Spontaneous Oscillatory Activity of Starburst Amacrine Cells in the Mouse Retina

Jerome Petit-Jacques\textsuperscript{1,2}, Béla Völgyi\textsuperscript{1,2}, Bernardo Rudy\textsuperscript{2,3} and Stewart Bloomfield\textsuperscript{1,2}

Departments of Ophthalmology\textsuperscript{1}, Physiology & Neuroscience\textsuperscript{2}, and Biochemistry\textsuperscript{3}

New York University School of Medicine
New York, NY 10016

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Correspondence to: Dr. Stewart Bloomfield
Dept. Ophthalmology
NYU School of Medicine
550 First Avenue
New York, NY 10016
Tel: 212-263-5770
Fax: 212-263-8072
Email: blooms01@med.nyu.edu
Abstract

Using patch clamp techniques we investigated the characteristics of the spontaneous oscillatory activity displayed by starburst amacrine cells in the mouse retina. At a holding potential of ~70 mV, oscillations appeared as spontaneous, rhythmic inward currents with a frequency of ~3.5 Hz and an average maximal amplitude of ~120 pA. Application of TEA, a potassium channel blocker, increased the amplitude of oscillatory currents by more than 70%, but reduced their frequency by about 17%. The TEA effects did not appear to result from direct actions on starburst cells, but rather a modulation of their synaptic inputs. Oscillatory currents were inhibited by CNQX, an antagonist of AMPA/kainate receptors, indicating that they were dependent on a periodic glutamatergic input likely from presynaptic bipolar cells. The oscillations were also inhibited by the calcium channel blockers cadmium and nifedipine, suggesting that the glutamate release was calcium dependent. Application of AP4, an agonist of mGluR6 receptors on on-center bipolar cells, blocked the oscillatory currents in starburst cells. However, subsequent application of TEA overcame the AP4 blockade, suggesting that the periodic glutamate release from bipolar cells is intrinsic to the inner plexiform layer in that, under experimental conditions, it can occur independent of photoreceptor input. The GABA receptor antagonists picrotoxin and bicuculline enhanced the amplitude of oscillations in starburst cells pre-stimulated with TEA. Our results suggest that this enhancement was due to a reduction of a GABAergic feedback inhibition from amacrine cells to bipolar cells and the resultant increased glutamate release. Finally, we found that some ganglion cells and other types of amacrine cell also displayed rhythmic activity, suggesting that oscillatory behavior is expressed by a number of inner retinal neurons.

Keywords: amacrine cells, oscillations, retina; glutamate
INTRODUCTION

Spontaneous and stimulus-induced neuronal rhythmicity and the resulting cell ensemble oscillations have been described throughout the CNS (Llinás 1988; Llinás et al. 1994). In the visual system, rhythmicity of cortical activity, which has been proposed to bind local features within an image, appears to reflect not just intracortical processing, but oscillatory signaling received through the lateral geniculate nucleus (LGN) of the thalamus (Doty and Kimura 1963; Ghose and Freeman 1992, 1997; Neuenschwander and Singer 1996; Castelo-Branco et al. 1998). In retina, rhythmic ganglion cell discharges first appear prenatally in the form of spontaneous propagating waves, which underlie activity-dependent development of circuits both within retina and the LGN (Meister et al. 1991; Goodman and Shatz 1993; Wong 1993). In the adult, precise oscillatory discharge patterns have been described for ganglion cells in a number of species under conditions of constant ambient light as well as in response to changes in luminance (Kuffler 1953; Doty and Kimura 1963; Steinberg 1966; Ariel et al. 1983; Neuenschwander et al. 1999). These oscillations show a wide range of frequencies and appear dependent on stimulus size and contrast.

It is now clear that oscillatory activity within the retina is not restricted to ganglion cells. The oscillatory potentials (OP) of the ERG consist of both fast and slow rhythmic components and thereby indicate prominent and widespread oscillations within the retina (Steinberg et al. 1983; Wachtmeister 1998). Although originally thought to be part of the major ERG wave components, it is now clear that OPs reflect postsynaptic activity as evidenced by their sensitivity to glutamate and dopamine (Wachtmeister 1981; Jaffe et al. 1987). Moreover, OPs are attenuated or abolished by the inhibitory transmitters GABA and glycine and are enhanced by the amacrine cell peptide somatostatin (Wachtmeister 1980; Wachtmeister 1983). Together, these drug effects suggest that the OPs reflect rhythmic activity generated within the proximal retina. This idea is supported
by the finding that bipolar cell axon terminals display calcium-dependent spontaneous
membrane oscillations (Burrone and Lagnado 1997; Zenisek and Matthews 1998; Ma
and Pan 2003), which may lead to pulsatile transmitter release and rhythmic activity of
postsynaptic amacrine and ganglion cells. Indeed, the oscillatory activity displayed by
certain amacrine cells in fish retina is thought to be synaptically driven (Djamgoz et al.
1989; Sakai and Naka 1990, 1992). In contrast, subthreshold oscillations in wide-field
amacrine cells in the fish retina (Solessio et al. 2002) as well as rhythmic spiking of
dopaminergic amacrine cells in mouse (Feigenspan et al. 1998) survive cell isolation,
indicating an intrinsic generating mechanism.

Here, we report spontaneous, rhythmic activity recorded from the starburst
amacrine cells, a unique subtype that releases both acetylcholine and GABA and
thereby functions as both an excitatory and inhibitory retinal interneuron (Agardh and
Ehinger 1983; Brecha et al. 1988; O’Malley and Masland 1989). Our pharmacological
data indicate that this oscillatory activity is derived from pulsatile, calcium-dependent
 glutamate release from presynaptic bipolar cell axon terminals. These oscillations
appear to reflect a synaptic generating mechanism intrinsic to the proximal retina as they
can be induced experimentally in the absence of photoreceptor signaling.
MATERIALS AND METHODS

Mouse retina-eyecup preparation

All animal procedures complied with National Institutes of Health guidelines for the ethical use of animals. Kv3.1/3.2 double knockout mice were generated and bred in the laboratory of Dr. Bernardo Rudy (Ozaita et al. 2004). ICR wild-type and Kv3.1/3.2 double knockout mice (25-60 days old) were deeply anesthetized with an intraperitoneal injection of pentobarbital (0.08g/g body weight). Lidocaine hydrochloride (20 mg/ml) was applied locally to the eyelids and surrounding tissue. A flattened retinal-scleral eyecup preparation developed for rabbit by Hu et al. (2000) was adopted and modified for the mouse. Briefly, the eye was removed under dim red illumination and hemisected anterior to the ora serrata. Animals were sacrificed immediately after enucleation by cervical dislocation. The lens and vitreous humor were removed and the resultant eyecup preparation was placed on the base of a submersion-type recording chamber. Several radial incisions were made peripherally and the retina was flattened in the chamber vitreal side up. The chamber was mounted on a microscope stage within a Faraday cage and superfused (1-2 ml/min) with an oxygenated mammalian Ringer solution composed of (in mM): NaCl 120, KCl 5, NaHCO₃ 25, Na₂HPO₄ 0.8, NaH₂PO₄ 0.1 MgSO₄ 1, CaCl₂ 2, D-Glucose 10. A pH of 7.4 was maintained by bubbling with 95% O₂ - 5% CO₂ at room temperature of 20-22 °C.

Electrophysiological recordings

Recordings were made in the whole cell patch mode with an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA). Cells were visualized with near infrared light (>775 nm) at 80X magnification with a Nuvicon tube camera (Dage-MTI, Michigan City, IN) and differential interference optics (DIC) on a fixed-stage microscope (BX51WI;
Olympus, Tokyo, Japan). Currents were recorded under voltage clamp, filtered at 1 kHz, sampled at 20 kHz, and stored directly on the computer’s hard drive using a Digidata 1200 A/D interface (Axon Instruments). For the characterization of voltage responses, neurons were recorded in the fast current-clamp mode of the amplifier. The resting potential of neurons was adjusted to –70 mV with small injections of direct current.
pCLAMP (v. 8.02; Axon Instruments) was used for data acquisition with data analysis performed off-line using Minianalysis (v. 6.0.1; Synaptosoft, Decatur, GA) and Origin (v. 6.1; OriginLab, Northampton, MA) software packages. The average oscillatory current was calculated by averaging all oscillations that occurred during a one minute long recording. The activation phase (baseline to peak) and the relaxation phase (peak to baseline) of the average current were fitted with first-order exponentials using Clampfit (v. 8.02; Axon Instruments) to calculate the time constants.

Patch electrodes (3-5 MΩ) were pulled from standard wall borosilicate glass tubing (World Precision Instruments, Sarasota, FL) with a Flaming/Brown type micropipette puller (Sutter Instruments, Novato, CA). Pipettes were filled with a K-glucuronate internal solution composed of (in mM): K-glucuronate 144, MgCl₂ 3, EGTA 0.2, HEPES 10, ATP-Mg 4, GTP-Tris 0.5, pH 7.3 with KOH, and biocytin (0.2% w/v, Sigma, St. Louis, MO). All recordings were made under ambient dim light.

**Biocytin labeling**

Neurons were labeled by allowing biocytin to diffuse from the micropipette during patch recordings. After electrophysiological experiments were completed, retinas were fixed in a cold (4°C) solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH=7.3) overnight. Retinas were then washed in phosphate buffer and soaked in a solution of 0.18% hydrogen peroxide in methyl alcohol for one hour. This treatment completely abolished the endogenous peroxidase activity. Retinas were then washed in
phosphate buffer and reacted with the Elite ABC kit (Vector Laboratories, Burlingame, CA) and 1% Triton X-100 in sodium phosphate-buffered saline (9% saline, pH=7.5). Retinas were subsequently processed for peroxidase histochemistry using 3,3’-diaminobenzidine (DAB) as the chromagen, dehydrated and flat-mounted for light microscopy.

Statistical analyses

Data were analyzed using Student’s t-test statistic. Presentation of data is in the form mean ± standard error (SEM) throughout.
RESULTS

*Characteristics of starburst amacrine cells of the mouse retina*

Recordings were made from on-center starburst amacrine cells, whose somata were displaced to the ganglion cell layer (GCL) and whose dendritic arbors stratified within sublamina *b* of the inner plexiform layer (IPL). We were unable to specifically label starburst amacrine cells in our mouse preparation prior to recordings and so we typically targeted small, round somata in the GCL. Following electrophysiological recordings, biocytin was injected into all cells to confirm their identity by post hoc histology. Overall, about 60% of our recordings were from starburst cells, with the remainder from other amacrine cell types as well as small ganglion cells.

Starburst amacrine cells in the mouse showed the typical symmetric dendritic morphology described in other species (Famiglietti 1983; Tauchi and Masland 1984; Bloomfield and Miller 1986). This included four to five primary dendrites that first branched into thin, wavy, intermediate segments that divided further into a dense plexus of distal branches showing numerous varicosities (Fig. 1A). We found that starburst amacrine cells displayed robust and stereotypic electrophysiological properties. This included an average membrane capacitance of $22.61 \pm 0.40 \text{ pF}$ and input resistance of $195.6 \pm 5.9 \text{ M}\Omega$ ($n=70$). By comparison, the non-starburst cells in our sample showed a significantly larger average membrane capacitance ($33.67 +/- 1.04 \text{ pF}$) and membrane input resistance ($340.5 +/- 26.4 \text{ M}\Omega$, $n=20$), despite having apparently similar soma sizes. Another characteristic feature of starburst cell physiology was revealed by their membrane voltage response to extrinsic current steps. Membrane depolarization triggered by pulses larger than +50 pA tended to saturate, so it was not possible to depolarize the membrane to potentials more positive than -20 mV (Fig. 1B). It has been suggested that this may reflect the presence of Kv3 potassium channels in the soma and
proximal dendrites, which create a shunt when activated and thereby limit the extent of depolarization (Ozaita et al. 2004). Under our recording conditions, starburst cells showed no spontaneous or evoked spiking, consistent with previous studies using whole cell recording techniques (Taylor and Wässle 1995; Peters and Masland 1996). Large depolarizing current pulses did evoke a characteristic small, transient response component (Fig. 1B). However, this component never reached potentials more positive than 0 mV and was not blocked by application of TTX.

Possibly the most robust and characteristic properties of starburst cell activity were the spontaneous, oscillatory currents. Oscillations were recorded in voltage clamp at −70 mV and appeared as spontaneous rhythmic inward currents. We observed a range of amplitudes for the oscillatory activity (Fig. 1C). Some starburst cells showed relatively low amplitude oscillations that could not be easily differentiated from baseline noise and miniature synaptic events. In contrast, other starburst cells displayed large amplitude oscillations coupled with lower baseline activity. Cells with large or small amplitude oscillations occurred in approximately equal numbers and were recorded under the same control conditions. Further, we found that drug effects (presented below) were similar for all oscillatory activity, irrespective of their amplitude. Therefore we have not differentiated large from small amplitude oscillations in the analyses below.

Under control conditions, oscillations had a mean frequency of 3.52 ± 0.14 Hz, and an average maximal amplitude of 121.36 ± 8.74 pA (n=33). Interestingly, the oscillations present in mouse starburst cells resemble those described in a subset of displaced amacrine cells in the adult ferret retina (Aboelela and Robinson 2004), although the frequency of the oscillations in the ferret was considerably lower.
Effects of TEA on oscillatory currents in starburst cells

During the course of a recent study, we used TEA to examine the role of Kv3 potassium channels in starburst cell activity (Ozaita et al. 2004). A surprising result was that low doses of TEA (1 mM) produced a large and reproducible increase in the amplitude of oscillatory activity (Fig. 1C). The average maximal amplitude of oscillations was increased more than 70% from 125.2 ± 14.4 pA in control Ringer to 214.8 ± 33.2 pA in TEA (n=9) (Fig. 2B). In addition, TEA reduced the frequency of oscillations by approximately 17%, from an average 3.48 ± 0.16 Hz in Ringer to 2.88 ± 0.17 Hz (Fig. 2A).

Figure 2C illustrates the average oscillatory current representing all oscillations in 9 cells during a one-minute recording at –70 mV, before and after application of TEA. In addition to the increase in the amplitude of the average oscillatory current, TEA significantly accelerated the activation phase (baseline to peak). In TEA, the time constant of the average current activation phase was reduced by half from 18.9 ± 1.6 ms in control to 9.9 ± 0.6 ms (n=9). Although TEA also accelerated the relaxation phase (peak to baseline) of the average current, the change was not statistically significant (τ relaxation = 20.7 ± 2.3 ms in control and 14.8 ± 1.3 ms in TEA) (Fig. 2D). Overall, TEA affected both the amplitude and kinetics of starburst cell oscillations. It should also be noted that TEA decreased the frequency of miniature synaptic events. When miniature events were counted outside oscillations, it was found that TEA significantly diminished their number by more than 35% (from 2.0 ± 0.28 Hz in control to 1.29 ± 0.17 Hz in TEA, n=9, p<0.01), without affecting their amplitude.

Starburst cells in the mouse retina display a high density of voltage-gated Kv3 channels that are responsible for large outward potassium currents (Ozaita et al. 2004). Therefore, the effects of TEA on the oscillatory currents could reflect direct actions on the Kv3 channels in starburst cells. To test this idea, we examined the effects of TEA on
oscillations in starburst cells from Kv3.1-Kv3.2 double knock-out (DKO) animals. As shown in Figure 2E, TEA remained effective in increasing the amplitude of oscillatory currents in starburst cells from DKO animals; the average maximum amplitude in TEA was 215.2 ± 79.4 pA (n=4). These data indicate that TEA effects on the oscillations in wild-type animals were not due to a blockade of Kv3 channels in starburst cells.

Oscillatory currents in starburst amacrine cells are synaptically mediated

In the next series of experiments, we examined whether the oscillatory currents in starburst cells were dependent on the excitatory synaptic drive from presynaptic bipolar cells. Bipolars cells form glutamatergic synapses onto starburst amacrine cells at which AMPA/kainate ionotropic receptors are localized (Brandstätter et al. 1998; Thoreson and Witkovsky 1999; Morgans 2000; Brandstätter and Hack 2001; Firth et al. 2003; Yang 2004). Application of CNQX, a specific blocker of AMPA/kainate receptors (Mayer and Armstrong 2004), reversibly blocked the oscillatory currents and miniature synaptic events (Fig. 3A). On average, CNQX (10 µM) reduced the oscillation frequency by 81% under control conditions and reduced the frequency of oscillations pre-stimulated by TEA by 76% (Fig. 3B). In addition, CNQX decreased the maximal amplitude of oscillations in control retinas by 90% and the oscillations enhanced by TEA by 89% (Fig. 3C). Clearly, these data indicate that oscillatory currents in starburst amacrine cells are dependent on the glutamatergic drive, likely from bipolar cells to starburst cells.

Oscillatory currents are sensitive to blockers of calcium channels

Since bipolar cells have different calcium channels, whose activation participates in the modulation of glutamate release, we examined the effects of calcium blockers on the spontaneous oscillations in starburst cells (Tachibana 1999; Pan 2000, 2001;
Berntson et al. 2003). Application of cadmium ions produced a nearly complete blockade of both basal and TEA-enhanced oscillatory currents, leaving only the miniature synaptic events (Fig. 4A). The frequency of basal oscillations as well as those enhanced with TEA was inhibited by more than 98% by CdCl₂ (Fig. 4B). The maximal amplitude of basal oscillations and those enhanced by TEA were reduced by cadmium to a similar degree, 85% and 90%, respectively (n=4) (Fig. 4C). Oscillatory currents enhanced by TEA were also largely inhibited by application of nifedipine (30 µM), a specific blocker of L-type calcium channels. Nifedipine blocked more than 74% of the oscillations amplitude, without significantly affecting their frequency (n=2, data not shown). Taken together, these data are consistent with a calcium-dependent glutamate release underlying the oscillatory activity of starburst amacrine cells.

Effect of AP4 on the oscillatory currents of starburst amacrine cells

The mGluR6 metabotropic glutamate receptors are expressed in the retina at the synapse between photoreceptors and on-center bipolar cells (Nomura et al. 1994; Ueda et al. 1997). When activated, these receptors lead to a closure of cation channels that result in a hyperpolarization of on-center bipolar cells and a reduction in their excitatory drive of proximal neurons (Nakajima et al. 1993; Tian and Slaughter 1994, 2003; Thomsen 1997; Gerber 2003). Application of AP4, an agonist of these receptors, produced a sustained blockade of the basal oscillatory currents until it was washed out (Fig. 5A). In contrast, after enhancement of the oscillations with TEA, application of AP4 produced only a transient inhibition (Fig. 5A). Within 6 minutes of AP4 application, the oscillations were totally recovered while still in the presence of the drug. At its maximal effect, AP4 reduced the average frequency of oscillations enhanced by TEA by 83% and maximal amplitude by 76% (n=4) (Fig. 5B,C). Again, the maximal inhibition by AP4 occurred within 3 minutes, but recovery was complete by about 6 minutes (Fig. 5D).
contrast, we examined the effects of AP4 for time periods of up to 10 minutes and never saw a reversal of the blockade of basal oscillations until we returned to control conditions.

**Effect of GABA blockers on oscillatory currents**

The excitatory drive from bipolar cells to starburst amacrine cells can be modified by GABAergic feedback inhibition from amarine cells (Wässle et al. 1998; Matsui et al. 2001; Pan 2001; Shen and Slaughter 2001; Völgyi et al. 2002; Freed et al. 2003). In addition, starburst cell activity may be altered by direct inhibition from neighboring amacrine cells. We therefore examined the effects of GABA receptor blockers on the oscillatory currents. Application of picrotoxin (PTX, 50 µM), a mixed GABA\(_A\) and GABA\(_C\) receptor antagonist, had no effect on basal oscillations under control conditions (Fig. 6A). However, PTX did dramatically increase the amplitude of the oscillations already enhanced with TEA (Fig. 6B). As shown in Figure 6C, PTX increased the amplitude of the average current without affecting significantly the kinetics of the activation and relaxation phases. Interestingly, PTX produced a reduction in the mean frequency of oscillations by 37%, concomitant with the 72% increase in average maximal amplitude (n=5) (Fig. 6D). Application of the GABA\(_A\) receptor antagonist, bicucculline (BMI, 10 µM), produced effects very similar to those of PTX, including a reduction in the average frequency (32%) and an increase in the average maximal amplitude (32%) of the oscillatory currents (n=3) (Fig. 6E).

**Other types of inner retinal neurons display oscillatory currents**

As aforementioned, we encountered other types of amacrine cells and ganglion cells during the course of experimentation. Many of these also showed spontaneous oscillations, but with characteristics different from those of starburst cells. For example, a
type of wild-field amacrine cell we encountered in the mouse displayed oscillatory currents that were broader, more frequent, and with more constant amplitudes than those displayed by starburst cells (Fig. 7A,B). In general, ganglion cell oscillations were more irregularly shaped and with slightly lower amplitudes than those recorded in the starburst cells (Fig. 7C). Figure 7D illustrates the different kinetics of the average oscillatory current recorded in wide-field amacrine cells from those recorded in starburst or ganglion cells. These data indicate that oscillatory currents, although highly variable across cells, are expressed by a number of neurons in the inner retina.
DISCUSSION

Our data show that starburst amacrine cells generate robust, spontaneous 3-4 Hz membrane oscillations in the adult mouse retina. It is well documented that the cholinergic starburst cells in the neonate display subthreshold oscillations crucial to the generation of spontaneous waves, which underlie activity-dependent development of the visual system (Meister et al. 1991; Feller 1999; Zhou 1998). However, these oscillations, which occur every 1-2 minutes, rely on cholinergic circuitry as well as excitatory, glycinergic circuitry which subsequently becomes inhibitory in the adult. Moreover, these oscillations disappear within several days postnatally and are thus quite distinct from the oscillatory currents reported here. In contrast, our results indicate that the oscillatory activity in the adult results from periodic, calcium-dependent release of glutamate from presynaptic bipolar cell axon terminals. Taken together, these data suggest strongly that the oscillatory activity we recorded in starburst cells does not reflect propagating spontaneous waves as seen during development.

Oscillations are dependent on glutamatergic input from bipolar cells

The finding that application of CNQX largely blocked the oscillatory currents suggests that they are dependent on activation of AMPA/kainate receptors postsynaptic to bipolar cell terminals. Cadmium ions and nifedipine also blocked the oscillatory currents, indicating that calcium channels are involved in the regulation of periodic glutamate release from bipolar cells. The persistent, pulsatile release of glutamate from presynaptic terminals has been found to be secondary to oscillations in intracellular calcium that are maintained by a calcium-induced calcium release process in various types of neuron including retinal bipolar cells (Aniksztejn et al. 1995; Burrone et al. 2002; Cherubini et al. 1991; Llobet et al. 2003; Pasti et al. 2001). In bipolar cells, calcium ion entry via calcium channels organized in clusters could trigger the release of calcium from
internal stores that ultimately control the exocytosis of glutamate vesicles (Burrone et al. 2002; Llobet et al. 2003). Also, a resonant mechanism combining calcium channel activation/inactivation and calcium-activated potassium channel activation/deactivation could underlie a cyclic entry of calcium into the synaptic terminal (Vigh et al. 2003). Calcium influx through T- and L-types of voltage-dependent calcium channels has been shown to underlie the spontaneous membrane oscillations in rat bipolar cells (Ma and Pan 2003). A similar generating mechanism has been suggested for the oscillatory activity of CA3 neurons in the hippocampus (Aniksztejn et al. 1995). Here, spontaneous oscillatory currents were synaptically driven by presynaptic glutamatergic inputs and were dependent on calcium entry likely via high voltage-activated calcium channels (Bacci et al. 1999).

Our finding that oscillations in starburst cell activity is dependent on bipolar cell input is consistent with observations in fish retina that oscillations in some amacrine cells arise from the activity of local circuits (Djamgoz et al. 1989; Sakai and Naka 1990, 1992). In contrast, Solessio et al. (2002) described intrinsic oscillatory activity in isolated wide-field amacrine cells of the fish that are generated from the interplay between calcium and potassium currents. Intrinsic rhythmic spike activity has also been described in isolated dopaminergic amacrine cells in the mouse (Feigenspan et al. 1998). Together, these data indicate that both synaptic circuitry and intrinsic membrane properties can play roles in generating the oscillatory activity of different amacrine cell types across a number of species.

*Actions of TEA are presynaptic to starburst amacrine cells*

Low concentrations of TEA produced a robust enhancement of the oscillatory currents in starburst cells. This suggests the involvement of TEA-sensitive potassium channel subfamilies, such as BK, Kv1, and/or Kv3 (Rudy et al. 1999). It has been
shown recently that starburst cells express Kv3 channels (Tian et al. 2003; Ozaita et al. 2004), raising the possibility that the TEA effects on oscillatory activity were due to direct actions on starburst cells. However, two of our findings argue against this. First, in our voltage clamp experiments, starburst cells were held at –70 mV, below the threshold of activation of Kv3 channels (Rudy et al. 1999). Second, we found that the oscillatory activity recorded from starburst cells in Kv3.1/3.2 double knock-out mice was still enhanced by TEA. Together, these data suggest strongly that TEA enhancement of the oscillations reflected actions on the large conductance calcium-activated (BK) and/or Kv1.1 potassium channels found in bipolar cell axon terminals (Sakaba et al. 1997; Pinto and Klumpp 1998; Gribkoff et al. 2001). In this scenario, application of TEA would block the potassium channels in bipolar cell terminals leading to a membrane depolarization and activation of voltage-gated calcium channels. The increase in internal calcium ion concentration triggered a calcium-induced release of glutamate. In addition to this increased release, perhaps TEA synchronized the release of multiple vesicles from one or more active zones (Sharma and Vijayaraghavan 2003; Singer et al. 2004), leading to a larger overall release of glutamate from the synaptic endings. Although TEA increased the amplitude of the average current, it also accelerated both the activation and the relaxation phases consistent with a modification of the release mechanism. The finding that TEA slightly decreased the frequency of oscillations while increasing their amplitude suggests that larger vesicles were released less frequently, consistent with a modification of release synchronization. Further, the finding that TEA decreased the frequency of miniature synaptic events suggests a modification of the recruitment of a finite pool of synaptic vesicles. Perhaps TEA enhancement of the oscillations reflected the fusion of individual vesicles or an increased simultaneous release of individual vesicles as clusters.
Oscillations in starburst cells can occur independent of photoreceptor synaptic drive

Application of AP4, a specific agonist of the mGluR6 receptors (Nakajima et al. 1993; Thomsen 1997) at photoreceptor to on-center bipolar cell synapse, eliminated the oscillatory currents in starburst cells. Interestingly, whereas AP4 produced a sustained inhibition of basal oscillations, its effect on TEA-enhanced oscillations was only transient. This suggests that the sustained depolarization induced by TEA could overcome the hyperpolarization of bipolar cells induced by AP4 and thereby trigger periodic glutamate release. Moreover, these data show that the underlying pulsatile glutamate release can occur, at least under experimental conditions, when photoreceptor synaptic drive is absent. This suggests that the machinery underlying periodic glutamate release and the resultant starburst cell oscillations is located at the bipolar cell level and can occur without periodic glutamate release from photoreceptor terminals. It is important to note here that our recordings were made from starburst-b amacrine cells for which morphological and physiological data indicate innervation strictly via the ON retinal pathway (Famiglietti 1983; Bloomfield and Miller 1986; Taylor and Wässle 1995; Peters and Masland 1996). Thus, the oscillatory activity under conditions of ON pathway blockade with AP4 cannot be due to an emergent photoreceptor signaling to the starburst cells via the OFF pathway.

Feedback inhibition affects starburst cell oscillations

Both GABA_A and GABA_C receptors are localized to mammalian bipolar cell axon terminals (Wässle et al. 1998; Pan 2001; Bloomfield and Dacheux 2001), which mediate the feedback inhibition from amacrine cells that regulates glutamate release (Euler and Masland 2000; Matsui et al. 2001; Shen and Slaughter 2001; Völgyi et al. 2002; Zhang et al. 2002). Our finding that both picrotoxin and bicuculline increased the amplitude of the TEA-enhanced oscillatory currents in starburst cells is consistent with an enhanced
release of glutamate from bipolar cell terminals due to a reduced feedback inhibition. Like TEA, the enhanced oscillatory currents produced by the GABA blockers was concomitant with a reduced frequency, again suggesting improved coordination of glutamate release from a finite vesicle pool in bipolar cell terminals. Interestingly, we found that GABA blockers had little effect on the amplitude of basal oscillations. Perhaps, under our basal experimental conditions, the glutamate release from bipolar cells is insufficient to generate a significant GABAergic feedback inhibition of the bipolar cell terminal. In essence, the GABA blockers are ineffective because there is no inhibition to block. In this scenario, only after glutamate release is increased by TEA is there a sizeable feedback inhibition generated to modulate further release. In any event, these results indicate that the oscillatory activity of starburst cells is plastic as it can be modulated by the established feedback circuitry in the IPL.

Significance of oscillations in the retina

Our results add to a growing list of reports of oscillatory activity within the retina. At a cellular level, it has been long known that ganglion cell spike discharges show both spontaneous and light-evoked oscillations (Kuffler 1953; Doty and Kimura 1963; Steinberg 1966; Ariel et al. 1983; Neuenschwander et al. 1999). It is now clear that spontaneous oscillatory activity is not restricted to the ganglion cells, but occurs in both bipolar cells and amacrine cells as well (Djamgoz et al. 1989; Burrone and Lagnado 1997; Zenisek and Matthews 1998; Ma and Pan 2003). These rhythmic activities are reflected in the prominent oscillatory potentials of the ERG (Wachtmeister 1998). Our results indicate not only a variety of cells in the inner mouse retina with oscillatory activity, but a variety of kinetics as well. These data are consistent with the wide range of frequencies found for the oscillatory activity reported for ganglion cells in a number of
preparations (Neuenschwander et al. 1999). Thus, the inner retina appears to contain groups of independent oscillators, possibly differentiated by neuronal subtype.

Clearly, the subthreshold oscillatory activity of bipolar and amacrine cells can produce periodic release of neurotransmitter that, in turn, will produce oscillatory activity in postsynaptic cells, particularly ganglion cells (Arai et al. 2004). Indeed, our data indicate that it is the periodic release of glutamate that underlies the robust oscillations in starburst amacrine cells. Interestingly, the periodic, spontaneous and light-evoked discharges of neighboring ganglion cells have been found to be highly synchronized (Arnett and Spraker 1981; Mastronarde 1983; Meister et al. 1995; Neuenschwander et al. 1999; Hu and Bloomfield 2003). It is thought that electrical coupling and/or synchronized synaptic release play a role. This suggests that the subthreshold oscillations in presynaptic amacrine cells may also be synchronized, possibly due to inputs from common bipolar cells. It will be of interest to determine whether the oscillatory currents in neighboring starburst amacrine cells are synchronous as well.

Synchronous sub-threshold oscillations distributed among a network of cells, when combined with stimulus-driven inputs, will result in light-evoked synchronous activity as cells will tend to reach spike threshold together. Synchronous activity of retinal neurons, as suggested elsewhere in the CNS, can serve to increase stimulus efficacy, encode additional information and/or bind information about local visual features (reviewed by Singer et al. 1997). It has also been shown that oscillatory activity, possibly propagated via electrical synapses, can distribute signals over long distances, thereby coordinating the activity of large cell networks (Neuenschwander and Singer 1996). Overall, these data suggest that the spontaneous oscillatory activity of inner retinal neurons may serve to organize ensembles of cells that can coordinate via synchronous activity when activated by appropriate visual stimuli.
At the cortical level, it has been posited that synchronized oscillations in cell assemblies act to bind temporal and spatial visual features within the image (Singer and Gray 1995). These rhythmic cortical discharges reflect, at least in part, synchronous oscillations within the LGN (Alonso et al. 1996). Moreover, the temporal precision of the geniculate cell spiking appears to be due to the synchronized oscillatory activity derived from the retina (Neuenschwander and Singer 1996). In this scheme, the widespread, spontaneous oscillatory activity created in the inner retina appears to be the initial step in entraining stimulus-induced rhythmicity conserved throughout the visual system.
GRANTS

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FIGURE LEGENDS

Figure 1. Characteristics of starburst amacrine cells in mouse retina.
A: photomicrograph of a starburst amacrine cell in the mouse retina labeled with biocytin, showing the characteristic dendritic arborization. B: representative current clamp recording from a starburst amacrine cell. Steps of current were injected in the cell for 600 ms and the resulting membrane voltage responses were recorded under whole-cell patch clamp. Between pulses, the cell was maintained at a voltage of −70 mV by constant injection of a small amount of current (indicated by the arrow at the left of the traces). The voltage traces are in response to injection of −100, -50, 0, +50, +200, +300 and +400 pA current pulses. Note the saturation of the membrane depolarization for current pulses greater than +50 pA. The dotted line represents the 0 mV level. C: examples of two variations of oscillatory currents recorded in starburst amacrine cells. Oscillatory currents were recorded in voltage clamp at −70 mV. Nearly half of the cells displayed low amplitude oscillations in control Ringer (left panel) that emerged from the baseline noise and the miniature events. The other half of the cells exhibited larger amplitude oscillations in control Ringer (right panel) that were well distinguishable from the baseline noise and the miniatures events. Both types of oscillations were enhanced by application of low doses of TEA. Holding current was −10 pA (left and right panels).

Figure 2. Effects of TEA on the oscillatory currents. A: the mean oscillatory current frequency is shown for 9 starburst cells in control Ringer and in the presence of 1 mM TEA. Vertical bars represent SEM. The asterisks indicate a statistically significant difference, p<0.0005. B: the average maximal amplitude of oscillatory currents is shown for starburst cells in control Ringer and in the presence of TEA (1 mM). The double asterisks indicate a statistically significant difference, p<0.05. C: all oscillatory current traces acquired during a one minute long recording were averaged for each of the 9
cells. The mean of the 9 average traces was then calculated for control Ringer (representing the mean of a total of 1881 oscillations, black trace) and for TEA (representing the mean of a total of 1557 oscillations, gray trace). Note that TEA increased the amplitude of the average oscillatory current and made its activation phase faster. D: average oscillatory currents were obtained from each of the 9 cells and the kinetics were obtained by fitting the average traces of current. For each of the 9 average currents the activation phase was fitted with a first-order rising exponential. The relaxation phase was fitted with a first-order decay exponential. Correlation factors were > 0.95. Bar graphs comparing the time constants for activation and relaxation of the average oscillatory current in control Ringer and TEA. Note that TEA significantly accelerated the activation phase. The asterisks indicate a statistically significant difference, p<0.001. E: traces of current recorded at –70 mV in a starburst cell are shown for different conditions. The records were obtained from the retina of a Kv3.1-Kv3.2 double knock-out animal. Note that TEA, like in the wild-type, increased the amplitude of oscillations in the knock-out retina. Holding current was +5 pA.

Figure 3. CNQX blocks oscillatory currents in starburst cells. A: current traces recorded at –70 mV in a starburst cell under sequential drug conditions. Note the disappearance of the oscillations as well as the miniature events in the presence of CNQX. Holding current was –10 to 0 pA. B: bar graphs comparing the average frequency of oscillations in different experimental conditions. Vertical bars represent SEM. The asterisk and the double asterisk symbols indicate statistically significant differences, p<0.001. C: bar graphs comparing the average maximal amplitude of oscillatory currents under various drug conditions. Symbols indicate a statistically significant difference, p<0.05.
Figure 4. Blockers of calcium channels inhibit oscillatory currents in starburst cells.  

A: oscillatory currents recorded at −70 mV and sequentially under different experimental conditions. Cadmium totally blocked both basal and TEA-enhanced oscillations. Holding current was +25 to +50 pA.  

B: bar graphs comparing the average frequency of oscillatory currents under the different experimental conditions. Vertical bars represent SEM. The asterisk and the double asterisk symbols indicate statistically significant differences, p<0.01.  

C: bar graphs comparing the average maximal amplitude of oscillatory currents under the various experimental conditions. Vertical bars represent SEM. The + and the ++ symbols indicate statistically significant differences of p<0.05 and p<0.01, respectively. It should be noted that for these data, TEA here did not enhanced the maximum amplitude of oscillations from control values. This is likely due to the fact that TEA was applied sequentially after CdCl₂ wash out. However, CdCl₂ was apparently difficult to washout as evidenced by the continued blockade of basal oscillations and thereby likely reduced the potency of TEA on the oscillations amplitudes.

Figure 5. AP4 blocks oscillatory currents in starburst cells.  

A: current traces recorded at −70 mV in a starburst cell under sequential drug conditions. Note the disappearance of the basal oscillations in the presence of AP4 and the transient effect of AP4 in the presence of TEA. Holding current was 0 to +8 pA.  

B: bar graphs comparing the average frequency of oscillations under different experimental conditions. The label “TEA + AP4 early” refers to the averaged time after AP4 application that the maximal AP4 effect was achieved. The label “TEA + AP4 late” refers to the averaged, earliest time of maximal recovery of the oscillations. Vertical bars represent SEM. The asterisk and the double asterisk symbols indicate statistically significant differences, p<0.01 and p<0.05, respectively.  

C: bar graphs comparing the average maximal amplitude of oscillatory currents under various experimental conditions. Conventions are the same
as in B. The asterisk symbols indicate a statistically significant difference, p<0.01. D: scatterplot of the average frequency and the average maximal amplitude of oscillatory currents of 4 cells as a function of time from the beginning of AP4 application. For each experimental condition, the average time for 4 cells is shown. Error bars represent SEM. Note the transient effect of AP4 in the presence of TEA.

Figure 6. GABA blockers modulate oscillatory currents in starburst cells. A: comparison of the oscillatory currents recorded in control Ringer and after exposure to picrotoxin (PTX). Application of PTX had no effect on the small, basal oscillations. Holding current was +20 pA. B: in contrast, PTX enhanced the oscillatory currents pre-stimulated with TEA. Holding current was +5 to +25 pA. C: the mean of the average currents for 4 cells in TEA (black trace) and in TEA + PTX (gray trace). While PTX increased the average current amplitude, it had little effect on the kinetics. D: bar graphs comparing the effects of PTX on the average frequency (upper panel) and the average maximal amplitude (lower panel) of oscillatory activity. Vertical bars represent SEM. The asterisk and the double asterisk symbols indicate statistically significant differences, p<0.005 and p<0.05 respectively. E: bar graphs comparing the effects of bicuculline (BMI) on the average frequency (upper panel) and the average maximal amplitude (lower panel) of oscillatory potentials. The + and the ++ symbols indicate statistically significant differences, p<0.05. Note that PTX and BMI had very similar effects on oscillation frequency and amplitude.

Figure 7. Oscillatory currents found in various types of cells in the mouse retina. A: typical oscillatory currents recorded at –70 mV from a starburst cell of the mouse retina. Holding current was +58 pA. B: oscillatory currents recorded from a wide-field amacrine cell at –70 mV. Note that the oscillations occur more frequently and have
longer durations than those of starburst cells. Holding current was 0 pA. C: oscillatory currents recorded at –70 mV in a ganglion cell. Oscillations are also wider than those recorded from starburst cells. Holding current was –30 pA. D: all oscillatory current traces during a one minute long recording were averaged and the mean of the average traces was calculated for 9 starburst cells (black trace), 4 ganglion cells (light gray trace) and 2 wide-field amacrine cells (gray trace). The baselines were superimposed in order to compare the average currents. Note the longer duration and slower kinetics of the wide-field amacrine cell oscillation.
Figure 1
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Figure 4
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Figure 7