Serotonergic Modulation of Afterhyperpolarization in a Neuron That Contributes to Learning in the Leech

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INTRODUCTION

Modulation of neuronal excitability makes an important contribution to learning and memory (see review by Zhang and Linden 2003) and dysfunctions in excitability are thought to contribute to age-related cognitive disorders (see review by Disterhoft and Oh 2006). As with synaptic plasticity, modulation of excitability can be mediated by multiple cellular mechanisms and one important mechanism involves changes in the afterhyperpolarization (AHP) following an action potential. Decreases in AHP lead to an increase in excitability by reducing the post action potential refractory period, permitting an increase in the number or rate of action potential initiations. These changes in the firing properties of a neuron could make an important contribution to learning and memory and modulation of AHP has been shown to contribute to learning in a variety of vertebrate and invertebrate species (Alkon et al. 1985; Brosh et al. 2006; Fournier et al. 2001; Stackman et al. 2002).

In the medicinal leech (Hirudo medicinalis), sensitization of the whole body shortening reflex is accompanied by an increase in excitability in the S-cell (Burrell and Sahley 2005). The S-cell is an interneuron that forms a network of electrically coupled neurons that extends throughout the leech CNS (see Fig. 1A). Although the S-cell receives afferent input from both touch- and pressure-sensitive mechanosensory cells, it is active during whole body shortening, lesions of the S-cell network do not affect the leech’s capacity to shorten (Shaw and Kristan Jr 1999). However, lesion experiments have demonstrated that the S-cell is critical for initiation of sensitization (Burrell et al. 2003; Modney et al. 1997; Sahley et al. 1994), indicating that the interneuron plays a role in learning-related behavioral plasticity. The S-cell is also thought to contribute to expression of the sensitized response, given that S-cell activity increases during sensitization and this activity correlates with the intensity of the sensitized shortening response, whereas no such correlation is observed in the nonsensitized state (Burrell and Sahley 2005; Burrell et al. 2001; Modney et al. 1997). Sensitization-induced enhancement of S-cell firing is thought to be mediated, at least in part, by an increase in excitability. Increased S-cell excitability during sensitization is mediated by serotonin (5-HT), most likely through the activation of a 5-HT₁₇-like receptor (Burrell and Sahley 2005; Crisp and Muller 2006).

The biophysical basis for sensitization/5-HT–mediated increases in excitability is not known. Enhanced S-cell excitability is expressed as both an increase in the number of action potentials elicited by a fixed stimulus and a decrease in the amount of current needed to elicit a single action potential (Burrell and Sahley 2005; Burrell et al. 2001; Crisp and Muller 2006). Therefore the mechanisms involved are likely to be complex. In the following set of experiments the potential role of AHP modulation contributing to 5-HT–induced increases in excitability is examined using both electrophysiological and computational modeling techniques. The currents that may mediate AHP are also examined using pharmacological blockers of ion channels. Some of the data presented herein were previously reported in abstract form (Burrell and Crisp 2006).
not include spontaneous excitatory postsynaptic potentials that could affect the data. In addition, the pre- and posttreatment measurements of AHP/ADP in each neuron were taken from the average of multiple recordings (three to eight sweeps for each AHP/ADP measurement) to ensure that an accurate measurement of the afterpotentials (all AHP/ADP traces shown are from these averaged recordings).

Two protocols were used to measure 5-HT modulation of excitability. The effect of 5-HT on the frequency–current (F–I) relationship was examined by applying depolarizing current pulses (500 ms) at 0.2-nA intervals (range: 0.2–3.0 nA) to S-cells in normal saline and those that had been treated with 5-HT (10 μM, 5 min; Wallen et al. 1989; Zhong et al. 2006). Excitability was also measured as the number of action potentials elicited during a 1-nA, 500-ms depolarizing current injection. Input resistance was tested by injecting a 1-nA, 500-ms hyperpolarizing current pulse. Both excitability and input resistance measurements were made with the membrane potential held at ~50 mV. Analog signals were digitized (Digidata 1322; Axon Instruments/Molecular Devices; Sunnyvale, CA) and transferred to a computer using data acquisition software (Axoscope; Axon Instruments/Molecular Devices) for storage and analysis.

All drugs were prepared just before the experiment from stock solutions maintained at ~20°C. For serotonin (5-HT) experiments, S-cell AHP, excitability, and input resistance were initially assessed in normal saline. The ganglion was then perfused for 5 min with 10 μM 5-HT (5-HT-HCl; Sigma), followed by a 5-min wash in normal saline before 5-HT, excitability, and input resistance were retested. The post-5-HT recordings were carried out in normal saline because 5-HT induces spontaneous movements in the ganglion, which are not conducive to intracellular recordings. Fortunately, the effects of 5-HT on S-cell excitability last ~1 h (Burrell et al. 2001). In Ca2+-free experiments, S-cell AHP was recorded in normal saline and following a 10-min perfusion of Ca2+-free saline. This same protocol (initial test in normal saline, restet after 10 min in treatment solution) was used in experiments in which ion channel blockers were used—specifically apamin, charybdoxin, iberiotoxin, saxitoxin, scyllatoxin, and α-tubocurarine (Sigma). In one set of occlusion experiments, S-cells were pretreated with tubocurarine prior to the application of 5-HT. The effect of 5-HT on the tubocurare-pretreated S-cell was examined in a manner identical to earlier 5-HT experiments, except that pretreatment measurements of AHP and excitability (made at ~50 mV) were carried out in the presence of 500 μM tubocurarine and posttreatment measurements were made following 5-min perfusion with 500 μM tubocurarine + 10 μM 5-HT.

**Hodgkin–Huxley modeling of the S interneuron**

Using the simulator SNAP 8 (Ziv et al. 1994), a spherical soma (10-μm diameter) was modeled. The integration time step (dt) was 10 μs, and parameters were defined with the temperature-correction value adjusted such that impulse activity in the model resembled that observed in recordings from the S-cell at room temperature. Membrane currents were determined as \( I_{\text{ion}} = \sigma_{\text{ion}} (V - E_{\text{ion}}) \) for Na\(^+\), K\(^+\), Ca\(^{2+}\), and a leak current, but Ca\(^{2+}\)-dependent currents were determined as \( I_{\text{Ca}} = \sigma_{\text{Ca}} (V - E_{\text{Ca}}) f(Ca^{2+}) \). Although the dynamics underlying cellular calcium pools are very complex (Blaustein 1988), we used a simplified model of calcium regulation (Ziv et al. 1994), where

\[
f(Ca^{2+}) = \frac{[Ca^{2+}]}{0.1 + [Ca^{2+}]}
\]

and

\[
\frac{\partial [Ca^{2+}]}{\partial t} = 5 \left( 0.4 \left( \sum_i L_i Ca^{2+} \right) - [Ca^{2+}] \right)
\]

Conductances included: voltage-dependent Na\(^+\) conductance (\(g_{Na}\)); delayed rectifier K\(^+\) conductance (\(g_K\)); a high-voltage-
activated \( V \geq -20 \text{ mV} \) Ca\(^{2+}\) conductance \( (g_{\text{Ca}}) \); a Ca\(^{2+}\)-dependent 
K\(^{+}\) conductance with fast activation and inactivation dynamics \( (g_{\text{h,AHP}}) \); a Ca\(^{2+}\)-dependent K\(^{+}\) conductance with slow activation and inactivation dynamics similar to previously described currents underlying the medium afterhyperpolarization \( (g_{\text{mAHP}}) \); a Na\(^{+}\)-driven afterdepolarization current \( (g_{\text{ADP}}) \); and a nonspecific leak conductance \( (g_{\text{leak}}) \). These conductances were calculated using Hodgkin–Huxley type equations with parameters similar to those previously used to model leech neurons (Baccus et al. 1998), but modified to resemble current-clamp data (subsequently described). Conductances were defined as 
\[ g_{\text{Na}} = g_{\text{Na,m}} m^3 h \] 
\[ g_{\text{K}} = g_{\text{K,m}} m^4 \] 
\[ g_{\text{Ca}} = g_{\text{Ca,m}} m \] 
\[ g_{\text{ADP}} = g_{\text{ADP,m}} m, g_{\text{AHP,m}} = g_{\text{AHP,m}} m + g_{\text{mAHP}} = g_{\text{mAHP}} \] (Baccus et al. 1998), and 
\[ g_{\text{leak}} = g_{\text{leak},m} \] 
where \( g_{\text{ion}} \) refers to the maximum conductance of current \( I_{\text{ion}} \) as defined in Table 1. State variables \( m \) and \( h \) were calculated as 
\[ \frac{\partial m}{\partial t} = 1.000[\alpha_{m}(1 - m) - \beta_{m} m] \] 
and 
\[ \frac{\partial h}{\partial t} = 1.000[\alpha_{h}(1 - h) - \beta_{h} h], \] 
respectively. The effects of 5-HT on the S-cell were approximated by 
decreasing \( g_{\text{ADP}} \) to 10 and \( g_{\text{mAHP}} \) to 0.125. This model can be accessed at ModelDB (http://senselab.med.yale.edu/modeldb/default.asp).

**RESULTS**

**S-cell AHP and its modulation by 5-HT**

The AHP following a single S-cell action potential has two components: a transient component observed immediately following the action potential (fast AHP or fAHP) and a later component, the medium AHP (mAHP) that develops after the fAHP and clearly has a slower rate of decay. No slow AHP (sAHP) was observed following a single S-cell action potential or following action potential trains of 10 spikes at 25 Hz or trains of 20 spikes at 50 Hz (Fig. 2, A and C). For both spike trains, significant AHP was observed between the action potentials within the train, but the posttrain AHP quickly decayed with the membrane potential returning to pretrain levels within 1 s. When the membrane potential at the S-cell soma was held at progressively more hyperpolarized potentials using direct holding current, the magnitude of both the fAHP and mAHP increased (Fig. 2B). However, when the soma membrane potential was held at -60 mV, the afterpotential reversed and an ADP was now observed (Fig. 2B). This reversal was routinely observed between -55 and -60 mV and the observed ADP increased with greater hyperpolarization (not shown).

5-HT significantly reduced S-cell AHP when compared with saline-treated S-cells. 5-HT (10 \( \mu \)M) reduced both the fAHP and the mAHP of the S-cell by about 1 mV (Fig. 3, A and B). In addition, when the ADP was measured following action potentials initiated at -60 mV, there was a significant increase in the ADP level (Fig. 3C). Decreased AHP as a result of 5-HT treatment was accompanied by enhanced excitability (Fig. 3D), consistent with the hypothesis that 5-HT–mediated increases in excitability are mediated, at least in part, by a reduction in AHP. In experiments where excitability was measured in terms of the frequency of action potential initiations as a function of the injected current \( (F-I) \) relationship, the slope of the \( F-I \) plot in the 5-HT–treated S-cells was greater than that of S-cells tested in normal saline (Fig. 3E). As observed in earlier experiments (Burrell and Sahley 2005; Burrell et al. 2001; Crisp and Muller 2006), 5-HT did not alter the resting potential or input resistance of the S-cell (data not shown).

**Contribution of Ca\(^{2+}\)-dependent K\(^{+}\) channels to S-cell AHP**

In many neurons, AHP is mediated by Ca\(^{2+}\)-dependent K\(^{+}\) channels. To begin to examine whether this is the case in the leech S-cell, AHP in these interneurons was approximated by decreasing \( g_{\text{Ca}} \) in the ADP level (Fig. 3C). The threshold for action potential initiation appears to be hyperpolarized in the 0 Ca\(^{2+}\)-free saline; ADP was now observed (Fig. 2B). This reversal was routinely observed between -55 and -60 mV and the observed ADP increased with greater hyperpolarization (not shown).

<table>
<thead>
<tr>
<th>( I )</th>
<th>( g, \text{nS} )</th>
<th>( E, \text{mV} )</th>
<th>( m )</th>
<th>( P )</th>
<th>( h )</th>
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<tbody>
<tr>
<td>Na(^{+})</td>
<td>350</td>
<td>50</td>
<td>0.03(V + 28)</td>
<td>4</td>
<td>( 0.045 e^{(V+33-V_{m})} )</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>( \beta_{m} = 2.7 e^{-(V+18)} )</td>
<td></td>
<td>( 1 + e^{-(V+25-V_{m})} )</td>
</tr>
<tr>
<td>K(_{DR})</td>
<td>6</td>
<td>-68</td>
<td>0.024(V - 17)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \beta_{m} = 0.125 e^{(V-60-V_{m})} )</td>
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</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.01</td>
<td>120</td>
<td>1.5(V - 20)</td>
<td>1</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>( \beta_{m} = 1.5 e^{(V-25-V_{m})} )</td>
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<tr>
<td>ADP</td>
<td>0.025</td>
<td>50</td>
<td>0.024(V - 17)</td>
<td>1</td>
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<td>( \beta_{m} = 0.125 e^{(V-60-V_{m})} )</td>
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<tr>
<td>fAHP</td>
<td>30</td>
<td>-68</td>
<td>0.024(V - 17)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>mAHP</td>
<td>0.25</td>
<td>-68</td>
<td>0.024(V - 17)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Leak</td>
<td>0.5</td>
<td>-47</td>
<td>( \beta_{m} = 0.125 e^{(V-60-V_{m})} )</td>
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**Table 1. Parameters used to model the dynamic membrane properties of the S-cell**

(1 mV = 0.001 V, 1 \( \text{nS} = 10^{-9} \text{A/V} \), 1 mV m = 0.001 mV m, 1 \( \mu \)M = 10^{-6} M)
precise reason for this change is not known, one possible explanation is the surface potential effect (also called local potential effect) in which extracellular Ca\(^{2+}\) ions screen negative charges in close proximity to voltage-gated Na\(^+\) channels and that the absence of these screening charges causes Na\(^+\) channels to open at more hyperpolarized potentials (Campbell and Hille 1976; Frankenhaeuser and Hodgkin 1957).

Given that S-cell AHP was clearly Ca\(^{2+}\) dependent, a variety of selective Ca\(^{2+}\)-dependent K\(^+\) channel blockers were tested. Charybdotoxin (ChTX, 100 nM), a blocker of both large-conductance (BK) and intermediate-conductance (referred to as K\(_{C3.1}\) or SK4) Ca\(^{2+}\)-dependent K\(^+\) channels (Garcia et al. 1997; Ishi et al. 1997; Joiner et al. 1997), completely blocked the fAHP and significantly reduced the mAHP amplitude in the S-cell (Fig. 5, A–C). In every ChTX experiment an ADP was observed at the same time point that the fAHP had been observed prior to treatment with the channel blocker, consistent with the idea that the AHP obscures an underlying ADP. In addition to the effects on AHP, ChTX treatment also produced a significant broadening of the action potential (Fig. 5, B and D). The effects of ChTX on both the fAHP and action potential width suggest the involvement of a BK-like channel, which is known to contribute to fAHP and the repolarization phase of the action potential (Sah and McLachlan 1992; Shao et al. 1999; Storm 1987). However, iberiotoxin, a selective BK channel blocker, had no effect on S-cell AHP (Fig. 5E).

A second Ca\(^{2+}\)-sensitive K\(^+\) channel that has been observed to contribute to AHP are the SK channels (SK1–3), typically contributing to the mAHP. Apamin is the classic SK channel blocker that is typically effective at nanomolar concentrations, although this drug had no effect on S-cell AHP even when concentrations >1 μM were used (Fig. 6D). This is consistent with reports that apamin does not affect the Ca\(^{2+}\)-dependent AHP in other leech neurons (Merz 1995). Another SK channel blocker, scyllatoxin, also had no effect on S-cell AHP (500 nM; data not shown). However, relatively high concentrations of tubocurare have been shown to block SK channel currents (Park 1994; Stocker et al. 2004) and 500 μM tubocurare significantly reduced mAHP in the S-cell (Fig. 6, A–C). Tubocurare had little or no effect on the fAHP (Fig. 6, B and C), consistent with the blockade of an SK-like current. From this pharmacological evidence it is not clear whether S-cell mAHP is mediated by an SK-like channel that is simply insensitive to apamin and scyllatoxin or whether this represents a completely different I\(_{K,Ca}\).

**ADP is inhibited by saxitoxin**

The presence of an ADP at hyperpolarized membrane potentials (Fig. 2B) and at normal membrane potentials when the AHP was blocked (Figs. 3 and 4) suggests the presence of an inward current whose effect on the postspike membrane potential (or afterpotential) is masked by the AHP currents. Nevertheless, such a postspike inward current could modulate the size of the AHP and therefore influence excitability. For example, a decrease in the inward current that mediates the ADP would be expected to cause the AHP to increase. Na\(^+\) currents may contribute to the ADP, and thus S-cells were treated with saxitoxin (STX; 50 μM), which has been reported to block persistent Na\(^+\) currents in leech neurons (Johansen and Kleinhans 1987). At −50 mV, STX significantly increased the amplitude of both the fAHP and mAHP (Fig. 7, A, B, and E), consistent with what would be expected if the ADP were reduced. Given the effects of STX on AHP at nonhyperpolarized membrane potentials, STX would be expected to cause a decrease in the amplitude of the ADP observed at hyperpolarized membrane potentials. This, in fact, was observed and the size of the ADP at −60 mV was significantly reduced following application of STX (Fig. 7, C–E).

**Modeling the effects of 5-HT on AHP and excitability**

To test whether the observed reduction of AHP in the presence of 5-HT was sufficient to enhance excitability a...
A model was developed with Hodgkin–Huxley-type fast sodium and delayed rectifier potassium currents, two afterhyperpolarization currents (fAHP and mAHP), an afterdepolarization current, a leak current, and simple calcium dynamics. To examine the sensitivity of the model to changes in parameters, repetitive firing was examined while $g_{Na}$ (as defined in Table 1) was varied from 10 to 400% for each active conductance (Fig. 8). The model was most sensitive to perturbations of the voltage-gated sodium, the medium AHP, and the calcium conductances. Firing frequency of the modeled neuron in response to a 200-ms current injection decreased from 60 to 10 Hz when the fast sodium conductance was decreased by 50%. When the sodium conductance was increased by 400%, the cell became unstable and failed to fire impulses in response to stimulation. Decreases in the maximum conductance of the mAHP current or the calcium current resulted in an increase in the firing rate, whereas increases in either conductance produced a marked decrease in firing. The only exception to this trend occurred when the maximum calcium conductance was decreased by 90%, in which case the firing rate dropped dramatically. Surprisingly, decreases in the fAHP current actually reduced excitability with a 90% reduction in the fAHP conductance producing a 42% decrease in repetitive firing. Changes in the ADP conductance had a relatively small effect on the firing rate. Decreasing the maximum ADP conductance by 90% reduced repetitive firing by only 8%, whereas increasing the maximum conductance by as much as 400% increased the firing rate by only 16%. Linear regressions of model firing frequency as a function of conductance perturbation were statistically significant for the ADP conductance ($R^2 = 0.95$; $P < 0.01$), the fAHP conductance ($R^2 = 0.66$; $P < 0.05$), the KDR conductance ($R^2 = 0.80$; $P < 0.05$), and the mAHP conductance ($R^2 = 0.89$; $P < 0.01$), of which only the mAHP line had a negative slope.

FIG. 3. 5-HT inhibits S-cell AHP and increases S-cell excitability. A: AHP following a single action potential in normal saline (black trace) and following 5-HT treatment (gray trace). Scale bars are 20 mV and 20 ms. B: boxed region in A shown at expanded time- and voltage scales. Scale bars are 2 mV and 2 ms. Dashed line represents the resting potential prior to the S-cell spike. C: comparison of changes in AHP in a group of 5-HT–treated S-cells ($n = 7$) vs. a saline-treated control group ($n = 5$). 5-HT significantly reduced the peak amplitude of the fAHP and mAHP and increased the ADP compared with saline-treated cells (one-tailed $t$-test, $P < 0.05$). D: 5-HT increased the number of action potentials elicited by a 500-ms, 1-nA current pulse (traces). This increase in excitability was significantly different when compared with the saline control group (one-tailed $t$-test, $P < 0.05$). Scale bars are 10 mV, 1 nA, and 100 ms. E: the slope of the frequency–current ($F–I$) plot was greater in 5-HT–treated neurons ($n = 5$) compared with S-cells tested in normal saline ($n = 4$).

FIG. 4. S-cell AHP is Ca$^{2+}$ dependent. A: a comparison of the S-cell AHP in normal saline (black trace) and then following treatment in Ca$^{2+}$–free saline (gray trace). Note the appearance of an ADP in the Ca$^{2+}$–free trace (*). Elimination of the AHP due to treatment in Ca$^{2+}$–free saline was observed in a total of 3 different S-cells. Scale bars are 10 mV and 20 ms. B: same traces as in A, but at an expanded timescale. Dashed line represents the resting potential prior to the S-cell spike. Scale bars are 10 mV and 2.5 ms.
mAHP (2-tailed \(t\)-test, \(P < 0.05\)). ChTX actually reversed the fAHP measured at potential prior to the S-cell spike. Scale bars are 10 mV and 2 ms. (open bars) and in iberiotoxin (filled bars, treated with 5-HT (Fig. 3D)). Note that the same amplitude comparable to the increase observed when real S-cells are to a 200-ms current injection by 25\% (Fig. 9B). These same perturbations of the AHP currents also increased the number of impulses the model fired in response to a 200-ms current injection by 25\% (Fig. 9A), which is comparable to the increase observed when real S-cells are treated with 5-HT (Fig. 3D). Note that the same amplitude current was used to evoke a spike train in both the top and bottom traces of Fig. 9B. The increase in firing frequency observed in the “5-HT” model relative to the “saline” model was partially dependent on stimulus amplitude. For example, when the stimulus current amplitude was decreased by just 7\%, the effect of imposing the 5-HT conditions was a 50\% increase in repetitive firing. In contrast, as the stimulus amplitude was increased, the model cell approached its maximum firing rate and the effect of the 5-HT conditions decreased. For example, when stimulus amplitude was increased by 50\%, only a 14\% increase in repetitive firing was observed under the 5-HT conditions (relative to saline). This observation from the model suggests that the effect of decreases in mAHP amplitude on repetitive firing is frequency dependent, such that decreases in mAHP amplitude would enhance the response of the S-cell to low-intensity stimuli with little impact on the response to high-intensity stimuli (such as a sensitizing stimulus). A better understanding of what role (if any) the 5-HT–induced decrease in impulse threshold (Burrell et al. 2001; Crisp and Muller 2006) plays in the enhancement of repetitive firing will be necessary before it can be determined whether the frequency-dependence prediction can be tested in the physiological S-cell.

**Tubocurare occludes 5-HT’s effects on mAHP and excitability**

To further examine whether the excitatory effects of 5-HT are mediated by a modulation of AHP, S-cells were treated with tubocurare prior to application of 5-HT. Tubocurare was chosen for these occlusion experiments because it selectively inhibits the mAHP component (Fig. 6) and the S-cell model demonstrated that repetitive firing was most sensitive to changes in the mAHP (Fig. 8); therefore one would expect tubocurare to be the most effective at blocking the 5-HT–mediated increases in excitability. Tubocurare blocked both the 5-HT–mediated decrease of the mAHP and the increase in excitability normally observed following 5-HT treatment (Fig. 10). The occlusion of 5-HT’s effect on the mAHP by tubocurare was not complete and there was still a small (<0.5-mV) decrease in the mAHP following 5-HT treatment (Fig. 10B). This residual decrease in the mAHP may reflect a reduction of the later component of the ChTX-sensitive portion of the AHP (Fig. 5) or unmasking of the ADP. Tubocurare did not block the 5-HT–mediated decrease in the fAHP, given that there were no differences in the level of fAHP change between the 5-HT– and curare + 5-HT–treated groups (Fig. 10, A and B). These results demonstrate that 5-HT–mediated increases in excitability were due largely to decreases in the level of the mAHP. In addition,
these experimental results confirm findings from the model that decreases in the level of the mAHP can profoundly change excitability.

**DISCUSSION**

Modulation of AHP plays a critical role in altering the pattern of repetitive firing in a neuron and therefore the output of that neuron. Decreased AHP enhances the number or frequency of action potentials elicited by a stimulus as well as the temporal precision of spike firing by a neuron, increasing the reliability of signal transmission within a circuit (Sourdet et al. 2003; Stocker et al. 2004). Such changes make an important contribution to processing and storage of information (e.g., learning and memory) and modulation of AHP has been observed during learning in a number of different vertebrate (Brosh et al. 2006; Hammond et al. 2006; Moyer et al. 1996, 2000; Oh et al. 2003; Saar et al. 1998; Shreurs et al. 1997, 1998; Stackman et al. 2002; Thompson et al. 1996) and invertebrate learning preparations (Alkon et al. 1985; Antonov et al. 2001; Burrell and Sahley 2005; Burrell et al. 2001; Cleary et al. 1998; Gainutdinov et al. 1998; Straub and Benjamin 2001). Furthermore, dysfunctions in AHP have been implicated in learning and memory deficits that occur as a result of aging or aging-related diseases such as Alzheimer’s disease (Disterhoft and Oh 2006). Understanding the processes that mediate modulation of AHP, the resultant changes in excitability/activity, and the contributions that such changes have on neuronal circuits can play an important part in understanding the physiological basis of learning and memory and, potentially, how to treat deficits in cognitive function.

In the leech, sensitization-type learning of the whole body shortening reflex is accompanied by enhanced excitability in the S-cell, which is thought to play an important role during learning in this behavior (Burrell et al. 2003; Modney et al. 1997; Sahley et al. 1994). This increase in excitability is mediated, at least in part, by 5-HT and drugs that block 5-HT modulation of the S-cell also prevent sensitization-induced increases in S-cell excitability and increases in the shortening response (Burrell and Sahley 2005; Burrell et al. 2001). 5-HT–mediated increases in S-cell excitability are due to the activation of 5-HT receptors that stimulate a cyclic AMP/protein kinase A second-messenger system (Burrell and Sahley 2005) and pharmacological experiments have indicated the involvement of a 5-HTγ–like receptor (Crisp and Muller 2006). Increased S-cell excitability is manifested in two ways: 1) by an increase in the number of spikes evoked by a fixed stimulus and 2) by a decrease in the amount of injected current needed to elicit one action potential (Burrell and Sahley 2005; Burrell et al. 2001; Cleary et al. 1998; Gainutdinov et al. 1998; Straub and Benjamin 2001). Furthermore, dysfunctions in AHP have been implicated in learning and memory deficits that occur as a result of aging or aging-related diseases such as Alzheimer’s disease (Disterhoft and Oh 2006). Understanding the processes that mediate modulation of AHP, the resultant changes in excitability/activity, and the contributions that such changes have on neuronal circuits can play an important part in understanding the physiological basis of learning and memory and, potentially, how to treat deficits in cognitive function.

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**FIG. 6. Tubocurare inhibits S-cell mAHP. A: a comparison of AHP in normal saline (black trace) and then AHP in the same neuron following treatment with tubocurare (gray trace). Scale bars are 20 mV and 25 ms. B: boxed region in A shown at expanded time- and voltage scales. Scale bars are 2 mV and 10 ms. C: average size of the AHP in normal saline (open bars) and in tubocurare (filled bars; labeled “curare”). Tubocurare significantly reduced the peak amplitude of the mAHP (2-tailed t-test, \( P < 0.05; n = 4 \)), but did not affect the fAHP. D: average size of the AHP in normal saline (open bars) and in apamin (filled bars). Apamin had no significant effect on either fAHP or mAHP.**
thought to contribute to the decrease in the amount of current necessary to initiate activity (Burrell and Sahley 2005). A reduction in the S-cell AHP—the focus of this paper—would be expected to contribute to increases in repetitive firing.

The S-cell AHP has both fast and medium components, but no slow AHP is observed even when the S-cell is fired at near its maximum rate. Both the fast and medium AHP components are Ca\(^{2+}\) dependent, given that no AHP was observed when action potentials were elicited in Ca\(^{2+}\)-free saline. ChTX, a blocker of BK Ca\(^{2+}\)-dependent K\(^{+}\) channels, completely inhibited the fAHP and produced significant broadening of the action potential. These results are consistent with the blockade of a BK-like conductance, which is known to contribute to both the repolarization phase of an action potential and to the fAHP (Sah and McLachlan 1992; Shao et al. 1999; Storm 1987). However, iberiotoxin, a selective BK channel blocker, had no effect on fAHP. It is possible that leech neurons possess BK channels, but that these channels lack the iberiotoxin binding site. BK channels in the cockroach are blocked by ChTX and iberiotoxin (Derst et al. 2003), but Drosophila BK channels are not blocked by either of these toxins (Meera et al. 1997; Toro et al. 1998).

ChTX significantly reduced the mAHP as well and this result is consistent with data from cockroaches and honey bees where ChTX-sensitive currents exhibited a fast-decaying component that would correspond with the fAHP and a slower-decaying component that would correspond with the mAHP (Derst et al. 2003; Perk and Mercer 2006). BK channels have also been observed to contribute mAHP in vertebrate neurons (Storm 1989; Williamson and Alger 1990). Alternatively, the ChTX-mediated decrease in mAHP could indicate the presence of an additional Ca\(^{2+}\)-dependent K\(^{+}\) conductance contributing to the mAHP. Intermediate Ca\(^{2+}\)-dependent K\(^{+}\) channels (referred to as K\(_{Ca}\)-3.1 or SK4) are also blocked by ChTX, but are insensitive to iberiotoxin and apamin (Ishii et al. 1997; Joiner et al. 1997). However, K\(_{Ca}\)-3.1/SK4 currents have been observed only in blood cells and not in neurons, although antibody staining has detected K\(_{Ca}\)-3.1/SK4 channels in the peripheral nervous system (Boettger et al. 2002). The mAHP (but not the fAHP) was also inhibited by tubocurare, which can block SK1-3 channels (Park 1994; Stocker et al. 2004). However, apamin and scyllatoxin, which are both selective SK channel blockers, had no effect on any S-cell AHP component. It is not clear whether S-cell mAHP is mediated by an SK-like channel that is simply insensitive to

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**FIG. 7.** Saxitoxin reduces the ADP resulting in an increase in the AHP. 

**A:** a comparison of AHP recorded at \(-50\) mV in normal saline (black trace) and then AHP in the same neuron in saxitoxin (gray trace). Scale bars are 10 mV and 20 ms. 

**B:** boxed region in A shown at expanded time- and voltage scales. Dashed line represents the resting potential prior to the S-cell spike. Scale bars are 10 mV and 2 ms.

**C:** a comparison of ADP recorded at \(-60\) mV in normal saline (black trace) and then AHP in the same neuron following treatment with saxitoxin (gray trace). Scale bars are 10 mV and 20 ms.

**D:** boxed region in A shown at expanded time- and voltage scales. Dashed line represents the resting potential prior to the S-cell spike. Scale bars are 2 mV and 2 ms.

**E:** saxitoxin significantly increases the fAHP and mAHP (2-tailed \(t\)-test, \(P < 0.05; n = 4\)) and significantly decreases the ADP (2-tailed \(t\)-test, \(P < 0.05\)).
apamin and scyllatoxin or whether this represents a completely different Ca$^{2+}$-gated K$^+$ current.

When the soma membrane potential is hyperpolarized to −60 mV or lower, an ADP is reliably observed. The AHP appears to “reverse” somewhere between −55 and −60 mV, which is too low to be caused by a reversal of a Cl$^-$/H$^+$ current (Blomeley and Bracci 2005; Colino and Halliwell 1987; Grunnet et al. 2004; Lorenzon and Foehring 1992; Wallen et al. 1989; Zhong et al. 2006). 5-HT also increased the ADP observed at hyperpolarized membrane potentials and, as the STX data demonstrate, inhibition of the ADP-mediating currents can increase the size of both the fAHP and mAHP. Therefore 5-HT may be reducing S-cell AHP by inhibiting the Ca$^{2+}$-dependent K$^+$ currents that mediate the AHP, although a contribution by the ADP current(s) cannot be ruled out. Resolving which mechanisms are responsible for 5-HT modulation of S-cell AHP will require voltage-clamp recordings of these specific currents. That 5-HT modulation of excitability is this complex is not surprising; studies of 5-HT–mediated increases in excitability in Aplysia revealed modulation of three different currents (Jacklet et al. 2006).

Experiments with the S-cell model support the hypothesis that a reduction in AHP could mediate 5-HT–induced potentiation of excitability. Decreases in the maximum conductances of AHP currents to produce a change in the AHP that resembles the effects of 5-HT on the S-cell is sufficient to produce an increase in repetitive firing comparable to the physiological effects of 5-HT as previously described (Burrell et al. 2001; Crisp and Muller 2006). The model also allowed for modification of individual currents, permitting us to observe how they might contribute to repetitive firing of the S-cell. For example, changes in the mAHP current alone produced significant changes in the firing rate of the modeled S-cell. However, changes in the ADP current had relatively modest effects on repetitive firing. This does not preclude the persistent Na$^+$ current thought to mediate ADP from contributing to changes in excitability; it simply suggests that the contribution is relatively small compared with that of mAHP currents. It is also possible that the

![FIG. 8. Sensitivity analysis showing changes in repetitive firing of the model S-cell as the maximum conductance ($g_{max}$) of each current was modified from 10 to 400%. Firing frequency during a 200-ms current injection is shown as a function of conductance perturbation (percentage maximum conductance as listed in Table 1). The model showed greatest sensitivity to perturbations of the fast sodium conductance ($Na^{+}$), medium AHP conductance (mAHP), and voltage-gated calcium conductance (Ca$^{2+}$). Perturbations in the ADP conductance had little effect on repetitive firing; perturbations in the mAHP conductance produced a dramatic change in repetitive firing, and perturbations in the fAHP conductance had an intermediate effect. Interestingly, the mAHP and fAHP conductances had opposite effects on repetitive firing; decreases in the mAHP conductance increased excitability, whereas decreases in the fAHP conductance decreased excitability. Note that the x-axis is plotted on a logarithmic scale.](http://jn.physiology.org/lookup/tif/55/V99/F2/fig8)

![FIG. 9. Computational model of 5-HT–induced changes in excitability in the S-cell. Voltage records were taken from a single compartment soma with Hodgkin–Huxley style sodium, potassium, and leak currents; two calcium-activated potassium currents (mAHP and fAHP); an afterdepolarization sodium current; and a calcium current. A: AHP following a single action potential in normal saline (black trace) and under “5-HT” conditions, in which the mAHP and fAHP maximum conductances were reduced by 50 and 66%, respectively. Scale bars are 10 mV and 10 ms. Resting membrane potential (dotted line) is −43 mV. B: the same “5-HT” treatment condition described in A also increased the number of impulses the model fired in response to a 200-ms current pulse of a fixed amplitude (traces). Scale bars are 20 mV and 25 ms.](http://jn.physiology.org/lookup/tif/55/V99/F2/fig9)
ADP contributes to other aspects of repetitive firing, such as reducing the variability in the timing of spikes within a train of action potentials (improving the regularity of firing; Vervaeke et al. 2006). One surprising finding from the model is that decreases in the fAHP current decreased excitability in the modeled S-cell. However, this is consistent with recent studies showing that BK channel activation, the channel thought to contribute to fAHP, actually enhances repetitive firing in hippocampal CA1 pyramidal neurons (Gu et al. 2007). The BK current accomplishes this by minimizing both Na\(^{+}\)/H\(^{+}\)-channel inactivation and voltage-gated K\(^{+}\)/H\(^{+}\)-channel activation (e.g., delayed rectifiers).

Both the 5-HT–mediated decrease in mAHP and increase in excitability were blocked by pretreatment of the S-cell with tubocurare. Two important conclusions can be drawn from this occlusion experiment: first, that 5-HT reduces mAHP by acting on the same ion channels that are sensitive to tubocurare, potentially an SK-like Ca\(^{2+}\)-dependent K\(^{+}\) channel, and, second, that decreases in the mAHP are necessary for the 5-HT–mediated increase in S-cell excitability. That 5-HT–mediated decrease in mAHP was not completely occluded by tubocurare is not surprising, given that both the ChTX-sensitive components of the AHP and the STX-sensitive ADP contributed to the afterpotential during the period that corresponds with the mAHP and both of these components appeared to be modulated by 5-HT. It is also not surprising that occlusion of the 5-HT–mediated decrease in mAHP also prevented the increased excitability normally produced by 5-HT treatment. Data from the model show that S-cell excitability is very sensitive to changes in the mAHP, indicating that the channels that mediate the mAHP are playing a critically important role in regulating excitability.

These findings, however, beg the following question. If the decrease in mAHP is sufficient to account for 5-HT’s effect on excitability, what is the role of the 5-HT–mediated decrease in the fAHP and increase in the ADP? In the case of the ADP, it is possible that modulation of this component does not affect excitability per se, but instead acts to reduce the variability in the timing of spikes within a train of action potentials (improving the regularity of firing), an effect ascribed to the persistent Na\(^{+}\)/H\(^{+}\) currents (Vervaeke et al. 2006) that are thought to contribute to S-cell ADP. In the case of the fAHP, results from the model indicate that S-cell excitability is very sensitive to changes in the mAHP, indicating that the channels that mediate the mAHP are playing a critically important role in regulating excitability.

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such increases in excitability to within specific physiological limits.

Properties of the afterpotential play a critical role in regulating the firing pattern of a neuron and represent a potentially important form of neuroplasticity. Most studies focus on either the AHP or ADP; however, as this and other studies show (Magee and Carruth 1998; Young et al. 2003), the afterpotential can be the net sum of both inward and outward currents. The simultaneous presence of both AHP-producing and ADP-producing currents may provide greater flexibility in controlling the excitability of the neuron with multiple modulators (e.g., 5-HT, dopamine, neuropeptides, etc.), potentially acting on one or more of the ion channels that mediate the AHP/ADP (e.g., Ca\(^{2+}\)-dependent K\(^+\) channels, persistent Na\(^+\) channels, etc.). Another advantage of both AHP and ADP currents contributing to the afterpotential is that the combination may increase the dynamic range of the afterpotential, similar to a synapse that can be facilitated or depressed. Given these properties, modulation of AHP/ADP may play an important role in the learning-related neuroplasticity.

**Acknowledgments**

We thank Dr. Brenda Moss for helpful comments while this manuscript was being prepared and A. Keay (St. Olaf College ‘07) for assistance during the initial development of the S-cell model.

**Grants**

This work was supported by National Science Foundation Grant IBN-0432683 to B. D. Burrell; the Council for Spinal Cord Injury/Traumatic Brain Injury; a Center of Biomedical Research Excellence/Division of Research Resources of the National Institutes of Health Grant P20 RR-015567 to B. D. Burrell; and a Behrent Family Endowment for undergraduate research (St. Olaf College). Resources of the National Institutes of Health Grant P20 RR-015567 to B. D. Burrell; and a Behrent Family Endowment for undergraduate research (St. Olaf College).

**References**


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