Published in final edited form as:
Channels (Austin). 2009 ; 3(1): 46–56.

Regulation of intrinsic excitability in hippocampal neurons by activity-dependent modulation of the Kv2.1 potassium channel

Durga P. Mohapatra[1,2], **Hiroaki Misonou**[2], **Sheng-Jun Pan**[2], **Joshua E. Held**[4], **D. James Surmeier**[4], and **James S. Trimmer**[1,2,3,*]

[1] Department of Neurobiology, Physiology and Behavior, College of Biological Sciences, University of California, Davis, CA 95616 USA

[2] Department of Pharmacology, School of Medicine, University of California, Davis, CA 95616 USA

[3] Department of Physiology and Membrane Biology, School of Medicine, University of California, Davis, CA 95616 USA

[4] Department of Physiology and Institute of Neuroscience, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611

Abstract

Kv2.1 is the prominent somatodendritic sustained or delayed rectifier voltage-gated potassium (Kv) channel in mammalian central neurons, and is a target for activity-dependent modulation *via* calcineurin-dependent dephosphorylation. Using hanatoxin-mediated block of Kv2.1 we show that, in cultured rat hippocampal neurons, glutamate stimulation leads to significant hyperpolarizing shifts in the voltage-dependent activation and inactivation gating properties of the Kv2.1-component of delayed rectifier K^+ (I_K) currents. In computer models of hippocampal neurons, these glutamate-stimulated shifts in the gating of the Kv2.1-component of I_K lead to a dramatic suppression of action potential firing frequency. Current-clamp experiments in cultured rat hippocampal neurons showed glutamate-stimulation induced a similar suppression of neuronal firing frequency. Membrane depolarization also resulted in similar hyperpolarizing shifts in the voltage-dependent gating properties of neuronal I_K currents, and suppression of neuronal firing. The glutamate-induced effects on neuronal firing were eliminated by hanatoxin, but not by dendrotoxin-K, a blocker of Kv1.1-containing channels. These studies together demonstrate a specific contribution of modulation of Kv2.1 channels in the activity-dependent regulation of intrinsic neuronal excitability.

Keywords

Voltage-gated potassium channel; hippocampal neuron; calcineurin; phosphorylation; neuronal excitability; hanatoxin; homeostatic plasticity

*Correspondence to: Dr. James S. Trimmer, Department of Neurobiology, Physiology and Behavior, College of Biological Sciences, 196 Briggs Hall, University of California, One Shields Avenue, Davis, CA 95616-8519, Tel.: (530) 754-6075; Fax: (530) 754-6079; jtrimmer@ucdavis.edu.
Dr. Mohapatra's present address: Department of Pharmacology, University of Iowa Roy J. and Lucille A. Carver College of Medicine, 51 Newton Road, Iowa City, IA 52242
Dr. Misonou's present address: Department of Biomedical Sciences, University of Maryland Baltimore, 650 W. Baltimore St., Baltimore, MD 21201

Introduction

Homeostatic processes that operate at both synaptic and cellular levels regulate overall activity in neuronal networks. Without these mechanisms, changes in synapse number and strength could threaten the stability of neuronal networks.[1] One mechanism for achieving homeostasis is to link synaptic activity to intrinsic excitability through activity-dependent modulation of voltage-gated ion channels. Voltage-gated potassium (Kv) channels play a crucial role in regulating neuronal excitability.[2] Different Kv channels are localized to presynaptic terminals, axons, somata and dendrites[3, 4], where they perform specific functions in maintaining and dynamically regulating membrane excitability.[5, 6] Two major classes of Kv currents in central neurons are the sustained delayed rectifier current I_K, and the transient A-type current I_A.[7] Kv2.1 channels constitute the major component of the somatodendritic sustained or delayed rectifier current I_K[8–11], and play a major role in regulating membrane excitability and Ca^{2+} influx in hippocampal and cortical neurons during high-frequency repetitive firing.[8, 12–14] The Kv2.1 channel is abundantly expressed in mammalian brain neurons[3], and specifically localized in high-density clusters in somata and proximal dendrites[15, 16] as well as the axon initial segment.[17]

Recently we found that the phosphorylation state and clustered localization of Kv2.1 in rat brain and in cultured hippocampal neurons are dynamically regulated in response to altered neuronal activity[18], ischemia[19], and muscarinic modulation.[20] These stimuli also lead to calcineurin-dependent changes in the voltage-dependence of activation of hippocampal neuronal I_K.[18–20] Similar modulation of voltage-dependent channel gating properties is induced by Ca^{2+}/calcineurin-dependent dephosphorylation of recombinant Kv2.1 in heterologous cells[20], at a large number of cytoplasmic serine phosphorylation sites.[21]

Here, we address the role of modulation of the Kv2.1-based neuronal I_K in regulating firing of hippocampal neurons. We first define the signaling pathway whereby glutamate stimulation leads to modulation of neuronal I_K, and then use a realistic model of neuronal firing to determine the potential effects of such modulation. We use current-clamp experiments and specific neurotoxin blockade to determine the contribution of Kv2.1 modulation to the changes in neuronal firing observed upon glutamate treatment. Together these studies demonstrate a critical role for calcineurin-mediated modulation of Kv2.1 in the suppression of the neuronal firing following high levels of neuronal activity

Results

Glutamate-stimulation of hippocampal neurons has diverse effects on voltage-dependent activation and inactivation of I_K via Ca^{2+}/calcineurin-dependent intracellular signaling

We previously found that seizures in rats *in vivo*, or glutamate stimulation of cultured rat hippocampal pyramidal neurons, led to calcineurin-dependent dephosphorylation of Kv2.1, and dispersion of Kv2.1 clusters.[18] Initial experiments showed that glutamate stimulation of cultured neurons also led to significant hyperpolarizing shifts in voltage-dependent activation of neuronal I_K.[18] However, the potential impact of these changes on neuronal function could not be accurately deduced without a more comprehensive analysis of the effects of glutamate stimulation on the gating properties of neuronal Kv2.1 channels. To electrophysiologically isolate I_K from I_A, neurons held at −80 mV were subjected to a conditioning step to −10 mV (30 ms) to eliminate the majority of fast-activating and inactivating component of I_A, briefly repolarized, and test-pulses delivered to evoke I_K currents. Under control conditions, I_K in cultured hippocampal neurons has half-maximal activation ($G_{1/2}$) and inactivation ($Vi_{1/2}$) voltages of +16.8 ± 0.3 mV and −28.5 ± 0.5 mV (n = 5), respectively (Fig. 1C; Table 1). These parameters were stable and not significantly different over an experimental time course of up to 20 min after the completion of the protocol for steady-state inactivation, in the absence of

further experimental manipulation. However, treatment of neurons with 10 μM glutamate for 10 minutes after the completion of the protocol for steady-state inactivation, and subsequent wash-out for 2 min, significantly increased the I_K amplitude (Fig. 1A–B), and yielded ≈25 mV hyperpolarizing shifts in the voltage-dependence of both activation and inactivation gating ($G_{1/2}$ = −8.6 ± 0.9 mV; $Vi_{1/2}$ = −54.7 ± 0.5 mV, n = 5, Fig. 1C; Table 1). Glutamate treatment also led to the suppression of most of the I_A component, and an increase in the amplitude of I_K component, as evident from the current traces at −10 mV conditioning pulse before each test pulse (Fig. 1A). Recovery from inactivation at −80 mV was also slowed. In control neurons the recovery from inactivation was quite fast and was complete within 1 sec (half-maximal recovery (τ_{recv}) = 0.2 ± 0.001 sec, n = 5), whereas in glutamate-treated neurons, recovery was incomplete even at 10 sec (τ_{recv} = 4.1 ± 0.3 sec, n = 5; p<0.001) (Fig. 1D).

We previously showed that glutamate-treatment of cultured rat hippocampal neurons led to dephosphorylation and lateral translocation of Kv2.1.[18] This involved activation of ionotropic glutamate receptors, leading to increased $[Ca^{2+}]_i$ and activation of the protein phosphatase calcineurin, and resulting in Kv2.1 dephosphorylation.[18] Here, we investigated whether the same signaling pathway underlies the glutamate-induced modification in the functional properties of I_K in cultured hippocampal neurons. Glutamate-induced hyperpolarizing shifts in the voltage-dependent gating parameters of I_K were abolished when the neurons were treated with glutamate in Ca^{2+}-free extracellular buffer, and also almost completely blocked by pre-treatment of neurons with APV, a specific antagonist of NMDA receptors (Fig. 2). Glutamate-induced shifts in I_K gating were also blocked by intracellular dialysis with a complex of cyclosporine A (CsA) and cyclophilin A (CypA), which specifically antagonizes the activity of calcineurin, but were not blocked by a low concentration of extracellular okadaic acid, which antagonizes the activity of the protein phosphatases 1A and 2A (Fig. 2). Extracellular perfusion of neurons with the Ca^{2+}-ionophore ionomycin also led to hyperpolarizing shifts in I_K gating (Fig. 2). These results suggest that the glutamate-induced hyperpolarizing shifts in I_K gating are mediated by a pathway identical to that which regulates Kv2.1 channel dephosphorylation and localization[18], namely activation of ionotropic glutamate receptors, increased $[Ca^{2+}]_i$, and activation of calcineurin.

Glutamate-induced modulation of I_K in hippocampal neurons is achieved primarily through effects on Kv2.1 channels

To identify the component of neuronal I_K sensitive to glutamate-induced gating modulation, we tested the effects of externally applied hanatoxin (HaTx), a Kv2.1 blocker/gating-modifier neurotoxin isolated from the venom of the Chilean tarantula.[22, 23] We first tested the blocking efficiency of bath applied HaTx on Kv2.1 channels expressed stably in HEK293 cells. Extracellular perfusion of 100 nM HaTx onto these cells for 10 min led to an ≈93% decrease in the Kv2.1 current amplitude (from 3.31 ± 0.49 nA to 0.21 ± 0.04 nA) at 0 mV, and an ≈62% decrease (from 11.7 ± 1.9 nA to 4.2 ± 0.4 nA) at +50 mV (n = 4, p< 0.05; Fig. 3A–B). That HaTx inhibition of Kv2.1 currents in HEK cells is dependent on membrane potential is consistent with previous studies of HaTx effects on Kv2.1 expressed in *Xenopus* oocytes.[22, 23] The voltage-dependent block of Kv2.1 currents by HaTx also led to a ≈25 depolarizing shift in the voltage-dependent activation gating of the channels ($G_{1/2}$ = +16.6 ± 0.9 mV for control vs +41.8 ± 0.6 mV for HaTx-treated, n = 4, Fig. 3C). HaTx-treatment (100 nM, 10 min) of cultured rat hippocampal neurons led to a significant decrease in peak I_K current amplitude, also dependent on membrane potential (Fig. 3D). This reduction in peak current amplitude occurred without significant changes in the voltage-dependence of I_K gating (Fig. 3E; Table 1). Pretreatment of neurons with 100 nM HaTx also eliminated the bulk of the glutamate-induced effects on the voltage-dependence of I_K activation and inactivation gating (from ≈25 mV without HaTx to ≈5 mV with HaTx; Fig. 3D–E; Table 1). That the glutamate-induced effects on I_K gating were lost upon blockade of Kv2.1 with HaTx indicates that the glutamate-

induced shifts in the voltage-dependent activation and inactivation properties of the I_K in hippocampal neurons are primarily contributed by the Kv2.1 component of this current.

Modeling the effects of Kv2.1 dephosphorylation on hippocampal neuronal firing

Because Kv2.1 is the major component of neuronal I_K in hippocampal neurons, altered activation and inactivation of Kv2.1 upon glutamate stimulation might change neuronal action potential firing. To test this we first developed detailed models of the effect of glutamate-mediated Kv2.1 current modulation on hippocampal neuron firing using NEURON 5.6 software.[24] We incorporated the data on the biophysical properties of the Kv2.1 component of I_K as we recorded from control and glutamate-treated neurons (see previous section), and kept all else constant in this hippocampal neuron model that resembles previously used models.[25, 26] Compared to control neurons, decreased action potential firing frequencies were observed in the glutamate-treated neurons at a number of different levels of current injections (Fig. 4A, C). Glutamate treatment also resulted in a fraction of Kv2.1 currents being active at resting membrane potentials, and during interspike intervals (Fig. 4B), which might contribute to increase in the duration of interspike intervals (Fig. 4A). As such the glutamate-stimulated modulation of Kv2.1 would be predicted to suppress hippocampal neuronal firing.

Glutamate-induced modification of the voltage-dependent gating of Kv2.1 contributes to suppression of action potential firing in cultured hippocampal neurons

To test the predictions obtained from these computer models of hippocampal neuron firing, we performed current-clamp experiments on cultured hippocampal neurons under control and glutamate-stimulated conditions. Under control conditions, neurons showed frequent spontaneous action potential firings presumably due to the high spontaneous activity of cultured neurons.[27] Firing was consistent over time periods of 15–17 min with continuous perfusion of ACSF. Glutamate treatment (10 μM, 10 min), led to a brief period of increased spontaneous firing, followed by a membrane depolarization to approximately −20 mV, at which point firing ceased (Fig. 5A). After glutamate washout, the neurons re-established a normal resting membrane potential within 2 min, but sustained the decreased spontaneous firing (Fig. 5A–B).

Injection of different amplitudes of currents into neurons led to a consistent frequency of action potential firing that was stable for more than 15 minutes (not shown). However, treatment with 10 μM glutamate for 10 min led to a significant decrease in action potential firing frequency (Fig. 6A, C), similar to that predicted from computer modeling (Fig. 4A, C). Similar experiments using 5 μM glutamate treatment for 10 min also led to significant suppression of firing (Fig. 6D, F). Note that treatment of cultured hippocampal neurons with 5 μM glutamate for 10 min had also resulted in large (20–25 mV) hyperpolarizing shifts in the voltage-dependent gating properties of I_K (Table 1). No significant differences in the membrane potential (V_m), input resistance (R_{input}), action potential amplitude ($AP_{amplitude}$), and action potential duration ($AP_{duration}$) were observed before and after glutamate treatment (Table 2). However, a decrease in the latency to the first spike was observed in glutamate-treated neurons (77.4 ± 4.2 ms and 34.9 ± 3.9 ms for 5 μM and 10 μM glutamate-treated neurons versus 87.2 ± 4.5 ms for control neurons), as measured with 20 pA current injections. The action potential rise-time was also decreased in glutamate-treated neurons (11.7 ± 0.3 ms for 10 μM glutamate-treated neurons versus 15.0 ± 0.6 ms for control, measured with 20 pA current injections).

We next directly investigated whether the glutamate-induced suppression of neuronal firing was dependent on the modified voltage-dependent gating parameters of Kv2.1 channels by using external HaTx treatment to block Kv2.1 currents in cultured hippocampal neurons (Fig. 3D–E). Treatment of control (i.e. unstimulated) neurons with 100 nM HaTx for 10 min led to an increase in firing frequency (Fig. 6B, C, E, F), consistent with a previous report on the effect

of anti-sense knock-down of Kv2.1 in hippocampal slices on pyramidal cell firing.[8] Interestingly, glutamate stimulation of HaTx-treated neurons led to a further significant increase in firing frequency (Fig. 6B, C, E, F), as opposed to the suppression of firing observed upon glutamate treatment of neurons in the absence of HaTx (Fig. 6B, C, E, F). Treatment of neurons with dendrotoxin-kappa (DTXκ, 1 μM for 10 min), a potent and selective blocker of Kv1.1-containing Kv channels subunits[28], also led to an increase in firing frequency in control neurons (Fig. 7A–B), consistent with previous reports on DTX treatments leading to increased action potential firing frequencies of rat CA1 hippocampal neurons[29], and striatal medium spiny neurons.[30] DTXκ treatment had no effect on the glutamate-induced hyperpolarizing shifts in the voltage-dependent I_K gating properties (not shown). Glutamate stimulation (5 μM for 10 min) of DTXκ-pretreated neurons still yielded a significant decrease in firing frequency (Fig. 7A–B). These data together demonstrate the important role of the HaTx-sensitive Kv2.1 currents in mediating the glutamate-induced suppression of neuronal firing.

The close correlation between predications derived from model hippocampal neurons, and experimental data from cultured hippocampal neurons, combined with the use of HaTx to block Kv2.1, suggest that dephosphorylation-induced changes specific to Kv2.1 gating contribute to the suppression of neuronal excitability observed upon glutamate treatment.

Sustained membrane depolarization also leads to modulation of the voltage-dependent gating of Kv2.1-based I_K and suppression of neuronal firing

We next studied whether membrane depolarization in itself was sufficient to modulate Kv2.1-based I_K and suppress firing in cultured hippocampal neurons. After achieving the whole-cell patch-clamp mode and recording I_K, the membrane potential of the neurons was depolarized to −20 mV for 10 min in the patch-clamp amplifier, followed by a return to the holding potential (−80 mV). This protocol resulted in ≈20–25 mV hyperpolarizing shifts in the voltage-dependent gating properties of the I_K, which were antagonized by including 1 mM $CdCl_2$ in the extracellular buffer to block Ca^{2+} entry, or by pre-treatment with intracellular CsA and CypA complex to block calcineurin activity (Fig. 2). Under current-clamp conditions, similar membrane depolarization (−20 mV for 10 min) led to significant suppression of spontaneous firing (Fig. 8A–B), as well as suppression of action potential firing upon injection of different amplitudes of currents into cultured hippocampal neurons (Fig. 8C–D). No significant changes in the V_m and R_{input} of neurons were observed during the resting period after 10 min of membrane depolarization to −20 mV (Table 2). We also subjected the neurons to short (≈30 sec) episodes of membrane depolarization (to −20 mV) interspersed with 1 min resting periods and recorded the spontaneous activity throughout the experiments. This type of episodic depolarizing stimulus led to a significant graded suppression of spontaneous spiking frequencies after subsequent stimuli (Fig. 8E–F). Although it would be interesting to see a similar graded change in the Kv2.1/I_K gating properties with step-wise short (≈30 sec) depolarizing pulses, it is not possible to do since the time duration (≈6.5 min) required to record the I_K activation gating parameters would not reflect the actual change with individual 30 s time-step depolarizing pulses. Altogether, these data show that multiple stimuli resulting in an increase in the Ca^{2+}/calcineurin activity in hippocampal neurons can affect Kv2.1 gating and lead to changes in neuronal firing.

Discussion

The studies presented here show that glutamate treatment of hippocampal neurons leads to pronounced changes in voltage-dependent gating of the Kv2.1 channels that constitute the majority of delayed rectifier current I_K. That altered properties of I_K are due to dephosphorylation of Kv2.1 is supported by a number of experimental observations. Kv2.1 undergoes calcineurin-dependent dephosphorylation in response to altered neuronal activity,

membrane depolarization, and neuromodulation[18–20], with parallel changes in the voltage-dependent gating of I_K (Fig. 1, 2). Blocking Kv2.1 currents with HaTx prevented the glutamate-induced changes in I_K gating (Fig. 3), showing that the Kv channels that comprise the minority non-Kv2.1 component of I_K are refractory to glutamate-dependent modulation (Fig. 3D–E).

We showed here that glutamate treatment of hippocampal neurons, through activation of Ca^{2+} permeable ionotropic glutamate receptors, leads to increased $[Ca^{2+}]_i$ that is both necessary and sufficient for the calcineurin-dependent modulation of I_K gating properties. This conclusion is drawn from our results showing that under Ca^{2+}-free extracellular conditions, or upon treatment with the specific NMDA receptor antagonist APV, effects of glutamate on voltage-dependent gating of I_K currents in neurons were eliminated (Fig. 2). Treatment of neurons with the Ca^{2+} ionophore ionomycin or increasing $[Ca^{2+}]_i$ through sustained membrane depolarization mimicked the glutamate-induced effects on I_K gating (Fig. 2), showing that, in the absence of glutamate stimulation, increased $[Ca^{2+}]_i$ is sufficient to induce modulation of neuronal of I_K. A central role for calcineurin in mediating the effects of increased $[Ca^{2+}]_i$ is evident from our experiments showing that dephosphorylation of Kv2.1[18–20] and, as shown here, modulation of I_K gating properties, in response to diverse stimuli that raise $[Ca^{2+}]_i$ is in all cases blocked by pre-treatment of neurons with specific calcineurin inhibitors. A recent study revealed that endogenous glutamate released in response to ischemia is also sufficient to induce Kv2.1 dephosphroylation and translocation. [31] Similar results have also been obtained for recombinant Kv2.1 in HEK293 cells.[20] Our previous studies show that at least eight cytoplasmic phosphoserine residues are sensitive to calcineurin-dependent dephosphorylation and contribute incrementally to modulation of the voltage-dependent gating properties of Kv2.1.[21] Specific modulation of these phosphorylation sites in various combinations presumably underlies the graded changes in I_K gating properties observed in response to different types and/or magnitudes of stimuli.[32]

Sustained activation of Kv2.1 has recently been suggested to be pro-apoptotic leading to increased neuronal cell death.[33] The enhanced Kv2.1 activity is a result of increased p38 MAP kinase-mediated phosphorylation of S800 in the cytoplasmic Kv2.1 C-terminus that leads to enhanced Kv2.1 expression.[33] S800 is constitutively phosphorylated on Kv2.1 purified from rat brain and in HEK293 and COS cells.[21, 34] This site is not regulated by calcineurin-dependent dephosphorylation of Kv2.1 in HEK293 cells, and mutation of S800 to Ala or Asp does not affect Kv2.1 gating.[21] As such the events that are associated with Kv2.1 activation during apoptosis appear to occur independently of those induced by glutamate stimulation as applied here. Treatment of cultured rat hippocampal neurons with high levels of glutamate (200 μM) for long periods (20 min) yields time-dependent increase in the density of I_K and decreased cell viability[35], through an as yet unknown mechanism. We previously found that reversible calcineurin-dependent Kv2.1 dephosphorylation as induced by ischemic insult is neuroprotective.[19] However, sustained activation of Kv2.1, as occurs upon induction of apoptosis[33], or prolonged treatment with high levels of glutamate[35], might lead to excessive K^+ efflux, and subsequent induction of apoptotic or excitotoxic cell death, as we suggested previously.[19]

The potential impact of modulating Kv2.1 channel gating in suppressing excitability of hippocampal neurons was proposed initially based on preliminary computer simulations.[36] Our more detailed modeling analyses presented here show that, using biophysical parameters of Kv2.1 activation and inactivation derived from control and glutamate-treated hippocampal neurons, the hyperpolarizing shift in the voltage-dependent gating of Kv2.1 channels alone was sufficient to dramatically reduce hippocampal neuronal firing in response to a broad range of excitatory current injections (Fig. 4). The computer simulations were predictive of experiments in cultured hippocampal neurons, which show drastic suppression of firing frequency upon glutamate treatment (Fig. 6). Similar suppression of action potential firing was

previously reported for kainate treatment of retinal ganglion neurons[37], which also express Kv2.1.[38] That glutamate-induced suppression of firing in cultured hippocampal neurons was reversed upon functional blockade of Kv2.1 currents with HaTx (Fig. 6), supports that modulation of Kv2.1 channels largely mediates the suppression of neuronal excitability. We should note that HaTx has some blocking activity against Kv4 channels[23], which underlie the neuronal I_A that prevent back propagation of action potentials into dendrites.[39] Activation of synaptic NMDA receptor-mediated Ca^{2+} entry in hippocampal neurons leads to increased phosphorylation of Kv4 channels and inhibition of I_A[40], presumably *via* phosphorylation-induced endocytosis of Kv4 channels[41], which results in increased dendritic excitability and induction of long-term potentiation.[39] Indeed our results also show that glutamate treatment led to the suppression of I_A currents (Fig. 1A). Increased firing of cultured rat hippocampal neurons is observed upon blockade of Kv4 channels with Heteropodatoxin-3.[42] However, our results here show a drastic reduction of neuronal firing frequency upon glutamate treatment (Fig. 6A, C, D, F), which suppresses I_A currents (Fig. 1A), but increases the amplitude and voltage-dependent gating properties of Kv2.1 (Fig. 1A–C). We did observe an increased firing frequency in HaTx-treated neurons (Fig. 6B, C, E, F), presumably due to blocking effects of HaTx on both Kv2.1[8] and Kv4[42] channels. Given these results and a recent report showing activity-dependent suppression of Kv4 channel function and increased neuronal excitability[6], it seems unlikely that Kv4 channels mediate the glutamate-induced, calcineurin-dependent suppression of neuronal excitability documented here. Although glutamate stimulation could affect the activity of other ion channels, the sufficiency of Kv2.1 modulation in model neurons in mimicking the experimentally observed suppression of neuronal excitability, and the loss of such suppression in HaTx-treated neurons, taken together argue that Kv2.1 contributes to the glutamate-induced suppression of firing.

While the model neurons accurately predicted the effects of glutamate on firing frequency, we should note that the model predicted an opposite effect of glutamate with regards to the latency to the first action potential following current injections (model: increased latency; cultured neurons: decreased latency). The differences between model and cultured neurons are presumably due to inhibitory effects of glutamate on Kv4 channels, which has been previously shown to yield decreased latency to the first spike.[42] That HaTx did not inhibit the glutamate-induced effects on the decreased latency to the first spike mediated by Kv4 channels, but eliminated the suppression of firing, supports that the effects of HaTx on the latter are mediated through Kv2.1 channels. Note however that glutamate induced an increase in the latency to the first spike in DTXκ-treated neurons (Fig. 7) suggesting that Kv1.1-containing channels also contribute to determining the latency to first spike.

Such activity-dependent effects on neuronal firing represent a form of homeostatic plasticity, whereby enhanced synaptic or intrinsic activity subsequently depresses intrinsic neuronal excitability.[43] That glutamate treatment and membrane depolarization lead to similar modulation of Kv2.1/I_K gating and suppresses neuronal firing is consistent with a general contribution of Kv2.1 modulation to homeostatic suppression of neuronal firing under conditions of hyperexcitability. The graded suppression of neuronal firing properties observed upon episodic membrane depolarization may correlate with a graded regulation of Kv2.1 gating properties upon dephosphorylation of individual phosphorylation sites on the channel protein.[21, 32] Several classes of ion channels, including Ca^{2+}-dependent K^+ channels[44], voltage-dependent Na^+ channels[45, 46], and HCN channels[47] have also been implicated in mediating changes in excitability associated with homeostatic plasticity. Our studies, together with those previously performed on cortical pyramidal neurons[48], argue that activity-dependent modulation of Kv2.1/I_K is also an important determinant of this type of plasticity. Modulation of Kv2.1 with subsequent effects on neuronal excitability may also contribute to changes in excitability seen in spreading depression[49] and ischemia/hypoxia, when excess glutamate may accumulate.[50] Furthermore, the broad expression of Kv2.1 in mammalian brain suggests that

this Kv2.1-dependent form of homeostatic plasticity may be found throughout the nervous system.

Materials and methods

Materials

Glutamate, aminophosphonovaleric acid (APV), ionomycin, okadaic acid (OA), dendrotoxin-kappa (DTXκ), 4-aminopyridine (4-AP), cyclosporin A (CsA), and cyclophilin A (CypA), were obtained from Sigma Chemical (St. Louis, MO). HPLC-purified hanatoxin (HaTx) was a generous gift from Dr. Kenton J. Swartz, NINDS, Porter Neuroscience Institute, NIH, Bethesda, MD. All the drugs were diluted in the extracellular buffer, except for CsA and CypA, which were dissolved in the pipette solution to achieve the final desired concentrations.

Culture of rat embryonic hippocampal neurons

Hippocampal neurons isolated from E18 or E19 rat embryos were cultured for 12–15 days on poly-L-lysine coated glass cover-slips or plastic tissue culture dishes as described previously.[18]

Culture of HEK293 cells stably expressing recombinant rat Kv2.1

Generation of the Human Embryonic Kidney (HEK) 293 cells (ATCC, Manassas, VA) stably expressing recombinant rat Kv2.1 was described previously.[21] Cells were maintained in Dulbecco's Modified Eagle's Media (DMEM) with 100 units/ml of penicillin and streptomycin, Glutamax (Invitrogen), and 10% Fetal Bovine Serum (FBS; Hyclone), along with 500 μg/ml of active G418 (Invitrogen), and plated 24 hours before use in electrophysiological experiments.

Electrophysiological recordings and data analyses

Voltage-clamp—Outward potassium currents were recorded from cultured rat hippocampal neurons in whole-cell mode using an EPC10 patch-clamp amplifier (HEKA Electronik, Lambrecht/Pfalz, Germany), at room temperature (23 – 25 °C) as described.[20] Patch pipettes were pulled from borosilicate glass tubes (Sutter Instrument, Novato, CA) to give a tip resistance of 1.5 – 3.0 MΩ when filled with the pipette solution. The extracellular buffer contained (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose, pH 7.3. The pipette solution contained (in mM) 140 KCl, 5 NaCl, 2 $MgCl_2$, 1 $CaCl_2$, 5 EGTA and 10 HEPES, pH 7.3. The free Ca^{2+}, Mg^{2+}, and EGTA concentrations in the pipette solution were calculated using the Max-Chelator software (Stanford, CA), and are 0.027 mM, 1.6 mM, and 2.6 mM respectively. Glutamate and HaTx were dissolved in the extracellular buffer to achieve the final desired concentration. Control and drug-added extracellular buffer were applied to the bath for 10 min immediately after recording the currents for activation, steady-state inactivation, and recovery from inactivation using a polytetrafluroethylene glass multiple-barrel perfusion system. In voltage-clamp experiments using HaTx, the first set of currents (for activation and steady-state inactivation) were recorded soon after obtaining the whole-cell configuration, then HaTx was applied in the extracellular buffer for 10 min and next set of currents were recorder. Further, HaTx and glutamate were applied together in the extracellular buffer for 10 min and the final set of currents were recorded.

For voltage-dependent current activation experiments, neurons were held at −80 mV and step depolarized to +80 mV for 200 ms with depolarizing 10 mV increments. A pre-pulse at −10 mV for 30 ms was given before each test-pulse to inactivate the majority of transient outward K^+ currents. For steady-state inactivation experiments, the cells were held at −100 mV and step depolarized to +40 mV for 10 s with 10 mV increments (conditioning steady-pulse)

followed by a test pulse at +10 mV (test-pulse) for 200 ms. The interpulse interval was 20 s. The Nernst K^+ equilibrium potential E_K was calculated as −84 mV. For calculating the voltage-dependent activation and steady-state inactivation of I_K currents, peak current amplitudes from the later 100 ms pulse at each test-pulse were taken. The activation and inactivation curves were generated using established protocols[19, 20] and the voltage-dependent parameters are given in Table 1. To determine the recovery time period from steady-state inactivation of I_K, the cells were held at −80 mV and depolarized to +50 mV for 500 ms, followed by subsequent pulses again at +50 mV with different interval durations in 200 ms increments with an intermediate holding at −80 mV. 2 mM 4-AP was used in the extracellular buffer to block I_A. Peak I_K amplitudes from the second pulse were normalized to the current obtained in the respective first pulse and plotted against the respective interval time.

PULSE 8.65 software (HEKA Elektronik) was used for acquisition and analysis of currents. IGOR Pro4 (WaveMetrix, Lake Oswego, OR), and Origin7 software (OriginLab, Northampton, MA) were used to perform least-squares fitting and to create figures. Data are presented as mean ± SEM or fitted value ± SE of the Boltzmann equation fit. Paired or unpaired Student's *t*-tests (Origin; OriginLab) were used to evaluate significance of changes in mean values. *P* values <0.05 were considered statistically significant.

Current-clamp—Action potential firing patterns in cultured rat hippocampal neurons were recorded in current-clamp mode of standard patch-clamp technique at room temperature (23 – 25 °C). Patch pipettes were pulled from borosilicate glass tubes to give a tip resistance of 1.5 – 4.5 MΩ when filled with pipette solution. Membrane voltages were recorded either with an Axopatch 1D patch-clamp amplifier and monitored with a PC running pClamp6 (Axon Instruments), or using PULSE 8.65 and Patchmaster 2.10 software in an EPC10 patch-clamp amplifier (HEKA Electronik). After achieving whole-cell configuration in voltage-clamp mode and compensating capacitance, membrane voltages were recorded in current-clamp mode by holding the neurons at their inherent membrane potentials (−57 mV to −68 mV). The extracellular buffer (ACSF) contained (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4, and 10 glucose, pH 7.3, and was continuously bubbled with CO_2 during the experiment. The pipette solution contained (in mM) 140 KCl, 5 NaCl, 2 $MgCl_2$, 1 $CaCl_2$, 3 Mg-ATP, 0.2 Na-GTP, 5 EGTA and 10 HEPES, pH 7.3. Glutamate, HaTx, and DTXκ were diluted in the ACSF to achieve the final desired concentration. Control and drug-containing ACSF were applied to the bath using a polytetrafluorethylene glass multiple barrel perfusion system. Glutamate was applied to the bath immediately after recording the firing by injecting different amplitude of currents, and after 10 min next set of current-injected firings were recorded. In experiments using HaTx, the first set of firings was recorded soon after 10 min treatment of neurons with HaTx in extracellular buffer. Further, HaTx and glutamate were applied together in the extracellular buffer for 10 min and the next set of firings was recorded. Origin7 software (OriginLab) was used to perform least squares fitting and to create figures. Data are presented as mean ± SEM. Paired or unpaired Student's *t*-tests were used to evaluate significance of changes. *P* values <0.05 were considered statistically significant.

Generation of current-clamp data by computer simulation

Simulations were performed using NEURON 5.7.[24] Because the employed electrophysiological protocols were similar to those used by Hodgkin and Huxley[51, 52], our simulations used a mathematical model with similar features. First, Kv2.1 channel gating was assumed to conform to a Hodgkin-Huxley-like formalism $I = G_{max} n^2(t,V)[(a)h(t,V) + (1 - a)](V\text{-}E_K)$, where I is the current, G_{max} is the maximum conductance, V is the transmembrane voltage, E_K is the K^+ equilibrium potential, and n and h are the gating particles that control activation and inactivation of the Kv2.1 channel, respectively. Both n and h were functions of time and transmembrane voltage that satisfied the partial differential equations,

$$\partial n/\partial t=[n(\infty,V)-n(t,V)]/\tau n(V)$$
$$\partial h/\partial t=[h(\infty,V)-h(t,Vh)]/\tau h(Vh)$$

where

$$n(\infty,V)=1/\{1+\exp[(V-Vnh)/Vnc]\}$$
$$\tau n(V)=Cn/\{\exp[(V-V\tau nn)/V\tau c1]+\exp[-(V-V\tau n2)/V\tau c2]+\tau n\phi$$
$$h(\infty,V)=(1-P)/\{1+\exp[-(V-Vhh)/Vhc]\}+P$$
$$\tau h(V)=Cn/\{\exp[(V-V\tau hh)/V\tau c1]+\exp[-(V-V\tau h2)/V\tau c2]+\tau h\phi$$

and where *Vnh* is the half-maximal activation of the steady-state activation curve; *Vnc* is the slope of the steady-state activation curve; C*n* is assumed to be the maximal activation time constant taking into account that $\tau n\phi$ is close to zero; $\tau n\phi$ is the activation time constant at maximal positive or negative potentials; *Vτn2* is the voltage at which the time constant for activation is maximum; *Vτc1* is the slope of the activation curve for the negative side of the *Vnh; Vτc2* is the slope of the activation curve for the positive side of the *Vnh; Vhh* is the half-maximal steady-state inactivation voltage; *Vhc* is the slope of the steady-state inactivation curve; C*h* is assumed to be the maximal inactivation time constant; $\tau h\phi$ is the inactivation time constant at maximal positive or negative potentials; *Vτh2* is the voltage at which the time constant for inactivation is maximum; *P* is the fraction of non-inactivating current.

This formalism was coded in a NEURON mod file and then virtual channels were distributed in the membrane of cylindrical soma (35 μm length, 25 μm diameter) of a model hippocampal pyramidal neuron with a single bifurcating apical dendrite and two basal dendrites. To model currents generated from somatic point clamp recordings, two different Hodgkin-Huxley-style Kv2.1 channel models were created to represent control and glutamate-modulated conditions. The Kv2.1 density was assumed to be zero in the all compartments except the soma. Manually adjusting values of various parameters in steady-state activation, steady-state inactivation, activation time constant and inactivation time constant formulae led to a reasonably accurate simulation of experimental data for both control (with $G_{1/2}$ = +16 mV and $Vi_{1/2}$ = −25 mV) and glutamate-modulated conditions (with $G_{1/2}$ = −10 mV and $Vi_{1/2}$ = −55 mV). A spiking model of a hippocampal pyramidal neuron was constructed by adding Nav1, Cav1, Cav2, Kv2, Kv3, Kv4 and Kv7 (KCNQ), SK, BK, KIR2 and HCN channel models that were based upon standard experimental measurements reported in the literature. These mod files and the details of this model are available upon request. Action potential firing patterns from this model neuron were predicted upon somatic injection of different current amplitudes ranging from 0 to 500 pA over a period of 500 ms from a holding potential of −65 mV using the NEURON utilities in current-clamp mode. The control and glutamate-induced altered biophysical properties of I_K were simulated separately to obtain the action potential firing patterns upon altered Kv2.1 channel activity. Upon publication, this model will be available on the Neuron Model Database website (http://senselab.med.yale.edu/modeldb/).

Acknowledgments

This work was supported by NIH/NINDS grants NS42225 (to J.S.T.) and NS34696 (to D.J.S.). D.P.M. was supported in part by a postdoctoral fellowship grant from the Epilepsy Foundation through the generous support of the American Epilepsy Society and the Milken Family Foundation. We thank Dr. Kenton J. Swartz, for the generous gift of hanatoxin; Dr. Elva Diaz and Ms. Evgenia Kalashnikova for providing some of the cultured rat hippocampal neurons; Dr. Phillip A. Schwartzkroin for critical reading and constructive suggestions on the manuscript.

Abbreviations

Kv voltage-gated potassium channel

I_K delayed rectifier K^+ current

HaTx hanatoxin

DTXκ dendrotoxin-kappa

ACSF artificial cerebrospinal fluid

$[Ca^{2+}]_i$ intracellular calcium

NMDA N-methyl-D-aspartate

References

1. Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci 2004;5:97–107. [PubMed: 14735113]
2. Pongs O. Voltage-gated potassium channels: from hyperexcitability to excitement. FEBS Lett 1999;452:31–5. [PubMed: 10376673]
3. Trimmer JS, Rhodes KJ. Localization of voltage-gated ion channels in mammalian brain. Annu Rev Physiol 2004;66:477–519. [PubMed: 14977411]
4. Vacher H, Mohapatra DP, Trimmer JS. Localization and targeting of voltage-dependent ion channels in mammalian central neurons. Physiol Rev 2008;88:1407–47. [PubMed: 18923186]
5. Dodson PD, Forsythe ID. Presynaptic K+ channels: electrifying regulators of synaptic terminal excitability. Trends Neurosci 2004;27:210–7. [PubMed: 15046880]
6. Jerng HH, Pfaffinger PJ, Covarrubias M. Molecular physiology and modulation of somatodendritic A-type potassium channels. Mol Cell Neurosci 2004;27:343–69. [PubMed: 15555915]
7. Hille, B. Ionic channels of excitable membranes. Sunderland, MA: Sinauer; 2001.
8. Du J, Haak LL, Phillips-Tansey E, Russell JT, McBain CJ. Frequency-dependent regulation of rat hippocampal somato-dendritic excitability by the K+ channel subunit Kv2.1. J Physiol 2000;522:19–31. [PubMed: 10618149]
9. Guan D, Tkatch T, Surmeier DJ, Armstrong WE, Foehring RC. Kv2 subunits underlie slowly inactivating potassium current in rat neocortical pyramidal neurons. J Physiol 2007;581:941–60. [PubMed: 17379638]
10. Murakoshi H, Trimmer JS. Identification of the Kv2.1 K+ channel as a major component of the delayed rectifier K+ current in rat hippocampal neurons. J Neurosci 1999;19:1728–35. [PubMed: 10024359]
11. Pal S, Hartnett KA, Nerbonne JM, Levitan ES, Aizenman E. Mediation of neuronal apoptosis by Kv2.1-encoded potassium channels. J Neurosci 2003;23:4798–802. [PubMed: 12832499]
12. Bekkers JM. Distribution and activation of voltage-gated potassium channels in cell-attached and outside-out patches from large layer 5 cortical pyramidal neurons of the rat. J Physiol 2000;525:611–20. [PubMed: 10856116]
13. Kang J, Huguenard JR, Prince DA. Voltage-gated potassium channels activated during action potentials in layer V neocortical pyramidal neurons. J Neurophysiol 2000;83:70–80. [PubMed: 10634854]

14. Malin SA, Nerbonne JM. Delayed rectifier K+ currents, IK, are encoded by Kv2 alpha-subunits and regulate tonic firing in mammalian sympathetic neurons. J Neurosci 2002;22:10094–105. [PubMed: 12451110]
15. Lim ST, Antonucci DE, Scannevin RH, Trimmer JS. A novel targeting signal for proximal clustering of the Kv2.1 K+ channel in hippocampal neurons. Neuron 2000;25:385–97. [PubMed: 10719893]
16. Trimmer JS. Immunological identification and characterization of a delayed rectifier K+ channel polypeptide in rat brain. Proc Natl Acad Sci U S A 1991;88:10764–8. [PubMed: 1961744]
17. Sarmiere PD, Weigle CM, Tamkun MM. The Kv2.1 K+ channel targets to the axon initial segment of hippocampal and cortical neurons in culture and in situ. BMC Neurosci 2008;9:112. [PubMed: 19014551]
18. Misonou H, Mohapatra DP, Park EW, Leung V, Zhen D, Misonou K, et al. Regulation of ion channel localization and phosphorylation by neuronal activity. Nat Neurosci 2004;7:711–8. [PubMed: 15195093]
19. Misonou H, Mohapatra DP, Menegola M, Trimmer JS. Calcium- and metabolic state-dependent modulation of the voltage-dependent Kv2.1 channel regulates neuronal excitability in response to ischemia. J Neurosci 2005;25:11184–93. [PubMed: 16319318]
20. Mohapatra DP, Trimmer JS. The Kv2.1 C terminus can autonomously transfer Kv2.1-like phosphorylation-dependent localization, voltage-dependent gating, and muscarinic modulation to diverse Kv channels. J Neurosci 2006;26:685–95. [PubMed: 16407566]
21. Park KS, Mohapatra DP, Misonou H, Trimmer JS. Graded regulation of the Kv2.1 potassium channel by variable phosphorylation. Science 2006;313:976–9. [PubMed: 16917065]
22. Lee HC, Wang JM, Swartz KJ. Interaction between extracellular Hanatoxin and the resting conformation of the voltage-sensor paddle in Kv channels. Neuron 2003;40:527–36. [PubMed: 14642277]
23. Swartz KJ, MacKinnon R. An inhibitor of the Kv2.1 potassium channel isolated from the venom of a Chilean tarantula. Neuron 1995;15:941–9. [PubMed: 7576642]
24. Hines ML, Carnevale NT. The NEURON simulation environment. Neural Comput 1997;9:1179–209. [PubMed: 9248061]
25. Lazarewicz MT, Migliore M, Ascoli GA. A new bursting model of CA3 pyramidal cell physiology suggests multiple locations for spike initiation. Biosystems 2002;67:129–37. [PubMed: 12459292]
26. Traub RD, Spruston N, Soltesz I, Konnerth A, Whittington MA, Jefferys GR. Gamma-frequency oscillations: a neuronal population phenomenon, regulated by synaptic and intrinsic cellular processes, and inducing synaptic plasticity. Prog Neurobiol 1998;55:563–75. [PubMed: 9670218]
27. Furshpan EJ, Potter DD. Seizure-like activity and cellular damage in rat hippocampal neurons in cell culture. Neuron 1989;3:199–207. [PubMed: 2560392]
28. Wang FC, Bell N, Reid P, Smith LA, McIntosh P, Robertson B, et al. Identification of residues in dendrotoxin K responsible for its discrimination between neuronal K+ channels containing Kv1.1 and 1.2 alpha subunits. Eur J Biochem 1999;263:222–9. [PubMed: 10429207]
29. Metz AE, Spruston N, Martina M. Dendritic D-type potassium currents inhibit the spike afterdepolarization in rat hippocampal CA1 pyramidal neurons. J Physiol 2007;581:175–87. [PubMed: 17317746]
30. Shen W, Hernandez-Lopez S, Tkatch T, Held JE, Surmeier DJ. Kv1.2-containing K+ channels regulate subthreshold excitability of striatal medium spiny neurons. J Neurophysiol 2004;91:1337–49. [PubMed: 13679409]
31. Misonou H, Thompson SM, Cai X. Dynamic regulation of the Kv2.1 voltage-gated potassium channel during brain ischemia through neuroglial interaction. J Neurosci 2008;28:8529–38. [PubMed: 18716211]
32. Misonou H, Menegola M, Mohapatra DP, Guy LK, Park KS, Trimmer JS. Bidirectional activity-dependent regulation of neuronal ion channel phosphorylation. J Neurosci 2006;26:13505–14. [PubMed: 17192433]
33. Redman PT, He K, Hartnett KA, Jefferson BS, Hu L, Rosenberg PA, et al. Apoptotic surge of potassium currents is mediated by p38 phosphorylation of Kv2.1. Proc Natl Acad Sci U S A 2007;104:3568–73. [PubMed: 17360683]

34. Park KS, Mohapatra DP, Trimmer JS. Proteomic analyses of Kv2.1 channel phosphorylation sites determining cell background-specific differences in function. Channels 2007;1:59–61. [PubMed: 18690023]
35. Zhao YM, Sun LN, Zhou HY, Wang XL. Voltage-dependent potassium channels are involved in glutamate-induced apoptosis of rat hippocampal neurons. Neurosci Lett 2006;398:22–7. [PubMed: 16434141]
36. Surmeier DJ, Foehring R. A mechanism for homeostatic plasticity. Nat Neurosci 2004;7:691–2. [PubMed: 15220926]
37. Taschenberger H, Grantyn R. Interaction of calcium-permeable non-N-methyl-D-aspartate receptor channels with voltage-activated potassium and calcium currents in rat retinal ganglion cells in vitro. Neuroscience 1998;84:877–96. [PubMed: 9579791]
38. Pinto LH, Klumpp DJ. Localization of potassium channels in the retina. Prog Retin Eye Res 1998;17:207–30. [PubMed: 9695793]
39. Birnbaum SG, Varga AW, Yuan LL, Anderson AE, Sweatt JD, Schrader LA. Structure and function of Kv4-family transient potassium channels. Physiol Rev 2004;84:803–33. [PubMed: 15269337]
40. Hoffman DA, Johnston D. Downregulation of transient K+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. J Neurosci 1998;18:3521–8. [PubMed: 9570783]
41. Kim J, Jung SC, Clemens AM, Petralia RS, Hoffman DA. Regulation of dendritic excitability by activity-dependent trafficking of the A-type K+ channel subunit Kv4.2 in hippocampal neurons. Neuron 2007;54:933–47. [PubMed: 17582333]
42. Varga AW, Yuan LL, Anderson AE, Schrader LA, Wu GY, Gatchel JR, et al. Calcium-calmodulin-dependent kinase II modulates Kv4.2 channel expression and upregulates neuronal A-type potassium currents. J Neurosci 2004;24:3643–54. [PubMed: 15071113]
43. Desai NS. Homeostatic plasticity in the CNS: synaptic and intrinsic forms. J Physiol Paris 2003;97:391–402. [PubMed: 15242651]
44. Nelson AB, Krispel CM, Sekirnjak C, du Lac S. Long-lasting increases in intrinsic excitability triggered by inhibition. Neuron 2003;40:609–20. [PubMed: 14642283]
45. Carr DB, Day M, Cantrell AR, Held J, Scheuer T, Catterall WA, et al. Transmitter modulation of slow, activity-dependent alterations in sodium channel availability endows neurons with a novel form of cellular plasticity. Neuron 2003;39:793–806. [PubMed: 12948446]
46. Pratt KG, Aizenman CD. Homeostatic regulation of intrinsic excitability and synaptic transmission in a developing visual circuit. J Neurosci 2007;27:8268–77. [PubMed: 17670973]
47. Brager DH, Johnston D. Plasticity of intrinsic excitability during long-term depression is mediated through mGluR-dependent changes in I(h) in hippocampal CA1 pyramidal neurons. J Neurosci 2007;27:13926–37. [PubMed: 18094230]
48. Desai NS, Rutherford LC, Turrigiano GG. Plasticity in the intrinsic excitability of cortical pyramidal neurons. Nat Neurosci 1999;2:515–20. [PubMed: 10448215]
49. Somjen GG. Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. Physiol Rev 2001;81:1065–96. [PubMed: 11427692]
50. Hertz L, Zielke HR. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. Trends Neurosci 2004;27:735–43. [PubMed: 15541514]
51. Hodgkin AL, Huxley AF. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J Physiol 1952;116:449–72. [PubMed: 14946713]
52. Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 1952;117:500–44. [PubMed: 12991237]

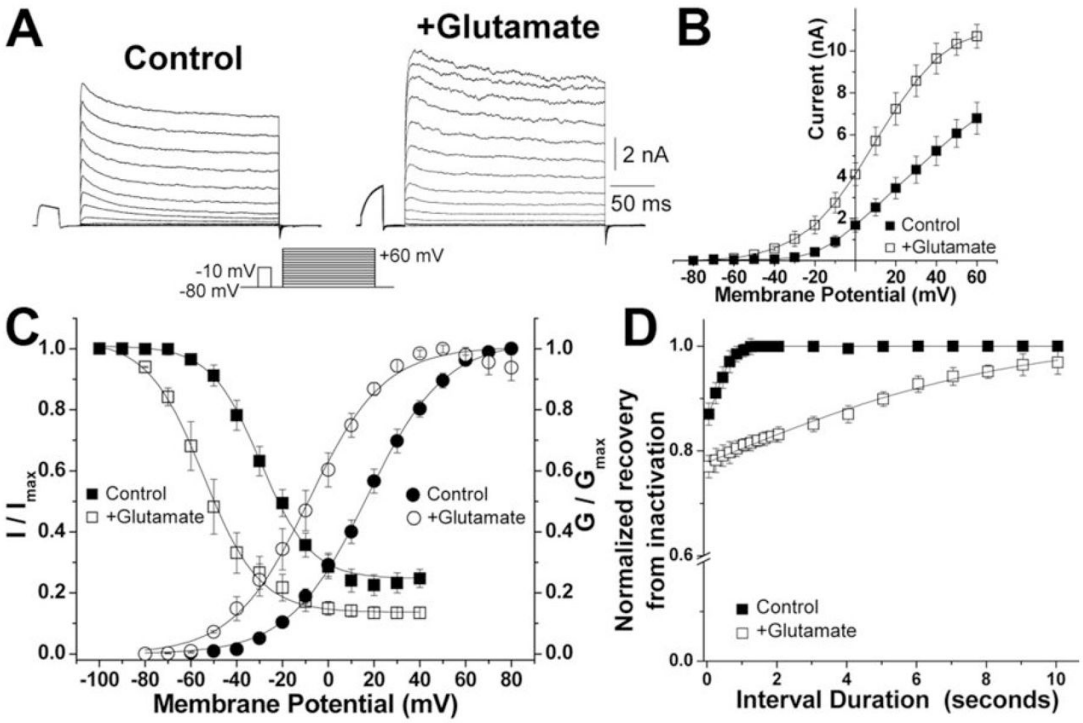

Fig. 1.

Glutamate treatment alters steady-state activation and inactivation properties of I_K in cultured rat hippocampal neurons. (A) Representative whole-cell current recordings obtained with the depicted pulse protocol before and after the extracellular perfusion of 10 μM glutamate for 10 min. The interpulse interval between each pulse was 20 sec. (B) Current-voltage relationship of peak currents obtained from the experiments in panel (A). (C) Voltage-dependent activation and steady-state inactivation curves for I_K from control and glutamate-treated neurons. (D) Time-course of recovery from steady-state inactivation of I_K currents from neurons before and after the extracellular perfusion of 10 μM glutamate for 10 min. The holding potential was −80 mV.

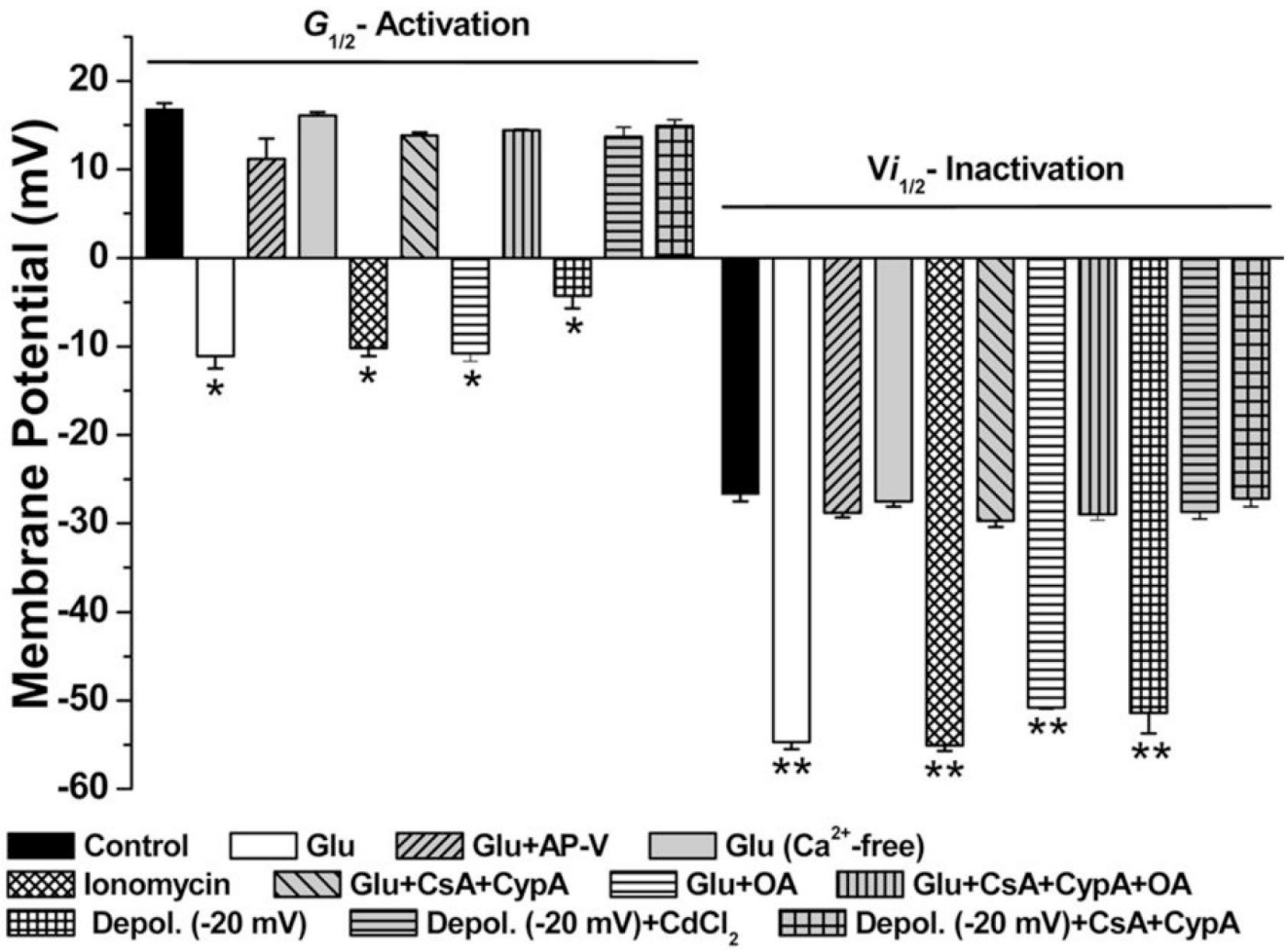

Fig. 2.

Ca^{2+}/calcineurin-dependent modulation of the half-maximal voltage-dependent activation ($G_{1/2}$) and steady-state inactivation ($Vi_{1/2}$) potentials of I_K currents from cultured rat hippocampal neurons with different drug treatments. Glutamate (10 μM), AP-V (5 μM), Ionomycin (1 μM), Okadaic acid (OA, 0.1 μM), and cadmium chloride ($CdCl_2$, 1 μM) were applied extracellularly, whereas cyclosporin A (CsA; 1 μM) and cyclophilin A (CypA; 1 μM) were applied intracellularly by dissolving in the pipette solution. Data are presented as mean ± SEM (n = 5, 5, 5, 6, 7, 5, 4, 6, 4, 4, and 4 for Control, Glu, Glu+APV, Glu[Ca^{2+}-free], Ionomycin, Glu+CsA+CypA, Glu+OA, Glu+CsA+CypA+OA, Depol.[−20 mV], Depol.[−20 mV]+$CdCl_2$, and Depol.[−20 mV]+CsA+CypA respectively). Asterisks indicate significant difference in the $G_{1/2}$ potentials (*) and $Vi_{1/2}$ potentials (**) as compared to respective values under control conditions (p<0.05).

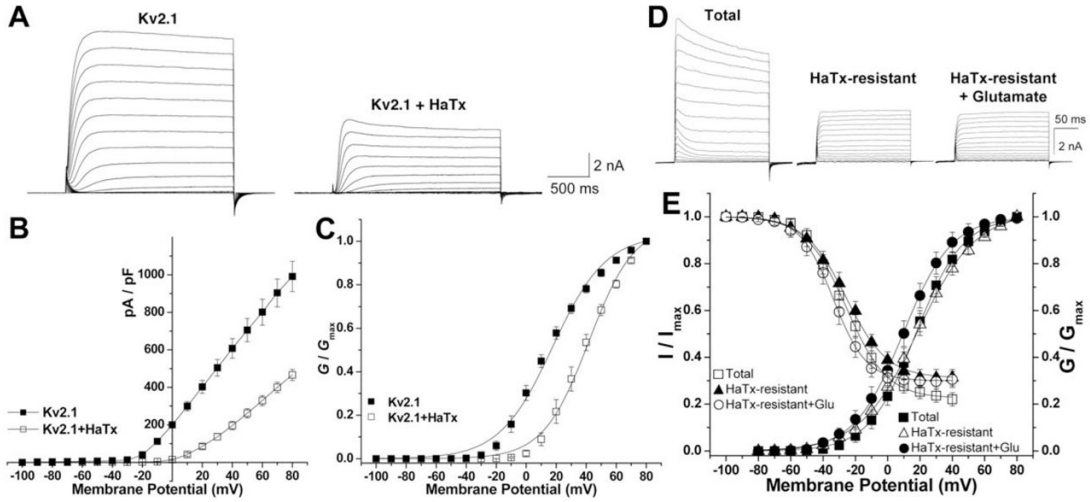

Fig. 3.

Block of Kv2.1 currents by hanatoxin (HaTx) eliminates the glutamate-induced shifts in the voltage-dependent gating properties of I_K. (A) Representative whole-cell current recordings from HEK293 cells stably expressing recombinant rat Kv2.1 channels, from a holding potential of −100 mV with 10 mV incremental depolarizing potentials to +80 mV before and after extracellular application of 100 nM HaTx for 10 min. (B–C) Current density plot (B) and steady-state activation curves (C) of recordings obtained from experiments as shown in panel (A). Data are presented as mean ± SEM. (D–E) Glutamate-induced shifts in I_K are specific to currents carried by Kv2.1 channels. (D) Representative whole-cell current recordings obtained from a cultured rat hippocampal neuron following the pulse protocol depicted in Fig. 1A, before and after 10 min superfusion of 100 nM hanatoxin (HaTx), followed by superfusion of 10 μM glutamate along with 100 nM HaTx. (E) Steady-state activation and inactivation curves for I_K recorded from neurons as shown in panel (A). The voltage-dependent biophysical parameters are detailed in Table 1.

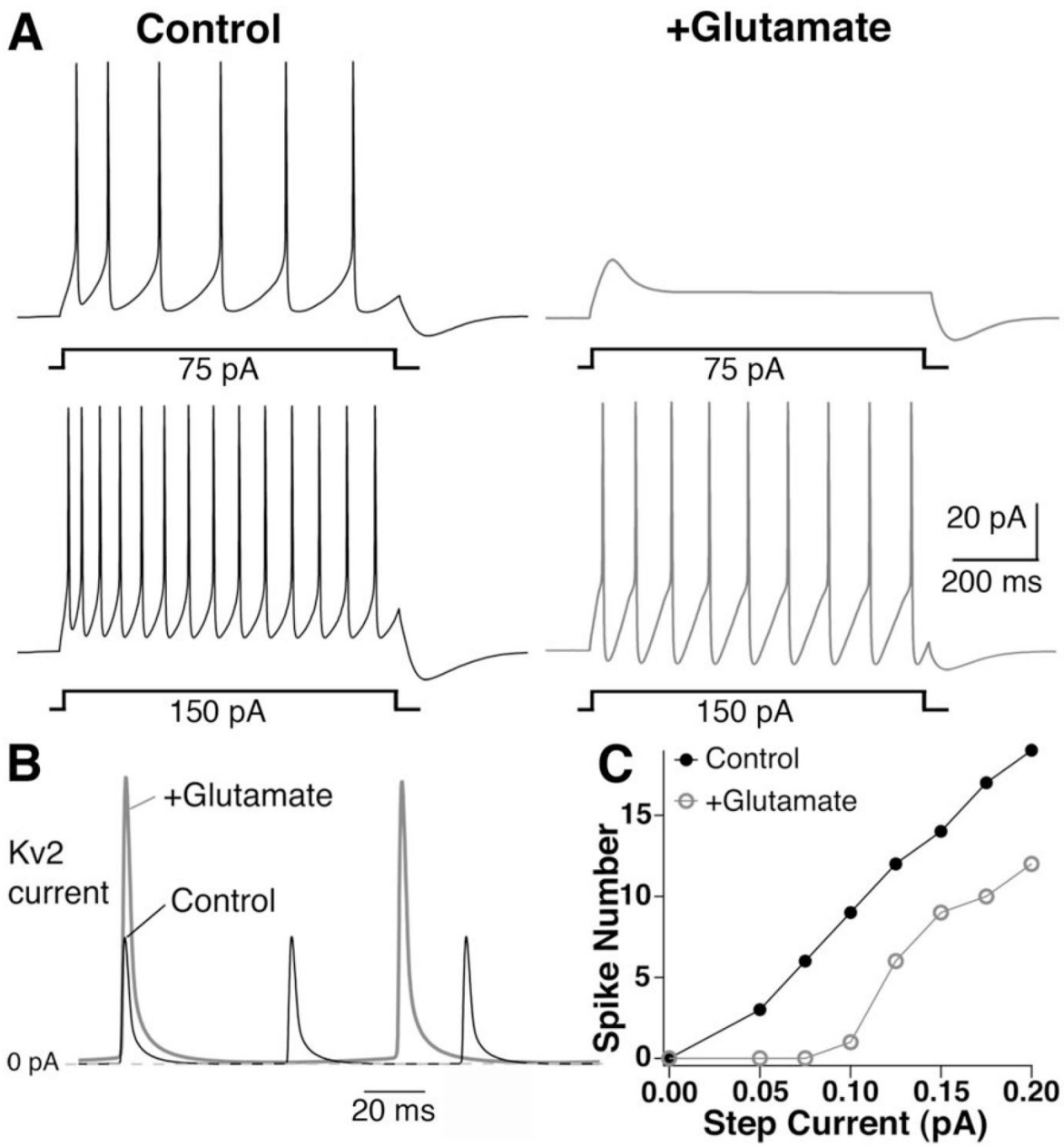

Fig. 4.

Glutamate-induced alterations in the activation and inactivation properties of Kv2.1/I_K lead to decreased action potential firing frequency in model hippocampal neurons. (A) Action potential firing patterns in an idealized hippocampal pyramidal neuron. Firing patterns were obtained by injecting 75 pA (upper panel) and 150 pA (lower panel) current from a membrane potential of −70 mV into model hippocampal pyramidal neurons using Kv2.1 functional parameters obtained from control or glutamate-treated neurons. Parameters of all other ion channels were kept constant. (B) Kv2.1 current in control (thin black line) and glutamate-stimulated (thick grey line) model neurons invoked by action potential waveforms obtained from a 150 pA current injection. (C) Frequency-intensity plot of action potential spikes generated by the hippocampal pyramidal neuron model upon step-wise current injections.

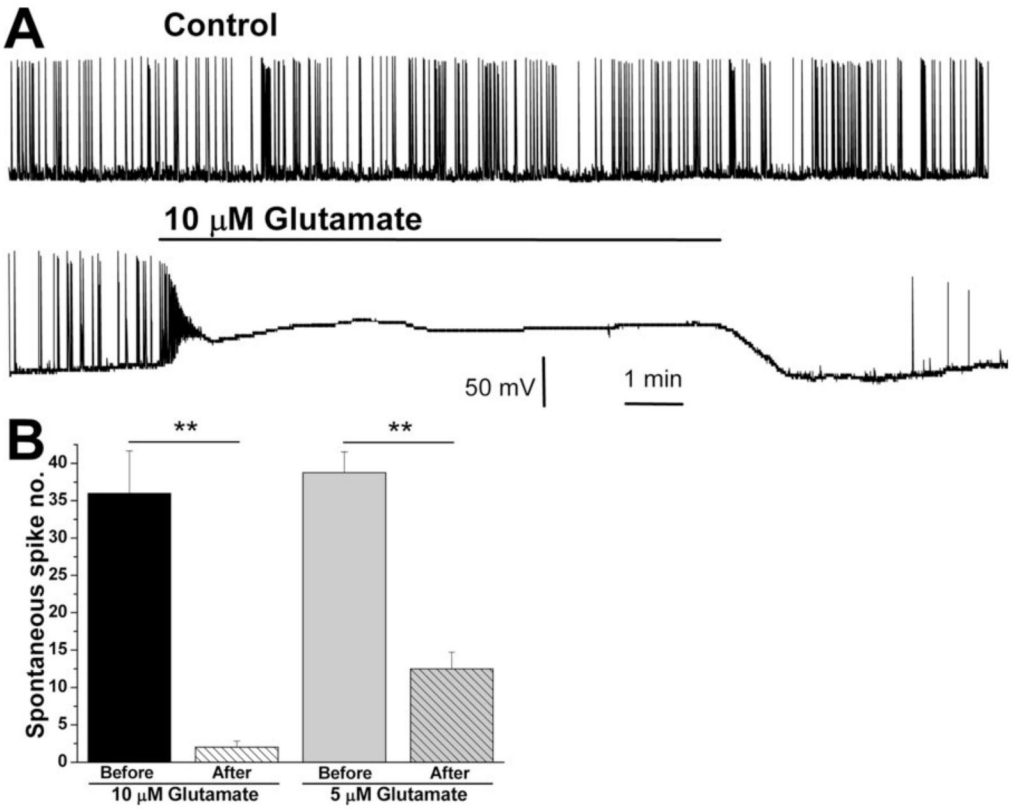

Fig. 5.

Glutamate treatment of cultured rat hippocampal neurons leads to suppression of spontaneous firing. (A) Representative traces of spontaneous firing patterns in cultured hippocampal neurons for a period of 17 min with continuous bath perfusion of ACSF (upper panel), and with 10 μM glutamate application in ACSF for 10 min following an initial ACSF perfusion for 2.5 min and post-glutamate ACSF wash of 4.5 min (bottom panel). (B) Quantitation of spontaneous firing before and after glutamate treatment as shown in panel (A). Data are presented as mean ± SEM of the number of spontaneous spikes per minute, before and after glutamate treatment (n = 4). *indicates significant difference in the number of spontaneous spikes ($p<0.05$).

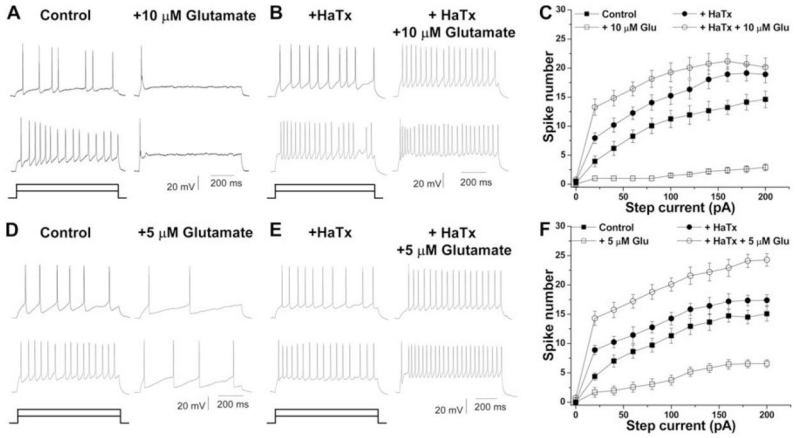

Fig. 6.

Decreased action potential firing in cultured rat hippocampal neurons upon glutamate-treatment is dependent on Kv2.1. (A, D) Representative traces of action potential firing in cultured hippocampal neurons upon injection of 20 pA (upper panel) and 100 pA (lower panel) currents, respectively, over a period of 1 sec, and before and after the treatment of 10 μM glutamate (A) or 5 μM glutamate (D) for 10 min, followed by wash-out for 2 min. (B, E) Representative traces of action potential firing patterns in cultured hippocampal neurons upon superfusion of 100 nM HaTx for 10 min (before clamping) with injection of 20 pA (upper panel) and 100 pA (lower panel) currents, respectively, over a period of 1 sec, and before and after the treatment with 10 μM (B) or 5 μM (E) glutamate for 10 min, followed by wash-out for 2 min. (C, F) Frequency-intensity plots of action potential spikes generated upon step-wise current injections from experiments in (C) panels (A–B), and (F) panels (D–E). Data are presented as mean ± SEM (n = 5 and 3 for experiments from panels A & D and panels B & E respectively).

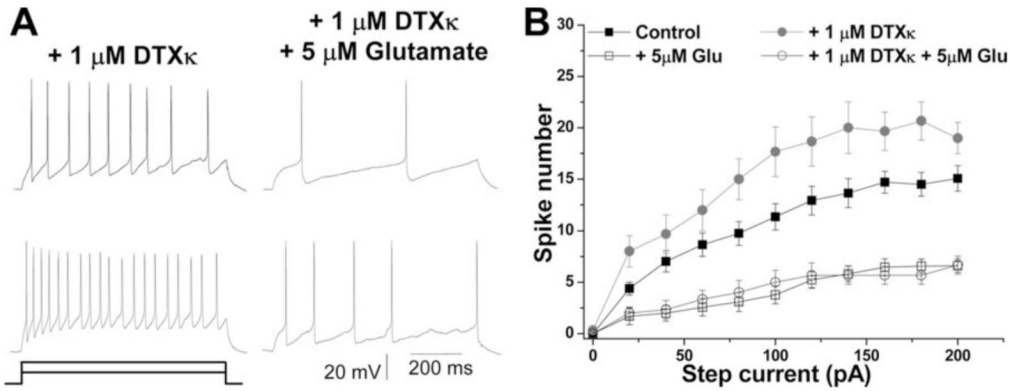

Fig. 7.

Effects of dendrotoxin-kappa (DTX3) on action potential firing in cultured hippocampal pyramidal neurons without or with glutamate treatment. (A) Representative traces of action potential firing in cultured hippocampal pyramidal neurons pre-treated with 1 μM DTXκ for 10 min, upon injection of 20 pA (upper panel) and 100 pA (lower panel) currents, respectively, over a period of 1 sec, and before and after treatment of 5 μM glutamate along with 1 μM DTXκ for 10 min. (B) Frequency-intensity plot of action potential spikes generated upon step-wise current injections from experiments in panel (a). Frequency-intensity relationship from untreated and 5 μM glutamate-treated neurons as shown in Fig. 6F are plotted alongside for comparison. Data are presented as mean ± SEM (n = 3).

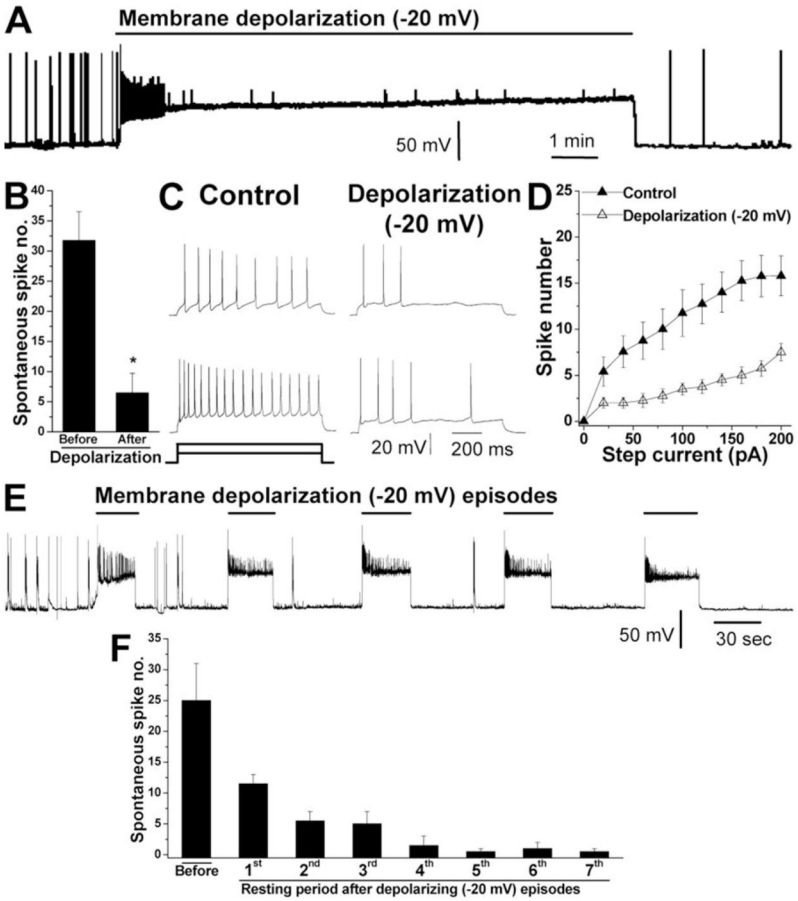

Fig. 8.

Membrane depolarization in cultured rat hippocampal neurons leads to suppression of action potential firing frequencies. (A) Representative trace of spontaneous firing in a cultured hippocampal neuron for a period of 18 min with membrane depolarization to −20 mV for 10 min following an initial holding period of 3 min, and post-depolarization period of 5 min, with continuous perfusion of ACSF. (B) Quantitation of spontaneous spikes before and after membrane depolarization to −20 mV as shown in panel (A). Data are presented as mean ± SEM of the number of spontaneous spikes per minute, before and after membrane depolarization (n = 3). *indicates significant difference in the number of spontaneous spikes ($p<0.05$). (C, D) Decreased action potential firing in cultured hippocampal neurons upon membrane depolarization (−20 mV). (C) Representative traces of action potential firing patterns in cultured hippocampal neurons upon injection of 20 pA (upper panel) and 100 pA (lower panel) currents, respectively, over a period of 1 sec, and before and after membrane depolarization (−20 mV) for 10 min. (D) Frequency-intensity plot of action potential spikes generated upon step-wise current injections from experiments in panel (C). Data are presented as mean ± SEM (n = 3). (E) Representative trace of spontaneous firing patterns in a cultured hippocampal neuron for a period of ≈8 min with episodic membrane depolarization to −20 mV for 30 sec interspersed with 1 min intervals of resting potential (≈ −63 mV), with continuous perfusion of ACSF. (F) Quantitation of spontaneous spikes under resting conditions before and after episodic membrane depolarization to −20 mV as shown in panel (E). Data are presented as mean ± SEM of the number of spontaneous spikes per minute, from every 1 min resting interval (n = 2).

Table 1

Voltage-dependent gating properties of I_K currents in cultured rat hippocampal neurons without or with treatment with different drugs

Drug treatments	Activation parameters		Inactivation parameters		
	$G_{1/2}$ (mV)	k	$Vi_{1/2}$ (mV)	k	n
Control	+16.8 ± 0.3	16.6 ± 0.3	−28.8 ± 0.5	11.4 ± 0.6	5
10 μM Glutamate-10 min	−8.6 ± 0.9[a]	14.2 ± 1.4	−54.7 ± 0.5[a]	11.3 ± 0.4	5
5 μM Glutamate-10 min	−4.1 ± 0.6[a]	15.2 ± 0.7	−52.2 ± 0.6[a]	10.7 ± 0.5	4
100 nM HaTx-0min	+15.4 ± 0.4	14.5 ± 0.2	−25.7 ± 0.5	12.6 ± 0.6	4
100 nM HaTx-10min	+16.2 ± 0.5	17.9 ± 0.3	−25.8 ± 0.8	12.7 ± 0.4	4
100 nM HaTx-10min +10 μM Glutamate-10 min	+10.1 ± 0.4[a]	15.1 ± 0.2	−32.7 ± 0.3[a]	11.2 ± 0.2	4

[a] significantly different from the control values.

Abbreviation: HaTx, hanatoxin.

Table 2

Electrophysiological properties of cultured rat hippocampal neurons under current-clamp conditions

Drug treatment	V_m (mV)	R_{input} (mΩ)	$AP_{amplitude}$ (mV)#	$AP_{duration}$ (ms)#	n
Control - 2 min	−63.8 ± 1.6	139.8 ± 11.3	79.4 ± 4.8	8.1 ± 0.6	4
- 15 min	−62.9 ± 1.3	147.4 ± 10.5	76.2 ± 3.6	7.9 ± 0.9	4
Before 10 μM Glutamate	−63.3 ± 2.1	146.2 ± 15.1	73.9 ± 5.8	7.5 ± 0.6	5
After 10 μM Glutamate	−59.7 ± 1.0	153.2 ± 20.7	64.8 ± 3.4	6.4 ± 0.5	5
Before 5 μM Glutamate	−64.9 ± 2.1	140.5 ± 10.8	73.5 ± 4.1	7.7 ± 0.8	4
After 5 μM Glutamate	−61.8 ± 2.8	155.0 ± 17.1	78.0 ± 5.0	6.9 ± 0.7	4
Before Membrane Depolarization (−20 mV)	−62.9 ± 3.5	139.3 ± 13.6	78.3 ± 8.6	7.4 ± 0.8	3
After Membrane Depolarization (−20 mV)	−58.2 ± 2.3	176.3 ± 23.6	85.6 ± 7.2	5.9 ± 0.7	3

#Calculated from the first action potential generated in response to a 20 pA current injection