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Dopamine regulation of $GABA_A$ receptors contributes to light/dark modulation of the ON-cone bipolar cell receptive field surround in the retina

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Summary

Cone-bipolar cells are interneurons that receive synaptic input from cone photoreceptor cells and provide the output of the first synaptic layer of the retina. These cells exhibit center-surround receptive fields, a prototype of lateral inhibition between neighboring sensory cells in which stimulation of the receptive field center excites the cell whereas stimulation of the surrounding region laterally inhibits the cell. This fundamental sensory coding mechanism facilitates spatial discrimination and detection of stimulus edges. However, although it is well established that the receptive field surround is strongest when ambient or background illumination is most intense, e.g., at midday, and that the surround is minimal following maintained darkness, the synaptic mechanisms that produce and modulate the surround have not been resolved. Using electrical recording of bipolar cells under experimental conditions in which the cells exhibited surround light responses, and light and electron microscopic immunocytochemistry, we show in the rabbit retina that bright light-induced activation of dopamine D_1 receptors located on ON-center cone-bipolar cell dendrites increases the expression and activity of $GABA_A$ receptors on the dendrites of the cells and that surround light responses depend on endogenous $GABA_A$ receptor activation. We also show that maintained darkness and D_1 receptor blockade following maintained illumination and D_1 receptor activation result in minimal $GABA_A$ receptor expression and activity and greatly diminished surrounds. Modulation of the D_1/$GABA_A$ receptor signaling pathway of ON-cBC dendrites by the ambient light level facilitates detection of spatial details on bright days and large dim objects on moonless nights.

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AUTHOR CONTRIBUTIONS

A.C., M.I. Y.C. and S.C.M. performed the experiments and analyzed the data. A.C., M.I., Y.C. and S.C.M. conceptualized the study, designed experiments, and discussed results and implications. A.C., M.I. and S.C.M. wrote the manuscript.

Keywords

receptive field surround; lateral inhibition; dopamine D_1 receptors; $GABA_A$ receptors; bipolar cells

Introduction

Many neurons in the visual, auditory, and somatosensory systems inhibit nearby cells via lateral connections. This lateral inhibition between neighboring sensory cells produces receptive fields (RFs) with a center-surround organization in which stimulation of the RF center excites a cell but stimulation of the surrounding region laterally inhibits the cell, a fundamental sensory coding characteristic that enhances spatial discrimination and the detection of stimulus edges.

Cone-bipolar cells (cBCs) receive input from cone photoreceptor cells (Fig. 1A) and provide excitatory signals to ganglion cells (GCs), the output neurons of the retina. Classic recordings of *in vivo* GCs and of cBCs in intact *in vitro* retinas have established that both cell types exhibit a center-surround RF organization with two characteristic features. First, following maintained (30 min) bright background illumination, stimulation of the RF surround alone using an annulus or ring of light produces a response that is opposite in polarity to that produced by center (spot) stimulation alone, a phenomenon called “surround activation,” and surround stimulation in the presence of center stimulation using spot and annulus together reduces the amplitude of the center response, a phenomenon called “surround antagonism” [1–8]. Second, although cBC and GC surround responses are strongest under maintained bright background illumination, surround strength progressively decreases as the light level decreases so that the surround is minimal following maintained darkness [7–12]. This adaptive process likely contributes to modulation of the spatial discrimination ability of human and monkey observers by the ambient light level [13]. In addition, evidence indicates that the greatly weakened surround that is observed following maintained darkness does not arise from the shift from cone to rod pathway function [9, 11, 14]. However, although these classic surround light responses depend on the ambient light level, the mechanisms and neural pathways (Fig. 1A) that produce cBC surrounds following maintained bright illumination and the light/dark adaptive processes that regulate surround strength remain unclear and controversial.

Intracellular cAMP and protein kinase A (PKA), by regulating the expression and activity of $GABA_A$ receptors ($GABA_A$Rs) [15], modulate the effectiveness of GABA inhibition. Because in the retina 1) bright light increases the release of dopamine, which modulates a variety of cellular processes such as gap junction permeability, glutamate receptor sensitivity, and voltage-gated channels [16], 2) cBC dendrites express $GABA_A$Rs [17–20] and dopamine D_1 receptors (D_1Rs), which increase intracellular cAMP and PKA when activated by dopamine [16, 21, 22], and 3) following maintained bright illumination D_1, but not D_2R-like antagonists reduce rabbit GC surround responses [23], we studied whether D_1Rs and $GABA_A$Rs on the dendrites of ON-center cBCs (ON-cBCs), a type of cBC, mediate light/dark modulation of the surround (Fig. 1B). Using a retinal preparation in

which ON-cBCs exhibited surround light responses, we found that light-induced activation of ON-cBC dendritic D_1Rs increases ON-cBC surround strength by enhancing the expression and activity of $GABA_A$Rs on their dendrites (Fig. 1B). Our measurements also suggest that the dendrites of horizontal cells (HCs), which receive input from cones (Fig. 1A), provide a GABA feedforward signal that tonically depolarizes ON-cBC dendrites. As a result, ON-cBC hyperpolarizing surround light responses may reflect a reduction in $GABA_A$R-mediated excitation.

RESULTS

Role of Dopamine D_1Rs in ON-cBC Surround Light Responses

To study the mechanisms that underlie light/dark modulation of the ON-cBC RF surround, it is essential to utilize a preparation, such as intact retina, in which cells exhibit surround light responses. However, because BC somata are located in the middle of the retina, it is extremely difficult when using intact retinas to obtain electrical recordings from them and not feasible to selectively apply GABA onto their dendrites (see below). Therefore, we used retinal slices for BC recording experiments because they offered easy access to BC somata and dendrites. A limitation of slicing the retina is that it physically eliminates many neural connections, especially those involving lateral interactions, and limits neural and transmitter interactions. Moreover, we found that maintained bright background illumination greatly reduced the viability of rabbit retinal slices. Therefore, to study the mechanisms that underlie light/dark modulation of the surround, we maintained rabbit retinal slices in the dark. Furthermore, because increases in background light intensity enhance dopamine release in the retina [16], we added dopamine (5 μM) to the superfusate to mimic the effect of maintained bright illumination in the intact retina.

Whole-cell patch-clamp recordings of ON-cBCs (Fig. 2A-top traces, Fig. 2B) in this dopamine-bathed retinal slice preparation show that the cells exhibited surround antagonism (13 out of 18 cells) and surround activation (10 out of 18 cells). Moreover, the center and surround of ON-cBCs were relatively small in spatial extent (center diam < 150 μm; surround outer diam. < 350 μm). It is worth noting that following maintained bright illumination of monkey and amphibian eyecups, an *in vitro* preparation that includes intact neural retina and pigment epithelium (which enhances viability and light responsiveness), cone-driven BCs exhibited center and surround light responses - including both surround activation and antagonism - and their RF center and surround were also similarly small in spatial extent [6, 7]. The control data in Fig. 2 thus show that most rabbit ON-cBCs in dopamine-bathed slices exhibited center and surround responses that were similar in many respects to those of bright light-adapted cone-driven ON-BCs in more intact retinal preparations.

Separate experiments revealed that the strength of ON-cBC surround light responses depends on dopamine D_1R – but not D_2R-like - activation. When the superfusate contained both dopamine and SCH23390 (selective D_1R antagonist), ON-cBCs exhibited depolarizing (RF center) responses to thin and wide centered bar stimuli and to bars flashed in the peripheral portion of their RFs, with little, if any, evidence of surround antagonism or surround activation (Fig. 2A-traces second row from top, Fig. 2B), as was observed when

slices were superfused without dopamine to mimic the effect of maintained darkness (Fig. 2A-traces third row from top, Fig. 2B). When the superfusate contained both dopamine and spiperone (selective D2R family antagonist). ON-cBCs exhibited both surround antagonism and activation (Fig. 2A-bottom traces, Fig. 2B), and the spatial extent of center and surround were similar to those observed for dopamine control cells. These results show that most ON-cBCs exhibited both surround antagonism and activation when D_1Rs were activated but not when D_1Rs were blocked or not activated. In addition, the lack of effect of spiperone suggests that D_2-like receptors, such as cone D_4Rs, do not play a role in ON-cBC surround responses following maintained bright illumination.

Interestingly, the average peak amplitude of ON-cBC responses to light stimulation (intensity = 40 lux) was significantly ($p < 0.05$) larger (~60%) in SCH23390-treated slices (ave. 7.86 ± 2.13 (SEM) mV; n=5) and in slices superfused in the dark without dopamine ($p < 0.01$, ave. 8.30 ± 1.46 mV; n=6) than observed in slices in which D_1Rs were strongly activated (i.e., containing dopamine alone or both dopamine and spiperone) and in which the cells exhibited surround light responses (4.62 ± 0.6 mV; n=14), suggesting that the light responses obtained during D_1R activation were produced by opposing excitatory and inhibitory mechanisms. In addition, the average resting membrane potential of ON-cBCs was more hyperpolarized ($p < 0.01$) in both the presence of SCH23390 (−47.6 ± 1.4 mV, n=5) and in the absence of dopamine (−46.7 ± 1.1 mV, n=6) than in slices in which D_1Rs were strongly activated (i.e., containing dopamine alone or both dopamine and spiperone) and in which the cells exhibited surround responses (−40.2 ± 1.0 mV; n=14) (see Discussion).

Activation of ON-cBC Dendritic D_1Rs Increases $GABA_A$R Activity of ON-cBC Dendrites

If maintained bright illumination – compared to maintained darkness - produces a sustained increase in dopamine activation of ON-cBC dendritic D_1Rs so that $GABA_A$R activity of the dendrites is strongly enhanced, there should be a clear difference in ON-cBC dendritic $GABA_A$R activity following D_1R activation compared to D_1R blockade or to the absence of D_1R activation. We therefore measured the $GABA_A$R activity of ON-cBC dendrites by puffing GABA onto the dendrites following Co^{2+} (2 mM) blockade of synaptic transmission. Because mammalian ON-cBC dendrites may express $GABA_C$Rs [19], the superfusate also included TPMPA (selective $GABA_C$R antagonist), so that GABA responses could be definitively attributed to $GABA_A$R activity. We found that ON-cBC dendrites exhibited clear $GABA_A$R activity when slices were maintained in dopamine-containing medium (Figs. 3A, D). Conversely, dendrites exhibited minimal $GABA_A$R activity following > 30-min SCH23390-treatment (Figs. 3B, D) or in a medium without dopamine (Figs. 3C, D). Because GABA was puffed directly onto the dendrites following synaptic blockade and because light stimulation increases extracellular dopamine and D_1R activation [16], the results suggest that light-evoked endogenous activation of D_1Rs on ON-cBC dendrites increases $GABA_A$R activity of the dendrites.

A Reduction in Tonic $GABA_AR$-mediated Excitation Underlies ON-cBC Hyperpolarizing Surround Light Responses

Because activation of D_1Rs on ON-cBC dendrites modulates the surround light responses (Fig. 2) and $GABA_AR$ activity (Fig. 3) of the dendrites, we next determined whether endogenous $GABA_AR$ activity modulates the RF surround. Addition of APB, which selectively blocks cone to ON-BC signaling [24], revealed a hyperpolarizing response to wide centered bar stimuli (Fig. 4A, 14 out of 19 cells (= 74%)), as reported for cone-driven ON-BCs in the amphibian retina [7, 25]. This result suggests that most light-adapted mammalian ON-cBCs exhibit hyperpolarizing surround responses mediated by a neural pathway that does not involve direct input from cones or feedback to cones. Moreover, the opposite-polarity surround responses revealed by APB were greatly reduced by gabazine ($GABA_AR$ antagonist) (Figs. 4A, B), demonstrating for the first time in vertebrate retina that endogenous $GABA_AR$ activation mediates the surround of a majority of ON-cBCs. Interestingly, APB, but not SCH23390 (Fig. 2A), blocked the hyperpolarizing response waveform at light offset (11 out of 14 ON-cBCs), suggesting that hyperpolarizing OFF-responses of ON-cBCs [6, 7] originate in cones. As occurred with ON-cBCs, APB application eliminated the depolarizing responses of rod-BCs to centered bar stimuli. However, in contrast to ON-cBCs, when retinal slices were bathed in 5 μM dopamine APB did not reveal rod BC surround responses or an effect of gabazine (7 out of 7 recorded cells; Fig. 4C).

To study whether ON-cBC hyperpolarizing surround light responses revealed by APB result from an increase or decrease in $GABA_AR$ conductance, we made I–V measurements of ON-cBCs using a medium that contained APB (blocks glutamate-gated conductance of ON-cBCs) and dopamine. For each cell, we made measurements before and during light stimulation and before and during gabazine application (Fig. 4D). We found that light stimulation (880 ± 124 pS, n=5) and gabazine (840 ± 111 pS, n=5) significantly decreased ($p < 0.05$, paired t-test) the mean slope conductance of ON-cBCs compared to APB alone (i.e., before light stimulation and gabazine application) (1,180 ± 135 pS, n=5) (Fig. 4D). In every cell, the conductance in APB alone exceeded the conductances during hyperpolarizing light responses and following gabazine application. Moreover, light stimulation following gabazine application in the dark did not further decrease the cell conductance. These results suggest that GABA tonically excites ON-cBCs following maintained D_1R activation and that hyperpolarizing surround light responses revealed by APB can be attributed to reduced $GABA_AR$ activity.

Light/dark Modulation of D_1Rs Regulates $GABA_AR$ expression of ON-cBC Dendrites

We performed immunocytochemistry experiments to determine whether the background illumination conditions and D_1Rs regulate ON-cBC dendritic $GABA_AR$ expression. As reported for mammalian retinas [8, 17–20], we found that ON-cBC dendrites express $GABA_ARs$ following maintained bright illumination. We also report the novel findings that ON-cBC dendritic $GABA_AR$ expression is greatly diminished following maintained darkness due to minimal activation of their D_1Rs and significantly enhanced following maintained bright illumination due to light-induced activation of their D_1Rs (Figs. 5, 6).

Following maintained bright illumination, staining for the α1 and β2/3 subunits of $GABA_A$ Rs were similar [17–19], revealing narrow bands composed of clustered puncta just beneath cone pedicles (Fig. S2). Although staining for the β2/3-subunit was somewhat less intense than that of the α1 subunit, the following results concerning the effects of background illumination and D_1R activation are based on β2/3-subunit immunoreactivity, because this subunit, and not the α1-subunit, contains phosphorylation and regulatory sites that modulate $GABA_AR$ expression and activity [15].

Double immunostaining of $GABA_ARs$ with cell-type specific markers demonstrated that $GABA_AR$-IR was located significantly more on the dendrites of ON-cBCs (Figs. 5A, 5B), but not rod BCs (Fig. 5D), following maintained bright illumination, than following maintained darkness or following maintained bright illumination with SCH23390. Quantification of co-localized signals supports these results (Figs. 5C, S3).

Immuno-electron microscopic data revealed that in the rabbit puncta of $GABA_AR$-IR were located in the dendritic tips of ON-cBCs and HCs within the invagination of the cone synaptic (“triadic”) terminal following maintained bright illumination (Fig. 6A), as reported [17–19], but the number and size of $GABA_AR$-IR puncta were significantly reduced following maintained darkness (Fig. 6B, 6D) and following maintained bright illumination when dopamine D_1Rs were blocked (Fig. 6C, 6D). $GABA_ARs$ were also found on vesicles within cone pedicles following maintained bright illumination (Fig. 6A), as reported in fish [26], and following maintained darkness and maintained bright illumination when D_1Rs were blocked (Figs. 6B, C).

DISCUSSION

Roles of ON-cBC Dendritic $GABA_ARs$ and D_1Rs in Light/Dark Modulation of the RF Surround

The findings reported here show in the rabbit retina the following: (1) endogenous activation of dopamine D_1Rs and $GABA_ARs$ strengthens the RF surround of ON-cBCs (Figs. 2, 4); (2) activation of D_1Rs on the dendrites of ON-cBCs increases the $GABA_AR$ activity of the dendrites (Fig. 3); (3) $GABA_AR$ expression increases on the dendrites - including dendritic tips - of ON-cBCs due to bright light-induced activation of D_1Rs located on the dendrites (Figs. 5, 6); (4) following maintained darkness when D_1R activation is minimal, ON-cBC surround light responses and ON-cBC dendritic $GABA_AR$ activity are strongly reduced (Figs. 2, 3) and $GABA_AR$ expression is significantly lower on the dendrites and dendritic tips of ON-cBCs (Figs. 5, 6); (5) the hyperpolarizing surround responses of most ON-cBCs are mediated by a neural pathway that does not involve feedback to cones (Fig. 4); (6) the input resistance of ON-cBCs increases during $GABA_AR$-mediated hyperpolarizing surround light responses (Fig. 4D); and (7) following maintained bright illumination when D_1R activation is strong, rod BCs do not produce surround light responses and their dendrites do not exhibit $GABA_AR$ activity or express $GABA_ARs$ (Figs. 4C, 5D; STAR Methods). Together, these results show that the increase in $GABA_AR$ expression and activity of the dendrites – including dendritic tips - of ON-cBCs following maintained bright light-induced activation of ON-cBC dendritic D_1Rs strengthens the RF surround of ON-cBCs (Fig. 1).

Conversely, following maintained darkness when D_1R activation is minimal, $GABA_AR$ function is substantially reduced and surround strength is greatly diminished.

The relatively slow time course of D_1R-mediated regulation of $GABA_AR$ expression and activity, which likely involves receptor trafficking, is consistent with measurements of the time course of light/dark-induced changes in surround strength. Observations from *in vivo* mammalian GCs and from GCs in intact *in vitro* non-mammalian retinas indicate that light/dark-induced changes in GC surround strength occur slowly (up to 30 minutes or more) depending on the extent and rate of change of the background light [9, 10, 27, 28]. For example, following 30 minutes of bright illumination GC surround strength becomes minimal only after ~30 minutes of complete darkness, and following maintained darkness surround strength reaches full strength – including both surround activation and antagonism - following 30 minutes of subsequent bright illumination. We observed a change from full to minimal $GABA_AR$ expression and activity of ON-cBC dendrites ~30 min following SCH23390 application (see STAR Methods; Figs. 2, 3, 5, 6). These results are consistent with the idea that a sudden extreme change in background light level from bright to dark (or dark to bright) switches surround strength from robust - including both surround activation and antagonism - to minimal over the course of 30 min during which $GABA_AR$ expression and activity decrease from full strength to minimal.

However, unlike in the laboratory where sudden extreme changes in background light intensity are often used, ambient light in the natural outdoor environment typically changes gradually over the course of day and night. Experimental evidence suggests that changes in surround strength in response to gradual changes in background illumination require minutes, rather than tens of minutes, to reach steady state [8, 10, 27–29]. Thus, if surround strength signals the ambient light level as suggested [29, 30], then modulation of the surround from its strongest state during bright illumination (midday) until it is minimal (middle of the night) results from changes in $GABA_AR$ expression and activity that need to keep pace with the rotation of the Earth, *i.e.*, that occur gradually over the course of ~12 hours (Fig. 1B).

Although $GABA_AR$ activity itself, which increases the chloride conductance, can produce surround antagonism by reducing glutamate-mediated center responses (*i.e.,* by shunting inhibition), opposite-polarity hyperpolarizing surround responses (i.e., surround activation) that are $GABA_AR$-mediated require that the E_{Cl} of ON-cBC dendrites is more positive than that of the somata or synaptic terminals. The finding that the conductance of ON-cBCs decreased during $GABA_AR$-mediated hyperpolarizing surround light responses (Fig. 4D) suggests that GABA tonically excites ON-cBCs following maintained D_1R activation, *i.e.,* following maintained illumination. As a result, brief surround light stimulation brighter than the background decreases this GABA excitation, producing a hyperpolarization. We found that the absence or blockade of D_1R activation hyperpolarized the resting membrane potential of rabbit ON-cBCs by ~7 mV (Fig. 2), supporting the idea that GABA depolarizes ON-cBCs following maintained bright illumination when D_1R activation is strongest but not following maintained darkness when D_1R activation is minimal. This result is consistent with the observation that the resting membrane potential of cone-driven ON-BCs in amphibian eyecups became progressively more negative as the maintained background

illumination decreased in intensity [31]. Our conductance measurements (Fig. 4D) are not consistent with a scenario in which light-evoked inhibitory $GABA_AR$-mediated signals produce ON-cBC hyperpolarizing surround responses. If this were so, light stimulation and gabazine application in the presence of APB would increase and decrease the conductance of the cells, respectively.

Although some reports suggest that $GABA_AR$ activity depolarizes ON-cBC dendrites [32, 33], especially in the case of a specific ON-cBC subtype [34], other work does not support this view. However, the strong dependence of surround strength on the intensity and duration of background illumination, especially the finding that *in vivo* mammalian GC surround activation requires brighter maintained illumination than does surround antagonism [8, 10, 27. 29], and the findings here that the expression and activity of ON-cBC dendritic $GABA_ARs$ and the $GABA_AR$-mediated conductance of ON-cBCs increase as background illumination and D_1R activation increase, suggest that previous studies did not find that GABA depolarizes ON-cBC dendrites because the experiments were performed in the dark [34, 35] when surround activation does not occur. We note that one study of ON-BCs in rat retinal slices [36], done with gramicidin perforated patch recording (to preserve E_{Cl}) under light-adapted conditions, found no evidence of GABA depolarizations. However, the data in that report came primarily from rod BCs, rather than ON-cBCs, and it was not documented that the ON-BCs exhibited surround activation or any light responses at all. As noted in Results, ON-cBCs in bright light-adapted rabbit retinal slices produced weak, if any, light responses and did not exhibit surround activation.

Neural Pathways that Contribute to the RF Surround of ON-cBCs

The cellular source of the GABA that activates ON-cBC dendritic $GABA_ARs$ following maintained bright illumination is not yet resolved. However, the following findings together strongly suggest that HC dendrites (adjacent to ON-cBC dendrites within the cone synaptic terminal invagination [17–19]) contribute to ON-cBC surround responses [2, 3, 5, 37–40] by directly releasing GABA onto ON-cBC dendrites and activating their $GABA_ARs$ [31, 32, 41]: 1) synaptic blocking experiments indicate that activation of ON-cBC dendritic D_1Rs increases the $GABA_AR$ activity of ON-cBC dendrites (Fig. 3); and 2) APB experiments show that hyperpolarizing surround light responses of most ON-cBCs are mediated by a reduction in tonic GABA excitation and a pathway that does not involve direct cone input to ON-cBCs or feedback to cones (Fig. 4). Moreover, the observation that HC dendritic tips, which express D_1Rs [16], express $GABA_ARs$ following maintained bright illumination but not following maintained bright illumination during D_1R blockade or maintained darkness (Fig. 6) suggests that the background light level and D_1R activation modulate the expression and activity of HC dendritic and ON-cBC dendritic $GABA_ARs$ in a similar fashion. In addition, because GABA depolarizes HCs [42], it seems possible that the light-evoked D_1R/$GABA_AR$ pathway in HC dendrites enhances the tonic release of GABA onto ON-cBC dendrites via $GABA_AR$-mediated excitation of HCs. According to this view, HC light responses initiate ON-cBC surround responses but the effectiveness of the HC signal is strongly modulated by dopamine regulation of ON-cBC dendritic $GABA_ARs$ (Fig. 1B). Moreover, the D_1R-mediated decrease in HC coupling that occurs when background illumination increases [8, 16] may account for the decrease in RF surround size that has

been observed for GCs [8, 9, 29] and ON-cBCs (Fig. 2 here) when the background light level increases. Further HC studies are needed to test these ideas.

The data in Fig. 4 suggest that following maintained bright illumination most (~70%) rabbit ON-cBCs exhibit a $GABA_AR$-mediated surround that is not blocked by APB (i.e., driven by a pathway independent of direct cone input). This is consistent with the observation in amphibian retina that following maintained bright illumination ~67% of BCs receive surround input via a pathway that does not involve direct cone input (i.e., is APB-insensitive) [7]. Based on these findings, and also because *in vivo* immunoEM has shown that virtually all mammalian ON-cBC dendrites express $GABA_ARs$ [18], it seems plausible that in *in vivo* retina most (> 70%) ON-cBC dendrites express functional $GABA_ARs$ that mediate surround responses following maintained bright illumination. However, whereas our findings suggest that ON-cBC dendritic $GABA_AR$ activity plays a key role in ON-cBC surround responses following maintained bright illumination, our results do not reveal the relative weight of this surround mechanism. Non-$GABA_AR$ mechanisms, such as protons and ephaptic communication [8, 43–46], and other neural pathways, such as HC feedback to cones (Fig. 1A) [8, 47, 48], have been reported to contribute to ON-cBC surrounds. However, evidence is still needed to address whether these other mechanisms and neural pathways strongly contribute to ON-cBC surround activation and antagonism following maintained bright illumination but are weakly active following maintained darkness when surround responses are minimal [8].

Electrical recordings of *in vivo* mammalian ON-GCs and ON-BCs in intact *in vitro* retinas, as well as the findings here, have shown that the classic antagonistic RF surround: 1) originates in the outer retina; 2) is strongest following maintained bright illumination and minimal following maintained darkness; 3) is relatively sustained [8, 10, 11, 49, 50]; and 4) is D_1R-dependent (Figs. 2, 3, 5, 6 here) [23, 49]. The similarity in the characteristics of the classic antagonist surround of ON-cBCs and ON-GCs, post-synaptic targets of ON-cBCs, as well as the finding that D_1Rs mediate light/dark modulation of $GABA_AR$ expression of *in vivo* ON-cBC dendrites (Figs. 5, 6), suggest that dopamine regulation of ON-cBC dendritic $GABA_ARs$ contributes to light/dark modulation of the surrounds of *in vivo* ON-cBCs and ON-GCs. It is worth noting that some GC subtypes exhibit additional diverse surround mechanisms that may be produced in the inner retina. However, in contrast to the classic antagonist surround, inner retinal surrounds are relatively transient and D_1R-independent [8, 49–52].

Function of Light/dark Adaptive Regulation of the RF Surround

Because the RF surround enhances spatial discrimination and edge detection, differentiating regions of the visual scene that are brighter or dimmer than the background [8, 29], the bright light driven increase in D_1R activation, and the resultant increase in ON-cBC dendritic $GABA_AR$ expression and activity, may enhance the spatial discrimination ability of ON-center cBCs and their downstream targets such as ON-center GCs. Conversely, the greatly diminished function of $D_1Rs/GABA_ARs$ following maintained darkness weakens the surround of ON-cBCs, reducing their spatial discrimination ability. Moreover, our finding (Fig. 2) and reports that the RF centers and surrounds of cone-driven BCs and GCs shrink as

the maintained background light level increases [6–9, 29] suggest that the interaction between center and surround spatially differentiates progressively smaller regions of the visual scene as ambient illumination gradually increases, thereby enhancing the ability of BCs and GCs to discriminate fine spatial detail and edges. Light/dark adaptive modulation of the strength and size of the ON-cBC surround thus may contribute to modulation of the spatial discrimination ability of human and monkey observers by ambient illumination, as we and others suggested [8, 13, 29, 53]. The D_1R/$GABA_A$R signaling pathway of ON-cBC dendrites therefore serves as a light/dark adaptive regulatory mechanism that tunes synaptic and neural pathway function in the retina to visual performance needs under different ambient light levels, increasing the ability of animals to see fine spatial details on bright days and to see large dim objects on moonless nights.

STAR★METHODS

CONTACT FOR REAGENTS AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Stuart Mangel (mangel.1@osu.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals—Experiments were performed on adult pigmented Dutch-belted rabbits (2.5–4.0 kg) of either sex. Rabbits were maintained on a 12h/12h light-dark schedule (lights-ON at 6 am and lights-OFF at 6 pm) at a temperature of 22°C with food and water available ad libitum. Retinal eyecups were obtained following deep general (urethane, induction dose: 2.0 g/kg, 31% solution, i.p.) and local intraorbital (2% Xylocaine) anesthesia. Following enucleations, deeply anaesthetized rabbits were killed by injection of 3.5 M KCl into the heart. Animal care and use complied with NIH regulations and all USA guidelines, and all procedures involving the care and use of rabbits in this study were reviewed and approved by the Ohio State University Institutional Animal Care and Use Committee (PHS Animal Welfare Assurance No. A3261-01). All light and dark adaptation electrophysiological and immunocytochemical experiments in this study were carried out at midday.

METHOD DETAILS

Tissue preparation—Rabbits were kept dark-adapted for at least 1 hour before surgery and retinal slice preparation. Following enucleation, under dim-red illumination, a central rectangular portion of retina was cut close to the visual streak, placed photoreceptor side down on filter paper (Millipore, Billerica, MA) before isolating the retina. Thick (250-μm) slices were prepared with a tissue chopper (Stoelting, Wood Dale, IL) and placed in a custom electrical recording chamber for use over the course of 3 hours. The retinal slices were continuously superfused at 3 mL/min with bicarbonate-based Ames medium and maintained at 34°C and pH 7.4 by bubbling it with 95% O_2 and 5% CO_2. The osmolality was adjusted to 285 mOsm/kg.

Electrophysiology—The light and $GABA_A$R-mediated responses of cone and rod BCs in the superfused rabbit retina slice preparation were monitored using patch pipettes in the whole-cell configuration. The patch electrodes had resistances of 7–12 MΩ and were filled

with a solution that included (in mM): 91.3 K-Gluconate, 22.7 KCl, 4.1 $KHCO_3$, 5 HEPES, 5.0 (H^+)EGTA, 0.5 $CaCl_2$, 0.5 Na_3-GTP, 3 Mg-ATP, and 10 Na_2-Phosphocreatine. The pH was adjusted to 7.4 with 1N KOH, and the osmolality of the pipette solution was adjusted to 270 mOsm/kg. All membrane potential values in this study include a −10 mV correction for liquid junction potential. All chemicals and test drugs were purchased from Sigma-Aldrich (St. Louis, MO), except for gabazine, SCH23390 and TPMPA (all from Tocris, Bristol, UK), and Alexa Fluor 488 and peanut agglutinin (Invitrogen, Carlsbad, CA).

The $GABA_AR$ activity and E_{GABA} of ON-cBC dendrites were measured by pressure ejecting (6 psi, 100 msec) GABA (0.5 mM) from 6–8 MΩ pipettes [54]. GABA was applied onto ON-cBC dendrites in the OPL with synaptic transmission blocked with cobalt (2 mM) and in the presence of the $GABA_CR$ antagonist, TPMPA (50 μM), while the membrane potential of the cells was shifted between −90 and +10 mV using constant current pulses. The potential at which GABA did not evoke a response corresponded to E_{GABA}. In these experiments, the superfusate flowed over the slices toward the outer segments of cones and rods, so that GABA puffed onto ON-cBC dendrites did not reach the axon terminals of the cells. Dye included in the puff pipettes confirmed this (n = 4).

The I–V relationship of ON-cBCs was measured by clamping the cells at a holding potential of −40 mV and then stepping from −90 mV to +10 mV in 10-mV increments. This procedure was repeated for each cell (n = 5) following APB application in the dark and during light stimulation, and during application of both APB and GBZ. Steady-state current at each holding potential was then compared for the experimental conditions. Because of the presence of an outwardly rectifying current at step potentials more positive than −20 mV [55, 56], conductance calculations were restricted to holding potentials between −80 mV and −30 mV, the physiological range of the cells in which the relationship between current and voltage was relatively linear.

Identification of cell types—ON-cBCs and rod-BCs, both of which depolarize to small centered stimuli, were distinguished based on their different light response waveforms (Figs. 2, 4) and on differences in the characteristic morphology and location of Alexa488-injected axon terminals [57] (see Fig. S1 in Supplemental Information) and immunostained dendritic arbors (see Fig. 5),

Because we were able to distinguish ON-cBCs from rod BCs, our results show that the dendrites of ON-cBCs – but not rod BCs – express $GABA_AR$-IR and exhibit $GABA_AR$ activity following maintained bright illumination when D_1Rs are strongly activated. The finding that rabbit rod BCs do not exhibit surround responses under D_1R activation is consistent with previous indirect evidence in wholemount rabbit retina that AII amacrine cells, which receive direct synaptic input from rod BCs, do not exhibit surround responses under maintained illumination [58]. However, recent observations in mouse wholemount retina suggest that following maintained background illumination rod BCs produce hyperpolarizing surround responses to large (800-μm diam) spot stimuli due to cone-driven HC input to rods [59]. Further work is needed to determine whether the discrepancy has a technical basis or is due to a species difference.

Although we could distinguish ON-cBCs and rod BCs, it was more difficult to distinguish the 4–5 different rabbit ON-cBC subtypes [57] based on their light response waveforms recorded in a retinal slice preparation and the morphology and location of their Alexa488 fluorescent cell bodies and axon terminals. As a result, it is not possible at this time to address whether $GABA_AR$- and D_1R-dependent surround responses are characteristic of specific ON-cBC subtypes or rather characteristic of most ON-cBCs irrespective of subtype. In addition, it is worth noting that our ON-cBC results do not address whether OFF-cBC dendritic $GABA_ARs$ mediate OFF-cBC surround responses.

Light stimuli—Light stimuli, which were delivered to the retinal slices from a 2.54 cm-wide CRT monitor (green (545 nm light) phosphor) (Lucivid, MicroBrightField, Colchester, VT) that was positioned in a port of an Olympus Upright microscope (Model BX51WI), were used to map the sizes of the RF center and surround of ON-cBCs and to determine whether surround antagonism and activation contributed to ON-cBC light responses under control conditions when dopamine D_1Rs were activated, as well as following D_1R blockade. Because the light pathway to the slices was perpendicular to the direction of incident light to *in vivo* retinas, bar stimuli, rather than spots and annuli, were used. Centered single bars of various widths were used to assess the size and strength of the RF center and the size and strength of the antagonistic surround (i.e., difference in response size to wide compared to narrow bars). Two simultaneously flashed bars, which were equidistant from the RF center and separated by various distances, were used to stimulate different portions of the RF surround, thereby providing a measure of the strength of surround activation. Individual stimuli, which were non-saturating, were spaced apart by at least 15 sec and the entire series of stimuli were presented every 2 minutes to limit light adaptation. The stimuli were programmed with VisionWorks for Electrophysiology software (Vision Research Graphics, Durham, NH).

Immunocytochemical labeling—Rabbits were bright light (intensity = ~1,000 lux)- or dark (intensity = ~0.0001 lux)-adapted for 60 min. Following deep general and local anesthesia, as described above, physiological saline with or without SCH23390 (10 μM) was injected intraocularly under light-adapted conditions or physiological saline without SCH23390 was injected under dark-adapted conditions. Thirty minutes later rabbits were perfused via the cardiac circulation with fixative solution that contained 4% paraformaldehyde and 0.01% glutaraldehyde in 0.1 M phosphate buffer (PB). Their eyecups were then immersed in 4% paraformaldehyde or in a combination of 4% paraformaldehyde and 0.01% glutaraldehyde for light microscopic or electron microscopic immunocytochemistry, respectively. The retinas were cryoprotected by immersing them in increasing concentrations (10, 20 and 30%) of sucrose in 0.1 M PB, embedded and frozen. Vertical cryosections (20-μm thick) were then obtained and processed for immunostaining using the indirect fluorescence method. When two different monoclonal antibodies from the same species (mouse) were used [60], intrinsic IgG in the tissue was blocked with mouse IgG $F(ab)_2$ monoclonal antibody (1:100, 115-006-006, Jackson Immunoresearch, West Grove, PA) following blocking with normal mouse serum at a dilution of 1:10 and normal goat serum at a dilution of 1:5. A mouse monoclonal antibody that specifically recognizes the β2/3-subunit of $GABA_ARs$ (clone BD17, MAB341, Millipore) was used at a dilution of

1:100 to label $GABA_A$Rs. Double-labeling with this $GABA_A$R antibody and antibodies that label both ON-cBCs and rod-BCs (1:500, mouse anti-$G\alpha_o$, clone 2A, MAB3073. Millipore) or only rod-BCs (1:100, anti-PKC_α mc5, Sigma) revealed whether $GABA_A$Rs are located on ON-cBC dendrites under various experimental conditions. After incubation in primary antiserum, the sections were washed in 0.1 M PB and incubated for 2 h in one of the following fluorescent secondary antisera as appropriate: Alexa fluor(R) 488 (1:200, A21202) donkey anti-mouse IgG antibody and Alexa Fluor(R) 647 donkey anti-mouse IgG antibody (1:200, A31571),(Invitrogen, Carlsbad, CA). Retinas in all experimental conditions were always processed in parallel and in an identical manner.

Immuno-electron microscopy—Immuno-EM was performed as previously described [61]. Briefly, after cryoprotection, 3×3-mm pieces of retina were repeatedly frozen with liquid nitrogen and thawed to permeabilize membranes in order to enhance antibody penetration. Tissue was then blocked with 30% normal donkey serum and 1% bovine serum albumin in 5% sucrose PB (0.1 M). Next, the $GABA_A$R antibody was applied and incubated overnight for 3 d in 4°C, after which the biotinylated secondary antibody (Biotin-SP AffiniPure Donkey Anti-Mouse IgG, 715-065-150, Jackson Immnoresearch) for primary mouse IgG was applied at a dilution of 1:200 for 1 h at RT. The tissue was then washed with Tris buffer (0.1 M, pH 7.3) and quenched with 30% hydrogen peroxide and 5% methanol in 5% PBS. Next, ABC and DAB reactions were carried out sequentially (ABC and DAB kits, Vector Labs, Burlingame, CA). The DAB reaction product was silver-intensified the next day, and then gold-substituted, after which the gold particles were fixed with thio-sulfate and ammonium-tetraamine. OsO_4 in cacodylate buffer was then used for post-fixation. The tissue was then dehydrated by immersion in a graded (30–99.9%) acetone series and embedded in resin (Ted Pella, Redding, CA) overnight at 60°C.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data collection and analysis—Values are expressed as mean ± standard error of the mean. Electrophysiological data were acquired using Clampex Software and analyzed using Clampfit 10.1 (Molecular Devices), GraphPad Prism (GraphPad Software, Inc.), and R (R Foundation for Statistical Computing, Vienna, Austria). One-tailed t-tests (Figs. 2, 3, 5) or paired t-tests (Fig. 4) with the assumption of normality were used in all statistical analyses, except where noted. Details of the specific statistical tests used and the meaning of “n” for each experiment can be found within figure legends and associated text in Results. Data were considered significantly different at $p < 0.05$.

Fluorescent image acquisition and analysis of histological data—Immunolabeled images were acquired with a Zeiss 510 confocal microscope at the highest resolution in tiff format. ZEN software (Carl Zeiss, Germany) was used to reconstruct 3D images from a z-stack series of contiguous optical sections obtained at 0.30 μm intervals. When collecting images of different fluorophores, each fluorophore was excited sequentially and each emission channel was acquired independently to minimize bleed-through artifacts and dye cross-reactivity. This procedure resulted in z-stack sets of different fluorophores that contained the same number of sections. All of these images were then imported into NIH-ImageJ software to assess the relative strength of co-localized signals. Co-localized signals

were extracted and converted into a binary code by the Colocalization plugin of ImageJ. Using the same thresholding procedure for all experimental conditions, intensity measurements were obtained sequentially from the corresponding sections of the z-stack sets of different fluorophores. Images of labeled cells were divided manually into regions of interest (i.e., ON-BC dendrites), so that the number of co-localized pixels in each region of interest, which were more intense than a predefined threshold value, could be counted. Use of the JACoP plugin for ImageJ confirmed that the $GABA_A$R-IR puncta were located on ON-BC dendrites (Fig. S3) [62]. Final images were adjusted in intensity using Adobe Photoshop CS5 (Adobe, CA).

DATA AND SOFTWARE AVAILABILITY

Data are available on request from the Lead Contact, Stuart Mangel (mangel.1@osu.edu).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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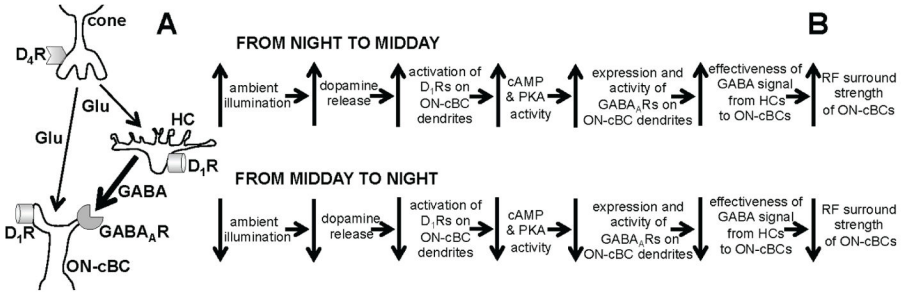

Figure 1. Schematic diagram showing signaling between cone, cone bipolar cell and horizontal cell (A) and model of how light/dark regulation of the dopamine D_1R/$GABA_A$R pathway of ON-cone bipolar cell dendrites modulates surround light responses (B)

(A) Cones use glutamate (Glu) to signal cone bipolar cells (cBCs) and horizontal cells (HCs). The dendrites of cBCs and HCs have both dopamine D_1Rs and $GABA_A$Rs. Evidence also suggests that HCs provide a feedforward GABA signal to cBC dendrites (shown) and an inhibitory feedback signal to cones (not shown), but the roles of these pathways in producing cBC surrounds remain unclear. (B) Model that accounts for how both the effectiveness of the GABA feedforward signal to ON-cBC dendrites and the strength of ON-cBC surround responses are modulated by gradual changes in the ambient (background) light level. As ambient illumination slowly increases during the morning, reaching a peak at midday, D_1R activation increases, which in turn augments intracellular PKA, so that the expression and activity of $GABA_A$Rs on ON-cBC dendrites - including dendritic tips - are enhanced. As a result, the effectiveness of the $GABA_A$R-mediated feedforward signal from HC dendrites to ON-cBC dendrites increases, enhancing the strength of surround antagonism and activation. Conversely, as background illumination slowly decreases during the afternoon and evening, reaching darkness at night, D_1R activation is reduced. This in turn decreases intracellular PKA, substantially lowering $GABA_A$R expression and activity so that ON-cBC surround strength is minimal. Experiments and findings in this study primarily address whether light/dark regulation of ON-cBC dendritic D_1Rs and $GABA_A$Rs modulates the strength of ON-cBC surround light responses.

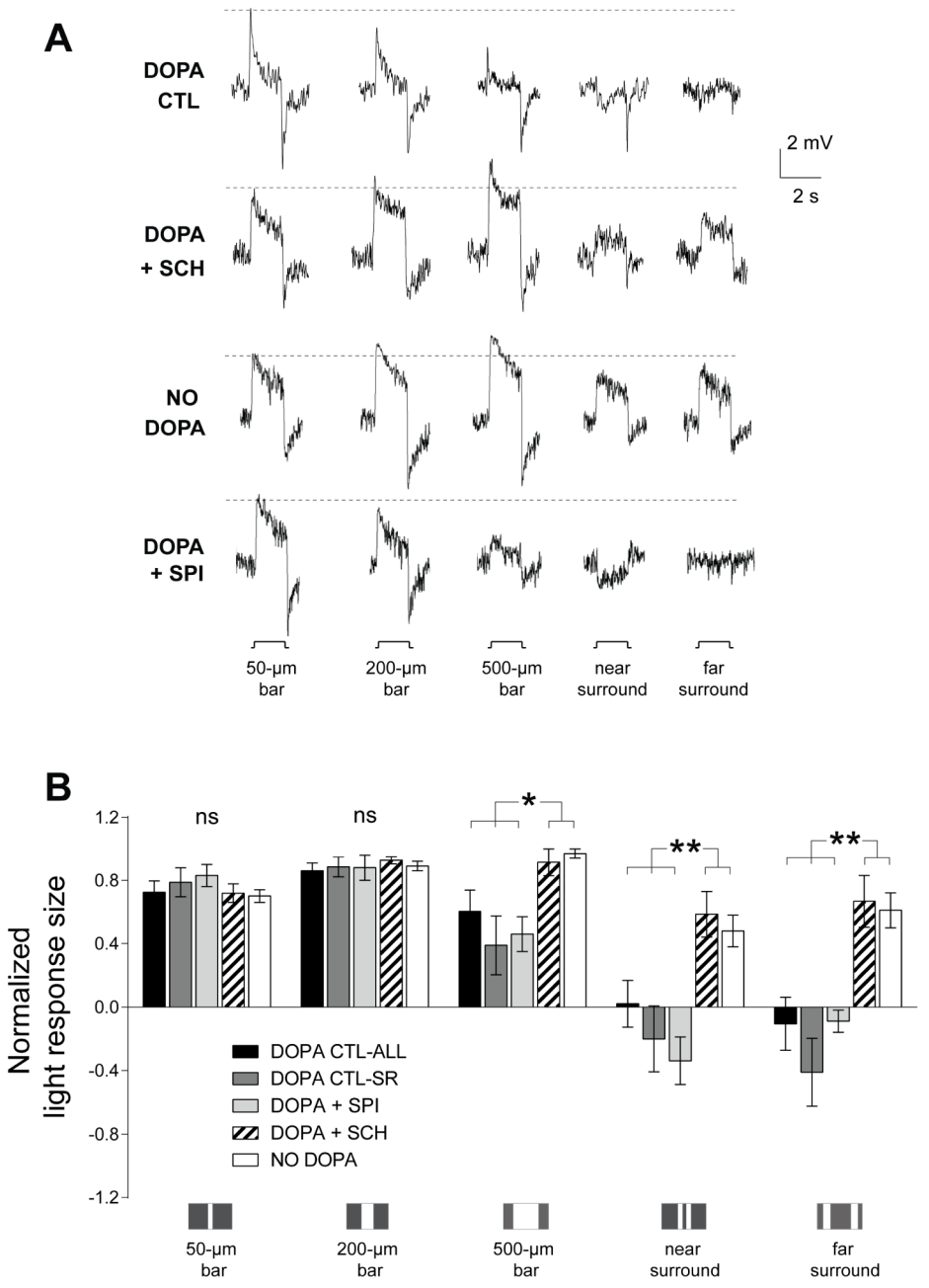

Figure 2. ON-cBC surround light responses depend on dopamine D_1R activation

(A) In dark-adapted rabbit retinal slices superfused with an Ames medium that contained dopamine (5 µM) to mimic the effect of maintained (30 min) bright illumination, ON-cBCs (top traces-control, DOPA CTL) exhibited both surround antagonism (i.e., the amplitude of center responses was reduced by simultaneous surround stimulation, as occurred in response to 500-µm (center and surround stimulation) vs. 50-µm (center stimulation only) wide bars), and surround activation (i.e., response to surround stimulation alone was opposite in polarity to the response produced by center stimulation alone, as occurred in response to near surround stimulation). In separate experiments, when the dopamine medium also contained

SCH23390 (SCH; 5 μM) for ≥30 min (traces second row from top) or when slices were superfused without dopamine (NO DOPA, traces third row from top) to mimic the effect of maintained darkness, ON-cBCs exhibited minimal surround antagonism and activation. When the dopamine medium also contained spiperone (SPI; 5 μM) for ≥30 min (bottom traces), ON-cBCs exhibited both surround antagonism and activation. On-cBCs with sustained and transient light responses were observed under all four experimental conditions. (B) Average normalized peak response size of ON-cBCs to centered single bar stimuli of various widths (50-, 200- and 500-μm) and to near and far surround stimulation revealed that, with respect to both surround antagonism ($p < 0.05$) and surround activation ($p < 0.01$), the entire population of dopamine control cells (DOPA CTL-ALL, n=18), the sub-population of dopamine control cells that exhibited clear surround light responses (DOPA CTL-SR, n=10), and the entire population of cells bathed in both dopamine and spiperone (DOPA + SPI, n=5) were significantly different from the population of SCH-treated ON-cBCs (DOPA + SCH, n=5) and from the cells superfused without dopamine (NO DOPA, n=6). (A, B) Surround responses were evoked by two simultaneously flashed bar stimuli equidistant from the RF center. Near surround stimulation: distance between 50-μm wide bars = 100 μm. Far surround stimulation: distance between 100-μm wide bars = 500 μm. (A) The dotted horizontal lines adjacent to the response traces denote the peak response amplitude of the cells to the smallest centered stimulus (50-μm wide bar).

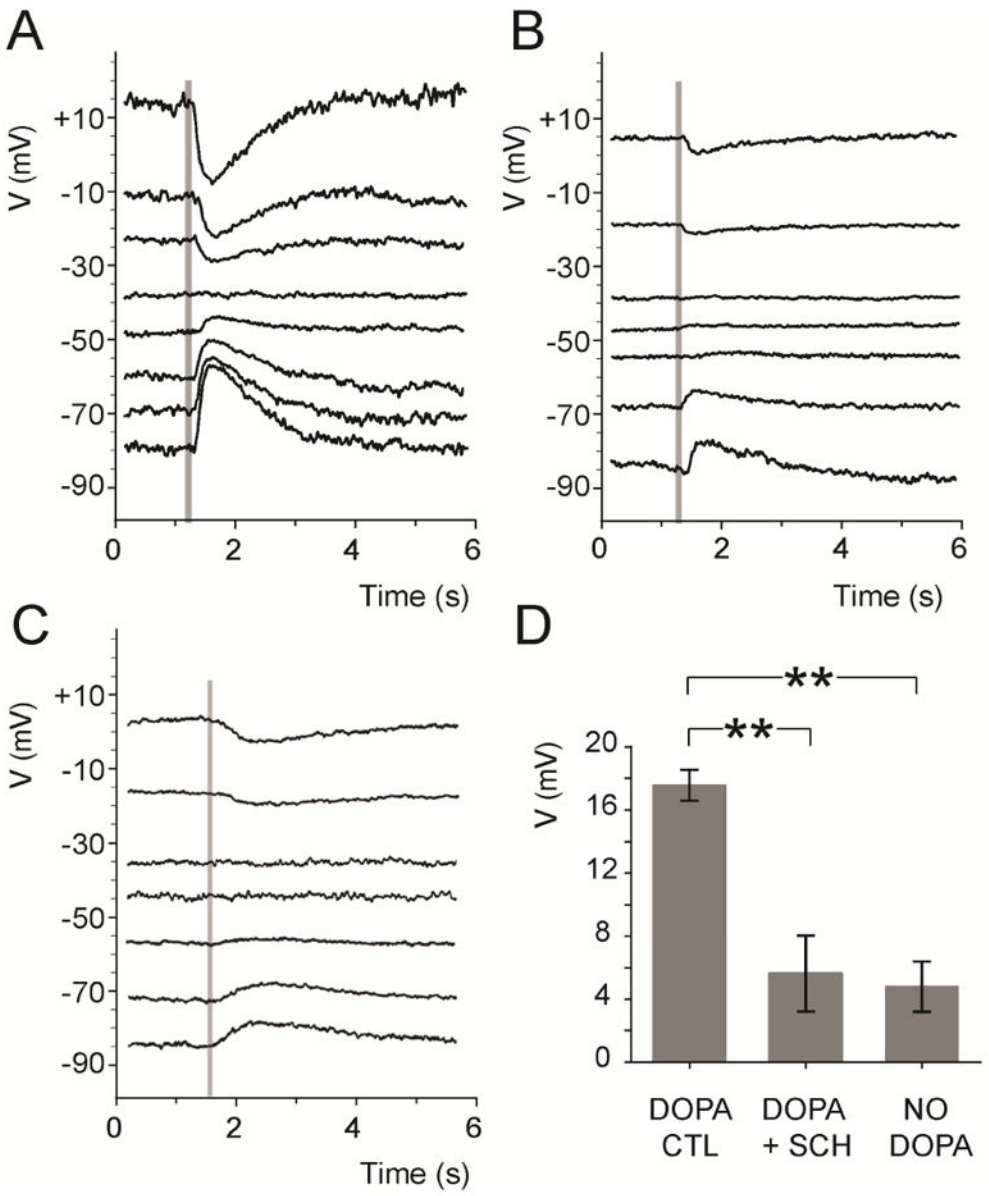

Figure 3. $GABA_AR$ activity of ON-cBC dendrites depends on endogenous activation of ON-cBC dendritic D_1Rs

In rabbit retinal slices superfused with medium that contained dopamine (5 μM) and TPMPA (50 μM) and in which synaptic transmission was blocked with cobalt (2 mM), GABA (0.5 mM) was puffed onto BC dendrites (time of puff is indicated by shaded vertical lines) by pressure ejection. GABA responses were clearly evident under dopamine (control) conditions (A) with an average GABA reversal potential (E_{GABA}) = −42.2 ± 2.6 (SEM) mV (n=5), but were much reduced in the presence of SCH (5 μM) (B) and when the superfusate did not contain dopamine (C). (D) When GABA was puffed at −70 mV, average peak $GABA_AR$ response size was significantly greater ($p < 0.01$) when the superfusate contained dopamine (DOPA CTL; 17.6 ± 1 mV; n=5) compared to when it contained dopamine and

SCH (DOPA + SCH; 5.6 ± 2.4 mV; n=5) or did not contain dopamine (NO DOPA; 4.8 ± 1.6 mV; n=5).

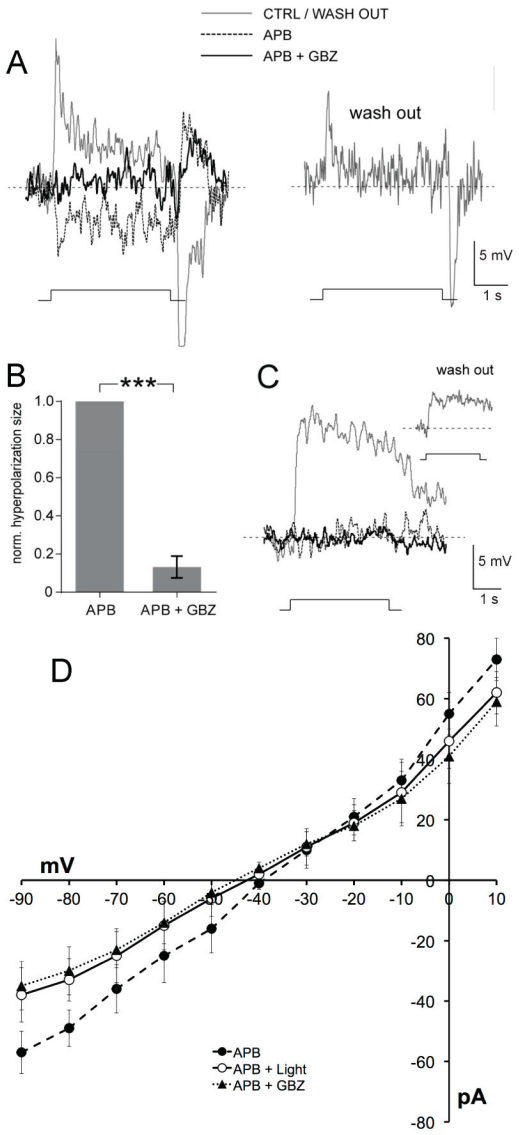

Figure 4. A reduction in tonic endogenous $GABA_AR$ excitation underlies the opposite-polarity, hyperpolarizing surround light responses of ON-cBCs

In the presence of dopamine (5 μM) and the glutamate analogue APB (i.e., L-AP4; 50 μM), a selective agonist of the mGluR6-Rs on both ON-cBC and rod BC dendrites that blocks cone to ON-cBC and rod to rod BC signaling [24], ON-cBCs (**A, B**), but not rod BCs (**C**), produced opposite-polarity (i.e., hyperpolarizing) surround responses to large (500-μm wide) centered bar stimuli that were blocked by gabazine (GBZ; 50 μM; 9 out of 9 cells). Washout of APB and GBZ showed recovery from their effects (**A, C**) (see Fig. S1 and "Identification of Cell Types" in STAR Methods). (**B**) For each ON-cBC, addition of GBZ to the APB-containing medium greatly reduced the average normalized size of opposite-polarity hyperpolarizing surround light responses ($p < 0.001$; paired t-test; n=9). (D) Comparison of the current-voltage relationship of ON-cBCs (n=5) following applications of APB alone in the dark and during surround light responses, and following application of both APB and gabazine in the dark. Average steady-state current at each holding potential (*i.e.,* current

measured near the end of the voltage pulses and when the amplitude of the light responses was relatively steady) is shown for all three experimental conditions.

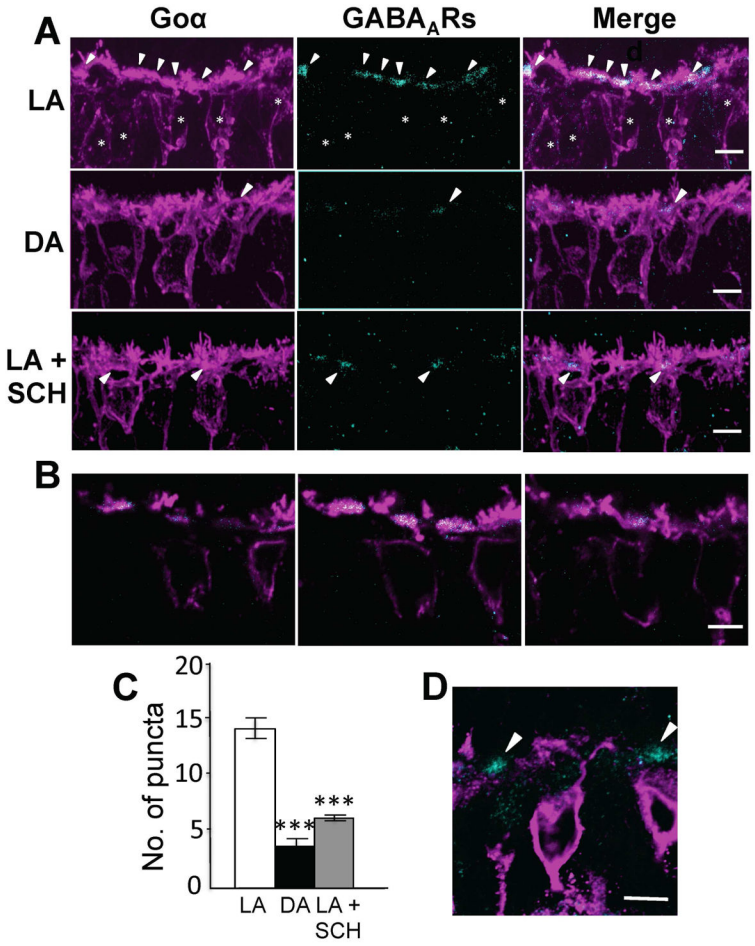

Figure 5. GABA$_A$R expression on rabbit ON-cBC dendrites is regulated by the illumination conditions and D$_1$R activation

(A, D) Projected images of several ON-BCs showing double immuno-staining of GABA$_A$Rs and Goα, which labels both ON-cBCs and rod BCs in the rabbit retina (A), and double immuno-staining of GABA$_A$Rs and PKCα, which labels rod BCs, but not ON-cBCs (D), revealed that GABA$_A$R-IR was located more on the dendrites of ON-cBCs (but not rod-BCs) following maintained bright light adaptation (LA) than following maintained dark adaptation (DA) or following maintained bright illumination with SCH (5 μM) (See also Fig. S2). Little evidence of GABA$_A$R-IR on rod-BC dendrites following maintained bright illumination (D) was observed. (B) Single confocal vertical optical sections (0.30-μm thick and at intervals of 0.60 μm) of the 3D-reconstructed rabbit ON-cBC dendrites shown in **A** indicate that GABA$_A$R-IR was located on the dendrites of ON-cBCs following maintained bright illumination. Immunostaining pattern in the absence of primary antibody confirmed the specificity of the GABA$_A$R labeling (data not shown). (C) Quantification showed that the average number of co-localized GABA$_A$R-IR signals on ON-cBC dendrites was significantly greater ($p < 0.01$) following maintained bright illumination compared to following maintained darkness or following maintained bright illumination with SCH. In addition, quantitative colocalization analysis confirmed that the GABA$_A$R-IR puncta were located on ON-BC dendrites following maintained bright illumination (Fig. S3). (A, B)

$GABA_A$Rs: cyan; ON-BCs (Goα): magenta; colocalized: white. (D) $GABA_A$Rs: cyan; rod BCs (PKCα): magenta. (A, B, D) Arrowheads denote colocalized puncta in A and B but denote $GABA_A$R-IR in D. (A) Asterisks denote ON-cBC somata. (A, B, D) Scale bars: 5 μm.

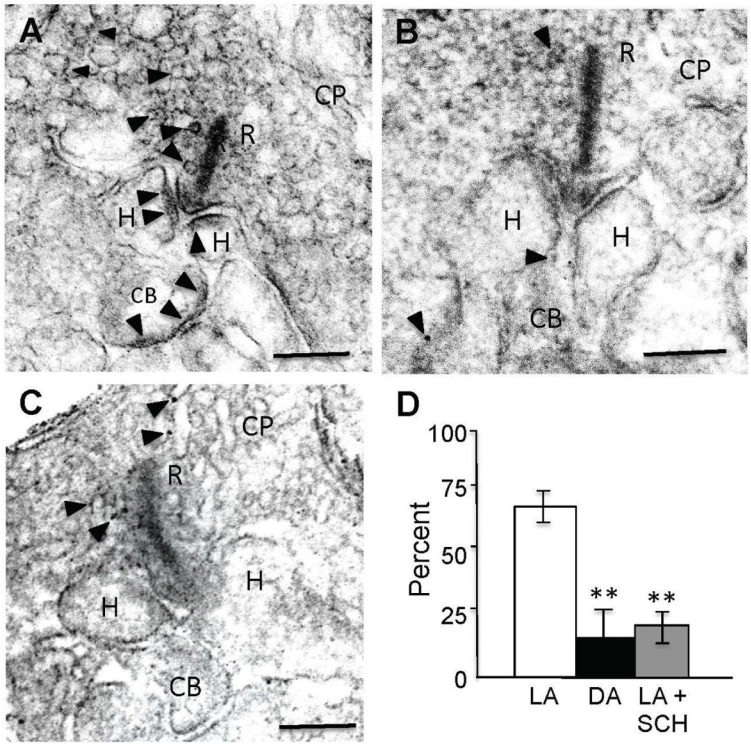

Figure 6. GABA$_A$R expression on the dendritic tips of ON-cBCs is increased by bright light-evoked dopamine D$_1$R activation

(**A–C**) GABA$_A$Rs were located at the plasma membrane of BC and HC dendritic tips following maintained bright illumination (A) but the number and size of clustered GABA$_A$R-IR puncta were reduced following maintained darkness (B) and following maintained bright illumination when D$_1$Rs were blocked with SCH (5 μM) (C). (D) Average percent of GABA$_A$R-IR puncta in invaginations of ON-cBC dendritic tips at cone ribbon (triadic) synapses for each of the three experimental conditions. The average percent values shown were obtained by first determining the average percent value for each retina and then calculating the average value for all of the retinas in each experimental condition (1 retina/rabbit, 3 rabbits per experimental condition). GABA$_A$R-IR was observed significantly more ($p < 0.01$; χ^2 test for both LA vs. DA and LA vs. LA + SCH) in invaginated ON-cBC dendritic tips following maintained bright illumination (light-adapted, LA; total of 33 triadic synapses) than following maintained darkness (dark-adapted, DA; total of 27 triadic synapses) and following maintained bright illumination when D$_1$Rs were blocked (LA + SCH; total of 23 triadic synapses). (A–C) Arrowheads: GABA$_A$R-IR puncta; R: synaptic ribbons; CP: cone pedicles; CB: ON-cBC dendrites; H: HC dendrites; Scale bars: 100 nm. See also Figure S2.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal antibody anti-β2/3 subunit of $GABA_A$ receptor; clone BD-17	Millipore	Cat#MAB341; RRID: AB_2109419
Anti-G-protein Goα; clone 2A	Millipore	Cat#MAB3073; RRID: AB_94671
Anti-PKCα, mc5	Sigma-Aldrich	Cat#P4334; RRID: AB_477345
Alexa Fluor®488 Donkey anti-mouse IgG antibody	Invitrogen	Cat#A21202; RRID: AB_141607
Alexa Fluor®647 Donkey anti-mouse IgG antibody	Invitrogen	Cat#A31571; RRID: AB_162542
Mouse IgG F(ab)2 monoclonal antibody	Jackson Immunoresearch	CAT#115-006-006
Biotin-SP AffinityPure Donkey anti-mouse IgG	Jackson Immunoresearch	Cat#715-065-150; RRID: AB_2307438
Chemicals, Peptides, and Recombinant Proteins		
SCH23390; dopamine D_1 receptor antagonist	Tocris	Cat#0925
Spiperone, dopamine D_2-like receptor antagonist	Sigma-Aldrich	Cat#S7395
Gabazine, $GABA_A$ receptor antagonist	Tocris	Cat#1262
TPMPA, $GABA_C$ receptor antagonist	Tocris	Cat#1040
Alexa Fluor 488 Hydrazide	Invitrogen	Cat#A10436
Lectin Peanut Agglutinin (PNA) from Arachis Hypogaea; Alexa Fluor ® conjugate	Invitrogen	Cat#L21409
Osmium tetroxide	Fisher Scientific	Cat#AC19118-0010
Gold Chloride Trihydrate	Fisher Scientific; MP Biomedicals	Cat#0215252601
Silver Nitrate	Fisher Scientific	Cat#BP2546100
Critical Commercial Assays		
Vectastain Standard Elite ABC Kit	Vector Labs	Cat#3333333
DAB Substrate Kit	Vector Labs	Cat#SK-4100
Epon812 Embedding kit	Electron Microscopy Science	Cat#50-980-391
Experimental Models: Organisms/Strains		
Dutch-belted rabbits	Myrtle's; Covance	N/A
Software and Algorithms		
VisionWorks for Electrophysiology	Vision Research Graphics, Durham, NH	http://www.vrg.com

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Clampfit 10.1	Molecular Devices	https://www.moleculardevices.com/systems/conventional-patch-clamp/pclamp-10-software
R	R Foundation for Statistical Computing, Vienna, Austria	https://www.r-project.org/about.html
GraphPad Prism, Version 6.01	*GraphPad Software, Inc.*	https://www.graphpad.com
ZEN Software	Carl Zeiss, Germany	https://www.zeiss.com/microscopy/us/products/microscope-software/zen.html
JACoP plugin for ImageJ	NIH	https://imagej.nih.gov/ij/plugins/track/jacop.html