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Small voltage changes at nerve terminals travel up axons to affect action potential initiation

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Abstract

Nerve terminals are generally considered the destination points for electrical signals, which propagate unidirectionally from the soma to nerve terminals. Here, we demonstrate that small hyperpolarizations or depolarizations (~10 mV), generated under physiological conditions in rat nerve terminals, backpropagate up the axon (~400 – 800 μm), and change the threshold for initiating action potentials and thus firing patterns. These results suggest a novel mechanism for information processing in neurons and neuronal circuits.

An action potential (AP) was recorded at the calyx of Held after applying a voltage pulse (~2 – 25 V, 0.1 ms) via a bipolar electrode positioned on the axon, ~400 – 800 μm away from the calyx (Fig. 1a, Supplementary Information I). As the stimulation intensity increased, the probability of generating an AP ($Prob_{AP}$) increased from 0 to 1. We identified the minimal stimulus threshold (V_T) that yielded a $Prob_{AP}$ of 1 (Fig. 1a), and set the stimulus intensity at ~3 – 10% (~0.5 – 1.0 V) above threshold, unless otherwise specified. A calyx is connected to a single axon[1], and the AP was generated near the stimulation electrode (Supplementary Information II).

A current injection which hyperpolarized the calyx by 11.2 ± 1.0 mV decreased $Prob_{AP}$ (Fig. 1b, left, stimulus ii) from 1.0 ± 0.0 to 0.02 ± 0.02 (n = 6, $p<0.01$). Increasing the stimulus ~10 – 20% above threshold restored AP firing during the hyperpolarization, ($Prob_{AP}$ = 1.0 ± 0.0, n = 4, $p < 0.01$, Fig. 1b, right), indicating that AP initiation failure, not propagation failure, caused the $Prob_{AP}$ reduction. This indicates that a hyperpolarization at the calyx can travel along the axon for ~400 – 800 μm to increase the AP initiation threshold.

To determine if a $Prob_{AP}$ reduction occurs with physiological hyperpolarizations, we used 100 APs at 100 Hz to induce an afterhyperpolarization[2] of 8.9 ± 0.8 mV (n = 18, Fig. 1c). Minimum $Prob_{AP}$ occurred at the afterhyperpolarization peak ($Prob_{AP}$ = 0.5 to 0, mean = 0.11 ± 0.05, n = 14), and recovered approximately parallel with recovery of the afterhyperpolarization (Fig. 1d). The $Prob_{AP}$ reduction was relieved using stimulus

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intensities 10 – 20% above threshold (n = 14, not shown), confirming that the $Prob_{AP}$ reduction was caused by AP initiation failure.

Three sets of evidence suggest that the afterhyperpolarization generated at the calyx reduces $Prob_{AP}$. First, when the afterhyperpolarization was offset by a depolarizing current at the calyx (Fig. 1e), the reduced $Prob_{AP}$ (0.02 ± 0.02, n = 5) was largely relieved ($Prob_{AP}$ = 0.89 ± 0.07, p<0.01). Second, the afterhyperpolarization is produced by a Na^+/K^+ ATPase localized to the calyx[2]. Blocking the afterhyperpolarization by removing ATP from the pipette solution (afterhyperpolarization = 2.1 ± 0.6 mV, n = 4) or by bath application of 100 μM ouabain (afterhyperpolarization = 2.8 ± 0.6 mV, n = 3) significantly increased the $Prob_{AP}$ (Control: 0.11 ± 0.05, n = 14; No ATP: 1.0 ± 0.0, n = 4; ouabain: 0.95 ± 0.01, n = 3, Fig. 1f). Third, the afterhyperpolarizations produced by AP trains, or matching hyperpolarizations generated by current injection at the same calyces, inhibited AP generation to similar extents (Fig. 1g,h, n = 3, p > 0.2). These results suggest that an afterhyperpolarization produced at the calyx is sufficient to cause the observed $Prob_{AP}$ reduction.

The afterhyperpolarization also occurs after trains at 10 – 20 Hz (20 – 100 APs, Fig. 1i), which can also cause AP failure (not shown). The afterhyperpolarization amplitude increased as the AP frequency and number increased (Fig. 1i). Thus, over a wide range of frequencies and train durations, an afterhyperpolarization occurs at the calyx and travels up the axon to inhibit AP initiation.

To determine whether depolarizations facilitate AP initiation, we adjusted the axon stimulus voltage to 3 – 10% below threshold (Fig. 2a). This subthreshold stimulus only produced an AP when it coincided with a 10 ms current injection that depolarized the calyx by ~10 – 15 mV ($Prob_{AP}$ = 1, n = 5, Fig. 2a). The 10 ms depolarization alone could not produce an AP ($Prob_{AP}$ = 0, n = 5, Fig. 2a). Thus, calyx depolarizations increase $Prob_{AP}$.

We next determined if the afterdepolarization that follows each AP[3] also increased $Prob_{AP}$. The afterdepolarization peaked ~10 – 20 mV above baseline within 5 – 10 ms after an AP and returned to baseline in ~70 – 100 ms (Fig. 2b, left). When a subthreshold stimulus was applied after an AP, the $Prob_{AP}$ was highest at the afterdepolarization peak, and decreased as the afterdepolarization decreased (Fig. 2b,c; n = 5). These results were from 7 – 10 day old rats (p7 – 10). Results similar to those shown in Figs. 2b–c and 1c–d, were also obtained with p13 – 15 rats (n = 4, not shown).

Three sets of evidence suggest that the afterdepolarization is generated at or near the calyx and travels up the axon to facilitate AP generation. First, an AP afterdepolarization, and matching depolarizations from current injections at the same calyces, produced similar facilitation (Fig. 2b,c; n = 5, p > 0.5). Second, local perfusion of riluzole (Supplementary Information I) onto the calyx reduced the afterdepolarization by 46.9 ± 5.6% (n = 4), and reduced $Prob_{AP}$ from 0.98 ± 0.02 (n = 4) before application, to 0.11 ± 0.04 (n = 4, Fig. 2d). This reduced $Prob_{AP}$ was largely relieved by a depolarizing current injection (Fig. 2d) which restored the afterdepolarization ($Prob_{AP}$ = 0.78 ± 0.02, n = 3). The unblocked portion of the afterdepolarization is unlikely to be due to unblocked current in the axon since riluzole bath

application at the same concentration (120 μM) inhibited the afterdepolarization to a similar extent (49.6 ± 4.0%, n = 3). Third, a delay of 1 – 3 ms occurred between the calyx depolarization and facilitation of axonal APs (Supplementary Information II, Fig. S4), suggesting that calyx depolarization travels up the axon. We conclude that afterdepolarizations generated at the calyx are sufficient to cause the observed axonal AP facilitation.

Synaptic plasticity could be generated when an afterdepolarization converts a subthreshold stimulus to a suprathreshold stimulus. For example, when a 10 ms, ~10 – 15 mV, depolarization in the calyx coincided with a subthreshold stimulus, during a 50 – 100 Hz subthreshold stimulation train, it initiated an AP, and subsequent subthreshold stimuli became suprathreshold due to the afterdepolarization generated by each AP (Fig. 2e). The afterdepolarization amplitude can decrease during prolonged stimulation, resulting in burst termination, followed by an afterhyperpolarization (Fig. 2e). Thus, a 10 ms depolarization at the calyx can convert a high frequency train of subthreshold stimuli to a train of APs. Similarly, during a subthreshold stimulation train, if a single stimulus was increased to suprathreshold level, not only this stimulus, but also subsequent stimuli become suprathreshold at high (≥ 50 Hz), but not at low (≤ 20 Hz) frequency stimulation (Fig. S5, Supplementary Information IV).

Next, we studied the combined impact of afterdepolarizations and afterhyperpolarizations on lower frequency trains. During prolonged 20 Hz stimulation, periods of AP failure interrupted firing (Fig. 2f, n = 9). The duration and frequency of failure periods increased as the stimulation voltage approached threshold (Fig. 2f). The membrane potential before each stimulus was depolarized early in the train, but gradually became hyperpolarized, allowing AP failure (Fig. 2g), suggesting that afterhyperpolarization develops *during* stimulation and overcomes the afterdepolarization. Upon AP failure, the afterdepolarization was removed and the hyperpolarization accelerated, thus prolonging the failure period (Fig. 2g). AP initiation recovered when the afterhyperpolarization returned near baseline (Fig. 2g). The AP failure ($Prob_{AP}$ = 0.64 ± 0.07) was relieved ($Prob_{AP}$ = 0.98 ± 0.02, n = 4) by a depolarizing current injection at the calyx that offset the hyperpolarization during the 20 Hz train (Fig. 2h). Thus, the afterhyperpolarization controls the AP failure duration. We conclude that during lower frequency stimulation, the firing pattern depends on the stimulation voltage, and the interaction between afterdepolarization and afterhyperpolarization.

We showed that afterhyperpolarizations and afterdepolarizations at calyces travel ~400 – 800 μm along the axon to influence AP generation (see also Supplementary Information III). These depolarizations and hyperpolarizations act alone or together. Their ultimate effect depends on stimulus frequency, duration and voltage. For the calyx of Held, the ~2 – 3 mm distance to the soma likely limits these effects. However, many CNS axons form boutons within 500 μm from the soma[4–7]. Small hyperpolarizations and depolarizations induced by action potentials[8,9] or neurotransmitters and neuromodulators[10,11] at conventional nerve terminals may thus reach the axon hillock and influence AP initiation. This possibility is supported by studies demonstrating that subthreshold membrane potential changes in the

soma propagate to conventional nerve terminals[4,5], and vise versa[12], with a length constant of ~200 – 600 μm.

In many studies, axonal APs have been initiated by a stimulating electrode. Inevitably, some axons will be closer to threshold, and a hyperpolarization and/or depolarization at terminals could greatly influence AP initiation in these axons. Caution is therefore necessary for the general assumption that the firing pattern is identical to the stimulation pattern. This could be critical when a single axon is activated with minimal stimulation (e.g., ref. 13), just above firing threshold, where an afterhyperpolarization could cause short-term depression by preventing AP initiation, and an afterdepolarizations could cause short-term facilitation by facilitating AP initiation.

The information a neuron carries is encoded by the frequency, duration and spike timing of AP firing, which determines the pattern of transmitter release and the forms of synaptic plasticity that may be generated[14,15]. When a neuron receives repeated excitatory inputs that are near the threshold for firing, the propagation of a hyperpolarization or depolarization from the nerve terminal to the site of AP initiation could be critical in determining the firing pattern. For example, a hyperpolarization induced by a high frequency AP train may prevent AP initiation by subsequent excitatory input (Fig. 1c), whereas an afterdepolarization may convert a series of high frequency subthreshold excitatory inputs to suprathreshold ones (Fig. 2e). The interaction between afterdepolarization and afterhyperpolarization may determine the firing pattern induced by lower frequency inputs (Fig. 2f–h). In summary, propagation of analog signals from nerve terminals to the AP initiation site may influence neuronal firing patterns.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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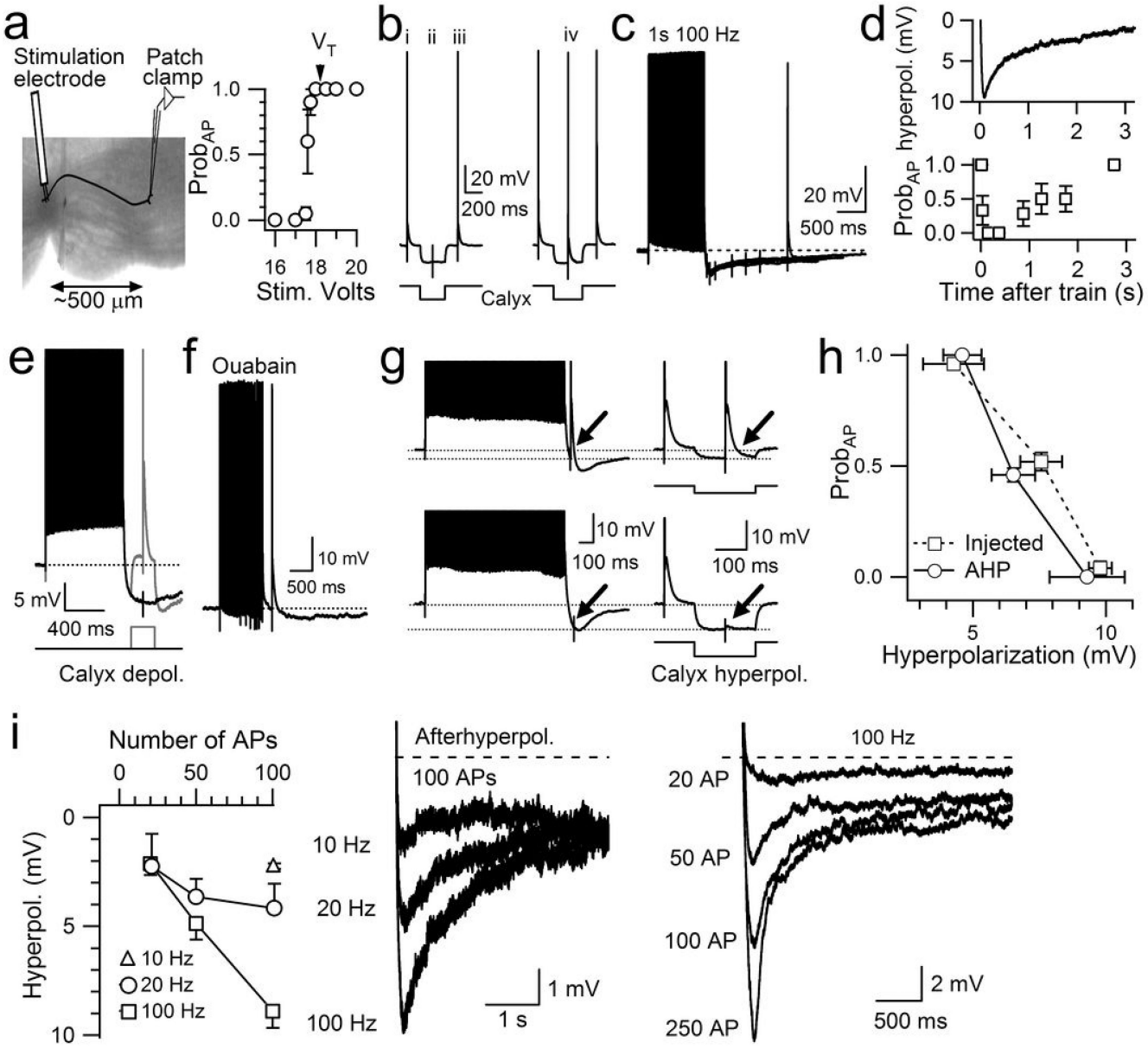

Figure 1. Afterhyperpolarization at the calyx reduces the $Prob_{AP}$ at the axon

a. Left: experimental setup. Right: $Prob_{AP}$ versus stimulation voltage from a calyx. V_T (voltage threshold).

b. Left: Stimulation ~3 – 10% above threshold generated APs in control (i, iii), but not during calyx hyperpolarization (ii).

Right: increasing stimulation voltage by ~15% restored firing during calyx hyperpolarization (iv).

c. During afterhyperpolarization induced by 100 APs at 100 Hz (applies to c–h), most stimuli failed to generate an AP (6 traces superimposed).

d. $Prob_{AP}$ closely paralleled afterhyperpolarization (calyx from panel c).

e. AP generation was inhibited during afterhyperpolarization (black), but restored by calyx depolarizing current injection (grey).

f. Ouabain (100 μM) reduced the afterhyperpolarization, and restored AP generation.

g. AP responses to stimuli (arrows) applied during small (upper) or large (lower) hyperpolarization induced by AP train (left) or calyx current injection (right).

h. $Prob_{AP}$ at various hyperpolarization voltages produced by AP trains (AHP) or calyx current injection (n = 3).

i. Left: afterhyperpolarization amplitude following 20 – 100 APs at 10 – 100 Hz (n = 3 – 11).

Middle: typical afterhyperpolarization following 100 APs at 20 – 100 Hz.

Right: afterhyperpolarization following 20 – 250 APs at 100 Hz.

Data are shown as mean ± s.e.m.

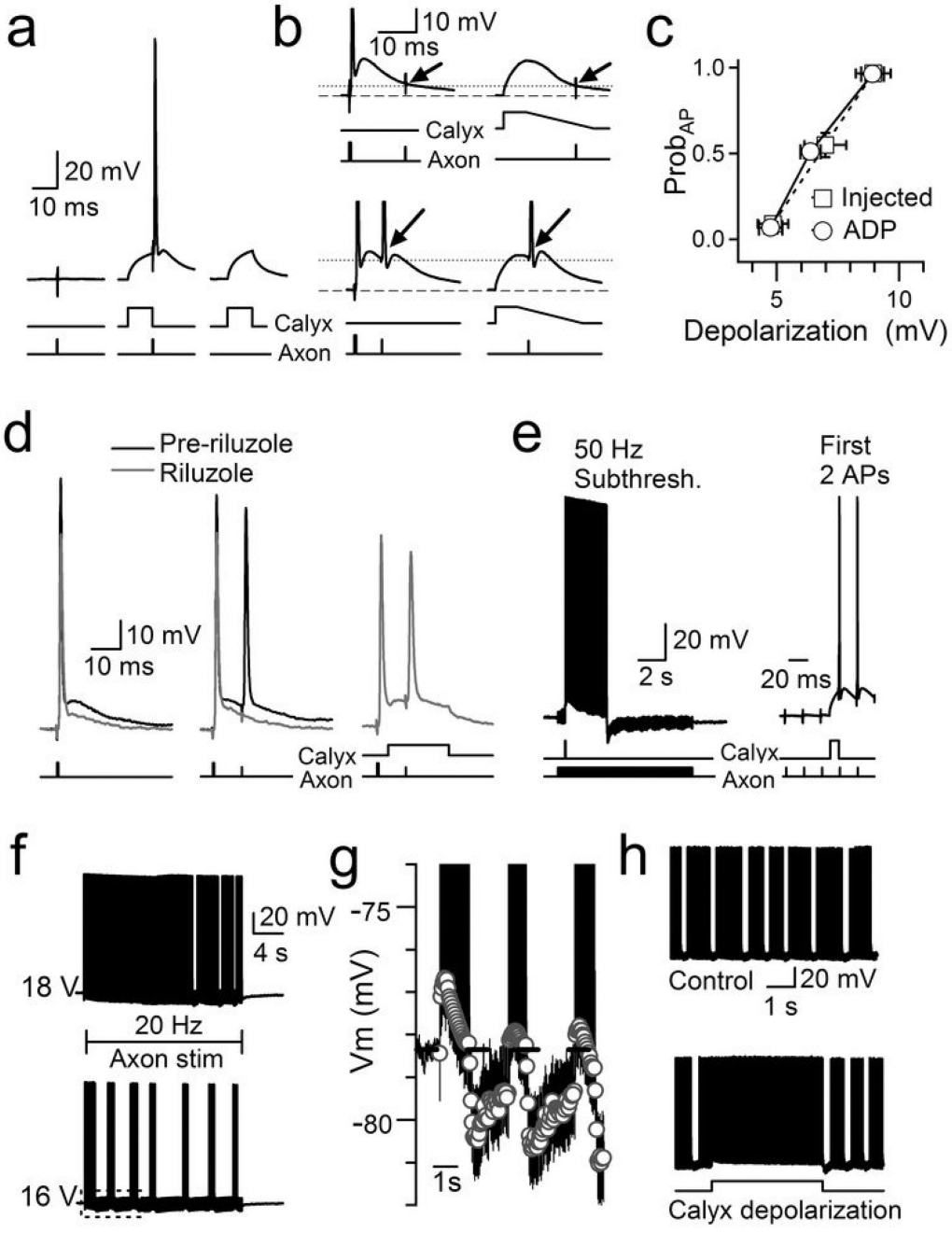

Figure 2. Afterdepolarizations at calyx increase axonal Prob$_{AP}$

a. AP response to subthreshold axonal stimulation alone (left), calyx depolarizing current injection alone (right) or both, combined simultaneously (middle).

b. AP response to subthreshold axonal stimulation (arrows) during small (upper) or large (lower) depolarization induced by an AP (left) or calyx current injection (right).

c. $Prob_{AP}$ at various depolarization voltages produced by an AP afterdepolarization (ADP) or calyx current injection (n = 5).

d. Left: afterdepolarization before and after local riluzole (120 μM) application.

Middle: AP response to subthreshold axonal stimulation during afterdepolarization before and after local riluzole application.

Right: calyx depolarization after riluzole treatment restored AP firing during afterdepolarization.

e. Left: a brief calyx depolarization (~12 mV, 10 ms) during 50 Hz subthreshold axonal stimulation generates an AP burst

Right: trace expanded to show AP response before and after calyx depolarization.

f. AP response to 20 Hz axonal stimulation. Stimulation voltage (18 or 16 V) was ~15% (top) or ~2% (bottom) above threshold.

g. Panel f, boxed segment (lower trace) enlarged. Circles indicate membrane potential (Vm) before each stimulus.

h. AP failures during 20 Hz stimulation (top) were prevented by calyx depolarizing current injection (bottom).