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Research report

Dopamine effect on the stimulus pattern related changes in response characteristics of R/G horizontal cells in carp retina

Yu Liu[a], Fu-Jun Luo[a], Pei-Ji Liang[b,*]

[a]*Key Laboratory of Neurobiology, Shanghai Institute of Physiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, China*
[b]*Department of Biomedical Engineering, College of Life Science and Biotechnology, Shanghai Jiao Tong University, 1954 Hua-Shan Road, Shanghai 200030, China*

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Abstract

Repetitive red flashes increased the R/G horizontal cells' red response amplitude and induced a hyperpolarization of the cells' dark membrane potential. These phenomena were eliminated in 6-OHDA pretreated retinas and restored by exogenous dopamine, which suggests the involvement of dopamine receptor activity changes instead of dopamine release changes. Furthermore, the phenomena persisted on D_1 receptor antagonist (SKF-83566) application, whereas they diminished on D_2 receptor antagonist (eticlopride) application, indicating that the mechanism is related to a D_2 receptor, possibly located on photoreceptors.

Theme: Sensory system

Topic: Retina and photoreceptor

Keywords: R/G HC; Stimulus pattern; Dopamine; D_1/D_2 receptors

Horizontal cells (HCs) are second-order neurons in the vertebrate retina that receive direct synaptic inputs from photoreceptors and respond to light with graded potentials. HCs release the inhibitory neurotransmitter γ-aminobutyric acid (GABA) upon depolarization. This forms a negative feedback pathway between HCs and photoreceptors, which contributes to the formation of color opponency that occurs in the chromaticity type HCs [19]. In teleost retina, cone driven HCs also receive extensive innervation from dopaminergic interplexiform cells (IPCs), which results in various modulatory effects in signal transmission in the outer plexiform layer and regulates the HCs' activity [6].

It was reported that HCs' light response properties could be affected by conditioning light projected onto the retina [1,10]. Morphological studies revealed that the responsiveness changes involved synaptic modifications, e.g. conditioning light induced spinule formation [8]. It was also suggested that these changes might be related to the corresponding changes in dopamine (DA) release, since conditioning light failed to induce observable synaptic changes when dopaminergic neurons were depleted or the retina was treated with haloperidol containing perfusate [4].

There are mainly two types of dopamine receptors expressed in the retina, i.e. D_1 and D_2 receptors. It has been demonstrated that in the retina, D_1 and D_2 receptors might distribute differentially and couple with distinct mechanisms thus exert different modulatory effects on retinal functions [17]. HCs mainly contain D_1 receptor, the activation of which will decrease the strength of electrical coupling between HCs via the cAMP pathway [14,15] and enhance the ion conductance of glutamate receptors on HCs [13]. On the other hand, the IPCs possess a D_2 receptor as autoreceptor, the activation of which results in inhibition of dopamine synthesis and release [9]. Additionally, recent evidence suggests the existence of D_2 receptor on photoreceptor and the dopamine inhibition effect on the intracellular cAMP in cone photoreceptors, which are

*Corresponding author. Tel./fax: +86-21-6407-0495.
E-mail address: pjliang@sjtu.edu.cn (P.J. Liang).

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related to the modulation of cone retinomotor movement [2,16].

One of the chromaticity-type HCs is R/G HC. It receives direct input from green cones and hyperpolarizes to green light; in the meantime, a red cone signal is provided into the R/G HC via a GABAergic feedback cascade, the R/G HC thus responds to dim and moderate red light with graded depolarization [21]. Since the color opponency of the R/G HC involves glutamatergic and GABAergic mechanisms, both of which are dependent on the light stimulus pattern, the cell's spectral properties can thus be regulated by various factors including conditioning light. In the present study, it was observed that in the R/G HCs, repetitive red flashes elicited a gradual hyperpolarization of the cell's dark membrane potential and increased its depolarizing red response amplitude. Furthermore, pharmacological studies revealed that DA, acting via the D_2 mechanism instead of the D_1 pathway, should be involved in the regulation of the cell's responsiveness.

Experiments were performed on isolated retina of adult carp (*Carassius auratus*; body length: 15–20 cm) following a procedure similar to a previous report [10]. Briefly, the fish was kept in an aquarium on a natural light–dark cycle. After being dark-adapted for 20–30 min, the animal was decapitated and the eye enucleated. The retina was isolated and then transferred, with the photoreceptor side up, into a recording chamber with a volume of 1.4 ml and perfused with oxygenated (95% O_2+5% CO_2) Ringer solution at a flow-rate of 4.5 ml/min. All the procedures were performed under dim red illumination. The Ringer solution contained (in mM): NaCl 116.0, KCl 2.4, $CaCl_2$ 1.2, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 30.0, glucose 10.0, and had a pH value of 7.7 at room temperature. In some experiments, the dopaminergic interplexiform cells were destroyed by injecting 10 μl 6-hydroxydopamine (6-OHDA) solution (9 mg/ml in 0.9% NaCl, with 2 mg/ml ascorbic acid to prevent possible oxidation of DA) into the right eye of the animal about a week before the experiment. As control, 10 μl 0.9% NaCl was injected into the left eye [20]. Exogenous DA was applied at a concentration of 20 μM in Ringer solution, together with 60 μM ascorbic acid as an antioxidant. SKF-83566, a selective D_1 receptor antagonist, dissolved in ethanol, was added to the Ringer solution at a concentration of 5 μM (with the final concentration of ethanol in Ringer solution lower than 0.5%). *S* (−)-Eticlopride hydrochloride (eticlopride), a selective D_2 receptor antagonist, was dissolved in Ringer solution at a concentration of 20 μM. All drugs applied were purchased from Sigma (St. Louis, MO, USA). The light beam, generated from a 100 W tungsten halogen light source (Osram, Germany), was set to present an 8-mm diameter diffuse light spot on the retina. The duration of light stimuli was controlled using a magnetic shutter (MS-101, Hirogo, Japan). Interference filters and neutral density filters were used for selecting the wavelength and intensity of the stimulus, respectively. The light intensities were calibrated using a photodetector (IL 1400A, International Light, USA). The unattenuated 701 nm light (log I=0) was 8.22×10^{13} quanta/(cm^2·s). All stimulus intensities are presented in relative log units in this paper. The microelectrode was pulled on a micropipette puller (PD-30, Narishige, Japan) and had a resistance of 100 MΩ when filled with 4 M K-acetate. A micromanipulator (PF-1, Narishige) was used to advance the electrode into the retina. The cell's responses were measured using an amplifier system (MEZ-8300, Nihon Kohden, Japan) and displayed on an oscilloscope (TDS 210, Tektronix USA). Raw data were sampled via an A/D interface (AD8111, Adlink Technology, Taiwan) and stored in an IBM-compatible PC for off-line analysis.

Red and green light stimuli of moderate intensities (log I=−2.89 for 701 nm and log I=−4.02 for 504 nm) were applied to elicit the R/G HC's hyperpolarizing green response and depolarizing red response of appropriate amplitudes. In order to study the effects of flickering light on the characteristics of the cells' photoresponses, we applied 30 repetitive stimuli of 500 ms duration at a rate of 1 Hz. Student's *t*-test was performed for statistical analysis.

It was found that repetitive red flashes remarkably altered the R/G HC's light responsiveness. As shown in Fig. 1A, the red flickering stimuli induced an increase in the amplitude of R/G HC's depolarizing response to the red light (11.5 vs. 13.8 mV, measured at the beginning and towards the end of the red flash sequence, respectively), which results in an end-flickering relative response amplitude (normalized against control) of 120%. It is interest-

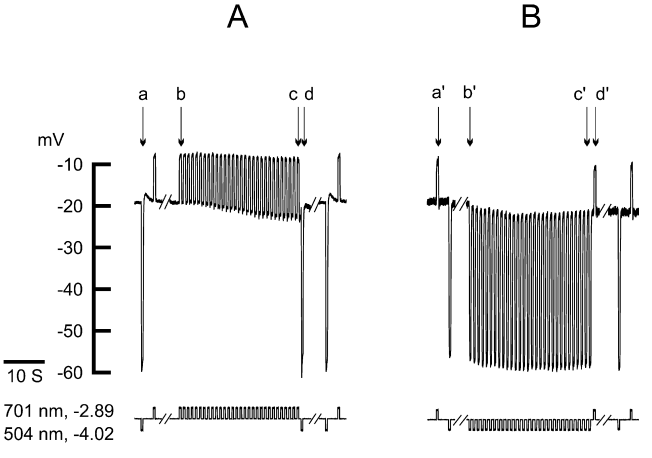

Fig. 1. Red flickering induced changes of R/G HC's responsiveness. (A) R/G HC's light response before, during and after a red (701 nm, −2.89) flickering sequence (upper panel: R/G HC's light response; lower panel: light flash). The repetitive red flashes increased the cell's red response amplitude (b, c) but induced no significant change of its response to green light (504 nm, −4.02; a, d). The cell's dark membrane potential was gradually hyperpolarized during the 30-s flickering period. (B) The same R/G HC's light response before, during and after a green (504 nm, −4.02) flickering sequence. No significant change was observed in the cell's response to green light during the period of repetitive flashes (b′, c′). The cell's response to red light was also unaffected (a′, d′).

ing that the response amplitude enhancement resulted from a progressive hyperpolarization of the cell's dark membrane potential rather than any change in the peak level of its depolarizing response. There was no observable change in the cell's response to green test flash induced by the red flickering (38.6 vs. 38.5 mV). Statistics from 21 recordings showed that the R/G HCs' red response enhancement (126±14.6%, mean±S.D.) and the hyperpolarization of the cells' dark membrane potential (1.9±0.90 mV) were both significant ($P<0.05$, paired *t*-test). After the red flickering sequence had ceased, the changes of the R/G HC's responsiveness recovered gradually in the dark or in the presence of green flickering light. On the other hand, the repetitive green flashes failed to induce any significant change of the R/G HC's response characteristics (as shown in Fig. 1B). Similar observations were made from a total number of 21 cells. Our further experiments were thus focused on the red flickering induced changes of R/G HC's responsiveness.

Previous evidences showed that retinal HCs receive extensive innervation from dopaminergic interplexiform cells [6] and DA release could be effectively regulated by light stimulation [5,11]. To investigate whether or not the DA modulatory effect was involved in the repetitive red flashes induced R/G HC responsiveness changes, we destroyed the dopaminergic interplexiform cells by intraocular injection of 6-OHDA. The experimental results revealed that the red flickering light failed to affect the R/G HC's responsiveness in those retinas pretreated with 6-OHDA (Fig. 2a). Consistent observations were made from all the preparations investigated. There was remarkable difference for the end-flickering relative response amplitude between the retinas injected with 0.9% NaCl and 6-OHDA (121±6.9 vs. 100±6.2%, respectively, $P<0.05$, $n=7$, unpaired *t*-test). The corresponding membrane hyperpolarizations were 1.6±0.54 and 0.2±0.38 mV, respectively ($P<0.05$).

Having the result that the flickering-induced responsiveness changes in the R/G HC is DA dependent, one would ask whether these phenomena are attributable to the changes in DA release or its receptor activity. We thus tried a protocol in which the 6-OHDA pretreated retina was perfused by DA-containing Ringer solution. As illustrated in Fig. 2, the results indicated that when the preparation was perfused using normal Ringer, repetitive red flashes caused little change in the R/G HC's light response (5.8 vs. 6.2 mV before and after the red flickering) and as in the cell's dark membrane potential. The administration of 20 μM DA depolarized the R/G HC by about 16 mV and increased its red response from 6.2 to 8.2 mV. Prominent response enhancement from 8.2 to 10.3 mV, with the relative amplitude being 125%, occurred during exposure to the repetitive red flashes. Consistently, the cell's dark membrane potential hyperpolarized noticeably. In all five recordings made in 6-OHDA pretreated retinas, DA application effectively restored the changes in the response

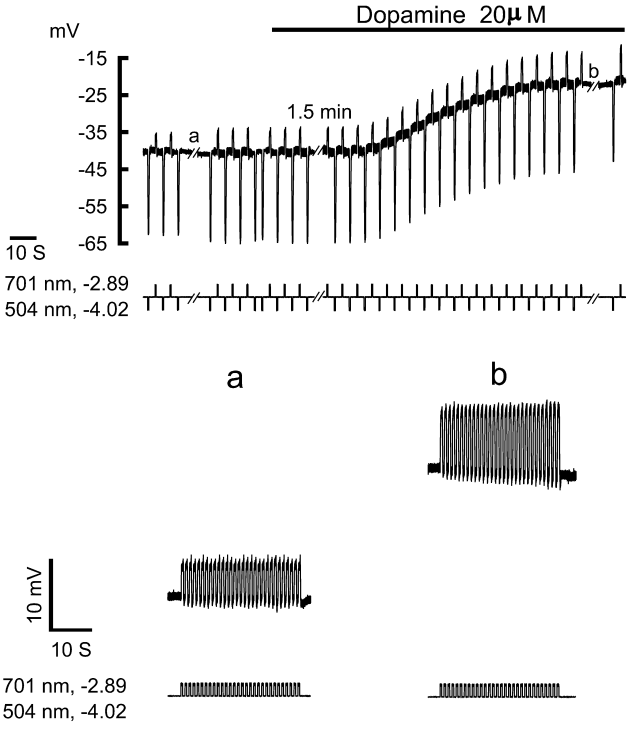

Fig. 2. DA-depletion abolished the red flickering effect. In 6-OHDA pretreated retina, repetitive red flashes induced no changes in the R/G HC's responsiveness (a); when 20 μM exogenous DA was applied, the membrane potential was depolarized and the amplitude of the cell's red response was increased; the red flickering effect on the R/G HC was restored by the application of 20 μM exogenous DA (b). During drug application, red and green test flashes were given with light-on duration being 500 ms and the inter-flash interval being 2.5 s.

amplitude (with the end-flickering relative response amplitude being 129±6.1%) and the dark membrane potential (with hyperpolarization being 1.5±0.62 mV), both of which were significant ($P<0.05$, unpaired *t*-test).

These results clearly indicated that the dopaminergic pathway played an important role in red-flickering-induced changes in the R/G HC's responsiveness, and the alteration is most likely due to the DA receptor activity changes instead of DA release changes. It has been reported that the DA receptors in the carp retina are essentially of D_1 and D_2 subtypes, which are distributed differentially and exert distinct functions in the retina [17,22]. To identify which subtype(s) of DA receptor contribute(s) to the stimulus-pattern-related changes in the R/G HC's response characteristics, the following protocols were applied. SKF-83566, a selective D_1 antagonist, was used. In normal Ringer, the repetitive red flashes increased the R/G HC's red response from 6.3 to 7.9 mV, with the relative amplitude being 125% (Fig. 3a). However, the red flickering-induced enhancement of the R/G HC's response to red flash persisted during the drug application (4.7 vs. 6.1 mV, with the relative amplitude being 130%), an accompanying moderate hyperpolarization of the dark membrane potential (2.0 mV) was also observed (Fig. 3b). During the drug adminis-

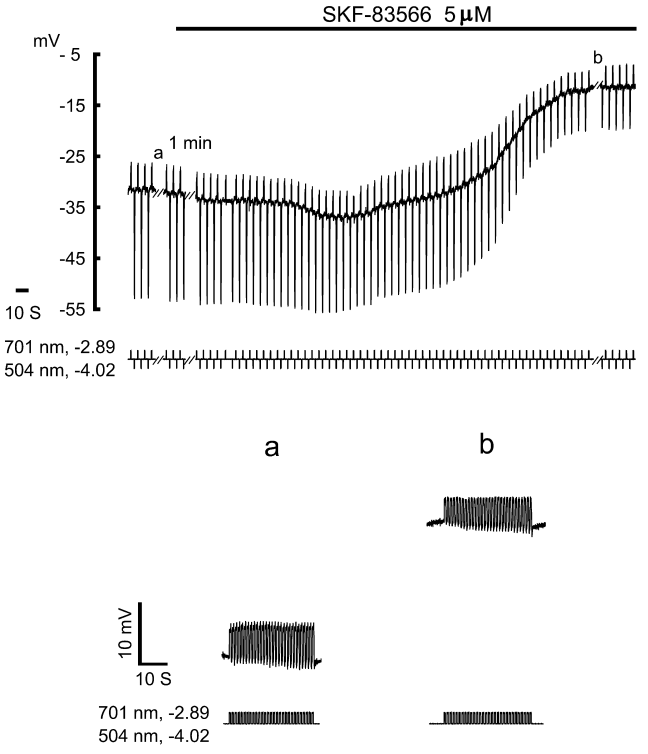

Fig. 3. SKF-83566 failed to influence the red flickering effect. Red-flickering-induced R/G HC's depolarizing response enhancement and dark membrane potential hyperpolarization in normal Ringer's solution (a); the application of 5 μM SKF-83566 depolarized R/G HC's membrane potential and decreased its response to light; the red flickering effect persisted in the presence of 5 μM SKF-83566 (b). During drug application, red and green test flashes were given with light-on duration being 500 ms and the inter-flash interval being 2.5 s.

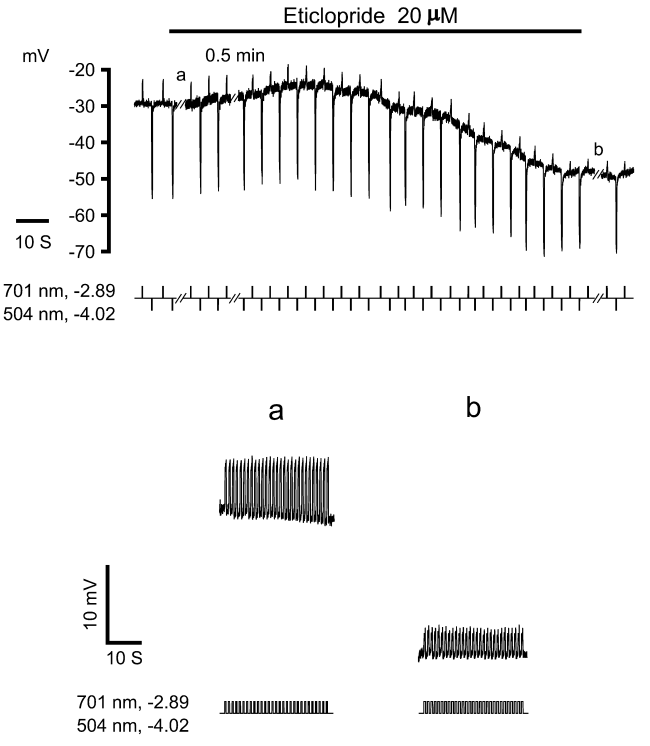

Fig. 4. Eticlopride eliminated the red flickering effect. Red-flickering-induced R/G HC's depolarizing response enhancement and dark membrane potential hyperpolarization in normal Ringer's solution (a); the application of 20 μM eticlopride hyperpolarized R/G HC's membrane potential and decreased its response to light; the flickering-induced responsiveness changes were completely diminished by application of 20 μM eticlopride (b). During drug application, red and green test flashes were given with light-on duration being 500 ms and the inter-flash interval being 2.5 s.

tration, the relative response enhancement and hyperpolarization of dark membrane potential in exposure to repetitive red light were 124±5.2% and 1.1±0.64 mV, respectively (n=4). No statistical significance was tested in SKF-83566 solution as compared to control (126±14.6% and 1.9±0.90 mV) (P>0.05, unpaired t-test). It is therefore suggested that D_1 receptor is not necessarily involved in the flickering related effect on the R/G HC's responsiveness.

Eticlopride, a chemical which antagonizes D_2 receptor, was also tested. In normal Ringer, the repetitive red flashes increased the R/G HC's red response from 6.1 to 8.0 mV with the relative amplitude being 131% and hyperpolarized the cell's dark membrane potential by 1.9 mV (Fig. 4a). The application of 20 μM eticlopride hyperpolarized the R/G HC by around 18 mV and suppressed its response amplitude from 6.2 to 3.2 mV. The red flickering-induced red response enhancement and dark membrane potential hyperpolarization were completely eliminated (101% and 0.0 mV, respectively) (Fig. 4b). The statistical result was that the red flickering light could no longer elicit the cell's responsiveness changes when 20 μM eticlopride was applied, the corresponding response enhancement and the membrane potential hyperpolarization were 101±2.1% and 0.2±0.49 mV respectively (n=5), the depression effect was significant as compared to control (P<0.05, n=5, unpaired t-test).

A large number of evidence has demonstrated that D_1 and D_2 receptors played different roles in retinal function. Dopamine, through activation of D_1 receptor on HCs, has been reported to decrease the strength of electrical coupling between HCs [14] and enhance the ion conductance of glutamate receptors on HCs [13]. It was also found that light-induced spinule formation of HCs relied on DA effect via D_1 receptor pathway [3,4]. On the other hand, activation of D_2 receptor on dopaminergic IPCs inhibits the DA release [9] and thus exerts the opposite effect to the D_1 receptor. Coincidently, in our experiments, blocking the D_1 pathway depolarized the R/G HC while antagonizing the D_2 receptor hyperpolarized the cell, which indicates that DA acting through distinct receptor pathways differentially modulated the response characteristic of the R/G HC.

It was previously reported that during light adaptation, the spectral sensitivity of the R/G HCs gradually changed in concord with a progressive development in the depolarizing component [4]. One argument is that repetitive flashes somewhat resemble the effect of light adaptation.

However, the light adaptive effect is not necessarily responsible for the flickering-elicited response changes of the R/G HCs, because only red flickering but not green flickering stimuli influenced the R/G HCs' response characteristics. The flickering-related process thus differs from that of light adaptation, at least in the sense that the flickering effect is pathway dependent. More importantly, the light adaptation process involves the D_1 receptor mechanism [12]. But in our experiment, the selective D_1 receptor antagonist SKF-83566 had no effect on flickering-induced changes in the R/G HC's responsiveness. In contrast, the phenomenon was blocked by the administration of eticlopride, which suggested that D_2 receptor was involved.

It is believed that stimulation of the D_2 receptor, primarily as an autoreceptor on the retinal dopaminergic neurons, reduces the DA release. However, the flickering effects observed in the present study can hardly be attributed to the modulation of DA release since the phenomenon could be restored when exogenous DA was applied to the 6-OHDA pretreated retina where the endogenous DA release was abolished. Thus, the DA release is not necessarily involved, although the flickering light might actually cause DA release [5].

Therefore, activation of D_2 receptor on the neurons other than the IPCs was required for the flickering effect. The most suitable candidate is probably the D_2 receptor on the cone photoreceptor [2,16], activation of which will inhibit the intracellular cAMP and protein kinase A (PKA) [16], and thus regulate the activity of $GABA_A$ receptor [7]. Since R/G HC's depolarizing red response involves GABAergic synaptic transmission from HCs to cone photoreceptors, activation of the D_2 receptor will inhibit PKA and dephosphorylate the $GABA_A$ receptor, which might render the negative feedback pathway a willing state being sensitive to flickering light. It was reported that in the hippocampus, tetanus stimulation was effective in inducing long-term potentiation of $GABA_A$ receptor-mediated IPSPs via the PKA pathway [18]. It is likely that a similar mechanism applies to the GABAergic synapse between HCs and cones, which explains the red flickering-induced enhancement of the feedback efficacy, and thus the changes in the R/G HCs' responsiveness.

Taken together, our electrophysiological evidence confirms the existence of a D_2 receptor in the outer plexiform layer of carp retina. The activation of the D_2 receptor might be related to the synaptic plasticity of GABAergic pathway between HCs and cone, which is responsible for the red flickering induced responsiveness changed in the R/G HC.

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References

[1] A. Akopian, J. McReynolds, R. Weiler, Short-term potentiation of off-responses in turtle horizontal cells, Brain Res. 546 (1991) 132–138.
[2] A. Dearry, B. Burnside, Dopaminergic regulation of cone retinomotor movement in isolated teleost retinas. I. Induction of cone contraction is mediated by D_2 receptors, J. Neurochem. 46 (1986) 1006–1021.
[3] M.B. Djamgoz, J.E. Downing, M. Kirsch, D. Prince, H.J. Wagner, Plasticity of cone horizontal cell functioning in cyprinid fish retina: effects of background illumination of moderate intensity, J. Neurocytol. 17 (1988) 701–710.
[4] M.B. Djamgoz, M. Kirsch, H.J. Wagner, Haloperidol suppresses light-induced spinule formation and biphasic responses of horizontal cells in fish (roach) retina, Neurosci Lett. 107 (1989) 200–204.
[5] C.-J. Dong, J.S. McReynolds, Comparison of the effects of flickering and steady light on dopamine release and horizontal cell coupling in the mudpuppy retina, J. Neurophysiol. 67 (1992) 364–372.
[6] J.E. Dowling, Dopamine: a retinal neuromodulator, Trends Neurosci. 9 (1986) 236–240.
[7] A. Feigenspan, J. Bormann, Facilitation of GABAergic signaling in the retina by receptors stimulating adenylate cyclase, Proc. Natl. Acad. Sci. USA 91 (1994) 10893–10897.
[8] S.N. Haamedi, M.B. Djamgoz, Effects of different patterns of light adaptation on cellular and synaptic plasticity in teleost retina: comparison of flickering and steady lights, Neurosci. Lett. 206 (1996) 93–96.
[9] K. Harsanyi, S.C. Mangel, Activation of a D_2 receptor increases electrical coupling between retinal horizontal cells by inhibiting dopamine release, Proc. Natl. Acad. Sci. USA 89 (1992) 9220–9224.
[10] J.-F. Hu, Y. Liu, P.-J. Liang, Stimulus pattern related plasticity of synapses between cones and horizontal cells in carp retina, Brain Res. 857 (2000) 321–326.
[11] M. Kirsch, H.J. Wagner, Release pattern of endogenous dopamine in teleost retinae during light adaptation and pharmacological stimulation, Vision Res. 29 (1989) 147–154.
[12] M. Kirsch, H.J. Wagner, M.B. Djamgoz, Dopamine and plasticity of horizontal cell function in the teleost retina: regulation of a spectral mechanism through D_1-receptors, Vision Res. 31 (1991) 401–412.
[13] A.G. Knapp, J.E. Dowling, Dopamine enhances excitatory amino acid-gate conductances in cultured retinal horizontal cells, Nature 325 (1987) 437–439.
[14] E.M. Lasater, J.E. Dowling, Dopamine decreases conductance of the electrical junctions between cultured retinal horizontal cells, Proc. Natl. Acad. Sci. USA 82 (1985) 3025–3029.
[15] S.C. Mangel, J.E. Dowling, Responsiveness and receptive field size of carp horizontal cells are reduced by prolonged darkness and dopamine, Science 229 (1985) 1107–1109.
[16] C. Ribelayga, Y. Wang, S.C. Mangel, Dopamine mediates circadian clock regulation of rod and cone input to fish retinal horizontal cells, J. Physiol. 544 (2002) 801–816.
[17] M. Schorderet, J.Z. Nowak, Retinal dopamine D_1 and D_2 receptors: characterization by binding or pharmacological studies and physiological functions, Cell. Mol. Neurobiol. 10 (1990) 303–325.
[18] T. Shew, S. Yip, B.R. Sastry, Mechanisms involved in tetanus-induced potentiation of fast IPSCs in rat hippocampal CA1 neurons, J. Neurophysiol. 83 (2000) 3388–3401.
[19] W.K. Stell, D.O. Lightfood, T.G. Wheeler, H.F. Leeper, Goldfish

retina: functional polarization of cone horizontal cell dendrites and synapses, Science 190 (1975) 989–990.
[20] T. Teranishi, K. Negishi, S. Kato, Dopamine modulate S-potential amplitude and dye-coupling between external horizontal cells in carp retina, Nature 301 (1983) 243–246.
[21] X.-L. Yang, Photoreceptor input converging onto C-type horizontal cells of the retina of crucian carp, Sci. Sin. (Series B) 30 (1987) 33–43.
[22] S. Yazulla, Z.S. Lin, Differential effects of dopamine depletion on the distribution of [^{3}H]SCH 23390 and [^{3}H]spiperone binding sites in the goldfish retina, Vision Res. 35 (1995) 2409–2414.