Activation of InsP$_3$ receptors is sufficient for inducing graded intrinsic plasticity in rat hippocampal pyramidal neurons

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Inositol 1,4,5-trisphosphate (InsP$_3$) receptors (InsP$_3$R), activated by their endogenous agonist InsP$_3$ are calcium release channels that critically contribute to the excitatory nature of neuronal endoplasmic reticulum (ER) (Berridge 2009; Verkhratsky 2005). Cytosolic mobilization of InsP$_3$ and the subsequent activation of InsP$_3$Rs play important roles in several physiological processes including synaptic plasticity, calcium waves, calcium homeostasis, gene transcription, and integration of intraneuronal biochemical signals (Berridge 2002; Berridge et al. 2000; Choe and Ehrlich 2006; Foskett et al. 2007; Kato et al. 2012; Ross 2002; Berridge et al. 2000; Choe and Ehrlich 2006; Foskett et al. 2007; Kato et al. 2012; Taylor and Tovey 2010). The synaptic plasticity literature has focused on establishing necessity and sufficiency as two essential features in causally relating a signaling molecule to plasticity induction, an approach that has been surprisingly lacking in the intrinsic plasticity literature. Consequently, we found that this InsP$_3$-induced plasticity depended on hyperpolarization-activated cyclic nucleotide-gated channels. Moreover, this calcium-dependent form of plasticity was critically reliant on the release of calcium through InsP$_3$Rs, the influx of calcium through N-methyl-D-aspartate receptors and voltage-gated calcium channels, and on the protein kinase A pathway. Our results delineate a causal role for InsP$_3$Rs in graded adaptation of neuronal response dynamics, revealing novel regulatory roles for the endoplasmic reticulum in neural coding and homeostasis.

endoplasmic reticulum; HCN channels; hippocampus; inositol trisphosphate receptors; intrinsic plasticity

INOSITOL 1,4,5-TRISPHOSPHATE (InsP$_3$) receptors (InsP$_3$R), activated by their endogenous agonist InsP$_3$, are calcium release channels that critically contribute to the excitatory nature of neuronal endoplasmic reticulum (ER) (Berridge 2009; Verkhratsky 2005). Cytosolic mobilization of InsP$_3$ and the consequent activation of InsP$_3$Rs play important roles in several physiological processes including synaptic plasticity, calcium waves, calcium homeostasis, gene transcription, and integration of intraneuronal biochemical signals (Berridge 2002; Berridge et al. 2000; Choe and Ehrlich 2006; Foskett et al. 2007; Patterson et al. 2004; Rose and Konnerth 2001; Ross 2012; Taylor and Tovey 2010; Verkhratsky 2005). InsP$_3$R-related signaling mechanisms are extremely complex, with several upstream activators, subtype-dependent modulation by cytosolic calcium, structural and functional interactions involving numerous calcium sources and ion channels, state-depen-
to activate numerous intracellular signaling pathways (Berridge 2002; Cahalan 2009; Lefkimmiatis et al. 2009; Mattson et al. 2000; Verkhratsky 2005), activates a putative neuroprotective response by reducing neuronal excitability. Although this study demonstrated the necessity of InsP3R in inducing a form of intrinsic plasticity, the sufficiency of InsP3R activation for altering neuronal intrinsic properties has remained unexplored. More generally, especially compared with the more extensive synaptic plasticity literature, the causal delineation of signaling components associated with intrinsic plasticity, by employing necessity and sufficiency as two distinct and essential tools, has been surprisingly lacking.

To fill this lacuna, and to disambiguate the precise roles of cytosolic InsP3 in neurophysiology, we asked if direct injection of InsP3 into hippocampal pyramidal neurons was sufficient to alter intrinsic response properties. Our results demonstrate that direct activation of InsP3Rs is sufficient to induce long-lasting plasticity in neuronal intrinsic response dynamics and uncover the signaling mechanisms underlying this plasticity. Importantly, we also show that this InsP3-induced form of plasticity is graded, whereby lesser activation of InsP3Rs led to lesser amount of plasticity, suggesting potential roles for this form of plasticity under physiological conditions. Together with and complementary to the earlier demonstration of the necessity of InsP3Rs in inducing a form of intrinsic plasticity (Narayanan et al. 2010), our demonstration that the specific activation of InsP3Rs is sufficient to induce graded intrinsic plasticity provides direct causal evidence for novel roles of InsP3 and intracellular stores in regulating neuronal integration, neural coding, and homeostasis through changes in intrinsic neuronal properties.

MATERIALS AND METHODS

Ethical approval. All experiments reported in this study were performed in strict adherence to the protocols cleared by the Institute Animal Ethics Committee of the Indian Institute of Science (Bangalore, India) and The University of Texas at Austin Institutional Animal Care and Use Committee. Experimental procedures were similar to previously established protocols (Narayanan et al. 2010; Narayanan and Johnston 2007, 2008) and are detailed below.

Surgery and slice preparation. Male Sprague-Dawley rats (4–10 wk old) were anesthetized by intraperitoneal injection of a combination of ketamine and xylazine. After onset of deep anesthesia, as determined by cessation of toe-pincher reflex, rats were transcardially perfused with ice-cold cutting solution containing (in mM) 210 sucrose, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 0.5 CaCl2, 7 MgCl2, 7 dextrose, and 3 sodium pyruvate (all from Sigma Aldrich). They were then decapitated, and the brain was removed quickly in the presence of ice-cold cutting solution. Near-horizontal slices (350 µm) were prepared from the hippocampus, using a VT1000P vibratome (Leica), while submerged in oxygenated ice-cold cutting solution. The slices were incubated for 15–25 min at 34°C in a holding chamber containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 2 CaCl2, 2 MgCl2, 10 dextrose, and 3 sodium pyruvate and then at room temperature for at least 1 h before recording. The holding chamber was continuously carbogenated with a mixture of 95% O2 and 5% CO2 gas.

Electrophysiology. Slices were visualized under a ×63 water-immersion lens through a Dohr contrast microscope (Carl Zeiss Axioexaminer). Somatic whole cell current-clamp recordings were made from CA1 pyramidal neurons using a Dagan BVC-700A amplifier. Data acquisition was done using custom-written software in the Igor Pro environment (Wavemetrics), with signals sampled at 10 kHz. During the entire course of the experiments the slices were perfused with carbogenated artificial cerebrospinal fluid (ACSF) at ~34°C containing (in mM) 125 NaCl, 3 KCl, 1.25 NaH2PO4, 25 NaHCO3, 2 CaCl2, 1 MgCl2, and 10 dextrose. Borosilicate glass electrodes, pulled (P-97 Flaming/Brown micropipette puller; Sutter) from capillaries of 1.5-mm outer diameter and 0.86-mm inner diameter (Sutter), with resistance of 3–7 MΩ were used for patch-clamp recordings. The intracellular pipette solution contained (in mM) 120 K-glucuronate, 20 KCl, 10 HEPES, 4 NaCl, 4 MgATP, 0.3 Na3-GTP, and 7 K₂-phosphocreatine, pH 7.3 with KOH. Series resistance was monitored and compensated online using the bridge-balance circuit of the amplifier. Experiments were discarded only if the initial resting membrane potential was more depolarized than ~60 mV, if series resistance rose above 30 MΩ, or if there were fluctuations in temperature during the course of the experiment. Unless otherwise stated, experiments were performed at the initial resting membrane potential of the cell. Voltages have not been corrected for the liquid junction potential, which was experimentally measured to be ~8 mV. Control recordings were performed at regular intervals to ensure that there were no time-dependent changes (over a 45-min period) in any of the intrinsic properties when electrophysiological recordings were performed in the absence of any pharmacological agent.

Pharmacological agents. Drugs used in the experiments were d-nyo-InsP3 (InsP3, Sigma Aldrich or Tocris Bioscience), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 10 µM (+)-bicuculline, 10 µM picrotoxin, 50 µM dl-2-amino-5-phosphonovaleric acid (d,l-APV), and 2 µM CGP55845 (all synaptic blockers from Allied Scientific), 50 µM NiCl2 (Sigma-Aldrich), 10 µM nimodipine (Tocris Bioscience), 1 mg/ml heparin (20,000–25,000 molecular weight; Calbiochem), 20 µM ZD7288 (Tocris Bioscience), 20 mM BAPTA (Life Technologies), 20 µM PKA inhibitor (PKI) (14-22) amide, myristoylated (PKAi peptide; Tocris Bioscience), and 500 nM KT5720 (Tocris Bioscience). For experiments with ZD7288, slices were first incubated in ACSF containing 100 µM ZD7288 for 10 min before the start of the recordings. Necessary care was taken and appropriate controls performed for each of the drugs used to ensure that there were no time-dependent changes initiated by just the presence of the drug in the bath or pipette. Through the course of the study, experiments with only 10 µM InsP3 were interleaved with experiments where any of the other pharmacological agents were also applied. This was to ensure that the InsP3 stock was not degraded and elicited the same levels of plasticity in the absence of the drug.

Data analysis. Physiologically relevant measurements from the recordings were computed by employing well-established analysis procedures (Narayanan et al. 2010; Narayanan and Johnston 2007, 2008). Specifically, for measuring input resistance (Rin), the response of neurons to pulse-current injection was analyzed and its steady-state voltage deflection plotted against the injected current to get the current-voltage (I–V) curve. It was fitted with a straight line whose slope formed the Rin of that neuron. α-Excitatory postsynaptic potentials (α-EPSPs) were evoked by current injections of the form I(t) = I0 + α(t − α), with α = 0.1 ms⁻¹. Temporal summation (Sα) was analyzed as the ratio of the first α-EPSP amplitude to the fifth α-EPSP amplitude from the voltage response to five α-excitatory postsynaptic current injections at 20 Hz. A linear chirp current (see Fig. 1B) spanning 15 Hz in 15 s was employed to estimate local α-EPSP (an important measure of frequency-dependent neuronal excitability (Narayanan and Johnston 2008)). The choice of the chirp rate at 1 Hz/s (15 Hz in 15 s) was dictated by experiments that demonstrated that a further increase in the chirp rate did not enhance the accuracy of the impedance-based measurements (Narayanan and Johnston 2007). To get the impedance amplitude profile (ZAP), the Fourier transform of voltage response of the neuron to the chirp stimulus was divided by the Fourier transform of injected current (Fig. 1F). The frequency at which this impedance amplitude profile reached
its maximum ($|Z|_{\text{max}}$) formed the resonance frequency ($f_{\text{r}}$). Resonance strength ($Q$) was defined as the ratio of $|Z|_{\text{max}}$ to the impedance magnitude at 0.5 Hz (Fig. 1F). The impedance phase profile (ZPP) was computed as the phase of the ratio of Fourier transform of voltage response to Fourier transform of the chirp stimulus. Total inductive phase ($\Phi_\text{L}$) was defined as the area under the inductive part of the ZPP. All data analyses were performed using custom-written software in Igor Pro (Wavemetrics), and statistical analyses were performed using the R computing package (http://www.r-project.org/).

RESULTS

The main objective of this study was to assess the specific role of InsP3 in altering intrinsic response dynamics (IRD) of CA1 pyramidal neurons. To fulfill this, somatic whole cell current-clamp recordings from these neurons were performed with different concentrations of d-myo-InsP3 in the recording pipette (Fig. 1, A and B). At the start and the end of the experiment, current pulses ranging from −50 to +50 pA (for 700-ms duration), with an increment of 10 pA, were injected to obtain the steady-state voltage response of the neuron. The response of the neurons to five α-excitatory postsynaptic currents (α-EPSCs) was also measured at these time points. For 45 min after the initial measurements, the response of the neuron to a chirp stimulus spanning 15 Hz in 15 s was continuously (2 per min) monitored (Fig. 1B). A hyperpolarizing test pulse of 100 pA was appended to the chirp stimulus to obtain an estimate of the input resistance. The neuron was maintained in subthreshold voltages through the entire course of the experiment to avoid synergistic interaction between backpropagating action potentials and InsP3 in the pipette (Ross 2012).

Inclusion of InsP3 was sufficient to induce persistent plasticity in neuronal intrinsic response dynamics. We recorded several measurements of neuronal IRD through the course of our experiment with 10 μM d-myo-InsP3 included in the pipette (Fig. 1). We found that the inclusion of InsP3 in the
The experiment with 10 tent for the 45-min time course of the experiment. We repeated temporal summation (InsP3-induced changes revealed a monotonic reduction in inductive phase (ΦP; Fig. 1H), accompanied by a reduction in maximal impedance amplitude (|Z|max; Fig. 1, E and F) and temporal summation (SRc; Fig. 1f). The temporal progression of InsP3-induced changes revealed a monotonic reduction in Rin (Fig. 1D) and a corresponding increase in fR (Fig. 1G), persistent for the 45-min time course of the experiment. We repeated the experiment with 10 μM d-myo-InsP3 included in the pipette for several neuronal recordings and found significant plasticity across all these measurements to be consistent across neurons (Fig. 2 and Table 1). Note that although InsP3 was present in the pipette through the course of the experiment, it is expected that cytosolic enzymes metabolize exogenously applied InsP3 (Berridge and Irving 1989; Irving and Schell 2001; Shears 1989), thereby restricting the period of InsP3R activation by exogenously applied InsP3.

InsP3-induced plasticity of IRD was graded and was dependent on InsP3 concentration. Although the results with 10 μM d-myo-InsP3 report its sufficiency to induce intrinsic plasticity in hippocampal neurons, we did not know if this form of plasticity was a graded or an all-or-none phenomenon. To address this, we repeated our experiment with various concentrations of InsP3 in the recording pipette and found that the magnitude of InsP3-induced plasticity in all IRD measurements was dependent on the concentration of InsP3 ([InsP3]). Specifically, increasing [InsP3] in the recording pipette significantly increased the percentage changes in Rin (Fig. 2, A, B, and E) and fR (Fig. 2, C–E), apart from imparting similar increases across other IRD-related measurements including sag ratio (Fig. 2F). These results suggested that InsP3-induced plasticity was graded, thereby pointing toward a potential role for InsP3 mobilization under physiological conditions in altering neuronal response properties.

InsP3-induced plasticity was expressed through changes in HCN channel properties. Which ion channel mediated the expression of InsP3-induced plasticity of IRD? The direction of

![Figure 2](https://example.com/fig2.png)

**Fig. 2.** InsP3-induced plasticity in intrinsic response dynamics was graded, with the gradation dependent on InsP3 concentration ([InsP3]i in the recording pipette. A: population plots of Rin measured at the beginning (blue) and the end (orange) of experiments where different [InsP3] were included in the recording pipette. The color codes for [InsP3] are as follows: cyan, 10 nM (n = 8); green, 100 nM (n = 6); red, 1 μM (n = 6); and black, 10 μM (n = 6). B: time courses of normalized Rin when experiments were performed with various [InsP3] in the recording pipette. C: population plots of fR measured at the beginning (blue) and the end (orange) of experiments where different [InsP3] were included in the recording pipette. D: time courses of normalized fR when experiments were performed with various [InsP3] in the recording pipette. E: %change in Rin and fR (at the end of the experiment, with reference to the beginning) plotted as a function of [InsP3]i in the recording pipette. For A–E, data are means ± SE and P values (when presented) are from paired Student’s t-tests. F: summary plot of %change in various measurements (from their respective baseline values) after 45 min into the recording with various [InsP3]. Data are medians and quartiles. *P < 0.05, Mann-Whitney test.

### Table 1. Measurements sensitive to changes in HCN channels

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Initial</th>
<th>45 Min</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP, mV</td>
<td>60.9 ± 0.4</td>
<td>56.3 ± 1.3</td>
<td>3.4 × 10⁻³</td>
</tr>
<tr>
<td>Rin, MΩ</td>
<td>76.0 ± 5.0</td>
<td>49.5 ± 5.8</td>
<td>5.1 × 10⁻¹</td>
</tr>
<tr>
<td>Sag, %</td>
<td>19.7 ± 2.2</td>
<td>22.9 ± 2.5</td>
<td>2.6 × 10⁻¹</td>
</tr>
<tr>
<td>fR, Hz</td>
<td>4.13 ± 0.3</td>
<td>5.77 ± 0.4</td>
<td>6.9 × 10⁻¹</td>
</tr>
<tr>
<td>Q</td>
<td>1.2 ± 0.05</td>
<td>1.4 ± 0.09</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>max, MΩ</td>
<td>80.6 ± 5.0</td>
</tr>
<tr>
<td>fL, rad·Hz</td>
<td>0.14 ± 0.02</td>
<td>0.12 ± 0.05</td>
<td>0.024</td>
</tr>
<tr>
<td>S*, %</td>
<td>1.14 ± 0.04</td>
<td>0.94 ± 0.05</td>
<td>1.4 × 10⁻¹</td>
</tr>
</tbody>
</table>

Values are means ± SE of measurements sensitive to changes in HCN channels, determined at the beginning (initial) and after 45 min of experiments where 10 μM inositol trisphosphate was included in the recording pipette. P values are reported for the paired Student’s t-test. RMP: resting membrane potential; Rin: input resistance; fR: resonance frequency; Q: resonance strength; |Z|max: maximum impedance amplitude; fL: total inductive phase; and S*, summation of α-excitatory postsynaptic potentials.
plasticity in the physiological measurements that we employed for characterizing neuronal response dynamics, along with their well-established sensitivity to HCN channels (Brager and Johnston 2007; Brager et al. 2013; Clemens and Johnston 2014; Fan et al. 2005; Magee 1998; 2000; Narayanan et al. 2010; Narayanan and Johnston 2007, 2008), pointed us to the hypothesis that the expressed form of plasticity is dependent on HCN channels. Furthermore, depletion of intracellular calcium stores is known to induce plasticity in HCN channels, through an InsP3,R-dependent mechanism (Clemens and Johnston 2014; Narayanan et al. 2010). Against this background, we tested our hypothesis that changes in HCN channels mediate the expression of plasticity in the presence of the channel blocker ZD7288 (Gasparini and DiFrancesco 1997; Magee 1998; Narayanan et al. 2010; Narayanan and Johnston 2007). We found that the InsP3-induced plasticity of IRD was abolished when recordings were performed in the presence of 20 μM ZD7288 in the recording pipette (Fig. 3). Specifically, there was no significant change in $R_n$ in the presence ZD7288, and it remained close to its initial value through the course of the experiment (Fig. 3, A–C). Additionally, there were no significant changes in $S_o$ (Fig. 3D) and the impedance amplitude and phase profiles (Fig. 3, E–G), consequently abolishing plasticity in $f_0$, $Q$, and $|Z|_{max}$ as well (Fig. 3, H–J). When we compared the percentage changes in various measurements in the presence of ZD7288, we found that they were significantly different from measurements in the control experiments where ZD7288 was absent (Fig. 3J). Together, plasticity in physiologically relevant measurements sensitive to changes in HCN

![Figure 3](https://example.com/fig3.png)

Fig. 3. InsP3-induced plasticity was abolished in the presence of ZD7288. Experiments depicted were performed with 10 μM InsP3 and 20 μM ZD7288 ($n = 8$) in the recording pipette. A: voltage response of a representative neuron to the pulse-current injections at the beginning (blue) and after 45 min (orange) of the experiment. $R_n$ values obtained from these traces are also shown. B: population plots of $R_n$ measured at the beginning (blue) and the end (orange) of experiments performed in the presence of ZD7288. C: time course of normalized $R_n$ in the presence (green) and absence (black; control) of ZD7288. D: voltage response of the neuron to the injection of a sequence of 5 alpha currents (20 Hz) at the beginning (blue) and the end (orange) of the experiment. The $S_o$ values are shown. E: voltage response of the neuron to the chirp stimulus at the beginning (blue; 0–5 min average) and the end (orange; 40–45 min average) of the experiment. F and G: impedance amplitude (F) and phase (G) profiles obtained from traces shown in E, also depicting the measurements obtained from these profiles: $f_0$, $|Z|_{max}$, $Q$, and $\Phi_1$. H: population plots of $f_0$ measured at the beginning (blue) and the end (orange) of experiments performed in the presence of ZD7288. I: time course of $f_0$ in the absence (black; top) and presence of ZD7288 (green; bottom). For B and C and for H and I, data are means ± SE and $P$ values (when presented) are from paired Student’s $t$-tests. J: summary plot of %change in various measurements (from their respective baseline values) after 45 min into the recording in the presence of ZD7288. Data are medians and quartiles. *$P < 0.05$, Mann-Whitney test.
channels (Fig. 2), coupled with pharmacological evidence through the use of ZD7288 (Fig. 3), provide clear lines of evidence in favor of our hypothesis that InsP₃-induced plasticity in IRD is mediated by changes in HCN channels.

InsP₃-induced plasticity of IRD was dependent on the elevation in cytosolic calcium concentration. Cytosolic InsP₃ is metabolized into different phosphate derivatives by a variety of cytosolic enzymes (Berridge and Irvine 1989; Irvine and Schell 2001), and there are several structural interactions between InsP₃ receptors and other signaling molecules (Fagni et al. 2000; Kato et al. 2012; Kennedy 2000). Furthermore, given the fast degradation of InsP₃ within the cell and the similarity of the time course of changes with depletion-induced plasticity in HCN channels (Brager et al. 2013; Clemens and Johnston 2014; Narayanan et al. 2010), we postulated that InsP₃-induced changes in the intrinsic response dynamics was plasticity consequent to an initial surge of calcium. Against this, is plasticity in IRD a consequence of InsP₃R-induced elevation in cytosolic calcium levels, or is it a consequence of some structural interactions or due to activation of calcium-independent biochemical signaling pathways such as those associated with phosphate derivatives of InsP₃ (Harwood 2005)? To answer this, we repeated our plasticity protocol (Fig. 1B) in the presence of 20 mM BAPTA in the recording pipette and found that InsP₃-induced plasticity of IRD was abolished when cytosolic calcium was chelated by BAPTA (Fig. 4). Specifically, there was no significant change in either \( R_{in} \) or \( f_R \), which also reflected in their temporal progression (Fig. 4, A–D), and in other IRD measurements (sag, \( Q \), \( |Z|_{max} \), \( S_{\alpha} \), and \( \Phi_L \); Fig. 4E). Together, these observations established that InsP₃-induced plasticity of IRD required an elevation in the cytosolic calcium concentration and was not due to some calcium-independent nonspecific effects of introducing InsP₃.

Plasticity in IRD was mediated by cytosolic influx of calcium through InsP₃Rs, with contributions from NMDA receptors and voltage-gated calcium channels. What sources contributed to the cytosolic calcium influx that resulted in InsP₃-induced plasticity in IRD? Apart from InsP₃Rs being the obvious candidate, synergistic interactions between several calcium sources (Berridge 2002; Berridge et al. 2000; Choe and Ehrlich 2006; Clemens and Johnston 2014; Narayanan et al. 2010; Ross 2012; Verkhratsky 2005) coupled with structural interac-

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**Fig. 4.** InsP₃-induced plasticity was abolished in presence of BAPTA. Experiments depicted were performed with 10 µM InsP₃ and 20 mM BAPTA (\( n = 5 \)) in the recording pipette. A: population plots of \( R_{in} \) measured at the beginning (blue) and the end (orange) of experiments performed in the presence of BAPTA. B: time course of normalized \( R_{in} \) in the presence (green) and absence (black; control) of BAPTA. C: population plots of \( f_R \) measured at the beginning (blue) and the end (orange) of experiments performed in the presence of BAPTA. D: time course of normalized \( f_R \) in the absence (black; top) and presence of BAPTA (green; bottom). For A–D, data are means ± SE and \( P \) values (when presented) are from paired Student’s t-tests. E: summary plot of %change in various measurements (from their respective baseline values) after 45 min into the recording in the presence of BAPTA. Data are medians and quartiles. \(* P < 0.05\), Mann-Whitney test.
tions between InsP$_3$Rs and other signaling molecules provide further routes for cytosolic calcium influx. From the perspective of interactions, InsP$_3$Rs are linked to PSD-95 and NMDA receptors (NMDARs) through various scaffolding proteins, and structural coupling and functional interactions between InsP$_3$Rs and voltage-gated calcium channels (VGCC) apart from several other signaling molecules are well established (Choe and Ehrlich 2006; Fagni et al. 2000; Foskett 2010; Kennedy 2000; Patterson et al. 2004). Therefore, we systematically tested the role of several calcium sources in mediating InsP$_3$-induced plasticity in IRD.

First, to assess the role of InsP$_3$Rs in mediating the plasticity, we repeated our experiments in the presence of 1 mg/ml heparin, a selective blocker of InsP$_3$. Incorporation of heparin in the recording pipette completely abolished the InsP$_3$ (10 μM)-induced plasticity in these neurons (Fig. 5, A–F), establishing that InsP$_3$-induced plasticity was a consequence of calcium release through InsP$_3$Rs. Whereas the demonstration of the dependence of depletion-induced intrinsic plasticity by blocking InsP$_3$Rs revealed the necessity of InsP$_3$Rs for inducing intrinsic plasticity (Narayanan et al. 2010), this abolishment of InsP$_3$-induced plasticity by InsP$_3$R blockers unveiled the sufficiency of InsP$_3$R activation for inducing intrinsic

![Fig. 5. InsP$_3$ receptors (InsP$_3$R), NMDA receptors (NMDAR), and voltage-gated calcium channels (VGCCs) contribute as calcium sources for InsP$_3$-induced plasticity. All experiments depicted were performed with 10 μM InsP$_3$ in the recording pipette. A and B: time courses of normalized $R_m$ (A) and $f_R$ (B) in the presence (green) and absence of 1 mg/ml heparin (black; control) in the recording pipette. C and D: summary plots of values of $R_m$ (C) and $f_R$ (D) at the beginning (blue) and at the end (orange) of experiments when various channels/receptors were blocked using specific pharmacological agents. For A–D, data are means ± SE. *P < 0.05, paired Student’s t-test. E and F: summary plots of values of % change in $R_m$ (E) and % change in $f_R$ (F) obtained when various channels/receptors were blocked using specific pharmacological agents (green) compared with the control (black) where no other pharmacological agent was employed. Data are medians and quartiles. **P < 0.05, Mann-Whitney test. Pharmacological agents indicated in C–F are defined as follows: InsP$_3$R, 1 mg/ml heparin in recording pipette (n = 6); AMPAR + GABAR, 10 μM (+)-bicuculline, 10 μM picrotoxin, 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione, and 2 μM CGP55485 in extracellular recording solution (n = 5); NMDAR, 50 μM 2-amino-5-phosphonovaleric acid (0.1-APV) in extracellular recording solution (n = 5); T Ca$_{2+}$ (T-type calcium channels), 50 μM NiCl$_2$ in extracellular recording solution (n = 5); L Ca$_{2+}$ (L-type calcium channel), 10 μM nimodipine in extracellular recording solution (n = 5); T+L Ca$_{2+}$, 50 μM NiCl$_2$ and 10 μM nimodipine in extracellular recording solution (n = 5). See text for definitions.

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plasticity. Next, we established that blocking AMPA and GABA receptors using a cocktail of pharmacological agents [10 μM CNQX, 10 μM (+)-bicuculline, 2 μM CGP55845, and 10 μM picrotoxin] did not have any effect on the InsP$_3$-induced plasticity, suggesting that baseline synaptic activity did not affect this form of IRD plasticity. However, blocking NMDARs using 50 μM D,L-APV, in the extracellular recording solution resulted in a differential effect on different IRD measurements, with a significant reduction in the magnitude of InsP$_3$-induced plasticity in $f_R$ but not in $R_{in}$ (Fig. 5, C–F). Although blocking L-type calcium channels (10 μM nimodipine in the extracellular recording solution) did not have any significant effect on the IRD plasticity, blocking T-type calcium channels (50 μM NiCl$_2$ in the extracellular recording solution) or both L- and T-type calcium channels resulted in a significant reduction of InsP$_3$-induced plasticity (Fig. 5, C–F). Together, these results suggested that InsP$_3$-induced plasticity was mediated by calcium influx through InsP$_3$R, with NMDARs and VGCCs providing additional modulatory effects on the plasticity.

**InsP$_3$-induced plasticity was dependent on the PKA signaling pathway.** Which downstream signaling pathway was responsible for the expression of InsP$_3$-induced plasticity? It has been previously reported that depletion of internal stores can activate the PKA pathway (LeKimmiatis et al. 2009) and induce an InsP$_3$-R dependent form of plasticity in HCN channels (Narayanan et al. 2010). Motivated by these, and to assess the role of the PKA pathway on InsP$_3$-induced plasticity in IRD, we repeated our protocol (Fig. 1B; 10 μM InsP$_3$) with two distinct PKA inhibitors. We found that inhibiting PKA, either by incorporating 20 μM PKAi in the recording pipette or by adding 500 nM KT5720 in the extracellular recording solution, significantly diminished the magnitude of plasticity in $f_R$ and $R_{in}$ (Fig. 6). These results underline a critical role for the PKA pathway in InsP$_3$-induced plasticity of IRD.

In summary, converging signaling mechanisms and similar plasticity in equivalent intrinsic measurements of depletion-induced (Narayanan et al. 2010) and InsP$_3$-induced forms of plasticity (Figs. 2–6) respectively establish necessity and sufficiency of InsP$_3$R’s for inducing intrinsic plasticity. Together, these results causally delineate specific roles for InsP$_3$Rs in inducing changes in neuronal intrinsic properties.

**DISCUSSION**

In this study, we demonstrated that specific activation of InsP$_3$Rs through their endogenous agonist InsP$_3$ is sufficient to induce long-lasting changes in neuronal intrinsic properties. Together with the earlier complementary demonstration of the necessity of InsP$_3$R’s for a form of intrinsic plasticity (Narayanan et al. 2010), this demonstration of sufficiency clearly demarcates a causal role of InsP$_3$Rs in regulating neuronal intrinsic properties, differentiating them from the activation of upstream signaling pathways that contribute to the mobilization of InsP$_3$. This is essential because the upstream signaling events, such as the activation of G protein-coupled receptors or store depletion, typically target several downstream mechanisms apart from the mobilization of InsP$_3$. Additionally, in establishing the graded nature of the plasticity by employing different concentrations of InsP$_3$, we also show that this InsP$_3$-induced form of plasticity is expressed through changes in HCN channels and is critically dependent on calcium release through InsP$_3$Rs and on the PKA pathway.

**Graded plasticity.** An important finding in the synaptic plasticity literature is that synaptic plasticity is not an all-or-none phenomenon but, instead, is graded (Berridge and Irvine 1989; Enoki et al. 2009; Montgomery and Madison 2002; O’Connor et al. 2005). Such activity- and state-dependent synaptic plasticity mechanisms allow neurons to operate over a large dynamic range, thereby increasing their information storage capacity (Montgomery and Madison 2002, 2004). In this context, a crucial finding from our study is that the InsP$_3$-induced plasticity in HCN channel properties is graded (Fig. 2). Plasticity in ion channels alters intrinsic response properties of a neuron and spatiotemporal integration of a neuron (Frick and Johnston 2005; Johnston and Narayanan 2008; Magee and Johnston 2005; Narayanan and Johnston 2012; Remy et al. 2010; Shah et al. 2010). Thus graded plasticity in HCN channels, in conjunction with graded synaptic plasticity, provides a neuron with multiple plasticity mechanisms, thereby immensely increasing the computational and storage capability of these neurons. Furthermore, because intracellular mobilization of InsP$_3$ can occur through several upstream signaling pathways, this diversity could contribute to differential mobilization of cytosolic InsP$_3$, leading to graded intrinsic plasticity under different physiological and pathophysiological conditions.

Several theoretical and experimental studies have explored the effect of specific levels of cytosolic calcium concentration on the polarity and magnitude of synaptic plasticity (Lisman 1989; Nishiyama et al. 2000; Shouval et al. 2002). In this work, we demonstrate that different concentrations of cytosolic InsP$_3$ and subsequent release of store calcium can induce graded plasticity in voltage-gated ion channels. Whereas graded synaptic plasticity is postulated to exhibit a biphasic dependence on cytosolic calcium, in striking contrast, our results show that intrinsic plasticity induced by different concentrations of cytosolic InsP$_3$ is not biphasic albeit being graded. Concurrent plasticity in synaptic and intrinsic properties has been hypothesized to play critical roles in neural coding, learning, memory, and homeostasis (Narayanan and Johnston 2012; Turrigiano 2011). With some forms of homeostatic mechanisms pointing toward modulation of intrinsic properties, it is imperative that graded forms of synaptic plasticity would require graded forms of intrinsic plasticity (Honnrueiah and Narayanan 2013; Narayanan and Johnston 2012; 2010; Turrigiano 2011). Our study suggests that such fine-tuning in intrinsic neuronal properties could be achieved by differential mobilization of store calcium through graded activation of InsP$_3$Rs. Whereas our results explore one form of calcium-dependent plasticity in intrinsic properties, other forms of calcium-dependent graded plasticity through changes in HCN channels through other pathways (Biel et al. 2009; Brager and Johnston 2007; Fan et al. 2005; Narayanan et al. 2010; Shah et al. 2010) and/or in other ion channels (Frick and Johnston 2005; Lujan et al. 2009; Magee and Johnston 2005; Remy et al. 2010; Shah et al. 2010; Turrigiano 2011) could play critical roles in maintaining homeostasis of physiological properties through collective forms of channelostasis (O’Leary et al. 2013; Rathour and Narayanan 2014; 2012a).

**Mechanisms.** Depletion of calcium stores by blocking sarco-(endo)plasmic reticulum Ca$^{2+}$-ATPase (SERCA) pumps results...
in changes in HCN channel properties (Brager et al. 2013; Clemens and Johnston 2014; Narayanan et al. 2010) mediated by calcium release through InsP₃Rs. Assessing the complementary sufficiency counterpart to these results on the necessity of InsP₃Rs for intrinsic plasticity, our study shows that calcium release through InsP₃Rs is sufficient to induce plasticity in HCN channels (Figs. 4 and 5). Whereas store depletion-induced plasticity could be regarded as a neuroprotective mechanism that expresses under several physiological instances (Berridge et al. 2000; Clemens and Johnston 2014; Narayanan et al. 2010; Ross 2012), potentially recruiting well-established structural interactions among InsP₃Rs, mGluRs, NMDARs, and VGCCs in this process (Fagni et al. 2000; Foskett et al. 2007; Kato et al. 2012; Naisbitt et al. 1999; Sala et al. 2001; Taylor and Tovey 2010; Tu et al. 1999; Tu et al. 1998; Xiao et al. 2000). Finally, InsP₃-induced plasticity was dependent on PKA activation (Fig. 6), similar to depletion-induced plasticity (Lefkimmiatis et al. 2009; Narayanan et al. 2010), suggesting a
convergent set of signaling pathways that contribute to depletion and InsP$_3$-induced plasticity.

Systematic investigations in the future should explore the diversity of signaling mechanisms involved in differential plasticity of HCN channels (Bender et al. 2007; Brager et al. 2013; Clemens and Johnston 2014; Fan et al. 2005; Narayanan et al. 2010; Narayanan and Johnston 2010; Shah et al. 2010), also accounting for specific localization of the various signaling molecules and their kinetics and binding interactions (Choe and Ehrlich 2006; Foskett et al. 2007; Kennedy et al. 2005; Koteleski and Blackwell 2010; Patterson et al. 2004; Rose and Konnerth 2001). In the context of our results, especially the differential signaling dependence of the different physiological measurements (Fig. 5–6), such investigations should also explore the specific impact of local dendritic release of calcium through InsP$_3$ receptors on localized plasticity of dendritic response properties (e.g., Frick et al. 2004; Losonczy et al. 2008), apart from systematically investigating the InsP$_3$R subunits involved in this form of plasticity (Hertle and Yeckel 2007; Nishiyama et al. 2000). Such analyses would provide a more holistic understanding on how intracellular stores alter neuronal response properties, on how localized ion channel plasticity could differentially alter measurements at various somatodendritic locations (Clemens and Johnston 2014; Narayanan et al. 2010; Rathour and Narayanan 2012b), and on how such plasticity mechanisms could synergistically contribute toward the maintenance of physiological homeostasis across the somatodendritic arbor (O’Leary et al. 2013, 2014; Rathour and Narayanan 2014; Turrigiano 2011).

Implications. Our results provide direct evidence for an InsP$_3$-induced reduction in neuronal gain, an increase in the optimal response frequency of the neuron, and a reduction in temporal summation of postsynaptic potentials (Fig. 2). Whereas these are consistent with established roles for HCN channels, there are other physiological implications for such plasticity in HCN channels. For instance, alterations in HCN channels would change the coupling across compartments through changes to transfer impedance (Cook et al. 2007; Hu et al. 2009; Kole et al. 2007; Ulrich 2002; Vaidya and Johnston 2013) and would change the intraneuronal synchronization frequency (Vaidya and Johnston 2013). Furthermore, changes in HCN channels can alter spike initiation dynamics, thereby allowing neurons to behave as coincidence detectors or as integrators (Das and Narayanan 2014). Finally, metaplasticity through HCN channels is well established (Honnurahia and Narayanan 2013; Narayanan and Johnston 2010; Nolan et al. 2004) and provides a link between HCN plasticity and synaptic plasticity. Therefore, synaptic plasticity that is dependent on InsP$_3$Rs (Bortolotto et al. 1999; Nishiyama et al. 2000) could synergistically interact with the metaplasticity introduced by HCN channel plasticity to play significant roles in neural coding and homeostasis (Honnurahia and Narayanan 2013; Narayanan and Johnston 2012; O’Leary et al. 2013; Rathour and Narayanan 2014; Turrigiano 2011). From this standpoint, it would also be important to explore if the release of presynaptic store calcium (Verkhratsky 2005) would alter HCN channels that are known to express in presynaptic terminals, as well (Bender et al. 2007; Huang et al. 2011), thereby providing additional roles for InsP$_3$-induced plasticity in regulating presynaptic release probability and synaptic maturation (Bender et al. 2007; Huang et al. 2011). Finally, although our focus has been limited to the hippocampus and to HCN channels, future studies should investigate the role of InsP$_3$ and its receptors in plasticity of other channels and in other neurons under physiological and pathological conditions.

In summary, our results further emphasize the critical role for InsP$_3$ and intracellular stores as synergistic integrators of several biochemical signals across the neuronal arbor (Berridge 1998; Berridge et al. 2000; Park et al. 2008; Patterson et al. 2004). These findings also constitute a novel addition to the several existing forms of interactions between the endoplasmic reticulum and the plasma membrane (Ashshad and Narayanan 2013; Berridge 2002; Choe and Ehrlich 2006; Clapham 2007; Foskett et al. 2007; Kato et al. 2012; Ross 2012) by providing direct evidence for the role of cytosolic InsP$_3$ in altering neuronal excitability and intrinsic response dynamics. This InsP$_3$-induced form of intrinsic plasticity underscores the necessity for an expansive reassessment of the already extensive roles of InsP$_3$ and intracellular stores in cell signaling, neuronal integration, neural plasticity, learning, memory, neural coding, and homeostasis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.A., D.J., and R.N. conception and design of research; S.A. and R.N. performed experiments; S.A. and R.N. analyzed data; S.A., D.J., and R.N. interpreted results of experiments; S.A. and R.N. prepared figures; S.A. and R.N. drafted manuscript; S.A., D.J., and R.N. revised manuscript; S.A., D.J., and R.N. approved final version of manuscript.

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