A Xenopus oocyte model system to study action potentials

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Action potentials (APs) are the functional units of fast electrical signaling in excitable cells. The upstroke and downstroke of an AP is generated by the competing and asynchronous action of Na+ and K+-selective voltage-gated conductances. Although a mixture of voltage-gated channels has been long recognized to contribute to the generation and temporal characteristics of the AP, understanding how each of these proteins function and are regulated during electrical signaling remains the subject of intense research. AP properties vary among different cellular types because of the expression diversity, subcellular location, and modulation of ion channels. These complexities, in addition to the functional coupling of these proteins by membrane potential, make it challenging to understand the roles of different channels in initiating and “temporally shaping” the AP. Here, to address this problem, we focus our efforts on finding conditions that allow reliable AP recordings from Xenopus laevis oocytes coexpressing Na+ and K+ channels. As a proof of principle, we show how the expression of a variety of K+ channel subtypes can modulate excitability in this minimal model system. This approach raises the prospect of studies on the modulation of APs by pharmacological or biological means with a controlled background of Na+ and K+ channel expression.

Introduction

The action potential (AP) is a self-regenerating electrical signal and is the basis for long-distance signaling in the nervous system, skeletal muscles, and the heart. APs are also observed in plants, where they are thought to control several physiological processes (Choi et al., 2016). The plasma membrane of living cells is electrically polarized, with an intracellular negative voltage with respect to the extracellular environment. An AP consists of a transient change of the membrane potential toward canceling its electrical polarity (depolarization), followed by the establishment of positive intracellular potentials (polarity reversion or antipolarization) and, subsequently, a return to the initial negatively polarized state (repolarization). Based on the seminal work of Hodgkin and Huxley (1939, 1952) on the squid giant axon, we know that at least two competing ionic currents are essential for the generation of APs. In general, the first current consists of an influx of positive charges, usually carried by Na+ ions, driving a change toward positive membrane potentials. The second current is mainly carried by K+ ions and flows in the opposite direction through a K+-selective pathway that is also activated by depolarization. Today, we know that these pathways are typically embodied in Na+- and K+-selective voltage-gated (NaV and Kv, respectively) ion channels (Hille, 2001).

Understanding how specific membrane transport pathways contribute to AP firing is the subject of considerable research effort (Bean, 2007). In excitable cells, a rich diversity of voltage-gated and non-voltage-gated channels and ion exchangers shape the AP, determining properties such as AP threshold, duration, amplitude, and firing frequency (Ballerini et al., 1997; Hille, 2001; Wang and Huang, 2006; Bean, 2007; Jan and Jan, 2012). The silencing of certain ion channel genes (Mancia et al., 2003; Peters et al., 2005; Speca et al., 2014) or inhibition of specific channels by toxins or drugs (Bekkers and Delaney, 2001; Pathak et al., 2016; Hu and Bean, 2018) have been useful subtractive methods contributing to understanding the role of specific ion channel pathways in AP firing. Alternatively, an additive approach could be useful to understanding the role of different channels incorporated into a background of the minimal components necessary for APs. Computational models often use an additive design to study membrane excitability in silico (Rudy and Silva, 2006; Marder and Taylor, 2011). To advance this goal in living cells, we turned to the Xenopus laevis oocyte expression system (Gurdon et al., 1971) because it is a highly used tool for the study of membrane transport proteins foreign to the amphibian oocyte and allowed us to select for the simultaneous expression of specific proteins (Dascal, 1987; Sigel, 1990).

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To date, there are more than 8,000 published works on the properties of ion channels expressed in Xenopus oocytes describing various experimental approaches, including techniques such as two-electrode voltage clamp (TEVC), patch clamp, or cut-open oocyte voltage clamp. However, investigation of the capacity of Xenopus oocytes to generate all-or-none APs is only sparsely addressed in the research literature. The present study explores a relatively simple and inexpensive adaptation of conventional TEVC recordings in Xenopus oocytes as an approach to the study of APs. We build from prior demonstrations of regenerative APs in oocytes (Shapiro et al., 2012, 2013; Corbin-Leftwich et al., 2016) and test the sufficiency of Na⁺ channel expression alone, as well as the addition of non-voltage-gated and/or voltage-gated K⁺ conductances to document the reliability of AP generation and the impact of K⁺ channel diversity on APs. Furthermore, we demonstrate that APs recorded in Xenopus oocytes can induce repetitive firing. The results are important in their ability to replicate key features of APs and explore the minimal components needed for AP generation. This work has wide applications to the study of APs, including the role of specific ion channel subunits, the impact of ion channel mutations and modulation, pharmacologic screening of small molecules, and comparative physiology of ion channels from diverse species. The approach also has educational value for inquiry-based learning and helps clarify common misconceptions about APs.

**Materials and methods**

**Preparation of oocytes and RNA injections**

RNA preparation, Xenopus laevis oocyte isolation and preparation, and oocyte injection were performed using published methods (Heler et al., 2013; Corbin-Leftwich et al., 2016). Animal protocols were approved by Institutional Animal Care and Use Committees at the University of Richmond, Virginia Commonwealth University, and the University of the Pacific and conform to the requirements in the Guide for the Care and Use of Laboratory Animals from the National Academy of Sciences. Some oocytes were prepared from ovarian lobules purchased from Xenopus 1, and some oocytes were purchased from EcoCyte Laboratory Animals from the National Academy of Sciences. Some oocytes were prepared from ovarian lobules purchased from Xenopus 1, and some oocytes were purchased from EcoCyte Bio-science. Results from many batches of oocytes were combined. Each oocyte was injected with 24–46 nl RNA and studied 2–4 d later. Oocytes were maintained at 14–17°C in a solution of (in mM) 96 NaCl, 2 KCl, 1.8 CaCl₂, 1 MgCl₂, 5 HEPES, 5 sucrose, and 2.5 Na pyruvate, pH 7.4, with 50 U/ml penicillin G and 50 µg/ml streptomycin or 25 µg/ml gentamycin.

**Electrophysiology**

Ionic currents were recorded in two electrode voltage clamp (TEVC) from oocytes with Warner OC-725B or OC-725C amplifiers using maximum clamp speed and gain (Warner Instruments). Voltage and current recording pipettes were filled with a filtered solution containing 1 M KCl, 10 mM EGTA, 10 mM HEPES, pH 7.4. To obtain AP recordings, we avoided voltageclamping the oocyte by decreasing the amplifier’s feedback gain (Corbin-Leftwich et al., 2016). As in many TEVC systems, our amplifiers consisted of two voltage-clamping circuits. One controls the intracellular potential, holding it at 0 mV with respect to a reference (“ground”). The second circuit (“bath clamp”) controls the extracellular potential, setting it to the negative of the command voltage (Vₘₑₚ). Thus, an oocyte is voltage-clamped at −70 mV by setting the extracellular voltage to +70 mV. Our approach was, therefore, to make one of these two circuits as inefficient as possible, without hampering the ability to depolarize the cell. To achieve this, a simple modification was made. A 50-megaohm resistor was added between the current (feedback) electrode and the headstage to decrease the ability of the amplifier to voltage clamp the interior of the oocytes at 0 mV. To depolarize the membrane, we added a second resistor (10-megaohm) connected in series to a diode and placed in parallel to the first resistor. The diode was oriented with the cathode pointing toward the cell (Fig. 1, A and B). This arrangement allowed us to readily depolarize the membrane, but not efficiently repolarize it (Figs. S1 and S3). A switch was added to bypass this circuit, allowing us to toggle between the “loose” voltage clamp mode for AP recordings and the traditional TEVC mode for current recordings.

In the loose-clamp mode, we were able to readily depolarize the membrane (Fig. S2). For AP triggering, brief depolarizing pulses (0.5–1 ms) were used to elicit single APs, and longer duration stimuli, sometimes in the form of ramps, were used to generate multiple APs, as noted in the figure legends.

Experiments were conducted at room temperature (20–23°C) in a recording chamber that was perfused continuously with a standard 2 Kᵋ solution containing (in mM) 2 KCl, 98 NaCl, 2 MgCl₂, and 5 HEPES, pH 7.4. A 10 Kᵋ solution (10 KCl with 90 Na or N-methyl-d-glucamine Cl) was used to study inwardly rectifying K⁺ currents in voltage clamp (Fig. 4). Alternative solutions are noted in figure legends. Data were recorded and analyzed on computers equipped with Digidata 1320A A/D hardware and Clampex and Clampfit software (Molecular Devices). Data were sampled at 10–50 kHz and filtered at 1–2 kHz. Some experiments used a USB-6251 multifunction acquisition board (National Instruments) controlled by an in-house program coded in LabVIEW (National Instruments; details available from C.A. Villalba-Galea upon request). For these, the electrical signals were filtered at 100 kHz, oversampled at 500 kHz to 2 MHz, and stored at 5–25 kHz for offline analysis. All data were transferred to Excel (Microsoft) and Origin (OriginLab Corp.) for additional analysis and the production of figures; some data were analyzed using custom Java-based software (details available from C.A. Villalba-Galea, upon request). For box-and-whisker plots (Figs. 3 and 4), the ends of the box are the upper and lower quartiles, and the median is marked by a horizontal line inside the box. The whiskers are the maximum and minimum values, and the mean is designated with a solid data point inside the box. Significant differences in resting membrane potential (Vᵢₒᵦ) measurements (Fig. 4) were determined by ANOVA followed by a Tukey–Kramer multiple comparisons test. P < 0.05 was considered significant.

**Plasmid constructs**

Ion channel plasmids used in this study included rat Na⁺,1.4 (a subunit), rat Naₐβ₁, human Kₗ.7.1, human Kₗ.7.2, human Kₗ.7.3, rat K₄.2.1, Shaker with fast inactivation-removed (Shaker Δ6–46, hereafter ShakerΔ), rat K₄.2Δ2–40 (slows inactivation and...
enhances channel expression), rat Kv2.1, and mouse TREK-1 E306A. Mutations or deletions were introduced and confirmed by published methods (Heler et al., 2013). The mass of each RNA injected is noted in each figure legend. The Naβ1 subunit was always coinjected with the Naα subunit to enhance channel expression and produce fast Na+ channel gating (Makita et al., 1994; Isom et al., 1995).

Online supplemental material
Figs. S1 and S2 show the performance of the loose TEVC clamp. Fig. S3 shows how the kinetics of membrane repolarization in loose TEVC is impacted by an outwardly rectifying, voltage-gated K+ conductance. Fig. S4 shows evidence for a contribution from native oocyte Cl− conductance to the repolarization of APs. Fig. S5 shows that Kv channels impact VREST but not VTHR in regulating AP excitability.

Results
We expressed Na+ and K+ channels in Xenopus oocytes to determine the minimal requirements for inducing all-or-none regenerative electrical activity in the membrane of a large, non-excitable cell. Fig. 1 shows the loose-clamp electronics used to measure the APs in oocytes (Fig. 1, A and B) and demonstrates APs recorded in oocytes with high expression of Nav1.4 (α and β subunits) and Kv currents (Fig. 1 C).

The resistor-diode device described in Methods allowed depolarization of the membrane, but not its repolarization (Fig. S1, A and B; and Fig. S2). Also, the expression of a Kv channel resulted in a rapid repolarization of the membrane after depolarization (Fig. S1, D and E) with a repolarization rate that was a function of the depolarizing pulse amplitude (Fig. S3 C). In oocytes expressing NaV and Kv channels, we were able to elicit APs once a threshold potential was surpassed by the application of 1-ms depolarizing pulse.
pulses. We attributed the fast upstroke of this electrical response and the subsequent rapid repolarization to the activity of NaV and Kv channels, respectively. These APs produced in Xenopus oocytes expressing NaV and Kv channels had a quick rising phase, were all or none (see inset to Fig. 1C), and reached a depolarized potential of approximately +20 mV under the conditions tested. The repolarizing or decaying phase of the AP was mainly driven by the large outward current from the ShakerΔ channels. We also observed that repolarization of Xenopus oocyte APs was often followed by an afterhyperpolarization (Fig. 1C) as long as Kv channels were used in the RNA injection mixture. The same type of recordings in uninjected oocytes produced electrotonic responses, displaying only slow, nonundershooting membrane potential changes (Figs. S1 and S3).

APs in Xenopus oocytes also demonstrate refractory periods (Fig. 1D). We observed a relative refractory period when a second AP could be initiated only after applying a stimulus stronger than that used to elicit the first AP (Fig. 1D, left). An absolute refractory period was determined as the shortest time interval during which a second AP could not be elicited, despite increases in stimulus magnitude (Fig. 1D, right). Thus, the APs in Xenopus oocytes demonstrate the hallmark features of APs that are critical to setting the firing rate of excitable cells.

**Are NaV channels alone sufficient to produce an AP?**

For the generation of an AP, both a depolarizing conductance and a repolarizing voltage-dependent conductance are thought to be required. Membrane repolarization was slow but steady in uninjected oocytes that were depolarized by a step pulse (Fig. S1B). This result suggests that the expression of NaV channels in oocytes combined with endogenous conductances in oocytes should produce a transient response, resembling an AP. To test this hypothesis, we injected oocytes with only cRNAs encoding NaV α and β subunits. Fig. 2 shows that long-lasting, all-or-none depolarizing membrane potential changes could be generated with sufficiently large NaV channel expression (even in the absence of K+ channel RNA in the injection mixture). Similar to uninjected cells, an oocyte expressing only a small inward NaV current (~1 µA at the peak inward current in TEVC; Fig. 2A, inset) generated only passive membrane depolarizations (electrotonic potential) in loose clamp (Fig. 2A), and cells with low levels of NaV current expression failed to trigger an AP (Fig. 2B). However, for oocytes with inward NaV currents greater than ~20 µA, APs were often elicited (Fig. 2C and D). With the highest expression of NaV currents measured (~40 µA or more), all-or-none responses reached a peak depolarization near the Nernst potential for NaV (approximately +40 mV; Fig. 2D). We speculate that, given the large size of the oocytes, a high density of current was required to overcome the background repolarizing conductances in the oocyte and depolarize the entire cell.

Another important factor to be considered was the $V_{\text{REST}}$. In the absence of exogenous K+ channels, we were able to manipulate $V_{\text{REST}}$ by setting $V_{\text{CMD}}$ to more negative potentials. Thus, we used this procedure to study the role of NaV channel modulation by $V_{\text{REST}}$ in AP triggering because NaV channels are known to undergo closed-state inactivation, represented as $h_{\infty}$ in the equations of Hodgkin and Huxley (1952); see also Armstrong (2006). As a consequence, the number of NaV channels that can be activated increases as the membrane potential becomes more negative. On the other hand, hyperpolarization also decreases the rate of activation of channels, as described by Cole and Moore (1960). Consistent with this idea, we observed that AP triggering had a bell-shaped dependence on $V_{\text{REST}}$ (Fig. 2E and F). In oocytes showing less than 20 µA of maximum NaV current, AP triggering was more effective when $V_{\text{REST}}$ was between −52.6 and −62.5 mV (Fig. 2E). In oocytes displaying more than 20 µA, triggering an AP was more effective when $V_{\text{REST}}$ was between −62.6 and −67.5 mV (Fig. 2F). This small change in the optimal $V_{\text{REST}}$ for AP triggering is consistent with a role for NaV channel closed-state inactivation in cellular excitability.

Note that we routinely recorded ionic currents in the TEVC mode as a way to estimate the relative magnitude of currents resulting from the mixtures of ion channel RNAs injected. However, we are under no illusions that the voltage control in TEVC was adequate at all test potentials (Baumgartner et al., 1999). To achieve AP generation and test for the minimal requirements by continually increasing NaV currents, we conceded this limitation of TEVC. In sum, to reliably eliciting an all-or-none regenerative change in membrane potential in Xenopus oocytes, the critical issue is that the estimated peak, inward, voltage-gated NaV current must be quite large (~20 µA) and $V_{\text{REST}}$ must be optimized for NaV channel availability.

**Are non-voltage-gated K+ conductances sufficient to support AP firing?**

The ability to generate APs in oocytes in the absence of exogenous K+ channel expression indicates that native resting conductances in oocytes are sufficient to repolarize the NaV-only AP. Furthermore, some of the resting conductances must hyperpolarize the membrane sufficiently to reset the voltage sensors of the NaV channels and increase their availability to produce the all-or-none AP. The hyperpolarizing actions of the NaV/K+ ATPase pump would be of insufficient magnitude to perform these functions; ouabain inhibition of the pumps in cardiac muscle induces a membrane depolarization of only 5 mV (Miura and Rosen, 1978). An alternative explanation is that the oocyte plasma membrane possesses a sufficient number of its own K+ or Cl− channels, although their contributions cannot be distinguished when the expression of foreign channels is very high. We observed evidence that endogenous Cl− conductances, activated by the NaV-dependent depolarizations (Sanguinetti et al., 1996; Weber, 1999), contribute to the repolarization of the AP generated from NaV channels alone (Fig. S4). In oocytes expressing NaV currents greater than ~30 µA, we observed (in five of eight cells) APs with repolarizing phases that lasted from 390 to 2,970 ms, and we observed that reduction of external Cl− lengthened the repolarization phase (Fig. S4). In addition, “leak” K+ channels of the Kp type are present in Xenopus oocytes (Weber, 1999) and may also contribute to repolarization.

We tested the impact of experimentally induced Kp channel expression on NaV-generated APs in Xenopus oocytes in the absence of exogenous voltage-gated K+ channel expression (Fig. 3). Along with the NaV channels, we coexpressed TREK-1 channels possessing an E306A mutation so that these Kp channels would...
be constitutively active in the absence of thermal, chemical, or mechanical stimuli (Honore et al., 2002), which might simultaneously impact Na, or endogenous channels. Notably, oocytes injected with RNA for Na, channels and only TREK-1 K+ channels demonstrated reliable APs (Fig. 3). Compared with oocytes expressing the slowly activating Kv7.2/7.3 channels, the expression of constitutively active TREK channels resulted in faster APs, although they lacked an undershoot after membrane repolarization (Fig. 3 A). TREK-1 conductances influenced the duration of the AP depolarization; as K+ current amplitude increased, the AP half-width decreased (Fig. 3 B). As described previously for large Na+ currents recorded in TEVC, large TREK-1 K+ currents introduced error in the voltage clamp recording, and so the values reported for TREK-1 current amplitudes (Fig. 3 B, inset; and Fig. 3 D) are estimates, with the largest values possessing the greatest error.

Increasing expression of Ksp channels also increased the magnitude of the polarization of the V REST (Fig. 3 C). Of course, the oocyte V REST measured in TEVC is expected to be more depolarized than the true V REST because two micropipettes are penetrating and thus damaging the membrane. Despite this drawback, under the same conditions of measurement, the impact of Ksp channels on V REST in Xenopus oocytes is similar to that of voltage-gated channels, with Kv7.2/7.3 channels used for comparison (Fig. 3 C). Fig. 3 D plots all of the V REST data for oocytes expressing TREK-1 (from the box plot in Fig. 3 C) so that the relationship between the variable expression of TREK-1 currents and V REST may be determined. Based on the impact of TREK-1 expression on AP duration and V REST, Ksp Channels support AP firing by facilitating Na+, channel deactivation and minimizing inactivation, even in the absence of exogenous K+ channels.

K+ channel expression enhances excitability in Xenopus oocytes

We also studied the impact of K+ channels on AP generation in oocytes (Fig. 4). We coexpressed Na+ channels with ShakerΔ plus K1,2,1 and used the loose-clamp approach to deliver depolarizing current ramps, which elicited a series of APs (Fig. 4 A). The APs eventually ceased because of the cumulative inactivation of Na+ channels. Block of K+ channels by external Ba2+ (30 or 100 µM) reduced the latency to fire APs and, sometimes, increased the number of APs fired during the current ramp (Fig. 4, B–D). This occurred with a reversible Ba2+-dependent depolarization of the V REST (Fig. 4 E). Using TEVC, we confirmed the presence of a
Ba²⁺-sensitive Kᵥ current in these cells by elevating the external K⁺ concentration from 2 mM to 10 mM Kₒ (Fig. 4 F). We tested if the enhanced excitability following Ba²⁺ block of Kᵥ channels could be explained by a change in threshold (VTHR), but we found no evidence for this (Fig. S5). These results highlight the exquisite sensitivity of AP generation to the difference between VREST and threshold for the all-or-none response.

**An additive approach to studying the impact of Kᵥ channel diversity on APs**

A diversity of AP waveforms could be achieved using the expression of different channel types (Fig. 5). When Naᵥ channel RNA was coinjected with only a Kᵥ4-type of K⁺ channel RNA, the oocyte APs were long and lacked a detectable undershoot after membrane repolarization (Fig. 5 A). The rapidly activating and inactivating Kᵥ4 channels did not confer a rapid phase of repolarization, and the K⁺ conductance was not sustained long enough to contribute to an undershoot. However, when Naᵥ channel RNA was coinjected with only a Kᵥ7-type of K⁺ channel RNA, the oocyte APs were shorter in duration and demonstrated a prominent undershoot after membrane repolarization (Fig. 5, B and E). By comparison to Kᵥ4, the more sustained activity of the Kᵥ7 channels would be expected to contribute more to the repolarization and afterhyperpolarization. The combined expression of Kᵥ4 and Kᵥ7 types of channels gave rise to an intermediate-duration AP with a prominent undershoot. The kinetics of the Kᵥ7 channel seem to dominate over the Kᵥ4 channel, perhaps because the Kᵥ4 currents are more transient. Neither Kᵥ4 nor Kᵥ7 K⁺ currents, alone nor combined, were sufficient to generate a fast AP with kinetics similar to neuronal APs. However, coexpression of Naᵥ channels with ShakerΔ resulted in fast APs (Fig. 5 D). When combined with the Kᵥ7 type of channels, the impact of ShakerΔ predominates and the APs are fast (Fig. 5 F). Importantly, when studying the additive effects of different K⁺ conductances on AP properties, the K⁺ channel currents were also observed in TEVC so that the presence of each current could be confirmed for each unique RNA. In sum, our results demonstrate the ability to induce diverse APs in oocytes, and future studies could use this additive approach to study in greater detail the contributions of different channel types to the AP waveform.

**Can oocytes generate repetitive AP firing?**

Oocytes had the capacity to generate multiple APs in response to sustained stimuli, multiple stimuli, or in some cases, no direct stimulus. For example, very high expression of Naᵥ channels along with very high expression of K⁺ channels could result in repetitive firing.
in spontaneous APs, as the $V_{\text{m}}$ hovered near the membrane’s threshold for firing (Fig. 6 A). Combinations of $K_{\text{v}}2.1$ plus ShakerΔ (Fig. 6 A) supported spontaneous APs. High expression of $K_{\text{v}}7$ channels could also support spontaneous APs, but with a different firing rate (Fig. 6 B). In addition, delivery of a sustained, suprathreshold depolarizing pulse from a loose clamp could excite a train of APs in oocytes expressing $K_{\text{v}}2.1$ plus ShakerΔ (Fig. 6 C). In sum, AP patterns induced in Xenopus oocytes represent an emergent property of the expression and interactions of a variety of ion channel types.

**Discussion**

The determinant of AP formation is the high expression of voltage-gated Na$^+$ and K$^+$ channels in a spatially compact and restricted area such as in the axon initial segment (Kole and Stuart, 2012). The high expression of Na$^+$ and K$^+$ channels in Xenopus oocytes affirmed these minimal channel requirements for inducing a rapid, all-or-none, regenerative electrical event. The findings in this paper extend the work of Shapiro et al. (2012), who demonstrated the ability of infrared radiation to depolarize a cell, and of Corbin-Leftwich et al. (2016), who studied the impact of a Kv7 modulator on cellular excitability. Unique to the present study is the assessment of membrane potentials generated in loose clamp by high expression of Na$^+$ channels (Fig. 2), examination of the role of non-voltage-gated K$^+$ conductances in the genesis of APs (Figs. 3, 4, and S5), demonstration of the diversity of AP waveforms that can be generated in oocytes (Figs. 5 and 6), and documentation of reliable methods necessary to achieve the AP recordings (Figs. 1, S1, S2, and S3). Collectively, these findings provide valuable information about the parameters of APs induced in oocytes that can be used in diverse research and educational settings.

Notably, our study used skeletal muscle Nav1.4 α subunits, because plasmid DNA constructs for neuronal Na$^+$ channels are remarkably unstable in bacterial hosts. However, if methods are successfully used to prepare neuronal Na$^+$ plasmid DNA without rearrangements or uncontrolled mutations (Feldman and Lossin, 2014), it is reasonable to expect that neuronal Na$^+$ channels (e.g., Na$^+$,1.1, 1.2, and 1.6) could be used to generate APs in oocytes as well. This remains to be tested in future studies.
In neurons, the axon hillock (Fuortes et al., 1957) and the axon initial segment (Araki and Otani, 1955) are sites of AP initiation (Colbert and Johnston, 1996), although this functional polarization may be a vertebrate specialization (Kole and Stuart, 2012). The unique role of these structures is governed by the very high expression of voltage-gated Na⁺ and K⁺ channels in a spatially restricted area. Although there are differences in the channel density and geometry of the neuronal structures and the Xenopus oocyte, a requirement for very high expression of Na⁺ channels is a cornerstone of the present study. The genesis of all-or-none events that resemble APs in excitable cells required the coexpression of at least one type of voltage-gated or non-voltage-gated K⁺ conductance. Indeed, coexpression of the TREK-1 type of K2P channels with Nav channels was sufficient to allow rapid APs (Fig. 3). These results compare favorably with the conclusions from a mixed computer simulation/biological experiment in which virtual Na⁺ currents were introduced to HEK cell patch-clamp recordings with biologically expressed K2P channels to assess the ability to fire APs without the introduction of voltage-gated K⁺ channels (MacKenzie et al., 2015). Although background Kv channels may have been present in the HEK cells, and the study examined in silico but not biological Na⁺ channels, K2P channels generated the hyperpolarized VREST needed for APs, and they contributed to repolarization of the AP (MacKenzie et al., 2015). Likewise, in cerebellar Purkinje cells, high expression of K2P channels supports rapid AP firing (Brickley et al., 2007). The results of the present study support the hypothesis that the minimal requirement for an AP includes a sufficiently large K⁺ conductance, although it need not be a voltage-activated conductance. Mechanistically, our results suggest that a Kᵦ conductance is sufficient to promote the deactivation of Na⁺ channels, at least for the capability of firing single APs.

Kᵦ channels have a complex relationship with cellular excitability. Neurons with naturally large Kᵦ currents tend to have strongly hyperpolarized VREST values (Hibino et al., 2010), which may suppress excitability (Leao et al., 2012; Li et al., 2013), and genetic overexpression of Kᵦ channels can be used to silence neuronal electrical activity (Nitabach et al., 2002). Correspondingly, genetic inhibition of Kᵦ.2.1 reduces membrane hyperpolarization and promotes rhythmic firing in ventricular myocytes (Miake et al., 2002). The dampening of excitability by Kᵦ channels may seem paradoxical, because membrane hyperpolarization promotes recovery from inactivation of Na⁺ channels. However, a membrane that is strongly hyperpolarized at rest may not generate a large enough depolarization in a spatially restricted area to engage the regenerative cycle of depolarization and Na⁺ channel activation, even when Na⁺ channels are available. Kᵦ channels thus influence cellular excitability not simply by maintaining a hyperpolarized VREST or altering VTH, but by regulating the relationship between VREST and Na⁺ channel availability (Figs. 4 and S5).

Kᵦ channel expression promoted spontaneous firing (Fig. 6 A) and high-frequency firing in response to long current injections (Fig. 6 C), probably by finely regulating the Na⁺ channel availabil-
An unexpected benefit that we experienced in exploring this AP model is the facilitation of student learning about the properties of APs. Undergraduate and graduate students alike benefit from the hands-on recording and analysis of APs, an experience that is not tractable for many students when using cultured cells or brain slices and patch-clamp methods. The approach offers the enjoyment of interacting with the data in real time and in a living cell; the ability to use the model for hypothesis-testing promotes inquiry-based learning, which deepens understanding and increases knowledge retention (Kober, 2015; Waldrop, 2015). The minimal AP model can certainly help to clarify common misconceptions about APs. For example, students, faculty, and researchers alike often believe that the value of −50 or −55 mV (as shown in textbooks) is the value of “threshold” in all cells. This minimal AP model, therefore, could certainly help investigators recognize the impact of ion channel composition and resting potential on threshold and appreciate that it is neither a set value for every excitable cell nor invariable in an individual cell. Academics also often believe that all neurons fire APs. The present approach helps students formulate their own explanation for why certain neurons do not show spiking behavior in native systems (Butler et al., 1992; Baden et al., 2013) and what is distinctive about an excitable membrane. This helps encourage discussion and understanding about how channel localization and clustering may affect AP firing and facilitates appreciation for the structure and function of the axon hillock, initial segments, and nodes of Ranvier.

Figure 6. Oocyte recordings may demonstrate multiple APs. 3 d before AP recordings, oocytes were injected with a mixture of 10 ng Na1.4 α and 2.5 ng Na1.4 β plus coexpressing K+ channel RNAs, as noted. (A) Spontaneous APs in oocytes coexpressing ShakerΔ (0.65 ng RNA) and Kv 2.1 (10 ng RNA). This is representative of four similar recordings from oocytes injected with the same mixture of RNAs. (B) Oscillatory APs were also spontaneously evoked in oocytes coexpressing Kv7.1 (2.5 ng RNA). This is representative of five similar recordings from oocytes injected with the same mixture of RNAs. (C) High-frequency AP firing in oocytes coexpressing ShakerΔ (0.65 ng RNA injected) and Kv 2.1 (10 ng) generated by a 1-s suprathreshold depolarizing stimulus applied from a loose clamp. Representative of four cells.
In conclusion, this work demonstrates reliable, useful, and simple approaches to induce APs in Xenopus oocytes with many applications for research and teaching.

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