rods (2 ± 1 (s.e.m.)) and cones (3 ± 1) near the recorded cones during the day and subjective day (Figs. 1A, 2A), indicating weak rod-cone coupling. During the night and subjective night (Figs. 1B, 2A), tracer staining was found in numerous rods (1,265 ± 277) and cones (102 ± 19), indicating strong rod-cone coupling. The average diameter of the tracer coupled rods and cones during the night and subjective night was ~ 500 μm. The extent and the day/night difference in tracer coupling were not affected by dim light (−5 log *Io* 500 ms-light flashes at 0.125 Hz for > 60 min) adaptation in the mesopic range (i.e. intensities to which both rods and cones normally respond) (Figs. 1E, 1F, 2B). In contrast, tracer remained in the injected cone in both the day and night following bright light (-2 log *Io* 500 ms-light flashes at 0.125 Hz for > 60 min) adaptation in the photopic range (i.e. intensities to which cones, but not rods, normally respond) (Figs. 1G, 1H, 2B), indicating that bright light, but not dim light, adaptation can override the effects of the clock.

Because the circadian clock in the fish retina increases dopamine release and activates D_2-like receptors in the subjective day (Ribelayga et al., 2002, 2004), we examined the effects of dopamine ligands on the extent of rod-cone tracer coupling during the subjective day. Application of the selective D_2-like receptor antagonist spiperone (10 μM) for > 1 hr during

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the subjective day increased rod-cone tracer coupling to a similar extent to that observed at night (Figs. 1C, 2A). In contrast, application of the D_1-like receptor antagonist SCH23390 (10 μM) during the subjective day had no effect on rod-cone coupling, that is, the tracer was detected in the injected cone only ($n = 3$, data not shown). In addition, the extent of tracer coupling was also restricted to the injected cone during the subjective night following the application of the D_2-like receptor agonist quinpirole (1 μM) (Fig. 1D, 2A). We therefore conclude that the circadian clock in the fish retina decreases rod-cone coupling in the day by activating D_2- like, and not D_1-like, receptors.

Cone light responses in the day and night

To determine whether changes in the extent of tracer coupling between photoreceptors alter cone light responses, whole-cell patch-clamp recordings from the inner segments of individual cones in intact goldfish retinas were obtained during the subjective day, subjective night, day, and night. The light responses of dark-adapted cones during the day and subjective day were similar to those previously reported (Palacios et al., 1998). Light response threshold was ~ −5.5 log *Io*, response amplitude increased with increasing light intensity, and response duration was similar to stimulus duration (Figs. 3A, 4A, 4C, 5). In contrast, dark-adapted cones at night responded to light in the scotopic range (i.e. intensities to which rods, but not cones, normally respond) with a response threshold of ~ −7.5 log *Io* (Figs. 3A, 4A, 4C), indicating the presence of substantial rod input to cones. Moreover, cone responses at night were significantly slower and smaller in amplitude (Fig. 3A). That is, response duration was significantly longer than stimulus duration (Figs. 3A, 5A, 5E), and response latency and time-to-peak were significantly longer than occurred in the day (Figs. 3A, 5C and 5D) ($p < 0.001$ for each response measure, Tukey *post-hoc* test). In addition, the length constant λ, a measure of the cone receptive field size that was derived from the spot size-response data (see Experimental Procedures) shown in Fig. 4D, was larger at night (48 ± 2 μm) compared to the day (10 ± 1 μm, $p < 0.001$, Student's t-test). These observations are consistent with the tracer labeling results and indicate that rods signal cones during the subjective night, but not during the subjective day, demonstrating that rod-cone gap junctions are functionally open at night, but not in the day. Moreover, the receptive field size measurements show that the spatial tuning of photoreceptor cells is increased at night due to the extensive coupling between rods and cones at night.

Because cone light responses are slower in the subjective night, compared to the subjective day (Figs. 3A, 5C, 5D), we examined whether the dominant rod signal to cones at night, but not in the day, can account for the circadian difference in cone light response kinetics, by comparing the response kinetics of cones and rod horizontal cells during the subjective night. As shown in Fig. 5A, the response waveforms of cones and rod horizontal cells to light stimuli of similar intensity closely match during the subjective night, but not during the subjective day, a finding that strongly suggests that the circadian difference in cone light response kinetics is primarily due to the increase in rod-cone coupling at night, that is, to the fact that cone responses at night, but not in the day, are dominated by the slower rod signals. Thus, although circadian control of cone phototransduction, ionic mechanisms, and voltage dependent conductances may play a role in the day/night difference in cone response kinetics, the primary means by which the circadian clock regulates cone light response

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kinetics is by controlling rod-cone coupling. This conclusion is also supported by the finding that the cone dark resting potential did not significantly change in the day and night under dark-adapted conditions: – 30.1 ± 0.7 mV during the day (n = 10), – 33.3 ± 1.1 mV during the subjective day (n = 15), – 32.6 ± 1.6 mV during the night (n = 14), and – 33.2 ± 1.2 mV during the subjective night (n = 6). Measurements of cone I-V curves also demonstrate that the cone resting potential was similar in the subjective day and night (Fig. 5F). Finally, the cone input resistance, which was derived from its I-V curve (Fig. 5F), was lower at night (394 ± 34 MΩ) compared to the day (1,359 ± 134 MΩ, $p < 0.001$, Student's t-test), a finding consistent with an increase in photoreceptor coupling at night.

Application of spiperone for > 1 hr during the subjective day decreased response threshold by ~ 2 log units and modified other response characteristics so that cone light responses resembled those observed at night (Figs. 3A, 4A, 5C–E) ($p < 0.01$ compared to the day and $p > 0.05$ compared to the night for each response measure, Tukey *post-hoc* test). In contrast, application of quinpirole for > 1 hr during the subjective night increased response threshold by ~ 2 log units and modified other response characteristics so that cone light responses resembled those observed during the day (Figs. 3A, 4A, 5C–E) ($p < 0.05$ compared to the night and $p > 0.05$ compared to the day for each response measure, Tukey *post-hoc* test). The half-saturating intensity was significantly greater during the day (−3.96 ± 0.36 log *Io*) and at night in the presence of quinpirole (−4.05± 0.32 log *Io*) than at night under control conditions (−5.55 ± 1.27 log *Io*; $p < 0.05$) and during the subjective day in the presence of spiperone (−5.25 ± 1.66 log *Io*; $p < 0.05$). There was no difference between night and subjective day + spiperone ($p > 0.05$) and day and subjective night + quinpirole ($p > 0.05$, Tukey *post-hoc* test) (Fig. 4A). Finally, application of SCH23390 during the subjective day did not affect the light responses of dark-adapted cones (n = 7, data not shown).

Following dim light adaptation for > 60 min during the day and night, cone light responses were similar to those observed under dark-adapted conditions during the day and night, respectively (Figs. 3B and 4B). The half-saturating intensity was significantly different between the day (−3.82 ± 0.76 log *Io*) and night (−5.60 ± 1.39 log *Io*; $p < 0.01$, Tukey *post-hoc* test). Together with the effects of dim light adaptation on tracer coupling, these observations demonstrate that light in the mesopic range does not affect rod-cone coupling. Thus, under normal conditions at night (i.e. ambient light level in the scotopic to mesopic range) the retinal clock, and not dim (scotopic to mesopic) light, increases rod-cone coupling around dusk and decreases rod-cone coupling at dawn.

Following bright light adaptation for > 60 min, cone light responses were similar in both the day and night ($p > 0.05$ for each response measure, Tukey *post-hoc* test), indicating that bright light adaptation overrides circadian control. Specifically, as observed under dark-adapted conditions during the day, response amplitude increased with increasing light intensity, and response duration was similar to stimulus duration (Fig. 3B, 5D and 5E). The half-saturating intensity was not significantly different between day (−3.47 ± 0.41 log *Io*) and night (−3.58 ± 0.41 log *Io*; $p > 0.05$) but day and night under bright light-adapted conditions were both different from night under dim light-adapted conditions ($p < 0.001$, Tukey *post-hoc* test) (Fig. 4B). These observations are consistent with the "masking effect" of bright light on circadian systems, including the retinal clock (Green and Besharse, 2004

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for review), but do not indicate that bright light adaptation normally alters rod-cone coupling.

Because previous studies have reported that bright light stimulation of dark-adapted amphibian retinas introduces a “rod plateau potential” into cone light responses by slightly increasing rod-cone coupling (Yang and Wu, 1989; Krizaj et al., 1998), we examined whether the duration of cone responses to bright stimuli (i.e. > -2 log *Io*) in the day depended on whether the retinas were previously dark-adapted or bright light-adapted. We found that the duration of cone light responses was longer under dark-adapted conditions due to the presence of a long-lasting (several seconds) hyperpolarizing after-potential or plateau potential (Figs. 5B, 5E). However, the after-potential was eliminated when bright light stimulation continued for > 5 minutes (Fig. 5B). Because after-potentials are believed to be rod-driven, these observations suggest that the introduction of a plateau potential in the cone response to bright light stimulation following dark adaptation is due to a transient increase in rod-cone coupling. Moreover, bright light stimulation of previously light-adapted (≥60 min) goldfish retinas in the day did not evoke a long-lasting after-potential in cones, and more generally, had no effect on cone light responses or on tracer coupling between rods and cones (Figs. 1–4). These results thus suggest that bright light stimulation in the day of fish and amphibian retinas produces a small, transient increase in rod-cone coupling if the retinas are dark-adapted immediately prior to the light stimulation. However, bright light stimulation in the day of previously bright light-adapted retinas, which normally occurs in midday, does not alter rod-cone coupling.

Cone spectral sensitivity in the day and night

In addition to the clear day/night differences in cone light response threshold and kinetics, the spectral sensitivity of dark-adapted cones was different in the day, compared to the night (Fig. 6A). That is, based on their spectral sensitivity properties under dark-adapted conditions during the day or subjective day, recorded cones could be distinguished into three types: L (λ_{max} ~ 608 nm), M (λ_{max} ~ 539 nm), and S (λ_{max} ~ 451 nm) (Palacios et al., 1998; Govardovskii et al., 2000). In contrast, at night all dark- adapted cones were most sensitive to green wavelength light (λ_{max} ~ 535 nm). The spectral sensitivity of dark-adapted cones at night closely resembled that of goldfish rods (Govardovskii et al., 2000) for λ < 600 nm, but exhibited a higher sensitivity than rods to λ > 600 nm (Fig. 6A), suggesting that red cones contribute to the rod-dominated spectral sensitivity of cones at night. The threshold stimulus (500 nm) intensity of the rod signal in cones at night was ~ 0.08 Rh*/rod/0.5 s on the retina, indicating that cones respond to light in the low scotopic range at night, when the background intensity is in the low scotopic range. In addition, the spectral sensitivity curve of cones recorded during the subjective day in the presence of spiperone was very similar to the spectral sensitivity curve obtained during the subjective night (Fig. 6A). The spectral sensitivity curves of 2 green cones and 1 red cone in the presence of quinpirole at night were similar to those of green and red cones, respectively, during the subjective day (data not shown). Finally, SCH23390 applied during the day did not affect cone spectral sensitivity (n = 1 red cone, data not shown). Together these data indicate that endogenous activation of D_2-like receptors in the day eliminates rod input to cones.

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Although all dark-adapted cones at night were most sensitive to green wavelength light (λ_{max} ~ 535 nm), bright light-adapted cones at night (i.e. following bright light adaptation for > 60 min) could be distinguished into three types: L (λ_{max} ~ 608 nm), M (λ_{max} ~ 539 nm), and S (λ_{max} ~ 451 nm) based on their spectral sensitivity (Fig. 6B), as observed for dark-adapted cones during the day.

Mouse rod-cone tracer coupling

In order to determine whether the day/night difference in rod-cone coupling observed in goldfish occurred in a mammalian retina as well, we investigated whether the extent of tracer coupling between photoreceptors in mice, as measured by intercellular propagation of Neurobiotin after “cut-loading” (see Experimental Procedures), depends on the time of day under dark-adapted conditions. Goldfish retinas were processed at the same time as a control. In goldfish retinas, the diffusion of Neurobiotin, as revealed by the fluorescence intensity of Alexa488, was restricted to the edge of the cut during the day (Fig. 7A, 7G, length constant (λ) = 4.8 ± 0.1 μm), but was observed as far as 200 μm from the cut at night (Fig. 7B, 7G, λ= 36.0 ± 0.9 μm) or during the day in the presence of spiperone (Fig. 7C, 7G, λ= 39.0 ± 1.1 μm; $p < 0.001$, Tukey *post-hoc* test). In mouse, fluorescence could be seen in cells that were adjacent to the cut during the day (Fig. 7D, 7H, λ= 4.5 ± 0.1 μm). Based on their morphology, these cells were mostly cones (Fig. 7D2). In contrast, fluorescence was detected in both cones and rods during the night as far as 60 μm from the cut (Fig. 7E, 7H, λ= 18.6 ± 0.7 μm) and during the day in the presence of spiperone (Fig. 7F, 7H, λ= 14.1 ± 0.4 μm; $p < 0.001$, Tukey *post-hoc* test). The exponential decrease in fluorescence intensity as a function of distance from the cut in all cases examined (Figs. 7G, 7H) indicates that Neurobiotin entered the photoreceptors via the cut and not from other sites. Moreover, the qualitatively similar day/night difference observed in goldfish with tracer injections into single cones (Figs. 1, 2) and with cut-loading substantiates the use of cut-loading as a technique to investigate the extent of photoreceptor coupling in the mammalian retina. These findings thus indicate that in mammals, as well as in fish, the retinal circadian clock controls rod-cone coupling by activating D_2-like receptors during the day, so that rod-cone coupling is weak during the day, but remarkably robust at night.

DISCUSSION

The data presented here thus demonstrate that the circadian clock in the goldfish retina, and not the retinal response to the level of ambient illumination, regulates rod-cone electrical coupling by activating D_2-like receptors in the day, so that coupling is weak during the day but robust at night. The results further indicate that rod input reaches fish cone horizontal cells at night via rod-cone gap junctions, demonstrating that the increased strength of rod-cone electrical coupling at night shapes in part the light responses of second order neurons that are part of the cone circuit (Wang and Mangel, 1996; Ribelayga et al., 2002, 2004). Moreover, our observations on both goldfish and mouse photoreceptor cells strongly suggest that the retinal clock controls the strength of rod-cone coupling in most, if not all, mammalian and non-mammalian retinas that have both rod and cone photoreceptors (duplex retinas). Circadian clock control of the strength of the electrical synapses between rods and cones thus serves as a synaptic switch that allows rod input to reach the cone circuit at night,

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but not in the day. We have also observed that repetitive dim light stimulation in the scotopic to mesopic range did not alter the effects of the clock on rod-cone coupling and on cone light responses (Figs. 1–4), a finding that indicates that the retinal clock, and not the retinal response to the normal visual environment at night, controls rod-cone coupling.

Based primarily on studies of individual, dissociated rods and cones, it has been accepted that rods, but not cones, respond to very dim light stimuli and operate at low light levels at night. Our results, however, demonstrate that cones in the intact retina respond to very dim light stimuli at night, but not in the day. In fact, the threshold light response of cones (Fig. 4) and cone horizontal cells at night was in the low scotopic range, indicating that cones and cone horizontal cells can detect very dim light signals from rods at night. The clock-induced increase in rod-cone coupling at night may not have been previously observed because prior studies did not investigate cone light responses or rod-cone coupling in an intact retina preparation at night under dark-adapted conditions. Our results are the first to describe the light responses of dark-adapted cones at night in an intact retina and show that these responses are dramatically different from those observed when retina preparations are sliced, bright light-adapted, or studied in the day. The limited rod-cone tracer coupling that we observed under dark-adapted conditions in the day (Figs. 1, 2, 7) is in agreement with previous reports of weak rod-cone coupling under dark-adapted conditions in the day in amphibian (Yang and Wu, 1989; Krizaj et al., 1998) and primate (Hornstein et al., 2005) retinas.

When the retinal clock increases the strength of rod-cone coupling at night, rod input dominates both cone and cone horizontal cell light responses because rods outnumber cones in the goldfish retina by 8 to 15-fold (Stell and Harosi, 1976), so that cone light signals are shunted by the strong coupling between rods and cones at night. This accounts for the lack of cone input to goldfish rod horizontal cells during the subjective night (Wang and Mangel, 1996; Ribelayga and Mangel, 2007). In addition, the observation that the spectral sensitivity of all cones is similar to that of rods at night (Fig. 6) is consistent with the findings that the spectral sensitivity of H1 cone horizontal cells (Wang and Mangel, 1996) and of H2 and H3 cone horizontal cells (Wang and Mangel, unpublished observations) are similar to that of rods during the subjective night, but not during the subjective day.

The finding that rod input dominates cone responses to dim light stimuli during the subjective night, but that rod input cannot be detected in cones during the subjective day (Figs. 3A, 4A, 4C, 5, 6A), indicates that the retinal clock controls the coupling strength of rod-cone gap junctions and demonstrates that rod-cone gap junctions are functionally open at night, but not in the day. Moreover, the finding that the average ratio of coupled rods to cones during the night and following spiperone application during the subjective day was ~ 12 (Fig. 2A), a value that is in close agreement with the ratio of rods to cones in the goldfish retina, is consistent with a clock-mediated increase in rod-cone coupling at night. Although it is possible that the clock increases the conductance of cone-cone and/or rod-rod gap junctions at night, in addition to increasing rod-cone coupling, the increase in the conductance of rod-cone gap junctions at night effectively increases electrical and cellular communication between cones and cones and between rods and rods, as well as between rods and cones.

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In fish, the present results and previous findings (Wang and Mangel, 1996; Ribelayga et al., 2002, 2004; Iuvone et al., 2005) indicate that the circadian clock in the retina regulates the light responses of cones and cone horizontal cells in part according to the scenario illustrated in Fig. 8. The retinal clock increases dopamine release from dopaminergic interplexiform cells (Dowling, 1987) during the subjective day by decreasing the synthesis and release of melatonin, which inhibits dopamine release (Ribelayga et al., 2004; Iuvone et al., 2005). The resultant increased level of extracellular dopamine then increases activation of the D_2-like receptors on rods and cones (Witkovsky, 2004), which in turn results in a decrease in intracellular cAMP and in protein kinase A activity in the photoreceptor cells. In contrast, during the subjective night the retinal clock reduces extracellular dopamine levels and D_2-like receptor activation, so that intracellular cAMP levels and protein kinase A activity increase. Indirect evidence based on previous studies of goldfish cone horizontal cells in the subjective day and night (Ribelayga et al., 2002, 2004) suggests that an increase in protein kinase A activity at night increases the conductance of rod-cone gap junctions so that rod input dominates cones and cone horizontal cells at night. Based on the finding that rod-cone coupling in mouse retinas is decreased during the subjective day due to endogenous activation of D_2-like receptors (Fig. 7), as occurs in fish, it seems likely that a similar scenario as that shown in Fig. 8, also occurs in mammalian retinas. In fact, previous studies have suggested that rod signals reach horizontal cells via rod-cone gap junctions in the cat (Nelson, 1977) and monkey (Verweij et al., 1999). However, the means by which the clock in the mammalian retina increases dopamine release during the subjective day is unclear, that is, the melatonin rhythm may generate the dopamine rhythm (Iuvone et al., 2005) or a circadian clock in dopaminergic cells may directly control dopamine metabolism (Ruan et al., 2006). Finally, as proposed previously (Ribelayga and Mangel, 2003, 2007), although the clock-driven increase in extracellular dopamine during the day is sufficient to activate the high affinity D_2-like receptors on rods and cones (Figs. 1–4, 6, 7 here), it is not sufficient to activate the low affinity D_1-like receptors on horizontal cells, which instead are activated by the higher levels of extracellular dopamine produced by bright light during the day. Thus, there may be two complementary dopamine systems in the retina, a circadian clock system that activates high affinity D_2-like receptors at dawn and decreases their activation at dusk, and a bright light system that activates low affinity D_1-like receptors during the day.

Functional and clinical significance

In addition to providing a means by which scotopic signals from rods reach the cone circuit at night, the circadian clock-induced increase in rod-cone coupling at night has at least three other highly significant functional implications. First, in addition to signaling cones at night via rod-cone electrical synapses, many rods converge onto rod bipolar cells (or in non-mammalian retinas many rods converge onto bipolar cells that also receive cone input (Stell et al., 1977)) at highly nonlinear chemical synapses (Field and Rieke, 2002; Copenhagen, 2004), so that the rod pathway pools dim light signals over a large spatial domain (Warrant, 1999). Because the intrinsic noise in each photoreceptor cell is independent of the noise in other photoreceptors, but local correlations within the visual scene produce shared photoreceptor signals, photoreceptor coupling reduces photoreceptor noise more than it reduces their output signals (Lamb and Simon, 1976; Tessier-Lavigne and Attwell, 1988; Copenhagen, 2004), especially for low spatial frequency signals (DeVries et al., 2002;

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Laughlin, 2002). The increased coupling between photoreceptors at night will therefore enhance the signal to noise ratio and the reliability of the rod light response before the signal and noise are distorted by the rod to rod bipolar cell nonlinear synapse. The increase in photoreceptor coupling at night will thus tune the retina to detect large dim objects, an idea that is supported by the finding that cone receptive field size increases at night (Fig. 4D). Viewed from this perspective, circadian control of rod-cone coupling enhances the detection of large dim objects at night and small objects during the day. Moreover, the finding that dark-adapted fish cones at night respond to light in the low scotopic range indicates that extensive coupling between photoreceptors does not impede the transmission of very dim light signals from rods to cones, even though the detection of spatial detail is reduced. In addition, circadian control of rod-cone coupling likely mediates in part the circadian rhythm in visual sensitivity, a phenomenon of retinal origin that has been observed in many vertebrate species, including fish, rat and human (Barlow, 2001).

Second, because functioning electrical synapses can synchronize coupled neurons (Connors and Long, 2004; Bennett and Zukin, 2004), the increase in the strength of rod-cone electrical coupling at night may synchronize the neural and metabolic activity of rods and cones on a daily basis. Moreover, because both rods and cones express circadian clock genes (Green and Besharse, 2004; Iuvone et al., 2005), the strong electrical coupling between rods and cones at night may synchronize the oscillator activity of the circadian clocks in individual rods and cones, thus minimizing phase differences in their oscillations (Connors and Long, 2004).

Finally, photoreceptor survival may depend on the daily increase in rod-cone coupling at night. Specifically, because gap junction channels are large enough to allow not only the flow of electric current, but also the diffusion of intracellular signaling molecules, nutrients, and small metabolites between coupled cells (Bennett and Zukin, 2004; Connors and Long, 2004), our findings suggest that metabolic exchange between rods and cones occurs at night on a daily basis. In fact, cone survival may depend on the presence of healthy rods, as suggested by the delayed death of neighboring cones in rod-cone dystrophy (i.e. retinitis pigmentosa) (Delyfer et al., 2004; Burns and Arshavsky, 2005). Cone survival might depend on the diffusion of nutrients and protective factors from coupled healthy rods (Striedinger et al., 2005) and/or cones might die due to the diffusion of pro-apoptotic factors from coupled dying rods (Ripps, 2002).

In summary, the circadian clock in the retina, and not the retinal response to the level of ambient illumination, controls the extent and strength of rod-cone coupling by activating dopamine D_2-like, but not D_1-like, receptors in the day, so that rod-cone coupling is weak during the day but remarkably robust at night. The clock-controlled increase in rod-cone electrical coupling at night not only provides a highly sensitive pathway for dim light signals from rods to directly enter the cone circuit, but also enhances the reliability of the rod light response and therefore the sensitivity of the rod to rod bipolar cell pathway to large dim light stimuli. The retinal circadian clock thus produces a synaptic switch between rod pathway function at night and cone pathway function in the day. Viewed as an optical device, the retinal clock controls the aperture or receptive field size of photoreceptor cells through which light signals reach the post-photoreceptor retina. It opens wide the aperture at night,

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enhancing the detection of large dim objects, and narrows the aperture during the day, facilitating the detection of small objects.

EXPERIMENTAL PROCEDURES

Goldfish and mouse neural retina preparations

The care and use of goldfish and mice were in accordance with federal and institutional guidelines. Goldfish (*Carassius auratus*), ~ 5 inches long, were housed in a 12-hr light/12-hr dark cycle (with lights-on at 3 a.m.) under constant conditions of temperature (22°C) for at least 2 weeks before an experiment. Fish were dark adapted for at least an hour before surgery. In the case of circadian experiments, fish were dark-adapted for 24–72 hrs before surgery. The circadian time (CT) was thus defined by the projected Zeitgeber time (ZT) from the previous 12-hr light/12-hr dark cycle. Fish were deeply anaesthetized with methanesulfanate (MS222, 150 $mg.L^{-1}$), an eye enucleated and the intact neural retina isolated, as described (Wang and Mangel, 1996; Ribelayga et al., 2002, 2004). Surgery was done using night-vision infrared goggles. The intact neural goldfish retina with photoreceptor side up was superfused at 1 $mL.min^{-1}$ in a 2 mL chamber with saline that contained (in mM) 130 NaCl, 20 $NaHCO_3$, 2.5 KCl, 10 glucose, 1 $MgCl_2$, and 0.7 $CaCl_2$ continuously gassed with 5% CO_2/ 95% O_2 to maintain pH at 7.5. In some experiments, the selective D_2-like antagonist spiperone, the D_2-like agonist quinpirole or the D_1 antagonist SCH23390 (Sigma, St Louis, MO) was dissolved in the superfusate and applied for at least 1 hr before electrical recording/tracer iontophoresis.

Adult *CBA/CaJ* mice from Jackson Labs (Bar Harbor, ME) were housed in a 12-hr light/12-hr dark cycle (with lights-on at 6 a.m.) for at least 2 weeks before an experiment. Dark-adapted mice were anesthetized with ketamine (100 mg/kg, *ip*), decapitated and both eyes enucleated. Neural mouse retinas were isolated under dim red light (long pass filter 650 nm) and placed in saline that contained (in mM) 120 NaCl, 25 $NaHCO_3$, 5 KCl, 10 glucose, 1 $MgSO_4$-$7H_2O$, 1 NaH_2PO_4, 0.1 glutamine, and 2 $CaCl_2$. The retinas were then incubated at 37°C in an atmosphere containing 5% CO_2 and 95% O_2 to maintain pH at 7.3.

Patch-clamp recording

Whole-cell patch clamp recordings from cone inner segments in intact goldfish neural retinas were obtained under continuous dark-adapted conditions in the subjective day and subjective night of a circadian cycle and in the day and night of a 12-hr light/12-hr dark cycle. Cone responses to dim full-field white and spectral light stimuli were measured to assess the light responsivity of the cones and determine whether they received rod input. Recordings (current-clamp configuration with I = 0) were obtained under visual control with a 3900A amplifier (Dagan Corporation, Minneapolis, MN) using pCLAMP software and digitized with a Digidata 1322A interface (Molecular Devices, Sunnyvale, CA). Signals were filtered at 1 kHz with a four-pole Bessel filter and sampled at 1 kHz. The preparation and electrode tips were visualized under infrared (> 900 nm) light. Electrodes were fashioned from borosilicate glass capillaries (OD 1.2 mm, ID 0.69 mm, Sutter Instruments, Novato, CA). The pipette solution contained (in mM) 20 KCl, 100 K-D- gluconate, 7.48 $KHCO_3$, 5.0 HEPES, 1.0 $MgCl_2$, 4.0 Na_2-ATP, 0.1 Na_3-GTP, and 5 Na_2-phosphocreatine.

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The pH was adjusted to 7.3 with KOH. Biocytin (0.3%) was added fresh daily to a frozen sample of pipette solution. Addition of the tracer lowered the pH to 7.2. Osmolarity was ~ 260 mOsm with biocytin. The tip resistance measured in the bath was ~ 15 MΩ and the liquid junction potential was adjusted to 0. The seal resistance ranged from 1 to 20 GΩ. Following rupture, the series resistance was 20–30 MΩ. In some experiments, the membrane current was measured under voltage-clamp configuration. Cones were clamped at -35 mV and the voltage was stepped (200 ms duration every 400 ms) from -90 mV to +30 mV in 10 mV increments and the peak current measured. We recorded light responses from short-single, medium-single, medium-double, long-single, and long-double goldfish cones (Stell and Harosi, 1976).

Light stimulation

Light stimuli, 500 ms in duration, were provided by an optical bench that included a 100 W tungsten-halogen lamp light source, and narrow-band interference (from 400 to 700 nm) and neutral density filters (Melles Griot). The output of the optical bench reached the microscope through a fiber optic and was focused onto the retina. The unattenuated light intensity (*Io*) at the level of the retina was 200 μW.cm^{-2}. Intensity values indicated in the text are relative to *Io*. During all circadian and dark adaptation electrical recording/tracer injection experiments, background illumination was ~ 4.5 log units lower than daytime cone threshold and only one cone/retina was studied. In addition, in all circadian and dark adaptation tracer injection experiments, only light stimuli ranging from −9 log *Io* to −5 log *Io* were flashed to minimize alteration of the dark-adapted state. Dim and bright light adaptation were achieved using −5 and −2 log *Io* flashes (duration 500 ms, frequency 0.125 Hz), respectively, delivered for at least 60 min prior to patch-clamp recording and tracer injection, and was maintained until the end of light adaptation experiments. A 0.5 mV criterion response was used for spectral sensitivity measurements to minimize alteration of the dark-adapted state. The maximum, unattenuated light intensity of the stimulus at 500 nm was 5.01 x 10^{12} photons.cm^{-2}.sec^{-1}.

The receptive field of a cell that is part of a continuous two-dimensional network can be described by the length constant (λ) with $\lambda = \sqrt{(R_m/R_s)}$, where R_m is the membrane resistance of the network of cells, and R_s is the sheet or trans-junctional coupling resistance (Lamb and Simon, 1976). In order to measure the receptive field size of goldfish cones, we recorded the light responses of dark-adapted cones to light stimuli of different radii and of 500 ms duration. To compare cone receptive field size in the day and night, we chose a stimulus intensity of −5 log *Io*, because the spot intensity, which had to be the same in the day and night, had to be above cone threshold during the day (~ −5.5 log *Io*), and because repeated flashes of −5 log *Io* light stimuli do not affect rod-cone coupling during the day or night (see Figs. 1–4). Stimulus size was controlled using circular apertures mounted inside the microscope. These apertures were rotated into the light beam, producing spot stimuli 5 to 2,600 μm in radius that were centered on the outer segments of the recorded cones.

Tracer coupling

Individual cones in intact goldfish neural retinas were labeled by iontophoresis of the biotinylated tracer biocytin (0.3%) during whole-cell patch-clamp recording by maintaining the recorded cone at +20 mV for 10 min. Thirty min after biocytin injection, retinas were

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fixed in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 for 2 hrs at room temperature. Biocytin was visualized using streptavidin-conjugated cyanine 3 (Jackson ImmunoResearch, West Grove, PA).

Cut loading

Several perpendicular radial cuts were made with a razor blade in goldfish and mouse retinas immediately after isolation. The retinas were then incubated for 15 min in the bicarbonate-buffered saline solution that contained 0.05% Neurobiotin. Following cell loading and diffusion, the retinas were then washed in saline without tracer and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 1 hr. Neurobiotin was visualized with strepavidin-conjugated-Alexa488 (Molecular Probes, Eugene, OR). In some experiments, the retinas were isolated and incubated in saline with 10 μM spiperone for 30 min before the cuts were made. Spiperone was present during the subsequent steps as well as until fixation.

Imaging

Cells were imaged and photographed with a Zeiss 510 META laser scanning confocal microscope (Carl Zeiss, Inc., Thornwood, NY). Serial reconstructions of rods and cones were made from z-stacks of confocal images with LSM-5 Image Browser 3,2,0,115 (Carl Zeiss). Rods and cones could be clearly distinguished in z-stacks of wholemount sections based on the diameters of their somata, and thus counted with NIH ImageJ software. Quantification of the cut-loading labeling was performed with NIH ImageJ software.

Data analysis

Because under dark-adapted conditions we did not observe any tracer coupling, light response, or spectral sensitivity differences between day and subjective day, and night and subjective night, averaged data (Figs. 2A, 4A, 5C–E, 6A) were pooled into two groups, day-dark-adapted and night-dark-adapted. All statistical analyses were performed using Origin 7.0 software (OriginLab Corp., Northampton, MA).

Rod-cone tracer coupling—The numbers of cones and rods labeled with biocytin and counted under each experimental condition were averaged and expressed as the mean ± s.e.m. of n values. To determine whether, under dark-adapted conditions, time of day and drug (spiperone or quinpirole) treatment and/or photoreceptor type affected photoreceptor tracer coupling, statistical analyses were performed using a two-way ANOVA. The first factor was time of day/treatment (i.e. subjective day and day-dark-adapted, subjective night and night-dark-adapted, subjective day + spiperone, subjective night + quinpirole) and the second factor was photoreceptor type (i.e. rod or cone). ANOVA of the data presented in Fig. 2A revealed significant time of day/treatment [$F_{(3,66)} = 24.39$, $p < 0.001$], photoreceptor type [$F_{(1,66)} = 46.06$, $p < 0.001$], and photoreceptor type x time of day/treatment [$F_{(3,66)} = 17.11$, $p < 0.001$] effects. To determine whether, under light-adapted conditions, time of day and light intensity and/or photoreceptor type affected photoreceptor tracer coupling, statistical analyses were performed using a two-way ANOVA. The first factor was time of day/light intensity (i.e. day-dim light-adapted, night-dim light adapted, day-bright light-adapted, and night-bright light-adapted) and the second factor was photoreceptor type.

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ANOVA of the Fig. 2B data revealed significant time of day/light intensity [$F_{(3,20)}$ = 135.67, p < 0.001], photoreceptor type [$F_{(1,20)}$ = 75.88, p < 0.001], and photoreceptor type x time of day/treatment [$F_{(3,20)}$ = 80.09, p < 0.001] effects. *Post-hoc* analysis was performed using the Tukey test.

Light intensity-response relationships—Light response peak amplitude was normalized and plotted against stimulus intensity. Data points were fit to a Hill-type equation: $V = V_{max} . [I^n/(I^n + K^n)]$, where V is the response amplitude, V_{max} is the maximum response amplitude, I is the stimulus intensity, K is the stimulus intensity needed to generate a response with half-maximal amplitude, and n is the Hill coefficient. Nonlinear least-squares regression analysis was performed with n and K as free parameters. Results from the least-squares nonlinear regression analysis are given ± standard deviation (s.d.). One-way ANOVA of the data in Figs. 4A and 4B showed that the stimulus intensity needed to generate a response with half-saturating intensity (K) varied with the experimental conditions under both dark-adapted (Fig. 4A: $F_{(3,36)}$ = 7.17, p < 0.001) and light-adapted conditions (Fig. 4B: $F_{(3,32)}$ = 12.49, p < 0.001). Differences between the groups were tested using the Tukey *post-hoc* test.

Cone light response threshold—The response threshold (intensity of light that elicits a 0.5 mV response) determined at different times of the circadian cycle and of the regular light/dark cycle was averaged and expressed as the mean ± s.e.m. of n values. To determine whether time of day and/or cycle type affected cone response threshold, statistical analysis was performed using a two-way ANOVA. The first factor was time of day (i.e. 0–6, 6–12, 12–18, or 18–24 hr) and the second factor was cycle type (i.e. circadian or 12-hr light/12-hr dark cycle). ANOVA of the Fig. 4C data revealed that cone response threshold was highly dependent on the time of day [$F_{(3,62)}$ = 59.07, p < 0.001] but not on the cycle type [$F_{(1,62)}$ = 0.0216, p = 0.884]. No significant time of day x cycle type effect was found [$F_{(3,62)}$ = 0.789, p = 0.505].

Kinetics of cone light responses—Kinetics was defined as previously (Ribelayga and Mangel, 2007). Cone light response data from dark-adapted and light-adapted experiments were analyzed separately. Statistical analysis was performed using a two-way ANOVA. The between-group factor was day, night, subjective day + spiperone, or subjective night + quinpirole for the dark-adapted experiments and day or night for the light-adapted experiments. The intra-group factor was the stimulus light intensity. However, data analysis was limited to the light intensities for which data were available from all the groups (i.e. dark-adapted conditions: $-6 \leq \log I \leq -2$; light-adapted conditions: $-5 \leq \log I \leq -2$). Under dark-adapted conditions, significant difference between groups (p < 0.001 for each response measure) and between light intensities (p < 0.001 for each response measure) were detected. No interaction was found between group and light intensity (p > 0.05 for each response measure). ANOVA of the data from bright light adaptation experiments showed a significant effect of light intensity (p < 0.001) except for time-to peak (p = 0.068). No interaction was found between time of day and light intensity (p > 0.05 in each case).

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Receptive field size measurements-electrophysiology—The length constant (λ) of the recorded cells was estimated by plotting the normalized response amplitudes against the stimulus radius and fitting the data with the following equation:

$$V(r)=1-(1+r/\lambda)\,e^{(-r/\lambda)}$$

Where V(*r*) is the normalized amplitude of the response to a spot of light of radius *r* and λ is the length constant and free parameter (Lamb and Simon, 1976).

Receptive field size measurements-cut-loading—Data points were normalized to the maximum fluorescence intensity and fit to the equation: $Y = Y_0 + Y_{max} \cdot e^{(-x/\lambda)}$, where Y is the relative fluorescence intensity, Y_0 is the background fluorescence, Y_{max} is the maximal relative fluorescence, λ is the length constant, and x the distance from the cut. Nonlinear least-squares regression analysis was performed with Y_0, Y_{max}, and λ as free parameters. Results from the least-squares nonlinear regression analysis are given ± s.d. One-way ANOVA of the data in Figs. 7G and 7H showed that λ varied with the experimental conditions (Fig. 7G: $F_{(2,11)} = 2{,}120$, $p < 0.001$; Fig. 7H: $F_{(2,11)} = 943$, $p < 0.001$). Differences between the groups were tested using the Tukey *post-hoc* test.

Spectral sensitivity—Statistical analysis of cone spectral sensitivity was done using nonlinear least-squares regression of our experimental data with the published template for goldfish visual pigments (Govardovskii et al., 2000). Nomograms were generated from the template with λ_{max} = 516 nm (goldfish rod porphyropsin), 451 nm (goldfish blue cone pigment, S), 539 nm (goldfish green cone pigment, M), and 608 nm (goldfish red cone pigment, L) (Govardovskii et al., 2000). The modified nomogram under dark-adapted conditions at night was calculated by combining the rod and L-cone nomograms weighted by their relative difference in sensitivity at λ_{max}.

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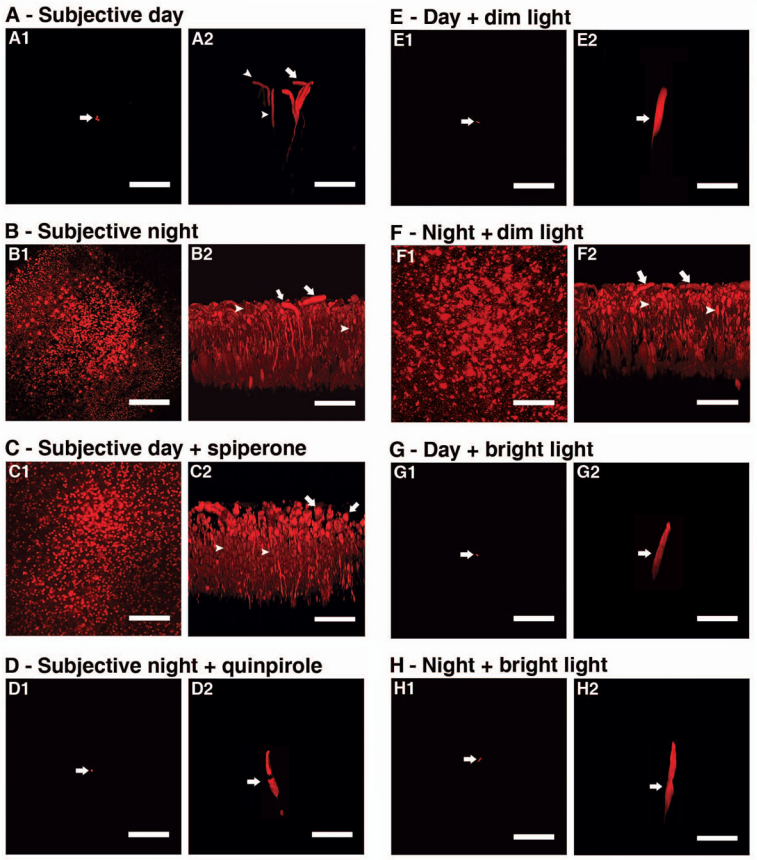

Figure 1. Rod-cone Tracer Coupling Varies with Time of Day

(A–H) Following iontophoresis of biocytin into individual cones, the tracer remained in a few cells (indicated by arrows in A1, D1, E1, G1, H1) near the injected cone during the subjective day (A), during the subjective night in the presence of the D_2-like receptor agonist quinpirole (1 μM, D), and following dim light adaptation for > 60 min in the day (E) and bright light adaptation for > 60 min in the day (G) and night (H), but diffused into many rods and cones during the subjective night (B), during the subjective day in the presence of the D_2-like receptor antagonist spiperone (10 μM, C), and following dim light adaptation for > 60 min in the night (F).

In each of A–H, confocal images of a whole-mount retina at the level of the rod inner segments are shown on the left and perpendicular views of the 3-D reconstruction of the photoreceptor cells from the same retina are shown on the right. Some cones (arrows) and rods (arrowheads) are indicated. Scale bars (A–H): 50 μm.

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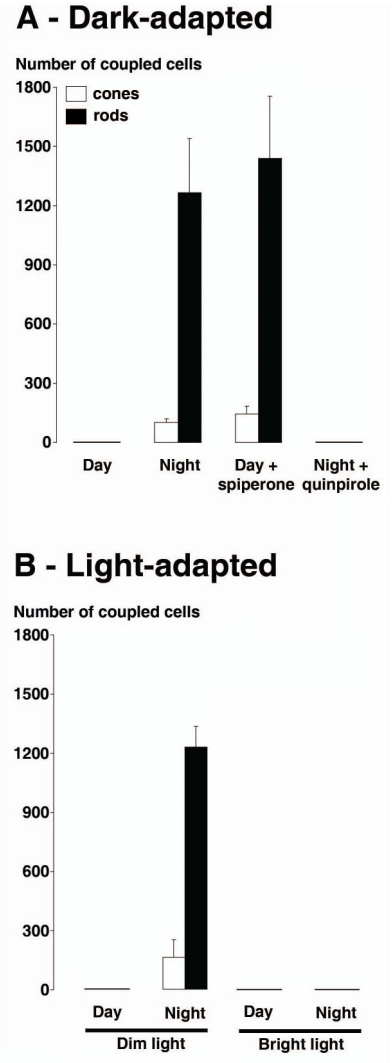

Figure 2. The Circadian Clock in the Goldfish Retina Controls Rod-cone Coupling by Activating Dopamine D_2-like Receptors in the Day

(A and B) Average numbers of stained cones (open bars) and rods (filled bars) following biocytin injections into individual cones (1 cone injected/retina) under dark-adapted conditions (A) during the day ($n = 11$) and subjective day ($n = 5$), night ($n = 4$) and subjective night ($n = 5$), subjective day in the presence of spiperone ($n = 6$), and subjective night in the presence of quinpirole ($n = 6$), and under dim light-adapted conditions (B-left) during the day ($n = 6$) and night ($n = 3$) and bright light-adapted conditions (B-right) during the day ($n = 2$) and night ($n = 3$). Under dark-adapted conditions, the number of tracer coupled rods and cones was significantly greater during the night ($p < 0.001$) and during the day following spiperone treatment ($p < 0.001$) than during the day under control conditions. Under dim light-adapted conditions, the number of tracer coupled rods and cones was significantly greater during the night ($p < 0.001$) compared to the day (Tukey *post hoc* analysis). Under bright light-adapted conditions, biocytin was restricted to the injected cone; no other cells were labeled. Error bars represent s.e.m.

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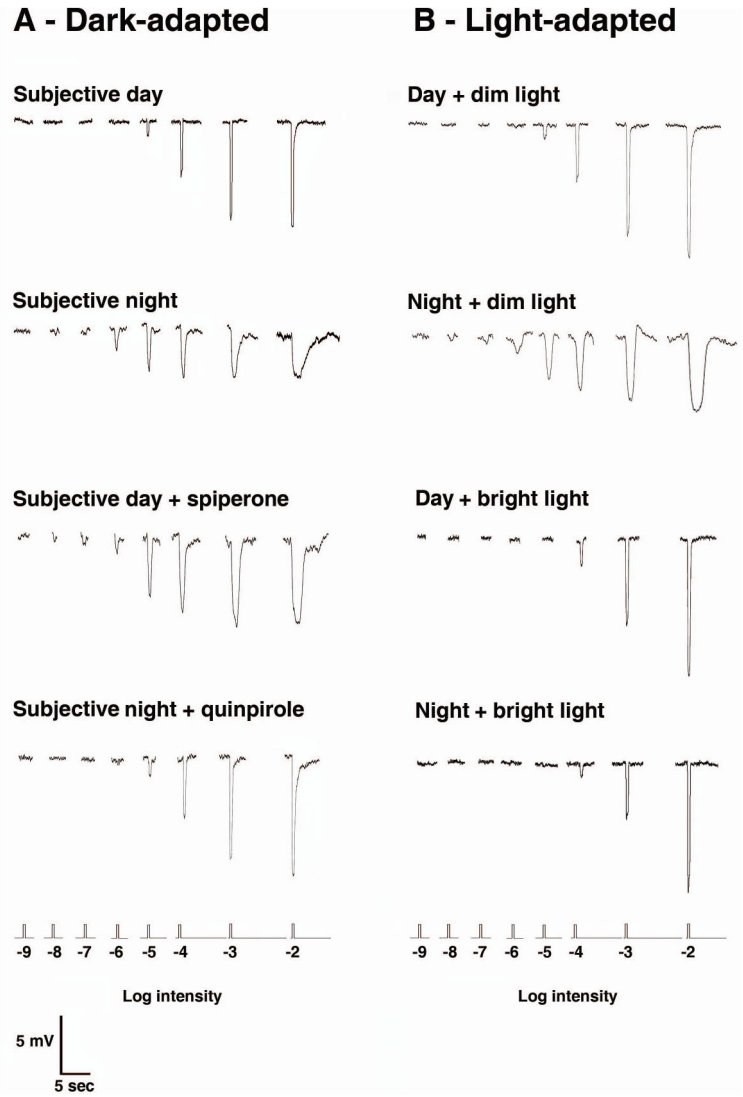

Figure 3. Dark-adapted Cones Receive Very Dim Light Signals from Rods at Night, but not in the Day

(A and B) Representative examples of cone responses to a series of full-field white light stimuli of increasing intensity recorded under dark-adapted conditions (A) during the subjective day, subjective night, in the presence of spiperone (10 μM) during the subjective day, and in the presence of quinpirole (1 μM) during the subjective night, and under dim and bright light-adapted conditions (B) during the day and night.

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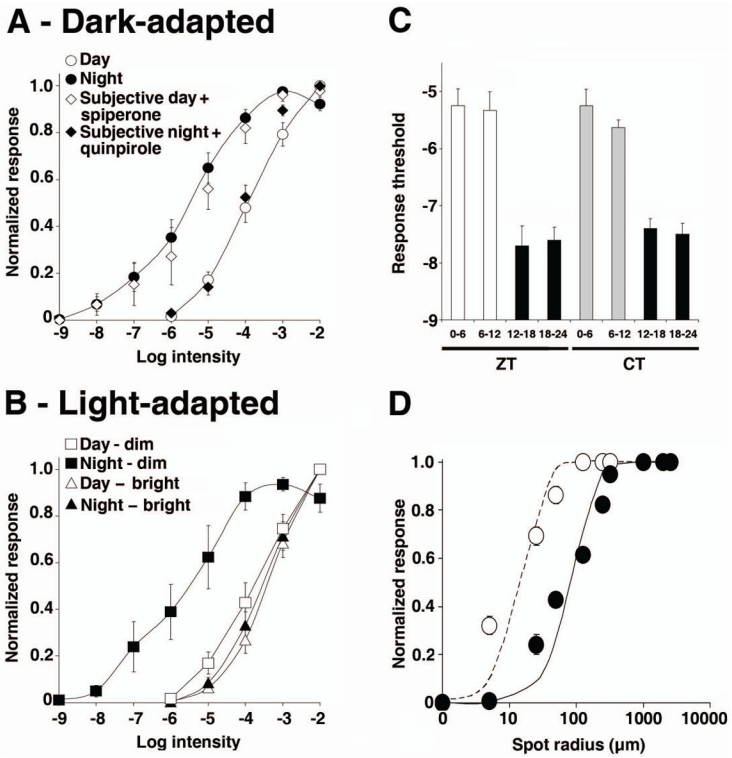

Figure 4. The Retinal Circadian Clock Regulates Cone Light Responses and Receptive Field Size by Activating D_2-like Receptors in the Day so that Rod Input is Dominant at Night, but not Present in the Day

(A and B) Average normalized intensity-response curves of cones (1 cone/retina) recorded under dark-adapted conditions (A) during the day ($n = 7$) and subjective day ($n = 9$) (open circles), night ($n = 7$) and subjective night ($n = 3$) (filled circles), in the subjective day in the presence of spiperone (open diamonds, $n = 5$), and in the subjective night in the presence of quinpirole (filled diamonds, $n = 9$), and under light-adapted conditions (B). Shown are values obtained under dim light-adapted conditions during the day (open squares, $n = 6$) and night (filled squares, $n = 6$), and under bright light-adapted conditions in the day ($n = 9$) and subjective day ($n = 2$) (open triangles) and night ($n = 6$) and subjective night ($n = 4$) (filled triangles).

(C) Average day/night and circadian rhythms of the cone light response threshold (i.e. intensity required to elicit a 0.5 mV response) under dark-adapted conditions. The average cone light response threshold (log intensity) was significantly higher during the day ($p < 0.001$) and subjective day ($p < 0.001$) than during the night and subjective night (Tukey *post hoc* analysis). Data points represent averages of 4 to 15 measurements. Error bars indicate s.e.m.

(D) Average normalized response amplitudes of dark-adapted cones plotted against stimulus radius for a stimulus of intensity -5 log *I*o. These data indicate that the receptive field size of cones is larger at night than in the day. Measurements were performed during the day (open circles, $n = 6$) and night (filled circles, $n = 6$).

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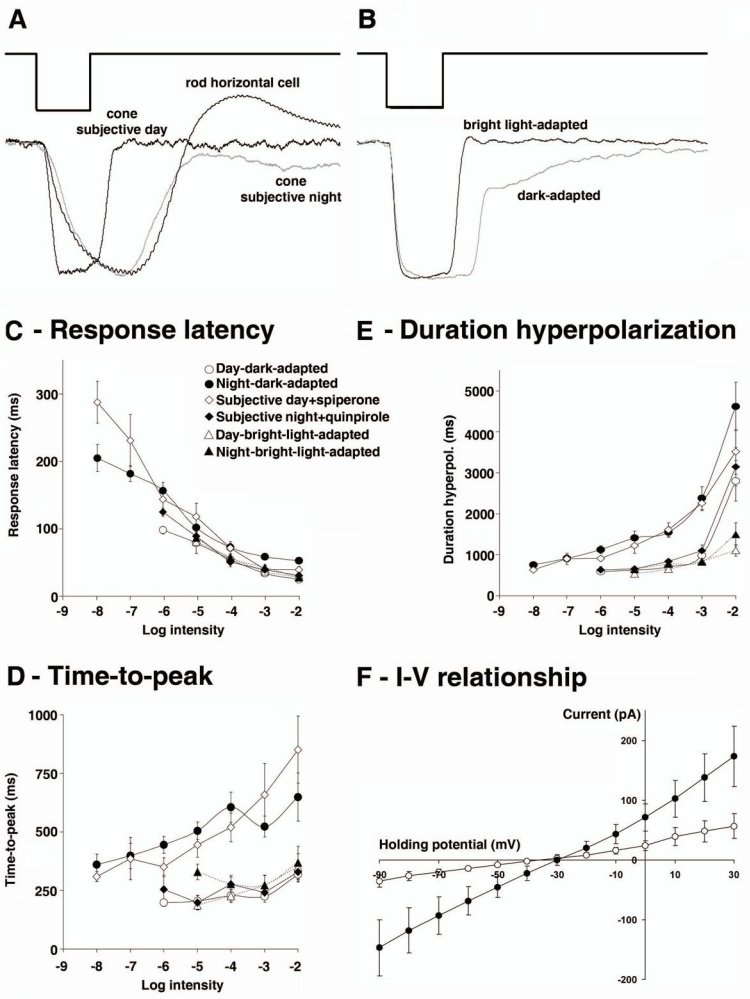

Figure 5. Kinetics of Cone Light Responses during the Day and Night under Different Lighting Conditions

(A) Representative examples of cone responses to a light stimulus flashed (500 ms) at intensity -5 log *Io* during the subjective day and subjective night (grey trace), and of a rod horizontal cell response to the same stimulus. The amplitude of each trace has been normalized relative to its peak for better comparison.

(B) Representative example of the responses of an individual cone to a light stimulus flashed (500 ms) at intensity – 2 log *Io* during the day immediately following 60 min of dark adaptation (grey trace), and following subsequent bright light adaptation (– 2 log *Io*, 500 ms stimuli at 0.125 Hz) for 6 min (black trace). Note that the cone response exhibited a prolonged plateau potential under dark-adapted conditions, but not following 6 min of bright light adaptation.

(C–E) Average latency (C), time-to-peak (D), and duration of the hyperpolarization (E) of cone light responses recorded under dark-adapted conditions during the night (filled circles; n = 10 to 19), day (open circles; n = 12 to 26), subjective day in the presence of spiperone (10 μM) (open diamonds; n = 5), and subjective night in the presence of quinpirole (1 μM)

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(filled diamonds, $n = 9$) or under bright light-adapted conditions during the day (open triangles; $n = 7$ to 11) and night (filled triangles; $n = 7$ to 10). Error bars represent s.e.m. (F) Relationship between membrane current and membrane potential of dark-adapted cones during the day (open circles, $n = 30$) and night ($n = 27$). The peak current was measured when cones were voltage-clamped at −35 mV and stepped (duration 200 ms every 400 ms) from -90 mV to +30 mV in 10 mV increments.

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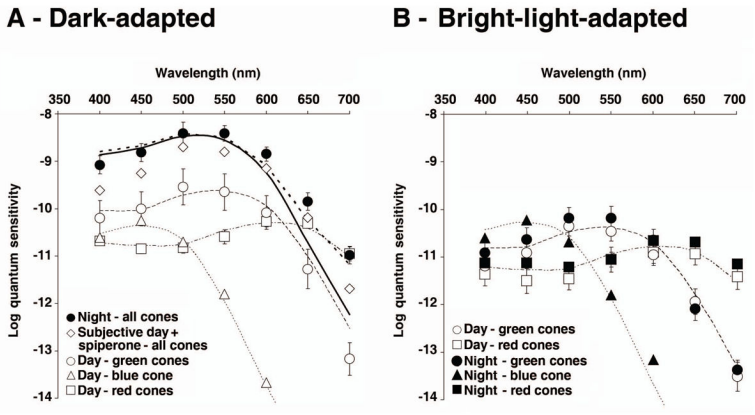

Figure 6. Circadian Variations in Cone Spectral Sensitivity

(A) Average spectral sensitivity of cones recorded under dark-adapted conditions during the day or subjective day fit one of three nomograms (thin dotted curves) corresponding to the three major known types of goldfish cone pigments: L, M, and S. Data points represent average sensitivity ± s.e.m. from recorded red cones (open squares; $n = 9$), green cones (open circles; $n = 6$) and blue cone (open triangle; $n = 1$). In contrast, the spectral sensitivity of all dark-adapted cones recorded at night peaked at ~ 535 nm (filled circles; $n = 10$). Although cone spectral sensitivity at night under dark-adapted conditions closely fits a rod nomogram (solid thick line) for 400 nm < λ < 600 nm, it does not fit the nomogram as well for λ > 600 nm. Rather, the data points closely fit a modified nomogram that combines goldfish rod and L-cone pigment nomograms (dotted thick curve; $\lambda_{max} = 537 \pm 3$ (s.d.) nm; $r^2 = 0.91$). Following application of spiperone (10 μM) (open diamonds; $n = 2$), cone spectral sensitivity in the subjective day resembled that observed during the subjective night and data points fit well the modified nomogram ($\lambda_{max} = 537 \pm 3$ nm; $r^2 = 0.96$).

(B) Following bright light adaptation at night or during the subjective night 3 groups of cones with different spectral sensitivities were observed: red cones (filled squares; $n = 4$), green cones (filled circles; $n = 5$) and blue cone (filled triangles; $n = 1$), whereas bright light adaptation during the day or subjective day did not affect the relative spectral sensitivity of the recorded cones (red cones: open squares; $n = 2$; green cones: open circles; $n = 6$) but slightly decreased the absolute sensitivity. Nomograms as in (A).

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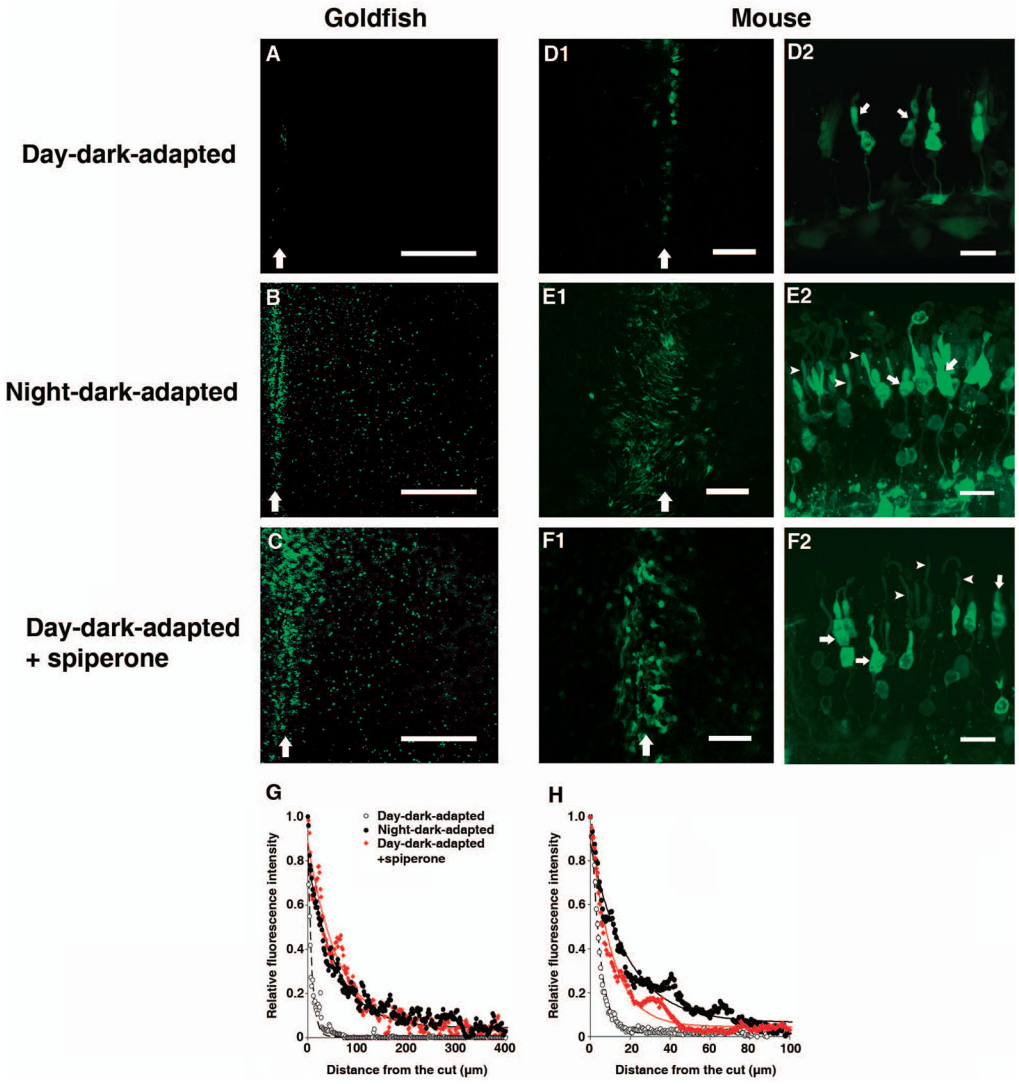

Figure 7. Day-night Difference in Photoreceptor Tracer Coupling in the Mouse Retina

(A–F) Representative examples of photoreceptor tracer coupling measured by intracellular propagation of Neurobiotin tracer after cut loading in goldfish (A–C) and mouse (D–F) retinas under dark-adapted conditions during the day (A and D), night (B and E), and in the presence of spiperone (10 μM) during the day (C and F). Similar results have been observed in 3 independent experiments (2 retinas/experiment). Shown are confocal images of whole-mount retinas at the level of the rod inner segments (A–C, D1, E1 and F1) and detailed perpendicular views of the 3-D reconstruction of the mouse photoreceptor cells adjacent to the cut at higher magnification (D2, E2 and F2). Large arrows (A-C, and D1-F1) indicate location of cut. Some cones (small arrows) and rods (arrowheads) are shown in D2-F2. Scale bars: 200 μm (A–C); 50 μm (D1, E1, F1); 10 μm (D2, E2, F2).

(G, H) Relative fluorescent intensity as a function of the distance from the cuts in goldfish (G) and mouse (H) retinas under the three experimental conditions tested in A–F. Averaged data from 4 experiments (1 retina/experiment) are shown.

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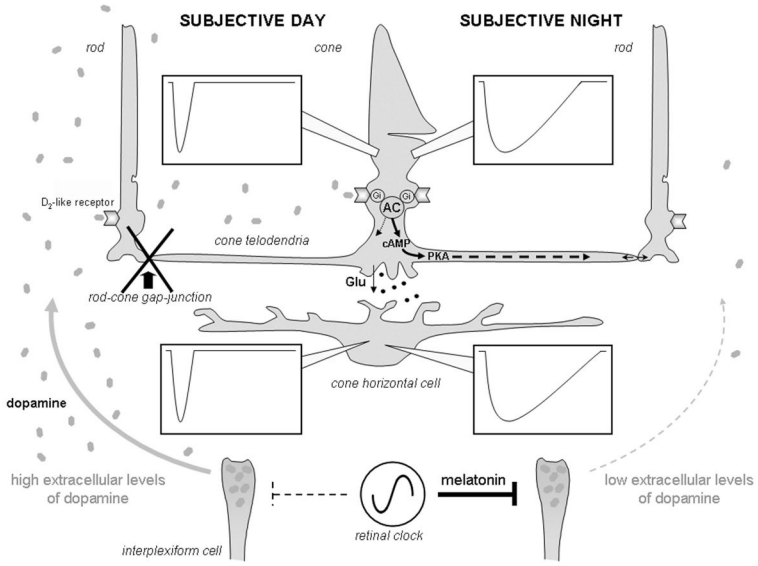

Figure 8. The Retinal Circadian Clock Controls Rod-Cone Coupling

The circadian clock in the retina increases dopamine release from dopaminergic neurons during the subjective day, thereby activating the D_2-like receptors on rods and cones so that the conductance of rod-cone gap junctions and the rod signal to cones and cone horizontal cells are decreased in the subjective day, compared to the subjective night. The traces shown are schematic representations of cone (top) and cone horizontal cell (bottom) responses to a 500 ms-light stimulus flashed at intensity −5 log *Io* during the subjective day (left) and subjective night (right). The cone traces were generated from the averaged response latency, time-to-peak, and response duration data shown in Fig. 5 and the cone horizontal cell traces were generated from similar averaged response kinetic data from Ribelayga et al., 2002, 2004, as well as from unpublished data. In each case, the three data points were connected by a computer-generated, smoothing curve. The amplitude of each trace was normalized relative to its peak for better comparison of response kinetics. See Discussion for details.