JOURNAL OF NEUROPHYSIOLOGY
Vol. 75, No. 2, February 1996. *Printed in U.S.A.*

**RAPID PUBLICATION**

# Cellular Short-Term Memory From a Slow Potassium Conductance

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## SUMMARY AND CONCLUSIONS

*1*. We use the dynamic clamp to add the slowly inactivating and slowly recovering $K^+$ conductance Kv1.3 to cultured stomatogastric ganglion neurons.

*2*. Introduction of Kv1.3 produced long delays to firing during depolarization. Additionally, the slow recovery from inactivation produced an increase in neuronal excitability after a depolarizing input that outlasted the input by many seconds. Finally, when introduced into bursting neurons, Kv1.3 produced a long-lasting depolarization-induced switch between tonic and burst firing.

*3*. These data demonstrate that the slow kinetics of a $K^+$ conductance can produce a form of cellular short-term memory that is independent of any changes in synaptic efficacy.

## INTRODUCTION

Most physiological and computational studies into the mechanisms of short-term memory have considered changes in synaptic efficacy to be the primary mechanism of memory storage (Hawkins et al. 1993). Another way to change the efficacy of a fixed synaptic input is to modify the intrinsic properties of the postsynaptic neuron. Here we show that the slow kinetics of a single $K^+$ conductance, Kv1.3, can produce long-lasting activity-dependent changes in neuronal excitability. This conductance produces a cellular short-term memory of a neuron's recent history of activation that can transform subthreshold inputs into suprathreshold inputs.

It has been proposed that slowly inactivating potassium currents produce long delays to firing in a number of neurons, including sympathetic (Wang and McKinnon 1995), hippocampal pyramidal (Storm 1988), thalamic (McCormick 1991), neostriatal (Nisenbaum et al. 1994), and cortical neurons (Foehring and Surmeir 1993; Schwindt et al. 1988). In hippocampal neurons, a slowly inactivating potassium current appears to cause changes in neuronal excitability that last many seconds (Storm 1988). These studies have relied on changes in the firing properties of neurons during the presumed blockade of a single conductance by pharmacological agents. Here we provide compelling additional evidence by effectively adding a slowly inactivating potassium conductance to a neuron that does not have these properties.

We use the dynamic clamp (Sharp et al. 1993) to introduce a computer-generated slowly inactivating $K^+$ conductance, the delayed rectifier Kv1.3, into cultured neurons. This conductance exhibits state-dependent cumulative inactivation that develops over a period of several seconds and requires >20 s to recover from full inactivation (Marom and Levitan 1994). Modeling studies in which this current was added to simple neurons with only Hodgkin-Huxley $Na^+$ and $K^+$ conductances and a leak conductance suggested that Kv1.3 could produce persistent changes in neuronal excitability after strong excitatory inputs (Marom and Abbott 1994). We now ask how this conductance affects the response to depolarizing inputs when expressed in real neurons possessing a complicated array of inward and outward conductances (Turrigiano et al. 1995). Cultured stomatogastric ganglion (STG) neurons represent an ideal system for these studies. First, they are electrotonically compact, and so space clamp errors due to introduction of Kv1.3 into the soma will be minimized. Second, STG neurons in culture fire either tonically or in bursts, depending on the relative magnitudes of inward and outward conductances (Turrigiano et al. 1995). This allows us to test the effects of introduction of Kv1.3 into neurons possessing similar intrinsic conductances, but exhibiting very different firing patterns.

## METHODS

### *Cell culture and physiology*

STG neurons from the spiny lobster *Panulirus interruptus* were cultured and recordings were obtained as previously described (Turrigiano et al. 1994, 1995). After 2–3 days, cultures were moved to the recording setup and continuously superfused with room temperature *P. interruptus* saline [which contained (in mM) 478 NaCl, 12.9 KCl, 13.7 $CaCl_2$, 10 $MgSO_4$, 3.9 $Na_2SO_4$, 6 tris base, 5.1 maleic acid, pH 7.5]. Somatic recordings were obtained using sharp electrodes (10–15 MΩ, 3 M $K_2SO_4$ + 20 mM KCl) and an Axoclamp 2A in DCC mode (switching frequencies of 4–5 kHz). Experiments were repeated a minimum of three times in different preparations with similar results.

### *Dynamic clamp*

The dynamic clamp is a system designed to allow computer-generated conductances to be added to biological neurons (Sharp et al. 1993). Current injection through the microelectrode was controlled by a 386-based computer running the dynamic clamp software. The membrane potential ($V$) of the neuron was sampled and the current due to Kv1.3 calculated by the software on the basis of $V$ and the equations describing activation and inactivation of Kv1.3 (see below). This calculated current then was injected into the neuron through the microelectrode. The membrane potential was sampled and injected current values updated at a rate of 2–3 kHz. This was equivalent to a conductance change in the soma of the neuron with the characteristics of the modeled Kv1.3. The magnitude of the conductance was varied in some experiments by changing the value of $g$ (see below) in the equations of the dynamic clamp software.

### *Modeling of Kv1.3*

The Kv1.3 conductance was modeled (Marom and Abbott 1994) by expressing the Kv1.3 membrane current in the standard Hodgkin-Huxley (1952) form for an inactivating current, $I = gn^4h(V - E_K)$, where $g$ is the maximal conductance and $E_K$ is the $K^+$ equilib-

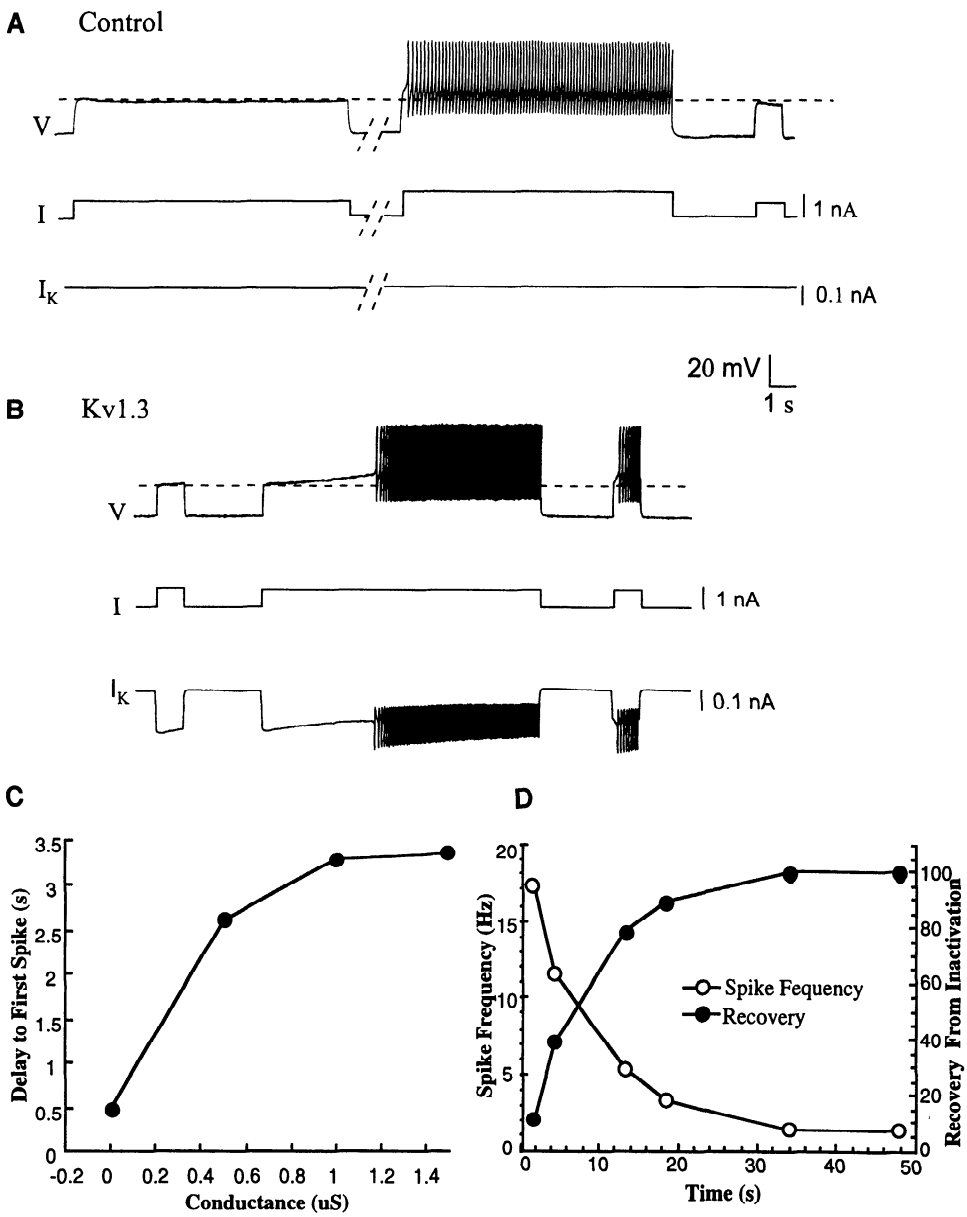


FIG. 1. Addition of Kv1.3 to a tonically firing neuron. *A*: control. A test current pulse was given before and after a stronger conditioning pulse. *B*: Kv1.3 conductance was turned on before beginning of pulses, and a test pulse was delivered before and after a longer conditioning pulse. *V*, membrane potential; *I*, DC current injection through the microelectrode; $I_k$, current due to Kv1.3 conductance injected through microelectrode; dashed line, −45 mV. *C*: delay to first spike during a current pulse as a function of the Kv1.3 maximal conductance in microsiemens. *D*: recovery from increased excitation as a function of time after a conditioning pulse. ○, spike frequency during test pulse; ●, recovery of Kv1.3 current from inactivation as a function of time after a conditioning pulse.

rium potential. The activation variable n obeys the equation $\tau dn/dt = n_\infty - n$ with $n_\infty = \alpha n/(\alpha_n + f\beta_n)$ and $\tau = 1/(\alpha_n + \beta n)$. The fits to $\alpha_n$ and $\beta_n$ are $\alpha_n = 210s^{-1}(V + 8.3)/\{1.0 - \exp[-(V + 8.3)/9.8]\}$ and $\beta_n = 1.5s^{-1} \exp[-(V + 23.6)/20.7]$ with $V$ in millivolts. The factor $f = 8.33$, not normally present in Hodgkin-Huxley models, was included to fit the effects of an extra fully activated but closed state (Marom and Levitan 1994). The cumulative inactivation of the Kv1.3 conductance is a state-dependent rather than voltage-dependent process. The equation describing the inactivation variable $h$ is therefore $dh/dt = k_1 (1 - h) - k_0 n^4 h$, where $k_1$ and $k_0$ are voltage-independent rate constants with $k_1 = 0.05s^{-1}$ and $k_0 = 1.4s^{-1}$.

## RESULTS

Tonically firing cultured STG neurons have delays of ≤500 ms from the initiation of depolarization to the first spike (Fig. 1*A*, *middle*). Addition of Kv1.3 to such a neuron using the dynamic clamp dramatically increased the delay to first spike when the neuron was depolarized to the same potential as in control (Fig. 1*B*, *middle*). At the beginning of the depolarization, Kv1.3 was strongly activated, producing a hyperpolarizing current that prevented the neuron from firing (Fig. 1*A*, $I_k$). Kv1.3 inactivated slowly, resulting in a slow depolarization that eventually brought the neuron over threshold for spike generation. The time to first spike increased as the magnitude of the Kv1.3 conductance was increased, and saturated at delays of 3–4 s (Fig. 1*C*).

As well as producing long delays to firing, Kv1.3 produced persistent changes in excitability after strong depolarizing inputs. Under control conditions, the same amplitude subtheshold current pulse produced identical results before and after a stronger "conditioning" pulse (Fig. 1*A*). In contrast, with Kv1.3 expressed in the neuron, a current step that was subthreshold before the conditioning pulse was able to elicit rapid firing after conditioning (Fig. 1*B*). Comparison of the magnitude of the Kv1.3 current elicited by the

test pulse before and after the conditioning pulse reveals that Kv1.3 had not completely recovered from inactivation after 3 s, resulting in a larger depolarization after conditioning than before. Measurement of the spike frequency during test pulses as a function of time after the conditioning pulse shows that the depolarization-induced change in excitability persists for ≤25–30 s (Fig. 1*D*, ○). Measurement of the magnitude of the Kv1.3 current elicited by the test pulses as a function of time after the conditioning pulse (Fig. 1*D*, ●) shows that Kv1.3 recovered from inactivation with a similar time course, suggesting that the persistent change in excitability after conditioning is due to the slow recovery from inactivation of Kv1.3.

In hippocampal neurons, a slowly inactivating potassium conductance is thought to contribute to the integration of synaptic inputs over time due to cumulative inactivation of the conductance by successive inputs (Storm 1988). A similar effect can be produced by introducing Kv1.3 into STG neurons. These neurons normally receive rhythmic inputs at a frequency of 0.5–1 Hz. When depolarizing inputs were delivered at 0.5 Hz, the number of spikes produced by each pulse increased over the first several cycles (Fig. 2*A*). This effect was exaggerated by introduction of Kv1.3 (Fig. 2*B*). A plot of spikes/pulse against pulse number shows that Kv1.3 increases the number of preceding inputs that influence the spike frequency during a given pulse (Fig. 2*C*).

A number of neuron types reported to possess slowly inactivating potassium conductances can fire in bursts, including thalamic neurons (McCormick and Feeser 1990) and a subset of cortical pyramidal neurons (Chagnac-Amitai and Connors 1989). We therefore wished to determine the effects of Kv1.3 when introduced into burst firing STG neurons. An example is shown in Fig. 3. Under control conditions, this neuron fired several spikes when initially depolarized and then began to burst regularly (Fig. 3*A*). Addition of Kv1.3 changed the firing properties of the neuron during short depolarizing pulses from bursting to tonic firing (Fig. 3*B*). During a longer conditioning pulse, this neuron underwent a transition from tonic firing at the beginning of the pulse to burst firing after ≈1 s. The same depolarizing pulse delivered after the termination of the conditioning pulse elicited not tonic firing, but burst firing. This effect persisted for many seconds past the termination of the conditioning pulse.

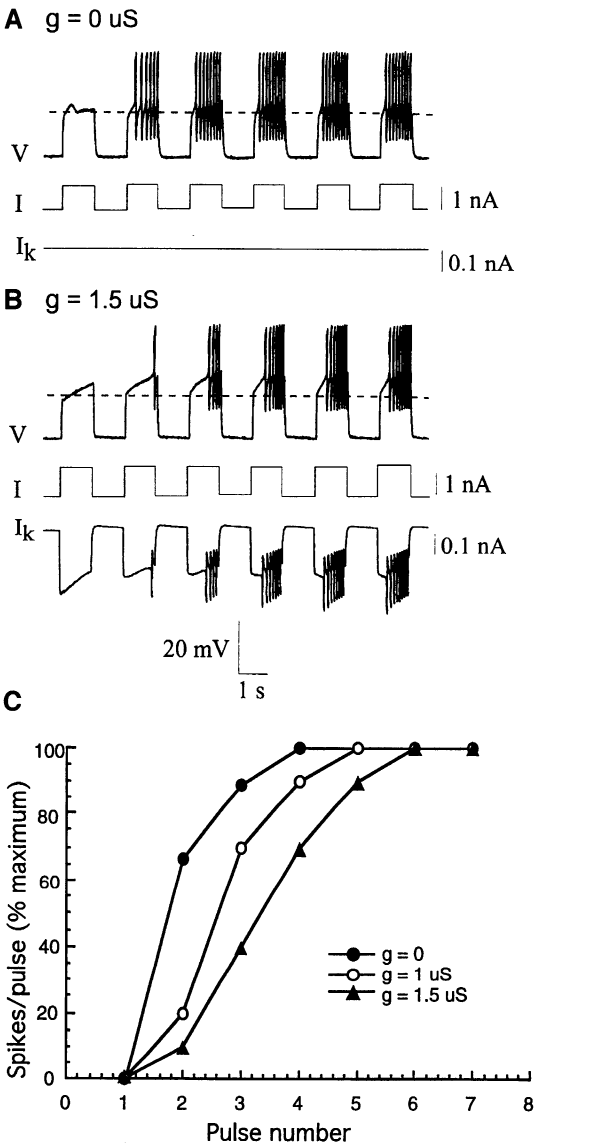


FIG. 2. Response of a neuron to repetitive depolarizing current pulses. *A*: control. *B*: addition of 1 μS Kv1.3. Dashed line indicates −47 mV. *C*: plot of spikes/current pulse as a function of pulse number for 3 different values of Kv1.3 conductance.

## DISCUSSION

The aim of this paper is to demonstrate that a $K^+$ conductance with slow kinetics can confer upon neurons a memory of past activation lasting many seconds that depends only on the intrinsic properties of the neuron. Addition of the slowly inactivating and slowly recovering $K^+$ conductance Kv1.3 to STG neurons produced several qualitative changes in their response to depolarizing inputs, including long delays to firing, persistent changes in neuronal excitability, and persistent depolarization-induced transitions between tonic firing and burst firing. These data show that the response of a neuron expressing Kv1.3 to a fixed synaptic input will depend critically on the recent history of activity in that neuron. For tonic firing neurons, a subthreshold input can be converted into a suprathreshold input following strong depolarization. For burst firing neurons, an input that elicits tonic firing can be converted into an input that elicits burst firing. Although we have confined our analysis to Kv1.3, in principle other conductances with kinetics that are slow relative to the kinetics and timing of synaptic inputs could produce similar effects.

The ability to add a conductance to a neuron is a powerful tool for determining how the biophysical properties of a conductance will affect neuronal firing properties. The dynamic clamp gives the ability to titrate precisely the magnitude of a conductance introduced into a neuron so that it does not swamp the other conductances expressed, something that is difficult to do using mRNA expression. The dynamic clamp offers an alternative approach to pharmacological blockade, which often results in nonspecific changes in neuronal properties and can only be used where specific channel blockers are available.

Long delays to firing and slow integration of synaptic

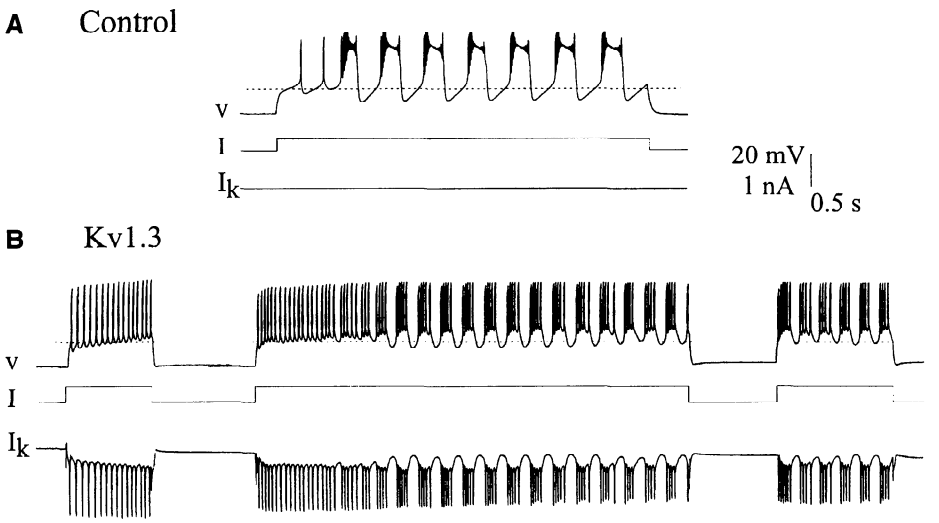


FIG. 3. Addition of Kv1.3 to a bursting neuron. *A*: control. *B*: Kv1.3 conductance was turned on before the beginning of the current pulses (maximal conductance = 10 $\mu$S), and a test pulse was delivered before and after a longer conditioning pulse. Dashed line indicates −45 mV.

inputs are two neuronal properties that have been attributed to the effects of slowly inactivating $K^+$ conductances, based on experiments in which these conductances have been blocked pharmacologically (McCormick 1991; Nisenbaum et al. 1994; Storm 1988). Kv1.3 has the additional property of slow recovery from inactivation (Marom and Abbott 1994; Marom and Levitan 1994). It is this slow recovery from inactivation, with a time constant of 20 s, that produces the persistent changes in firing properties after depolarizing inputs. This cellular memory could have dramatic effects on the properties of the circuits in which such neurons participate, including enhancing synaptic plasticity that requires postsynaptic depolarization (such as long-term potentiation) and controlling transitions between oscillatory and nonoscillatory network activity.

We thank M. O'Neil for technical assistance with the dynamic clamp software.

Work supported by National Institute of Mental Health Grant MH-46742 to E. Marder, National Science Foundation Grants IBN-9543166 to G. G. Turrigiano and DMS-9503261 to L. F. Abbott.

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Received 21 August 1995; accepted in final form 31 October 1995.

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