Plasticity in the intrinsic excitability of cortical pyramidal neurons

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During learning and development, the level of synaptic input received by cortical neurons may change dramatically. Given a limited range of possible firing rates, how do neurons maintain responsiveness to both small and large synaptic inputs? We demonstrate that in response to changes in activity, cultured cortical pyramidal neurons regulate intrinsic excitability to promote stability in firing. Depriving pyramidal neurons of activity for two days increased sensitivity to current injection by selectively regulating voltage-dependent conductances. This suggests that one mechanism by which neurons maintain sensitivity to different levels of synaptic input is by altering the function relating current to firing rate.

A common feature of learning and development is that the number and/or strength of a neuron's synapses can vary substantially over time. During development, synapses are continually being formed and eliminated[1], and many learning mechanisms such as long-term potentiation and depression (LTP and LTD) rely on experience-dependent modification of synaptic strengths[2]. As a result, the synaptic input received by a neuron may vary over time by orders of magnitude. Although such synaptic change is crucial for the formation, maintenance and proper functioning of neural circuits, it raises the question of how cortical neurons keep from falling silent or keep their firing rates from saturating if the average synaptic input falls too low or rises too high. Without a mechanism that allows a neuron to remain responsive to its input in this most basic sense, the neuron would be unable to transmit information or to participate effectively in the correlation-based synaptic modifications needed for the development and maintenance of neural circuits[1].

Several possible solutions to this problem have been proposed[3,4]. Among these are the ideas that the ability of a synapse to undergo Hebbian modification depends upon its history of use[5–7], and that global mechanisms stabilize neuronal activity by regulating either the total synaptic strength[8–11] or the intrinsic excitability[4,12] of a neuron. In keeping with the latter two ideas, recent studies on cortical neurons have shown that cortical firing rates are regulated by activity in a homeostatic manner[10,13–15]. This is accomplished in part by scaling the strength of excitatory synapses up or down as a function of activity[10], but the question of whether changes in intrinsic excitability also contribute to this homeostatic regulation of firing rates has not been addressed.

The dynamics of neural circuits arise through a complex interplay between synaptic inputs and the intrinsic electrical properties of individual neurons, and the magnitude and distribution of ionic conductances can influence processes as diverse as synaptic integration[16,17], the pattern and rate of firing[18] and synaptic plasticity[19,20]. Recent experimental work on both invertebrate and vertebrate preparations has suggested that activity is important in modulating intrinsic neuronal excitability[18,21–26]. In invertebrate preparations, activity can switch neurons between tonic firing and burst firing modes by regulating the balance of inward and outward currents[18], and in a variety of preparations, activity is important in regulating the magnitude of individual ionic conductances[18,21–26].

We asked whether long-lasting changes in activity influence the intrinsic excitability of cortical neurons, and whether this regulation is consistent with a role in stabilizing firing rates. We found that preventing cortical neurons from firing for two days dramatically increased their intrinsic excitability. In response to injected current, activity-deprived neurons fired much more rapidly and did so in response to smaller current injections. This increase in excitability was mediated by selective regulation of the magnitudes of sodium and persistent potassium currents; the former increased, whereas the latter decreased. These findings demonstrate that the history of activity of a cortical neuron helps to determine its intrinsic excitability. This may allow a neuron to adjust the way it transduces synaptic input to maintain its responsiveness during periods of intense change in synapse number and strength.

Results

We used primary cell cultures of postnatal rat visual cortical neurons to examine effects of long-lasting changes in activity on the intrinsic electrical properties of pyramidal neurons. Over time *in vitro*, neurons in these cultures form excitatory and inhibitory synaptic connections and develop spontaneous activity driven by synaptic input[10,13,14]. Recordings were obtained after 7–9 days *in vitro*. To block spontaneous activity in test cultures, cultures were incubated with the sodium channel blocker tetrodotoxin (TTX). Except where noted, the duration of the activity blockade was 48 hours. The TTX was then washed out, and whole-cell patch recordings were obtained from pyramidal neurons in both treated and sister control cultures.

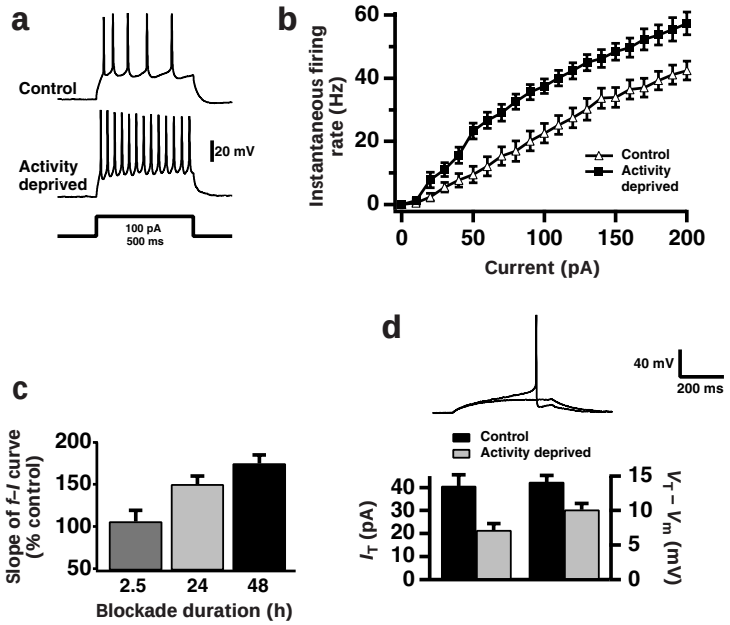

Fig. 1. Chronic activity blockade increased the firing frequency and lowered the spike threshold of pyramidal neurons. **(a)** Sample spike trains evoked by a somatic current injection in neurons grown under control and activity-deprived conditions. The neurons shown came from sister cultures and had very similar resting potentials and input resistances (control, –61 mV, 1.0 G□; activity deprived,–62 mV, 0.9 G□). **(b)** Average *f–I* curves for control (n = 18) and activity-deprived (n = 18) neurons. The plot shows initial instantaneous firing frequency (frequency of first spike interval) versus amplitude of current injection. **(c)** The size of the increase in the initial slope of the *f–I* curve varied with the duration of activity blockade. **(d)** Activity blockade reduced the spike threshold. Top, an example control neuron just below threshold (passive trace) and just above threshold (action potential). The minimum current needed to evoke an action potential was designated the threshold current, I_T; the maximum potential reached when the current injection was just subthreshold was designated the threshold potential, V_T. Bottom, average values of threshold current and threshold potential (n = 9, each condition); the latter is plotted relative to the resting potential of V_m= –60 mV.

Activity blockade increases intrinsic excitability

Chronic activity blockade increased the excitability of pyramidal neurons. To measure firing rates, we delivered constant current pulses of variable amplitude and 500 ms duration through the somatic pipet while monitoring membrane potential. All current injections were applied from a resting potential of –60 mV, maintained by injecting a small DC current. Synaptic transmission was blocked pharmacologically. Neurons that were activity deprived for two days fired much more rapidly than did neurons from sister control cultures, both initially and later on in the spike train (**Fig. 1a**). Average frequency versus current (*f–I*) curves constructed by measuring the initial firing frequency (inverse of period between first and second spikes) for each current amplitude clearly show the differences between the two conditions (**Fig. 1b**). The slope of the initial, linear part of the curve for activity-deprived neurons (0.40 ± 0.02 Hz/pA, n = 18) was approximately double that for control neurons (0.23 ± 0.01 Hz/pA, n = 18). Moreover, at each current amplitude above 20 pA, the average frequency of activity-deprived neurons was significantly larger than the average control frequency ($p < 0.05$, *t*-test); most differences were highly significant ($p < 0.005$, *t*-test). Most of these neurons showed relatively little spike frequency adaptation, and the average increase in firing frequency for later spike intervals was comparable to the initial increase. For example, activity blockade increased the firing frequency in response to a current injection of 150 pA by 60–80% in each of the first five spike intervals.

We determined the time dependence of these effects by varying the duration of activity deprivation (**Fig. 1c**). Blocking activity for only 2.5 hours produced no discernible shift in the *f–I* curve. However, 24-hour activity blockade increased the slope of the *f–I* curve by 50 ± 11%, a significant but smaller increase in slope than that produced by 48-hour activity blockade (74 ± 11%). These data indicate that this process is slow and cumulative, with a time course similar to that of the activity-dependent changes in synaptic strengths previously measured in these cultures[10].

Activity blockade not only increased firing frequency, but also lowered the spike threshold. Threshold current was determined by increasing the amplitude of the current injection in one-picoamp steps and noting the current at which a spike was first elicited, and threshold voltage was determined by the highest voltage evoked by the largest subthreshold current step (**Fig. 1d**). Threshold current decreased from 41 ± 5 pA for control neurons to 21 ± 3 pA for activity-deprived neurons ($p < 0.05$, n = 9 each condition, *t*-test), and the threshold potential decreased from –46 ± 1 mV for control neurons to –50 ± 1 mV for activity-

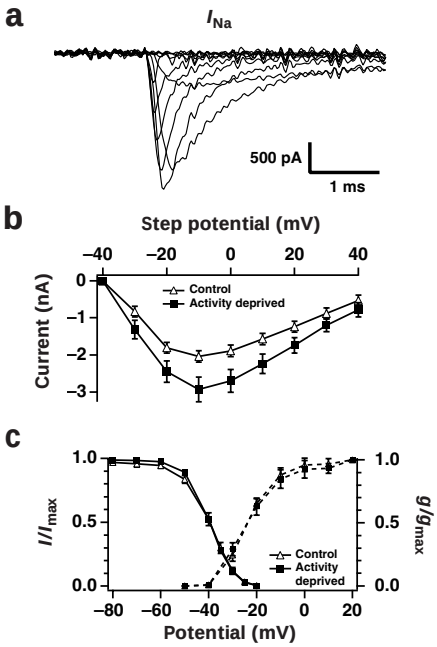

Fig. 2. The amplitude of I_{Na} was increased by activity deprivation, but the voltage dependence was unaltered. **(a)** I_{Na} evoked in an activity-deprived neuron by depolarizing voltage steps from a holding potential of –60 mV to step potentials between –40 and +40 mV, in increments of 10 mV. **(b)** Average current–voltage plots constructed by evoking currents as in **(a)** and measuring peaks (n = 21 control, n = 22 activity deprived). **(c)** Average activation and inactivation curves of I_{Na} (see Methods). Activation (g/g_{max}) is given as chord conductance normalized to the value at +20 mV; inactivation (I/I_{max}) is given as current normalized to that elicited from a holding potential of –100 mV.

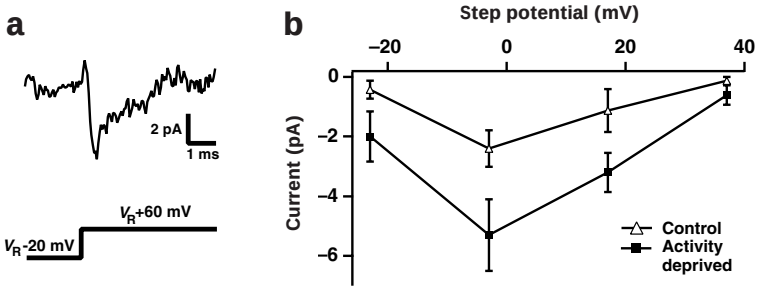

Fig. 3. Cell-attached patch recordings of sodium currents confirmed that activity deprivation increases their amplitude. **(a)** I_{Na} evoked in a somatic patch by stepping the potential from 20 mV below to 60 mV above the resting potential. Trace shown is an average of 20 individual traces. **(b)** Average peak currents elicited in somatic patches by depolarizing voltage steps from a holding potential 20 mV more negative than rest (n = 8 or 9 for each condition). In the plot, voltage steps are converted into step potentials by assuming a resting potential of –63 mV, which is approximately the average measured in these cells.

deprived neurons ($p < 0.01$, $n = 9$ each condition, t-test). In addition, peak voltage reached during the first spike increased by 5.5 ± 1.8 mV in response to activity deprivation ($p < 0.002$, $n = 18$ each, t-test).

These changes in excitability were not mediated by changes in passive neuronal properties. The whole-cell capacitance, resting input resistance and resting potential of neurons grown under activity-deprived or control conditions were comparable (**Table 1**). Earlier work indicates that activity blockade for two days does not affect cell size, survival or passive properties[10,13,14].

Activity regulates ionic conductances

Given that passive properties were unchanged, the increased excitability following activity blockade must be the result of activity-dependent changes in voltage-gated currents. We examined this idea by isolating the major ionic currents expressed in these neurons using standard voltage-clamp protocols and pharmacological manipulations. In all of the experiments described below, glutamatergic and GABAergic synaptic transmission was blocked pharmacologically, and leak and capacitive currents were subtracted offline. Except where noted, the measurements were made using whole-cell voltage clamp.

The average amplitudes of sodium currents (I_{Na}) were significantly increased by chronic activity blockade. To block potassium and calcium currents, I_{Na} was measured in the presence of internal cesium, external tetraethylammonium (TEA), 4-aminopyridine (4-AP) and cadmium. I_{Na} was evoked by voltage clamping pyramidal neurons at –60 mV and then delivering a series of depolarizing voltage steps (**Fig. 2a**). We found that, on average, activity blockade significantly increased peak sodium current amplitude (control, 2.04 ± 0.16 nA, $n = 21$; TTX, 2.93 ± 0.33 nA, $n = 22$; $p < 0.025$, t-test; **Fig. 2b**). Results for this current as well as for those described below were not altered if currents were scaled by that neuron's capacitance before averaging to take differences of size into account (see **Fig. 6**).

Whereas the amplitude of I_{Na} was regulated by activity, its voltage dependence was not. Neither activation nor inactivation characteristics of I_{Na} were altered by activity blockade (**Fig. 2c**). This suggests that activity regulates sodium currents by changing channel density, though we cannot rule out changes in channel properties that do not affect voltage dependence (for example, a voltage-independent change in open-channel probability, such as that produced by cAMP-dependent phosphorylation[27]).

Because I_{Na} is such a large and fast current, isolating it using whole-cell techniques is a stringent test of voltage clamp and requires that special care be taken to insure an adequate clamp. Our data met a number of criteria that suggest a satisfactory voltage clamp: the peak I_{Na} current occurred at a potential of –10 mV, consistent with earlier studies on similar neurons[28,29]; the activation time decreased with larger voltage steps, as expected; when sodium was included in the internal pipet solution, the measured reversal potential (42 ± 2 mV) was within a few millivolts of its predicted value (38 mV); and repetitively applying the same voltage step produced overlapping traces that differed in peak amplitude by only a few percent and in time to peak by no more than 50–100 □s. To confirm the whole-cell results using an independent method not subject to space-clamp errors, we repeated our measurements of sodium current amplitude using cell-attached patch recordings (**Fig. 3**). Under conditions in which potassium currents were blocked, tight seals were formed on somatic patches with diameters of approximately 1 □m; sodium currents were elicited by first holding the patches at 20 mV more negative than the resting potential and then stepping to potentials 40–100 mV more positive than the resting potential. In 8 of 10 control cells and 9 of 11 activity-deprived cells, we detected a measurable current with kinetics and voltage dependence appropriate for a sodium current (**Fig. 3a**). In agreement with the whole-cell recordings, activity blockade significantly increased the sodium current amplitude ($p < 0.05$, t-test), from a peak of 2.4 ± 0.6 pA in control cell patches to a peak of 5.3 ± 1.2 pA in activity-deprived cell patches (**Fig. 3b**).

In contrast to sodium currents, calcium currents (I_{Ca}), the other major inward currents expressed by these neurons, were not affected by chronic activity blockade. Using whole-cell voltage clamp, we measured these currents by blocking sodium and potassium currents by a combination of internal cesium and external TEA, 4-AP and TTX. The remaining currents could be completely blocked by 0.4 □M $CdCl_2$. Depolarizing voltage steps were applied to pyramidal neurons from a holding potential of –80 mV (**Fig. 4a**). The resulting currents peaked within a few milliseconds and then decayed toward a steady state, as expected for mixed currents generated by a number of channel types. Neither the peak nor the sustained values of I_{Ca} were significantly affected by activity blockade ($n = 12$, each condition; **Fig. 4b**).

The outward currents in these cells were made up of two principal components that, together, accounted for more than 80% of the total outward current (**Fig. 5a**). One was TEA-sensitive and persistent and had the characteristics of a delayed rectifier potas-

Table 1. Passive cell properties.

	Control neurons	Activity-deprived neurons
C_m (pF)	23.4 ± 1.3	24.9 ± 1.1
R_{in} (M□)	817 ± 64	843 ± 64
V_m (mV)	–63.2 ± 1.0	–63.7 ± 0.6

Resting input resistance, R_{in}, and resting potential, V_m, were measured in current clamp on the same cells used to construct f–I curves and determine threshold values ($n = 26$ for each condition). Whole-cell capacitance, C_m, was measured in voltage clamp on cells used to measure sodium currents (control, $n = 21$; activity-deprived, $n = 22$). No differences were significant.

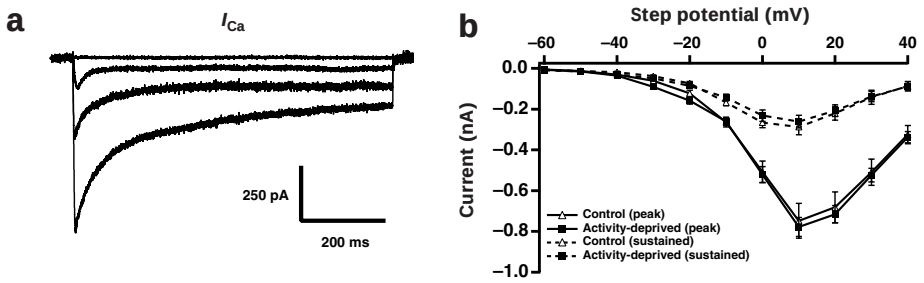

Fig. 4. Calcium currents were unaffected by activity blockade. **(a)** I_{Ca} evoked in a control neuron by depolarizing to step potentials of –50, –20, +40 and +10 mV (in order of increasing size) from a holding potential of –80 mV. **(b)** Average current–voltage plots constructed by evoking currents as in **(a)** and measuring both the peak current (solid lines) and the current 700 ms after the start of the step (dashed lines). For each condition, $n = 12$.

sium current (I_{TEA}); the other was TEA-insensitive, 4-AP-sensitive and fast-inactivating, and had the characteristics of an A-type potassium current[30,31] (I_A). These were measured with sodium currents blocked by TTX, but with calcium currents still present to maintain calcium-dependent potassium currents. Some varieties of calcium-dependent potassium currents likely contribute to I_{TEA} as they are TEA-sensitive and activate at potentials similar to those of the delayed rectifier[32]. I_{TEA} was measured from a holding potential of –60 mV; to isolate this current, currents elicited in 10 mM TEA were subtracted from those elicited without TEA. I_A was measured in the presence of TEA by subtracting currents elicited from a holding potential of –40 mV from those elicited from a holding potential of –80 mV.

Whereas the current–voltage plot of the inactivating current I_A was very similar for neurons grown under activity-deprived and control conditions ($n = 11$ for each), the amplitude of I_{TEA} significantly decreased from 3.83 ± 0.50 nA to 2.50 ± 0.40 nA for the largest voltage step used ($p < 0.05$, t-test, $n = 12$ for each; **Fig. 5b**). As this is a persistent, hyperpolarizing current, this decrease could help mediate an increase in excitability. At the same time, there was no significant change in the voltage dependence of either current (**Fig. 5c**). As in the case of I_{Na}, we do not know whether the decrease in I_{TEA} was produced by a change in the number of channels or in the properties of single channels.

In addition to these two currents, a persistent outward current, I_P, not blocked by 10 mM TEA, was found in neurons in these cultures. The current–voltage plot of I_P was very similar to that of I_{TEA}, and, like I_{TEA}, its voltage dependence was unaltered by activity blockade, whereas its amplitude decreased from 1.48 ± 0.33 nA to 0.58 ± 0.18 nA for the largest voltage step ($p < 0.02$, t-test, control, $n = 11$; activity deprived, $n = 12$ neurons). This residual outward current has not yet been characterized. One possibility is that it is a calcium-dependent potassium current, some of which are TEA resistant[32]; another possibility is that I_P is a delayed rectifier current, as 10 mM TEA may be insufficient to block all delayed rectifier currents.

Composite data on the effects of activity blockade on average current densities for the major inward and outward currents measured are shown in Fig. 6. Individual current measurements were scaled by the capacitance before averaging, demonstrating that the results are independent of neuron size. Inward and outward current densities were differentially regulated by activity blockade: sodium currents increased in magnitude and some outward currents decreased in magnitude, whereas other outward currents and calcium currents were unaffected. To ask whether these changes in current densities can account for the changes in excitability, we built a simple, conductance-based neuronal model based on our physiological data (see supplementary material, *Nature Neuroscience* web site). Changing the balance of conductances in the model neuron to match those produced by activity deprivation reproduced the experimentally measured increase in neuronal excitability, suggesting that these changes are sufficient to account for the effect.

Discussion

Our results demonstrate that depriving cortical pyramidal neurons of activity for two days increases their intrinsic excitability by modifying the balance of inward and outward currents expressed. In other words, these neurons respond to loss of activity by becoming more sensitive to remaining inputs. By boosting neuronal excitability in response to reduced activity, this process may contribute to the activity-dependent stabilization of firing rates we demonstrated previously[10]. This suggests that stability in neuronal firing rates is maintained throughout the lifetime of a neuron, in part, through the activity-dependent regulation of ionic conductances. This process could serve to compensate for changes

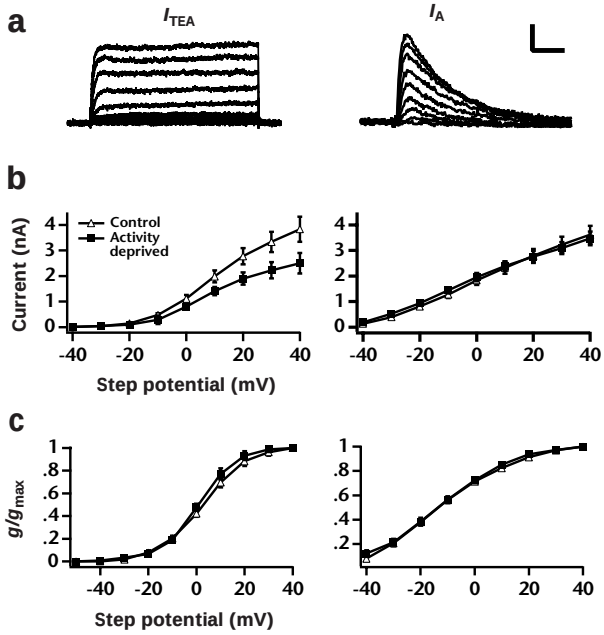

Fig. 5. Persistent outward currents were reduced by activity blockade, but inactivating currents were unaltered. **(a)** Major outward currents (I_{TEA} and I_A) elicited in an activity-deprived neuron by depolarizing voltage steps to step potentials between –40 mV and +40 mV, in increments of 10 mV. Scale bar, vertical 0.5 nA, horizontal 150 ms (I_{TEA}) and 10 ms (I_A). **(b)** Average current–voltage plots for outward currents. Each curve represents an average of data from 11 or 12 cells. Legend in graph on left applies to all graphs in **(b)** and **(c)**. **(c)** Conductance values at each step potential normalized to the value at +40 mV.

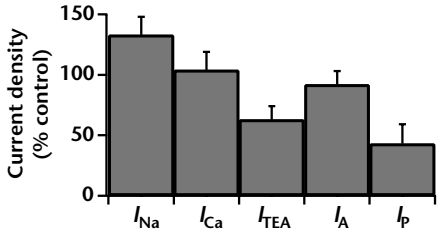

Fig. 6. Current densities were differentially regulated by activity. Before averaging, currents from each cell were scaled by that cell's whole-cell capacitance to convert currents to current densities. Data for peak I_{Na} elicited by a step to –10 mV, peak I_{Ca} elicited by a step to +10 mV, and peak outward currents (I_{TEA}, I_A, and I_P) elicited by steps to +20 mV. Average values for activity-deprived neurons are plotted as a percent of those for control neurons: I_{Na}, 88 ± 6 pA/pF; I_{Ca}, 34 ± 4 pA/pF; I_{TEA}, 119 ± 16 pA/pF; I_A, 119 ± 10 pA/pF and I_P, 39 ± 9 pA/pF.

in excitatory drive produced by changes in the number or strength of synaptic inputs[1–3] or by the dramatic shifts in sensory input that accompany events such as eye opening[1]. In each of these cases, large decreases or increases in input to a cortical neuron could cause it to fall silent or its firing rate to saturate without regulatory mechanisms aimed at preserving its responsiveness.

An important unanswered question regards the aspect of activity that regulates neuronal excitability. Several possibilities exist that are difficult to distinguish in these experiments. Upregulation of postsynaptic sensitivity following activity blockade could result from lack of activation of postsynaptic receptors or from a drop in the postsynaptic firing rate. Both of these possibilities would offer effective and general means for maintaining a neuron's responsiveness to input in a changing environment. A third possibility is that the upregulation of sodium current resulted from prolonged blockade of sodium channels with TTX. This possibility seems less likely, however, because it does not explain the coordinated changes in inward and outward currents produced by activity blockade.

Activity blockade produces a pattern of change in voltage-dependent conductances distinct from developmental changes. During comparable periods of development *in vivo*, sodium, potassium and calcium currents are still changing in magnitude[21,22,33]. Work on mammalian central neurons indicates that, in normal development, sodium[28], sustained potassium[34,35] and high-voltage-activated calcium[36,37] current densities increase in the early postnatal period, whereas transient potassium current densities decrease[35]. In contrast, we find that although sodium currents increase in size in response to activity blockade, persistent potassium currents decrease, and calcium and transient potassium currents are unaltered. Our findings are consistent with earlier studies on amphibian and non-cortical mammalian preparations showing that blocking activity can increase sodium channel number and mRNA[38,39] and decrease persistent potassium currents[26,40] (E.C. Tayag, S. Ge & C.E. Niesen, *Soc. Neurosci. Abstr.* **23**, 681.5, 1997). These considerations suggest that activity blockade does not modify conductances through a simple change in the kinetics of a normal developmental process.

Activity-dependent plasticity of intrinsic electrical properties is not the only form of homeostatic plasticity present in neural circuits. We have shown that excitatory synaptic strengths are reduced by increased activity and enhanced by activity blockade[10]. In addition, inhibition is regulated by activity in the opposite direction as excitation[13]. As these forms of synaptic homeostasis all increase the excitability of pyramidal neurons following activity blockade, they are complementary to the regulation of intrinsic conductances demonstrated here. These data demonstrate that multiple mechanisms exist to regulate the sensitivity of neurons to their inputs and raise the interesting question of how tightly these different regulatory mechanisms may be coupled.

The ability of activity to produce selective changes in a complex array of voltage-dependent ionic conductances could endow this process with great flexibility. Theoretical studies predict that if ionic conductances are differentially regulated as a function of a neuron's history of activity, the synaptic contribution from dendritic regions receiving unequal stimulation may be equalized[41], temporal structure of firing patterns may be dramatically changed, as from tonic firing to bursting[18], and neurons may adjust the distribution of their firing rates to match the statistics of their inputs[42]. These studies suggest that, as well as regulating the mean firing rate of a neuron, activity-dependent changes in intrinsic excitability could be important in modifying the integrative properties of cortical neurons.

Methods

Cortical cultures. Visual cortical cultures were prepared from rat pups at postnatal days 4–6 as described[13]. Experiments were done after 7–9 days *in vitro*. All data were obtained in parallel on treated and age-matched sister control cultures. Activity was blocked with 0.5 μM TTX; the TTX was refreshed after 24 h.

Whole-cell patch recordings. These recordings were obtained from pyramidal neurons in artificial cerebrospinal fluid at room temperature as described[10,13] (see below for solutions). Recordings with resting potentials more positive than –55 mV or series resistances larger than 10 MΩ were excluded. Junction potentials for the various solutions were measured to be 3–8 mV and were left uncorrected. All average data are reported as mean ± s.e. for the number of neurons indicated. In all experiments, synaptic transmission was blocked with 20 μM 6-cyano-7-nitoquinoxaline-2,3-dione (CNQX), 20 μM bicuculline and 50 μM D(–)-amino-7-phosphonovaleric acid (AP5).

Current-clamp experiments to measure firing rates and threshold were done with an Axoclamp 2B amplifier. Voltage-clamp experiments to measure individual currents were done with an Axopatch 1D or an Axopatch 200B amplifier. Neurons were held at –60 mV. For each cell, series resistance and whole-cell capacitance were estimated using the amplifier's built-in circuitry. To minimize errors associated with series resistance, it was compensated 70–80%. A number of 10-mV hyperpolarizing voltage steps were delivered to each neuron from a holding potential of –60 mV and were used to subtract leak and capacitive currents offline. Recordings were analog filtered at 2–5 kHz and digitized at 5-20 kHz. Individual ionic currents were elicited by stepping the membrane potential from a fixed holding potential to a family of depolarized step potentials. Sodium activation and inactivation curves were measured with the sodium concentration of the solutions adjusted to give a reversal potential of +38 mV. The activation curve was constructed by measuring the maximum chord conductance produced by depolarizing voltage steps from a holding potential of –60 mV. The inactivation curve was constructed by measuring the maximum current produced by a voltage step to –10 mV from holding potentials between –100 and –20 mV.

Cell-attached patch recordings. These recordings were obtained from somatic patches of pyramidal neurons in artificial cerebrospinal fluid at room temperature (see below for solutions). Recordings were done with an Axopatch 1-D amplifier and patch pipets (4 MΩ) with tip diameters of approximately 1 μm. Data were analog filtered at 2 kHz and digitized at 20 kHz. Somatic patches were held at potentials 20 mV hyperpolarized with respect to each cell's resting potential, and then sodium currents were elicited by stepping the potential 40, 60, 80 and 100 mV above rest. Each depolarizing voltage step was repeated 20 times, and the average peak current response was measured. These currents were determined to be mediated by sodium because potassium currents had been blocked pharmacologically and because these currents had the correct kinetics (activating within 1 ms and inactivating rapidly) and voltage dependence (peaking near 0 mV) to be sodium currents. For every depolarizing voltage step, 15 small (10 mV) hyperpolarizing steps were

applied, to be used offline for leak and capacitive current subtraction. After recordings were complete, patches were ruptured, and attempts were made to record each cell's resting potential. These did not yield accurate measurements, presumably because cells were dialyzed too quickly by the sodium-rich pipet solution. Instead, voltages are presented relative to the average resting potential measured in these cultures. Given a standard deviation of 4 mV in the measured resting potential, average voltages should be within ± 4 mV of the calculated value.

Solutions. The external solution for experiments on firing rates, threshold and potassium currents contained 126 mM NaCl, 3 mM KCl, 2 mM $MgSO_4$, 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$ and 14 mM dextrose; the pH was buffered to 7.4 by bubbling continuously with 5% CO_2/95% O_2. To measure potassium currents, we blocked sodium currents with 0.1 □M TTX. The external solution for whole-cell sodium current experiments contained 33 mM NaCl, 97 mM choline chloride, 3 mM KCl, 2 mM $MgCl_2$, 20 mM HEPES/Na-HEPES, 1.6 mM $CaCl_2$, 0.4 mM $CdCl_2$, 10 mM TEA-Cl, 5 mM 4-AP and 14 mM dextrose; the pH was adjusted to 7.4 with NaOH. The external solution for calcium current experiments was similar to that for sodium currents except that the KCl and the $CdCl_2$ were replaced by equal amounts of TEA-Cl and $CaCl_2$, respectively, and 0.1 □M TTX was added. In all experiments, the osmolality was 305–315 mOsm.

The internal solution for experiments on firing rates, threshold and potassium currents contained 130 mM $KMeSO_4$, 10 mM KCl, 10 mM HEPES/K-HEPES, 2 mM $MgSO_4$, 0.5 mM EGTA and 3 mM ATP; the pH was adjusted to 7.3 with KOH. The internal solution for whole-cell sodium current experiments contained 120 mM $CsMeSO_4$, 10 mM KCl, 10 mM HEPES/Na-HEPES, 10 mM EGTA and 3 mM ATP; the pH was adjusted to 7.3 with NaOH. In measuring sodium activation and inactivation curves and the sodium reversal potential, 8 mM NaCl was added. The internal solution for calcium current experiments was similar to that for sodium currents except that the KCl was replaced by an equal amount of TEA-Cl. In all experiments, the osmolality was adjusted to 289–291 mOsm with sucrose.

The external solution for cell-attached patch recordings contained 130 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 20 mM HEPES/Na-HEPES, 2 mM $CaCl_2$, 14 mM dextrose and 10 mM sucrose; the pH was adjusted to 7.4 with NaOH. The pipet solution contained 120 mM NaCl, 30 mM TEA-Cl, 10 mM HEPES/Na-HEPES, 2 mM $CaCl_2$, 3 mM KCl, 1 mM $MgCl_2$ and 5 mM 4-AP; the pH was adjusted to 7.4 with NaOH.

Note: Supplementary material may be found on the Nature Neuroscience web site at http://neurosci.nature.com/web_specials.

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