Light-Evoked Oscillatory Discharges in Retinal Ganglion Cells Are Generated by Rhythmic Synaptic Inputs

Itaru Arai, Yoshiyuki Yamada, Tomomitsu Asaka, and Masao Tachibana

Department of Psychology, Graduate School of Humanities and Sociology, The University of Tokyo, Tokyo 113-0033, Japan

Submitted 19 February 2004; accepted in final form 25 March 2004

Arai, Itaru, Yoshiyuki Yamada, Tomomitsu Asaka, Masao Tachibana. Light-evoked oscillatory discharges in retinal ganglion cells are generated by rhythmic synaptic inputs. J Neurophysiol 92: 715–725, 2004; 10.1152/jn.00159.2004. In the visual system, optimal light stimulation sometimes generates γ-range (ca. 20–80 Hz) synchronous oscillatory spike discharges. This phenomenon is assumed to be related to perceptual integration. Applying a planar multi-electrode array to the isolated frog retina, Ishikane et al. demonstrated that dimming detectors, off-sustained type ganglion cells, generate synchronous oscillatory spike discharges in response to diffuse dimming illumination. In the present study, applying the whole cell current-clamp technique to the isolated frog retina, we examined how light-evoked oscillatory spike discharges were generated in dimming detectors. Light-evoked oscillatory (~30 Hz) spike discharges were triggered by rhythmic (~30 Hz) fluctuations superimposed on a depolarizing plateau potential. When a suprathreshold steady depolarizing current was injected into a dimming detector, only a few spikes were evoked at the stimulus onset. However, repetitive spikes were triggered by a γ-range sinusoidal current superimposed on the steady depolarizing current. Thus the light-evoked rhythmic fluctuations are likely to be generated presynaptically. The light-evoked rhythmic fluctuations were suppressed not by intracellulr application of N-(2,6-dimethyl-phenylcarbamoylmethyl)triethylammonium bromide (QX-314), a Na⁺ channel blocker, to the whole cell clamped dimming detector but by bath-application of tetrodotoxin to the retina. The light-evoked rhythmic fluctuations were suppressed by a GABA_A receptor antagonist but potentiated by a GABA_C receptor antagonist, whereas these fluctuations were little affected by a glycine receptor antagonist. Because amacrine cells are spiking neurons and because GABA is one of the main transmitters released from amacrine cells, amacrine cells may participate in generating rhythmically fluctuated synaptic input to dimming detectors.

INTRODUCTION

In the visual system where retinotopic map is formed, each neuron responds to a light stimulus that falls on its receptive field. However, it was found that synchronized spike discharges were evoked in cortical neurons without overlapping receptive fields when they were stimulated with an optimal visual stimulus (Eckhorn et al. 1988; Engel et al. 1990; Gray and Singer 1989; König et al. 1995; Ts’o and Gilbert 1988; Ts’o et al. 1986). The synchronized activity was frequently associated with γ-range (ca. 20–80 Hz) oscillatory spike discharges (Eckhorn et al. 1988; Engel et al. 1990; Gray and Singer 1989). It has been suggested that synchronous oscillations, which could establish dynamic relations among a cluster of neurons, may be a neuronal mechanism underlying perceptual integration (for review, see Singer and Gray 1995). Although several types of neuronal models have been proposed for synchronous oscillations (review; Jefferys et al. 1996; Ritz and Sejnowski 1997), critical experiments to evaluate these hypotheses seem to be hindered by technical difficulties and by anatomical complexity of cortical networks.

Retinal ganglion cells also generate synchronous oscillatory spike discharges (cat: Neuenschwander and Singer 1996; frog: Ishikane et al. 1999). The retina has the advantage of easier access to neurons in vitro and simpler anatomical structure than the visual cortex. In our previous study, light-evoked spike discharges were recorded from dimming detectors, off-sustained type ganglion cells, in the frog retina with a planar multi-electrode array (Ishikane et al. 1999). When the retina was stimulated with full-field, sinusoidally modulated illumination (temporal frequency at 0.25 Hz), synchronized spike discharges accompanied with oscillations (~30 Hz) were detected even in cell pairs more than a few millimeters apart. The synchronized activities were decreased under the conditions where oscillatory spike discharges were suppressed. For example, the stimulated area was restricted to the receptive field size of dimming detectors (~1 mm diam) or GABA_A receptor antagonists were applied to the retina. These results suggest that oscillatory spike discharges may facilitate the establishment of synchrony among dimming detectors and that wide-range neural interactions may be essential for the oscillatory spike discharges in dimming detectors. However, the analysis of spike discharge patterns was not enough to elucidate the mechanisms underlying the oscillatory activities of dimming detectors.

In the present study, we applied the whole cell current-clamp technique to dimming detectors in the isolated frog retina and recorded the membrane potential changes induced by light stimulation and current injection. It was found that dimming detectors did not have intrinsic properties to generate oscillations but received rhythmically fluctuated synaptic input that triggered oscillatory spike discharges. The presynaptic neuronal mechanism seems to include GABAergic and spiking neurons, probably amacrine cells. Part of the present work has been reported elsewhere in an abstract form (Arai et al. 2002, 2003).

METHODS

Preparation

Procedures for making whole-mount retinal preparations were nearly identical to those previously described (Ishikane et al. 1999). In

Address for reprint requests and other correspondence: M. Tachibana, Dept. of Psychology, Graduate School of Humanities and Sociology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan (E-mail: L.tmasao@L.u-tokyo.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
brief, a bullfrog (Rana catesbiana, body wt: ~180 g), which had been dark-adapted overnight, was double-pithed and eyes were enucleated in a dark room in accordance with “A Manual for the Conduct of Animal Experiments in The University of Tokyo” and “Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, The Physiological Society of Japan”. Under the stereomicroscope equipped with infrared (IR) converters (Night Viewer CS100, Hamamatsu Photonics, Hamamatsu, Japan), the cornea and lens were ablated and the vitreous humor was absorbed into a piece of paper. The eyecup was cut into a few pieces, and the retina was isolated carefully from the pigmented epithelium in an oxygenated control external solution. Then the isolated retina was transferred into a recording chamber on the stage of a microscope equipped with IR differential interference optics (Eclipse E600FN, Nikon, Yokohama, Japan), which was stretched in the middle part of the recording chamber and held down with nylon fibers attached to a U-shaped platinum weight. The retina was superfused with oxygenated external solution not only from the ganglion cell side but also from the photoreceptor side through the dialysis membrane. Recordings were carried out in a light-tight Faraday cage at room temperature (~25°C).

**External solutions**

The control external solution contained (in mM) 100 NaCl, 10 D-glucose, 2.5 KCl, 18 NaHCO3, 1 CaCl2, and 1.6 MgCl2 and 2 mg/l phenol red (242 mosM). The superfusate was continuously bubbled with 95% O2-5% CO2 to keep the pH at 7.2 and was fed into the recording chamber (3 ml in volume) at a rate of 1.3 ~ 1.7 ml/min. In some experiments, we used another solution that consisted of (in mM) 93 NaCl, 10 D-glucose, 2.5 KCl, 25 NaHCO3, 2 CaCl2, and 1.6 MgCl2 and 2 mg/l phenol red (245 mosM). Because photoresponses of dimming detectors were similar in either external solution, data were pooled and analyzed together.

Pharmacological agents were added to the control external solution and were bath-applied. Tetrodotoxin (TTX) was purchased from Wako (Osaka, Japan). Picrotoxin and strychnine were from Sigma (St. Louis, MO). L- (+)-2-amino-4-phosphonobutyric acid (L-AP4), (3-aminopropyl) diethoxyethyl phosphinic acid (CGP), (2- (+)-)bicuculline methochloride (bicuculline), D- (+) -2-amino-5-phosphono-pentanoic acid (D-AP5), 2,3-dioxyo-6-nitro-1,2,3,4-tetrahydrobenzo[f] quinoxaline-7-sulfonamide (NBQX), and (1,2,5,6-tetrahydropyridin-4-yl) methylphosphinic acid (TPMPA) were from Tocris (Bristol, UK). Picrotoxin and NBQX were dissolved in dimethyl sulfoxide (DMSO) for stock solutions. The final concentration of DMSO after dilution with the control external solution was always kept <0.1% (vol/vol).

**Whole cell recordings**

Patch pipettes for whole cell recordings were pulled with a horizontal puller (P97, Sutter Instruments, Novato, CA). The electrode resistance was 6 ~ 8 MΩ in the control external solution when pipette was filled with a pipette solution.

Two pipette solutions were used in the present study. Pipette solution A contained (in mM) 90 K-glucurate, 20 HEPES, 5 EGTA, 0.5 CaCl2, 5.5 MgCl2, 0.5 NaGTP, and 0 NaATP and 0.8 mg/ml Lucifer yellow dipotassium salt (227 mosM). Pipette solution B contained (in mM) 92 K-glucurate, 20 HEPES, 5 EGTA, 0.5 CaCl2, 2 MgCl2, 3.5 MgSO4, 0.5 NaGTP, and 5 NaATP and 0.8 mg/ml Lucifer yellow dipotassium salt (224 mosM). In the control external solution, $E_C$ was calculated to be ~56 and ~77 mV in pipette solutions A and B, respectively. In some experiments, N,N(2,6-di-methyl-phenylcarbamoylmethyl)triethylammonium bromide (QX-314), (5 or 10 mM) was added to the pipette solution to block Na+ channels intracellularly (Connors and Prince 1982). The pH was titrated to 7.2 with KOH. Liquid junction potential was corrected for all recordings.

The inner limiting membrane prevented a clear view of the array of ganglion cell bodies and the access of electrode tip to the cell surface. Therefore a small hole (~100 µm diam) was pierced in the inner limiting membrane with two cleaning pipettes. One pipette was filled with the control external solution, and the other with a collagenase solution (0.5 ~ 1 mg/ml of collagenase dissolved in the control external solution). The solutions were ejected from the cleaning pipettes by a positive pressure, and each cleaning pipette attached to a micromanipulator was independently moved against the inner limiting membrane in the opposite direction.

Light-evoked responses were recorded in the current-clamp mode with EPC-9/2 (HEKA, Lambrecht, Germany), which was controlled by the Pulse software (HEKA). The fast and slow capacitance components were mostly cancelled automatically. The membrane potential was low-pass filtered at an appropriate corner frequency (1 ~ 4 kHz), which was always set to one-fifth of the sampling frequency.

**Light stimulation**

The retina was illuminated from the photoreceptor side through the transparent dialysis membrane and a slide glass attached to the bottom of the recording chamber. A beam-slit ter cube was placed under the condenser lens of the microscope. The diffuser, lenses, and ND filters were put between the beam-slit cube and a light-emitting diode (LED, emission maximum at 530 nm). The illuminated area was ~7 mm in diameter and was large enough to cover the entire retinal preparation.

Two types of light stimulation were used; a sinusoidally modulated light (temporal frequency at 0.25 Hz) and a step light. The waveform of light stimulation was computer-controlled by changing the amount of the current supplied to the LED, which was driven in the range where the relationship between the supplied current and the light intensity (I) was linear. Appropriate ND filters were inserted into the light path to adjust the intensity of light stimulation. The mean intensity and contrast ($I_{max} - I_{min}$/max + Imin) of the sinusoidally modulated light ranged between 7.4 mlx and 1.5 klx, and between 0.55 and 0.87, respectively. Both values were optimally adjusted to evoke the oscillatory activities for each dimming detector every time when recordings were started. Before application of the sinusoidally modulated light, the retina was adapted to a background light (a constant current was supplied to the LED), the intensity of which was set to the mean value of the sinusoidally modulated light. The step light was applied with or without the background light.

**Cell morphology**

Cells were whole cell clamped with a patch pipette filled with the solution containing Lucifer yellow. Their morphology was visualized under epifluorescence illumination after the recording, and the images taken by a CCD camera (DXM1200; Nikon) were saved in a computer.

**Cell identification and data analysis**

Frog retinal ganglion cells are classified into at least four types based on their photoresponses; the dimming detector, the moving-edge detector, the contrast detector, and the convexity detector (Lettin et al. 1959). It has been reported that the dimming detector has a larger soma (>20 µm diam) than the other types (~10 µm diam) (Kock et al. 1989; Stirling and Merrill 1987). Thus we searched for a cell with a large soma in the ganglion cell layer and recorded photoresponses in the whole cell current-clamp mode.

When a step light was applied to the retina, a majority of large ganglion cells generated a few or no on-transient spike discharges
with a hyperpolarization and off-sustained spike discharges with a large depolarization. These ganglion cells had similar morphological and physiological properties and thus were identified as dimming detectors (see RESULTS). In a few ganglion cells with a large cell body, the step light induced on-transient spike discharges without hyperpolarization and off-sustained spike discharges with a moderate depolarization. These ganglion cells responded with repetitive firings to current injection and had bi-stratified dendritic fields. Thus these cells were not classified as dimming detectors, and their data were not included for the present analysis. Some large ganglion cells that showed on-off transient spike discharges to the step light were classified into other cell types and their data were discarded.

The data obtained from dimming detectors were analyzed off-line with the Igor Pro software (Wavemetrics, Lake Oswego, OR). Pooled data were expressed as means ± SE. Statistical difference was assessed with two-tailed, paired and unpaired Student’s t-test as appropriate, with P < 0.01 considered significant.

RESULTS

Light-evoked responses and morphology of dimming detectors

An example of dimming detectors filled with Lucifer yellow is shown in Fig. 1, A and B. Because an axon was always observed between the ganglion cell layer and the inner limiting membrane (Fig. 1B), the cell could be easily identified as a ganglion cell but not a displaced amacrine cell, which has no axon. The cell body of dimming detectors was 33.4 ± 1.5 μm in diameter (n = 23, Fig. 1A). As the focusing plane of the objective lens was advanced from the ganglion cell layer toward the inner nuclear layer, three to five thick dendrites extended downward from the soma and then branched out into the inner plexiform layer (IPL) near the amacrine cell layer [sublamina a, where off type neurons make synaptic contacts (Famiglietti et al. 1977)]. Dye coupling with neighboring cells was not observed. The dendritic field was >700 μm in diameter.

Dimming detectors generated off sustained discharges to a full-field diffuse light when the light intensity was decreased abruptly (Fig. 1C) or gradually (Fig. 2A). The resting membrane potential was approximately −70 mV in the dark. When a step light was applied, the membrane potential was hyperpolarized to approximately −80 mV. During this period, the fluctuations of the membrane potential observed in the dark were suppressed (Fig. 1C). E_C was calculated to be −56 mV under the present recording condition. Thus light-evoked hyperpolarization may not be solely ascribed to the inhibitory input because it should depolarize the membrane potential toward E_C. When the step light was turned off, the membrane potential was depolarized to a plateau (approximately −60 mV), and spikes were generated repetitively.

Autocorrelation analysis

To investigate the temporal properties of light-evoked spike discharges in dimming detectors, the retina was stimulated with a full-field diffuse light, the intensity of which was sinusoidally modulated at the temporal frequency of 0.25 Hz. It has been reported that this stimulus frequency generates oscillatory spike discharges most efficiently in frog dimming detectors (Ishikane et al. 1999). When a background light was turned on, the membrane potential was slightly hyperpolarized and then gradually returned to the resting level in the dark. As the light intensity was sinusoidally modulated, the dimming detector hyperpolarized during the rising phase of the light stimulus, and then depolarized during its decay phase (Fig. 2A, top). On top of the depolarizing plateau, spikes with a regular interval (box b) were generated following a burst of spikes (box a). The interspike interval was variable during the burst (Fig. 2A, middle, box a) and then became regular (Fig. 2A, bottom, box b).

Autocorrelograms were calculated from the spike trains during the bursting period (Fig. 2B1) and the regularly spiking period (Fig. 2B2), separately. The central peak at 0 time shift indicates the number of spikes generated during each period when the retina was stimulated by 30 consecutive trials (1 trial...
corresponded to 1-cycle stimulation at 0.25 Hz). Auto-correlation analysis of the spike train during the bursting period revealed the presence of two side peaks (at approximately ±15 ms), which corresponded to the bursting spikes (Fig. 2B1). Power spectral density (PSD) analysis of the autocorrelogram showed no peaks in the γ range (ca. 20 ~ 80 Hz) during the burst (Fig. 2D, thin line). On the other hand, a rhythmic or oscillatory firing pattern was clearly observed in the autocorrelogram calculated from the regularly spiking period (Fig. 2B2). Periodic peaks of spike coincidences occurred at approximately 35 ms intervals. PSD analysis revealed a peak at ~30 Hz (26.5 ± 0.64 Hz, n = 21, Fig. 2D, thick line). This frequency is similar to that reported by Ishikane et al. (1999), who applied a planar multi-electrode array to the isolated frog retina to record spike discharges from dimming detectors.

Raw autocorrelograms (Fig. 2B, I and 2) include both stimulus and neural-connection dependent components (Perkel et al. 1967). To evaluate the stimulus-dependent component, a
shuffled autocorrelogram was calculated from the regularly spiking period (Fig. 2C). The shuffled autocorrelogram was nearly flat and there were no clear peaks. This was also supported by PSD analysis (Fig. 2D, gray line). Thus the oscillatory pattern appeared in the raw autocorrelogram (Fig. 2B2) has a neural origin. Oscillations developed with a delay after the first spike appeared for each cycle of sinusoidal illumination.

Oscillations were observed irrespective of the type of pipette solutions. The calculated $E_C$ was positive ($-56$ mV for pipette solution A) or negative ($-77$ mV for pipette solution B) to the resting potential (approximately $-70$ mV). Therefore oscillations observed in dimming detectors would not be directly related to the intracellular concentration of $\text{Cl}^-$.

**Rhythmic fluctuations of the membrane potential during dimming stimulation**

To gain insight into the mechanism how oscillatory spike discharges were generated in dimming detectors, changes of the membrane potential during dimming illumination were examined closely. The bottom trace of Fig. 2A (box b) illustrates a temporally expanded waveform of the voltage response, where spikes were generated regularly (Fig. 2A, top, box b). Interspike intervals were usually $35$ ms and sometimes twice or three-times as long as $35$ ms. Spikes were triggered in a depolarizing phase of the membrane fluctuations. The membrane potential fluctuated rhythmically even when a failure of spike discharge occurred.

To examine the correlation between spike discharges and membrane potential fluctuations, the upward zero-crossing time of each spike was aligned to time 0 and the membrane potential was averaged in the range of $\pm 120$ ms (Fig. 2E, calculated from A, box b). This analysis demonstrates the presence of rhythmic fluctuations of the membrane potential, the frequency of which was close to that of spike discharges ($\sim 30$ Hz). The abrupt potential change around time 0 illustrates the averaged waveform of action potentials with overshoot, whereas the small spine-like waveforms around the top of each rhythmic depolarization are the reduced action potentials due to averaging of spikes evoked with a temporal jitter. Rhythmic fluctuations of the membrane potential probably caused oscillatory spike discharges in dimming detectors.

**Effects of current injection to dimming detectors**

Two possible origins of the rhythmic fluctuations can be imagined. The first possibility is that active membrane properties of dimming detectors may have the ability to generate the rhythmic fluctuations, which would be triggered by a signal such as sustained depolarization. It has been reported that some CNS neurons produce the oscillatory activities by their own active membrane properties (Bevan and Wilson 1999; Boehler et al. 2000; Dossi et al. 1992; Gray and McCormick 1996; Llinás et al. 1991; Pedroarena and Llinás 1997). The second possibility is that dimming detectors may simply follow the rhythmic synaptic input, which would be generated in the distal layer(s) of the retina. It has been reported that network inter-action produces oscillations in various CNS regions (Buhl et al. 1998; Eeckman and Freeman 1990; Sugai et al. 1999; Whittington et al. 1995).

To determine a possible source of the rhythmic fluctuations in dimming detectors, we injected a depolarizing current into the soma of a dimming detector through a recording electrode (Fig. 3A) under the condition where most of chemical synaptic transmission in the retina had been blocked (t-AP5, NBQX, picrotoxin, strychnine and t-AP4 to block the transmission via NMDA receptors, non-NMDA receptors, GABA receptors, glycine receptors, and group III metabotropic glutamate receptors, respectively).

Injection of a small (i.e., subthreshold) sustained depolarizing current evoked no rhythmic fluctuations of the membrane potential (Fig. 3A, left). Injection of larger (i.e., suprathreshold) depolarizing currents evoked only a few spikes at the onset of the current injection but failed to generate the rhythmic fluctuations (Fig. 3A, right 3 panels). When a sinusoidal current (25 Hz) was added to the sustained current, the dimming detector sometimes evoked a spike in the rising phase of each sinusoidal membrane depolarization (Fig. 3B, right 3 panels). As the amount of sustained depolarization was increased while the intensity of the sinusoidal current was fixed, the rate of spike failure decreased.

This experiment indicates that steady depolarization is not enough to generate oscillatory spike discharges intrinsically and that dimming detectors are able to induce spikes following the sinusoidal current inputs, resulting in oscillatory spike discharges. Therefore it is not likely that the active membrane properties of dimming detectors may be the origin of rhythmic fluctuations.

**Contribution of $\text{Na}^+$ channels to rhythmic fluctuations**

Dimming detectors, similar to other ganglion cells, receive synaptic inputs from amacrine and bipolar cells. Ishikane et al. (1999) have shown that dimming detectors generate oscillatory spike discharges only when a retinal area much wider than the receptive field is stimulated with a dimming light. Because amacrine cells are assumed to contribute to lateral interaction in the inner retina, it is likely that amacrine cells may be involved in the system which generates the rhythmic fluctuations in dimming detectors.

In the retina, the transmitter release from both photoreceptors and bipolar cells is regulated by graded potential changes without $\text{Na}^+$ spikes (Werblin and Dowling 1969). However, amacrine cells, which receive synaptic inputs from bipolar and amacrine cells, generate $\text{Na}^+$ spikes accompanied with graded potential changes in response to light stimulation. Thus some functions of amacrine cells may be suppressed by a $\text{Na}^+$ channel blocker.

To clarify the effect of $\text{Na}^+$ spike elimination from amacrine cells on the rhythmic fluctuations of dimming detectors, $\text{Na}^+$ spikes of the whole cell clamped cell were blocked by intracellular application of QX-314 (5 or 10 mM) through a recording pipette in advance. Spikes disappeared soon after the whole cell mode was established, and thus the examined cell was identified as a dimming detector based on the properties of photoresponses (sustained hyperpolarization to light increment and sustained depolarization to light decrement) and morphology (the soma size and dendritic pattern revealed by Lucifer yellow staining; see Fig. 1, A and B).

Figure 4A is an example of photoresponses recorded from QX-314-filled dimming detectors. During the decay phase of
sinusoidally modulated illumination, the dimming detector showed depolarization accompanied with obvious fluctuations of the membrane potential [Fig. 4A, top (box) and middle]. PSD analysis of the fluctuations revealed a peak at the frequency of 22 Hz (Fig. 4A, bottom), indicating the presence of the rhythm. The mean frequency of the rhythm was 28.0 ± 0.73 Hz (n = 31), which was not significantly different from that calculated as 26.5 ± 0.64 Hz, n = 21 (P = 0.149, 2-tailed, unpaired Student’s t-test). When both Na⁺ and K⁺ conductances were blocked by introducing QX-314 and Cs⁺ into the whole cell clamped dimming detectors, the rhythmic fluctuations were evoked by light stimulation (n = 9, data not shown). Thus it is not likely that Na⁺ or K⁺ conductance of dimming detectors is essential for generating rhythmic fluctuations.

To block Na⁺ spikes of amacrine cells, TTX (0.5 or 1 μM) was bath-applied to the retina. The QX-314-filled dimming detector still responded to light stimulation (Fig. 4B), indicating that the signal transmission of photoreceptor → bipolar cell → dimming detector was little affected by TTX. However, the fluctuations of the membrane potential during depolarization were obscured [Fig. 4B, top (box) and middle]. PSD analysis confirmed that the rhythmic fluctuations disappeared (Fig. 4B, bottom). After extensive washout of TTX, the rhythmic fluctuations recovered and a peak reappeared obviously ~22 Hz in the power spectrum (Fig. 4C). When the power at the peak frequency of the rhythm in control solution was compared with that in the TTX solution, it was confirmed that TTX significantly suppressed the rhythmic fluctuations in dimming detectors (0.189 ± 0.079 of control condition, n = 5; P < 0.01).

This series of experiments indicates that Na⁺ spikes are essential for generating the rhythmic fluctuations in dimming detectors. It is probable that Na⁺ spikes may originate from amacrine cells, which contribute to lateral interaction in the inner retina (see DISCUSSION).

Inhibitory synaptic transmission crucial for generating the rhythmic fluctuations

It has been proposed that inhibitory neurons may be involved in generating synchronous oscillatory spike discharges in CNS (for review, see Ritz and Sejnowski 1997). Because GABA and glycine are the major inhibitory transmitters released from amacrine cells (Kalloniatis et al. 1996), it is likely that GABA and/or glycine may participate in generating the rhythmic fluctuations in dimming detectors. Actually, Ishikane et al. (1999) reported that oscillatory spike discharges of dimming detectors were totally suppressed by application of bicuculline, a GABA_A receptor antagonist. However, they
recorded spike discharges from dimming detectors extracellularly with a planar multi-electrode array, and thus it is not obvious whether bicuculline suppressed the rhythmic fluctuations or not. Therefore we examined the effects of bicuculline on the light-evoked rhythmic fluctuations in QX-314-filled dimming detectors under the current clamp. Bath-applied bicuculline (10 μM) reduced the rhythmic fluctuations (Fig. 5B) observed in control solution (Fig. 5A). PSD analysis revealed that the peak at 30 Hz in control solution (Fig. 5A, bottom) disappeared in the presence of bicuculline (Fig. 5B, bottom). The effects of bicuculline partially recovered after washout (data not shown). Similar results were obtained from other five cells. These results suggest that GABA_A receptor-mediated synaptic transmission may be included in the system that generates the rhythm presynaptically (see Discussion).

It has been reported that GABA_C receptors also exist in the frog retina (Du and Yang 2000; Vitanova et al. 2001). To investigate whether GABA_C receptors contribute to the rhythmic fluctuations in dimming detectors, TPMPA (100 μM), a selective antagonist of GABA_C receptors, was bath-applied to the retina (Fig. 5, C and D). In contrast to the suppressive effect of bicuculline, the rhythmic fluctuations during the depolarizing plateau were drastically enhanced by TPMPA (Fig. 5D). PSD analysis proved enormous enhancement of the rhythm (Fig. 5D, bottom). Similar effects were observed in other seven cells.

When spike discharges were recorded from dimming detectors with a QX-314-free pipette, TPMPA decreased the number of spike failure for each rhythmic depolarization. Furthermore, doublets or triplets of spikes were frequently observed for each rhythmic depolarization (n = 3, data not shown).

Although the presence of GABA_B receptors has not yet been demonstrated in the frog retina, we examined a possible contribution of GABA_B receptors to the rhythmic fluctuations. When CGP (100 μM), a selective antagonist of GABA_B receptors, was bath-applied, no significant changes were observed in the rhythmic fluctuations of QX-314-filled dimming detectors (n = 4, P = 0.349; Fig. 7).

To clarify the contribution of glycinergic amacrine cells to the rhythmic fluctuations, strychnine (1 μM), a selective antagonist of glycine receptors, was bath-applied to the retina (Fig. 6). The rhythmic fluctuations of the QX-314-filled dimming detector observed in control solution (Fig. 6A) remained

---

**FIG. 4.** Effects of Na⁺ channel blockers on the rhythmic fluctuations. N-(2,6-dimethyl-phenylcarbamoylmethyl)triethylammonium bromide (QX-314, 5 mM) was applied intracellularly to the cell under the whole cell clamp. Responses to sinusoidally modulated light stimulation (0.25 Hz) were recorded in control (A), in the TTX (1 μM) solution (5 min after the bath application; B), and after washout (1.5 h; C). The mean intensity and contrast of illumination were 3.79 mIx and 0.57, respectively. Top: light stimulation (top) and voltage responses (bottom). Middle: expanded waveforms obtained from the box region shown above. Bottom: averaged PSDs. PSD was calculated for each response (0 ~ 3,800 ms) to 1 cycle of stimulation, and then PSDs obtained by 20-cycle stimulation were averaged.
in the presence of strychnine (Fig. 6B). PSD analysis showed a tendency of decrease in the power ±30 Hz in the presence of strychnine (Fig. 6B, bottom), but the effect of strychnine was not statistically significant (n = 5, P = 0.179; Fig. 7). Therefore glycinergic amacrine cells may have little, if any, contribution to the system that generates the rhythm.

Figure 7 summarizes the effects of antagonists for inhibitory receptors on the rhythmic fluctuations in dimming detectors. The peak value of PSD around 30 Hz in the presence of each antagonist was normalized to that in control solution. It is evident that the rhythmic fluctuations were suppressed by GABA A receptor antagonist (Fig. 7A, Bic: n = 6, P < 0.01) while enhanced enormously by GABA C receptor antagonist (Fig. 7B, TPMPA, n = 8, P < 0.01). Significant effects were not observed when GABA B or glycine receptor antagonist was applied (Fig. 7A, CGP: n = 4, P = 0.349, Stry: n = 5, P = 0.179).

DISCUSSION

In the present study, we examined how oscillatory spike discharges were generated in dimming detectors of the frog retina. Whole cell current-clamp technique was applied to the isolated whole-mount retina, and voltage responses to light stimulation and current injection were recorded from dimming detectors. It was found that oscillatory (~30 Hz) spike discharges were triggered by rhythmic (~30 Hz) fluctuations of the membrane potential superimposed on the depolarizing plateau, which was induced by diffuse dimming illumination (Fig. 2). Oscillatory spike discharges were not evoked by the injection of a steady depolarizing current into the soma of a dimming detector but were induced by the addition of sinusoidal current to the steady depolarizing current (Fig. 3). Blockade of Na + channels in the retina by TTX suppressed the rhythmic fluctuations without affecting the basic light-evoked responses of dimming detectors (Fig. 4). Pharmacological experiments revealed the involvement of GABAergic synaptic transmission in generating the rhythmic fluctuations in dimming detectors (Figs. 5–7).

Contribution of amacrine cells to generation of the rhythmic fluctuations

The current injection experiment indicates that dimming detectors may not have the intrinsic ability to generate the rhythmic fluctuations (Fig. 3). The synaptic input to dimming detectors is likely to be rhythmic. The presynaptic mechanism that can generate the rhythm seems to be characterized by susceptibility to Na + channel blockers and involvement of GABAergic synaptic transmission.

SUSCEPTIBILITY TO Na + CHANNEL BLOCKERS. The rhythmic fluctuations of dimming detectors were not affected by the intracellular application of QX-314 but suppressed by the bath application of TTX (Fig. 4). Therefore Na + spikes in presynaptic cells are essential for the generation of the rhythmic fluctuations in dimming detectors.

The cells presynaptic to dimming detectors are bipolar and amacrine cells. Recently it was reported that subsets of bipolar cells had TTX-sensitive Na + channels (rat: Pan and Hu 2000;
However, these Na\textsuperscript{+}/H\textsuperscript{+} channels were mainly localized to the dendrite and soma of bipolar cells, and only a few Na\textsuperscript{+}/H\textsuperscript{+} channels were located at the axon terminal. Thus Na\textsuperscript{+}/H\textsuperscript{+} channels of bipolar cells may not contribute to the signal processing in the inner retina but may be used to amplify the signal from photoreceptors (Pan and Hu 2000; Zenisek et al. 2001). It is well accepted that amacrine cells generate Na\textsuperscript{+}/H\textsuperscript{+} spikes (Barnes and Werblin 1986). Na\textsuperscript{+}/H\textsuperscript{+} spikes can conduct along the dendrites of amacrine cells with less attenuation than the graded potential, resulting in the establishment of wide-range interaction in the inner retina (Shields and Lukasiewicz 2003).

INVOLVEMENT OF GABA\textsubscript{A} ERGIC SYNAPTIC TRANSMISSION. The rhythmic fluctuations of dimming detectors were suppressed by bicuculline but not by strychnine (Figs. 5 and 6). Vitanova et al. (2001) have shown that GABA\textsubscript{A} receptors mainly distribute in the inner plexiform layer of the frog retina, where amacrine cells make synapses. The release of GABA from amacrine cells is triggered by Na\textsuperscript{+} spikes (goldfish: Watanabe et al. 2000; tiger salamander: Shields and Lukasiewicz 2003). Therefore GABA\textsubscript{ergic} amacrine cells are likely to play an important role in the network that generates the rhythm in the inner retina. This hypothesis is compatible with the observation that oscillatory potentials of local electoretinogram to a flash light are originated from the inner plexiform layer of the mudpuppy retina (Wachtmeister and Dowling 1978).

One may imagine that dimming detectors receive direct rhythmic GABA\textsubscript{A}ergic input from amacrine cells and that the GABA\textsubscript{A}ergic transmission does not participate in generating the rhythm per se. However, the rhythmic activities of dimming detectors were not affected by the concentration of Cl\textsuperscript{−} in the pipette solution although the calculated $E_{\text{Cl}}$ was different between the pipette solutions A (−56 mV) and B (−77 mV). If dimming detectors received direct rhythmic GABA\textsubscript{A}ergic inputs, it would be difficult to detect the rhythmic fluctuations of dimming detectors when the pipette solution A was used for recordings because the light-evoked depolarizing plateau (approximately −60 mV, Fig. 1C) was close to $E_{\text{Cl}}$ (−56 mV). Therefore it is likely that GABA\textsubscript{A}ergic synaptic transmission may be involved in the presynaptic network that generates the rhythm.

Ishikane et al. (1999) have shown that wide-field dimming illumination is essential for the generation of synchronous oscillatory spike discharges among dimming detectors. A possible mechanism for the wide-range interaction would be the signal transmission via gap junctions. It has been reported in the cat and rabbit retinas that ganglion cells can interact with one another via gap junctions (Vaney 1991; Xin and Bloomfield 1997). However, in the present study, the dye coupling was not observed when dimming detectors were intracellularly stained with Lucifer yellow (Fig. 1). A preliminary experiment with neurobiotin has not yet been successful to show the tracer coupling. Even if gap junctions existed among dimming detectors, the pore size of gap junctions would be too small and the coupling ratio would be too low to contribute to the oscillatory potentials.
wide-range interaction and/or the generation of the rhythm, though we cannot deny a possible contribution of gap junctions among dimming detectors to the narrow-range synchronization (Brivanlou et al. 1998). On the other hand, amacrine cells in various species are known to make extensive gap junctions each other (for review, see Vaney 1994). Furthermore, wide-field amacrine cells are found in the frog retina (Vigh et al. 2000). Although circumstantial, these pieces of evidence (susceptibility to Na⁺ channel blockers, involvement of GABAergic synaptic transmission, and wide-range interaction) suggest that amacrine cells may contribute to generating rhythmic input to dimming detectors.

Functions of GABA<sub>C</sub> receptors in the inner retina

Application of TPMPA drastically enhanced the amplitude (or power) of the rhythmic fluctuations but did not change obviously their frequency (Figs. 5 and 7). Thus GABA<sub>C</sub> receptor-mediated interactions may not participate in the system that regulates the frequency of the rhythm.

It has been suggested that the GABA<sub>C</sub> receptor-mediated negative feedback from amacrine cells to bipolar cells may be used to control the gain of glutamatergic transmission (tiger salamander: Dong and Werblin 1998; mouse: Matsui et al. 2001). GABA<sub>C</sub> receptors distribute mainly in the inner plexiform layer, especially at the axon terminal of bipolar cells (rat: Wässle et al. 1998; frog: Du and Yang 2000; frog and turtle: Vitanova et al. 2001). Therefore application of TPMPA probably increased the release of glutamate from bipolar cells in the frog retina (Figs. 5 and 7). This, in turn, would enhance the inhibitory feedback or feedforward activities of amacrine cells and/or the excitatory direct input to dimming detectors, resulting in the potentiation of the oscillatory activities of dimming detectors.

Models that may generate the rhythmic fluctuations in dimming detectors

Three types of neuronal models have been proposed for synchronous oscillations (for review, see Jefferys et al. 1996; Ritz and Sejnowski 1997). The first type includes a feedback loop between excitatory and inhibitory neurons (Eckman and Freeman 1990). Because most bipolar and amacrine cells are assumed to be excitatory and inhibitory, respectively, and because bipolar cells make reciprocal synapses with amacrine cells (Dowling 1968; Dowling and Werblin 1969; Hartveit 1999), this type of model would be a neuronal base of oscillations in dimming detectors. In this case, the rhythm is produced locally at the axon terminal of bipolar cells, and thus gap junctions among amacrine cells would help to synchronize the locally generated rhythm (Deans et al. 2001; Traub et al. 2001).

The second type hypothesizes an inhibitory neuron that can induce oscillations by the intrinsic properties. Oscillator neurons (or chattering cells) can make a rhythm in the network (Gray and McCormick 1996). It has been reported that oscillatory membrane potentials were produced by injecting a sustained depolarizing current into GABAergic wide-field amacrine cells in the white bass retina (Sollessio et al. 2002; Vigh et al. 2003). It is possible that some amacrine cells in the frog retina would produce the rhythm intrinsically and send their output to bipolar cells and/or amacrine cells.

The third type is composed of the network where inhibitory neurons are mutually connected. Oscillations are generated when the network receives tonic excitatory inputs (Whittington et al. 1995). It has been shown that retinal amacrine cells make inhibitory synapses each other (Dowling 1968; Roska et al. 1998; Watanabe et al. 2000). If the inhibitory network of amacrine cells received sustained excitatory inputs from bipolar cells, the network would generate the rhythm. Further studies are required to specify the neuronal basis of rhythm generation in the inner retina.

Functional significance of oscillations in dimming detectors

It has been suggested that the activities of dimming detectors in the frog retina are related to the escape behavior (Maturana et al., 1960). The large shadow of a big animal that moves toward a frog produces a large dark image on the frog retina. Applying a planar multi-electrode array to the retina isolated from frog, Ishikane et al. (1999) demonstrated that a large dimming stimulus evoked synchronous oscillatory spike discharges in a large cluster of dimming detectors, whereas small multiple dark spots failed to induce synchronous oscillations. Tachibana et al. (2003) showed that the stimulus pattern that induced synchronous oscillations in dimming detectors could trigger the escape behavior in frogs. However, it is not yet certain whether synchronization or oscillations is essential for triggering the escape behavior. Further studies are required to elucidate the functional significance of oscillations in dimming detectors.

ACKNOWLEDGMENTS

We thank JL. Du for participation in early experiments and H. Ishikane, M. Gangi, N. Hosoi, and J. Hasegawa for discussion and comments on this manuscript.

GRANTS

This work was supported by Grant-in-Aid for Scientific Research (12053212) and the Special Coordination Funds for Promoting Science and Technology (The NRV Project) from the Ministry of Education, Science, Sports and Culture to M. Tachibana.

REFERENCES


ORIGIN OF OSCILLATIONS IN RETINAL GANGLION CELLS


J. Neurophysiol • VOL 92 • AUGUST 2004 • www.jn.org 725


