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 rhythm 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 action potentials were initiated 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.

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 4 hours (control = stimulation, P < 0.001, paired t test, n = 16). Stimulation produced no change in resting potentials (V₉₀) or input resistances (Rᵢₚₚ) (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 provoked

<table>
<thead>
<tr>
<th>Days in culture</th>
<th>Activity (%)</th>
<th>V₉₀ (mV)</th>
<th>Rᵢₚₚ (megohms)</th>
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<td>Tonic Bursts</td>
<td>Other</td>
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<td></td>
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<tr>
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<tr>
<td>4</td>
<td>23</td>
<td>66</td>
<td>11</td>
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Table 1. Percentage of neurons that fire tonically or in bursts after 2, 3, or 4 days in culture.

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 2 or 3 days in culture, showing their response to depolarizing and hyperpolarizing current. Dashed line indicates V₉₀. 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.

Fig. 2. Change in the rebound 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 V₉₀ (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 both conditions, the activity of the neurons was determined for a range of current injections.

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 V₉₀ (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 both conditions, 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 15.3 ± 2.7 to 8.0 ± 2.8 mV, and this reversed (16.4 ± 1.7 mV) after about 30 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 an eliciting calcium burst. 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 (15).

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.

REFERENCES AND NOTES

6. Methods were as reported (5), except that cultu- res were maintained and recorded at room temperature. Only neurons with membrane poten- tials (V_m) between −45 and −65 mV and input resistances (R_m) > 20 megohms were used. Neurons were visualized with an inverted micro- scope with modulation contrast optics. Sharp electrode recordings (0.6 M KSCN, +20 mV KC). 10 to 15 megohms) were made in physiological saline with an Axoclamp (IIA amplifier (Axon Instru- ments, Foster City, CA) in discontinuous current clamp mode. Data represent mean ± SEM for the number of experiments indicated.
Hyperpolarization activated an inward current (I_h) that produced a depolarizing "sag" in the membrane potential (Fig. 2A). I_h deactivated slowly on release from hyperpolarization, producing a 5- to 10-mV depolarization that was sufficient to activate sodium currents and trigger action potentials in tonically firing neurons or to activate calcium currents and trigger a rebound burst in bursting neurons (Fig. 1B).

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.

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.

Rebound bursts were eliminated by blocking I_h with external Ca^2+, which prevented rebound bursts but left depolarization-induced burst firing intact (n = 2), or by use of neurons that did not exhibit rebound bursts (n = 4).

To infuse BAPTA (1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid Sigma), we included 200 mM BAPTA in the recording electrodes, and dc hyperpolarizing current (−0.2 to −0.5 nA) was passed for 10 to 20 min, or until neuronal properties stabilized.

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