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Deletion of the L-Type Calcium Channel $Ca_V1.3$ but not $Ca_V1.2$ Results in a Diminished sAHP in Mouse CA1 Pyramidal Neurons

Amy E. Gamelli,[1] Brandon C. McKinney,[2,3] Jessica A. White,[1] and Geoffrey G. Murphy[1,3,4]*

ABSTRACT: Trains of action potentials in CA1 pyramidal neurons are followed by a prolonged calcium-dependent postburst afterhyperpolarization (AHP) that serves to limit further firing to a sustained depolarizing input. A reduction in the AHP accompanies acquisition of several types of learning and increases in the AHP are correlated with age-related cognitive impairment. The AHP develops primarily as the result of activation of outward calcium-activated potassium currents; however, the precise source of calcium for activation of the AHP remains unclear. There is substantial experimental evidence suggesting that calcium influx via voltage-gated L-type calcium channels (L-VGCCs) contributes to the generation of the AHP. Two L-VGCC subtypes are predominately expressed in the hippocampus, $Ca_V1.2$ and $Ca_V1.3$; however, it is not known which L-VGCC subtype is involved in generation of the AHP. This ambiguity is due in large part to the fact that at present there are no subunit-specific agonists or antagonists. Therefore, using mice in which the gene encoding $Ca_V1.2$ or $Ca_V1.3$ was deleted, we sought to determine the impact of alterations in levels of these two L-VCGG subtypes on neuronal excitability. No differences in any AHP measure were seen between neurons from $Ca_V1.2$ knockout mice and controls. However, the total area of the AHP was significantly smaller in neurons from $Ca_V1.3$ knockout mice as compared with neurons from wild-type controls. A significant reduction in the amplitude of the AHP was also seen at the 1 s time point in neurons from $Ca_V1.3$ knockout mice as compared with those from controls. Reductions in both the area and 1 s amplitude suggest the involvement of calcium influx via $Ca_V1.3$ in the slow AHP (sAHP). Thus, the results of our study demonstrate that deletion of $Ca_V1.3$, but not $Ca_V1.2$, significantly impacts the generation of the sAHP. © **2009 Wiley-Liss, Inc.**

KEY WORDS: hippocampus; neuronal excitability; afterhyperpolarization; mouse; voltage-gated ion channel

INTRODUCTION

In the hippocampus, as well as other brain regions, bursts of action potentials are followed by a prolonged postburst afterhyperpolarization (AHP) that serves to limit further firing to a sustained depolarizing input (Alger and Nicoll, 1980; Hotson and Prince, 1980; Schwartzkroin and Stafstrom, 1980; Madison and Nicoll, 1984). A reduction in the AHP in CA1 pyramidal neurons accompanies learning in a variety of hippocampal-dependent tasks in multiple species including rabbits, rats and mice (Moyer et al., 1996; Oh et al., 2003; Ohno et al., 2006). Similarly, a reduction in the AHP has also been seen in piriform cortical neurons following odorant-discrimination learning (Saar et al., 1998).

There are also numerous reports documenting an age-related increase in the AHP (for recent review see Disterhoft and Oh, 2007) and this age-related increase in AHP is correlated with a decline in performance during learning and memory tasks that require the hippocampus (Moyer et al., 2000; Tombaugh et al., 2005). Finally, genetic and pharmacological manipulations that functionally oppose the age-related increase in the AHP (Oh et al., 1999; Murphy et al., 2004) act to ameliorate cognitive impairments in spatial learning that are typically observed in aged rodents (Dachir et al., 1997; Murphy et al., 2004; 2006a). These experimental results have led to the suggestion that plasticity of the AHP is a neurobiological substrate of learning which is sensitive to aging (Wu et al., 2002).

The postburst AHP in CA1 hippocampal pyramidal neurons is often described as having three components: a fast, medium and a slow AHP. The fast AHP (fAHP) occurs immediately after individual action potentials and lasts only 1–5 ms. The current underlying the fAHP (termed I_c) is calcium and voltage dependent, is blocked by low concentrations of TEA (Lancaster and Nicoll, 1987; Storm, 1987), iberiotoxin (Zhang and McBain, 1995), and paxilline (Knaus et al., 1994; Shao et al., 1999) indicating that the underlying channels are BK-type channels. In addition, voltage-gated potassium channels that underlie the A-type current have been implicated in rapid action potential repolarization (Hoffman et al., 1997; Johnston et al., 2000).

The medium AHP (mAHP) is typically observed after a burst of action potentials and has a decay constant of ~100 ms. Currently there is some debate as to the exact current or currents that underlie the mAHP. There are numerous reports implicating an apamin sensitive calcium-activated potassium current (Stocker et al., 1999; Shah and Haylett, 2000; Pedarzani et al., 2001; Sailer et al., 2002; Stackman et al., 2002; Gu et al., 2005). It seems likely that the apamin sensitive current (I_{AHP}) is gated by the small-

[1] **Molecular & Behavioral Neuroscience Institute, University of Michigan Medical School, Ann Arbor, Michigan;** [2] **Medical Scientist Training Program, University of Michigan Medical School, Ann Arbor, Michigan;** [3] **Neuroscience Graduate Program, University of Michigan Medical School, Ann Arbor, Michigan;** [4] **Department of Molecular & Integrative Physiology, University of Michigan Medical School, Ann Arbor, Michigan**

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*Correspondence to: Geoffrey G. Murphy, University of Michigan, 5037 Biomedical Science Research Building, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200. E-mail: murphyg@umich.edu

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conductance calcium-activated potassium channel SK2 because knockout mice in which the gene that codes for SK2 (*Kcnn2*) is deleted do not exhibit an apamin sensitive current (Bond et al., 2004). In addition, there are a number of reports demonstrating that bath application of apamin reduces the amplitude of the mAHP in CA1 pyramidal neurons (Norris et al., 1998; Oh et al., 2000; Kramar et al., 2004; Kaczorowski et al., 2007). However, other reports have failed to find an affect of apamin on the mAHP (Storm, 1989; Gu et al., 2005) and further suggest that the mAHP is not calcium dependent but is instead dependent upon the activation of KCNQ (I_M) and HCN (I_h) channels (Gu et al., 2005).

The slow AHP (sAHP) has a rather long time constant (1–5 seconds) and is voltage-independent (Storm, 1990). The current that underlies the sAHP, the I_{sAHP}, is calcium dependent and is influenced by a wide variety of neuromodulators including serotonin and norepinephrine (e.g., Pedarzani and Storm, 1993). Despite numerous efforts, the channels that give raise to the sAHP remain elusive.

At present, the precise source of calcium required for the activation of the various calcium-activated potassium currents that underlie the postburst AHP remains unclear. Several studies using L-type voltage-gated calcium channel (L-VGCC) blockers have shown that blockade of these channels leads to a reduction in the currents underlying the AHP in hippocampal neurons, suggesting that the AHP is generated by calcium influx via L-VGCCs (Rascol et al., 1991; Marrion and Tavalin, 1998; Shah and Haylett, 2000; Power et al., 2002; Lima and Marrion, 2007). In addition, an increase in both the activity and expression of L-VGCC in the hippocampus occurs with aging (Landfield and Pitler, 1984; Thibault and Landfield, 1996; Veng and Browning, 2002) and application of the L-VGCC blocker nimodipine results in a reduction of the AHP in neurons from aging animals (Moyer et al., 1992).

Despite the evidence suggesting a link between calcium influx via L-VGCCs and the AHP in neurons from both young and aging animals, it is not known which L-VGCC subtype is involved in generation of the AHP. In the rodent hippocampus, two L-VGCC subtypes are expressed: $Ca_V1.2$ and $Ca_V1.3$ (Hell et al., 1993). $Ca_V1.2$ is preferentially expressed in pyramidal cell soma and dendritic fields of areas CA1-CA3 and in granule cell soma and fibers in the dentate gyrus (Tippens et al., 2008), while $Ca_V1.3$ is expressed in cell bodies and proximal dendrites in CA1-CA3 (Hell et al., 1993). In addition, $Ca_V1.3$ is evenly distributed throughout the cell body of the neuron, while $Ca_V1.2$ is found in clusters in this region (Hell et al., 1993) and is observed in dendrites, axons, and axon terminals (Tippens et al., 2008). Taken together, these data suggest possible different functional roles for $Ca_V1.2$ and $Ca_V1.3$. Currently, it is not possible to assess the relative contributions of $Ca_V1.2$ and $Ca_V1.3$ to neuronal excitability with respect to the AHP, due to the lack of isoform-specific agonists or antagonists. In light of the role ascribed to the AHP during learning and the now well-documented age-related changes in the AHP, we have completed a series of experiments using mice in which the gene encoding $Ca_V1.3$ (Platzer et al., 2000; Clark et al., 2003) or $Ca_V1.2$ was deleted (McKinney et al., 2008; White et al., 2008) to determine the impact of deletion of these two L-VCGG subtypes on the AHP and neuronal excitability. The results of our study demonstrate that deletion of $Ca_V1.3$, but not $Ca_V1.2$, has a significant impact on the generation of the AHP in CA1 pyramidal neurons.

MATERIALS AND METHODS

Mice

For the $Ca_V1.2$ study, conditional knockout mice with a forebrain-specific (hippocampus and forebrain) deletion of $Ca_V1.2$ were used (McKinney et al., 2008; White et al., 2008). For these experiments mice heterozygous for the floxed $Ca_V1.2$ exon two allele were maintained on a 129SvEv genetic background. Experimental animals were generated by crossing heterozygous floxed $Ca_V1.2$ mice ($Ca_V1.2^{f/+}$ mice) with transgenic mice that expressed Cre-recombinase (Chen et al., 2006). Cre-recombinase expression was regulated by the calcium calmodulin kinase IIα (CaMKIIα) promoter which reaches peak expression levels postnatally (Kelly et al., 1987; Sugiura and Yamauchi, 1992) and restricted Cre-recombinase expression to glutamatergic neurons of the forebrain (Chen et al., 2006). These mice (termed here as the CaMK-Cre mice) were maintained on a C57BL/6 background (10+ generations). Offspring from the F1 cross that were heterozygous floxed and Cre positive (i.e., $Ca_V1.2^{f/+}$, CaMK-$Cre^{Cre/+}$) were then intercrossed (nonsibling) with mice heterozygous floxed and Cre negative (i.e., $Ca_V1.2^{f/+}$, CaMK-$Cre^{+/+}$) to achieve homozygous conditional knockout mice ($Ca_V1.2^{f/f}$, CaMK-$Cre^{Cre/+}$) and wild-type mice ($Ca_V1.2^{+/+}$, CaMK-$Cre^{+/+}$) on an F2 129Sve:C57Bl/6 hybrid background. For ease of reading, conditional knockout mice are referred to as $Ca_V1.2^{-/-}$ and wild-type mice are referred to as $Ca_V1.2^{+/+}$ throughout the remainder of the text. For the $Ca_V1.3$ study, knockout mice with a global deletion of the gene encoding $Ca_V1.3$ were used (Platzer et al., 2000; Clark et al., 2003; McKinney and Murphy, 2006). Mice were maintained on a C57BL/6 background by successively crossing heterozygous offspring with C57BL/6 wild-type mice. Experimental animals were generated by crossing heterozygous $Ca_V1.3$ mice with wild-type 129SvEv mice. Heterozygous offspring from the F1 cross were then intercrossed (nonsibling) to achieve homozygous knockout mice ($Ca_V1.3^{-/-}$) and wild-type mice ($Ca_V1.3^{+/+}$) on an F2 129Sve:C57Bl/6 hybrid background. For all experiments approximately equal numbers of adult (~6 months) male and female mice were used. All comparisons were made between knockout mice and wildtype littermates and the experimenter was kept blind as to the genotype throughout the experiment. All experiments were conducted in accordance with the guidelines set forth by the University of Michigan Committee on Use and Care of Animals.

Slice Preparation

Mice were anesthetized with isoflurane and decapitated. Whole-brain (minus the cerebellum) 400 μm sagittal sections

TABLE 1.

Biophysical Properties of CA1 Pyramidal Neurons From $Ca_V1.2^{+/+}$ and $Ca_V1.2^{-/-}$ Mice

	RMP (mV)	IR (mΩ)	AP threshold (mV)	AP width (ms)	AP height (mV)
$Ca_V1.2^{+/+}$	58.9 ± 1.0	150.6 ± 8.6	52.3 ± 0.7	1.4 ± 0.1	84.6 ± 1.8
$Ca_V1.2^{-/-}$	56.8 ± 1.8	151.1 ± 11.0	52.7 ± 0.7	1.3 ± 0.1	88.1 ± 3.1

There was no significant difference in any property measured. RMP and AP threshold values were not corrected for liquid junction potential. All data are presented as mean ± S.E.M.

RMP, resting membrane potential; IR, input resistance; AP, action potential.

were cut on a vibratome and rapid microdissection of both hippocampi was completed in ice-cold (<1°C) oxygenated sucrose-based cutting solution containing the following (in mM): 2.8 KCl, 1 $MgCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 1 $CaCl_2$, 206 sucrose, 26 $NaHCO_3$, 10 D-glucose, 0.40 ascorbic acid. Slices were transferred to a holding chamber filled with artificial cerebrospinal fluid (aCSF) containing the following (in mM): 124 NaCl, 2.8 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$, 10 D-glucose, 0.40 ascorbic acid at room temperature and remained there for at least 1 hour before being individually transferred to a submersion chamber and continuously perfused (~1.5 ml/min) with oxygenated aCSF heated to 31°C.

Electrophysiology

Whole-cell recordings were made from CA1 pyramidal neurons using a Dagan 3,900A amplifier in bridge mode. Neurons were visualized with an Olympus BX51WI upright microscope equipped with infrared differential interference contrast optics. Patch-pipettes made from Clark Borosilicate Standard Wall glass (Warner Instruments) and pulled using a P-97 Flaming-Brown pipette puller (Sutter Instruments) with resistances of 5–8 MΩ were used and filled with the following internal solution (in mM): 120 potassium methylsulfate, 20 KCl, 10 HEPES, 4 Na_2-ATP, 2 $MgCl_2$, 0.3 GTP, 0.2 EGTA, 7 phosphocreatine. Seal resistances of (>2 GΩ were achieved before rupturing into whole-cell mode. Action potentials were measured from rest and were analyzed for spike threshold, amplitude, and width. Spike width was measured at 1/2 of the action potential amplitude. Cells were held at 5 mV below action potential threshold and the AHP was studied using a 100-ms current step sufficient to elicit five action potentials. AHP measurements were made using the average of 10 successive traces from each neuron (10 s intersweep interval). Total integral area of the AHP was calculated from the time that the voltage trace crosses the baseline from the depolarized state until it returned to baseline. The duration of the AHP was measured as the time required for the AHP to return to baseline for at least 10 ms (maximum duration 10 s), starting at the offset of the stimulus. The peak AHP amplitude was calculated as the maximum negative voltage deflection (occurring at any time point during the entire AHP), relative to resting potential. Latency was calculated as the time at which the AHP achieved peak amplitude. Amplitude of the AHP was also calculated at 200 ms and 1 s after the offset of the stimulus used to generate the AHP to assess alterations in the medium and slow AHP, respectively (Oh et al., 2003). Accommodation was studied at rest in these neurons using a series of 500 ms current injection of increasing intensity (−0.05 nA to 0.4 nA, 0.05 nA steps).

Data were acquired and analyzed using pClamp 9.2. The number of neurons recorded from an individual animal ranged from 1 to 5 (average = 2.4 ± 0.2). For analysis, when recordings from multiple neurons were obtained, the values from individual neurons for each animal were averaged such that each animal contributed a single data point to the group average and thus the sample size reported refers to the number of animals and not the number of neurons. All values are expressed as mean ± standard error of the mean (S.E.M.). Statistical analysis was performed using student *t*-tests or two-way repeated ANOVA using Fisher's post hoc tests where appropriate.

RESULTS

Deletion of $Ca_V1.2$ Does not Impact Generation of the AHP

Whole-cell current clamp recordings were made from 11 $Ca_V1.2^{+/+}$ mice (24 neurons) and 9 $Ca_V1.2^{-/-}$ mice (19 neurons). Conditional deletion of $Ca_V1.2$ did not significantly impact resting membrane potential or input resistance (Table 1). Action potential threshold, height, and width were also not altered in $Ca_V1.2$ knockout mice when compared with wild-type littermates (Table 1). Deletion of $Ca_V1.2$ did not significantly impact the AHP recorded in CA1 pyramidal neurons. There were no differences in any of the AHP measures examined. We examined peak amplitude ($Ca_V1.2^{+/+}$: 4.8 ± 0.6 mV; $Ca_V1.2^{-/-}$: 5.2 ± 0.5 mV), latency to peak amplitude ($Ca_V1.2^{+/+}$: 44.5 ± 3.0 ms; $Ca_V1.2^{-/-}$: 46.5 ± 6.2 ms), area ($Ca_V1.2^{+/+}$: 2,965.4 ± 595.3 mV·ms; $Ca_V1.2^{-/-}$: 2,527.7 ± 119.1 mV·ms), and duration ($Ca_V1.2^{-/-}$: 4,303.0 ± 450.0 ms; $Ca_V1.2^{-/-}$: 3,544.3 ± 371.8 ms) in neurons from $Ca_V1.2^{-/-}$ and $Ca_V1.2^{+/+}$ neurons (Fig. 1A–F). In addition, we examined accommodation in neurons from both groups (Fig. 2). There was no difference in number of action potentials fired in response to a range of current steps in neurons from $Ca_V1.2^{-/-}$ mice as compared with those from $Ca_V1.2^{+/+}$

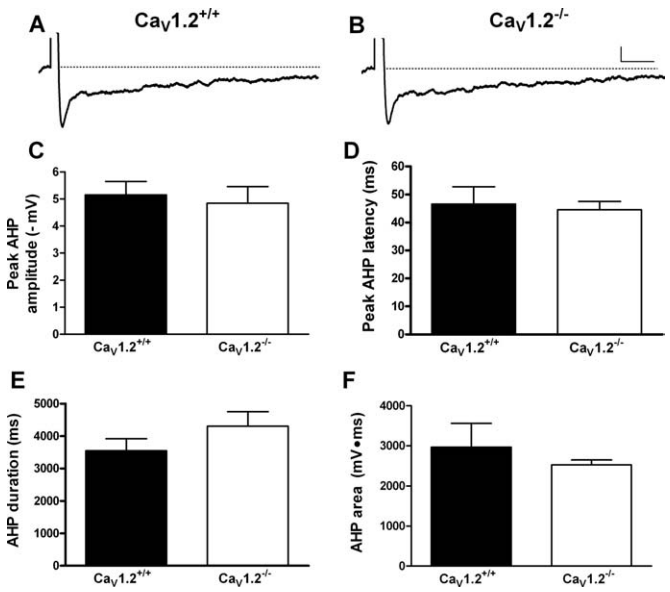

FIGURE 1. Deletion of $Ca_V1.2$ has no effect on the AHP in CA1 pyramidal neurons. Representative AHP traces (average of 10 successive sweeps) recorded from a CA1 neuron from a $Ca_V1.2^{+/+}$ (A) and $Ca_V1.2^{-/-}$ (B) mouse. There was no difference in any AHP measure, including peak amplitude (C), latency to peak (D), duration (E), or area of the AHP (F) between neurons from knockout and wild-type mice. Scale bar: 1 mV/500 ms. All data presented as mean ± S.E.M.

mice ($F_{[1,17]} = 2.0$, $P = 0.17$ main effect of genotype, two-way repeated measures ANOVA). In addition, we examined the peak and area of the AHP during these longer current steps and found no difference between $Ca_V1.2^{-/-}$ and $Ca_V1.2^{+/+}$ mice (data not shown).

Deletion of $Ca_V1.3$ Significantly Reduces the AHP

Whole cell recordings were made from 14 $Ca_V1.3^{+/+}$ mice (35 neurons) and 14 $Ca_V1.3^{-/-}$ mice (40 neurons). Similar to that observed in neurons from $Ca_V1.2^{-/-}$ mice, deletion of $Ca_V1.3$ did not have an effect on membrane properties such as resting membrane potential, input resistance, action potential threshold, or width when compared with wild-type littermates (Table 2). In addition we found no significant difference in the peak amplitude ($Ca_V1.3^{+/+}$: 4.3 ± 0.4 mV; $Ca_V1.3^{-/-}$: 4.2 ± 0.3 mV), latency to peak ($Ca_V1.3^{+/+}$: 54.8 ± 3.7 ms; $Ca_V1.3^{-/-}$: 49.4 ± 3.2 ms), or duration of the AHP ($Ca_V1.3^{+/+}$: 5,115.8 ± 309.8 μs; $Ca_V1.3^{-/-}$: 4,541.7 ± 320.2 ms) in CA1 pyramidal neurons (Fig. 3A–E). However, a significant difference was observed in the area of the AHP in neurons from $Ca_V1.3^{-/-}$ mice as compared with those from

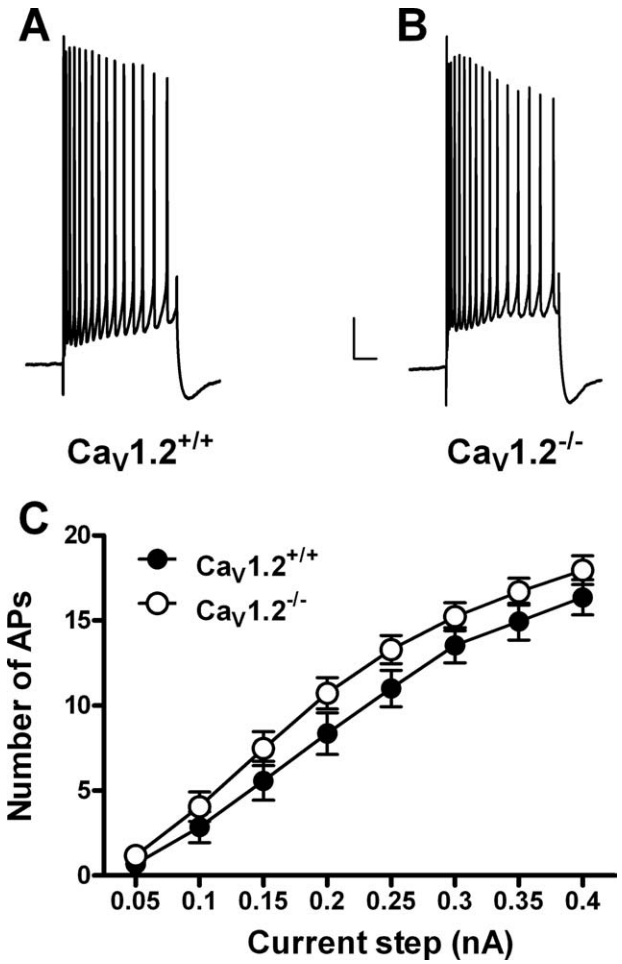

FIGURE 2. Deletion of $Ca_V1.2$ does not alter accommodation in CA1 pyramidal neurons. Representative accommodation traces from a neuron from a $Ca_V1.2^{+/+}$ (A) and $Ca_V1.2^{-/-}$ (B) mouse in response to a 0.35 nA current step (500 ms). No difference was seen in accommodation in neurons from either group (C); neurons from $Ca_V1.2^{+/+}$ (closed circles) and $Ca_V1.2^{-/-}$ (open circles) mice fired an equal number of action potentials in response to a given current step. Scale bar: 10 mV/500 ms. All data presented as mean ± S.E.M.

TABLE 2.

Biophysical Properties of CA1 Pyramidal Neurons From $Ca_V1.3^{+/+}$ and $Ca_V1.3^{-/-}$ Mice

	RMP (mV)	IR (mΩ)	AP threshold (mV)	AP width (ms)	AP height (mV)
$Ca_V1.3^{+/+}$	60.3 ± 0.7	150.3 ± 8.6	54.3 ± 0.4	1.4 ± 0.04	87.8 ± 2.1
$Ca_V1.3^{-/-}$	59.3 ± 0.7	155.6 ± 15.5	54.8 ± 0.5	1.4 ± 0.03	86.7 ± 2.6

Deletion of $Ca_V1.3$ did not significantly alter any of the properties that were measured. RMP and AP threshold values were not corrected for liquid junction potential. All data are presented as mean ± S.E.M.
RMP, resting membrane potential; IR, input resistance; AP, action potential.

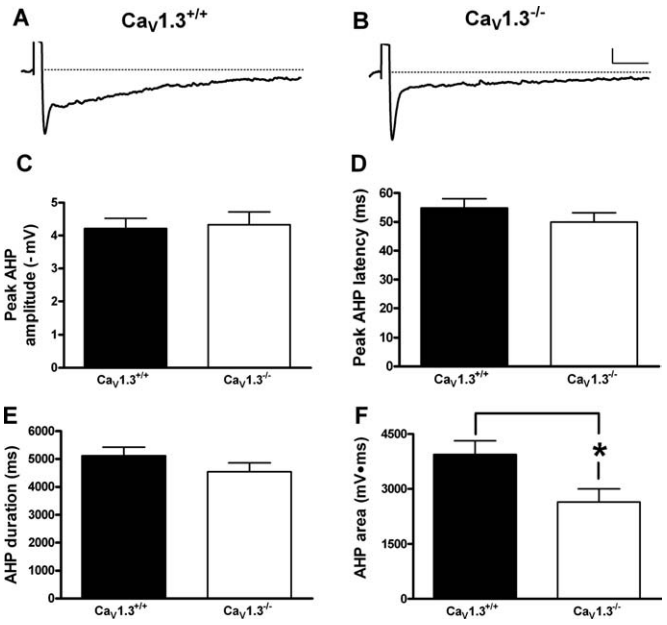

FIGURE 3. Deletion of $Ca_V1.3$ has a significant impact on generation of the AHP in CA1 pyramidal neurons. Representative AHP traces (average of 10 successive sweeps) in a neuron from a $Ca_V1.3^{+/+}$ (A) and $Ca_V1.3^{-/-}$ (B) mouse. No difference was seen in peak amplitude (C), latency to peak (D), or duration (E) of the AHP between neurons from $Ca_V1.3^{+/+}$ and $Ca_V1.3^{-/-}$ mice. However, a significant difference was seen in the area of the AHP (F) between neurons from both groups. *$P < 0.02$, unpaired *t*-test. Scale bar: 1 mV/500 ms. All data presented as mean ± S.E.M.

$Ca_V1.3^{+/+}$ mice (Fig. 3F). Neurons from $Ca_V1.3^{-/-}$ mice had a significantly smaller AHP area as compared with those from $Ca_V1.3^{+/+}$ mice ($Ca_V1.3^{+/+}$: 3,943.7 ± 370.6 mV·ms; $Ca_V1.3^{-/-}$: 2,647.2 ± 355.5 mV·ms, $P < 0.02$, unpaired *t*-test). As a change in the area of the AHP could result from alterations in either the medium or slow AHP, we measured the amplitude of the AHP at both 200 ms (measure of the medium AHP) and 1 s (measure of the slow AHP) after current stimulus offset. As shown in Figure 4A, there was a strong trend for a reduced AHP amplitude at 200 ms in neurons from $Ca_V1.3^{-/-}$ mice as compared with those from $Ca_V1.3^{+/+}$ mice, although this difference was not statistically significant ($Ca_V1.3^{+/+}$: 1.5 ± 0.2 mV; $Ca_V1.3^{-/-}$: 1.1 ± 0.1 mV, $P = 0.07$, unpaired *t*-test). Importantly, analysis of the AHP amplitude at 1 s (Fig. 4B) revealed a significantly smaller amplitude in neurons from $Ca_V1.3^{-/-}$ mice as compared with those from $Ca_V1.3^{+/+}$ mice ($Ca_V1.3^{+/+}$: 1.2 ± 0.1 mV; $Ca_V1.3^{-/-}$: 0.8 ± 0.09 mV, $P = 0.01$, unpaired *t*-test) further suggesting a preferential role for $Ca_V1.3$ in the generation of the sAHP. We also examined accommodation in neurons from both groups of mice (Fig. 5) and found no difference in accommodation in neurons from $Ca_V1.3^{-/-}$ mice as compared with those from $Ca_V1.3^{+/+}$ mice ($F_{[1,22]} = 0.32$, $P = 0.6$). Similar results were obtained when the interspike interval was analyzed (data not presented).

DISCUSSION

The principal finding of this study is that deletion of the L-type calcium channel pore-forming subunit $Ca_V1.3$ results in a significant reduction in the postburst AHP in pyramidal neurons within the CA1 region of the mouse hippocampus. In addition we found that conditional deletion of the gene encoding the $Ca_V1.2$ L-type calcium channel subtype has no impact on generation of the AHP. This study is the first to our knowledge to demonstrate the requirement for the $Ca_V1.3$, but not $Ca_V1.2$ subtype for generation of the AHP. Specifically, neurons from $Ca_V1.3^{-/-}$ mice had a significantly reduced AHP area as compared to neurons from $Ca_V1.3^{+/+}$ mice. In addition, there was a significant reduction of the AHP amplitude measured at 1 s after current stimulus offset in neurons from knockout mice as compared with wild-type controls demonstrating that deletion of $Ca_V1.3$ primarily impacts the sAHP. In addition, we observed a strong trend toward a reduction when similar measurements were made at 200 ms after the current stimulus offset, suggesting that deletion of $Ca_V1.3$ may also have a modest impact on the mAHP. These effects on the AHP in CA1 neurons from $Ca_V1.3^{-/-}$ mice were seen in the absence of any

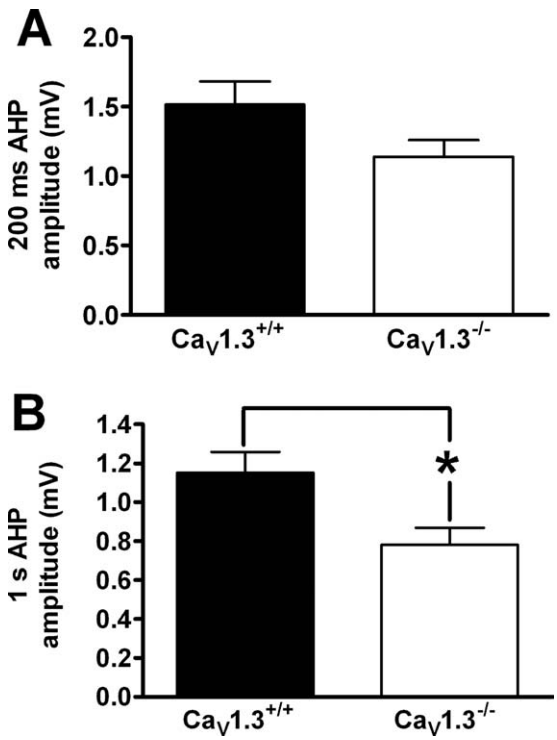

FIGURE 4. Deletion of $Ca_V1.3$ preferentially impacts the slow AHP. A strong trend was observed for a smaller AHP amplitude at 200 μs in neurons from $Ca_V1.3^{-/-}$ mice as compared to those from $Ca_V1.3^{+/+}$ mice, suggesting a modest reduction in mAHP in the knockout animals (A). A significant reduction was seen in the AHP amplitude at 1 s between neurons from $Ca_V1.3^{-/-}$ mice as compared neurons from $Ca_V1.3^{+/+}$ mice, demonstrating a reduction in sAHP in knockout animals (B). *$P = 0.02$, unpaired *t*-test. All data presented as mean ± S.E.M.

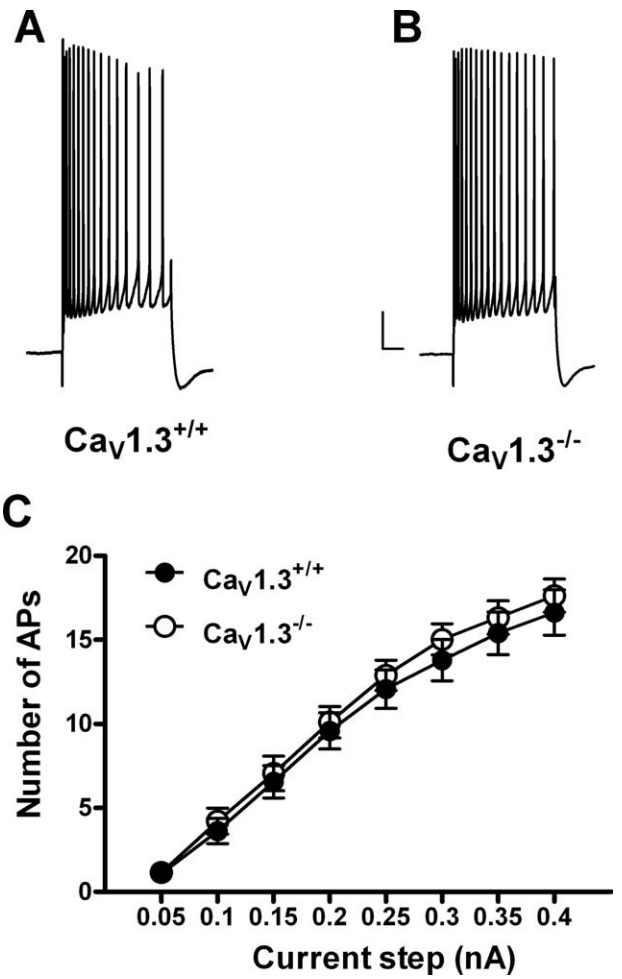

FIGURE 5. Deletion of $Ca_V1.3$ does not impact accommodation in CA1 pyramidal neurons. Representative accommodation traces from a neuron from a $Ca_V1.3^{+/+}$ (A) and $Ca_V1.3^{-/-}$ (B) mouse in response to a 0.35 nA current step (500 μs). No difference was seen in accommodation in neurons from either group (C); neurons from $Ca_V1.3^{+/+}$ (closed circles) and $Ca_V1.3^{-/-}$ (open circles) mice fired an equal number of action potentials in response to a given current step. Closed circles: $Ca_V1.3^{+/+}$, open circles: $Ca_V1.3^{-/-}$. Scale bar: 10 mV/500 ms. All data presented as mean ± S.E.M.

effects on basic membrane properties of these neurons. Conversely, we did not observe any significant changes in the AHP or spike accommodation in neurons from $Ca_V1.2^{-/-}$ mice. These results are significant in that they suggest that the calcium that is gated by $Ca_V1.2$ does not have functional access to the calcium activated potassium channels that underlie the sAHP. This might reflect the differential cellular distribution of the two different Ca_V calcium channel subtypes (Hell et al., 1993) and would suggest that $Ca_V1.2$ is not located in the same cellular compartment as the calcium activated potassium channels that generate the sAHP. Alternatively, it is possible that the both channels reside in the same cellular compartment but the diffusion distance between the $Ca_V1.2$ channels and the potassium channels that underlie the sAHP is sufficiently great that under normal physiological conditions (i.e., brief repetitive spiking) the relative contribution of $Ca_V1.2$ is negligible.

Interestingly, deletion of $Ca_V1.3$ had no effect on the peak amplitude of the AHP. In rats and rabbits, the peak AHP amplitude in CA1 pyramidal neurons is thought to be representative of the mAHP and is a result of overlapping activation of I_{AHP} and sI_{AHP}. In both rats and rabbits, the peak AHP occurs around ~100–200 ms, consistent with activation of both I_{AHP} and sI_{AHP} (Coulter et al., 1989; Oh et al., 2003). However, in the current study we found that the peak amplitude of the AHP in mouse CA1 pyramidal neurons occurs around ~50 ms. Recent studies have revealed that I_M is predominately active during this phase of the AHP (Peters et al., 2005; Kuo et al., 2007). Given that I_M is not a calcium-activated potassium current, it is not surprising that deletion of $Ca_V1.3$ did not impact the peak amplitude of the AHP.

Despite the impact of deletion of $Ca_V1.3$ on the sAHP, no concomitant reduction was seen in accommodation. This may seem surprising given previous reports (Madison and Nicoll, 1984; Storm, 1990) that the sAHP modulates accommodation in CA1 pyramidal neurons (but see also Jones and Heinemann, 1988; Colling et al., 1996; Oh et al., 2003). One possibility is that in our accommodation experiments the duration of the current steps was limited to 500 ms whereas many previous studies have used longer pulses ranging from 600 to 1,000 ms and thus had we used longer pulses this may have revealed a significant decrease in accommodation in the $Ca_V1.3$ knockout mice. This seems unlikely given that in mice this 500 ms window brackets the largest portion of the hyperpolarizing deflection. In addition, we have recently demonstrated that in principle neurons in the amygdala, deletion of $Ca_V1.3$ decreases the sAHP as well as spike accommodation (McKinney et al., 2009). The major difference between these two studies is the relative size of the sAHP. In the current study, the sAHP (measured at 1,000 ms) is ~1.2 mV whereas in the amygdala the sAHP is over double that ~−2.9 mV. Although the sAHP in our recordings is somewhat small when compared with that recorded in rats with sharp electrodes, it is in line with recent reports using mice with comparable recording methods (Weiss et al., 2005; Kaczorowski et al., 2007; Kaczorowski and Disterhoft, 2009). Therefore, it seems likely that in mouse CA1 hippocampal pyramidal neurons where the sAHP is relatively small, deletion of $Ca_V1.3$ has minimal impact upon spike accommodation.

Although deletion of $Ca_V1.3$ did result in a significant reduction of the sAHP, a residual sAHP was still observed in neurons from $Ca_V1.3^{-/-}$ mice. This finding is consistent with previous studies demonstrating the lack of complete abolition of the I_{sAHP} using pharmacological blockade of L-VGCCs (Shah and Haylett, 2000; Power et al., 2002; Lima and Marrion, 2007), suggesting that additional calcium sources may also contribute to generation of the sAHP. One possibility is that the residual sAHP observed in our study results from calcium influx through other voltage gated calcium channels. Indeed it has been previously reported that bath application of omega-conotoxin GIVA significantly reduced the I_{sAHP} (Shah

and Haylett, 2000) in rat pyramidal neurons in culture, suggesting that N-type calcium channels may also contribute to the sAHP (but see also Dutar et al., 1989). To date, little work has been done elucidating the calcium sources of the AHP in mouse CA1 pyramidal neurons; it is possible that in mouse hippocampal neurons N, P/Q, R, and/or T-type calcium channels play a greater role in generation of the AHP than previously observed in rat neurons.

Although our experiments with the $Ca_V1.2$ knockout mice indicate that $Ca_V1.2$ does not normally modulate the sHAP, an alternate possibility is that genetic ablation of $Ca_V1.3$ results in some sort of compensatory upregulation of $Ca_V1.2$. This seems unlikely as it has been previously reported that deletion of $Ca_V1.3$ in these mice does not lead to compensatory upregulation of $Ca_V1.2$ as measured by high affinity (+)[^{3}H]isradipine binding assays, in situ hybridization and Western blot analysis (Clark et al., 2003). In addition, $Ca_V1.2$ and $Ca_V1.3$ differ significantly in their gating properties and thresholds of activation (Xu and Lipscombe, 2001; Helton et al., 2005) as well as their cellular distributions (Hell et al., 1993). Thus, a compensatory shift would not only mean a change in expression levels but would also necessitate a significant change in biophysical properties and distribution for $Ca_V1.2$.

Finally, as is the case with all experiments that utilize knockout mice, some consideration regarding potential nonspecific changes need be made. In light of the fact that the identity of the channel (or channels) that mediate the sAHP remains unknown, it is possible that deletion of $Ca_V1.3$ resulted in a concomitant reduction in the expression or trafficking of the calcium activated potassium channels that underlie the sAHP. While our data do not specifically rule this out as a possibility, if this were the case, then the $Ca_V1.3$ knockout mice might be a useful tool in the establishment of the identity of these channels.

As previously mentioned, there are multiple reports documenting an age-related increase in the sAHP (Disterhoft and Oh, 2007). Furthermore, there is ample evidence that the age-related increase in the sAHP results from an increase in L-type calcium channel density (recently reviewed by Thibault et al., 2007). The best evidence to date is that an increase in the $Ca_V1.3$ pore-forming subunit is responsible for the age-related increase in the sAHP. Landfield and colleagues have demonstrated that magnitude of the age-related increase in L-type currents was correlated with mRNA levels of $Ca_V1.3$-as determined by RT-PCR-in young and aged animals (Chen et al., 2000) which supports earlier experiments from the same group utilizing in situ hybridization and ribonuclease protection assays, that demonstrated that there is an up-regulation of hippocampal $Ca_V1.3$ subunit mRNA in aged rodents (Herman et al., 1998). In addition, using antibodies directed against $Ca_V1.3$, Browning and colleagues have reported finding an age-related increase in expression of $Ca_V1.3$ protein within the CA1 region of the hippocampus in rats (Veng and Browning, 2002; Veng et al., 2003). Conversely, there does not appear to be an up-regulation of $Ca_V1.2$ protein in hippocampal tissue harvested from aged mice that are cognitively impaired (Murphy et al., 2006a,b). Although we did not examine aged animals in the present study, our results would support the hypothesis that the age-related increases observed in the sAHP are directly attributable to an increase in channel density of $Ca_V1.3$.

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