Modulation of Voltage-Gated Conductances of Retinal Horizontal Cells by UV-Excited TiO₂ Nanoparticles

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Abstract. This study examines the ability of optically-excited titanium dioxide nanoparticles to influence voltage-gated ion channels in retinal horizontal cells. Voltage clamp recordings were obtained in the presence and absence of TiO₂ and ultraviolet laser excitation. Significant current changes were observed in response to UV light, particularly in the -40 mV to +40 mV region where voltage-gated Na⁺ and K⁺ channels have the highest conductance. Cells in proximity to UV-excited TiO₂ exhibited a left-shift in the current-voltage relation of around 10 mV in the activation of Na⁺ currents. These trends were not observed in control experiments where cells were excited with UV light without being exposed to TiO₂. Electrostatic force microscopy confirmed that electric fields can be induced in TiO₂ with UV light. Simulations using the Hodgkin-Huxley model yielded results which agreed with the experimental data and showed the I-V characteristics of individual ion channels in the presence of UV-excited TiO₂.

Keywords: Electrostatic force microscopy; Hodgkin-Huxley model; Retinal horizontal cell; TiO₂ nanoparticle; Voltage-gated ion channel

Abbreviations: EFM, electrostatic force microscopy; HH, Hodgkin-Huxley; TiO₂, titanium dioxide; UV, ultraviolet.

1. Background

Cellular ion currents in excitable cells, such as neurons and muscle cells, are essential for the function of living organisms, as they allow the cells to communicate. These ion currents are possible due to an electrochemical gradient created by varying concentrations of ions inside and outside of the cell. K⁺ is approximately 30 times more concentrated intracellularly than extracellularly, while Na⁺ is approximately 10 times more concentrated extracellularly than intracellularly. This is primarily due to the Na⁺/K⁺ pump, which uses energy to transport 3 Na⁺ ions out of the cell for every 2 K⁺ ions into the cell. In addition, K⁺ is allowed to passively flow through leak channels. The unequal ion concentrations inside and outside the cell result in two opposing gradients – a concentration gradient and an electrical gradient. At equilibrium,

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4 when these two forces negate each other, the electric potential across the membrane is typically around -
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6 70 mV, known as the resting membrane potential (1).
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10 Because the cell membrane is comprised of a hydrophobic lipid bilayer, hydrophilic particles like ions
11 cannot pass directly through the membrane and instead must rely on a variety of channels. In addition to
12 the K^+ leak channels mentioned above, which open and close randomly, several other types of ion
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14 channels exist. Ligand-gated ion channels open upon binding to a specific receptor. Glutamate-gated
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16 channels, for instance, open when bound to the neurotransmitter glutamate and play a major role in
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18 excitatory signaling in the brain (2). Mechanically activated channels exist in the inner ear and open in
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20 response to sound waves (1). This study focuses on voltage-gated ion channels, which open in response to
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22 changes in membrane potential.
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28 Retinal horizontal cells are neuronal cells found in the retina that are responsible for enhancing visual
29 contrast (3). Horizontal cells from channel catfish retina were used in this study due to their large flat
30 morphology, which makes them ideal for whole cell patch clamp recordings. These cells display six types
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32 of ion currents. Na^+ current is activated in response to membrane depolarization. Its threshold is around -
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34 50 mV, and depolarizing voltages beyond the threshold of activation of these channels result in a positive
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36 current from Na^+ flow into the cell. This creates a positive feedback loop, further depolarizing the
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38 membrane. An L-type Ca^{2+} current, which activates at around -30 mV, also flows into the cell and
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40 contributes to membrane depolarization. Whereas Na^+ conductance only lasts several milliseconds (4),
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42 Ca^{2+} current can last much longer, though it is small in magnitude compared to Na^+ current (5, 6).
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50 Several K^+ currents are responsible for repolarizing the membrane. Transient outward K^+ current is
51 rapidly activated and consists of K^+ moving out of the cell during membrane repolarization. Delayed
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53 rectifying K^+ channels open slowly and play a large role in repolarization by enabling K^+ flow out of the
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55 cell to the point where the membrane is hyperpolarized. Anomalous rectifying K^+ current is activated by
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57 hyperpolarization and consists of K^+ flowing into the cell to bring the membrane voltage back to resting
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potential (1, 6). Leak currents are not voltage dependent and simply operate according to Ohm's law to maintain resting membrane potential.

A number of studies have been done on factors that can affect the activation voltage of voltage-gated ion channels (7-10), since the ability to influence the activation dynamics could have potential applications for a variety of diseases affecting voltage-gated ion channels, such as epilepsy or cardiac arrhythmia (11), human genetic diseases (12, 13), neuropathic pains (14), *etc.* A typical example of the modification of the activation dynamics of voltage-gated ion channels is the shift in current-voltage (I-V) characteristics. Theoretically, the shift in I-V characteristics influences significantly the cellular excitation (12) and inactivation mechanism (13). Bendahhou *et al* showed that the slow inactivation of the voltage-gated sodium channel is the primary cause of autosomal dominant skeletal muscle disorder (13). Thus, the importance of the ability to alter the dynamics of the voltage-gated ion channels cannot be over emphasized. Nanoparticles can be used as a tool in basic science research into these diseases, as they can be remotely optically activated to simulate abnormal ion channel dynamics such as the ones found in the above-mentioned diseases.

In this work, TiO₂ nanoparticles are used in conjunction with retinal horizontal cells to study the effects of light-induced electric field on the behavior of voltage-gated ion channels. Previous theoretical work has postulated on semiconductor nanoparticles' ability to influence voltage-gated ion channels with their polarization (15, 16). It has been shown that neurons grown on HgTe nanoparticle-containing films experience action potentials when irradiated with light (17). CdS nanoparticles have also been shown to cause ion channel activation when excited optically (18). Nanoparticles are, therefore, promising tools for controlling ion channel behavior. Since TiO₂ is more biocompatible than heavy metal materials, its potential as a tool to control I-V characteristics in living cells will be of interest to researchers.

Electrostatic force microscopy (EFM) and theoretical modeling are used in this work to confirm experimentally observed trends. While a number of studies has been done to characterize individual

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4 voltage-gated conductances in horizontal cells, a comprehensive theoretical description of ion channel
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6 behavior in these cells is lacking. Such a theoretical description could help reveal fundamental
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8 characteristics of ion channel behavior, especially under circumstance where such channel activity could
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10 be modulated by the influence of nanoparticles. In this work, an electrical model of a horizontal cell in the
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12 absence and presence of TiO₂ nanoparticles is proposed based on our experimental data. Both
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14 experimental results and theoretical modeling show that UV-induced electric fields of TiO₂ nanoparticles
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16 can influence the activation of voltage-gated ion channels as long as the QDs are in close proximity to ion
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18 channels.
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26 **2. Methods**

27 *2.1 Cell culture*

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33 Channel catfish were obtained from Keystone Hatcheries (Richmond, IL) and from Wing Lee Co.
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35 (Chicago, IL). Fish were dark adapted for at least an hour before use, anesthetized with a solution of
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37 approximately 1 g/gallon of ethyl 3-aminobenzoate methanesulfonate (Sigma Aldrich, St. Louis, MO) and
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39 2.5 g/gallon of sodium bicarbonate (Sigma Aldrich, St. Louis, MO), and sacrificed by cervical transection
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41 and pithing. Retinal horizontal cells were obtained with previously described protocols (19, 20). Both
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43 eyes were removed, sliced in half, and the posterior half was immersed in a solution containing
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45 approximately 1 mg/mL papain (Acros Organics, Fair Lawn, NJ) and 0.5 mg/mL L-cysteine (Sigma
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47 Aldrich, St. Louis, MO). After 30 min, retinas were removed and rinsed 8 times in Ringer's solution
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49 containing 126 mM NaCl, 4 mM KCl, 3 mM CaCl₂, 1 mM MgCl₂, 15 mM dextrose, and 10 mM HEPES,
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51 pH 7.4. The retinas were then dissociated by gentle agitation with a glass pipette. After around 15 seconds
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53 of agitation, the supernatant, containing primarily photoreceptors, was removed and discarded. The
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55 remainder of the retinas, containing primarily horizontal cells, was agitated for an additional 30 seconds.
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59 The resulting suspension was placed in 35-mm cell culture dishes containing 4 mL of Ringer's solution
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(approximately 2 drops of suspension per dish). The dishes were kept at 14°C until use and recordings were obtained on the same day.

2.2 Whole Cell Patch Clamp Recordings

Whole cell patch clamp recordings were obtained using a HEKA EPC 10 USB amplifier (Holliston, MA). Glass capillaries (Sutter Instruments, Novato, CA) were pulled to form pipettes with resistances of 4-8 MΩ and filled with intracellular solution containing 110 mM KCl, 0.5 mM CaCl₂, 5 mM EGTA, and 10 mM HEPES. The pH was adjusted to 7.0 using KOH. Once the pipette was brought into contact with the cell, suction was applied to create a gigaohm seal and rupture the membrane.

For recordings obtained with TiO₂, 140 mM TiO₂ nanoparticles (Sigma Aldrich, St. Louis, MO) were added to the dish after successful formation of the gigaohm seal. A 325-nm and 442-nm dual wavelength 100-mW IK Series He-Cd Laser (Kimmon Electric Company, Tokyo, Japan) was shined on the cell to excite the nanoparticles (Figure 1). Voltage pulses from -110 mV to 60 mV, each lasting 200 ms, were applied sequentially and the resulting current readings were recorded using HEKA Patchmaster acquisition software. When voltage-gated currents begin to flow through the membrane, the amplifier applies an equal and opposite current necessary to maintain the cell at the set voltage. Voltage-activated ion currents can thus be measured.

Recordings were obtained with and without laser excitation, and the minimum current during the first 15 ms was used to plot I-V curves, since the maximum inward current due to Na⁺ conductance occurs during this time period. A total of 12 different data sets were obtained from different cells. In addition, 12 control trials were performed in the absence of TiO₂ to confirm that any change in channel conductance was not caused by UV light itself.

2.3 Electrostatic Force Microscopy Studies of TiO₂

EFM was used to confirm that TiO₂ nanoparticles were, in fact, generating electric fields in response to UV light. TiO₂ nanoparticles were dropcast onto a gold-coated silicon substrate and imaged using Bruker Dimension Icon AFM (Billerica, MA). A SCM-PIT probe was used to first obtain a topological image of the sample in contact mode. The sample was then scanned in lift mode with a lift height of 10 nm and the interleaved amplitude data was used to observe any change in distance between the tip and the sample. Scans were obtained with and without illumination by a handheld laser.

3. Results

3.1 Whole Cell Voltage Clamp Recordings

A typical whole cell voltage clamp recording is shown in Figure 2. For applied voltages below -70 mV, there is net negative current, or an influx of positive ions into the cell. This inward current is caused primarily by inwardly rectifying K⁺ channels that are activated by hyperpolarizing voltages. At resting membrane potential (around -70 mV), the cell is at equilibrium and there is no net current flow. Progressive depolarizing steps lead to activation of the, voltage-gated activation of Na⁺ channels (inward currents), as Na⁺ rapidly flows into the cell due to the high concentration gradient, resulting in a large negative current which is represented by the green trace corresponding to V=0 mV in the inset of Figure 2b. Voltage-gated K⁺ channels are also opening at this time, but the Na⁺ conductance dominates, which explains the net negative current. At membrane potentials of greater than 30 mV, K⁺ currents dominate; current flow through Na⁺ channels is small near the Nernst equilibrium potential for Na⁺, so the net current is positive (1, 21). The large transients of positive or negative current at the beginning and end of the voltage pulse are capacitive currents caused by rearrangement of charges at the membrane (21), and they can be ignored for the purposes of this experiment.

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4 In the presence of TiO_2 , a noticeable shift in the voltage associated with maximum Na^+ conductance can
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6 be seen, as shown in a representative plot in Figure 3a, which is consistent with the Hodgkin-Huxley
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8 model discussed in Section 3.3. Whereas the maximum inward current occurred at around 0 mV prior to
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10 laser UV excitation, with laser excitation it shifted in the negative direction to around -10 mV. This shift
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12 was not observed in the control experiments performed in the absence of TiO_2 (Figure 3b). It is clear that
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14 the presence of both TiO_2 and UV light is necessary for the shift to occur, suggesting that it occurs due to
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16 the optical excitation and resulting polarization of TiO_2 .
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21 The effect of UV-excited TiO_2 on voltage-gated currents is also noticeable when looking at the current
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23 change at each voltage in response to laser light. Figure 4 shows the light-induced current change in the
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25 presence and absence of TiO_2 nanoparticles. In the -30 to +40 mV region, where voltage-gated Na^+ and
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27 K^+ channels display their highest conductance, the currents change by as much as 84 pA for $V=0$ mV,
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29 compared to 10 in the case of the control. The large standard deviations indicated by error bars in the case
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31 of TiO_2 -exposed cells likely reflect the fact that the distance between nanoparticles and ion channels
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33 varies from cell to cell, and thus the strength of the electric field sensed by the voltage-gated ion channels
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35 is not consistent. The relatively small light-induced current changes in the control experiment can be
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37 explained by natural fluctuation in currents that occurs in all cells even in the absence of any external
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39 factors (22).
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44 *3.2 UV-Induced Electric Fields in TiO_2*

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47 EFM measurements confirm the presence of electric fields in TiO_2 nanoparticles when exposed to light.
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49 Figure 5 shows that the interleave amplitude, or the scanning probe's distance from the sample, increased
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51 by around 0.4 nm upon illumination with the laser, suggesting that an electrostatic force repels the tip
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53 from the surface of the nanoparticle film (23). This is in agreement with previously-published studies on
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55 optically-induced electric fields in TiO_2 (24). While quantifying the electric field based solely on
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interleave amplitude shift involves multiple approximations and assumptions, EFM is nonetheless a useful tool for qualitatively observing electric field.

In addition to EFM measurements, theoretical modeling was used to confirm experimental results.

3.3 Hodgkin and Huxley Model

Hodgkin and Huxley proposed an electrical circuit model (HH model) of a cell membrane from a squid axon with two different types of voltage-gated ion channels in 1952 (25). In the HH model, current from an individual ion channel is described by equation with the membrane potential and a set of first order differential equations which can be empirically fitted well with the experimental data. That is,

$$I_i = g_i^{max} \times m_n(V, t) \times h_p(V, t) \times (V - E_i) \quad (1)$$

where i is the type of ion channel, E_i is the reverse potential of i type of ion channel, $m_n(V, t)$ and $h_p(V, t)$ are activation and inactivation constants, respectively, and n and p are power constants. These constants are given by:

$$\frac{dm_i}{dt} = \alpha_{m_i}(V) \times (1 - m_i(t)) - \beta_{m_i}(V) \times m_i(t) \quad (2)$$

$$\frac{dh_i}{dt} = \alpha_{h_i}(V) \times (1 - h_i(t)) - \beta_{h_i}(V) \times h_i(t) \quad (3)$$

where α 's and β 's are empirical parameters that are tunable to designated behavior of ion channels. The parameters used in this work are summarized in Table 1. The total ionic current in a cell is given by the sum of currents from all individual ion channels plus the capacitive current due to the change of the membrane potential in time:

$$I_{ion}^{total} = C_{membrane} \frac{dV}{dt} + \sum_i I_i(V, t) \quad (4)$$

With the presence of a nanoparticle that produces electrostatic field, this equation becomes

$$I_{ion}^{total} = C_{membrane} \frac{dV}{dt} + \sum_i I_i(V + V_{QD}, t) \quad (5)$$

To model the electric field from TiO_2 , a nanoparticle is assumed to be a sphere. When it is in an intense laser field, electron and hole pairs are created. As a result, it is polarized uniformly along the polarization direction of the laser. This phenomena is referred to as induced polarization, which is mathematically described by

$$\vec{P} = \alpha \vec{E} \quad (6)$$

where $\alpha = \left[1 - \frac{\sqrt{E_g}}{4.06}\right] \frac{M}{\rho} \times 4.4060 \times 10^{-35} \text{ F} \cdot \text{m}^2(25)$, $\vec{E}$ is the laser field, and E_g , M , and ρ are the band gap, molecular weight, and density of the nanoparticle, respectively. Then the surface charge, σ , is calculated by

$$\sigma = \vec{P} \cdot \hat{n} = P \cos(\theta) \quad (7)$$

The laser field is calculated from the intensity of laser used in the experiment, the total power of which is 100 mW, by the relationship between the time average of energy density of laser and the amplitude of electric field which is given by

$$|E|^2 = \frac{2I}{cn\epsilon_0} \quad (8)$$

where I is the intensity per unit area, c is the speed of light, n is the refractive index of the media, and ϵ_0 is vacuum permittivity.

Then, the electric field produced from a TiO_2 nanoparticle can be calculated by solving Gauss's law (26):

$$E_{QD}^{induced} = \frac{P_{induced}}{3\epsilon} \frac{R^3}{r^3} [2 \cos(\theta) \hat{r} + \sin(\theta) \hat{\theta}], r > R \quad (9)$$

where ϵ is the dielectric function of the media and R is radius of a nanoparticle. In water-based electrolytes, the screening effect has to be considered as an exponential decay:

$$E_{QD}^{electrolyte} = E_{QD}^{induced} e^{-\frac{(r-R)}{\lambda_D}} \quad (10)$$

λ_D is the Debye length which can be written by

	α_m	β_m	α_h	β_h	$g_{max} [nS]$	$E_i [mV]$	I_{ion}
Transient outward K^+ current (27, 28)	$\frac{24}{1 + e^{-\frac{V-50}{28}}}$	$8e^{-\frac{V}{36}}$	$0.5e^{-\frac{V}{60}}$	$\frac{0.4}{1 + e^{-\frac{V+40}{5}}}$	3.0	-80	$g_{Kv} m_{Kv}^3 h_{Kv} (V - E_{Kv})$
Delayed rectifying K^+ current (27-29)	$\frac{4.0(65 - V)}{e^{\frac{65-V}{50}} - 1}$	$48e^{\frac{45-V}{85}}$	$\frac{30}{1 + e^{\frac{V+92}{7}}}$	$\frac{16}{1 + e^{\frac{V+100}{15}}}$	22.0	-80	$g_A m_A^4 h_A (V - E_A)$
Anomalous rectifying K^+ current (27, 28)		$m_{Ka} = \frac{1}{1 + e^{\frac{V+60}{12}}}$			4.5	-80	$g_{Ka} m_{Ka}^5 (V - E_{Ka})$
Ca^{2+} current (29)	$\frac{5(70 - V)}{e^{\frac{70-V}{28}} - 1}$	$2e^{-V/15}$	$h_{Ca} = \frac{[K]}{[K] + [Ca]_{out}}$		30.0	$12.9 \log \left[\frac{[Ca]_{in}}{[Ca]_{out}} \right]$	$g_{Ca} m_{Ca}^4 h_{Ca} (V - E_{Ca})$

Na ⁺ current (27)	$\frac{20(38-V)}{e^{\frac{38-V}{25}} - 1}$	$200e^{-\frac{55+V}{18}}$	$1.0e^{-\frac{80+V}{8}}$	$\frac{0.8}{1 + e^{\frac{80-V}{75}}}$	5.0	55	$g_{Na}m_{Na}^3h_{Na}(V - E_{Na})$
Leakage current (25, 27)					0.5	-80	$g_l(V - E_l)$

Table 1. Summary of parameters used in HH model.

$$\lambda_D = \sqrt{\frac{\epsilon k_B T}{2N_A e^2 I}} \quad (11)$$

where k_B is Boltzmann constant, T is temperature, N_A is Avogadro's number, e is elementary charge, and I is ionic strength of the electrolyte, which is given by $I = \frac{1}{2} \sum_{i=1}^n c_i z_i^2$, and c_i is the molar concentration of the i th ion in the electrolyte and z_i is the charge of the i th ion.

Our simulation shows that in retinal horizontal cells the addition of an excited TiO₂ nanoparticle results in a left shift of the I-V characteristics along the voltage axis, in the case when the polarization axis of the nanoparticle is perpendicular to the membrane and the nanoparticle is 1 nm away from the ion channel. In Figure 6, I-V characteristics of a horizontal cell are plotted which represent the total ion currents in response to the applied membrane potential. Inset of Figure 6 represents the magnification near the local minima where Na⁺ channels play the major role. The voltage associated with the minimum current is shifted to the left in the presence of the excited nanoparticle compared to without nanoparticles. The shifts of I-V characteristics also appear in case of individual ion channels, as shown in Figure 7. For each ion channel, the I-V curves shifted to the left in the range that the specific channel is activated with addition of TiO₂. For example, the activation range of the voltage gated anomalous rectifying K⁺ channel is below potentials of -40 mV (30), and there is a clear shift of I-V characteristics to the left in this range.

The behavior of individual ion currents (Figure 7) in the presence of nanoparticles can be used to explain

the trends observed experimentally (Figure 3). The minor left-shift in I-V curve observed experimentally in the -110 mV to -70 mV range can be attributed to anomalous rectifying K^+ channels. The shift in the I-V curve in -40 mV to +20 mV range is due to voltage-gated Na^+ and Ca^{2+} channels, the conductances of which dominate in this range.

Although this simulation is specific to retinal horizontal cells, the parameters can be easily adjusted to predict the behaviors of other cell types in response to induced electric fields or other external stimuli. This is potentially relevant in medical applications for treatment of ion-channel related diseases.

4. Discussion

In this work, optically-excited TiO_2 nanoparticles are shown to influence the opening of voltage-gated ion channels in retinal horizontal cells by shifting the cell's I-V characteristics to more negative voltage values. This trend is especially strong in the physiologically relevant region of -30 mV to +40 mV, where voltage-gated Na^+ and K^+ channels display the highest conductance. EFM measurements and theoretical modeling confirmed that this is due to the electric fields generated by optically-excited TiO_2 nanoparticles located in close proximity to the ion channels.

The structural properties of TiO_2 can give insight into its influence on voltage-gated ion channel activation. TiO_2 is a semiconductor material with a large band gap of around 3.2 eV for the anatase form, which allows it to be used in a variety of applications involving light absorption, including sun protection, photocatalysts, and solar energy production (31). TiO_2 is an indirect bandgap material, meaning an electron excited to a higher energy band cannot recombine with a hole without first undergoing a phonon-aided change in momentum (32). Due to this structural property, TiO_2 rarely exhibits radiative recombination of electrons and holes as observed in direct bandgap materials, and as a result rarely emits

light. Instead, the separation of carriers contributes to the formation of a dipole moment and, subsequently, an electric field around the TiO₂ particle.

A shift in cellular activation voltage similar to one observed in this work has been previously reported in response to a variety of factors. It has been shown that protonation enhances Na⁺ conductance (8) and Ca²⁺ conductance (9) in retinal horizontal cells, resulting in a shift in voltage associated with maximum inward current. This is likely due in part to H⁺ screening the negative charges near the membrane, with the resulting change in electrochemical gradient leading to a shift in activation potential (33). Similarly, studies involving mutant K⁺ channels which were missing either the N or C terminus of the channel showed in a shift in activation voltage (7). While the exact mechanism of modulation of many voltage-gated channels is still under debate, it is largely believed that the voltage sensing occurs due to reorientation of positively and negatively charged subunits of the ion channel in response to potential changes, resulting in opening or closing of the channel (21). It is, therefore, reasonable that the presence of protons or the absence of a charged terminus would cause a change in the channel's conductance. In our case, the electric fields from optically-excited TiO₂ affect the channels in a similar manner by introducing an induced electrostatic potential, altering the orientation of the charged subunits and causing the channel to open or close.

The distance between a nanoparticle and the ion channel plays a large role in this phenomenon. The strength of the electric field generated by polarized TiO₂ decreases exponentially with distance due to screening by the electrolyte (26), in this case the Ringer's solution, so it is imperative for the TiO₂ to be in close proximity to the ion channel in order for the voltage shift to occur. For this reason, a TiO₂ concentration of 140 mM was used in our study. Despite this high concentration, the cells still exhibited the expected I-V characteristics (Figure 3), showing that on the time scale of the experiment the large amount of TiO₂ did not have adverse effects on cellular ion channel behavior. TiO₂ has low toxicity compared to many other semiconductor materials, hence its use as a UV light absorbent in a variety of products such as sunscreen and cosmetics (34). Nevertheless, it has been shown that prolonged (24 hours)

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4 exposure to TiO₂ nanoparticles can effect mitochondrial function (35), and two-year exposure to high
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6 concentrations of TiO₂ nanoparticles has been found to cause pulmonary lesions in rats (36). UV-
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8 photoactivated TiO₂ nanoparticles have also been shown to generate reactive oxygen species (ROS)
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10 which could make cells more susceptible to bacterial invasion (37) and cause other cell damage after
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12 several hours of exposure (38-40). While no adverse effects were observed on the time scale of the
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14 experiment, more research is needed before implementing photoactive TiO₂ in long-term studies or
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16 therapeutics. Future studies could also benefit from using ligand-functionalized nanoparticles, followed
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18 by washing away of unbound nanoparticles, which would minimize the TiO₂ concentration while insuring
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20 its proximity to membrane ion channels. Nevertheless, the method shown here is promising for basic
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22 science research and *in vitro* experiments into ion channel diseases.
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28 Among other methods of controlling voltage-gated ion channel activation is optogenetics, a field that
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30 relies on genetic modification of cells through introduction of foreign DNA in order to express light-
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32 activated proteins. Channelrhodopsins are light-activated cation channels that were discovered in algae
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34 (41) and have since been used extensively for optically controlling neuronal excitability. Numerous
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36 mutants of channelrhodopsins have since been created, including some allowing for greater selectivity for
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38 specific cations (42, 43), some that are activated by different wavelengths of light (44) and some that can
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40 also be optically deactivated as well as activated (45). Optogenetic tools clearly have an advantage of
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42 being extremely customizable. However, they involve genetic manipulation of cells through transfection,
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44 which often affects the long-term survival of cells, and the transfection protocols for less widely-used cell
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46 lines often require time-consuming optimization.
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52 Voltage-gated ion channels have also been controlled via photochromic ligands (PCLs), small molecules
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54 that bind to the inside of the ion channel and change conformation upon light absorption, thereby either
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56 blocking or allowing the flow of ions (46). Like optogenetics, this method allows for some tuning, since
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58 different PCLs are selective for different ion channels. However, delivery of the PLCs to the cell can be
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60 problematic, since extracellular PLCs can only enter the cell through specific channels which are not
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4 present in all cell types, requiring exogenous expression of these channels in cell types where they are not
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6 native. A variant of PCLs exists that covalently binds to extracellular regions of the ion channels it
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8 modulates, but this requires mutation of the ion channel (46).
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11 Thus, while some tools such as optogenetics and PCLs have the advantage of being highly customizable
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13 and specific in their ability to optically switch voltage-gated ion channels, the nanoparticle method
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15 described in this work is noninvasive and can be easily implemented with a variety of cell types. As
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17 mentioned earlier, HgTe (17) and CdSe (18) nanoparticles have been successfully used for this purpose.
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19 Other studies have used gold nanoparticles, which emit heat upon light absorption and thereby alter the
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21 membrane capacitance (47). Together, these studies show that voltage-gated ion channel characteristics
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23 can be controlled with a variety of nanoparticles and light wavelengths. While further optimization of the
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25 TiO₂ method is needed due to potential long-term adverse effects on cells, this method is nonetheless
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27 useful for altering I-V dynamics in short time scale experiments investigating diseases affecting voltage-
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29 gated ion channels. Moreover, these results indicate that it may be possible to modify or control the
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31 physiological behavior of cellular and neuronal ion channels, potentially opening the way to modifying
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33 ion channel behaviors in ways that impact cellular and neuronal functions in both diseased and healthy
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Figure Legends

Figure 1. Schematic of whole cell patch clamp recordings performed in this study. EGTA in the intracellular fluid serves as a Ca^{2+} chelator, effectively making the free Ca^{2+} concentration in nM range. A gigaohm seal is created by bringing the electrode-containing glass capillary to the cell membrane and applying suction until the membrane inside the pipette is ruptured. The membrane can then be maintained at a set voltage point (V_{cmd}) through a current generated by the amplifier.

Figure 2. Typical whole cell voltage clamp recordings. A series of 200-ms voltage pulses from -110 to +60 mV in magnitude are applied sequentially (a). The corresponding current traces are recorded (b). The initial portion contained within the red rectangle is enlarged (c) to better examine the voltage-activated Na^+ currents and the subsequent repolarizing K^+ currents which dominate the membrane

conductance in this range. The minimum current readings from the 15-25 ms range are plotted against the applied voltage to obtain an I-V curve (d).

Figure 3. Representative I-V plots obtained from whole cell recordings in the presence (a) and absence (b) of TiO₂. Black traces were obtained with the laser off and red with the laser on. A shift in the voltage associated with the maximum Na⁺ conductance can be seen in the presence of UV-excited TiO₂. This trend was observed in 6 out of the 12 cells tested.

Figure 4. Changes in current (current with laser off minus current with laser on) in response to UV laser excitation in the presence (red) and absence (black) of TiO₂. Cells exposed to UV-excited TiO₂ experience significant current shifts at membrane voltages in the range of -40 to +40 mV, where voltage gated Na⁺ and K⁺ channels are most conductive. Error bars represent standard deviations from 6 data sets.

Figure 5. Electric fields in TiO₂ nanoparticles induced by UV light can be detected via EFM. (a) shows the topology of dropcasted TiO₂ suspension on a Au-coated Si substrate. (b) shows the interleave amplitude of probe in the absence of UV light. (c) shows the interleave amplitude of the probe in the presence of UV light from a handheld laser. The corresponding interleave amplitude profiles along the dashed segment are displayed on the left.

Figure 6. I-V characteristics of a horizontal cell, which represent the total ion currents in response to the applied membrane potentials. Inset figure is the magnification near the local minima where Na⁺ channels play the major role. The minimum point with TiO₂ is shifted to the left compared to that without TiO₂.

Figure 7. I-V characteristics of individual ion channels in a horizontal cell. For each ion channel, the I-V curves shifted to the left in the range that the specific channel is activated with addition of TiO₂.

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Table 1

	α_m	β_m	α_h	β_h	g_{max} [nS]	E_i [mV]	I_{ion}
Transient outward K ⁺ current (27, 28)	$\frac{24}{1 + e^{-\frac{V-50}{28}}}$	$8e^{-\frac{V}{36}}$	$0.5e^{-\frac{V}{60}}$	$\frac{0.4}{1 + e^{-\frac{V+40}{5}}}$	3.0	-80	$g_{Kv}m_{Kv}^3h_{Kv}(V - E_{Kv})$
Delayed rectifying K ⁺ current (27-29)	$\frac{4.0(65 - V)}{e^{\frac{65-V}{50}} - 1}$	$48e^{\frac{45-V}{85}}$	$\frac{30}{1 + e^{\frac{V+92}{7}}}$	$\frac{16}{1 + e^{\frac{V+100}{15}}}$	22.0	-80	$g_Am_A^4h_A(V - E_A)$
Anomalous rectifying K ⁺ current (27, 28)		$m_{Ka} = \frac{1}{1 + e^{\frac{V+60}{12}}}$			4.5	-80	$g_{Ka}m_{Ka}^5(V - E_{Ka})$
Ca ²⁺ current (29)	$\frac{5(70 - V)}{e^{\frac{70-V}{28}} - 1}$	$2e^{-V/15}$	$h_{Ca} = \frac{[K]}{[K] + [Ca]_{out}}$		30.0	12.9 log	$g_{Ca}m_{Ca}^4h_{Ca}(V - E_{Ca})$
Na ⁺ current (27)	$\frac{20(38 - V)}{e^{\frac{38-V}{25}} - 1}$	$200e^{-\frac{55+V}{18}}$	$1.0e^{-\frac{80+V}{8}}$	$\frac{0.8}{1 + e^{\frac{80-V}{75}}}$	5.0	55	$g_{Na}m_{Na}^3h_{Na}(V - E_{Na})$
Leakage current (25, 27)					0.5	-80	$g_l(V - E_l)$

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7