Differential effect of brief electrical stimulation on voltage-gated potassium channels

Cameron MA, Al Abed A, Buskila Y, Dokos S, Lovell NH, Morley JW. Differential effect of brief electrical stimulation on voltage-gated potassium channels. J Neurophysiol 117: 2014–2024, 2017. First published February 15, 2017; doi:10.1152/jn.00915.2016.—Electrical stimulation of neuronal tissue is a promising strategy to treat a variety of neurological disorders. The mechanism of neuronal activation by external electrical stimulation is governed by voltage-gated ion channels. This stimulus, typically brief in nature, leads to membrane potential depolarization, which increases ion flow across the membrane by increasing the open probability of these voltage-gated channels. In spiking neurons, it is activation of voltage-gated sodium channels (NaV channels) that leads to action potential generation. However, several other types of voltage-gated channels are expressed that also respond to electrical stimulation. In this study, we examine the response of voltage-gated potassium channels (KV channels) to brief electrical stimulation by whole cell patch-clamp electrophysiology and computational modeling. We show that nonspiking amacrine neurons of the retina exhibit a large variety of responses to stimulation, driven by different KV-channel subtypes. Computational modeling reveals substantial differences in the response of specific KV-channel subtypes that is altered, transiently depolarizing the cell and giving rise to activation of voltage-gated ion channels (for review, see Ye and Steiger 2015).

In the majority of spiking neurons, voltage-activated sodium (NaV) channels are the primary target of electrical stimulation (Fried et al. 2009). Activation of these channels causes an influx of Na+ ions, depolarizing the neuron, and increasing the probability that an action potential will occur (Durand 1995; Hodgkin and Huxley 1952). However, NaV channels also respond to extracellular voltage changes, while producing the opposite effect, whereby activation allows the flow of K+ ions out of the cell (Cameron et al. 2013; Hodgkin and Huxley 1952), causing hyperpolarization of the neuron. During a synaptically evoked action potential, the activation of KV channels is delayed with respect to activation of NaV channels (Hodgkin and Huxley 1952). The membrane begins to depolarize due to influx of cations via ligand-gated ion channels, activating NaV channels and causing a substantial flow of Na+ ions into the cell. Concurrently, KV channels are activated, but, as they exhibit much slower kinetics at these membrane potentials, substantial outward K+ flow generally occurs after the NaV channels are fully activated, allowing repolarization of the membrane. Conversely, when considering external electrical stimulation, since the change in extracellular potential is usually so brief, the amplitude of the pulse must be very high,
meaning the magnitude of the change in transmembrane potential is very large (Irnich 1980). This likely changes the pattern of activation of these voltage-gated channels. We have previously shown, in amacrine cells of the retina, that the balance of activation between Kᵥ channels and Naᵥ channels can alter the polarity of the response with the Kᵥ channel involvement prevailing at high stimulation intensities (Cameron et al. 2013). Although many studies address the geometrical distribution of transmembrane potentials, and the effectiveness of different stimulus strategies in the retina (Bina-nagrov et al. 2014; Freeman et al. 2010; Fried et al. 2006; Habib et al. 2013; Jensen et al. 2003), we are not aware of any that have examined the response of voltage-gated Kᵥ channels to brief electrical stimulation, and the mechanism underlying their activation.

A large obstacle in electrical stimulation of neuronal tissue is achieving high-frequency responses (Tsi et al. 2009, 2011). Reliable action potential generation in retinal ganglion cells (RGCs) has only been produced up to 50 Hz, whereas action potential firing at over 200 Hz can be elicited by natural stimuli via synaptic input (Baccus 2007; Meister and Berry 1999). We postulate that simultaneous activation of Naᵥ and Kᵥ channels underlies these issues, with Kᵥ channels potentially limiting the excitability of target neurons. Additionally, the specific kinetics of each Kᵥ channel likely plays a large role in the response to stimulation. Certainly, in comparison to Naᵥ channels, Kᵥ channels show a wide variety of activation and inactivation time courses (Gutman et al. 2005). If certain channel classes could be implicated in inhibiting action potential generation in spiking neurons, there is a potential to pharmacologically block these channels to improve the efficacy of electrical stimulation.

Furthermore, a large percentage of mammalian neurons are non-spiking, including hair cells of the auditory and vestibular system, periglomerular cells of the olfactory bulb (Bufler et al. 1992), and the majority of retinal neurons (Baden et al. 2013). Consequently, the effect of electrical stimulation on nonspiking cells impacts primarily on the most highly expressed voltage-gated channels, the Kᵥ channels. Retinal neurons, which we investigate here, with the exception of RGCs, exhibit a minimal expression of Naᵥ channels in comparison to spiking neurons. Many amacrine cell types (Bloomfield and Völgyi 2007; Cohen 2001; Taylor 1996; Wässe et al. 1993), horizontal cells (Mojumder et al. 2007; Ueda et al., 1992), and bipolar cell types (Cui and Pan 2008; Ichinose et al. 2005; Pan and Hu 2000) do express Naᵥ channels, but the predominant voltage-activated current is K⁺ (Pinto and Klumpp 1998). Although many studies acknowledge the input of presynaptic cells to the RGC response, few studies have directly examined the influence of electrical stimulation on these cells. With the placement of human electrical retinal prostheses subretinally (Zrenner et al. 2011), or supratheroidally (Ayton et al. 2014), the activation of preganglionic cells is inevitable. With a better understanding of the mechanism of electrical stimulation in these nonspiking neurons, it may be possible to stimulate the retina in a more sophisticated way, using the existing circuitry for processing.

We have previously reported that nonspiking retinal neurons frequently show membrane hyperpolarization following external electrical stimulation (Cameron et al. 2013). This response can be blocked by the K⁺ channel blocker tetraethylammonium (TEA), suggesting it reflects the activation of Kᵥ channels. In this paper we further investigate the currents elicited by external electrical stimulation of amacrine cells using whole cell voltage-clamp electrophysiology and computational modeling. We find that electrical stimulation does indeed activate Kᵥ channels, with a few specific channel types that display unique temporal activation characteristics particularly sensitive to brief electrical stimulation.

METHODS
Animals. All procedures involving animals were performed in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes and were approved and monitored by the Western Sydney University Animal Care and Ethics Committee (Project numbers: A8966 and A10396). Wild-type C57BL/6J mice were purchased from the Animal Resources Centre (ARC, Canning Vale, Australia). Mice were bred on site, and only offspring (both male and female) >60 days were used. Animals were maintained under a 12:12-h light/dark cycle at ~300 lx illumination during the daytime. Retinae were excised, and all recordings taken, during the animal’s subjective day.

Tissue preparation. All tissue was prepared under normal laboratory lighting conditions. Animals were euthanized using cervical dislocation, eyes enucleated, cut along the ora serrata, and placed in artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 25 NaHCO₃, 3 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, and 10 glucose at room temperature within 1 min of euthanasia. The retinal slice preparation procedure was adapted from Arman and Sampath (2010). Briefly, retinæ were isolated from the surrounding eye tissue and placed in ~40°C low-melt agarose (Sigma-Aldrich, Australia) dissolved in HEPES buffered ACSF containing (in mM) 140 NaCl, 10 HEPES, 3 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, and 10 glucose. The agarose block was then submerged in ice-cold ACSF where it quickly solidified. A cube of agarose around the retina was then cut with a scalpel blade and glued with cyanoacrylate onto the cutting stage of a vibrating microtome (model 7000srm, Campden Instruments). The cutting chamber was filled with ACSF at room temperature, and 200-µm sections were taken from the entire retina (the extreme periphery was excluded). Sections were transferred to a Braincubator (PAYO Scientific, Sydney, AU) containing ACSF bubbled with 5% CO₂-95% O₂ (carbogen) at room temperature as previously described (Cameron et al. 2016). As needed, sections were then transferred to the recording chamber where they were infused with carbogen, bubbled ACSF at a rate of ~5–6 ml/min. A tissue anchor made of platinum with nylon threads (~500 µm apart) was used to keep the tissue in place.

Electrophysiology. Whole cell current- and voltage-clamp recordings were made from cells of the inner nuclear layer (INL) at room temperature, with patch electrodes of resistances 5.0 – 8.0 MΩ. Electrodes were filled with (in mM) 120 KMeSO₄, 10 KCl, 0.008 CaCl₂, 0.5 EGTA, 1 MgCl₂, 10 HEPES, 4 ATP-Na₂, and 0.5 GTP-Na₃, adjusted to pH 7.2 with KOH. In every case, morphological identification of the recorded cells was made with epifluorescent imaging of Alexa Fluor 488 Hydraz (70 µM, Invitrogen) included in the pipette solution. The series resistance (Rₛ) was monitored throughout the experiments and was in the range of 10–30 MΩ. Errors associated with Rₛ in voltage-clamp mode were compensated by 60–80% at 5- to 7-kHz bandwidth, using Rₛ compensation on a Multiclamp 700B amplifier (Molecular Devices). In current-clamp mode, the bridge was adjusted accordingly. 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 10 mV was corrected after recording. Pharmacological agents were dissolved in ACSF and used at the concentrations described in the results. All experiments were carried out under synaptic
block: 10 μM CNQX, 50 μM AP-5, 50 μM L-AP-4, 10 μM strychnine, and 100 μM picrotoxin.

Electrical stimulation. The retinal slices were stimulated with a 50-μm-diameter circular platinum (Pt) electrode, coated with 25-μm-thick Teflon (100-μm total diameter; AM Systems) placed behind the photoreceptor layer (wild-type) in a pseudo-“subretinal” configuration (Fig. 1). A large (120 μm × 3 mm) Pt wire, submerged in the extracellular fluid, ~1 cm from the retina, was used as the stimulus return. Electrical stimuli consisted of anodic-first, charge-balanced, constant-current, rectangular biphasic pulses of 100–200 μs per phase, without interphase delay.

Analysis. Analysis of electrophysiological traces was completed using Axograph software (Sydney, Australia) and current-voltage (I-V) relationships plotted using GraphPad Prism (San Diego, CA) software. Responding cells were classified as any cell exhibiting a stimulus-locked response to electrical stimulation. A paired Student’s t-test was utilized to compare means at 0.1 μM.

In addition, we conducted in silico voltage-clamp experiments on a number of Kv-channel subtypes. Formulations for the Kv1.3 (Douglass et al. 1990), Kv1.6 (Grupe et al. 1990), Kv2.2 (Schmalz et al. 1998), Kv3.1 (Kanemasa et al. 1995), Kv3.2 (Hernández-Pineda et al. 1999), and Kv3.3 (Desai et al. 2008) currents were obtained from Channelpedia (http://channelpedia.epfl.ch). To allow for a standardized comparison, maximal conductance was normalized to 10 mS/cm² and reversal potential to −95 mV. Models were implemented in Matlab (MathWorks) and solved using the ODE15s adaptive time stepping routine with a maximum step of 20 μs, relative error tolerance 10⁻⁶, and absolute error tolerance 10⁻⁵. Presented model outputs were sampled at 50 kHz, except at baseline holding potentials where a lower sampling rate of 1 kHz was used.

RESULTS

We sought to further describe the impact of very brief membrane depolarizations, both internal and external, on Kv-channel currents that cause the membrane hyperpolarization observed in current clamp (Cameron et al. 2013). Cells from the inner nuclear layer were recorded in retinal slices from C57BL/6 mice via whole cell patch clamp. A 50-μm platinum electrode was placed in the photoreceptor layer (pseudo-“subretinal” configuration) to mirror our previous studies as depicted in Fig. 1A (Cameron et al. 2013; Habib et al. 2013). Under conditions of synaptic block to exclude presynaptic inputs, brief electrical stimulation elicited intrinsic responses in 75% (32/43) of inner nuclear layer (INL) neurons. Anodic-first
biphasic pulses were applied for the data presented here, but cathodic-first biphasic pulses also elicited a similar pattern of response (data not shown). Amacrine cells were most likely to show a large-amplitude response, so we focused our attention on cell bodies situated near the border of the INL and inner plexiform layer (IPL). Current- and voltage-clamp data were recorded, and stimulation reliably elicited a hyperpolarizing response in current clamp (Fig. 1B), and an outward current in voltage clamp (Fig. 1C) which were both blocked by 30 mM TEA. This led us to hypothesize that both responses are driven by Kv channels.

We further investigated the origin of this outward current in an attempt to describe both the mechanism and the principal channels involved. We used a voltage-clamp protocol to determine the predominant ion involved in the current (Margalit and Thoreson 2006). We also compared the effect of delivering the stimulus intra- or extracellularly. Long voltage steps were applied to the patched cell from $-95\text{mV}$ to $15\text{mV}$ in $10\text{mV}$ increments for $4\text{s}$. After $3\text{s}$, during the voltage step, cells were treated with either a brief ($100–200\mu\text{s}$ biphasic) external stimulus as described above, or a brief ($100\mu\text{s}$) internal depolarizing stimulus, applied through the patch pipette. Intensity of the stimulus was adjusted for each cell, such that a response could be recorded. This varied greatly between cell types and ranged from 0.8 to 4 $\text{mC/cm}^2$, for the external stimulus, and from 150 to 300 $\text{mV}$, for the internal stimulus. Figure 2 compares the responses to these two stimuli; large outward currents were elicited by the series of voltage steps (Fig. 2A). Brief external electrical stimulation after $3\text{s}$ produced a transient outward current (as seen in Fig. 2A, box) that peaked in amplitude when the cell was held at $-29.2 \pm 0.06\text{mV}$. Similarly, brief depolarization of the cell via the patch pipette, superimposed on the existing voltage steps, produced the same pattern of transient outward currents as elicited by the external stimulus (Fig. 2C). This indicates that whether the cell is depolarized from the outside, or from the inside, does not affect the Kv-channel response pattern. Measurement of this response was conducted for both external and internal stimulation to compare the I-V curve 50 ms immediately before the stimulus (Fig. 2, D and E, black line), and at the peak of the transient outward current (Fig. 2, D and E). The resulting I-V curves demonstrate that brief electrical stimulation (either external or internal) causes a shift in the I-V curve to more negative potentials, but that this shift is restricted to holding potentials between $-60$ and $-20\text{mV}$.

We were also able to reproduce this shift in the I-V curve using the single-compartment ON-OFF amacrine cell model (Miller et al. 2006) incorporating somatic values for ion channel maximal conductance (Royer and Miller 2007). The cell was voltage clamped from a holding potential of $-65\text{mV}$ for 4 s and stepped from $-95\text{mV}$ to $15\text{mV}$ in $10\text{mV}$ steps. Three seconds after the onset of each voltage step, a $100\mu\text{s}$- $400\text{mV}$ internal step was applied to mimic external stimulus conditions (Fig. 2F). I-V curves were constructed by sampling responses 50 ms before and 0.5 ms after the onset of the internal step. Although the same shift was observed, the restriction of this response between $-60\text{mV}$ and $-20\text{mV}$ was not as apparent as in the in vitro data. The model suggests that the delayed

Fig. 2. External electrical stimulation (1.6 mC/cm$^2$) superimposed on voltage steps elicits transient outward currents immediately following stimulation, A, box: B and D. Quantification of these currents (I-V plots) reveals that they are more prevalent between $-60$ and $-20\text{mV}$ holding potentials, and cause a leftward shift in the I-V curve ($n = 5; P < 0.05$). C and E: a brief (0.1 ms) internal depolarizing step applied to recreate membrane depolarization caused by external stimulation elicits the same pattern of outward currents immediately following stimulation ($n = 12; P < 0.001$). F: the leftward shift in I-V curve following an internal stimulus could be replicated in an amacrine cell computational model. G–I: when specific classes of K$^+$ currents were examined in detail, contributions from the delayed rectifier, A-type, and Ca$^{2+}$-activated K$^+$ currents were observed.

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rectified K$^+$ current is the main contributor to the I-V curve shift, with lesser influences by the transient outward and Ca$^{2+}$-activated K$^+$ currents (Fig. 2, G–I), although this may reflect the relative expressions of each of these current types represented in this specific model. The dissimilarity in the expression levels of ionic channels between amphibians, which the model is based upon, and rodents is a likely cause of the difference between the main current contributing to the shift in I-V curve predicted by the mathematical model and suggested by electrophysiological recordings.

We hypothesized that the shift in the I-V curve reflects the transient positive shift in the membrane potential, caused by either the external environment surrounding the cell becoming more negative, by external electrical stimulation, or the internal environment of the cell becoming more positive, by transient depolarization of the cell via the patch pipette (see Fig. 7 for summary). However, this does not fully explain the restriction of this phenomenon between $-60$ and $-20$ mV. If the membrane potential is shifted by the brief stimulus, it would be expected that the curve would show a global leftward shift, as seen for the “delayed rectifier” current in the simulated data (Fig. 2G). However, this shift is restricted to negative potentials for A-type and Ca$^{2+}$-activated K$^+$ currents (Fig. 2, H and I). We explored the idea that inactivation of specific Kv channels could play a role in the restriction of this response, and considered that, contrary to the amacrine cell model presented above, these channels play a predominant role in the response in vitro. In the protocol described in Fig. 2, the transient electrical stimulus was applied 3 s after a voltage step was applied. As can be clearly seen from Fig. 2A, large outward currents, elicited by the long depolarizing steps, reduce in conductance over time (downward slope over the 4 s), demonstrating that they are inactivating. Therefore, if the response to the brief stimuli results from Kv channels that inactivate, at more depolarized potentials, more of these channels will be inactive by the time the brief stimulus is applied 3 s later. However, due to the fact that voltage-steps are applied from $-95$ to $+15$ mV in this protocol, we were able to observe the response of these channels before they were inactivated. This could explain why the amplitude of the electrically evoked response (at 3 s) is reduced when the cell is held above $-30$ mV, as a higher proportion of Kv channels are inactivated.

To directly assess the influence of a brief stimulus on non-inactivated channels, we applied a “tail current” protocol to compare a brief pulse (100 μs) to a 100-ms pulse. The rationale for using this protocol was to compare the effect of a very brief depolarizing stimulus to a longer duration depolarizing stimulus from a hyperpolarized condition where all channels will be available for activation. Additionally, since the brief electrical stimulus is over well before the current in Fig. 2, B and C, is recorded, it is likely that it represents the residual flow of ions through channels as they deactivate, i.e., a “tail current”. Figure 3 compares the tail currents elicited by a 100-ms depolarization to a 0.1-ms depolarization (magnitude of depolarization was adjusted until currents were apparent in both conditions). Tail currents were similar under both conditions; however, the kinetics and reversal potential varied. Tail currents from the 100-ms pulse decayed slower, indicating the involvement of a slow activating Kv channel, whereas the tail currents elicited by the brief pulse decayed quickly, suggesting the involvement of a fast(er) activating Kv channel. We observed differences in reversal potential between the two pulse lengths with the brief pulse giving a more depolarized reversal potential (approximately $-80$ vs. $-95$ mV; $P < 0.001$); this likely reflects the influence of other ions, small Na$^+$ and calcium (Ca$^{2+}$) currents, that are inactivated by the end of the 100-ms pulse.

TEA is known to block the majority of delayed-rectifier K$^+$ currents; however, at 30 mM it blocks a multitude of channel subtypes that possess various activation and inactivation characteristics (Gutman et al. 2005). To tease out which channels...
are involved, we applied the transient “A-type” K⁺ current blocker 4-aminopyrine (4-AP; 5 mM) to the I-V protocol (Fig. 4). Both the transient outward current evoked by the long voltage steps (Fig. 4A, black trace), and the brief stimulus-evoked current, were completely abolished by the 4-AP (Fig. 4, B and C; n = 4). These data suggest that the majority of the brief stimulus-evoked current derives from Kᵥ channels that can be blocked by both TEA (30 mM) and 4-AP (5 mM).

Considering the results from the mathematical modeling (Fig. 2), we used the Kᵥ-channel-specific blockers apamin (100 nM) and charybdotoxin (20 nm) to assess the influence of these channels. The brief stimulus-evoked current was partially blocked, in both voltage and current clamp, by the Kᵥ-channel blockers (Fig. 4, D and E; n = 5), indicating that these channels indeed play a role in the response. Complete block with TEA (30 mM) is shown for comparison (Fig. 4, D and E).

These data led us to the conclusion that more than one subtype of Kᵥ channel contributes to the hyperpolarizing response caused by brief electrical stimulation.

We returned our focus to computational modeling to determine which Kᵥ-channel subtypes are likely to be responsible for the largest responses to electrical stimulation. Our pharmacological data indicates that channels blocked by both TEA and 4-AP must have the greatest contribution to the response. From the literature, six Kᵥ-channel subtypes are blocked by both TEA (30 mM) and 4-AP (5 mM): Kᵥ1.3, Kᵥ1.6, Kᵥ2.2, Kᵥ3.1, Kᵥ3.2, and Kᵥ3.3 (Gutman et al. 2005). Using computational modeling we assessed the impact of brief internal stimulation on each of these channel subtypes (Fig. 5).

Channels were mathematically described using Hodgkin-Huxley type kinetics. Formulations for activation and inactivation gates of each channel [Kᵥ1.3 (Douglass et al. 1990), Kᵥ1.6 (Grupe et al. 1990), Kᵥ2.2 (Schmalz et al. 1998), Kᵥ3.1 (Kanemasa et al. 1995), Kᵥ3.2 (Hernández-Pineda et al. 1999), Kᵥ3.3 (Charybdoendoxin, 20 nm)] were used to assess the impact of brief internal stimulation on each of these channel subtypes (Fig. 5).
KV3.3 (Desai et al. 2008)] were obtained from Channelpedia. The protocol of Fig. 2F was replicated for each individual channel subtype. We were surprised to observe large differences in response between subtypes, with no response from KV2.2 and KV3.2, and only a small response from KV1.3. The largest responses were observed in KV1.6 and KV3.1, with KV3.3 showing a small, but very noticeable response (Fig. 5). As both the maximum conductance per unit area and reversal potential were standardized for all channels, from these data we can conclude that channel activation and inactivation kinetics are a strong predictor of response to a brief stimulus.

Furthermore, our in vitro recordings from individual cells show similar response characteristics to those seen in the modeling data for KV1.6, KV3.1, and KV3.3 (Fig. 6), which have been reported to be expressed in the retina (Hölter et al. 2007; Klumpp et al. 1995; Pinto and Klumpp 1998). This suggests the activation of retinal neurons is linked to KV-channel expression, and the relative expression of a certain kind of KV channel has the potential to shape the response of the cell to electrical stimulation.

**DISCUSSION**

We have characterized the response of KV channels in retinal neurons, to external electrical stimulation, showing that they produce a hyperpolarizing response in current clamp and an outward current in voltage clamp. Although we examined this response at relatively small magnitudes in this publication, we have previously shown a large influence of the KV-channel response on membrane potential in amacrine cells at higher stimulation intensities (Cameron et al. 2013). Here we focus on
the pattern of response to elucidate the mechanisms behind activation of these channels.

The pattern of response to brief stimuli is the same, irrespective of depolarization occurring via external or internal stimulation. This suggests that the reduction in transmembrane potential is caused by the extracellular environment of the cell becoming more negative leading to activation of voltage-gated channels (we present a summary of this proposed mechanism in Fig. 7). We therefore consider brief internal depolarization of the cell a good model to investigate the effects of rapid electrical stimulation on voltage-gated ion channel response. We suggest that the differences in intrinsic electrical activation/inactivation properties of neurons is greatly influenced by the relative expression of various Kv-channel types (Gutman et al. 2005).

Our data have highlighted the range of responses that can be elicited by different classes of Kv channels. Although this response was observed in the majority of INL cells, the sensitivity of this outward current varied greatly between cell types. This was most apparent when using the internal depolarization protocol, which allowed a more accurate comparison of response sensitivity. External electrical stimulation, especially in neuronal slices, is subject to many variables that contribute to the probability of activating voltage-gated channels, such as electrode placement, cell orientation, extracellular environment, and placement of the return electrode. However, internal stimulation is not affected by these variables, although it will be altered by the input resistance and channel expression of the cell. We observed a large variation in response to internal stimulation with some very sensitive cells responding to a 50-mV, 100-μs depolarizing pulse and some only having a small response to a 300-mV, 100-μs pulse. Our experimental data and modeling results suggest that dominance of specific channel types drives the response in these cells.

As Hodgkin and Huxley famously reported, in the physiological ranges of cell membrane potentials, NaV-channel kinetics are significantly faster than Kv channels, meaning the flow of K+ ions is delayed with respect to Na+ ions (Hodgkin and Huxley 1952). This is thought to be a result of hydrophilic Ser or Thr residues in the S2 and S4 segment of the voltage sensors in domains I–III, which speeds the NaV-channel kinetics by up to 3-fold (Lacroix et al. 2013). However, the Kv channel reported in the original Hodgkin and Huxley models is a relatively slow rectifying channel, and several other Kv channels show much faster activation kinetics (Gutman et al. 2005). Furthermore, Hodgkin and Huxley-based computational mod-
hyperpolarization of these cells via activation of KV channels inhibit spiking. However, given the inhibitory nature of most BK-channel blocker, charybdotoxin, the SK channel blocker, apamin, alone (data not shown), with served a reduction in the evoked current when blocking with BK and SK Ca\(^{2+}\) channel subtypes that are of most interest are KCa channels as specific pulse duration and amplitude depends on a balance more probable. Therefore, we suggest the effectiveness of a non-voltage-gated Ca\(^{2+}\) channel, or stimulated release of Ca\(^{2+}\), from intracellular stores (Adelman et al. 2012). We do not see any evidence for a large-amplitude inward Ca\(^{2+}\) current as a result of the brief electrical stimulation; however, it is possible such a current is masked by the stimulation artifact. Certainly, the reversal potential for the brief stimulus elicited in the tail current protocol (Fig. 3) suggests the influence of ions with a more positive reversal potential. However, this response to electrical stimulation does persist under Ca\(^{2+}\) channel block by CdCl\(_2\) (Cameron et al. 2013), implying SK channels do not drive the majority of this response.

Activation of BK channels by electrical stimulation could explain the issues with achieving high-frequency action potential generation in spiking cells (Tsai et al. 2011). BK channel activation is shifted toward negative potentials by intracellular Ca\(^{2+}\) concentration (Berkefeld et al. 2006), and thus repeated stimulation would produce an increase in intracellular Ca\(^{2+}\) levels (Helmchen et al. 1996), and cause BK channels to be activated at more hyperpolarized potentials. Since electrical stimulation appears to temporally shift this KV-channel response, the normal physiological function of these channels, to assist repolarization of the cell after an action potential, may be disrupted. In the instance of electrically stimulating spiking neurons, this increase in K\(^{+}\) conductance could have the effect of antagonizing the depolarizing effect of Na\(_V\) channels during spike generation and could explain the drop in probability of action potential generation over successive electrical challenges (Tsai et al. 2011).

Of the six channels investigated by computational modeling, five are expressed in the mammalian retina (Hölting et al. 2007; Klupp et al. 1995; Pinto and Klumpp 1998), with the exception being KV2.2. Indeed, the pattern of response from the single cells represented in Fig. 6 suggests that these channel types may play roles in different cell types, conferring altered sensitivity to electrical stimulation. KV3 channels play a large role in the fast afterhyperpolarization potential (AHP) to facilitate firing of action potentials at high frequencies, especially KV3.3 channels (Rudy and McBain 2001). However, in the context of electrical stimulation it is possible that the activation characteristics of these channels actually inhibit action potential generation. KV3.3 channels are only activated at approximately +10 mV (Gutman et al. 2005), and brief, high-amplitude electrical stimulation depolarizes the membrane to very positive potentials and likely exceeds the activation potential for these channels. Although the overshoot of action potentials often exceeds +10 mV, this potential would not be reached until ~0.5–1 ms into the action potential and thus KV3.3 plays a large role in the repolarization of the membrane. Indeed, it is possible that disrupting the order of activation of the voltage-gated channels leads, not only to reduced action potential probability, but reduced afterhyperpolarization, causing prolonged Na\(_V\)-channel recovery time and reducing the frequency of action potential firing. The functions of KV1.3 and KV1.6 are less defined in the literature, but both are thought to exhibit C-type inactivation, a slower form of KV-channel inactivation (Cuello et al. 2010).

Less dealt with in the literature is the modulatory role of KV channels during internal or external electrical stimulation with brief pulses. Mathematical modeling suggests that KV channels have a minor (≤1%) effect on the rheobase of the strength-duration curve and the action potential conduction velocity of a myelinated nerve excited by a single stimulus pulse (Bostock 1983). On the other hand, in more complex in silico stimulation paradigms, low-threshold “slow” KV channels mediated the reduction in responses to external stimuli observed in auditory nerve fibers subjected to a preconditioning subthreshold pulse train (Miller et al. 2011). 4-AP-sensitive KV channels are thought to mediate the interspike interval of action potential responses to pacing trains in the rabbit saphenous nerve unmyelinated fibers (Zhu et al. 2013), and A-type and inwardly rectifying IR channels have been suggested to modulate the frequency response of auditory thalamic neurons to sinusoidal
intracellular stimulation (Tennigkeit et al. 1999). Both “jamming” of action potential transmission and inhibition of action potential initiation have been suggested as potential mechanisms underlying the effects of high-frequency electrical stimulation in the central nervous system (Benabid et al. 2005). Direct modulation of Kv by electrical stimulation could underlie these mechanisms.

Through pharmacological studies and mathematical modeling we have shown that external electrical currents are capable of directly activating K⁺ channels, rather than just secondary to stimulus induced membrane potential depolarization associated with Na⁺ or Ca²⁺ influx. We therefore propose that extracellular electrical stimulation can modify the well-characterized natural neuromodulatory roles of K⁺ channels in excitable cells such as modulating AHP, action potential duration, and rate adaptation. Although no obvious pattern of activation/inactivation characteristics predicts the differential response to brief electrical stimulation between the channel subtypes investigated here, it is likely the interaction between activation potential, activation and inactivation kinetics that predicts the response.

The impact of this Kv-channel response is particularly important to the nonspiking cells of the retina. Electrical stimulation of the retina following photoreceptor degeneration in diseases such as retinitis pigmentosa and age-related macular degeneration has become a promising therapeutic strategy for the restoration of vision. RGCs are the cells that must ultimately be activated (directly or indirectly) by a retinal prosthesis to produce visual sensation. However, several classes of neuron, presynaptic to the ganglion cells, survive photoreceptor degeneration and are possible targets for electrical stimulation. We have shown here and previously that electrical stimulation does have a measurable effect on nonspiking retinal neurons (Cameron et al. 2013). This study has extended our previous finding to characterize the channels and temporal dynamics that respond best to brief electrical stimulation. Although spikes are not elicited, it is clear that even small changes in membrane potential in the presynaptic cell could have a large effect on neurotransmitter release, and their subsequent impact on spiking cells. Importantly, we have shown that the expression levels of these Kv-channel subtypes is varied, giving differential responses to electrical stimuli. Given the ON/OFF parallel channels of information flow in the retina, being able to selectively activate or inhibit a certain class of cells could be advantageous for the production of a coherent perceptual response to be elicited.

With a greater number of patients being implanted with devices whose therapeutic function depends on delivery of electrical stimulation, it is critical to characterize the interactions between external currents and ion channels in-depth. Understanding the effects of electrical stimulation on excitable cells will lead to optimized stimulus delivery protocols, with higher therapeutic efficacy and greater cell stimulation specificity.

DISCUSSIONS

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.C., A.A., and J.M. conceived and planned the experiments; M.C. and A.A. wrote the main manuscript text and prepared figures; M.C., A.A., J.M., Y.B., N.L., and S.D. reviewed and edited the manuscript.

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