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Activity-dependent regulation of excitability in rat visual cortical neurons

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Abstract

A neuron's electrical properties are produced by a variety of voltage- and time-dependent ionic conductances. Here we examine how activity deprivation affects the excitability and ionic currents of cultured cortical pyramidal neurons. Blocking activity for 48 h results in a marked increase in excitability, mediated by changes in sodium and persistent potassium currents. This finding suggests that neurons can control their firing rates by tuning their distribution of ionic conductances in response to changes in activity. © 1999 Published by Elsevier Science B.V. All rights reserved.

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The dynamics of neural circuits are determined by the complex interplay between synaptic connections and the intrinsic electrical properties of individual neurons. While the regulation by activity of synaptic transmission has received extensive attention – both experimental and theoretical – the regulation of intrinsic ionic conductances has been much less well studied. Despite this imbalance, there exist very good reasons for examining how experience, in the form of activity, shapes a neuron's distribution of ionic conductances: throughout life there is a continual turnover in the membrane of the channels underlying these conductances, and during development neurons can change size and shape. Modulation of ionic conductances in these ways

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can profoundly alter the electrical properties of individual neurons, and modeling studies have demonstrated that network dynamics are very sensitive to the intrinsic properties of the network's constituent parts. This sensitivity suggests that to achieve and maintain appropriate electrical activity, neurons must continually and selectively adjust the magnitude of their conductances [2]. To address one aspect of this issue, we here study how long-lasting deprivation of activity affects the intrinsic excitability and ionic currents of neocortical neurons.

Experiments were performed on pyramidal neurons in primary cell culture. As previously described [3], these cultures were prepared by removing visual cortex from postnatal (P4-6) Long-Evans rat pups, dissociating cells using an enzymatic procedure, and plating neurons in a monolayer on top of astrocyte feeder layers. Cultures were maintained for 6–8 days in vitro before use. During this time neurons in these cultures formed synaptic connections and developed spontaneous electrical activity. To test the effects of activity deprivation, tetrodotoxin (TTX) was added to test cultures two days before each experiment; the TTX served to block all activity. After the incubation period, the TTX was washed out and whole cell patch recordings were obtained from pyramidal neurons in both test and sister control cultures. In some experiments, *F–I* curves were constructed for cells in the two conditions by measuring firing rates in response to depolarizing square current injections from a fixed membrane potential (-60 mV). In other experiments, individual ionic currents were measured by holding neurons in voltage clamp and using standard voltage step protocols and pharmacological blocking agents to isolate them.

We found that activity blockade increased the excitability of neurons substantially, as measured by the *F–I* curves. For the largest current step used (200 pA), neurons in TTX-treated cultures initially fired at a rate almost 40% faster than did neurons in control cultures. The slope of the initial, linear part of the curve was significantly different for TTX-treated neurons (0.47 ± 0.05 Hz/pA) than for control neurons (0.19 ± 0.02 Hz/pA). This increase in the rate of firing of the first two spikes was mirrored by an increase in the rate for subsequent spikes, and was accompanied by a decrease in the threshold (rheobase) current from 44.6 ± 7.5 pA to 22.4 ± 1.1 pA. Other neuronal properties, including resting input resistance and whole cell capacitance, were unchanged. Taken together, these data demonstrate that pyramidal neurons increase their intrinsic excitability in response to activity blockade, and this effect is not simply the product of changes in passive cell properties.

Voltage clamp experiments revealed that the increase in excitability was at least partly mediated by activity-dependent changes in the amplitudes of transient sodium and persistent potassium currents (Fig. 1). Sodium currents were measured by stepping neuron membrane potentials from a holding potential of -60 mV to step potentials between -40 and $+60$ mV, with potassium and calcium currents blocked pharmacologically. While TTX treatment did not affect the shape of the resulting sodium *I–V* curves or the reversal potential, it did significantly increase the average peak amplitude of these currents, by roughly one third. Potassium currents were measured in a similar way, using depolarizing voltage steps from holding potentials

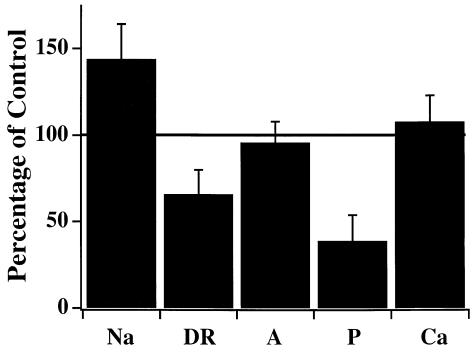

Fig. 1. Activity blockade increases sodium currents while decreasing persistent potassium currents. Plotted are the average peak amplitudes of currents in TTX-treated neurons elicited by depolarizing voltage steps from near rest to −10 mV (sodium: Na), +40 mV (TEA-sensitive potassium: DR; A-type potassium: A; TEA-insensitive, persistent potassium: P), or 0 mV (total calcium: Ca). The numbers are plotted as a percentage of the values for control cells.

between −80 and −40 mV; here sodium currents were blocked by TTX, but calcium currents remained present (to include the effects of calcium-dependent potassium currents). The outward currents were divided into three classes: tetraethylammonium (TEA)-sensitive, persistent currents (mainly delayed rectifier); TEA-insensitive, transient currents (A-type); and TEA-insensitive, persistent currents (a mixed current with an I–V curve similar to that of the delayed rectifier). Both kinds of persistent currents were significantly reduced by activity blockade, but the transient current was unaltered. Calcium currents were measured by delivering positive voltage steps from holding potentials between −80 and −50 mV, with sodium and potassium currents blocked by a combination of TTX, TEA, 4-aminopyridine, and cesium. No significant differences between control and TTX-treated cells in calcium amplitudes were detected.

In order to determine if the measured changes in current densities were sufficient to account for the measured change in excitability, we constructed a conductance-based model neuron using our physiological data. The model neuron is similar to one employed recently by Wang [6]; equations for currents not included in Wang's model were obtained from other published sources [4,7]. The model contains two compartments, one to represent the soma and initial axon segment, the other to represent the dendrites. While our basic results can be obtained from a single-compartment model, we included two compartments because we found that the weak electrotonic coupling between them was necessary to reproduce the firing patterns of our cultured cells. The somatic compartment contains a leak current (I_L), a full range of voltage-gated currents [sodium (I_{Na}), delayed rectifier (I_{DR}), A-type (I_A), high- and low-voltage-activated calcium (I_{Ca})], coupling with the other compartment, and a term representing a somatic current injection (I):

$$C_M \, dV_S/dt = -I_L - I_{Na} - I_{DR} - I_A - I_{Ca} - (g_C/p)(V_S - V_D) + I. \quad (1)$$

In Eq. (1), V_S is the somatic potential, C_M is the membrane capacitance, g_C is a coupling parameter between the two compartments, and p is the ratio of the somatic area to the total area. The dendritic compartment, with a potential given by V_D, contains a leak current, a calcium current (imaging experiments with the calcium indicator dye fura-2 indicate substantial calcium currents in the dendrites of these cells), and a coupling term:

$$C_M \, dV_D/dt = -I_L - I_{Ca} - [g_C/(1-p)](V_D - V_S). \quad (2)$$

All of the voltage-gated currents are described by the standard Hodgkin–Huxley formalism, $I_X = g_X m^p h^q (V - E_X)$, where g_X is the maximal conductance, m and h are gating variables with exponents p and q, and E_X is the reversal potential. The gating variables all obey first-order kinetics. The equations describing the rate constants are identical to those given in the references cited above, with one exception: both the activation and inactivation curves for sodium were shifted up by $+5$ mV to obtain better fits to our sodium I–V curves. The maximal conductances and reversal potentials were chosen to match our voltage-clamp data. The coupling parameters g_C and p were adjusted to give patterns of action potentials in response to somatic current injections that resembled those of the experimental data; the qualitative results did not depend strongly on either parameter.

We found that increasing the sodium current density and decreasing the delayed rectifier density by the percentages specified by our experimental data had a pronounced effect on the excitability of the model neuron (Fig. 2). The F–I curve for the model with altered currents was shifted considerably upwards from that of the model with control currents; the threshold current was reduced; and the initial spike height was increased. All of these effects are compatible with the experimental data. Moreover, the qualitative results were relatively robust, meaning they could be obtained with a variety of parameter choices. While it is true that factors other than those that we measured in our voltage-clamp experiments may contribute to the

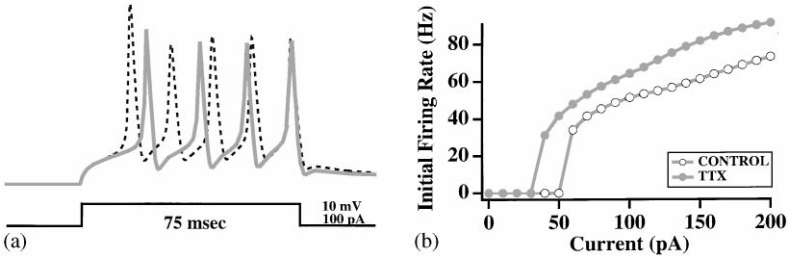

Fig. 2. Model neuron reproduces qualitative behavior. (A) Action potentials elicited in model neurons by a square current injection. Solid line is for a model neuron with the sodium and delayed rectifier densities of control neurons; dashed line is for a model neuron with the densities appropriate to TTX-treated neurons. (B) F–I curves for model neurons with control and TTX-treated current densities.

increased excitability, the computational results suggest that the changes in the magnitudes of the sodium and persistent potassium currents account for much of the effect.

Finally, an important question to consider is what purpose this activity-dependent process might serve. One possibility is that it exists to allow neurons to avoid falling silent or having their firing rates saturate in response to long-lasting changes in the average amount of synaptic input they receive – such as those, for example, that accompany development. A more subtle possibility is that this process acts to allow neurons to respond not only to changes in average input but also to changes in the statistics of that input. In particular, the distribution of ionic conductances might be adjusted so that neurons can use their entire range of firing frequencies in order to encode whatever input they receive [1]. The regulatory process we have discovered, by making activity-deprived cells more excitable, would certainly serve the first possibility, and is compatible with the second. It is important to point out that a similar sort of regulatory mechanism would involve adjusting synaptic strengths based on a neuron's history of activity; and indeed we have, using this same culture system, discovered evidence of such a process [5]. These data suggest that intrinsic and synaptic properties are conjointly regulated to achieve stable and flexible circuit dynamics.

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References

[1] C. Koch, M. Stemmler, Information maximization in single neurons, Soc. Neurosci. Abstr. 24 (1998) 1500.
[2] E. Marder, L.F. Abbott, G.G. Turrigiano, Z. Liu, J. Golowasch, Memory from the dynamics of intrinsic membrane currents, PNAS 93 (1996) 13481–13486.
[3] L.C. Rutherford, A. DeWan, H.M. Lauer, G.G. Turrigiano, BDNF mediates the activity-dependent regulation of inhibition in neocortical cultures, J. Neurosci. 17 (1997) 4527–4535.
[4] R.D. Traub, R.K.S. Wong, R. Miles, H. Michelson, A model of a CA3 hippocampal pyramidal neuron, J. Neurophysiol. 66 (1991) 635–650.
[5] G.G. Turrigiano, K.R. Leslie, N.S. Desai, L.C. Rutherford, S.B. Nelson, Activity-dependent scaling of quantal amplitude in neocortical neurons, Nature 391 (1998) 892–896.
[6] X.-J. Wang, Calcium coding and adaptive temporal computation in cortical pyramidal neurons, J. Neurophysiol. 79 (1998) 1549–1566.
[7] X.-J. Wang, J. Rinzel, M.A. Rogawski, A model of the T-type calcium current and the low-threshold spike in thalamic neurons, J. Neurophysiol. 66 (1991) 839–850.

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