Reproducing Retinal Rod Bipolar Cell Light Response by Mathematical Model including Neurotransmitter Receptors

Shingo Nishiyama[1], Yukari Hosoki[1], Chieko Koike[2] , and Akira Amano[1]

Abstract— **Detailed mathematical model of retinal cells is useful for the quantitative understanding of the subcellular processes of the visual system. Retinal bipolar cells receive information from photoreceptor cells, horizontal cells and amacrine cells, thus it can be considered as information integration system. Despite its importance, bipolar cell model including inputs and outputs has not been proposed. In this paper, we propose a rod bipolar cell model which can reproduce voltage response of light. The model includes TRPM1 channel which receives signal from photoreceptor cells, GABA channel which receives signal from surrounding amacrine cells, and cell body model which is based on the model proposed by Ishihara et al. The model was evaluated with several light signals, where experimentally obtained photoreceptor cell responses were used as TRPM1 channel input. Resulting bipolar cell membrane potential showed good agreement with the reported experimental results.**

I. INTRODUCTION

Since visual impairment elevates risk of functional disability on the Quality of Life, several studies including stem cell-based therapies and artificial retina, have been developed as treatment for retinal diseases. In this respect, there is need for quantitatively understanding of the physiological characteristics of the retinal cellular functions and the related neural networks. The retina contains several different types of neurons, interconnected to make the retinal network: photoreceptors, horizontal cells, bipolar cells, amacrine cells, ganglion cells, and Muller cells.

Bipolar cells receive inputs from photoreceptors, horizontal cells, and amacrine cells, and besides, send output to amacrine cells and ganglion cells. Therefore, bipolar cells take a significant role in signal transmission, that is, integration of several signal inputs. Rod bipolar cell (RBC) dominantly receives input from rod photoreceptor cells, and relays signal to brain via amacrine cells and ganglion cells. Membrane potential response of RBC to light can be characterized by the fast depolarization phase and the later hyperpolarization phase (Fig.1)[1].

Ishihara et al. proposed a mathematical model of bipolar cell body based on the physiological characteristics of resting state. Although the model contains ion channels reported to exist on cell body, neurotransmitter receptors, which receive and transmit signal of light response, were not included. Here, we propose a retinal rod bipolar cell model which can reproduce light response. The model is constructed by introducing two neurotransmitter receptor models and a simple amacrine cell model to the Ishihara model.

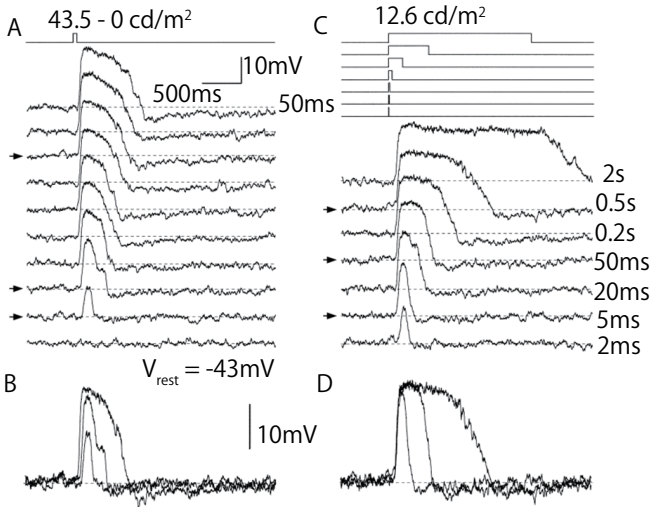

Fig. 1. Light-induced voltage responses of a rod bipolar cells on rat retinal slice (Euler, 2000[1]). Perforated patch clamp recordings were performed. A: responses of a current-clamped rod bipolar cell to 50 ms light stimuli of different light intensities (From top: 43.5, 27.4, 19.1, 16.1, 12.6, 9.4, 6.4, 3.5, 0.6, and 0 $[cd/m^2]$, respectively). B: superimposed 3 traces from A (arrows) for comparison: with increasing stimulus intensity, the amplitude of both the depolarization and the hyperpolarization increases. C: responses to stimuli (12.6 $[cd/m^2]$) of different durations. D: superimposed 3 traces from A (arrows) for comparison.

II. BACKGROUNDS

A. Bipolar Cell

Several subtypes of bipolar cell (BC) are distinguished by stratification of axon ramifying in inner plexiform layer, and connection patterns with other retinal neurons, such as photoreceptors, horizontal cells, amacrine cells, and ganglion cells. BC can also be classified by the cell response to light stimulus: ON-type response which depolarize with light, and OFF-type response which hyperpolarize with light. For mammals, bipolar cells are mainly divided into three types: ON-type cone bipolar cell (ON CBC), OFF-type cone bipolar cell (OFF CBC), and rod bipolar cell (RBC).

RBC shows only ON-type response, and does not have any direct connections to ganglion cells. Many studies have been performed on the RBC, since more than 50% of bipolar cells in retina are RBCs. RBC is known to receive glutamate from rods, GABA from horizontal cells at dendrite, and GABA and glycine from amacrine cells at axon. At the axon of RBC, there are connections to several classes of

[1]College of Life Sciences, Ritsumeikan University, Shiga, 525-8577 Japan
[2]College of Pharmaceutical Sciences, Ritsumeikan University, Shiga, 525-8577 Japan

978-1-4244-7929-0/14/$26.00 ©2014 IEEE

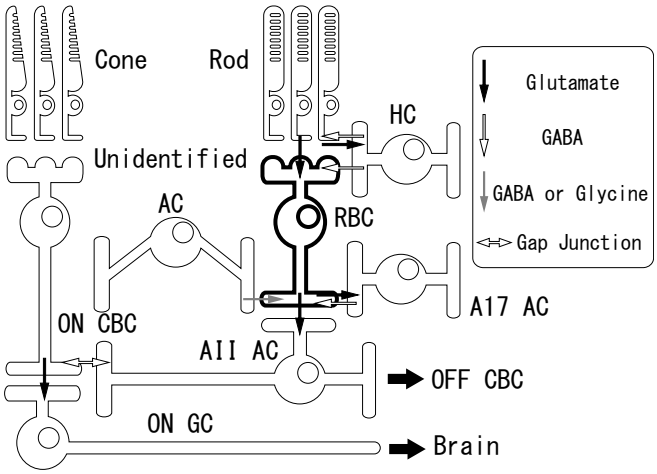

Fig. 2. Retinal Cells. Abbreviations; ON Cone Bipolar Cell (ON CBC), OFF Cone Bipolar Cell (OFF CBC), Rod Bipolar Cell (RBC), Horizontal Cell (HC), AII Amacrine Cell (AII AC), A17 Amacrine Cell (A17 AC), Unidentified Class Amacrine Cell (Unidentified AC), ON Ganglion Cell (ON GC).

amacrine cell, and they may take different rolls of regulation due to specific activity pattern[15]. Besides, RBC releases glutamate depending on membrane potential to amacrine cells, such as AII amacrine cell (AII AC) and A17 amacrine cell (A17 AC) (Fig.2)[2].

B. Light-dependent glutamate input from photoreceptor

Rod photoreceptor cells release glutamate depending on its membrane potential, and the glutamate is received by RBCs. With incident light, rod becomes hyperpolarized which decreases glutamate secretion at synapse. Under dark condition, abundantly existing glutamate at synaptic cleft, activates mGluR6, metabotropic glutamate receptor on the dendrite of ON-type bipolar cell. Activated mGluR6 produces G protein signal which inhibits the non-selective cation channel TRPM1 channel opening. After light onset, glutamate decreases as explained above, and declines inhibiting signal of G protein; therefore, TRPM1 opens and causes cation influx. Thus, light response results in depolarization of ON-type bipolar cell [3].

C. Lateral inhibition from horizontal cell and amacrine cell

GABA and glycine release from horizontal cell and amacrine cell, activate chloride-permeable ionotropic receptors. Role of GABA from horizontal cell is still controversial since the chloride concentration at dendrite is unclear. However, at axon, chloride reversal potential (E_{Cl}) is known to be lower than the resting potential of intact cell. Thereby, opening of the receptors causes chloride influx which hyperpolarizes the cell. Lateral inhibition is considered to modulate light response of bipolar cell.

D. Ishihara Model

Passive membrane model of RBC was proposed by Oltedal [12], however the only existing detailed active model is the one proposed by Ishihara et al. [6].

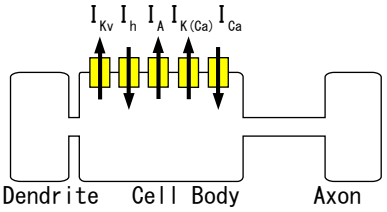

Fig. 3. Simple diagram of Ishihara model

The model proposed by Ishihara, contains five ion channels of bipolar cell soma and an intracellular calcium mechanism (Fig.3). The model can reproduce the experimental data of dissociated goldfish bipolar cell [5]. I_{Kv} is outward potassium current blocked by tetraethylammonium (TEA), and activates above -60 [mV]. I_A is current mainly carried by potassium, and activates above -30 [mV]. $I_{K(Ca)}$ activates above -10 [mV] and becomes maximum at +40 [mV]. 4 [mM]-Co, 1.6 [mM]-Ba, 35 [mM]-TEA, and 30 [μM]-quinine block the current. I_{Ca} activates above -40 [mV], and becomes maximum at +10 [mV]. I_h activates below resting membrane potential, moreover, has relatively-slow activation kinetics without inactivation. The model of spatial distribution of intracellular calcium is also introduced; intracellular calcium is assumed to interact with high affinity buffers and low affinity buffers, in both intracellular surface part and intracellular deep part.

III. PROPOSED MODEL

In this study, we added two neurotransmitter receptor models: glutamate sensitive current and GABA sensitive current, to the previously proposed Ishihara model. Since lateral inhibition signal is required for GABA sensitive current, we also incorporated a retinal tissue model from which GABA concentration is calculated.

A. Model Structure

The detailed structure of the lateral inhibition system to RBC still remains unclear except for the local feedback from A17 AC [4]. However, as mentioned above, there may be different regulation by some classes of amacrine cell; we assumed that the signal from other bipolar cells could be transmitted via amacrine cells. Also by assuming the flat light exposure, we can assume that the cell activities are homogeneous in the lateral direction.

The constructed tissue model is shown in Fig.4 where the lateral inibition signal from amacrine cell is represented by a first order delay system whose input is the membrane potential of bipolar cell. Note that, we omitted lateral inhibition signal path from horizontal cells, since the signal can be merged with the glutamate signal from rod.

B. Glutamate sensitive current

RBC has mGluR6, metabotropic glutamate receptor, on its postsynaptic dendrite surface. Metabotropic glutamate receptor is one of the G protein-coupled receptor(GPCR), and has seven transmembrane regions, outside ligand-binding

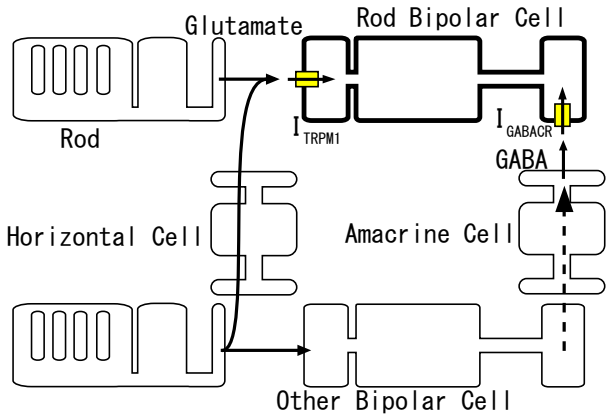

Fig. 4. Schematic diagram of proposed model.

site, and inside coupling site with G protein. According to the reports of Kunishima [8], mGluR6 makes homodimer, whose orientation modulation is essential to activate G protein. Thus, mGluR6 acts with two glutamate molecules. Since activation of G protein inhibits TRPM1, we described deactivation against glutamate as Hill equation with Hill coefficient of two.

Rapid depolarization phase is reported for the light response of retinal slice [1]. We used a simple gating model for the channel kinetics, and manually adjusted its rate constants to reproduce the light response of fast membrane potential rising. The parameters where manually adjusted to reproduce light response of bipolar cell. Reversal potential E_{TRPM1} was set to -11.5 [mV] according to the I-V curve reported by Yamashita [13]. Although the glutamate application response of isolated ON-type bipolar cells saturates at 200 [μM], according to Shiells [14], $K_{Glu} = 50$ [μM] was used to adjust membrane potential to appropriate physiological level on retinal organization. $\overline{g_{TRPM1}}$ was manually adjusted to 1.65 to reproduce the inward current of -30 [pA] at -30 [mV] (Yamashita [13]). The relation between the glutamate and the channel current is shown in Fig.5(a). The channel current I_{TRPM1} was modeled by the following equations.

$$\alpha_{mTRPM1} = 40.0 \left(1 - \frac{[Glu]^2}{[Glu]^2 + {K_{Glu}}^2} \right) \quad (1)$$

$$\beta_{mTRPM1} = 40.0 \left(\frac{[Glu]^2}{[Glu]^2 + {K_{Glu}}^2} \right) \quad (2)$$

$$\frac{dm_{TRPM1}}{dt} = \alpha_{mTRPM1} \cdot (1 - m_{TRPM1}) - \beta_{mTRPM1} \cdot m_{TRPM1} \quad (3)$$

$$g_{TRPM1} = \overline{g_{TRPM1}} \cdot m_{TRPM1} \quad (4)$$

$$I_{TRPM1} = g_{TRPM1} \cdot (V - E_{TRPM1}) \quad (5)$$

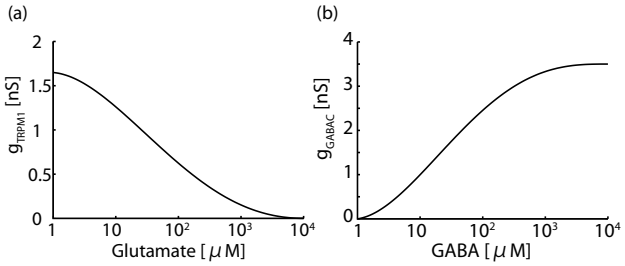

Fig. 5. Relationship between (a) g_{TRPM1} and glutamate concentration, and (b) g_{GABAC} and GABA concentration.

C. GABA sensitive current

GABA receptor family is composed of three subtypes, and two of them: $GABA_A$ receptor and $GABA_C$ receptor express on RBC. Both receptors are known to be ionotropic receptors, and have function that channel opens with GABA binding to their binding sites. Its permeable ion is chloride, additionally, the reversal potential of chloride ion (E_{Cl}) is lower than the resting membrane potential. Thus, GABA effects on RBC is causing hyperpolarization.

Distrubution pattern of the receptors expression differs in species. In mouse, $GABA_A$ receptor exists mainly at dendrite, and $GABA_C$ receptor at axon [9][10]. We modeled lateral inhibition at axon with $GABA_C$ receptor. While the number of binding sites of the $GABA_C$ receptor is not clear, Hill coefficient is reported between three and five [11]. Thus we used four as its Hill coefficient. Its kinetics were manually adjusted to reproduce the GABA puff application response of isolated mouse RBC [10]. Similarly, $\overline{g_{GABAC}}$ and K_{GABA} were manually adjusted to 4.5 and 100 [μM], respectively. Reversal potential (E_{Cl}) was calculated to be -70 [mV], by using the intracellular chloride concentration of 7.9 [mM], extracellular chloride concentration of 110 [mM]. The relation between GABA and the current is shown in Fig.5(b). The channel current I_{GABACR} was modeled by the following equations.

$$\alpha_{mGABAC} = 300.0 \left(\frac{[GABA]^4}{[GABA]^4 + {K_{GABA}}^4} \right) \quad (6)$$

$$\beta_{mGABAC} = 0.8 \left(1 - \frac{[GABA]^4}{[GABA]^4 + {K_{GABA}}^4} \right) \quad (7)$$

$$\frac{dm_{GABAC}}{dt} = \alpha_{mGABAC} \cdot (1 - m_{GABAC}) - \beta_{mGABAC} \cdot m_{GABAC} \quad (8)$$

$$g_{GABAC} = \overline{g_{GABAC}} \cdot m_{GABAC} \quad (9)$$

$$I_{GABACR} = g_{GABA} \cdot (V - E_{Cl}) \quad (10)$$

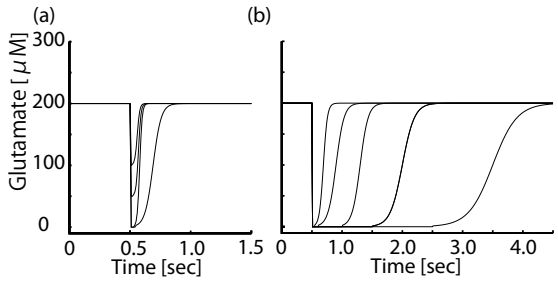

Fig. 6. Glutamate concentration used for the input of the proposed model under (a) various light intensities, and (b) various light duration.

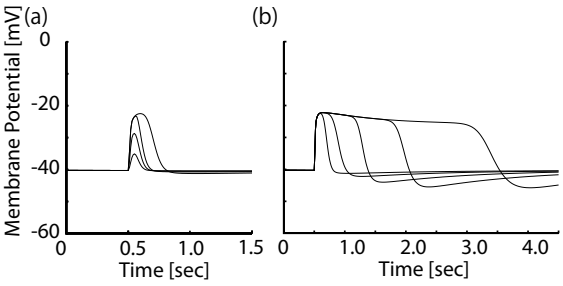

Fig. 7. Membrane potential responses of the proposed model with Fig.6 signal.

D. Amacrine Cell

We modeled the membrane potential of amacrine cell $Vm_{Amacrine}$ as first-order delay system of the RBC membrane potential as follows. According GABA secretion is modeled to be proportional to the amacrine cell membrane potential.

$$\frac{dVm_{Amacrine}}{dt} = k1 \cdot (Vm - Vm_{Amacrine}) \tag{11}$$

$$GABA = k2 \cdot (Vm_{Amacrine} - V_{rest}) \tag{12}$$

k_1 and k_2 represents time constant and amplitude, respectively. We used $k_1 = 6.0$, $k_2 = 0.2$ to reproduce the cell light response.

IV. EXPERIMENTAL RESULTS

We reproduced the RBC light response [1] with our proposed model by providing several glutamate signal. For the input glutamate signal, we assumed that the glutamate release from photoreceptor depends on the rod membrane potential. By refering to the reported rod membrane potential trace with different intensities (Fig.6(a)) and different interval light stimulation (Fig.6(b)), we provided mathematically generated rod membrane potential signal . The resulting membrane potential of the proposed model are shown in Fig.7(a) and (b). Compared to the light response shown in Fig.1, the model well reproduced the membrane potential.

We can find some undershoot appearing with increasing light duration in the hyperpolarization phase (Fig.7(b)), which is reported in the experimental data [1].

V. CONCLUSIONS

We proposed a RBC model by incorporating the neurotransmitter receptor channel models to the previously proposed RBC cell body model. The model could well reproduce the reported experimental data. To improve reproducibility of the model, description of detailed TRPM1 cascade, the essential function of ON response, would be needed. Besides, we described amacrine cell simply; however, the model of amacrine cell, which can reproduce its own physiological characteristics, is also needed for this respect.

ACKNOWLEDGMENT

We thank professor Akinori Noma (Ritsumeikan Univ.) for model construction advice.

REFERENCES

[1] Thomas Euler and Richard H. Masland. Light-Evoked Responses of Bipolar Cells in a Mammalian Retina. The Journal of Neurophysiology. 83:1817-1829, 2000

[2] Stewart A. Bloomfield and Ramon F. Dacheux. Rod Vision: Pathways and Processing in the Mammalian Retina. Progress in Retinal and Eye Research, Vol.20, No. 3, pp. 351 to 384, 2001

[3] Chieko Koike, Takehisa Obara, Yoshitsugu Uriu, Tomohiro Numata, Rikako Sanuki, Kentarou Miyata, Toshiyuki Koyasu, Shinji Ueno, Kazuo Funabiki, Akiko Tani, Hiroshi Ueda, Mineo Kondo, Yasuo Mori, Masao Tachibana and Takahisa Furukawa. TRPM1 is a component of the retinal ON bipolar cell transduction channel in the mGluR6 cascade. PNAS, vol. 107, 332-337, 2010

[4] Andrés E. Chávez, Joshua H. Singer and Jeffrey S. Diamond. Fast neurotransmitter release triggered by Ca influx through AMPA-type glutamate receptors. Nature. Vol 443, 2006, doi:10.1038/nature05123.

[5] Akimichi Kaneko and Masao Tachibana. A Voltage-Clamp Analysis of Membrane Currents in Solitary Bipolar Cells Dissociated from Carassius Auratus. J. Physiol. 358, pp. 131–152, 1985

[6] Ishihara, Kamiyama, and Usui, *"ion current model of retinal bipolar cell"*(Mathematical model of bipolar cell), IEICE D Vol.J80-D2 No.12 pp.3181-3190, 1997

[7] S. Barnes and B. Hill. Ionic channels of the inner segment of tiger salamander cone photoreceptors. J. Gen. Physiol, Vol.94: 719-743, 1989.

[8] Naoki Kunishima, Yoshimi Shimada, Yuji Tsuji, Toshihiro Sato, Masaki Yamamoto, Takashi Kumasaka, Shigetada Nakanishi, Hisato Jingami and Kosuke Morikawa. Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. Nature, Vol 407, 2000.

[9] Carolina Varela, Itziar Igartua, Enrique J. De la Rosa, Pedro De la Villa. Functional modifications in rod bipolar cells in a mouse model of retinitis pigmentosa. Vision Research, 43 (2003), 879–885.

[10] Carolina Varela, Román Blanco, Pedro De la Villa. Depolarizing effect of GABA in rod bipolar cells of the mouse retina. Vision Research, 45 (2005) 2659–2667.

[11] Joachim Bormann. The 'ABC' of GABA receptors. TiPS - January 2000 (Vol. 21) 16-19.

[12] Leif Oltedal, Svein Harald Mørkve, Margaret Lin Veruki, Espen Hartveit. Patch-clamp investigations and compartmental modeling of rod bipolar axon terminals in an in vitro thin-slice preparation of the mammalian retina. Journal of Neurophysiology, 2007, Vol. 97, no. 1171–1187.

[13] Masayuki Yamashita and Heinz Wassle. Response of Rod Bipolar Cells Isolated from the Rat Retina to the Glutamate Agonist 2-Amino-4-phosphonobutyric Acid (APB). The Jounal of Neuroscience, 1991, 11(8): 2271–2382.

[14] R.A. Shiells and G. Falk. Responses of rod bipolar cells isolated from dogfish retinal slices to concentration-jumps of glutamate. Visual Neuroscience (1994), 11, 1175–1183.

[15] Erika D. Eggers, Reece E. Mazade, and Justin S. Klein. Inhibition to retinal rod bipolar cells is regulated by light levels. J Neurophysiol 110: 153 - 161, 2013.