Published in final edited form as:
Eur J Neurosci. 2011 December ; 34(11): 1756–1765. doi:10.1111/j.1460-9568.2011.07899.x.

Bidirectional pattern-specific plasticity of the slow AHP: role for HVA Ca^{2+} channels and I_h

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Abstract

A burst of action potentials in hippocampal neurons is followed by a slow afterhyperpolarization (sAHP) that serves to limit subsequent firing. A reduction in the sAHP accompanies acquisition of several types of learning, whereas increases in the sAHP are correlated with cognitive impairment. The present study demonstrates *in vitro* that activity-dependent bidirectional plasticity of the sAHP does not require synaptic activation, and depends on the pattern of action potential firing. Whole-cell current-clamp recordings from CA1 pyramidal neurons in hippocampal slices from young rats (p14–24) were performed in blockers of synaptic transmission. The sAHP was evoked by action potential firing at gamma-related (50 Hz, gamma-AHP) or theta frequencies (5 Hz, theta-AHP), two firing frequencies implicated in attention and memory. Interestingly, when the gamma-AHP and theta-AHP were evoked in the same cell, a gradual potentiation of the gamma-AHP (191±32%) was observed that was blocked using Ca^{2+} channel blockers nimodipine (10 μM) or ω-conotoxin MVIIC (1 μM). In experiments that exclusively evoked the sAHP with 50 Hz firing, the gamma-AHP was similarly potentiated (198±44%). However, theta-burst firing pattern alone resulted in a decrease (65±1%) of the sAHP. In these experiments, application of the h-channel blocker ZD7288 (25 μM) selectively prevented enhancement of the gamma-AHP. These data demonstrate that induction requirements for bidirectional AHP plasticity depend on the pattern of action potential firing, and result from distinct mechanisms. The identification of novel mechanisms underlying AHP plasticity *in vitro* provides additional insight into the dynamic processes that may regulate neuronal excitability during learning *in vivo*.

Keywords

intrinsic excitability; intrinsic plasticity; afterhyperpolarization; theta-burst; gamma-related firing; h-channels; L-type Ca^{2+} channels; N-type Ca^{2+} channels; P/Q-type Ca^{2+} channels

INTRODUCTION

Action potentials (APs) in hippocampal neurons are followed by a post-burst afterhyperpolarization (AHP) that is dependent upon several outward Ca^{2+}-dependent K^+ currents, most notably the I_{AHP} and sI_{AHP} (Storm, 1990; Sah, 1996), but see Gu and colleagues (2005). The AHP serves to limit firing in response to sustained excitation

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Disclosure Statement: There are no real or perceived conflicts of interest.

(Madison & Nicoll, 1984; Lancaster & Nicoll, 1987; Schwindt *et al.*, 1988), and is highly sensitive to changes in intracellular Ca^{2+} (Storm, 1987; Lancaster & Zucker, 1994; Velumian and Carlen, 1999). Modulation of the AHP by alterations in intracellular Ca^{2+} can be achieved through several pathways, including Ca^{2+} entry through L-type (Rascol *et al.*, 1991; Moyer *et al.*, 1992; Gamelli *et al.*, 2009), N-type (Shah & Haylett, 2000), and P/Q-type (Pineda *et al.*, 1999) voltage-dependent Ca^{2+} channels, and through calcium-induced calcium release (Shah & Haylett, 2000). Elucidating the mechanisms underlying plasticity of the AHP is important because reductions in the slow AHP (sAHP) are posited as a general mechanism for increasing neuronal excitability, which is involved in learning (Disterhoft *et al.*, 1986; Disterhoft *et al.*, 1988; Coulter *et al.*, 1989; Disterhoft *et al.*, 1996; Moyer *et al.*, 1996; Thompson *et al.*, 1996; Saar *et al.*, 1998; Moyer *et al.*, 2000; Saar *et al.*, 2001; Oh *et al.*, 2003; Tombaugh *et al.*, 2005; Disterhoft & Oh, 2006; Ohno *et al.*, 2006; Zelcer *et al.*, 2006; Disterhoft & Oh, 2007; Kaczorowski & Disterhoft, 2009). Preliminary studies suggest that the pattern of action potential firing can influence the magnitude of the AHP (Kaczorowski *et al.*, 2003), but few studies have explored how differences in activity patterns may induce long-lasting changes in the sAHP.

Theta-burst firing has been shown previously to induce a long-lasting activity-dependent reduction of the sAHP/I_{sAHP} (Kaczorowski et al., 2007) that mimics changes observed after learning (see Disterhoft and Oh, 2007 for review). Although theta-burst firing is often thought to be a more physiologically relevant activity pattern (Ranck, 1973; Larson & Lynch, 1986; Larson *et al.*, 1986; Otto *et al.*, 1991), evidence suggests some CA1 pyramidal neurons exhibit gamma-related firing between 40 – 80 Hz *in vivo* (Barnes *et al.*, 1990; Soltesz & Deschenes, 1993; Senior *et al.*, 2008; Colgin *et al.*, 2009) that may play a role in spatial and temporal encoding and memory (Barnes *et al.*, 1990; Lisman & Idiart, 1995; Csicsvari *et al.*, 2003; Lisman, 2005; Senior *et al.*, 2008; Colgin *et al.*, 2009). Although prior work suggests high-frequency somatic action potential firing in response to depolarizing constant current pulses enhances the sAHP (Borde *et al.*, 1995), a systematic study of the effect of gamma-related firing (using brief current pulses at fixed frequency, 50 Hz) on plasticity of the sAHP in CA1 neurons has not been performed. Moreover, a direct comparison of these two activity patterns matched for the number of action potentials generated has not been performed to date. Therefore, the present report investigated how two different activity patterns (gamma-related firing and theta-burst firing), which have been observed *in vivo* during spatial navigation and memory tasks (Barnes *et al.*, 1990; Otto *et al.*, 1991; Senior *et al.*, 2008; Colgin *et al.*, 2009; Colgin & Moser, 2010), would interact to modulate the sAHP within the same cell, as well as in isolation, and to explore underlying mechanisms.

The primary candidate mechanisms underlying activity-dependent modulation of the sAHP are notably the I_{AHP} and sI_{AHP} (Borde *et al.*, 1995; Borde *et al.*, 1999; Borde *et al.*, 2000; Le Ray *et al.*, 2004; Kaczorowski *et al.*, 2007), as well as alterations in voltage-gated and store-operated Ca2+ influx (Borde *et al.*, 1995). In addition to I_{AHP} and sI_{AHP}, activity-induced increases in intracellular Ca^{2+} are likely to activate signaling pathways that can modify the AHP (Pedarzani *et al.*, 1998), although other mechanisms are possible (Pedarzani and Storm, 1996). These modifications are thought to occur through Ca^{2+}-triggered activation of kinases or adenylyl cyclases (Jonas & Kaczmarek, 1996).

The hyperpolarization-activated mixed cation channel (h-channel) is a strong candidate for Ca^{2+}-dependent modulation, and the hyperpolarization-activated mixed cation current (I_h) has been shown to regulate neuronal excitation by altering the size and kinetics of the AHP (Storm, 1990; Maccaferri *et al.*, 1993; Maccaferri *et al.*, 1994; Poolos *et al.*, 2002; Gu *et al.*, 2005). Additionally, I_h is modulated by intracellular cAMP (DiFrancesco & Tortora, 1991), which has been shown to induce a shift in the activation curve of I_h to more depolarized

potentials (DiFrancesco, 1993; Pape, 1996). Moreover, the magnitude and source of intracellular Ca^{2+} increases has been shown to affect I_h (Fan *et al.*, 2005). The present work combines pharmacology and electrophysiology to demonstrate pattern-specific differences in the amount or source of Ca^{2+} and/or I_h modulation contribute to AHP plasticity independent of synaptic activation.

MATERIALS AND METHODS

Transverse Hippocampal Slices and Internal Solutions

All animal procedures were approved by the Northwestern University Animal Care and Use Committee. Male P14–28 Wistar rats were used. After deep halothane anesthesia, the rat was decapitated and the brain quickly removed and placed into ice-cold artificial cerebral spinal fluid (aCSF, in mM): 125 NaCl, 25 glucose, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$ (pH 7.5, bubbled with 95%O_2/5%CO_2). A blocking cut was made to obtain near-horizontal slices to maintain intact CA1 apical dendrites within the hippocampal slice. Slices (300 μm) of the medial hippocampus and adjacent cortex were made using a Leica vibratome (Wetzlar, Germany). The slices were first incubated at 34°C in bubbled aCSF for 30 minutes, and then held at room temperature in bubbled aCSF for 1–4 hours before use.

For whole-cell recordings, electrodes prepared from thin-walled capillary glass were filled with 115 mM potassium methylsulfate (KMeth)-based internal solution and had a resistance of 3–5 MΩ. Internal solution also contained 20 KCl, 10 Na-PCr, 10 HEPES, 2 MgATP, 0.3 NaGTP and 0.10% Biocytin. KOH was used to adjust the pH to 7.3. KMeth-based solution was used based on previous work showing this internal anion best recapitulates the AHP compared to perforated-patch configuration, and the appropriate controls were conducted (Kaczorowski et al, 2007). Unless otherwise stated, chemicals were obtained from Sigma (St. Louis, MO). Potassium methylsulfate was purchased from ICN Biomedical (Aurora, OH).

Electrophysiological Recording

Slices were transferred to a recording chamber mounted on a Zeiss Axioskop (Oberkochen, Germany) where they were submerged in oxygenated aCSF at 33–34°C. Neurons were visualized with infrared differential interference contrast video microscopy. High-resistance seals (>1 GΩ) were obtained under visual control on the somata of CA1 pyramidal neurons; brief suction was applied to the patch in order to gain whole-cell access to the neuron. Once in whole-cell configuration, cells were evaluated on a number of criteria and accepted for use only if they had a resting membrane potential (V_m) < −58 mV, access resistance (R_s) < 40 MΩ, input resistance (R_N) > 30 MΩ, and an action potential amplitude greater than 70 mV relative to action potential threshold (see data acquisition and analysis). All recordings were done in current-clamp mode using a Dagan current-clamp amplifier; cells were held at −66 mV by manually adjusting the holding current by less than ±100 pA. The electrode capacitance and series resistance (R_s) were monitored, compensated, and recorded frequently throughout the duration of the recording. In all experiments, $GABA_A$ receptors, ionotropic glutamate receptors, and muscarinic acetylcholine receptors were blocked by the addition of SR95531 (2 mM), kynurenic acid (2 mM) and atropine (1 μM) to the standard aCSF solution.

Stimulation and Recording Protocols

In the first set of experiments, AHPs were triggered by alternating between two distinct patterns of somatic current injection in the same cell (once every 3 minutes or once per minute for a total of 30 minutes, Figure 1A, B). Gamma-related firing consisted of a train of 50 action potentials at 50 Hz (gamma-AHP, black), and a theta-burst firing that consisted of

10 bursts of 5 action potentials at 100 Hz with an inter-burst frequency of 5 Hz (theta-AHP, grey). Under no circumstances were action potential failures or bursts (doublets) observed in response to brief current step used to evoke the AHP. Note that the stimulation described herein results from somatically generated action potentials, and occurs in the presence of synaptic blockers.

In the second set of experiments, cells were exposed to one of the two stimulation patterns once every minute for a total of 40 minutes (Figure 3A). All current injections used to trigger action potentials were brief (2 ms) somatic current injections (1 nA). In general, data collection began within 10 minutes after membrane rupture. In all experiments, initial measurements of the R_N were performed directly following capacitance and series resistance compensations, approximately 2–3 minutes after gaining access to the neuron. R_N was monitored at least once every 3 min for the duration of the recordings and determined by Ohm's Law from the voltage response to an 800 ms, −50 pA current step.

Data acquisition and Analysis

Data were transferred to a computer using an ITC-16 digital-to-analog converter (InstruTech, Port Washington, NY). IgorPro (Wavemetrics, Lake Oswego, OR) software was used for acquisition and analysis. Statistical tests were performed using SigmaPlot software (Systat Software Inc., San Jose, CA). Significance was determined by repeated measures ANOVA and Fisher's least significant difference t-tests where appropriate (unless otherwise noted). All results are reported as mean ± SEM.

The AHP was defined as the membrane potential beginning at the peak negative value (relative to the initial baseline) following the last spike (Figure 1A, upward arrow) and ending when the voltage had decayed to 95% of that peak value (see Kaczorowski et al., 2007). The integral of the AHP was calculated and shown as the area under the red dotted line (Figure 1A, inset). For ease of comparisons, the integral of the AHP was defined as the sAHP, given that the mAHP comprises less than 100 ms of the entire AHP lasting for tens of seconds (Storm, 1990; Stocker *et al.*, 1999). Our prior work shows that > 90% of the integral of the AHP triggered by either gamma-related or theta-burst firing is blocked by the broad spectrum calcium channel blocker Cd^{2+} (200 μM) or noradrenaline (10 μM), indicating that the majority of the AHP integral is driven by the Ca^{2+}-dependent sI_{AHP} (Kaczorowski *et al.*, 2007). The apamin-sensitive AHP contributes minimally to the integral of the AHP using the present stimulation protocols and recording solutions (Kaczorowski, 2006; Kaczorowski *et al.*, 2007).

RESULTS

Whole-cell recordings were obtained from CA1 pyramidal neurons in rat hippocampal slices. All cells were regular spiking neurons that demonstrated a prominent AHP (a negative membrane potential relative to baseline) in response to action potential firing.

Differential effects of activity patterns on AHP plasticity

First, the AHP was assessed in response to two activity patterns, a gamma-related 50 Hz train (gamma-AHP) and theta-burst pattern (theta-AHP) of action potentials (n = 19). Analysis of results revealed that the integral of the gamma-AHP was less than the theta-AHP (gamma-AHP, −9.6 mV·s ± 1; theta-AHP, −11.8 mV·s ± 1; $t_{18} = 3.93$, $p = 0.001$, paired-t-test). AHPs measured at 10 min served as the baseline in subsequent comparisons aimed at examining the effects of repeated stimulation using gamma-related and theta-burst activity patterns on AHP plasticity.

Next, the AHP was assessed over time in response to two alternating activity patterns, a gamma-related 50 Hz train (gamma-AHP) and theta-burst pattern (theta-AHP) of action potentials (Figure 1B). When AHPs were evoked once per minute (Figure 1C), the integral of the gamma-AHP significantly increased to 186 ± 31% of baseline across 40 min ($F_{6,132}$ = 3.72, p = 0.002). Post-hoc tests revealed a significant difference between the AHP integral measured at baseline, −9.6 ±1 mV·s, relative to 40 min, −15.8 ± 3.2 mV·s (t_{18} = 3.66, p = 0.002). Surprisingly, saturation of the gamma-AHP was not observed over the duration of the experiment. In contrast to the gamma-AHP, the integral of the theta-AHP was comparable to baseline across 40 min (119 ± 12%; baseline, −11.8 ±1 mV·s; 40 min, −12.0 ± 1.2 mV·s; $F_{6,132}$ = 1.75, p = 0.13). Analysis of the results revealed a significant difference in the % change in AHP from baseline of the gamma-AHP compared to the theta-AHP at 40 minutes (t_{36} = 2.24, p = 0.032). Note that plasticity of the AHP was not observed in experiments when AHPs were triggered once every three minutes (n = 8, see Figure 1D), which were modeled after the low-activity protocol reported previously (Kaczorowski et al., 2007). Taken together, these data suggest repeated firing at gamma-frequency results in the activity-dependent enhancement of the sAHP and reflects a decrease in intrinsic excitability.

Blockade of L-type or N- and P/Q-type Ca^{2+} channels disrupt potentiation of the gamma-AHP

Previous work has shown that high-frequency firing in response to depolarizing constant current pulses increases the amplitude of the AHP, as well as I_{AHP} and sI_{AHP}, that depends on L-type Ca^{2+} channels or release from internal stores (Borde *et al.*, 1999; Borde *et al.*, 2000; Le Ray *et al.*, 2004). Therefore, we hypothesized that the potentiation of the gamma-AHP observed herein may be sensitive to Ca^{2+} channel blockade as well. First, the immediate effect of N-P/Q-type Ca^{2+} channels on the AHP was assessed. Bath application of the N-P/Q-type Ca^{2+} channel blocker ω-conotoxin MVIIC (1 μM) had no general effect on the sAHP. Statistical analysis of the results using paired t-tests revealed no meaningful difference in the integral of the gamma-AHP or theta-AHP immediately (5–10 min) following bath application of ω-conotoxin MVIIC (gamma-AHP: aCSF −10.8 ± 2.0 mV·s vs. MVIIC −10.6 ± 1.5 mV·s, t_7 = 0.14, p = 0.90; theta-AHP: aCSF −13.2 ± 2.5 mV·s vs. MVIIC −11 ± 1.5 mV·s, t_7 = 1.88, p = 0.10). The integral of the gamma-AHP and theta-AHP in ω-conotoxin MVIIC were 114 ± 17% and 96 ± 8% of baseline (aCSF), respectively.

Next, the effects of both the N-P/Q-type, as well as L-type Ca^{2+} channels, on plasticity of the AHP using application of either ω-conotoxin MVIIC (1 μM) or nimodipine (10 μM) were examined. The gamma-AHP and theta-AHP measured in aCSF (10 min) were compared to measures in ω-conotoxin MVIIC or nimodipine at 40 minutes (Figure 2). Analysis of the results revealed ω-conotoxin MVIIC was sufficient to occlude potentiation of the AHP; no significant difference in the gamma-AHP was observed at 40 min in ω-conotoxin MVIIC compared to baseline (gamma-AHP; baseline, −10.8 ± 2.5 mV·s; MVIIC, −8.7 ± 1.5 mV·s, t_7 = 0.87, p = 0.41, paired t-test). Similar to previous work by Borde and colleagues (2000), blockade of Ca^{2+} via L-type Ca^{2+} channels using nimodipine was sufficient to block or mask potentiation of the AHP (gamma-AHP, 101 ± 11%, n = 5). These data suggest that potentiation of the gamma-AHP observed in normal aCSF after 40 min of repeated stimulation (186 ± 31% of baseline) was disrupted when either N-P/Q-type Ca^{2+} channels or L-type Ca^{2+} channels were blocked (92 ± 14% and 101 ± 11% of baseline, respectively). Given that no plasticity of the theta-AHP was observed in aCSF after 40 min of repeated stimulation (119 ± 12% of baseline), it was not surprising that neither ω-conotoxin MVIIC nor nimodipine differed from baseline (85 ± 15% and 114 ± 22% of baseline, respectively; Figure 2).

Pattern-specific bidirectional AHP plasticity in vitro

In a second set of experiments, AHPs were triggered using only 50 Hz or only theta-burst firing. As when both stimulation patterns were applied in the same cell (Figure 2), the gamma-AHP was increased to 198 ± 44% of baseline when measured after 40 min of repeated 50 Hz gamma-related stimulation (Figure 3). The integral of the gamma-AHP at 40 min was significantly increased compared to the gamma-AHP measured at 10 min (gamma-AHP, baseline, −14.5 ± 2.6 mV·s vs. 40 min, −25 ± 2.6 mV·s, $t_5 = 2.98$, $p = 0.041$, paired t-test). As predicted based on our prior work (Kaczorowski et al., 2007), a significant reduction in the amplitude of the theta-AHP to 65 ±19% baseline was observed after 40 min of theta-burst firing alone (theta-AHP, baseline, −18.5 ± 2.8 mV·s vs. 40 min, −10.2 ± 3.0 mV·s, $t_5 = 2.19$, $p = 0.049$, one-tailed paired t-test) (Figure 3B). One important observation made herein was that in the gamma-only stimulated experiments, the input resistance (R_N) gradually increased over the duration of recordings from 89 ± 7 MΩ to 151 ± 8 MΩ ($t_5 = 10.06$, $p = 0.001$, paired t-test). R_N was altered less in theta-only stimulated experiments, from 95 ± 7 MΩ to 122 ± 21 MΩ, and this change was not significant ($t_5 = 1.73$, $p = 0.16$, paired t-test). A reduction in voltage sag, evidenced by an increase in the sag ratio (Δsteady-state voltage deflection/Δpeak voltage deflection), was greatest following 40 min gamma-related stimulation (50 Hz firing) relative to theta-burst stimulation (gamma-AHP, 107 ±1% of baseline; theta-AHP 104 ±1.3% of baseline). Based on the role of I_h in generation of depolarizing sag in CA1 hippocampal neurons (Maccaferri *et al.*, 1993), these data hinted that alterations in I_h may contribute to plasticity of the sAHP that was directly tested by repeating these experiments in the presence of the h-channel blocker ZD7288.

H-current contributes to the size and shape of the AHP and plays a role in AHP plasticity

Prior to examining the effects of I_h on plasticity, the immediate effects of the h-channel blocker ZD7288 were first examined by comparing the gamma-AHP or theta-AHP (n = 5) in aCSF followed by bath application of ZD7288 (25 μM). Previous work suggests that blockade of I_h with ZD7288 or Cs^+ reduces the medium component of the AHP (Storm, 1989; Williamson & Alger, 1990; Maccaferri *et al.*, 1993; Gu *et al.*, 2005), but enhances the sAHP (Gu *et al*., 2005). Therefore, consistent with previous reports, it was not surprising that ZD7288 (25 μM) reduced the peak of the AHP in 5 of 5 cells tested (Figure 4; gamma-AHP: −5.3 ± 0.06 mV in aCSF vs. −4.9 ± 0.1 mV: theta-AHP, −6.1 ± 1 mV in aCSF vs. −4.8 ± 1.7 mV). Moreover, the blockade of I_h using ZD7288 increased the integral of the gamma-AHP and theta-AHP to ~200% of baseline (gamma-AHP, aCSF, −13.4 ± 0.8 mV·s vs. ZD7288, −27.5 ± 4.0 mV·s: theta-AHP, aCSF, −12.2 ± 1.8 mV·s vs. ZD7288, −25.5 ± 7.8 mV·s), as shown previously (Gu *et al*., 2005).

To test the hypothesis that plasticity of the AHP results from alterations in the availability and/or function of h-channels, the specific h-channel blocker ZD7288 was applied during repeated 50 Hz firing or theta-burst firing alone experiments (Figure 5). Blockade of h-channels prevented the enhancement of the gamma-AHP measured across 40 minutes of repeated 50 Hz gamma-related stimulation (72 ± 9% in ZD7288 compared to 198 ± 44% in aCSF, $t_9 = 3.08$, $p = 0.013$), and revealed a decrease in the integral of the gamma-AHP that was qualitatively similar to that observed following 40 min of repeated theta-burst firing. Note that blockade of h-channels had no effect on the integral of the theta-AHP relative to aCSF measured after 40 minutes of repeated theta-burst firing (54 ±14% in ZD7288 compared to 65 ±19% in aCSF at 40 min, $t_8 = 0.42$, $p = 0.69$). Therefore, it appears that when h-channels are not available for activation or modulation, the amplitude of the gamma-AHP in ZD7288 is comparable to the theta-AHP measured at 40 min in aCSF and ZD7288 (Figure 5). Although ZD7288 significantly increased R_N compared to control conditions (Figure 6), statistical comparisons revealed no significant difference in the magnitude of R_N in neurons exposed to gamma-related firing compared to theta-burst firing ($t_7 = 0.48$, $p =$

0.64). Blockade of h-channels using ZD7288 did not prevent the gradual increase in R_N, which increased from 179 ± 11 MΩ (10 minutes) to 300 ± 31 MΩ (40 minutes). This represents a 59% increase in R_N using ZD7288 compared to a 53% increase in aCSF. Therefore, the effect of ZD7288 on AHP plasticity did not result from a non-specific stabilization of input resistance. Rather, h-channel blockers prevented enhancement of the AHP in spite of the general increase in R_N that has been reported previously using KMeth-based internal solution (Kaczorowski et al., 2007). Further studies are required to determine the source of elevated R_N described here and elsewhere (Kaczorowski et al., 2007).

DISCUSSION

This study demonstrates that plasticity of the AHP is bidirectional, and the direction of plasticity depends on the pattern of action potential firing used to trigger the AHP. The sAHP is enhanced in response to high-frequency firing in the gamma frequency (50 Hz), or when alternated with theta-patterned stimulation. Remarkably, the results suggest that the availability of N-P/Q-type and L-type Ca^{2+} channels permit the activity-dependent enhancement of the gamma-AHP following repeated 50 Hz stimulation, but had no effect on the theta-AHP. Similarly, blockade of h-channels prevented plasticity of the gamma-AHP, but had no effect on plasticity of the AHP induced using theta-burst firing alone. Taken together, these data suggest activity-dependent alterations in N- and P/Q type Ca^{2+} channels and h-channels provide novel mechanisms to reduce neuronal excitability in response to gamma-related firing, as evidenced by an enhancement of the gamma-AHP. Future studies are necessary to determine whether or not activation of L-type and/or N-P/Q-type Ca^{2+} channels are coupled to alterations in I_h during the induction of gamma-AHP plasticity.

Contrary to plasticity induced by gamma-related activity, the induction and/or expression of AHP plasticity by theta-burst firing (theta-AHP) differed depending on whether theta-burst stimulation was used alone or in alternation with gamma-related firing. The theta-AHP was unchanged in experiments in which stimulation patterns were alternated, yet theta-burst patterned stimulation alone induced a reduction of the theta-AHP similar to previous work (Kaczorowski et al., 2007). Therefore, gamma-related firing appeared to prevent or mask the induction of theta-AHP plasticity. While a more detailed study is required to fully elucidate how different patterns of activity lead to changes in the sAHP, our results provide a framework to understand how these physiologically relevant firing patterns can alter the intrinsic excitability in principle neurons of the hippocampus – thus, providing additional computational power for information processing and storage.

AHP plasticity: new findings and comparison to previous reports

Previous reports have demonstrated that repeated suprathreshold somatic firing leads to an enhancement of the AHP (AHP-E) and potentiation of the sI_{AHP} (Borde *et al.*, 1995; Borde *et al.*, 1999; Borde *et al.*, 2000). However, recent work suggests the opposite, that suprathreshold somatic firing reduces the sAHP and the sI_{AHP} (Kaczorowski *et al.*, 2007). One interesting difference between these prior studies is the firing-frequency of the stimulation that was used; Borde and colleagues used high-frequency variable firing and Kaczorowski and colleagues (2007) used theta-burst firing to evoke the AHP. However, because Borde and colleagues (1995) allowed firing frequency and total action potential number to vary, it was premature to attribute differences in the direction of AHP plasticity to differences in firing frequency. Therefore, the present results extend earlier work to clarify the differential role of activity patterns in the regulation of the AHP (gamma-related versus theta-burst firing) using reproducible stimuli, matched in total action potentials, and evoked at fixed intervals. This approach allowed, for the first time, the direct comparison of different activity patterns on AHP plasticity.

Moreover, the present report demonstrates that plasticity of the sAHP is activity dependent. No change in sAHPs were observed in experiments where activity patterns were delivered less frequently (every 3 min compared to 1 min), thus resulting in 1/3 number of action potentials triggered. Because the present experiments were performed under blockers of fast excitatory and inhibitory transmission, for the first time, it is shown herein that the induction requirements for bidirectional sAHP plasticity do not depend on synaptic activation. Lastly, these data reveal the select involvement of N-P/Q-type Ca^{2+} channels (in addition to L-type Ca^{2+} channels reported by Borde *et al*. 2005) for plasticity of the gamma-AHP. These data suggest that activity-dependent potentiation of the AHP results from specific activity patterns (notably gamma-related firing), in part, from increases in Ca^{2+} influx via voltage-gated calcium channels and/or activation of their downstream targets, for example the sI_{AHP}.

Modulation of I_h alters neuronal excitability in hippocampal neurons

This study also shows that blockade of I_h using ZD7288 masked or prevented the potentiation of the gamma-AHP observed under control conditions, and suggests that the availability of h-channels are important for plasticity of the gamma-AHP. Although prior work has shown that long-term exposure to ZD7288 can have nonspecific effects on neuron excitability (Chevaleyre & Castillo, 2002), it is unlikely that such nonspecific effects could explain the present results given that 1) blockers of fast excitatory and inhibitory synaptic transmission were present, 2) the time required to observe significant and nonspecific effects of ZD7288 (50 μM, twice the concentration used in this study) reported previously (Chevaleyre and Castillo, 2002) was longer than most of the recordings in this study (40 min), and 3) ZD7288 had a select effect on plasticity of the gamma-AHP.

The I_h–dependent AHP plasticity induced by repeated 50 Hz gamma-related firing described in the present report differs from recent work by Fan and colleagues (2005). They report that either synaptic theta-burst firing or somatic theta-burst firing in CA1 hippocampal neurons can drive a decrease in R_N and reduce hippocampal neuronal excitability resulting from an NMDA receptor and CaMKII-dependent increase in I_h. Such an increase in I_h available at the resting membrane potential would have the opposite effect of ZD7288 on the AHP shown here and in previous work (Gu et al., 2005; Maccaferri et al., 1993; Storm, 1989; Williamson & Alger, 1990). Specifically, an increase in I_h is posited to increase the mAHP and reduce the sAHP. Moreover, the present results using somatic theta-burst firing show a trend towards an increase in R_N that may indicate a reduction in I_h, and an increase in hippocampal neuronal excitability (evidenced by a reduction in the theta-AHP). These differences may be due to our use of synaptic blockers, including kynurenate, which would block activation of postsynaptic NMDA receptors posited to underlie plasticity described by Fan and colleagues (2005).

The present results also differ in that here I show blockade of I_h with ZD7288 did not significantly alter the induction of intrinsic plasticity (e.g. increased neuronal excitability evidenced by a reduction in the sAHP) following repeated theta-burst firing compared to control experiments. Differences in the number of action potentials and the frequency of the theta-burst stimulation, which is used as the induction protocol, may be responsible for the differences in the mechanisms underlying theta-burst induced plasticity, or lack thereof. Fan and colleagues (2005) used one epoch of theta-burst firing (150 spikes over 2 minutes), whereas the present report triggered the AHP using theta-burst firing once a minute for 40 minutes (2000 spikes). Therefore, it is possible that the brief theta-burst firing stimulation used by Fan and colleagues (2005) was not sufficient to induce alterations in neuronal excitability reported here.

Bidirectional modulation of the AHP may result from differences in the source or magnitude of Ca^{2+} entry or downstream targets

The present work demonstrates that the pattern of activity in the form of postsynaptic firing, in the absence of synaptic input, is a critical determinant of the plasticity of the AHP. Pattern-specific differences in the induction and/or expression of AHP plasticity may arise from differential involvement of voltage-gated channels located in the dendrites. Because somatic action potentials in a theta-burst pattern are expected to backpropagate more effectively into the dendritic tree than gamma-related firing at 50 Hz (Spruston *et al.*, 1995), pattern specific differences in AHP plasticity may reflect differential activation and/or expression of voltage-gated channels in the dendrite. The unique activation of the distal dendrites by theta-burst firing could modify voltage-gated channels that are primarily expressed in the dendrites – and thus, more likely to be activated by theta-burst firing compared to gamma-related firing. An example of this was shown by Nolan and colleagues (2004) where modifications in h-channel expression substantially impacted synaptic plasticity in the distal portions of the dendrite, but not at synapses located in the proximal dendrite (Nolan *et al.*, 2004). Alternatively, the spatial and temporal characteristics of intracellular Ca^{2+} rise and buffering may differ between gamma-related and theta-burst firing. Pattern specific differences in Ca^{2+} rise and buffering would be expected to alter the sAHP magnitude in response to subsequent stimulation (Powell *et al.*, 2008; Goldberg *et al.*, 2009), as well as differentially affect Ca^{2+}-dependent plasticity similar to that described with regards to synaptic plasticity (Larson *et al.*, 1986; Huang & Kandel, 1994).

It is possible that when alternating stimulation is used that there are two opposing effects: an amplification of the sAHP caused by the 50 Hz train stimulation and a reduction in the sAHP induced by theta-burst firing. This could explain why the theta-AHP is reduced in isolation but not in experiments that alternate activity patterns. However, this speculation is inconsistent with the observation that the rate and amplitude of gamma-AHP potentiation is comparable in experiments where 50 Hz firing was delivered alone or interleaved with theta-burst firing (Figures 1 and 3). Lastly, it is possible that different activity patterns lead to changes in calcium buffering that influence the sAHP (Powell et al., 2008). While a more detailed study is required to fully elucidate how different patterns of activity lead to changes in the sAHP, our results provide a framework to understand these mechanisms.

Bidirectional modulation of the AHP provides a synapse-independent mechanism that shapes excitability of hippocampus in response to neural activity and experience

Activity-dependent bidirectional modulation of the sAHP occurred in response to somatically generated action potentials. Because neuronal output in response to somatic current injection is determined by activation of intrinsic voltage-gated or Ca^{2+}-activated ion channels, changes in the sAHP reported herein most likely reflect alterations in intrinsic postsynaptic excitability. Although $GABA_B$ receptors were not blocked in the present report, $GABA_B$-receptor activation is unlikely to occur in response to postsynaptic firing (in the absence of synaptic stimulation). Furthermore, the present experiments were conducted in the presence of fast excitatory and inhibitory synaptic blockers that essentially isolate the postsynaptic neuron from activating downstream neurons that could potentially provide a source of GABA for activation of these receptors. Taken together, these data confirm that synaptic activation is not a requirement for induction of the sAHP plasticity reported here. Although a more detailed analysis of the signal transduction pathways involved in pattern-specific sAHP plasticity are important, the present report provides novel insight into the differential role of HVA Ca^{2+} channels and I_h in bidirectional pattern-specific sAHP plasticity.

Examinations of learning-related AHP alterations in CA1 pyramidal neurons have been observed following learning paradigms that depend upon the hippocampus, including trace eyeblink conditioning in rabbit and rat (de Jonge *et al.*, 1990; Moyer *et al.*, 1996; Moyer *et al.*, 2000; Kuo *et al*., 2004), and spatial water maze training and contextual fear conditioning in rat and mouse (Oh *et al.*, 2003; Tombaugh *et al.*, 2005; Ohno *et al.*, 2006; Kaczorowski & Disterhoft, 2009; McKay *et al.*, 2009). Reductions in the AHP are posited to underlie, or at least facilitate, cellular mechanisms of hippocampus-dependent learning. In other studies, activation of metabotropic glutamate receptors have been shown to induce long-lasting suppression of the I_{AHP} and sI_{AHP} (Faber & Sah, 2002; Melyan *et al.*, 2002; Sourdet *et al.*, 2003; Ruiz *et al.*, 2005). Here we show somatic-theta burst pattern firing, that mimics activity patterns observed during learning tasks *in vivo*, is sufficient to induce a reduction in the sAHP.

Curiously, repetition of gamma-related firing resulted in an enhancement of the sAHP (i.e. decreased neuron excitability) that was qualitatively similar to that observed in hippocampal neurons from poor-learner mice expressing familial Alzheimer's Disease (FAD) mutations (Kaczorowski *et al.*, 2009). Remarkably, mice with FAD mutations are more susceptible to unprovoked seizures than their wild-type counterparts largely due hyperexcitable neurons in brain areas expressing beta-amyloid protofibrils (Minkeviciene *et al.*, 2009) and beta-amyloid plaques (Busche *et al.*, 2008). It is possible that gamma-related enhancement of the sAHP provides a mechanism to reduce disease-related neuron hyperexcitability that is capable of inducing seizures; with the caveat being this occurs at the expense of cognitive performance. Alternatively, gamma-related firing may restore basal levels of excitability following a learning event to prevent hyperexcitability following multiple learning episodes.

Overall, plasticity of intrinsic neuronal excitability is thought to increase the storage capacity of neurons and likely plays a critical role in learning and memory (Saar & Barkai, 2003; Zhang & Linden, 2003; Disterhoft *et al.*, 2004; Oh *et al.*, 2010). Therefore, the identification of novel mechanisms underlying AHP plasticity *in vitro* herein provides additional insight into the dynamic processes that likely influence learning and memory. The plasticity described here differs significantly from the induction of synaptic plasticity; synaptic stimulation at either gamma- or theta-frequencies induces rapid and long lasting potentiation of synaptic strength at Shaffer-collateral-CA1 synapses (Chen et al., 1999 and Larson *et al.*, 1986, respectively) whereas sAHP induced by postsynaptic firing develops gradually and the direction of plasticity differs based on the activity pattern used. Postsynaptic firing at 50 Hz, gamma-related frequency, results in a gradual decrease in neuronal excitability that is opposite to the enhancement in neuronal excitability observed following repeated postsynaptic theta-burst firing. Both synaptic plasticity and intrinsic plasticity have been postulated as cellular mechanisms critically involved in learning and memory. Since it has yet to be elucidated how learning-related changes in neuron excitability and synaptic strength develop and interact during the acquisition of various hippocampal-dependent learning and memory tasks that can require minutes (e.g. contextual fear conditioning) to several days (e.g. spatial watermaze and trace eyeblink conditioning) of training, the present findings provide new information on the mechanisms underlying AHP changes and provide a model to further how such changes relate to learning processes.

Acknowledgments

The authors wish to thank Dr. Gianmaria Maccaferri and Dr. Shannon Moore for their advice and critical reading of the manuscript. Caroline Cook provided advice on the illustrations. This work was supported by the National Institutes of Mental Health grant F31 MH-067445 awarded to C.C.K. and the Department of Physiology at the Medical College of Wisconsin, Milwaukee, WI.

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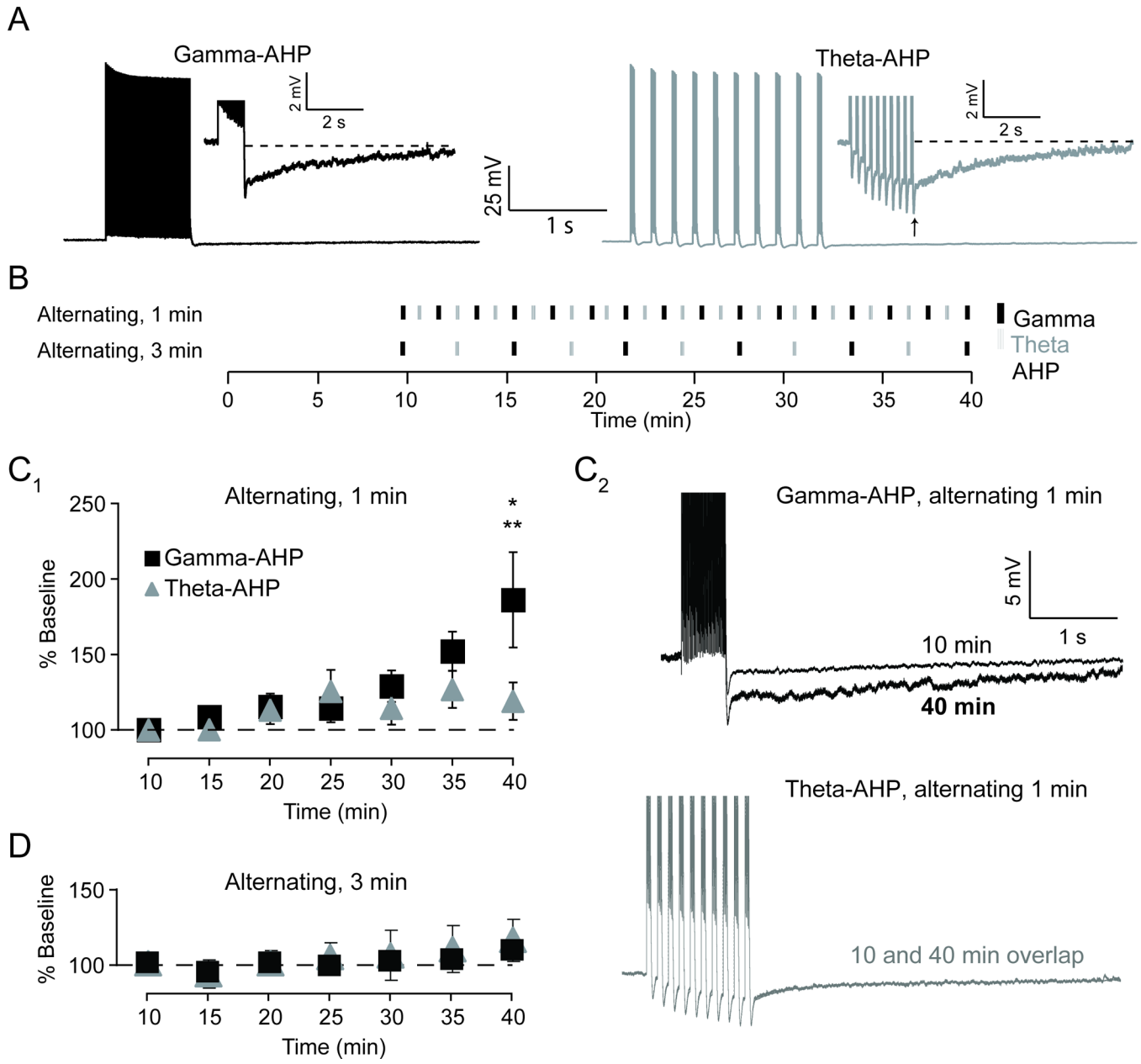

Figure 1. Alternating 50 Hz and theta-burst firing produced an increase in the afterhyperpolarization (AHP) triggered with 50 Hz firing (gamma-AHP) in CA1 pyramidal neurons from hippocampal slice of the rat

A, Typical example of the post-burst AHP triggered using a train of action potentials at 50 Hz (black) compared to using theta-burst pattern firing (grey). Arrow denotes the peak of the AHP. Inset shows AHP on an expanded scale. AHP integral was measured as area under the dashed line. B, Schematic diagram illustrates the stimulation protocols used to examine plasticity of the AHP. Alternating protocol used somatic action potential firing at 50 Hz (black) and theta-burst firing (grey) in an alternating fashion. This served as both the induction protocol and the stimulus used to evoke AHPs (gamma-AHP and theta-AHP, respectively). C_1, Plot showing alternating stimuli at 1 min intervals resulted in a gradual increase in the gamma-AHP relative to baseline (10 min). The gamma-AHP was significantly increased compared to baseline (* $p < 0.05$) and to theta-AHP measured at 40

minutes (** $p < 0.05$) in recordings from neurons exposed to alternating 50 Hz and theta-burst firing. C_2, Top, representative trace of the gamma-AHP triggered at an early time point (10 min; thin black) superimposed on the gamma-AHP triggered at a late time point (40 min; thick black). Bottom, representative trace of the theta-AHP triggered at an early time point (10 min; dotted grey) superimposed on the gamma-AHP triggered at a late time point (40 min; grey). D, Plot showing alternating stimuli at 3 minute intervals had no effect on integral of the gamma-AHP or theta-AHP relative to baseline (10 min).

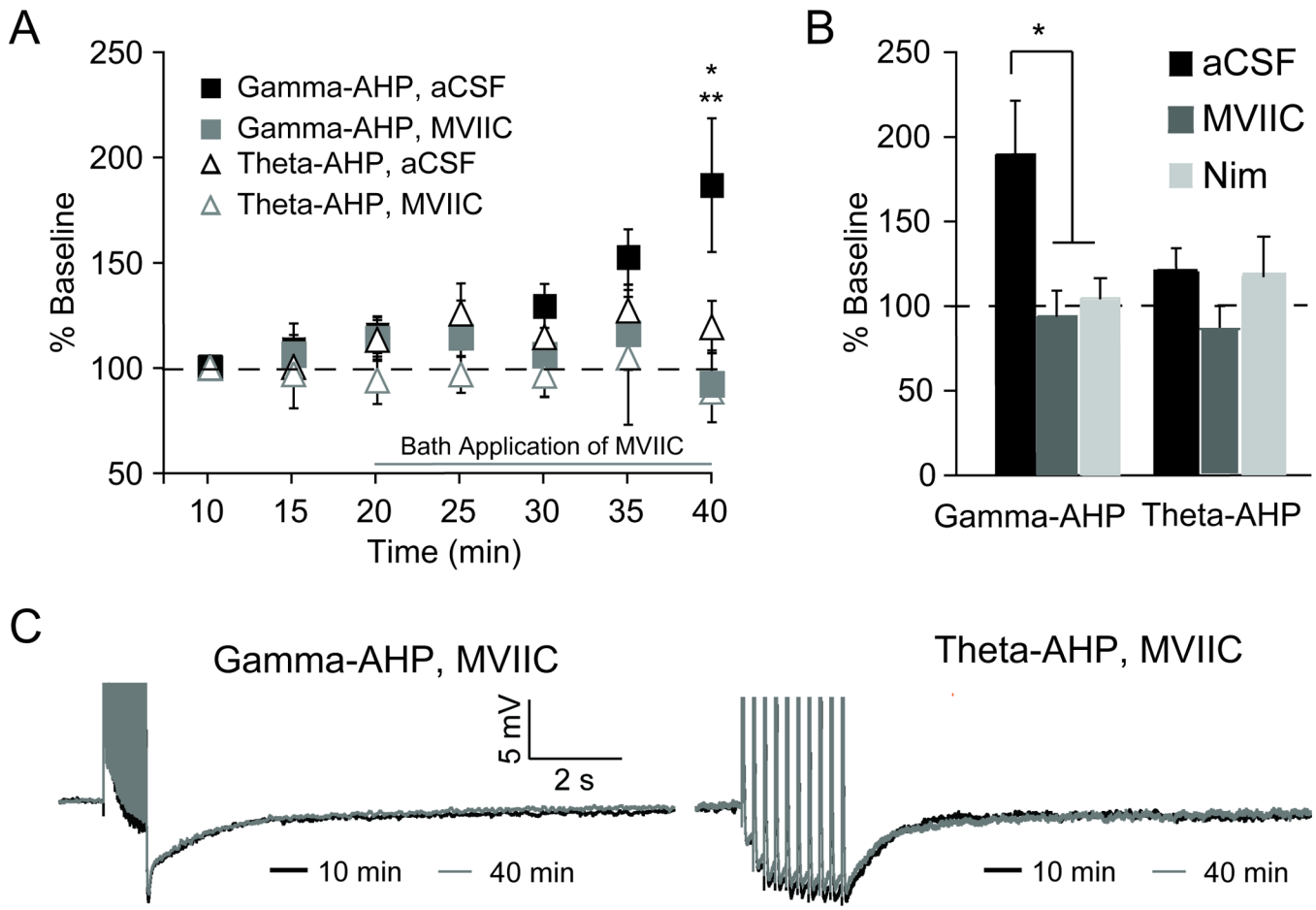

Figure 2. Potentiation of gamma-AHP is not observed using Ca^{2+} channel blockers

A, Plot showing that enhancement of the gamma-AHP (control aCSF, filled black squares) is absent in the presence of the N- and P/Q-type Ca^{2+} channel blocker ω-conotoxin MVIIC (1 μM) (grey filled squares). ω-conotoxin MVIIC (1 μM) did not alter the theta-AHP (open grey triangles) relative to control experiments (open black triangles). B, Summary graph illustrates enhancement of gamma-AHP, was significantly reduced or blocked by either ω-conotoxin MVIIC (grey) or the L-type Ca^{2+} blocker nimodipine (Nim, 10 μM, light grey, *$p < 0.05$). Neither ω-conotoxin MVIIC nor nimodipine altered the theta-AHP compared to baseline. C, Left, representative trace of the gamma-AHP triggered under control conditions at 40 min (black) superimposed on the gamma-AHP triggered with ω-conotoxin MVIIC at 40 min (grey). Right, representative trace of the theta-AHP triggered under control conditions at 40 min (black) superimposed on the theta-AHP triggered with ω-conotoxin MVIIC at 40 min (grey).

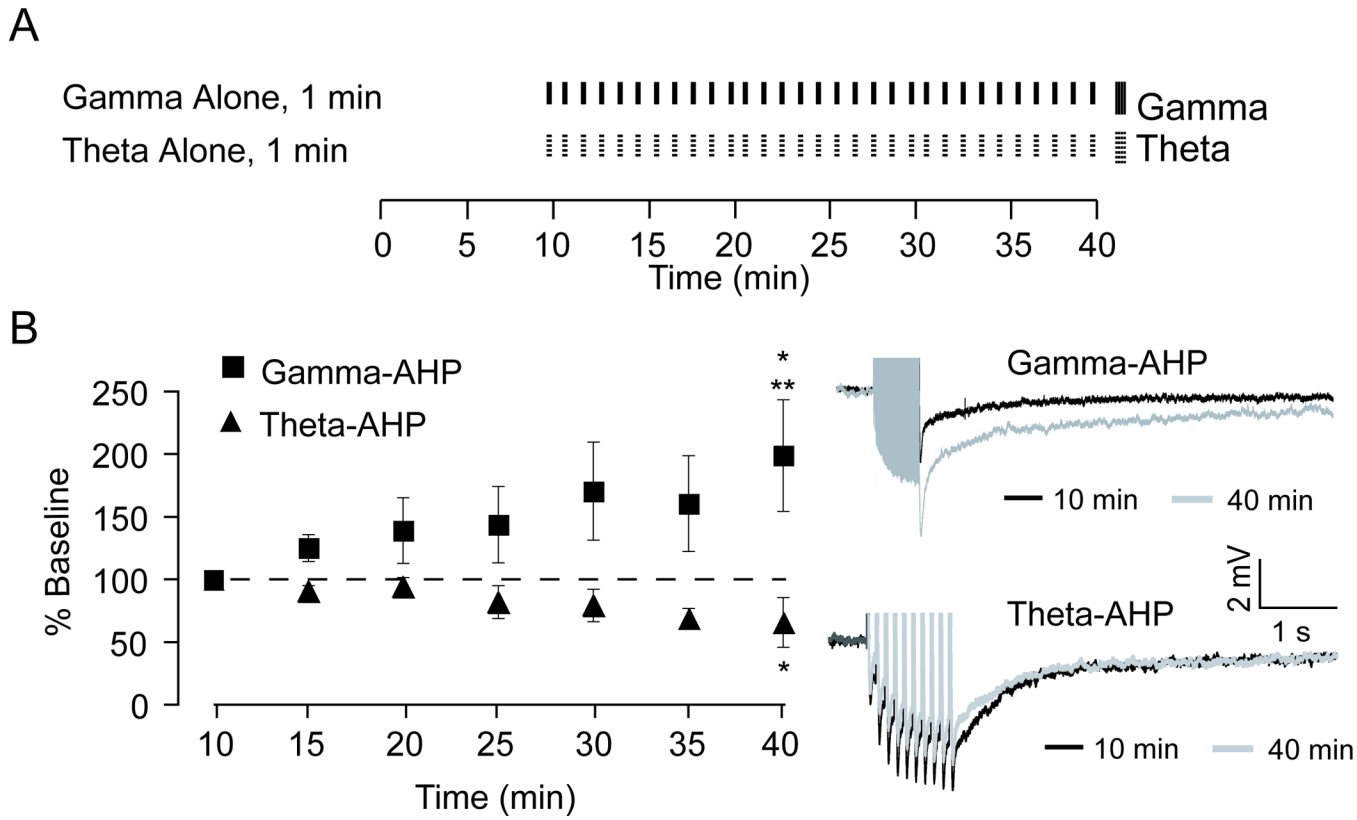

Figure 3. Bidirectional plasticity of the AHP depends on the firing pattern

A, Schematic diagram illustrates the stimulation protocols used to examine plasticity of the AHP. Neurons received either somatic action potential firing at 50 Hz (black) or theta-burst firing (dotted). B, Plot showing that 50 Hz firing alone produced a significant enhancement of the integral of the gamma-AHP measured at 40 min, relative to baseline (* $p < 0.05$) and to theta-AHP measured at 40 minutes (** $p < 0.05$). Theta-burst firing alone produced a significant reduction, relative to baseline, of the integral of the theta-AHP (* $p < 0.05$). Top right, representative trace of the gamma-AHP triggered at 10 min (black) superimposed on the gamma-AHP triggered at 40 min (grey). Bottom right, representative trace of the theta-AHP triggered at 10 min (black) superimposed on the theta-AHP triggered at 10 min (grey).

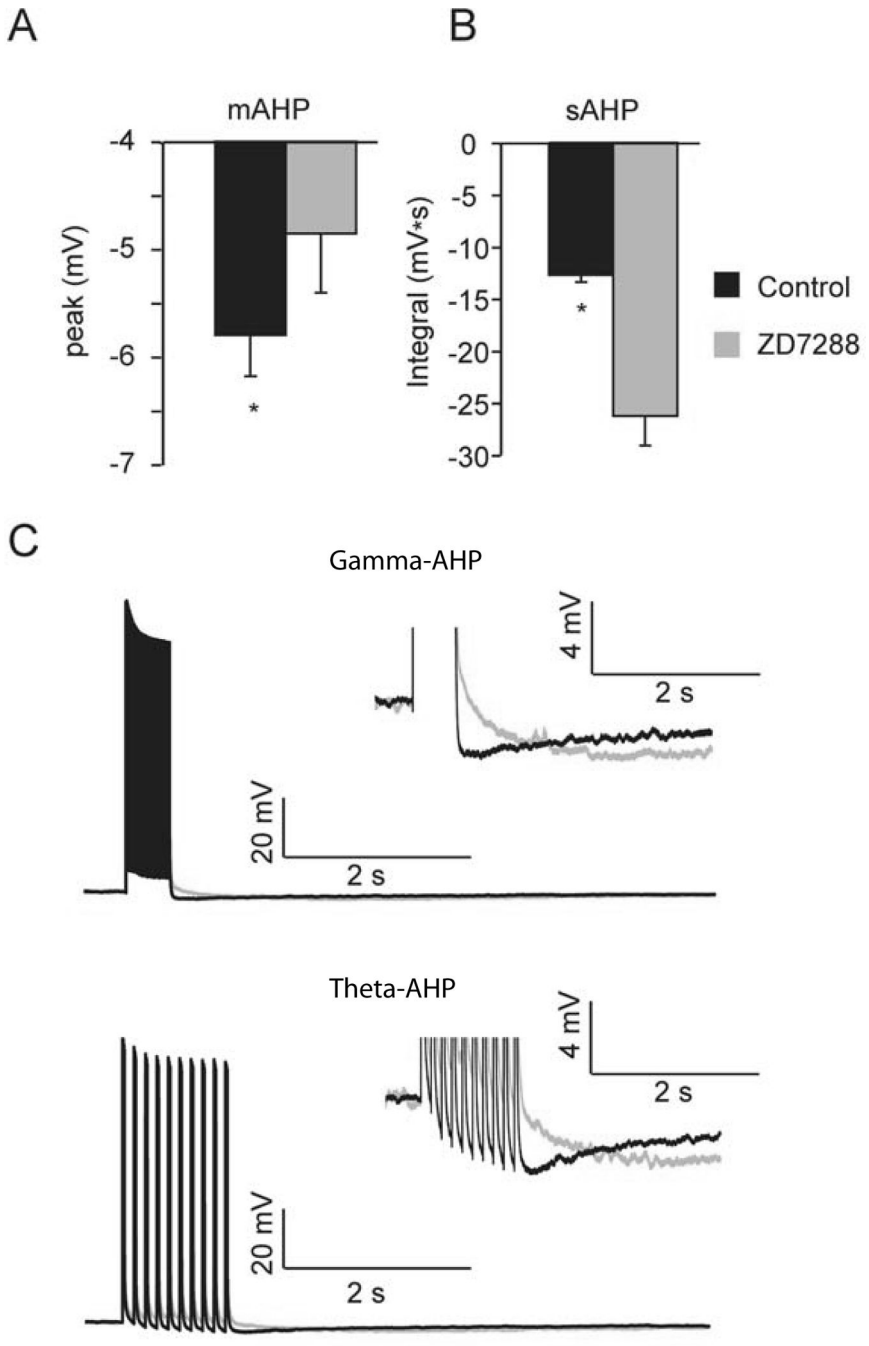

Figure 4. I_h contributes to the size and shape of the AHP

A, Summary graph illustrates the peak of AHP is significantly reduced in ZD7288 (theta-AHP and train-AHP pooled for comparison, *$p < 0.05$). B, Summary graph illustrates the integral of AHP is significantly increased in ZD7288 (*$p < 0.05$). C, Top, representative trace of the train-AHP triggered in aCSF (black) superimposed on the train-AHP triggered in ZD7288 (grey). Inset shows train-AHP on an expanded scale. Bottom, representative trace of the theta-AHP triggered in aCSF (black) superimposed on the theta-AHP triggered in ZD7288 (grey). Inset shows theta-AHP on an expanded scale. Data from train-AHP and theta-AHP were similar, and pooled for comparison in shown in panel A.

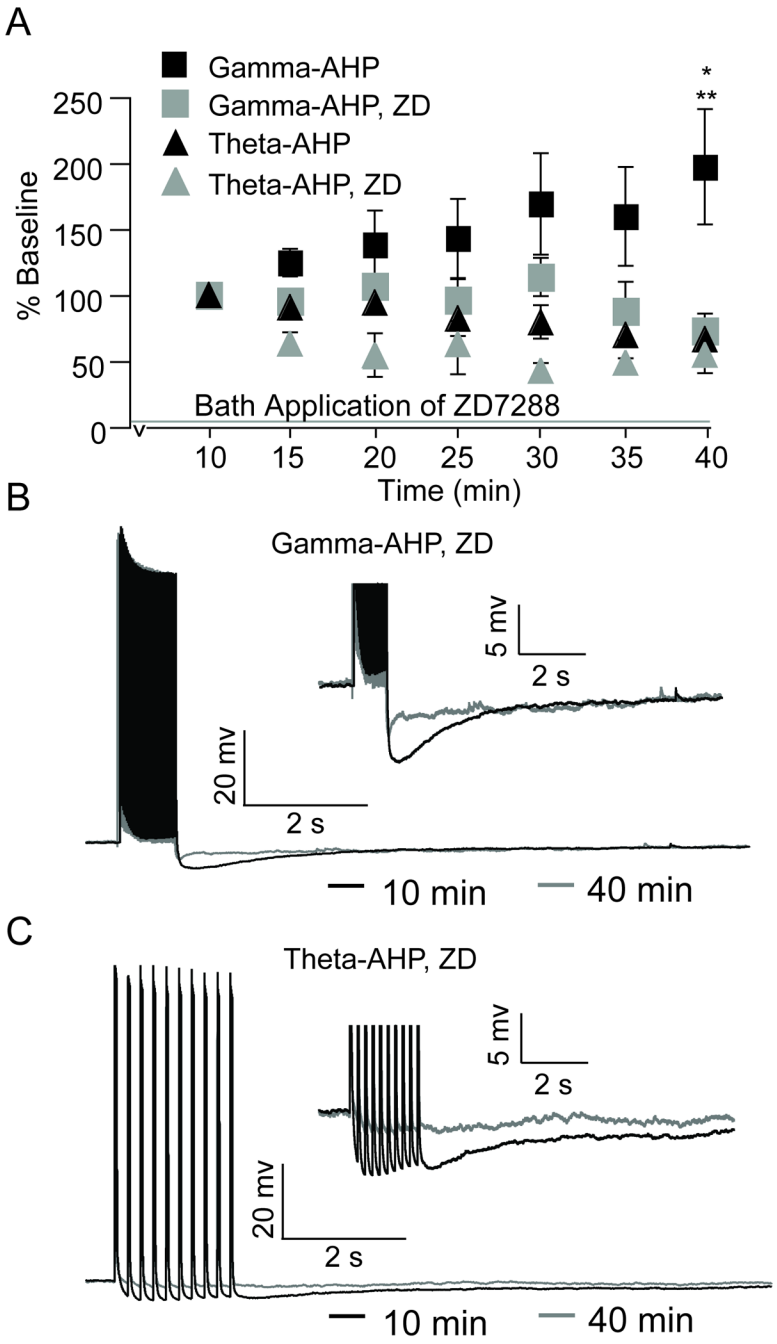

Figure 5. Enhancement of gamma-AHP is blocked by h-channel blockers

A, Plot showing that enhancement of the gamma-AHP (black squares), measured at 40 min, is blocked by the h-channel blocker ZD7288 (25 μM) (grey squares), whereas ZD7288 did not alter the theta-AHP (black triangles, control; grey triangles, ZD7288). B, Representative trace of the gamma-AHP triggered under ZD7288 at 10 min (black) superimposed on the gamma-AHP triggered under ZD7288 at 40 min (grey). Inset shows AHPs on an expanded scale. C, Representative trace of the theta-AHP triggered under ZD7288 at 10 min (black) superimposed on the theta-AHP triggered under ZD7288 at 40 min (grey). Inset shows AHPs on an expanded scale.

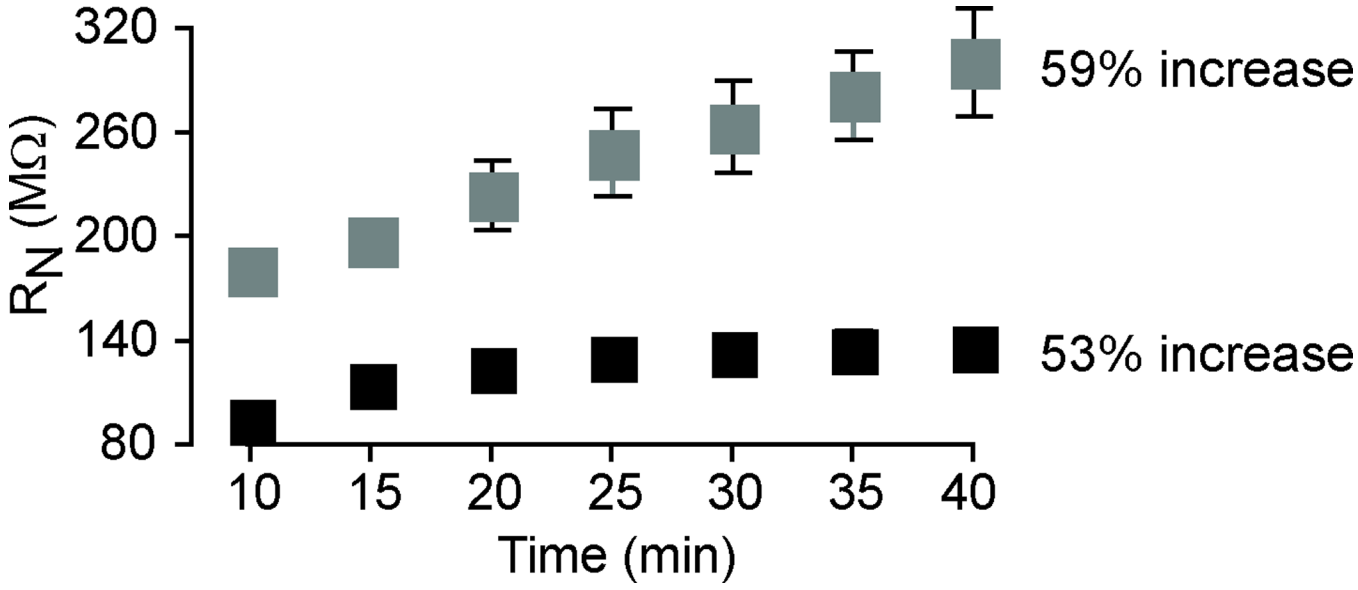

Figure 6. Input resistance (R_N) increases in the presence of H-channel blocker ZD7288

Time course and magnitude of enhancement of the R_N measured in neurons exposed to gamma-related firing under control conditions (black) and in ZD7288 (grey). The magnitude of R_N was significantly increased in ZD7288 at each time point (10 and 40 min) compared to the control condition ($p < 0.05$). However, the increase in R_N over time was comparable in both conditions.