deletion within the SH2 domain might represent a general mechanism by which cells regulate signaling and the cascade of connections that occur by means of phosphotyrosine-containing proteins and adaptors.

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Activity-Dependent Changes in the Intrinsic Properties of Cultured Neurons

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Learning and memory arise through activity-dependent modifications of neural circuits. Although the activity dependence of synaptic efficacy has been studied extensively, less is known about how activity shapes the intrinsic electrical properties of neurons. Lobster stomatogastric ganglion neurons fire in bursts when receiving synaptic and modulatory input but fire tonically when pharmacologically isolated. Long-term isolation in culture changed their intrinsic activity from tonic firing to burst firing. Rhythmic stimulation reversed this transition through a mechanism that was mediated by a rise in intracellular calcium concentration. These data suggest that neurons regulate their conductances to maintain stable activity patterns and that the intrinsic properties of a neuron depend on its recent history of activation.

The outputs of neural circuits depend both on synaptic connections and on the intrinsic electrical properties of individual neurons (*1*). Activity-dependent modification of synaptic strengths contributes to processes such as developmental segregation of inputs and learning (*2*) and has been well described. Less extensively studied has been the role of activity in shaping the intrinsic electrical properties of neurons [but see (*3*)]. These properties are determined by the balance of a neuron's ionic conductances, and modification of this balance can substantially change the output of the circuits in which a neuron participates (*1, 4*). Here we show that activity can alter the intrinsic electrical properties of neurons, which suggests that a neuron's physiological identity is influenced by the synaptic input it receives.

We studied stomatogastric ganglion (STG) neurons from the spiny lobster, *Panulirus interruptus*, that participate in two motor programs producing rhythmic movements of the teeth and foregut. This rhythmic activity depends both on modulatory and rhythmic inhibitory synaptic drives that cause STG neurons to fire bursts of action potentials when released from inhibition. When pharmacologically isolated, STG neurons do not fire in bursts but fire tonically (Fig. 1A) (*4*).

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What happens to the intrinsic electrical properties of STG neurons when chronically isolated from their normal inputs? To investigate this, we placed STG neuronal somata with short primary neurites into primary cell culture under defined conditions (*5*, *6*). After 2 days in culture, most STG neurons were silent at rest and fired action potentials tonically when depolarized and when released from hyperpolarization (Fig. 1B and Table 1). These properties were similar to those of STG neurons that were pharmacologically isolated in the ganglion (Fig. 1A). After 3 or 4 days in culture, however, most STG neurons fired in bursts when depolarized and produced a large slow-wave depolarization when released from hyperpolarization, which we term a rebound burst (Fig. 1B and Table 1). Thus, when chronically isolated from their normal inputs, STG neurons become capable of generating bursts endogenously.

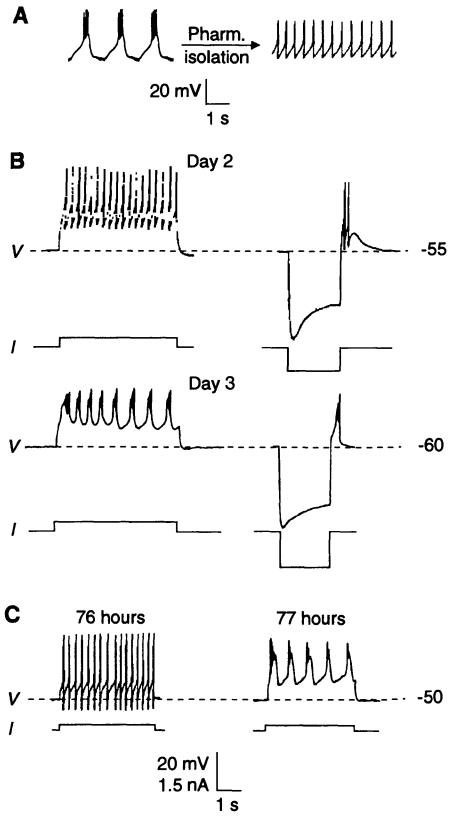

Fig. 1. Properties of STG neurons in the ganglion and isolated in primary cell culture. (**A**) Schematic illustration of the activity of STG neurons in the ganglion when connected to their normal synaptic and modulatory inputs and when pharmacologically isolated (*4*). (**B**) Intracellular recordings from two STG neurons after 2 or 3 days in culture, showing their response to depolarizing and hyperpolarizing current. Dashed line indicates V_m in millivolts. Here and in subsequent figures, *V* is the membrane potential and *I* is the current injected through the microelectrode. (**C**) Intracellular recording from an STG neuron in culture after 76 and 77 hours. Neuron was recorded continuously for 8 hours and its properties monitored every 15 min. Cultured neurons firing bursts had small action potentials because of the partial inactivation of sodium currents during the depolarizing phase of the burst.

The transition from tonic firing to burst firing of STG neurons in culture is caused by changes in the expression of intrinsic conductances. The densities of calcium currents contributing to burst firing increase, whereas outward current densities decrease (*7*). Long-term recordings from individual neurons showed that the spontaneous transition from tonic firing to burst firing could occur rapidly, in as little as 1 hour (Fig. 1C). This is because small conductance changes can move neurons between these two activity states (*7*).

The change in activity of isolated STG neurons suggests that the balance of conductances is regulated by synaptic inputs. Perhaps when isolated, STG neurons adjust their conductances and gain the ability to fire bursts endogenously to compensate for the loss of rhythmic drive. If so, supplying rhythmic drive to cultured neurons should reverse the transition from tonic firing to burst firing. To test this possibility, we took neurons on day 3 that fired bursts when depolarized and, by means of rhythmic hyperpolarizing current pulses, drove them to fire bursts on the rebound (Fig. 2A) (*8*). Repetitively driving neurons in this way produced a gradual reduction in the magnitude of the rebound burst (Fig. 2B). After 1 hour of stimulation, the rebound burst resembled the small-amplitude rebound of a tonically firing neuron, and this effect reversed after about 1 hour (*9*). On average, prolonged stimulation significantly reduced the rebound from 17.8 ± 1.6 to 8.5 ± 1.1 mV, and this reversed (16.1 ± 2.5 mV) after about 1 hour (control ≠ stimulation, $P < 0.001$, paired *t* test, $n = 16$). Stimulation produced no change in resting potentials (V_m) or input resistances (R_{in}) (*10*).

The magnitude of the change in the rebound burst depended on the frequency of stimulation. Interpulse periods of 12 s or longer produced no change in burst properties. Interpulse periods of 8 s or less progres-

Table 1. Percentage of neurons that fire tonically or in bursts after 2, 3, or 4 days in culture.

Days in culture	Activity (%)			V_m (mV)	R_{in} (megohms)
	Tonic	Bursts	Other		
2	64	26	10	−58 ± 2	55 ± 8
3	31	65	4	−56 ± 2	63 ± 7
4	23	66	11	−57 ± 3	55 ± 13

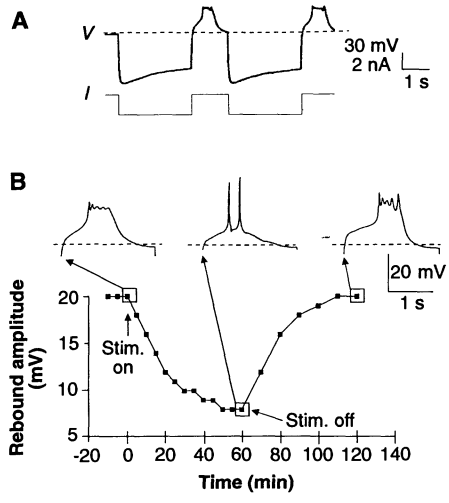

Fig. 2. Change in the rebound properties of STG neurons after rhythmic stimulation. (**A**) Stimulation paradigm: −2.5-nA pulses were delivered at 0.3 Hz to elicit repetitive rebound bursts. (**B**) Plot of rebound amplitude versus time during rhythmic stimulation for the neuron illustrated in (A). Rebound properties are shown for the time points surrounded by boxes. "Stim. on" indicates onset of rhythmic hyperpolarizing current pulses; "stim. off" indicates cessation of stimulation. Dashed line indicates V_m (−50 mV).

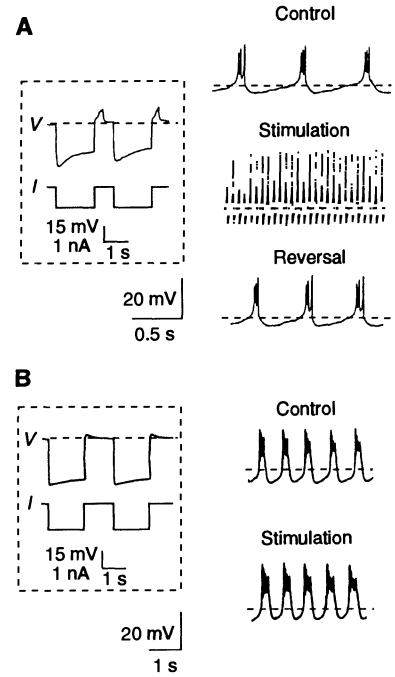

Fig. 3. Change in the depolarization-induced burst properties of STG neurons after rhythmic stimulation. (**A**) Hyperpolarizing current pulses (−1.5 nA, 0.33 Hz) were delivered to elicit rebound bursts; dashed line indicates V_m (inset). Control: activity before stimulation; stimulation: activity after 1 hour of hyperpolarizing current pulses; reversal: activity 1 hour after cessation of current pulses. For each condition, neuron was depolarized with 0.5 nA dc to elicit activity. Dashed lines indicate −40 mV. (**B**) Same stimulation paradigm delivered to a neuron that did not fire bursts on the rebound (inset). For each condition, the activity of the neurons was determined for a range of current injections.

sively decreased the amplitude of the rebound burst, and interpulse periods of 3 to 4 s produced the largest decrease (*11*). This frequency is within the range of the faster of the two STG rhythms in the intact ganglion (*4*).

In addition to changing the rebound properties of STG neurons, driving neurons to fire rebound bursts produced a long-lasting change in their response to depolarization. Driving a neuron for 1 hour transformed its response to depolarization from burst firing to tonic firing, and this effect reversed after about 1 hour (Fig. 3A). Rhythmic drive therefore eliminated the neuron's ability to fire bursts endogenously. Of 16 neurons that were driven to fire rebound bursts for 1 hour, 15 showed a large and persistent reduction in the amplitude of the slow wave underlying depolarization-induced bursts. On average, prolonged rhythmic drive reduced the amplitude of depolarization-induced bursts from 18.3 ± 2.7 to 8.0 ± 2.8 mV, and this reversed (16.4 ± 1.7 mV) after about 50 min (stimulation ≠ control, $P < 0.001$, paired t test, $n = 16$).

These data show that patterned input can profoundly alter the intrinsic properties of STG neurons. When isolated from rhythmic drive, they gained the ability to fire bursts endogenously, and when rhythmic drive was restored, this ability was reduced or eliminated. This suggests that STG neurons adjust their conductances in a homeostatic manner, so that they retain the capacity to fire in bursts despite changes in external inputs. Such a process requires an intracellular messenger, such as calcium, whose concentration is well correlated with activity. The stimulation-induced transition from burst firing to tonic firing depends on eliciting rebound bursts. One hour of hyperpolarizing pulses administered to neurons that fired bursts when depolarized but did not exhibit rebound bursts (Fig. 3B) did not change the depolarization-induced burst amplitude (control: 15.5 ± 1.1 mV; stimulation: 15.4 ± 2.0 mV, no significant difference, $P > 0.1$, paired t test, $n = 6$) (*12*). Rebound bursts were blocked by a reduction in extracellular calcium concentration (Fig. 4A) or by calcium-channel blockers, which indicates that they are carried in part by a calcium current. This suggests that a rise in intracellular calcium concentration may mediate the changes in the neurons' intrinsic properties. Calcium is known to mediate many activity-dependent processes, including changes in synaptic efficacy (*13*), neurite outgrowth (*14*), and the magnitude of ionic currents (*3*).

To test this possibility, we used intracellular injections of the calcium chelator BAPTA to buffer intracellular calcium (Fig. 4, B and C) (*15*). BAPTA infusion broadened the rebound burst but did not affect its amplitude. Driving neurons to fire rebound bursts for 1 hour with intracellular BAPTA produced no change in the rebound amplitude (Fig. 4C; control: 17.4 ± 0.9 mV; stimulation, 16.6 ± 1.3 mV, no significant difference, $P > 0.1$, paired t test, $n = 7$). These data indicate that a rise in intracellular calcium concentration is necessary to produce the change in intrinsic properties.

Recent theoretical work has noted that activity-dependent regulation of neuronal conductances provides a mechanism for maintaining stable properties in response to growth and to changing inputs as well as a mechanism for differentiating neurons according to the pattern of inputs they receive (*16*). In this model, the balance of inward and outward conductances depends on the intracellular calcium concentration and thus on activity. The model predicts that isolating neurons from their normal inputs or subjecting them to stimulation will change their intrinsic electrical properties. This prediction holds true for STG neurons isolated in primary cell culture.

Many neurons maintain stable functional characteristics over the entire lifetime of an organism, despite constant channel turnover, changes in size and shape, and changing inputs. Active regulation of intrinsic properties may be an important mechanism for achieving such stability. Our data suggest that neurons do not maintain a fixed balance of conductances but rather that this balance is adjusted to maintain relatively constant patterns of activity. This in turn suggests that synaptic inputs are instrumental in shaping a neuron's intrinsic properties. The regulation described here acts to stabilize neuronal activity. In contrast, Hebbian synaptic potentiation acts to modify activity and can be destabilizing (*17*). Together, these mechanisms provide a powerful tool for maintaining stability and flexibility in neural systems.

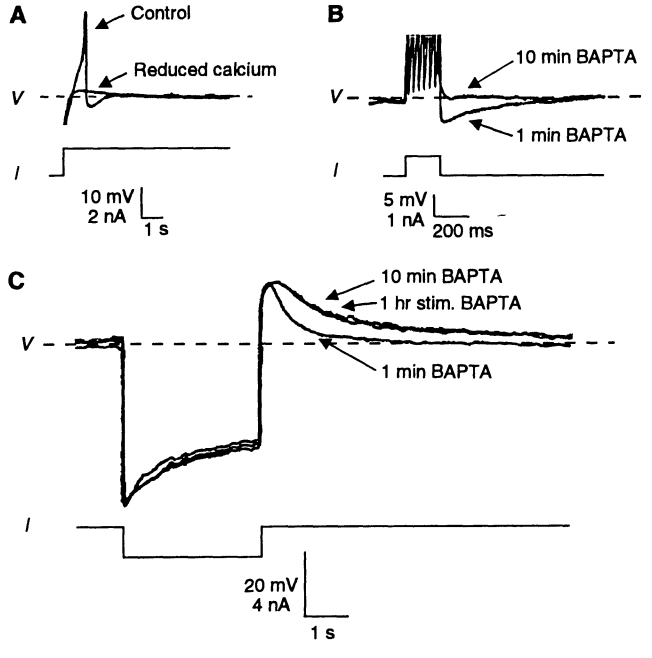

Fig. 4. Intracellular calcium buffering blocks the effects of stimulation. (**A**) Effect on the rebound burst of reducing extracellular calcium concentration from 13.6 to 0.136 mM. Dashed line indicates V_m (−45 mV). (**B**) STG neurons showing an afterhyperpolarization (ahp) produced by a calcium-dependent potassium current. This ahp was evident 1 min after penetration with a BAPTA electrode but was abolished after 10 min of BAPTA infusion. Dashed line indicates −40 mV. (**C**) Effect of intracellular BAPTA on the stimulation-induced change in rebound properties. Rebound is compared 1 min after penetration, after 10 min of BAPTA infusion, and after 1 hour of rhythmic hyperpolarizing current pulses (−2 nA at 0.33 Hz) that elicited repetitive rebound bursts. To compare the slow wave of the rebound, we held the neuron at −65 mV (dashed line), so that the rebound was below the spike threshold.

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9. Burst amplitudes were measured several minutes after termination of the stimulation in order to assess only stable changes in neuronal properties and not those produced by fast activation or inactivation properties of individual ionic currents.
10. Average V_m was −59 ± 2 mV before and after stimulation. Average R_{in} was 60 ± 7 megohms before and 60 ± 8 megohms after stimulation.
11. The same total hyperpolarizing current delivered dc rather than in pulses produced no stable change in burst properties (n = 4). Up to 6 hours of hyperpolarizing pulses delivered to neurons that fired tonically when depolarized produced little perceptible change in activity. Depolarizing current pulses produced mixed results, leading to a small decrease in burst amplitude in some cases and a small increase in others.
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Aberrant Neurites and Synaptic Vesicle Protein Deficiency in Synapsin II–Depleted Neurons

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Synapsin I and synapsin II are neuron-specific phosphoproteins that have a role in the regulation of neurotransmitter release and in the formation of nerve terminals. After depletion of synapsin II by antisense oligonucleotides, rat hippocampal neurons in culture were unable to consolidate their minor processes and did not elongate axons. These aberrant morphological changes were accompanied by an abnormal distribution of intracellular filamentous actin (F-actin). In addition, synapsin II suppression resulted in a selective decrease in the amounts of several synaptic vesicle–associated proteins. These data suggest that synapsin II participates in cytoskeletal organization during the early stages of nerve cell development.

As hippocampal neurons grow in culture, the cytoskeleton undergoes a sequential reorganization that begins with the spreading of a lamellipodial veil around the periphery of the cell (stage I) (*1*). Shortly thereafter, the veil consolidates to form an array of discrete minor neurites (stage II). The transition from stage I to stage II is associated with the establishment of a microtubule-rich domain within the shafts of the neurites and a restriction of the flattened actin-rich portion of the veil to the tips of the neurites, the site of the growth cone. Selective elongation of one of those neurites to form the axon (stage III) involves lengthening of the shaft while the growth cone is maintained in a relatively constant organizational state. This sequence occurs within 24 hours of plating (*1*). In these neurons, synapsin I (*2*) and synapsin II (*3*) are expressed before the establishment of synaptic contacts. The synapsins are associated with the cytoplasmic surface of synaptic vesicles (*4*) and have been shown to interact with actin and other cytoskeletal elements in vitro (*4*–*6*). To examine the possibility that the synapsins might function in the early stages of neuronal development, we suppressed the expression of synapsin II in hippocampal neurons.

Synapsin II became readily detectable in the cell bodies of untreated hippocampal neurons 4 hours after plating, a time that coincides with the initial neurite outgrowth stage (stage II) (*3*). Twenty-four hours after plating, nearly all hippocampal neurons had extended several minor processes and a single axon (Fig. 1A), and strong synapsin II staining was present in the cell body (Fig. 1B). Similar results were observed for synapsin I at this early stage of neuronal development (*2*).

Two nonoverlapping antisense oligonucleotides corresponding to rat synapsin II complementary DNA (cDNA) sequences from positions −13 to +10 (AS-RSII −13+10) and from positions −88 to −66 (AS-RSII −88−66) (*7*) reduced the amounts of synapsins IIa and IIb, the two isoforms of synapsin II, by about 75% after 24 hours of incubation (Table 1). On the other hand, sense oligonucleotides corresponding to the same rat synapsin II cDNA sequences, S-RSII −13+10 (Table 1) and S-RSII −88−66, did not affect synapsin II amounts in comparison with untreated control cultures.

When cultures were treated with S-RSII −13+10 (Fig. 1, C and D) or S-RSII −88−66, the ability of hippocampal cells

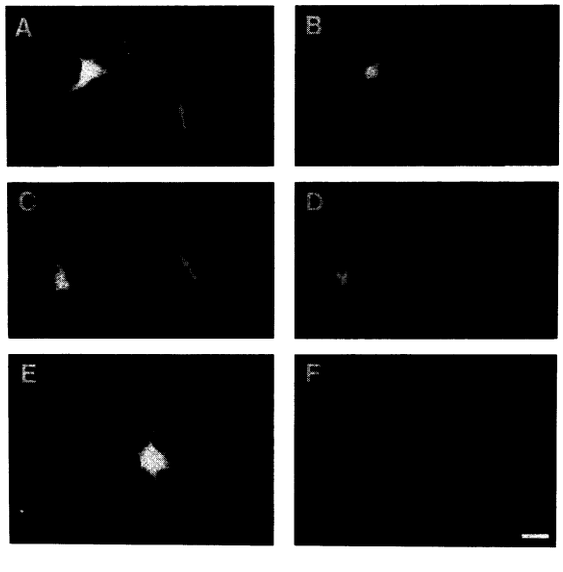

Fig. 1. Inhibition of neurite growth by synapsin II antisense oligonucleotides in cultured hippocampal neurons (*20, 21*). Control (**A** and **B**), sense-treated (**C** and **D**), and antisense-treated (**E** and **F**) hippocampal neurons were double-labeled with a monoclonal antibody against tubulin [(A), (C), and (E)] and an affinity-purified polyclonal antibody against synapsin II [(B), (D), and (F)]. No immunoreactivity for synapsin II was detected in the antisense-treated cell (F). Solid arrows, axons; outlined arrows, minor processes. Bar, 20 μm.

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