Coupling of L-Type Ca\(^{2+}\) Channels to \(K_{\text{V}7}/K_{\text{CNQ}}\) Channels Creates a Novel, Activity-Dependent, Homeostatic Intrinsic Plasticity

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Submitted 9 March 2008; accepted in final form 8 August 2008

Wu WW, Chan CS, Surmeier DJ, Disterhoft JF. Coupling of L-type Ca\(^{2+}\) channels to \(K_{\text{V}7}/K_{\text{CNQ}}\) channels creates a novel, activity-dependent, homeostatic intrinsic plasticity. J Neurophysiol 100: 1897–1908, 2008. First published August 20, 2008; doi:10.1152/jn.90346.2008. Experience-dependent modification in the electrical properties of central neurons is a form of intrinsic plasticity that occurs during development and has been observed following behavioral learning. We report a novel form of intrinsic plasticity in hippocampal CA1 pyramidal neurons mediated by the \(K_{\text{V}7}/K_{\text{CNQ}}\) and \(Ca_{\text{v}1/L}\)-type Ca\(^{2+}\) channels. Enhancing Ca\(^{2+}\) influx with a conditioning spike train (30 Hz, 3 s) potentiated the \(K_{\text{V}7}/K_{\text{CNQ}}\) channel function and led to a long-lasting, activity-dependent increase in spike frequency adaptation—a gradual reduction in the firing frequency in response to sustained excitation. These effects were abolished by specific blockers for \(Ca_{\text{v}1/L}\)-type Ca\(^{2+}\) channels, \(K_{\text{V}7}/K_{\text{CNQ}}\) channels, and protein kinase A (PKA). Considering the widespread expression of these two channel types, the influence of Ca\(^{2+}\) influx and subsequent activation of PKA on \(K_{\text{V}7}/K_{\text{CNQ}}\) channels may represent a generalized principle in fine tuning the output of central neurons that promotes stability in firing—an example of homeostatic regulation of intrinsic membrane excitability.

INTRODUCTION

Activity-dependent changes in synaptic strength and connectivity are central to current cellular models of learning and memory. However, certain types of learning tasks also are known to produce enduring changes in intrinsic membrane properties (Hansel et al. 2001; Moyer et al. 1996; Oh et al. 2003; Saar et al. 1998). This type of intrinsic plasticity reflects modulation of ion channels and affects not only synaptic throughput but also pattern, frequency, and timing of action potentials that are pivotal for encoding information within the neural network. Different forms of activity-dependent intrinsic plasticity have been described in cortical and hippocampal pyramidal neurons in recent years (Cudmore and Turrigiano 2004; Misonou et al. 2004; Xu et al. 2005). Despite the differences in the underlying mechanisms and time scales of expression, two themes have emerged: a rise in intracellular Ca\(^{2+}\) is required, and modulation of K\(^{+}\) channels is often involved.

Here we describe a novel, plastic behavior of CA1 pyramidal neurons: a brief spike train only seconds in duration induced a persistent increase in spike frequency adaptation—a gradual reduction in the firing frequency in response to sustained excitation—that lasted for ≤60 min. This form of intrinsic plasticity does not require synaptic activation and depends on the availability of the \(K_{\text{V}7}/K_{\text{CNQ}}\) (M-type) channels, a unique class of voltage-dependent K\(^{+}\) channels that exhibit subthreshold-active, slowly activating, and nonactivating gating properties (Brown and Adams 1980). In response to an increase in neuronal activity, as encoded by an enhanced Ca\(^{2+}\) influx through the \(Ca_{\text{v}1/L}\)-type Ca\(^{2+}\) channels and subsequent activation of protein kinase A (PKA), CA1 pyramidal neurons dynamically regulate their output by potentiating the \(K_{\text{V}7}/K_{\text{CNQ}}\) current. Such a mechanism thus confers an activity-dependent stabilization of firing during sustained excitation—a homeostatic intrinsic plasticity in CA1 pyramidal neurons.

METHODS

Animals

Five- to 10-wk-old, F344XBN male rats (Harlan; Indianapolis, IN) were used in the present study. All experiments were conducted in strict accordance with a protocol approved by the Animal Care and Use Committee of Northwestern University.

Hippocampus slice preparation

Rats were anesthetized with a mixture of ketamine and xylazine and transcardially perfused with ice-cold artificial cerebrospinal fluid (ACSF, see following text) followed by decapitation. The brain was rapidly removed, and a block containing the left hippocampus and surrounding structures was dissected out, attached to a mounting tray with cyanoacrylate glue, and immersed in ice-cold ACSF consisting of the following (in mM): 119 NaCl, 26 NaHCO\(_3\), 2.5 KCl, 1 NaH\(_2\)PO\(_4\), 1.3 MgCl\(_2\), 6H\(_2\)O, 2 CaCl\(_2\), 2H\(_2\)O, and 25 glucose and saturated with carbogen (95% O\(_2\)-5% CO\(_2\)). Transverse hippocampal slices (300 \(\mu\)m) were cut along the dorsal-ventral axis using a vibrating microtome (VT 1000s; Leica Instrument, Leitz, Nussloch, Germany). Slices were transferred to fresh ACSF and equilibrated at 34°C for 30 min and then maintained at room temperature (~22°C) for ≥30 min prior to electrophysiological recording and acute dissociation.

Perforated-patch recording in slice

Only slices from the middle third of the hippocampus were used in this study. Slices were transferred to a small volume (~0.5 ml) recording chamber that was mounted on a fixed-stage, upright microscope (Axioskop; Carl Zeiss, Thornwood, NY or BX51; Olympus, Melville, NY) equipped with infrared differential interference contrast...
To examine the gating properties of K<sub>ur</sub>/KCQ channels, voltage-clamp recordings were performed in the presence of Cs<sup>+</sup> (2 mM) to block inwardly rectifying K<sub>ur</sub>2 channels and HCN channels, 4-aminopyridine (4-AP, 2.5–5 mM) to block K<sub>v</sub>1-4 channels, and TTX (0.5 μM) to block Na<sub>c</sub> channels and spontaneous synaptic activity. Unless otherwise mentioned, current-clamp recordings were performed in a modified ACSF containing the following compounds to suppress synaptic activity and currents with voltage dependence known to overlap with that of K<sub>ur</sub>/KCQ current: Cs<sup>+</sup> (1 mM) to suppress inwardly rectifying K<sub>ur</sub>2 channels and HCN channels, 4-AP (100 μM) to suppress K<sub>v</sub>1 channels, SR95531 (5 μM) to block GABA<sub>A</sub> receptors, CGP35845 (1 μM) to block GABA<sub>B</sub> receptors, d-2-amino-5-phosphonopentanoic acid d-AP5, 50 μM, and CNQX (20 μM) to suppress excitatory synaptic transmission. Experiments performed in this modified ACSF without further addition of pharmacological agents are referred to as “controls” in this study. A 1-s test pulse with stimulus intensity that reliably elicited the same number of action potentials (range, 7–10 action potentials for neurons included in this study; Table 1) was delivered during every 60-s for 10–20 min to establish a measure for baseline excitability. A brief, suprathreshold conditioning pulse train (30 Hz, 3-s; each pulse within the train was 1.5 nA in amplitude and 2 ms in duration) was then delivered to generate the corresponding number of action potentials. Subsequently, membrane excitability was monitored once every 30-s for as long as the recordings remained in the perforated patch configuration. TEA, linopirdine, XE991, BAPTA-AM, SR33805, calciseptine, nimodipine, and ω-conotoxin MVIIIC were added to the modified ACSF for ≥15 min prior to CS presentation to ensure steady-state blockade. ω-conotoxin MVIIIC was added to the modified ACSF along with cytochrome c (1 mg/ml). For experiments involving H-89, slices were preincubated in H-89 for ≥30 min prior to recording.

**Single-cell RT-PCR analysis**

Acute dissociation was done with our standard procedure (Chan et al. 2004). Isolated, individual hippocampal CA1 neurons were aspirated into sterilized glass pipettes containing 1–2 μl of diethyl pyrocarbonate (DEPC)-treated water and 1.5 U/ml SUPERase-In (Ambion, Austin, TX). After aspiration of the neuron, the electrode tip was broken in a 0.6-ml presiliconized tube (Midwest Scientific, Valley Park, MO) containing 1.9 μl of DEPC-treated water, 0.7 μl of SUPERase-In (20 U/ml), 0.7 ml of oligo-dT (50 mM), 0.7 μl of BSA

![Image](http://jn.physiology.org/100/08/fig1.jpg)

**FIG. 1.** Protocol for inducing intrinsic plasticity. A: Alexa594 (10 μM) was included in the patch solution for a subset of neurons to confirm the integrity of the perforated-patch configuration. As illustrated, establishment of stable series resistance in the perforated-patch configuration for 25 min did not introduce Alexa594 into the cell. B: sudden membrane rupture, however, was accompanied by an instantaneous fluorescent signal in the soma. C: topographical location and morphological features of the recorded neuron. D, top: intrinsic membrane excitability was first assessed with a 1-s depolarizing current test pulse. A brief, suprathreshold conditioning pulse train (CS: 30 Hz, 3-s) was then delivered via the recording electrode. Subsequently, membrane excitability was monitored for as long as the recordings remained in the perforated patch configuration. Bottom: a representative voltage trace from one neuron illustrating that CS triggered the corresponding number of action potentials, followed by a pronounced postburst afterhyperpolarization (AHP). E: 1st temporal derivative of the 1st action potential triggered by the test pulse. Neither the threshold nor the maximum rate of rise of the action potential was altered immediately following CS presentation, suggesting that CS-induced enhancement of spike frequency adaptation is not due to an alteration in the Na<sub>c</sub> channel availability that had developed during CS.

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(IR-DIC) optics. The recording chamber was superfused with carbo-
gen-saturated ACSF with a flow rate of 2–3 ml/min. Experiments were performed at room temperature.

Patch electrodes were fabricated from filamented, thick-walled borosilicate glass pipettes and heat-polished to a resistance of 3–4 MΩ when filled with an internal solution consisting of the following (mM): 140 KMeSO<sub>4</sub>, 10 KCl, 10 HEPES, 2 Mg<sub>2</sub>ATP, 0.4 Na<sub>2</sub>GTP, and 10 Tris-phosphocreatine; pH adjusted to 7.25 with KOH. The final osmolality of this solution was ~290 mosM. Liquid junction potential (~7 mV) was not corrected for. Alexa594 (Invitrogen, Carlsbad, CA) was included in the initial experiments to verify the integrity of the membrane under perforated-patch configuration and identification of the recorded neuron (Fig. 1, A-C).

All the data presented were recordings conducted under perfo-
rated-patch configuration to prevent disruption of intracellular milieu and signaling. Recordings were made with an Axopatch 1C or a Multiclamp 700A amplifier (Molecular Devices, Union City, CA) operating in either voltage-clamp or current-clamp mode on the soma of CA1 pyramidal neurons. Solubilized amphotericin B (200–300 μg/ml) was added to the pipette solution for membrane perforation. Patch pipettes were front-filled with solution free of amphotericin B to assist seal formation. Seal resistances were typically >5 GΩ. Membrane rupturing was accompanied by an instantaneous appearance of fluorescent signal inside the cyto-
plasm (Fig. 1, A and B), and a sudden jump in the series resistance and whole cell capacitance, followed by a rapid decrease in the holding current and the K<sub>ur</sub>/KCQ current relaxation in voltage-
clamp configuration. As abrupt changes in the series resistance, whole cell capacitance, and holding current were predictive of the ensuing current rundown, these parameters were used as indicators of intactness of the perforated-patch, and Alexa594 was omitted in the subsequent experiments.

Electrophysiological records were acquired at 5 or 10 kHz with a Digidata 1322A interface (Molecular Devices) in conjunction with a PC, and filtered at 1 or 2 kHz, respectively, with a low-pass Bessel filter. Stimulus generation and data acquisition was performed using pClamp9 (Molecular Devices). Only data gathered from neurons with resting membrane potential less than −60 mV (RMP = −69 ± 1 mV; n = 107); input resistance >150 MΩ (R<sub>input</sub> = 228 ± 7 MΩ; data from all drug treatments combined), series resistance <40 MΩ (32.0 ± 0.7 MΩ), spike height >110 mV from baseline potential of −65 mV were accepted for further analysis.

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**FIG. 1.** Protocol for inducing intrinsic plasticity. A: Alexa594 (10 μM) was included in the patch solution for a subset of neurons to confirm the integrity of the perforated-patch configuration. As illustrated, establishment of stable series resistance in the perforated-patch configuration for 25 min did not introduce Alexa594 into the cell. B: sudden membrane rupturing, however, was accompanied by an instantaneous fluorescent signal in the soma. C: topographical location and morphological features of the recorded neuron. D, top: intrinsic membrane excitability was first assessed with a 1-s depolarizing current test pulse. A brief, suprathreshold conditioning pulse train (CS: 30 Hz, 3-s) was then delivered via the recording electrode. Subsequently, membrane excitability was monitored for as long as the recordings remained in the perforated patch configuration. Bottom: a representative voltage trace from one neuron illustrating that CS triggered the corresponding number of action potentials, followed by a pronounced postburst afterhyperpolarization (AHP). E: 1st temporal derivative of the 1st action potential triggered by the test pulse. Neither the threshold nor the maximum rate of rise of the action potential was altered immediately following CS presentation, suggesting that CS-induced enhancement of spike frequency adaptation is not due to an alteration in the Na<sub>c</sub> channel availability that had developed during CS.
TABLE 1. Effects of K\textsuperscript{+} and Ca\textsuperscript{2+} channel blockers and Ca\textsuperscript{2+} chelator on resting membrane potential and number of action potentials triggered by the test pulse

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>RMP, mV</th>
<th>$I_{\text{test}}$, pA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, modified ACSF</td>
<td>15</td>
<td>$-67 \pm 1$</td>
<td>$114 \pm 27$</td>
</tr>
<tr>
<td>Plain ACSF, no drugs</td>
<td>5</td>
<td>$-62 \pm 1$</td>
<td>$204 \pm 49^{*#}$</td>
</tr>
<tr>
<td>TEA, 0.5 mM</td>
<td>5</td>
<td>$-67 \pm 1$</td>
<td>$88 \pm 13$</td>
</tr>
<tr>
<td>Linopirdine, 1 $\mu$M</td>
<td>4</td>
<td>$-63 \pm 3$</td>
<td>$71 \pm 11$</td>
</tr>
<tr>
<td>Linopirdine, 3 $\mu$M</td>
<td>3</td>
<td>$-67 \pm 3$</td>
<td>$95 \pm 8$</td>
</tr>
<tr>
<td>XE991, 1 $\mu$M</td>
<td>8</td>
<td>$-67 \pm 1$</td>
<td>$87 \pm 16$</td>
</tr>
<tr>
<td>XE991, 3 $\mu$M</td>
<td>12</td>
<td>$-65 \pm 1$</td>
<td>$104 \pm 13$</td>
</tr>
<tr>
<td>BAPTA-AM, 20 $\mu$M</td>
<td>5</td>
<td>$-64 \pm 2$</td>
<td>$144 \pm 19^{**}$</td>
</tr>
<tr>
<td>SR38305, 5 $\mu$M</td>
<td>5</td>
<td>$-66 \pm 2$</td>
<td>$118 \pm 28$</td>
</tr>
<tr>
<td>Calciseptine, 1 $\mu$M</td>
<td>4</td>
<td>$-66 \pm 2$</td>
<td>$85 \pm 5$</td>
</tr>
<tr>
<td>Nimbodine, 1 $\mu$M</td>
<td>4</td>
<td>$-63 \pm 1$</td>
<td>$73 \pm 6$</td>
</tr>
<tr>
<td>$\alpha$-Conotoxin MVIIIC, 1 $\mu$M</td>
<td>4</td>
<td>$-66 \pm 1$</td>
<td>$71 \pm 33$</td>
</tr>
<tr>
<td>H89, 5 $\mu$M</td>
<td>6</td>
<td>$-62 \pm 4$</td>
<td>$84 \pm 8$</td>
</tr>
</tbody>
</table>

RMP, the resting membrane potential in plain ACSF (no drugs added), modified ACSF (+excitatory/inhibitory synaptic blockers, 4-aminopyridine (4-AP), Ca\textsuperscript{2+}) alone, or modified ACSF plus the specified drug. Note that following the RMP measurement that occurred at the beginning of each experiment, membrane potential was maintained at $-65$ mV throughout the remaining recording period with a constant current injection. Number of APs denotes the number of action potentials elicited by the 1-s depolarizing current. Periods of current deactivation/activation were fit with a double-exponential function.

Data analysis and statistics

Curve-fitting (using a least-squares criterion), data and statistical analyses, and plotting were done using Clampfit9 (Molecular Devices), IgorPro 5.05 (WaveMetrics, Lake Oswego, OR), and Statview (SAS Institute, Cary, NC). Data were presented as mean ± SE and compared statistically using Mann-Whitney or Wilcoxon signed-rank test as appropriate. $P \leq 0.05$ indicates statistical significance. Activation curves for K\textsubscript{\textalpha}/KCNQ channels were fit with a single Boltzmann function of the form: $g(V) = 1/(1 + \exp[V - V_{1/2}]/k])$, where $V_{1/2}$ is the half voltage of activation and $k$ is the slope factor. The time courses of current deactivation/activation were fit with a double-exponential function.

Solutions and channel ligands

MeSO\textsubscript{4} was purchased from MP Biomedicals (Solon, OH); d-AP5, CGP55845, nimodipine, XE991 hydrochloride, linopirdine hydrochloride, SR95531, and SR33805 were purchased from Tocris Cookson (Ballwin, MO); TTX, calciseptine, and $\alpha$-conotoxin MVIIIC from Alomone Laboratories; BAPTA-AM from Invitrogen; and H-89 from Calbiochem (San Diego, CA). All other drugs were purchased from Sigma (St. Louis, MO). Drugs were dissolved as stock solutions in either water or DMSO, aliquoted, and frozen at $-30^\circ$C before use. Each drug was diluted in the perfusate immediately before the experiment. When used, the final concentration of DMSO was always <0.1%.

RESULTS

Repetitive firing generates sustained reduction in intrinsic excitability

All recordings were performed using amphotericin-perforated patches (Fig. 1, A–C) to prevent disruption of intracellular milieu and signaling. Synthetic blockers (see METHODS) were included in all the experiments except where noted to eliminate changes in membrane properties arising from synaptic activation and recurrent network activity. A brief conditioning spike train (CS, 30 Hz for 3 s) was applied to mimic sustained firing in CA1 pyramidal neurons (Fig. 1D). Membrane excitability was monitored periodically before and after CS presentation.
(METHODS). Under the control condition, CS reliably induced a gradual and persistent suppression of membrane excitability \((n = 10/12); \text{Fig. 2, A and B})\). In contrast, the firing pattern of neurons in the absence of CS was stable across time (Fig. 2B). Input resistance was monitored regularly and did not show significant change before and after CS presentation (pre CS: \(168.0 \pm 19.8 \, \text{M} \Omega\); 25 min post CS: \(189.8 \pm 26.2 \, \text{M} \Omega\); \(P > 0.05\)). Neither spike threshold nor rate of rise of the first action potential—indices of \(\text{Na}_v\) channel availability—was altered immediately following CS (Fig. 1E). Thus it is unlikely that this form of intrinsic plasticity is due to a slowing of recovery from accumulated \(\text{Na}_v\) channel inactivation that developed during CS. To quantify the changes in the firing rate and pattern induced by CS, we formulated an index for spike frequency adaptation for all action potentials generated during each depolarizing step that accounts for changes in the interspike interval and the time that the neurons reach maximal adaptation (i.e., cease to fire as represented by an index of 1; Fig. 2C, legend). The plot of adaptation index against time revealed a leftward shift in the curve following CS (Fig. 2C), illustrating that CA1 pyramidal neurons adapted more readily and fired fewer action potentials following CS. This increase in spike frequency adaptation was associated with a slight elevation in spike threshold, particularly for action potentials generated later in the depolarizing step (Fig. 2D). Altogether, these measurements suggest that an activity-dependent modulation of a slowly or noninactivating subthreshold active conductance is responsible for mediating a long-lasting suppression of membrane excitability.

CS-induced intrinsic plasticity was also evident in plain ACSF without additional pharmacology, indicating that the underlying mechanisms could be initiated under more physiological conditions (number of spikes generated 25 min post CS was \(84.7 \pm 1.7\%\) of that generated prior to CS presentation, \(n = 5\); Table 1, Supplementary Fig. S1).

**K\(_v7\)/KCNQ channels are necessary for CS-induced intrinsic plasticity**

One possibility for the change in firing pattern following CS is a progressive enhancement of a \(\text{K}^+\) conductance. In support, when TEA (10 mM), a broad-spectrum \(\text{K}^+\) channel antagonist, was included in the bath solution, CS presentation did not induce a change in spike output (number of spikes generated 25 min post CS as compared with control, TEA: \(91.9 \pm 6.0\%\), \(P < 0.01\), \(n = 7\); Fig. 3E). Based on the gating properties, pharmacological criteria, and known channel function, the primary candidate for this form of intrinsic plasticity were the K\(_v7\)/KCNQ channels (Fig. 4, A and B, and Supplementary Fig. S2, A–C) (Marrion 1997). To test this hypothesis, linopirdine and XE991, selective K\(_v7\)/KCNQ channel blockers, were separately included in the bath solution. Both compounds dose-
CS-induced intrinsic plasticity requires Ca\textsubscript{2+} influx through the Ca\textsubscript{v,1}-type Ca\textsuperscript{2+} channels and PKA activity.

Ca\textsuperscript{2+} plays a pivotal role in inducing various forms of activity-dependent intrinsic plasticity (Cudmore and Turrigiano 2004; Fan et al. 2005; Frick et al. 2004; Misonou et al. 2004; Xu et al. 2005). We first assessed the dependence on Ca\textsuperscript{2+} of CS-induced intrinsic plasticity by including BAPTA-AM (20 \mu M), a membrane-permeable Ca\textsuperscript{2+} chelator, in the bath solution. BAPTA-AM prevented CS-induced sup-
expression of membrane excitability (25 min post CS, BAPTA-AM: 115.6 ± 5.3%; P < 0.005, n = 5. Fig. 6, A–C), suggesting that Ca2+ influx associated with CS is involved in initiating this form of plasticity. In hippocampal neurons, CaV1/L-type and CaV2/N- and P, Q-type Ca2+ channels constitute a major source for activity- and spike-induced Ca2+ influx (Christie et al. 1995; Eliot and Johnston 1994). When SR33805 (5 μM) or calcisepine (1 μM), two selective nondihydropyridine CaV1/L-type Ca2+ channel blockers (Avery and Johnston 1996; de Weille et al. 1991; Romey and Lazdunski 1994), were included in the bath solution, CS presentation did not induce a change in spike output (25 min post CS, SR33805: 97.7 ± 3.1%; P < 0.005, n = 5. Calcisepine: 104.0 ± 3.9%; P < 0.005, n = 4. Fig. 7, B and C, left; Supplementary Fig. S3, A and B). Similar results were obtained when nimodipine (1 μM), the classical dihydropyridine CaV1/L-type Ca2+ channel blocker, was included in the bath solution (25 min post CS, nimodipine: 102.3 ± 8.6%; P < 0.005; n = 4. Fig. 7, A–C, left). In contrast, ω-conotoxin MVIIIC (1 μM), a blocker of CaV2/N- and P, Q-type Ca2+ channels, reduced but failed to prevent CS-induced intrinsic plasticity (25 min post CS, ω-conotoxin MVIIIC: 75.3 ± 2.4%; n = 4; P > 0.05. Fig. 7, B and C, right; Supplementary Fig. S3C; see also Fig. 8). These data indicate that an elevation of intracellular Ca2+ level mediated by influx preferentially through the CaV1/L-type Ca2+ channels during CS serves as a trigger for the induction of this sustained reduction in membrane excitability.

We next performed voltage-clamp experiments to evaluate whether Ca2+ influx through the CaV1/L-type Ca2+ channels could modulate the Kv7/KCNQ current. Neurons were voltage-clamped at −30 mV to elicit a steady CaV1/L-type Ca2+ current (Tavalin et al. 2004; Xu and Lipscombe 2001). Bath application of BayK8644 (10 μM), a CaV1/L-type Ca2+ channel agonist, led to a potentiation of the Kv7/KCNQ current that was readily suppressed by XE991 (10 μM; Fig. 9, A and B). In the presence of BayK8644, the XE991-sensitive current showed no significant change in the voltage dependence of steady-state activation (Fig. 9E; cf. Supplementary Fig S2C). In contrast, bath applications of 300 nM and 10 μM, nimodipine suppressed the Kv7/KCNQ current by 46.9 ± 7.8 and 58.9 ± 9.0%, respectively (Fig. 9, C, D, and F). Using acutely dissociated CA1 pyramidal neurons, we verified that XE991 (10 μM) did not affect Ba2+ influx through the CaV1/2 class Ca2+ channels (Supplementary Fig. S4, A–C). Altogether, our experiments illustrate a form of intrinsic plasticity in response to repetitive firing that requires both the CaV1/L-type Ca2+ channels and the Kv7/KCNQ channels.

One mechanism that has been linked with potentiation of the Kv7/KCNQ current is activation of PKA by intracellular cyclic AMP (Sims et al. 1988) and subsequent phosphorylation of the Kv7.2/KCNQ2 subunits (Schroeder et al. 1998). To test the hypothesis that a PKA-dependent pathway is involved in CS-induced intrinsic plasticity, we examined the effect of H-89, a potent and selective PKA inhibitor, on plasticity induction. Preincubation of slices in H-89 (5 μM) blocked CS-induced suppression of membrane excitability (25 min post CS, H-89: 119.2 ± 13.0%; P < 0.0001, n = 6, Fig. 8, B–D). The most parsimonious explanation for our data is a model in which Ca2+ influx through CaV1/L-type Ca2+ channels during CS leads to a long-lasting potentiation of the Kv7/KCNQ current via PKA phosphorylation, thereby resulting in a persistent suppression of membrane excitability.

DISCUSSION

In this study, we describe a novel feedback mechanism that decreases intrinsic membrane excitability of CA1 pyramidal neurons following persistent activation. Our major finding is an activity-dependent, long-lasting potentiation of the Kv7/KCNQ current that lowers neuronal responsiveness to subsequent input as evidenced by an enhanced spike frequency adaptation. This change does not require synaptic activation, is triggered by Ca2+ influx through the CaV1/L-type Ca2+ channels, depends on PKA activity, and can be rapidly induced. Such Ca2+–dependent modulation of the Kv7/KCNQ channel function likely operates to stabilize the firing frequency of CA1 pyramidal neurons during periods...
of sustained excitation—a homeostatic tuning of intrinsic membrane excitability.

**KV7/KCNQ channels are well suited to regulate intrinsic excitability**

KV7/KCNQ channels are subthreshold-active K⁺ channels that give rise to the muscarine-sensitive, noninactivating M-current (Supplementary Fig. S2, B–D). Mutations in the genes that encode the KV7/KCNQ channel subunits cause congenital epilepsy and dominant hereditary deafness (Jentsch 2000), indicating a key role in the normal functioning of various neural circuits. With scRT-PCR, we demonstrated that hippocampal CA1 pyramidal neurons from adult rats express mRNAs for KV7.2/KCNQ2 and KV7.3/KCNQ3 (Fig. 4, C and D), subunits that encode a major portion of the neuronal KV7/KCNQ current. We also confirmed the presence of corresponding proteins with immunohistochemistry (Fig. 5). Unfortunately, our immunoperoxidase staining performed on fixed tissue sections does not allow for accurate estimation of the putative localization of the membrane-associated KV7/KCNQ channels. Immunolabelings performed on unfixed tissue preparations have shown that KV7.2/KCNQ2 and sometimes KV7.3/KCNQ3 proteins are concentrated at the axon initial segments and nodes of Ranvier, colocalizing with Naᵥ channels (Devaux et al. 2004; Pan et al. 2006). In many neurons, these sites correspond to the action potential initiation zones (Clark et al. 2005; Colbert and Johnston 1996; Colbert and Pan 2002; Khalilq and Raman 2006; Stuart and Hausser 1994). Corroborating with this immunolocalization pattern, two recent studies have demonstrated that functional KV7/KCNQ channel function is concentrated near the perisomatic region: focal application of XE991 to the perisomatic region but not to the distal dendrites enhanced temporal summation of excitatory postsynaptic potentials (Hu et al. 2007) and spike output (Hu et al. 2007; Yue and Yaari 2006) in CA1 pyramidal neurons. Furthermore, enhancing KV7/KCNQ channel function with retigabine decreased the amplitude of population spikes in CA1 that represents the synchronous discharge of a number of CA1 pyramidal neurons in response to repetitive synaptic stimulation (Hu et al. 2007). Modulation of the KV7/KCNQ channel function will thus profoundly affect the global integrative properties of hippocampal pyramidal neurons, altering information coding and throughput within the CA1 subfield by gating the timing and generation of action potentials (Fig. 2, A–C, and Supplementary Fig. S2A) (Hu et al. 2007).

Most of our experiments were carried out in a modified ACSF containing synaptic blockers, 4-AP, and Cs⁺/H¹¹⁰⁰¹. We have also verified that CS-induced intrinsic plasticity occurs in plain ACSF without additional pharmacology (Supple-

![Kv7.2 immunoreactivity](image1)

![Kv7.3 immunoreactivity](image2)

**Fig. 5.** Kv7.2/KCNQ2 and Kv7.3/KCNQ3 proteins in the hippocampus. Light micrographs depicting Kv7.2/KCNQ2 (top left) and Kv7.3/KCNQ3 (bottom left) immunoreactivities in the hippocampus and adjacent structures in rat sagittal sections. Specific labeling was found in the stratum pyramidale of the CA1 and CA3 subfields, granule cell layer of the dentate gyrus, thalamus (Th), and superior colliculus (SC). At higher magnification, Kv7.2/KCNQ2 and Kv7.3/KCNQ3 immunoreactivities were found to associate with the perisomatic region. Diffuse neuropilar labeling was also evident in both st. oriens and st. radiatum of the CA1 subfield.

![Graphs](image3)

**Fig. 6.** CS-induced intrinsic plasticity requires activity-dependent rise in intracellular Ca²⁺. BAPTA-AM, a membrane-permeable Ca²⁺ chelator, was applied for ≥15 min prior to CS presentation and maintained throughout the recordings. A and B: bath application of BAPTA-AM (20 μM; n = 5) prevented CS-induced intrinsic plasticity. C: histogram summarizing the effect of BAPTA-AM on CS-induced intrinsic plasticity.
Functional homomeric $K_v$7.2/KCNQ2 channels in CA1 pyramidal neurons

In many neuronal types, it is thought that the $K_v$7/KCNQ current is largely mediated by heteromeric channels of $K_v$7.2/KCNQ2 and $K_v$7.3/KCNQ3 subunits (Hadley et al. 2003; Shen et al. 2005; Wang et al. 1998). Our TEA dose-responsive profile of the $K_v$7/KCNQ current is compatible with this idea but further implicates the presence of a highly TEA-sensitive component (~30%), likely mediated by homomeric $K_v$7.2/KCNQ2 channels (Fig. 4, A and B; corresponding figure legend). Surprisingly, CS-induced intrinsic plasticity was sensitive to TEA at a concentration that preferentially blocks these homomeric channels (Fig. 8, A, C, and D). It is tempting to think that this form of intrinsic plasticity reflects an activity-dependent modulation of homomeric $K_v$7.2/KCNQ2 channels given that $K_v$7.2/KCNQ2 proteins are more prevalent at the axon initial segments and nodes of Ranvier than $K_v$7.3/KCNQ3 proteins in certain neuronal types (Devaux et al. 2004; Pan et al. 2006; Schwarz et al. 2006). However, linopirdine and XE991 dose-dependently suppressed CS-induced intrinsic plasticity (Fig. 3, A–C, and E) but do not discriminate between $K_v$7/KCNQ channels of different subunits. Thus the linkage between homomeric $K_v$7.2/KCNQ2 channels and CS-induced intrinsic plasticity still requires further investigation.
**Ca\textsubscript{v}1.2** channels account for the properties of Ca\textsuperscript{2+} influx that modulate K\textsubscript{v7}/KCNQ channels

Hippocampal pyramidal neurons express both high- (Ca\textsubscript{v}1.2) and low (Ca\textsubscript{v}1.3)-threshold activated L-type Ca\textsuperscript{2+} channels (Hell et al. 1993). Compared with other Ca\textsuperscript{2+} channel subtypes, Ca\textsubscript{v}1.2/L-type Ca\textsuperscript{2+} channels are relatively insensitive to voltage-dependent inactivation (Lipscombe et al. 2004), allowing them to reliably report ongoing neuronal activity in the form of sustained Ca\textsuperscript{2+} influx. As Ca\textsubscript{v}1.2 channels are far more sensitive to nimodipine than Ca\textsubscript{v}1.3 channels (IC\textsubscript{50} for nimodipine: Ca\textsubscript{v}1.2 ~140 nM; Ca\textsubscript{v}1.3 ~3 \mu M) (Xu and Lipscombe 2001), our finding that CS-induced intrinsic plasticity could be blocked by a low concentration of nimodipine (Fig. 7, A–C) suggests that Ca\textsubscript{v}1.2 channels are the primary source of Ca\textsuperscript{2+} influx that mediates K\textsubscript{v7}/KCNQ channel modulation. This preferential coupling may reflect the abundance of Ca\textsubscript{v}1.2 over Ca\textsubscript{v}1.3 channels in pyramidal neurons as well as their close spatial proximity to the K\textsubscript{v7}/KCNQ channels (Davare et al. 2001; Hell et al. 1993) as Ca\textsubscript{v}1.2 proteins have been reported to cluster in the perisomatic region of pyramidal neurons.

Could K\textsubscript{v7}/KCNQ channels and Ca\textsubscript{v}1.2/L-type Ca\textsuperscript{2+} channels belong to a multiprotein complex that includes key elements of signaling transduction pathways such as kinases? Several pieces of evidence suggest so. First, components of PKA have been co-purified with the K\textsubscript{v7.2}/KCNQ2 subunit by affinity chromatography (Cooper et al. 2000), implicating a physical association between these two proteins. Second, both the K\textsubscript{v7}/KCNQ channels and the Ca\textsubscript{v}1.2/L-type Ca\textsuperscript{2+} channels are known to associate with A-kinase anchoring proteins (AKAP) (Hoshi et al. 2003; Hulme et al. 2003). In particular, Ca\textsubscript{v}1.2 channels have been demonstrated to associate with AKAP79/150 (Gao et al. 1997; Oliveria et al. 2007)—the same AKAP that interacts with the K\textsubscript{v7.2}/KCNQ2 subunit (Hoshi et al. 2003). AKAP79/150 is present at extremely high levels within the central nervous systems, including the hippocampus (Glantz et al. 1992). Thus there lies a distinct possibility that the K\textsubscript{v7}/KCNQ channels and the Ca\textsubscript{v}1.2 channels are organized into a macromolecular complex that includes PKA, protein kinase C (PKC), and protein phosphatase 2B (PP2B)—enzymes that bind to AKAP79/150 (Carr et al. 1992; Coghlan et al. 1995; Klauck et al. 1996) that are also known to regulate the K\textsubscript{v7}/KCNQ channels (Hoshi et al. 2003; Marrion 1996; Schroeder et al. 1998)—to allow for rapid, localized, and efficient modulation of the K\textsubscript{v7}/KCNQ current following Ca\textsuperscript{2+} entry during neuronal activity. In addition to potentiating the K\textsubscript{v7}/KCNQ current, PKA has also been shown to increase Ca\textsuperscript{2+} transients through the Ca\textsubscript{v}1.2/L-type Ca\textsuperscript{2+} channels in CA1 pyramidal neurons (Hoogland and Saggau 2004)—an effect that could be mediated by both the Ca\textsubscript{v}1.2 (Davare et al. 2001) and Ca\textsubscript{v}1.3 (Qu et al. 2005) channels. This synergistic action would result in a positive feedback loop that amplifies the amount of Ca\textsuperscript{2+} influx to further modulate the K\textsubscript{v7}/KCNQ channels during sustained neuronal activity.

**Plausible signaling cascade in the Ca\textsuperscript{2+}-dependent potentiation of K\textsubscript{v7}/KCNQ channels**

K\textsubscript{v7}/KCNQ channels are not Ca\textsuperscript{2+} dependent in the sense that Ca\textsuperscript{2+} binding is not obligatory for channel opening (Adams et al. 1982). However, they are exquisitely sensitive to changes in the level of intracellular Ca\textsuperscript{2+} with reports of both an enhancement and a suppression (Cruzblanca et al. 1998; Kirkwood and Lismann 1992; Marrion 1996, 1997; Marrion et al. 1991; Tokimasa et al. 1996; Yu et al. 1994). Ca\textsuperscript{2+}-dependent modulation of the K\textsubscript{v7}/KCNQ channels is thought to occur in spatially restricted domains (Delmas and Brown 2005), and the distinct effects likely reflect the interactions of Ca\textsuperscript{2+} with different Ca\textsuperscript{2+}-sensing molecules present either on the K\textsubscript{v7}/KCNQ channels themselves or within the K\textsubscript{v7}/KCNQ channel macromolecular complex. Ca\textsuperscript{2+}-mediated suppression of the K\textsubscript{v7}/KCNQ current arises through Ca\textsuperscript{2+} binding to the...
calmodulin associated with the $K_v7/KCNQ$ channels (Gampfer and Shapiro 2003; Wen and Levitan 2002; Yus-Najera et al. 2002). On the other hand, two intracellular signaling pathways have been implicated in the potentiation of the $K_v7/KCNQ$ current. The first is mediated by arachidonic acid (AA) or its related metabolites (Moore et al. 1988; Schweitzer et al. 1990); the second, activation of PKA by cyclic AMP (Sims et al. 1988) and subsequent phosphorylation of the $K_v7.2/KCNQ2$ subunits (Schroeder et al. 1998). $Ca^{2+}$ influx through the $Cav_1/L$-type $Ca^{2+}$ channels can stimulate both pathways by activating the $Ca^{2+}$-stimulated form of phospholipase A2 (cPLA2) and adenyl cyclases (AC1 and AC8) to produce AA and cAMP, respectively. Both classes of enzymes are known to be present in the hippocampus (Kishimoto et al. 1999; Xia et al. 1991).

We suspect that multiple processes are involved in the induction and maintenance of CS-induced intrinsic plasticity, judging by its time course and kinetic features (Fig. 2B). The onset of CS-induced suppression of membrane excitability occurs quite rapidly, and in all cases, the decrease in spike output was apparent in the first few voltage traces acquired immediately following CS presentation (Fig. 1A). This again reinforces the notion that one molecular mechanism involved (e.g. phosphorylation) can be rapidly deployed. While our results support an involvement of the cAMP-PKA pathway (Fig. 8, B–D), CS-induced intrinsic plasticity nevertheless took $\approx 20$ min to reach plateau (Fig. 2B). Therefore we cannot rule out the possibility that other processes are recruited on a different and perhaps overlapping time scale to maintain such long-lasting suppression of membrane excitability. Enhancing $Ca^{2+}$ influx through the $Cav_v1/L$-type $Ca^{2+}$ channels with BayK8644 increases the $K_v7/KCNQ$ conductance without shifting its voltage dependence (Fig. 9E; cf. Supplementary Fig. S2C). At the moment, we cannot rule out the possibility that such increase in $K_v7/KCNQ$ conductance is mediated by an increase in the functional $K_v7/KCNQ$ channel density.

**Functional relevance to learning and memory**

Enduring changes in neuronal excitability following periods of activity or learning a behavioral task are features of central neurons thought to encode information at the cellular level. Reduction in the capacity to regulate intrinsic excitability, in fact, has been correlated to impaired learning in aging animals (Moyer et al. 2000; Tombreau et al. 2005). Cholinergic agonists and $Cav_v1/L$-type $Ca^{2+}$ channel blockers increase the excitability of hippocampal pyramidal neurons in vitro (Moyer et al. 1992; Weiss et al. 2000) and facilitate learning of hippocampus-dependent tasks in vivo (Deyo et al. 1989; Weiss et al. 2000). An attractive hypothesis waiting to be tested is that the facilitating effect of cholinergic or $Ca^{2+}$ treatment on learning is mediated in part by modulation of the $K_v7/KCNQ$ current. In support of this idea, a recent study showed that transgenic mice expressing dominant-negative $K_v7.2/KCNQ2$ subunits are impaired in learning hippocampus-dependent spatial tasks (Peters et al. 2005). Given the widespread and overlapping expression patterns of the $K_v7/KCNQ$ channels and the $Cav_v1/L$-type $Ca^{2+}$ channels, the interactions between them may serve as a generalized principle in fine tuning the activity of various neural circuits in a history-dependent manner. Understanding the ways in which neurons adjust their output in response to prior activity will be a pivotal step in advancing our knowledge of information storage at the cellular and network level, and ultimately provide a key to addressing higher brain functions such as learning and memory.

**GRANTS**

This work was supported by National Institutes of Health Grants R37 AG-08796, T532-MH-67564, and P50 NS-047085.

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KCNQ1-Dependent Modulation of M-type Calcium Channels by the Neuronal Calcium Sensor Orai1

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J Neurophysiol 100: 2599-2616, 2008

Abstract: The neuronal calcium sensor Orai1 appears to function as a calcium channel located at the plasma membrane. We hypothesized that the Orai1 channel might function as a calcium channel with KCNQ1, a voltage-gated potassium channel, to modulate M-type calcium channels. Our data support these hypotheses and suggest that KCNQ1 may regulate M-type calcium channels through its interaction with Orai1.


Abstract: KCNQ1 is a member of the family of voltage-gated potassium channels that is expressed in many cell types. It is known to be expressed in the brain and is involved in the regulation of neuronal excitability. In this study, we investigated the role of KCNQ1 in the modulation of M-type calcium channels in the brain. Our results indicate that KCNQ1 modulates M-type calcium channels through its interaction with Orai1, a calcium sensor that is also expressed in the brain.


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