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Responses of starburst amacrine cells to prosthetic stimulation of the retina

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Abstract— **Recent advances in the design and development of retinal implants have made these devices a promising therapeutic strategy for restoring sight to the blind. Over the last decade a plethora of studies have investigated the responses of the retinal ganglion cells (RGCs) to electrical stimulation under a variety of stimulus configurations. Similar to the RGCs, the amacrine cells also survive in large numbers following retinal neural degeneration. However, with the exception of two previous reports, where the responses of the amacrine cells were measured indirectly, these cells have thus far received little attention in the context of prosthetic stimulation. In this study we focused on the starburst amacrine cells (SACs), a particularly well–characterized amacrine cell among the approximately two–dozen types known to exist in the retina. Using whole–cell patch clamp recordings in the whole–mount rabbit retina, we investigated the temporal responses of the SACs following subretinal biphasic pulse stimulation. These cells responded to the stimuli with oscillatory membrane potentials that lasted for tens to hundreds of milliseconds, with the response amplitude increasing as a function of stimulus strength. Furthermore, the SAC responses originated primarily from the presynaptic inputs they receive, rather than through direct activation of these cells by the electrical stimuli.**

I. Introduction

Retinal implants currently under development aim to restore functional sight to the profoundly blind through electrical stimulation of the surviving neurons after retinal neurodegenerative diseases. Studies in blind human patients have established that such devices could elicit simple percepts [1], [2], [3], [4]. However, much of the neural mechanism underlying the electrically evoked response is not well understood. In the healthy retina the retinal ganglion cells (RGCs) receive up to 70% of their inputs from the amacrine cells [5], and the amacrine cells could outnumber the RGCs by as much as 15 to 1 [6]. Similar to the RGCs, following disease progression these cells continue to survive in large numbers [7]. Despite the overwhelming presence of amacrine cells relative to the RGCs, they have received little attention in the context of retinal prosthetic stimulation. Besides two prior reports [8], [9] demonstrating indirectly, through inhibitory postsynaptic current measurements at the RGCs, that the amacrine cells inhibit RGC responses following electrical stimulation, we know very little about how these cells respond to retinal prosthetic stimulation.

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There are two–dozen morphologically distinct types of amacrine cells [5]. With few exceptions, the functional roles of most amacrine cells have yet to be characterized. To circumvent this shortcoming, we focused on a particularly well–studied type – the displaced starburst amacrine cells (SACs). These cells are present in the ganglion cell layer in large numbers. They could generally be distinguished by the smaller somatic size relative to the RGCs. Using whole–cell patch clamp recording we investigated the temporal responses of the SACs to subretinal biphasic pulse stimulation under a variety of pulse configurations.

II. Methods

All procedures were approved and monitored by the University of NSW Animal Care and Ethic Committee. The retinal preparation procedure has been described in detail previously [10]. Briefly, NZ White rabbits weighing 2.0 – 2.5 kg were deeply anesthetized with ketamine (70 mg/kg) and xylazine (10 mg/kg). After enucleating the eyes, the animals were immediately euthanized with an overdose of pentobarbital. The eyes were hemisected, vitreous cleared, the inferior retina inclusive of the visual streak dissected, and kept in a holding chamber in darkness for periods ranging 1 – 10 hours prior to recording. The holding chamber contained Ames' Medium (Sigma Aldrich) supplemented with 1% (V/V) Penicillin/Streptomycin (Invitrogen), and aerated with 95% O_2 + 5% CO_2. Prior to recording, a small piece of the retina was separated from the underlying pigment epithelium and sclera then transferred photoreceptor–side down into an imaging chamber sitting on a fixed stage microscope.

Whole–cell current clamp recordings were made on the displaced starburst amacrine cells with patch electrodes of resistances 3.0 – 5.5 $M\Omega$. We filled the electrodes with (mM): 120 $KMeSO_4$, 10 KCl, 0.008 $CaCl_2$, 0.5 EGTA, 1 $MgCl_2$, 10 HEPES, 4 $ATP - Na_2$, and 0.5 $GTP - Na_3$, adjusted to pH 7.2 with KOH. In every case morphological identification of the recorded cells were made with epi–fluorescent imaging of Alexa Fluor 488 (Invitrogen) supplemented in the pipette solution. Recordings were performed in aerated Ames' Medium heated to 34 – 35 $^\circ C$ and perfused at 4 – 5 mL/min. Series resistance was compensated with the bridge–balance circuit on a Multiclamp 700B amplifier (Molecular Devices). Data were low–pass filtered at 10 kHz at the amplifier output and digitized at 50 kHz on a computer running pClamp 10 (Molecular Devices) connected to a Digidata 1440A data acquisition system (Molecular Devices). A liquid junction potential of 5 mV has been corrected for all results.

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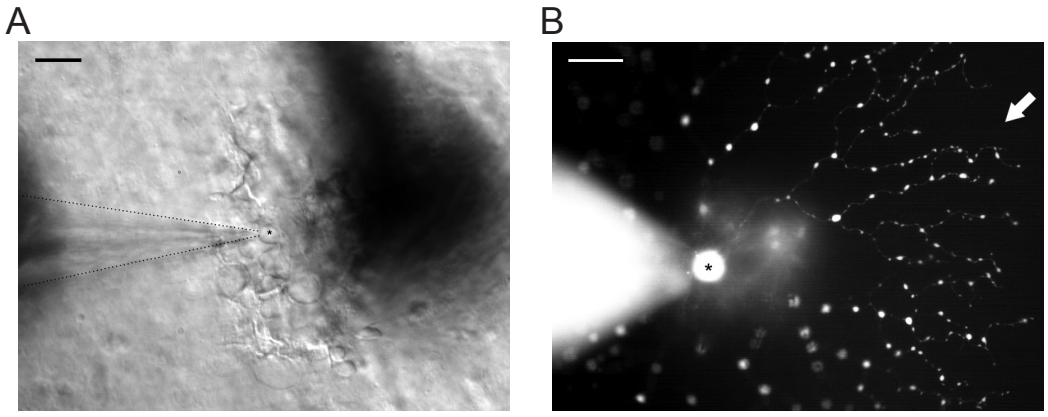

Fig. 1. Whole–cell patch clamp recording and electrical stimulation of a starburst amacrine cell (SAC). A. Responses of the cell (star) was recorded with whole–cell current clamp. The recording pipette is marked by the dashed lines. Note the shadows casted by the subretinal stimulation electrodes. B. The morphology of the SAC is characteristically distinct, with radially branching dendrites containing large varicosities at the distal end (arrow). Scale bar = 20 μm.

Synaptic inputs were blocked with a combination of drugs. The AMPA/kainate, NMDA, mGluR6, GABAergic, and glycinergic receptors were blocked with (in mM): 0.075 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 0.06 (+)-MK 801 hydrogen maleate (MK-801), 0.02 L-(+)-2-Amino-4-phosphonobutyric acid (L-AP4), 0.1 picrotoxin (pic), and 0.01 strychnine (stry), respectively. All pharmacological agents were from Tocris Bioscience or Sigma Aldrich.

The retinas were stimulated subretinally (at the photoreceptor–side) with a single electrode in a multielectrode array (MEA; Ayanda Biosystems) embedded within the imaging chamber base. The MEA contained 60 identical Pt electrodes with dimension 40 x 40 μm arranged in a square grid, with 200 μm center–to–center distance between adjacent electrodes. Eight electrodes connected in parallel at the perimeter of the MEA were used as the stimulus return. Electrical stimuli consisted of cathodic–first, charge–balanced, constant–current, rectangular biphasic pulses without inter–pulse delay. The pulse amplitude and width were defined as the height and duration, respectively, for one phase of the biphasic pulse.

We determined the cells' light responses by focusing a circular light spot with 160 μm diameter onto the photoreceptor layer. The spot was centered over the soma of the target cell. Experiments were performed under mesopic ambient lighting (approximately 7 $\mathrm{cd.sr/m^2}$). The cells were visualized with near–IR illumination ($\geq$ 820 nm) to prevent photoreceptor bleaching.

III. Results

A. Targeting and recording from the SACs

We recorded the responses of the displaced SACs following subretinal stimulation with whole-cell current clamp in the whole–mount retina (Fig 1A). We found, with experience, SACs could be targeted with a high success rate ($>$ 80%) by bright field microscopy alone. However, in every case we also morphologically ascertained the identity of the recorded cells with epi–fluorescent imaging. After 20 – 30 minutes of whole–cell recording the fluorescent dye (Alexa Fluor 488) within the recording pipette diffused throughout the cell, revealing the characteristic "starburst" morphology (Fig 1B). Radiating from the soma (star) were progressively branching dendrites, with the distal end containing numerous varicosities (arrow), where the synaptic outputs of the SACs are believed to occur [11].

Consistent with the previously reported light–evoked responses of the displaced SACs [11], [12], these cells (n = 12) responded to a 1 s stationary light spot with a transient depolarization followed by a plateau for the duration of the stimulus (Fig 2). At stimulus offset, the cells responded with a transient hyperpolarizing overshoot before returning to baseline.

B. SAC temporal responses to subretinal electrical stimulation

We investigated the temporal responses of the SACs (n = 12) to a variety of stimulus configurations. The SACs responded to the subretinal stimuli with an oscillatory trans–membrane potential (Vm) over tens to hundreds of milliseconds. Fig 3A illustrates the Vm responses of a representative SAC to a 0.1 ms biphasic pulse. For each pulse amplitude (0 – 100 μA) 20 repetitions are shown in gray. The black

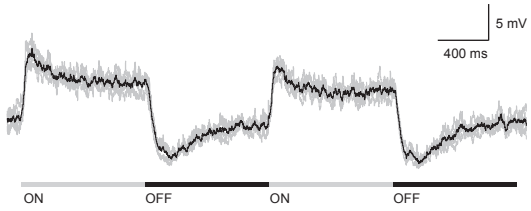

Fig. 2. Light response of a SAC to a stationary light spot with sequence: ON–OFF–ON–OFF. The stimulus was repeated five times (superimposed gray traces). The black trace represents the averaged responses.

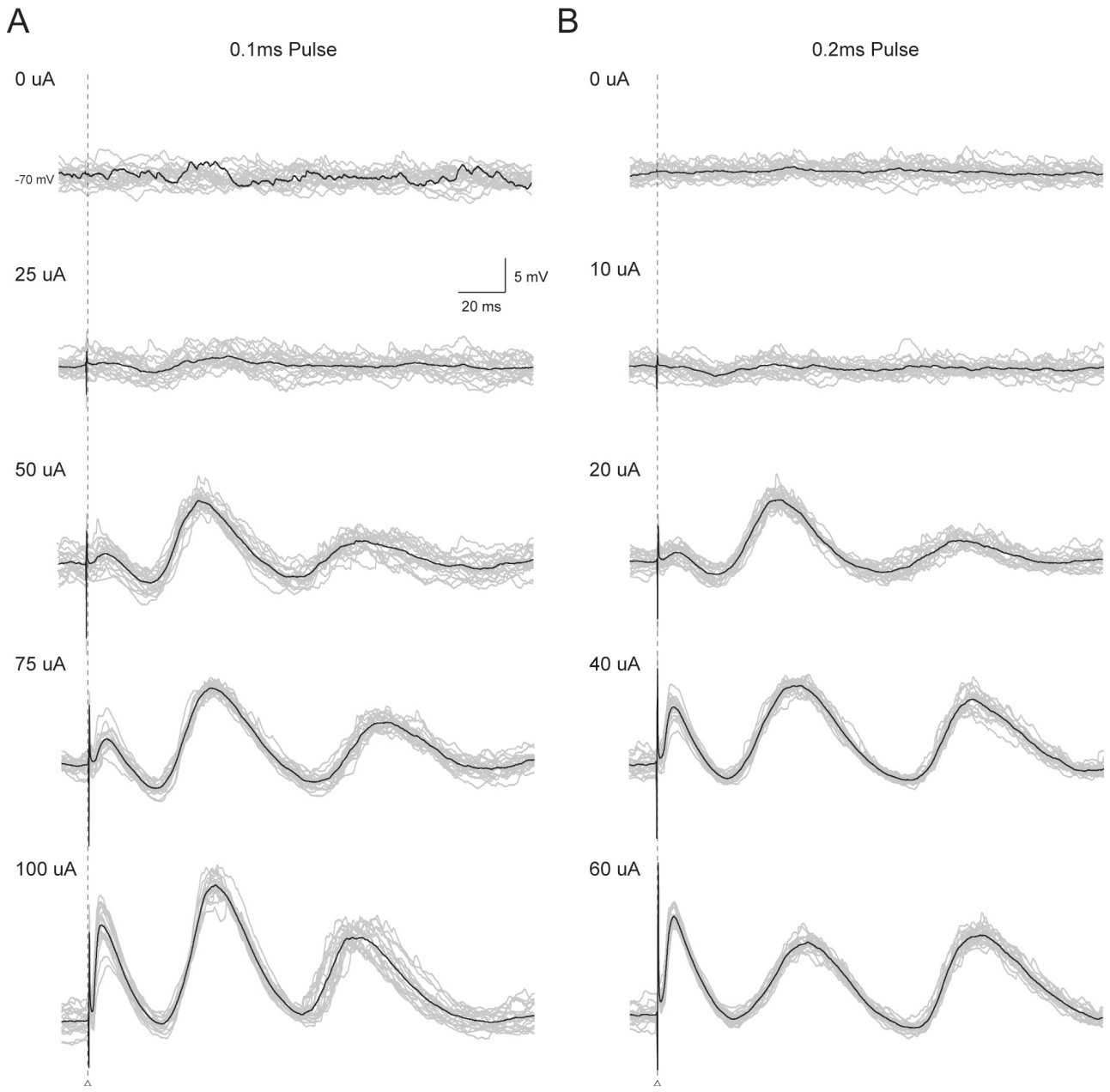

Fig. 3. Temporal responses of a SAC following subretinal biphasic pulse stimulation. With increasing stimulus strength the cell responded with oscillatory membrane potential of increasing magnitude. Individual trials (20 repetitions) and the average responses are shown in gray and black, respectively. A. 0.1 ms biphasic pulse. B. 0.2 ms biphasic pulse.

traces indicate the average across the repetitions. The peak magnitude for each phase of the Vm oscillation increased with increasing stimulus strength. We next tested longer stimulus pulses on these cells. When stimulated with 0.2 ms biphasic pulses (Fig 3B, same cell as 3A), the cell similarly exhibited oscillatory Vm of increasing amplitude as the stimulus strength was increased. Similar to the 0.1 ms pulse configuration, the oscillation could also last up to several hundreds of milliseconds. Finally, and importantly, we never observed electrically evoked spiking responses in the SACs.

C. Origin of SAC electrically evoked responses

Because of the extended duration of the SAC electrically evoked responses and the lack of spiking responses, we next examined the origin of the oscillatory Vm responses. In particular, we asked to what extent does the cells presynaptic to the SACs contribute to their responses, and how much of the responses were due to direct activation of voltage–gated currents in these cells?

Using identical stimulus configurations, we compared the SAC Vm under the control condition and with presynaptic input blocking using a mixture of presynaptic input blockers

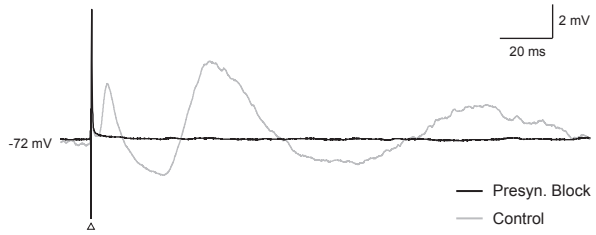

Fig. 4. Blocking presynaptic inputs eliminated all SAC responses to electrical stimulation. Both traces are average of ten repetitions.

(CNQX + MK-801 + L-AP4 + pic + stry). As demonstrated for one cell in Fig 4, blocking the presynaptic inputs completely eliminated the electrically evoked responses in every cell examined (n = 5). Similar observations were made when perfusing the retina with $CdCl_2$ (250 μM, n = 2), a non–selective calcium channel blocker, which also prevents synaptic vesicle release. These results suggest that the SACs derive most, if not all, of their electrically evoked responses from their presynaptic partners. Thus the electrical stimuli do not directly recruit these cells to any significant extent, at least with the single subretinally delivered cathodic–first biphasic pulses used here.

IV. Discussion

In this report we studied the responses of SACs following subretinal electrical stimulation with cathodic–first biphasic stimulus. The SACs responded to a single pulse with oscillatory membrane potential of increasing magnitude as the stimulus strength was increased. We further found the SACs to derive most of their electrically evoked responses from their presynaptic inputs, rather than through direct activation.

Consistent with previous studies [12], [13], [14], we did not observe spiking responses in these cells upon light stimulation. Following maturation in the developing retina, the SACs convert from spiking to non–spiking neurons by down-regulation of intrinsic excitability and an increase in network inhibition [12], [13]. This switch to passive responses likely also underlies the lack of direct electrically evoked responses in these cells following biphasic pulse stimulation. The SACs are not the only non–spiking neurons in the adult retina. However, unlike the other passive neurons, the SACs are easily amenable to investigations in the whole–mount retina, due to their presence in the ganglion cell layer, rather than deep within the retina. Therefore the SACs may be a good candidates for studying the dichotomy between passively responding neurons and the active cells, such as the RGCs, following electrical stimulation.

Amacrine cells are morphologically diverse [15]. Thus these cells likely subserve a similarly wide variety of functional roles [16]. The SACs constitute only one of the two dozen or so types of amacrine cells currently known to exists in the mammalian retina [5], [17]. This study analyzed the responses of one identified type of amacrine cells (the SACs). The soma of all other amacrine cells is located in the inner nuclear layer. It remains to be investigated whether this difference in somatic location, and if and how the differences in synaptic connectivity, and potentially also variations in intrinsic properties, affect the electrically evoked responses of the other amacrine cells comparing to the SACs.

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