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The critical role of persistent sodium current in hippocampal gamma oscillations

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Abstract

Gamma network oscillations in the brain are fast rhythmic network oscillations in the gamma frequency range (~30-100 Hz), playing key roles in the hippocampus for learning, memory, and spatial processing. There is evidence indicating that GABAergic interneurons, including parvalbumin-expressing basket cells (PVBCs), contribute to cortical gamma oscillations through synaptic interactions with excitatory cells. However, the molecular, cellular, and circuit underpinnings underlying generation and maintenance of cortical gamma oscillations are largely elusive. Recent studies demonstrated that intrinsic and synaptic properties of GABAergic interneurons and excitatory cells are regulated by a slowly inactivating or non-inactivating sodium current (*i.e.*, persistent sodium current, I_{NaP}), suggesting that I_{NaP} is involved in gamma oscillations. Here, we tested whether I_{NaP} plays a role in hippocampal gamma oscillations using pharmacological, optogenetic, and electrophysiological approaches. We found that I_{NaP} blockers, phenytoin (40 μ M and 100 μ M) and riluzole (10 μ M), reduced gamma oscillations induced by optogenetic stimulation of CaMKII-expressing cells in CA1 networks. Whole-cell patch-clamp recordings further demonstrated that phenytoin (100 μ M) reduced I_{NaP} and firing frequencies in both PVBCs and pyramidal cells without altering threshold and amplitude of action potentials, but increased rheobase in both cell types. These results suggest that I_{NaP} in pyramidal cells and

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Declarations of interest
None.

PVBCs is required for hippocampal gamma oscillations, supporting a pyramidal-interneuron network gamma model. Phenytoin-mediated modulation of hippocampal gamma oscillations may be a mechanism underlying its anticonvulsant efficacy, as well as its contribution to cognitive impairments in epilepsy patients.

Keywords

Optogenetics; Pyramidal-interneuron network gamma (PING); Parvalbumin-expressing interneurons; Antiepileptic drugs; Cognitive impairment

1. Introduction

Gamma waves are fast rhythmic network oscillations in the gamma frequency range (~30-100 Hz) (Buzsáki and Wang, 2012; Colgin, 2016; Sohal, 2016). They are commonly observed in many brain areas including the hippocampus, visual cortical areas, and entorhinal cortex (Buzsáki and Wang, 2012). Hippocampal gamma oscillations may play a key role in memory operations (e.g., memory formation and retrieval) and attentional selection (Colgin and Moser, 2010; Engel and Singer, 2001). Gamma oscillations may be necessary for normal cognitive functions in healthy brains, as compromised gamma oscillations in several major neurological and psychiatric disorders (e.g., Alzheimer's disease, epilepsy, and schizophrenia) are closely associated with cognitive dysfunction in those brain disorders (Cho et al., 2015; Dugladze et al., 2007; Mably and Colgin, 2018; Nakazono et al., 2018). Perisomatic inhibition critically contributes to network gamma oscillations in several experimental models (Buzsáki and Wang, 2012; Sohal, 2016). However, the cellular mechanisms underlying gamma oscillations and how they are disrupted in neurological diseases are not fully understood.

How do gamma oscillations arise from neuronal circuits in the hippocampus? At least two distinct models of cortical gamma oscillations have been proposed: circuit-based models and intrinsic membrane resonance models. In circuit-based models, GABAergic interneurons contribute to gamma oscillations through synaptic interactions with excitatory cells (e.g., CA1 pyramidal cells) (i.e., PING model, pyramidal-interneuron network gamma) or other GABAergic interneurons (i.e., ING model, interneuron network gamma) (Buzsáki and Wang, 2012). In the membrane resonance models, intrinsic resonance properties of the neuronal membrane related to cytoarchitecture and ion channel biophysics are thought to cause parvalbumin-positive basket cells (PVBCs) to preferentially fire at gamma frequencies and to autonomously produce intrinsic subthreshold membrane oscillations at gamma frequencies (Kang et al., 2018; Lee et al., 2018; Pike et al., 2000). Given that both intrinsic and synaptic properties of individual neurons in a neuronal network are critical factors for gamma oscillations, the two distinct models of gamma oscillations are not mutually exclusive.

While there is a general agreement that PVBC-mediated perisomatic inhibition is mechanistically critical for generation of gamma oscillations (Buzsáki and Wang, 2012), the ionic mechanisms underlying intrinsic and synaptic properties of hippocampal neurons are not fully understood. A slowly inactivating or non-inactivating sodium current, known as the

persistent sodium current (I_{NaP}), is known to regulate intrinsic and synaptic properties of both inhibitory and excitatory cells within and outside the hippocampus, including subthreshold membrane oscillations, resonant properties, repetitive firing, and synaptic amplification (Hsu et al., 2018; Kang et al., 2018; Stafstrom, 2007). Specifically, PVBCs manifest I_{NaP} -dependent intrinsic subthreshold membrane oscillations (Kang et al., 2018) and firing preference at gamma frequencies (Pike et al., 2000). The intrinsic oscillatory and resonant properties of PVBCs have been implicated in network gamma oscillations as previously proposed (Lee et al., 2018; Llinás, 1988; Pike et al., 2000). I_{NaP} , expressed in CA1 pyramidal cells, regulates synaptic amplification and contributes to spatial processing in pyramidal cells (Hsu et al., 2018). Given that intrinsic and synaptic properties of PVBCs and pyramidal cells are regulated by I_{NaP} and that those properties are key factors necessary for gamma oscillations, neuronal I_{NaP} in the hippocampus is expected to be critically involved in the generation or regulation of gamma oscillations. However, the role of I_{NaP} in hippocampal gamma oscillations remains largely unknown.

In the current study, we set out to test the function of I_{NaP} in hippocampal gamma oscillations by using pharmacological, optogenetic, and electrophysiological approaches. We used CA1 network oscillations, since prior studies showed that GABAergic interneurons and pyramidal cells in the CA1 manifest I_{NaP} -dependent synaptic or intrinsic properties that are critical to gamma oscillations (Hsu et al., 2018; Kang et al., 2018; Lee et al., 2018). We induced network gamma oscillations in the mouse hippocampus by selective optogenetic stimulation of Ca^{2+} /calmodulin-dependent protein kinase II-alpha (CaMKII-alpha)-expressing cells (e.g., CA1 pyramidal cells), and examined the actions of two I_{NaP} blockers, phenytoin and riluzole, on network gamma oscillations. Phenytoin and riluzole reduced hippocampal gamma oscillations. Whole-cell patch-clamp recordings demonstrated that both PVBCs and pyramidal cells manifested I_{NaP} , which were blocked by phenytoin, leading to a decrease in the intrinsic excitability in those cells. These results demonstrate cellular mechanisms underlying the modulation of hippocampal gamma oscillations by selective I_{NaP} blockers, which may be relevant for their anticonvulsant mechanism of action.

2. Materials and Methods

All experimental procedures were carried out in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of Arkansas for Medical Sciences and the University of Kentucky.

2.1. Animals

To express channelrhodopsin2 (ChR2) in CA1 pyramidal cells for optogenetic generation of gamma oscillations in the CA1 subregion, CaMKII-Cre mice (the Jackson Laboratory stock # 005359) were crossed with floxed ChR2 mice (the Jackson Laboratory stock # 012569) to produce mice expressing ChR2 in CaMKII-expressing cells (referred to as CaMKII-ChR2 mice in this study). To efficiently target PVBCs for whole-cell patch-clamp recordings, we crossed Ai9 Cre reporter mice (the Jackson Laboratory stock # 007905) with PV-Cre mice (the Jackson Laboratory stock # 008069) to produce mice expressing tdTomato in PV + cells (referred to as PV-TOM mice in this study) as previously described (Kang et al., 2018). Both

genders of 4 to 8-week-old CaMKII-ChR2 mice, PV-TOM mice, and C57BL/6 mice (The Jackson Laboratory stock # 000664) were used in the current study.

2.2. Brain slice preparation

The mice were deeply anesthetized with isoflurane and their brains were removed. The brains were submerged in cold, oxygenated (95% O₂ and 5% CO₂) slicing medium containing (in mM): 85 NaCl, 1.25 NaH₂PO₄, 4 MgCl₂, 0.5 CaCl₂, 24 NaHCO₃, 2.5 KCl, 75 sucrose, and 25 glucose. Horizontal hippocampal slices (450 µm or 300 µm thick for LFP recordings or whole-cell patch-clamp recordings, respectively) were cut using a vibratome (Leica VT 1200; Leica Microsystems, Buffalo Grove, IL, USA). The solution was continuously oxygenated with 95% O₂ and 5% CO₂. Slices were initially maintained at 33 °C for 30 min, then at room temperature (22 ± 1 °C) until they were used for electrophysiological recordings.

2.3. Optogenetics and LFP recordings

We used 450 µm-thick horizontal hippocampal slices from 4 to 8-week-old CaMKII-ChR2 mice. The slices were transferred to a submersion recording chamber. To increase oxygen diffusion to the deepest part of the hippocampal slices in the recording chamber, oxygenated artificial cerebrospinal fluid (ACSF) was supplied to both sides of the mounted slice by sandwiching the slice between two slice harps. ACSF contained (in mM) 126 NaCl, 2.5 KCl, 26 NaHCO₃, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, and 10 glucose. Slices were superfused with ACSF at a rate of 2.5ml/min. LFP recordings were obtained from the CA1 pyramidal cell layer with glass pipettes (1-2 MΩ) filled with ACSF at 33 °C using a MultiClamp700B amplifier (Molecular Devices: San Jose, CA, USA). Signals were sampled at 10 kHz, low-pass filtered at 3 kHz using a Digidata 1440A analog-to-digital digitizer (Molecular Devices: San Jose, CA, USA), and stored on a PC.

Focal 470 nm blue light was delivered through the epifluorescence port of a Nikon Eclipse FN-1 with high-power light guide coupled LED source (GCS-0470-50-A0510; Mightex, Toronto, Ontario, Canada), a light source control module (BLS-13000-1; Mightex, Toronto, Ontario, Canada), and Digidata 1440A (for analogue voltage signals). Blue light (470 nm) was delivered in 1.4 s ramps of increasing intensity through a 40 × objective to the CA1 (light power density from near zero to 1.36 mW/mm², 4.47 mW/mm², or 11.3mW/mm²). The power density was estimated from the field of view (550 µm) of 40 × objective (Fluor 40 × NA 0.8; Nikon Instrument Inc., Melville, NY, USA) and light power, which was measured under the 40 × objective using an optical power meter (PM100D; Thorlabs Inc., Newton, New Jersey, USA) and a microscope slide photodiode power sensor (S170C; Thorlabs Inc., Newton, New Jersey, USA). Blue light was always centered on the pyramidal cell layer of the CA1 where LFP recordings were performed so that blue light was delivered to proximal stratum radiatum, stratum pyramidale, and stratum oriens of the CA1. Such light ramps produced stable CA1 gamma oscillations for at least 1 h when CA1 gamma oscillations were evoked every 2 min.

2.4. Whole-cell patch-clamp recordings

As described previously (Kang et al., 2018), we used 300 μm -thick horizontal hippocampal slices from PTOM mice and C57BL/6 mice. The slices were transferred to a submersion recording chamber. Slices were superfused with one of two types of ACSF described below at a rate of 2.5 ml/min. Whole-cell patch-clamp recordings were performed with glass pipettes (3–5 M Ω) filled with one of two types of pipette solution described below at 33 $^{\circ}\text{C}$. Whole-cell patch-clamp recordings were performed using a MultiClamp 700B amplifier. Signals were sampled at 20 kHz, low-pass filtered at 10 kHz using a Digidata 1440A analog-to-digital digitizer, and stored on a PC.

I_{NaP} was studied using a high Cs^+ pipette solution similar to that described in published studies (Ottolini et al., 2017) (in mM: 140 CsF, 2 MgCl_2 , 1 EGTA, 10 HEPES, 4 Na_2ATP , 0.3 NaGTP , and 10.7 biocytin). All recorded neurons were later confirmed by their morphological properties (for pyramidal cells and PVBCs) and tdTomato positivity (for PVBCs). I_{NaP} was recorded in ACSF containing (in mM): 30 NaCl, 120 TEA-Cl, 10 NaHCO_3 , 1.6 CaCl_2 , 2 MgCl_2 , 0.2 CdCl_2 , and 10 glucose (this ACSF is referred to as I_{NaP} -ACSF in this study). We added synaptic receptor antagonists (NBQX, 10 μM ; APV, 40 μM ; SR95531, 10 μM ; CGP55845, 2 μM) to I_{NaP} -ACSF to block AMPA, NMDA, GABA_A , and GABA_B receptors, respectively. We targeted PV+ interneurons with large multipolar somata in the stratum pyramidale or the stratum oriens with epifluorescence microscopy for whole-cell patch-clamp recordings. Slow voltage ramps (from -80 mV to -20 mV , 50 mVs^{-1} ; one ramp per minute) were used to generate I_{NaP} in pyramidal cells and PVBCs in a way similar to that described in prior studies (Ottolini et al., 2017; Royeck et al., 2015). After stable current responses to the voltage ramps were established, tetrodotoxin (TTX, 1 μM) was bath applied to block voltage-gated sodium current in recorded neurons. The differences in current responses between non-TTX and TTX conditions were measured and referred to as I_{NaP} in this study. Series resistances were continuously monitored and recordings were discarded if the resistance increased $> 20\%$ or 20 M Ω .

To determine the effects of phenytoin on the intrinsic properties of pyramidal cells and PVBCs, whole-cell patch-clamp recordings were performed in current clamp mode using potassium gluconate-based pipette solution containing (in mM) 126 K-gluconate, 4 KCl, 10 HEPES, 4 ATP-Mg, 0.3 GTP-Na, 10 phosphocreatine, and 10.7 biocytin with a pH of 7.2 and osmolarity of 290 mOSM. Electrical recordings were performed at 33 $^{\circ}\text{C}$ in oxygenated ACSF containing (in mM): 126 NaCl, 2.5 KCl, 26 NaHCO_3 , 2 CaCl_2 , 2 MgCl_2 , 1.25 NaH_2PO_4 , and 10 glucose. The recording ACSF also included the four synaptic blockers (NBQX, 10 μM ; APV, 40 μM ; SR95531, 10 μM ; CGP55845, 2 μM). Series resistances were continuously monitored and recordings were discarded if the resistance increased $> 20\%$ or 20 M Ω . The resistances were continuously compensated using bridge balance. Action potentials (APs) were induced in current-clamp mode by injecting 1 s-long depolarizing current steps from -65 mV (for pyramidal cells) or -60 mV (for PVBCs) (from 0 to 700 pA in 50 pA increments for both cell types).

For simultaneous LFP recordings and whole-cell patch-clamp recordings from CA1 pyramidal cells or PVBCs we used the pipettes filled with ACSF (for LFP recordings) or similar potassium gluconate-based pipette solution (for whole-cell patch-clamp recordings)

to that used for experiments on intrinsic properties of CA1 neurons. Similar ACSF to that described in the Materials and Methods section 2.3 was used for the dual electrical recordings. CA1 pyramidal cells and PVBCs were voltage-clamped at 0 mV (for IPSC recordings) and -70 mV (for EPSC recordings), respectively.

2.5. Administration of pharmacological agents

As recommended by the manufacturer, pharmacological agents were dissolved in their proper diluents and stored at -20 °C. These drugs were then diluted in ACSF to the concentration described in this study just before use. APV, NBQX, and phenytoin were obtained from Alomone Labs (Jerusalem, Israel). CGP55845, SR95531, and tetrodotoxin were purchased from Tocris Bioscience (Ellisville, MO, USA). Riluzole was obtained from Sigma-Aldrich (St. Louis, MO, USA). Excitatory synaptic blockers (APV and NBQX), inhibitory synaptic blockers (CGP55845 and SR95531), phenytoin, or riluzole were bath applied as noted in the result section for LFP recordings (Figs. 2, 4, and 5). Since the inhibitory effects of phenytoin on hippocampal gamma oscillations were slowly manifested, brain slices were pretreated with either phenytoin or vehicle (DMSO) for 20 min before whole-cell patch-clamp recordings (Figs. 6–9). The brain slices were continuously supervised with the same ACSF during whole-cell patch-clamp recordings as used during the pretreatment.

2.6. Data analyses

The data were analyzed using Clampfit 10 software (Molecular Devices). The LFP was high-pass filtered at 1 Hz using a Bessel high-pass filter (8-pole). 1.4 s-long hippocampal gamma oscillations evoked by blue light were used to construct the power spectra of voltage records using pClamp 10 software as previously described (Govindaiah et al., 2018; Kang et al., 2018). Prominent peaks at gamma frequencies were shown in the power spectra. Maximal peak frequency was the frequency at which the highest peak in the power spectrum of CA1 gamma oscillations was observed. The power of gamma oscillations was measured by integrating the area under the curve of the power spectra from 30 to 100 Hz. In addition, we used continuous wavelet analysis to measure the instantaneous oscillation amplitude as described in published studies (Le Van Quyen et al., 2001; Oren et al., 2010). 1.4 s-long LFP records were processed using the Morlet analytic wavelet and the resultant transform signal was examined between 30 and 100 Hz using custom-written routines in MATLAB®, rel. 2018a, Natick, MA. For simultaneous LFP recordings and whole-cell patch-clamp recordings, the amplitudes of IPSCs in pyramidal cells and EPSCs in PVBCs were measured using the last five PSCs evoked by the 1.4 s ramps for each neurons.

The TTX-subtracted responses to voltage ramps were used to measure I_{NaP} peak current in each recorded cell as presented in Figs. 6, 8, and 10. To determine the voltage-dependent activation of I_{NaP} in both PVBCs and pyramidal cells, the TTX-subtracted current responses (I_{NaP}) were changed to conductance based on the following equation: $G(V) = I(V) / (V - V_{Na})$. V is the membrane potential and V_{Na} is the Na^+ equilibrium potential. $I(V)$ is the TTX-subtracted current response in a given membrane potential. The membrane potential for half-maximal Na^+ conductance (V_{50}) was estimated using the Levenberg Marquardt algorithm. AP threshold was defined as the membrane potential at which the derivative of the

membrane potential (dV/dt) first exceeded 4% of maximal velocity as previously described (Khaliq and Bean, 2010).

2.7. Identification of PVBCs and pyramidal cells

TdTomato+ cells and pyramidal cells were filled with pipette solution containing biocytin (10.7 mM) during whole-cell recordings. When the recordings were finished, hippocampal slices were placed in fixative solution consisting of 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (PB, pH 7.4) and stored at 4 °C for 24–48 h. Fixed hippocampal slices were washed in PB, cryoprotected in 30% sucrose solution and resectioned at 60 µm thickness using a Leica Cryostat CM-1950 (Leica Biosystems, Wetzlar, Germany). Biocytin-filled neurons were incubated in 1:500 diluted streptavidin conjugated with Alexa Fluor 488 (Invitrogen, Carlsbad, CA, USA) in 1x Tris-buffered saline (TBS), 0.3% Triton-X 100 for 2 h at room temperature. Stained brain slices were thoroughly washed in PB and mounted in series with Vectashield medium (Vector Laboratories, Burlingame, CA). Resections displaying biocytin-filled neurons were photomicrographed to acquire tile scans of z-stack images using Airyscan mode of Zeiss LSM880 confocal microscope (Zeiss, Oberkochen, Germany) at the Digital Microscopy Core Facility at the University of Arkansas for Medical Sciences. Z-stack projections were generated and stitched with other projected images to complete morphological reconstructions of recorded neurons using Zen 2.3 Blue software (Zeiss, Oberkochen, Germany). PVBCs and pyramidal cells were identified based on the following criteria: (1) PVBCs. We recorded from tdTomato+ cells in PV-TOM mice, which only express tdTomato in PV-expressing cells. Only tdTomato + neurons showing dense axonal arbors in pyramidal cell layer with basket-forming patterns were included in this study, as previously described (Kang et al., 2018; Lee et al., 2014). (2) Pyramidal cells. Only cells showing characteristic basal, apical, and distal dendrites with dense spines of CA1 pyramidal cells and CA3 pyramidal cells were included in this study as previously described (Lee et al., 2014; Hunt et al., 2018).

2.8. Statistics

Paired or unpaired two-tailed Student's t-tests were used when the data showed a normal distribution on the basis of the Shapiro–Wilk test. If the data did not show a normal distribution, Wilcoxon's signed rank (for paired data) or Mann–Whitney (for unpaired data) were used. ANOVAs were followed by Tukey–Kramer tests for mean comparisons. Data are presented as mean ± SEM. A p value less than 0.05 was considered as significant. Statistical analyses were performed using Origin Pro 2015 software (OriginLab Corporation), Prism 5 software (GraphPad Software Inc.), or SigmaPlot 13 (Systat Software Inc.).

3. Results

3.1. Gamma oscillations in the CA1 region are generated through synaptic interactions between pyramidal cells and GABAergic interneurons

Subregions of the hippocampus (e.g., CA1 and CA3) can generate intrinsic gamma oscillations through local neuronal circuits (Butler et al., 2016). To generate CA1 gamma oscillations we combined cell type-specific expression of ChR2 in CaMKII-expressing cells with focal sustained light simulations of the CA1 region (see the Methods section for

details). Hippocampal slices from CaMKII-ChR2 mice were used for optogenetic experiments. 1.4 s-long ramps of 470 nm blue light through a 40 × objective (4.47 mW/mm²) were delivered to the CA1 region as shown in Fig. 1A. Similar light ramps were previously used to trigger cortical gamma oscillations, depending on synaptic interactions within the cortical network (Adesnik and Scanziani, 2010; Crandall et al., 2015). Our LFP recordings from the pyramidal cell layer of the CA1 revealed that such light ramps evoked stable CA1 gamma oscillations for the duration of ramp stimulations in all tested hippocampal slices (see Fig. 1Ab for an example). In general agreement with prior studies (Bettterton et al., 2017; Butler et al., 2016), the maximal peak frequencies of power spectra constructed from the 1.4 s-long CA1 gamma oscillations were in the gamma range (Fig. 1Ac; from 53.4 Hz to 75 Hz; 63.1 ± 1.7 Hz, $n = 15$).

We next determined the effects of light power density on CA1 gamma oscillation frequency and power using three levels of light intensity: 1.36 mW/mm², 4.47 mW/mm² and 11.3 mW/mm², which were in a range similar to that described in prior *in vitro* slice optogenetic studies (Butler et al., 2016; Crandall et al., 2015; Dine et al., 2016). The three intensity levels of 470 nm blue light were applied to the CA1 subregion and gamma oscillations were recorded from the pyramidal cell layer. Our LFP recordings revealed that higher intensity ramp stimuli produced higher power CA1 gamma oscillations compared to those evoked by lower intensity ramp stimuli (Fig. 1B, E; 1.36 mW/mm²: 0.00249 ± 0.00076 mV, $n = 6$; 11.3 mW/mm², 0.0079 ± 0.00148 mV, $n = 6$; $p = 0.014$). In contrast, there were no differences in maximal peak frequencies of gamma oscillations among the three light intensity groups (Fig. 1C and D; 1.36 mW/mm²: 59.5 ± 3.2 Hz, $n = 6$; 4.47 mW/mm²: 59.9 ± 4.7 Hz, $n = 6$; 11.3 mW/mm²: 64.4 ± 2.0 Hz, $n = 6$; $p = 0.327$). These results suggest that ramp stimuli of blue light produce CA1 oscillations in the gamma frequency range regardless of light intensity level.

According to the PING model, hippocampal gamma oscillations arise through synaptic interactions between CA1 pyramidal cells and GABAergic interneurons (Butler et al., 2016; Buzsáki and Wang, 2012). Thus, we sought to examine whether inhibition of excitatory or inhibitory synaptic transmission reduces CA1 gamma oscillations. Our LFP recordings revealed that bath application of excitatory synaptic blockers (40 μM APV and 10 μM NBQX) reduced gamma oscillations (Fig. 2A, B, C; Control: gamma power, 0.00870 ± 0.00070 mV, $n = 4$; APV + NBQX: gamma power, 0.00070 ± 0.00061 mV, $n = 4$; $p < 0.005$). Similarly, bath application of inhibitory synaptic blockers (10 μM SR95531 and 2 μM CGP55845) strongly inhibited CA1 gamma oscillations (Fig. 2D, E, F; Control: gamma power, 0.00830 ± 0.00294 mV, $n = 6$; SR95531 + CGP55845: gamma power, 0.000395 ± 0.00012 mV, $n = 6$; $p < 0.05$). Together, the findings suggest that the observed CA1 gamma oscillations are generated via PING mechanisms.

If CA1 gamma oscillations are generated via the PING model, PVBCs should receive excitatory inputs, whereas pyramidal cells should receive inhibitory inputs during gamma oscillations. We performed simultaneous LFP recordings from pyramidal cell layer and whole-cell patch-clamp recordings from PVBCs or pyramidal cells to examine whether PVBCs and pyramidal cells receive EPSCs and IPSCs, respectively. EPSCs in PVBCs and IPSCs in pyramidal cells were recorded at -70 mV and 0 mV, respectively, since the

estimated reversal potentials of IPSCs and EPSCs were approximately -70 mV and 0 mV, respectively. Our simultaneous recordings revealed that 1.4 s-long light ramps produced large EPSCs in all PVBCs during gamma oscillations (Fig. 3A; -617.5 ± 79.8 pA, $n = 5$). The power spectra, constructed from 1.4 s-long EPSCs in the PVBC, showed predominant gamma frequency components (Fig. 3B). A crosscorrelogram constructed from EPSCs and gamma oscillations showed high crosscorrelation (peak positive correlation, 0.70 ± 0.05 , $n = 5$), suggesting that EPSCs in PVBCs and gamma oscillations were strongly phase-locked (Fig. 3C). Next, we turned to IPSCs in pyramidal cells and their relationship with gamma oscillations. Our simultaneous recordings showed that 1.4 s-long light ramps evoked IPSCs in ah pyramidal cells during gamma oscillations (Fig. 3D; 88.8 ± 33.2 pA $n = 3$). Similar to EPSCs in PVBCs IPSCs in pyramidal cells were also phase-locked with gamma oscillations (Fig. 3F; peak positive correlation, 0.41 ± 0.03 , $n = 3$), while light ramps evoked gamma frequency IPSCs in pyramidal cells (Fig. 3E). These data support that our gamma oscillations are generated via the PING model.

3.2. I_{NaP} blockers, phenytoin and riluzole, reduce CA1 gamma oscillations

I_{NaP} contributes to intrinsic subthreshold gamma oscillations, subthreshold resonant properties, and amplification of synaptic transmission (Hsu et al., 2018; Kang et al., 2018; Pike et al., 2000). These I_{NaP} -mediated intrinsic and synaptic properties are thought to critically contribute to coordinated network oscillations and hippocampus-dependent spatial processing (Hsu et al., 2018; Lee et al., 2018). Thus, we sought to determine whether commonly used selective I_{NaP} blockers (e.g., phenytoin and riluzole) disrupt CA1 gamma oscillations. Since phenytoin and riluzole are used to treat seizures and amyotrophic lateral sclerosis (ALS), respectively (Brodie, 2010; Petrov et al., 2017), therapeutic doses of both drugs were used in this study to determine the possible effects of those treatments on hippocampal network function (Diao et al., 2013; Lacomblez et al., 1996; Pothmann et al., 2014; Rambeck et al., 2006).

Our LFP recordings revealed that phenytoin reduced CA1 gamma oscillations. Specifically, we found a dosage-dependent reduction of gamma oscillations by phenytoin (Fig. 4). Bath application of a low concentration (40 μ M) of phenytoin was sufficient to reduce gamma oscillations (Fig. 4A, B, E, F; gamma powers in control: 0.01022 ± 0.00147 mV, $n = 10$; gamma powers in 40 μ M phenytoin: 0.00669 ± 0.00180 mV, $n = 10$; $p < 0.01$). Simultaneously, phenytoin caused a small but significant decrease in maximal peak frequencies of power spectra constructed from gamma oscillations (Fig. 4B, G, H; Control: 58.6 ± 2.7 Hz, $n = 10$; Phenytoin: ± 2.7 Hz, $n = 10$; $p < 0.01$). A higher concentration (100 μ M) of phenytoin resulted in a larger decrease in gamma power compared to that caused by 40 μ M phenytoin (Fig. 4C, D, E; gamma power in control: 0.01017 ± 0.00406 mV, $n = 7$; gamma power in 100 μ M phenytoin: 0.00279 ± 0.00112 mV, $n = 7$; $p < 0.001$). In addition, the higher concentration of phenytoin (100 μ M) resulted in a larger decrease in maximal peak frequency compared to that caused by 40 μ M phenytoin (Fig. 4G and H; Control: 60.7 ± 3.0 Hz, $n = 7$; Phenytoin: 48.1 ± 2.8 Hz, $n = 7$; $p < 0.01$). Furthermore, we examined the trend in normalized peak frequency and gamma power over 40 min for each condition (i.e., DMSO control, 40 μ M phenytoin, and 100 μ M phenytoin; Fig. 4E, G). We found that both time and phenytoin concentration, as well as their interaction, had a statistically significant

effect ($p < 0.001$ for the two independent variables, i.e., time and phenytoin concentration, and their interaction in Fig. 4E and G; two-way ANOVA with Tukey-Kramer posthoc test). In contrast, application of DMSO control ACSF produced negligible effects on network gamma oscillations (Fig. 4E, F, G, H). These results support the notion that I_{NaP} in CA1 neurons is necessary for network gamma oscillations.

Riluzole is structurally distinct from phenytoin, but is also commonly used as I_{NaP} blocker (Stafstrom, 2007). Thus, we examined whether riluzole also reduces CA1 gamma oscillations. Our LFP recordings revealed that bath application of riluzole (10 μ M) reduced gamma oscillations (Fig. 5A, B, C, D; gamma power in control: 0.00439 ± 0.00071 mV, $n = 6$; gamma power in riluzole: 0.00139 ± 0.00040 mV, $n = 6$; $p < 0.005$). In addition, riluzole caused a decrease in maximal peak frequencies similar to that induced by phenytoin (Fig. 5B, E, F; Control: 60.1 ± 1.7 Hz, $n = 6$; Riluzole: ± 1.9 Hz, $n = 6$; $p < 0.005$). Together, these results suggest that I_{NaP} is a critical “pacemaker” determining both the frequency and power of hippocampal gamma oscillations.

3.3. PVBCs manifest phenytoin-sensitive I_{NaP}

In agreement with the PING model, our current LFP recordings implicated GABAergic interneurons in CA1 gamma oscillations (Figs. 2 and 3). Among functionally distinct subtypes of GABAergic interneurons, PVBCs are thought to critically contribute to cortical gamma oscillations (Buzsáki and Wang, 2012; Freund and Katona, 2007). Since the I_{NaP} blocker, phenytoin, reduced hippocampal gamma oscillations, we sought to examine whether phenytoin reduces I_{NaP} in PVBCs, thus resulting in changes in intrinsic excitability.

To measure I_{NaP} in PVBCs we used slow depolarizing voltage ramps (-80 to -20 mV; 50 mVs $^{-1}$) in presence of synaptic blockers and other channel blockers (see the Methods section for details) before and during application of TTX, as seen in prior studies (Ottolini et al., 2017; Royeck et al., 2015). All PVBCs included in this and the following section were identified based on their expression of tdTomato (parvalbumin) and their axonal morphology (Fig. 6A; see Methods section for details). First, we measured I_{NaP} in PVBCs from slices pretreated with DMSO control ACSF. In all PVBCs tested, the voltage ramps consistently evoked TTX-subtracted inward current (i.e., I_{NaP}) (Fig. 6B, E; I_{NaP} peak amplitude: 175 ± 19.2 pA, $n = 6$). The membrane potential for half-maximal activation (V_{50}) was measured by fitting the measured conductance (see Fig. 6F for an example of conductance measured in the same PVBC as that used in Fig. 6B) by the Boltzmann equation (V_{50} in PVBCs: -47.5 ± 2.1 mV, $n = 6$). Second, we pretreated hippocampal slices with phenytoin (100 μ M) for 20 min before voltage-clamp recordings and continuously bath applied phenytoin for the entire recording. In 6 PVBCs from hippocampal slices pretreated with phenytoin, voltage-clamp recordings revealed that I_{NaP} in PVBCs was of lower amplitude than that from slices pretreated with DMSO control ACSF (Fig. 6C, D, E; I_{NaP} peak amplitude in phenytoin: -39.3 ± 8.2 pA, $n = 6$, $p < 0.005$). Specifically, the voltage ramps evoked negligible TTX-subtracted inward current in 3 PVBCs tested in hippocampal slices pretreated with phenytoin (see Fig. 6C, G for an example; I_{NaP} peak amplitude: -23.6 ± 5.7 pA, $n = 3$), while similar ramps evoked low amplitude I_{NaP} in the remaining 3 PVBCs (see Fig. 6D for

an example; I_{NaP} peak amplitude: -55.0 ± 7.8 pA, $n = 3$). These data suggest that PVBCs express phenytoin-sensitive I_{NaP} .

3.4. Intrinsic PVBC excitability is reduced by phenytoin

How does phenytoin-induced suppression of I_{NaP} in PVBCs translate to changes in excitability? To answer this question, whole-cell patch-clamp recordings from PVBCs were performed in current-clamp mode. Hippocampal slices were pretreated with DMSO control or phenytoin for 20 min before electrical recordings and were continuously superfused in the same ACSF during the recordings. Synaptic blockers were bath applied during the entire recording to exclude potential involvement of indirect effects of phenytoin on excitability through synaptic transmission. PVBCs were held at -60 mV and stimulated by 1 s-long current steps of positive current steps (from $+50$ to $+700$ pA, $+50$ pA increments). Phenytoin pretreatment resulted in a decrease in firing rates of PVBCs (Fig. 7A and B; two-way ANOVA, $p < 0.001$). Specifically, all PVBCs produced prolonged episodes of continuous high frequency action potentials (APs) in hippocampal slices pretreated with DMSO control ACSF (Fig. 7A and B; $+700$ pA steps: 149.0 ± 17.8 Hz, $n = 6$), which is in agreement with published studies (Kang et al., 2018; Lee et al., 2014). In sharp contrast, most PVBCs produced 1-2 APs transiently at the onset of step currents in hippocampal slices pretreated with phenytoin (Fig. 7A and B; $+700$ pA steps: 5.1 ± 3.9 Hz, $n = 6$; $p < 0.001$). Furthermore, phenytoin pretreatment increased rheobase in PVBCs (Fig. 7A, C; Phenytoin: 608.3 ± 53.8 pA, $n = 6$; Control: 341.6 ± 65.0 pA, $n = 6$; $p < 0.05$). However, there were no differences in AP properties (*e.g.*, threshold and amplitude) of PVBCs between DMSO control and phenytoin groups (Table 1). These results suggest that phenytoin reduces I_{NaP} and excitability in PVBCs.

3.5. Pyramidal cells also manifest phenytoin-sensitive I_{NaP}

Excitatory cells (*e.g.*, CA1 pyramidal cells) are also known to express I_{NaP} (Hsu et al., 2018), raising the possibility that phenytoin may reduce I_{NaP} in pyramidal cells, contributing to phenytoin-induced suppression of gamma oscillations. We directly tested this hypothesis using voltage-clamp recordings from CA1 pyramidal cells (Fig. 8). In order to measure I_{NaP} in pyramidal cells, we used slow depolarizing voltage ramps as described in section 3.3 (Fig. 8B and C). All pyramidal cells included in this and the following section were identified based on their location and morphology (Fig. 8A). Hippocampal slices were pretreated with DMSO or phenytoin for 20 min before electrical recordings and were continuously treated with the same solution during the recordings. In hippocampal slices pretreated with DMSO control ACSF, the voltage ramps evoked I_{NaP} in all pyramidal cells (Fig. 8B, D, E; I_{NaP} peak amplitude: -176.3 ± 17.3 pA, $n = 6$; V_{50} : -50.2 ± 0.6 mV, $n = 6$), as observed in prior studies (Royeck et al., 2015). As expected, phenytoin pretreatment reduced I_{NaP} in pyramidal cells (Fig. 8C, D, F; I_{NaP} peak amplitude in phenytoin: -62.4 ± 8.0 pA, $n = 5$, $p < 0.005$). Together, these data suggest that phenytoin reduces I_{NaP} in both pyramidal cells and PVBCs.

3.6. Intrinsic pyramidal cell excitability is also reduced by phenytoin

To determine the effects of phenytoin on CA1 pyramidal cell excitability, whole-cell patch-clamp recordings were performed in current-clamp mode. Pyramidal cells were held near

resting membrane potential (-65 mV) and stimulated by 1 s-long depolarizing current steps (from $+50$ to $+700$ pA, $+50$ pA increments). Hippocampal slices were pretreated with DMSO control ACSF or phenytoin for 20 min before electrical recordings. Phenytoin preincubation resulted in a decrease in pyramidal cell firing rates (Fig. 9A and B; two-way ANOVA, $p < 0.001$). Specifically, all pyramidal cells produced repetitive APs at theta to low gamma frequencies in hippocampal slices pretreated with DMSO control ACSF, (Fig. 9A and B; $+700$ pA steps: 37.0 ± 1.8 Hz, $n = 6$). In contrast, phenytoin pretreatment produced fewer APs in pyramidal cells transiently at the onset of step currents compared to those in the DMSO control group (Fig. 9A and B; $+700$ pA steps: 15.7 ± 1.3 Hz, $n = 9$; $p < 0.001$), with evidence of spike frequency adaptation (spike accommodation). As expected, phenytoin pretreatment also increased rheobase in pyramidal cells (Fig. 9A, C). However, there were no differences in AP properties (*e.g.*, threshold and amplitude) of pyramidal cells between DMSO control and phenytoin groups (Table 1). Collectively, these results suggest that phenytoin-mediated inhibition of intrinsic excitability in pyramidal cells and PVBCs contributed to the inhibitory effects of phenytoin on gamma oscillations.

3.7. CA3 pyramidal cells show I_{NaP}

Gamma network oscillations are also generated within upstream subregions of CA1 (*e.g.*, CA3). Interestingly, previous studies showed that phenytoin produced no reduction in kainate-induced CA3 gamma oscillations (Cunningham et al., 2004), raising the possibility that the CA3 subregion expresses negligible I_{NaP} . Thus, we examined whether CA3 pyramidal cells express I_{NaP} . We used slow depolarizing voltage ramps similar to those shown in Fig. 6. In hippocampal slices, the voltage ramps evoked I_{NaP} in all CA3 pyramidal cells (Fig. 10; I_{NaP} peak amplitude: -62.2 ± 19.8 pA, $n = 5$; V_{50} : -56.2 ± 1.7 mV, $n = 5$). The I_{NaP} peak amplitude of CA3 pyramidal cells was smaller than that of CA1 pyramidal cells (-176.3 ± 17.3 pA, $n = 6$, $p < 0.005$), whereas there were no differences in V_{50} between I_{NaP} in CA1 and CA3 pyramidal cells ($p > 0.05$). These data suggest that pyramidal cells in both CA1 and CA3 express I_{NaP} , although phenytoin might inhibit CA1, but not CA3 gamma oscillations (see the discussion section for a subregional difference in the effects of phenytoin).

4. Discussion

In this study, we examined the properties of I_{NaP} in hippocampal interneurons and the actions of commonly used I_{NaP} blockers (*i.e.*, phenytoin and riluzole) on excitability and hippocampal gamma oscillations. The key findings are: 1) CA1 gamma oscillations required involvement of both principal and inhibitory neurons, consistent with the PING model; 2) Phenytoin and riluzole (both at therapeutic concentrations) reduced hippocampal gamma oscillations; 3) Phenytoin blocked I_{NaP} in both PVBCs and pyramidal cells; 4) Phenytoin reduced repetitive firing and increased rheobase in both cell types. These results indicate not only that I_{NaP} in GABAergic interneurons and pyramidal cells makes significant contribution to neuronal excitability in the hippocampus, but also that neuronal I_{NaP} plays a key role in hippocampal gamma oscillations.

4.1. Synaptic and circuit mechanisms underlying CA1 gamma

Previous LFP recordings from CA1 showed slow and fast gamma (Colgin, 2016). It is thought that slow CA1 gamma is driven by CA3, whereas fast CA1 gamma is driven by entorhinal cortex (Colgin et al., 2009). A highly interconnected network of principal cells in CA3 and the neocortex with recurrent excitatory collaterals is crucial for spontaneous generation of gamma oscillations, whereas CA1 lacks such recurrent excitatory collaterals between pyramidal cells (Hájos and Paulsen, 2009), supporting the notion that CA1 gamma is driven by external inputs. Alternatively, CA1 itself can generate gamma oscillations (Butler et al., 2016; Craig and McBain, 2015). What was the origin of CA1 gamma described in this study? The evidence suggests that it arises intrinsically within CA1. First, prior studies showed that local optogenetic stimulation of pyramidal cells in CA1 generated gamma oscillations in mini-CA1 slices similar to CA1 gamma evoked in hippocampal slices with CA3, using a similar mouse line to that used in this study (*i.e.*, offspring of CaMKII Cre mice and floxed ChR2 mice; Butler et al., 2016). Second, previous studies showed that CA3 generated slow (~40 Hz) gamma oscillations using *in vitro* slice preparations (Craig and McBain, 2015; Fisahn et al., 1998; Gloveli et al., 2005; Hájos et al., 2004), whereas CA1 generates fast (~60 Hz) gamma oscillations (Butler et al., 2016; Craig and McBain, 2015). The frequency of CA1 gamma oscillations presented in this study was consistent with high gamma, supporting the notion that the CA1 gamma activity observed here arose not from CA3, but within CA1.

According to proposed models, PVBCs contribute to gamma oscillations through synaptic interactions with excitatory cells or other GABAergic inhibitory interneurons (Buzsáki and Wang, 2012). Our current findings, as illustrated in Figs. 2 and 3, are in agreement with the PING model (Butler et al., 2016; Mann et al., 2005; Varga et al., 2012). Butler et al. (2016) performed simultaneous whole-cell patch-clamp recordings (for EPSCs and IPSCs) or loose cell-attached recordings (for APs) along with LFP recordings in the CA1 region using a similar optogenetic approach to that described in this study. Based on phases of EPSCs, IPSCs, and APs in PV + interneurons and pyramidal cells relative to CA1 gamma network oscillations, they elegantly showed that PV + interneurons were excited likely by pyramidal cells and, in turn, pyramidal cells were inhibited likely by PV + interneurons through GABA_A-receptors with a decay time constant conducive to gamma oscillations. When pyramidal cells are relieved from PVBCs-mediated inhibition, the next cycle of excitation-inhibition between PVBCs and pyramidal cells resumes. Thus, cell type-specific firing at distinct phases of gamma oscillations is thought to be critically involved in their generation.

4.2. The contribution of I_{NaP} to intrinsic and synaptic properties of GABAergic interneurons and pyramidal cells

GABAergic interneurons are required for almost all cortical circuit functions (Pelkey et al., 2017). Prior studies suggest that intrinsic properties of GABAergic interneurons are critical factors for coordinated network oscillations. For example, subthreshold oscillations and resonant properties of PVBCs are thought to contribute to hippocampal theta and gamma oscillations, and I_{NaP} is thought to contribute to the intrinsic properties of PVBCs (Kang et al., 2018; Pike et al., 2000). However, this is the first direct evidence to our knowledge showing that PVBCs manifest I_{NaP} . In general agreement with published studies showing

that I_{NaP} critically contributes to firing patterns of central neurons including repetitive firing and burst firing (Lin and Onimaru, 2015), our current results showed that the selective I_{NaP} blocker, phenytoin, reduced repetitive firing in PVBCs and increased rheobase.

PVBCs in the CA1 region receive excitatory inputs from local excitatory cells (i.e., CA1 pyramidal cells) and excitatory cells in upstream regions (e.g., CA3 pyramidal cells), thus providing feedback and feedforward inhibition to CA1 local circuits (Lee et al., 2014). It is possible that excitation of CA1 PVBCs by local pyramidal cells and CA3 pyramidal cells is amplified via I_{NaP} -dependent mechanisms as previously shown in CA1 pyramidal neurons (Hsu et al., 2018). Since CA1 gamma oscillations are generated according to the PING model, the I_{NaP} -mediated amplification of synaptic transmission in PVBCs might be a key factor for CA1 gamma oscillations. These possibilities will need to be addressed in future studies using selective I_{NaP} blockers and selective downregulation of voltage-gated sodium channels in PVBCs.

I_{NaP} is also expressed in other major types of GABAergic interneurons in the hippocampus such as cannabinoid type 1 receptor-expressing basket cells, Shaffer collateral-associated cells, ivy cells, neurogliaform cells, and oriens-lacunosum moleculare interneurons (Govindaiah et al., 2018; Kang et al., 2018). Those distinct cell types are known to manifest I_{NaP} -dependent intrinsic subthreshold membrane oscillations at specific frequencies (e.g., theta and gamma), contributing to coordinated network oscillations (Govindaiah et al., 2018; Kang et al., 2018; Lee et al., 2018). Those findings are in general agreement with previous findings showing that these interneuron types participate in hippocampal theta and gamma oscillations (Bezaire et al., 2016; Ferguson et al., 2017; Fuentealba et al., 2010, 2008; Klausberger et al., 2005; Klausberger and Somogyi, 2008; Varga et al., 2012). However, the properties and functions of I_{NaP} in these interneuron cell types remain largely unknown, and should also be addressed in future studies.

I_{NaP} in glutamatergic principal cells (e.g., CA1 pyramidal cells) has been implicated in amplification of excitatory transmission, burst discharges, spontaneous firing by muscarinic receptor stimulation, and increased neuronal excitability in epilepsy (Hsu et al., 2018; Ottolini et al., 2017; Royeck et al., 2015; Yamada-Hanff and Bean, 2013; Yue et al., 2005). Riluzole and phenytoin were commonly used in published studies as I_{NaP} blockers to reveal the roles of I_{NaP} in intrinsic and synaptic properties of pyramidal cells (Hsu et al., 2018; Yue et al., 2005). In agreement with those findings, this report showed that phenytoin reduced I_{NaP} , causing an increase in rheobase and a decrease in repetitive firing in CA1 pyramidal cells. Thus, I_{NaP} in hippocampal GABAergic interneurons and pyramidal cells is critically involved in their intrinsic and synaptic properties.

The current results showed that blockade of I_{NaP} in hippocampal GABAergic interneurons and pyramidal cells by phenytoin reduced firing frequencies and increased rheobase in those cell types in response to depolarizing current steps without changing the properties of individual APs (e.g., thresholds and amplitudes). Reduced firing frequencies and increased rheobase in both cell types likely contributed to inhibition of gamma oscillations by phenytoin. It is also possible that I_{NaP} blockers produced changes in firing preference of both pyramidal cells and PVBCs at specific phases of gamma oscillations, contributing to

modulation of gamma oscillations by I_{NaP} blockers. These possibilities will need to be addressed in future studies using simultaneous LFP recordings and single-unit recordings from pyramidal cells and PVBCs. Given that phenytoin and riluzole are known to have additional effects on voltage-gated sodium channels (e.g. use-dependent blocking of voltage-gated sodium channels), glutamate release, and glutamate metabolism (Cheah et al., 2010; Yaari et al., 1986), we cannot exclude the possibility that the additional effects of those blockers on intrinsic and synaptic properties contributed to their inhibition of gamma oscillations.

4.3. CA3 gamma, sharp wave-ripples, and I_{NaP}

CA3, an upstream region of CA1, also generates gamma oscillations primarily through similar synaptic interaction between pyramidal cells and PVBCs to that described in CA1 PING model (Craig and McBain, 2015; Hájos and Paulsen, 2009; Traub et al., 2003). In this study, we found, for the first time, that CA3 pyramidal cells expressed I_{NaP} , raising the possibility that phenytoin also inhibits CA3 gamma. However, previous studies showed that phenytoin produced no reduction in kainate-induced CA3 gamma (Cunningham et al., 2004), suggesting a potential subregional difference in the functions of I_{NaP} in gamma oscillations between CA1 and CA3. Given that kainate-induced CA3 gamma is also generated via a PING mechanism (Craig and McBain, 2015; Hájos and Paulsen, 2009), it is unlikely that a difference in gamma models between this study and the studies by Cunningham et al. resulted in the discrepancy between the two studies. Based on our current results on I_{NaP} in CA1 and CA3 pyramidal cells it is possible that smaller I_{NaP} in CA3 pyramidal cells than that in CA1 pyramidal cells contributes to negligible inhibitory effects of phenytoin on kainate-induced CA3 gamma. It is also possible that I_{NaP} in CA3 PVBCs, which was not studied in this study, is smaller than that in CA1 PVBCs, thus contributing to negligible effects of phenytoin on CA3 gamma. Future studies are needed to test those possibilities. Such regional differences in I_{NaP} could have important implications in generation of gamma oscillations and gamma range communication between hippocampal subregions.

Hippocampal pyramidal cells and PVBCs are critically involved in sharp wave-ripples (SWRs) (Schlingloff et al., 2014; Ylinen et al., 1995). SWRs are LFP patterns composed of large amplitude sharp waves and associated fast oscillations called ripples (Buzsáki, 2015; Colgin, 2016). They are observed in animals during waking immobility, consummately behaviors, or non-REM sleep, supporting “offline” mnemonic functions (e.g., memory consolidation) (Buzsáki, 2015; Colgin, 2016). Distinct models of SWRs have been proposed based on the results from experimental and computational studies. In general, those models are largely divided into two groups: pyramidal cells/PV+ interneurons-based models and pyramidal cells-based models. According to pyramidal cells/PV + interneurons-based models, excitation of pyramidal cells by excitatory recurrent collateral system for CA3 SWRs (or by Schaffer collaterals for CA1 SWRs), excitation of PV + interneurons by pyramidal cells, and reciprocal inhibition of the activated PV + interneurons generate local ripples (Note that reciprocal inhibition of PV+ interneurons is not a key factor for PING model). In agreement with pyramidal cells/PV+ interneurons-based models selective brief (millisecond range) excitation of CA3 PV + interneurons or CA1 pyramidal cells induce

CA3 ripples (Schlingloff et al., 2014) or CA1 ripples (Stark et al., 2014), both through activation of PV+ interneurons, respectively. Given that I_{NaP} contributes to excitability of pyramidal cells and PVBCs, key players in SWRs, as described in this study it is most likely that I_{NaP} plays a role in SWRs, at least in part, through synaptic amplification and amplification of resonance properties in pyramidal cells/PV+ interneurons-based models (e.g., fast inhibitory neuronal oscillation model, known as FINO model, and pyramidal cell-interneuron-interneuron model, known as PYR-INT-INT model; Schlingloff et al., 2014; Stark et al., 2014). However, it is also possible that SWRs are insensitive to phenytoin in a way similar to CA3 gamma. Thus, future studies are needed to determine whether inhibitory effects of phenytoin on network oscillations can be extended to SWRs or not.

4.4. Functional relevance

Voltage-gated sodium channels are one of the major targets of commonly used antiepileptic drugs. The sodium channel blockers are thought to reduce seizures primarily through use-dependent blockade of voltage-gated sodium channels in central neurons. However, most sodium channel blockers (e.g., phenytoin and riluzole) are also known to reduce neuronal I_{NaP} (Stafstrom, 2007), raising the possibility that inhibition of I_{NaP} in central neurons contributes to the antiseizure effects of the blockers. Indeed, selective I_{NaP} blockers are known to reduce seizures in animal models of temporal lobe epilepsy (Anderson et al., 2014). Those results are in general agreement with our current findings indicating that the I_{NaP} blocker, phenytoin, reduced neuronal excitability in the hippocampus. Furthermore, there is evidence from animal research showing that I_{NaP} is upregulated in epilepsy (Ottolini et al., 2017; Royeck et al., 2015). Those studies also support the notion that I_{NaP} is a viable target for epilepsy treatments. Our current findings may also have implications for studies showing that phenytoin can cause cognitive deficits including memory impairment in epilepsy patients (Butlin et al., 1984; Thompson et al., 1981). Given that therapeutic doses of phenytoin reduce hippocampal gamma oscillations, which are involved in learning, memory, and spatial processing, it is likely that phenytoin-mediated downregulation of gamma oscillations could contribute to cognitive deficits in patients with epilepsy during phenytoin treatment, particularly at high concentrations.

Collectively, our current findings provide new insights into cellular and circuit mechanisms underlying the regulation of neuronal excitability and coordinated network oscillations by I_{NaP} blockers (i.e., phenytoin and riluzole). Our data on I_{NaP} in PVBCs will be of critical importance in computational models of coordinated network oscillations in the hippocampus. Based on results from the current studies, we predict that I_{NaP} in PVBCs and pyramidal cells may be critically involved in hippocampal network gamma activity, and suggest that it should be incorporated into existing large-scale computational models of the CA1 region such as that previously described (Bezaire et al., 2016). Such efforts will advance our understanding of cellular and circuit mechanisms underlying cortical network oscillations in healthy brains and abnormal network activity in brain disorders.

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Abbreviations:

ACSF	artificial cerebrospinal fluid
AMPA	α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP	action potential
APV	2-Amino-5-phosphonopentanoic acid
CaMKII-α	Ca ²⁺ /calmodulin-dependent protein kinase II- α
ChR2	channelrhodopsin2
CGP55845	(2 <i>S</i>)-3-[[<i>(1S)</i> -1-(3,4-Dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid hydrochloride
DIC	differential interference contrast
DMSO	dimethyl sulfoxide
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
<i>I</i>_{NaP}	persistent sodium current
ING	interneuron network gamma
NBQX	2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F) quinoxaline
LFP	local field potential
NMDA	<i>N</i> -methyl-D-aspartate
PING	pyramidal-interneuron network gamma
PVBC	parvalbumin-positive basket cell
SR95531	6-Imino-3-(4-methoxyphenyl)-1-(6 <i>H</i>)-pyridazinebutanoic acid hydrobromide
SWRs	sharp wave-ripples
TTX	tetrodotoxin

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HIGHLIGHTS

- Phenytoin and riluzole reduce hippocampal gamma oscillations in the CA1 subregion.
- Phenytoin blocks I_{NaP} in parvalbumin-expressing basket cells (PVBCs).
- Phenytoin increases rheobase and reduces firing frequency of PVBCs.
- Phenytoin blocks I_{NaP} in pyramidal cells.
- Phenytoin increases rheobase and reduces firing frequency of pyramidal cells.

Fig. 1. CA1 gamma network oscillations induced by optogenetic stimulation.

(A) Representative CA1 LFP gamma oscillations recorded with an extracellular glass pipette filled with ACSF in the CA1 pyramidal cell layer. The gamma oscillations were induced by an 1.4 s-long 470 nm blue light ramp (from near zero to 4.47 mW/mm²). Schematic of *in vitro* optogenetic experiments is shown in left column. A representative segment of gamma oscillations shown on a faster time base (a). The Morlet wavelet transform of LFP recordings is shown in (b). The 1.4 s-long gamma oscillations (middle top) was used to construct the power spectrum showing a predominant peak at 65.9 Hz (c). (B, C, D, E) Dose-response relationship. Three voltage traces of gamma oscillations induced by three levels of light ramps (from near zero to 1.36 mW/mm², 4.47 mW/mm², or 11.30 mW/mm²). These voltage records were used to construct power spectra showing similar peak frequencies regardless of light power as shown in C. Summary of peak frequencies and gamma power of hippocampal network oscillations evoked by the three levels of light ramps

are shown in D and E, respectively. Open circles and solid circles indicate values for individual LFP recordings and mean values of 6 LFP recordings, respectively. Note that higher amplitude light ramps produced higher gamma power without changes in peak frequencies. $*p < 0.05$. The number of slices tested is indicated by “*n*”.

Fig. 2. Synaptic interactions between pyramidal cells and interneurons contribute to gamma oscillations.

(A, B, C) The voltage traces recorded from CA1 pyramidal cell layer in response to light ramps were similar to those described in Fig. 1 before and during bath application of APV (40 μ M) and NBQX (10 μ M) (A). Representative sections of gamma oscillations shown on a faster time base (A, bottom). The 1.4 s gamma oscillations displayed in A were used for construction of the power spectra shown in B. Summary of population data showing inhibition of gamma by excitatory synaptic blockers. (C). (D, E, F) Gamma oscillations recorded from the CA1 pyramidal cell layer before and during bath application of SR95531 (10 μ M) and CGP55845 (2 μ M). Representative sections of gamma oscillations shown on a faster time base (D, bottom). The 1.4 s induced gamma oscillations shown in D were used to construct the power spectra shown in E. Summary of population data showing a decrease in gamma power by inhibitory synaptic blockers (F). Error bars indicate standard error of the mean (SEM) in these and subsequent figures. Open circles, individual gamma oscillations; solid circles, averages for C and F. * $p < 0.05$; *** $p < 0.005$.

Fig. 3. Excitation of PVBCs and inhibition of pyramidal cells during gamma oscillations.

(**A, B**) EPSCs in a PVBC (held at -70 mV) during gamma oscillations recorded from the CA1 pyramidal cell layer. The gray dotted lines in **A** and **D** indicate the peaks of gamma oscillations. The 1.4 s-long induced EPSCs in **A** were used to construct the power spectra shown in **B**. (**C**) Crosscorrelation of EPSCs and LFP during light stimulation or no light stimulation for the same pair of the PVBC and LFP used in **A**. (**D, E**) IPSCs in a pyramidal cell (held at 0 mV) during gamma oscillations recorded from the CA1 pyramidal cell layer. Note that the peaks of gamma oscillations precede the peaks of IPSCs, but the peaks of EPSCs precede the peaks of gamma oscillations (as shown in **A**), together suggesting that EPSCs in PVBCs precede IPSCs in pyramidal cells. These data support that gamma oscillations presented in this study were generated via PING mechanisms. The power spectrum shown in **E** was constructed from the 1.4 s induced IPSCs in **D**. (**F**) Crosscorrelation of IPSCs and LFP during light stimulation or no light stimulation for the same pair of the pyramidal cell and LFP used in **D**.

Fig. 4. Antioscillatory effects of phenytoin on hippocampal gamma oscillations.

(A, B, C, D) CA1 gamma oscillations evoked by light ramps before and during bath application of phenytoin (A, 40 μ M; C, 100 μ M). Light ramps were delivered to the CA1 region every 2 min for 40 min of LFP recording sessions. The power spectra in B and D were constructed from the 1.4 s-long LFP traces shown in A and C, respectively. (E) Normalized gamma power before and during bath application of phenytoin (10 min control period followed by 30 min drug period). Note that gamma oscillations were stable in DMSO control ACSF over 40 min, but oscillations were reduced by therapeutic concentrations of

phenytoin (*i.e.*, 40 μ M and 100 μ M). **(F)** Gamma power during drug application (30–40 min shown in E) and DMSO control period (30–40 min) normalized to gamma power prior to drug exposure (0–10 min). Numbers in the bars indicate numbers of hippocampal slices used for phenytoin or DMSO control experiments. **(G)** Normalized peak frequencies of gamma oscillations. Note that the peak frequencies were stable in DMSO control ACSF over 40 min, but they were reduced by phenytoin along with a decrease in gamma power. **(H)** Peak frequencies during drug application (30–40 min shown in G) and DMSO control period (30–40 min) normalized to peak frequencies prior to drug exposure (0–10 min). n.s., not significant; * $p < 0.05$. The number of slices tested is indicated by “*n*”.

Fig. 5. Riluzole reduces hippocampal gamma oscillations.

(A, B) CA1 gamma oscillations before and during bath application of riluzole (10 μ M). Representative segments of gamma oscillations shown on a faster time base (A, bottom). The power spectra shown in B were constructed from the 1.4 s LFP traces shown in A. (C) Normalized gamma power before and during bath application of riluzole (10 min control period followed by 30 min drug period). The data of DMSO control experiments are the same as those shown in Fig. 4. (D) Gamma power of drug period (30-40 min shown in E) normalized to gamma power prior to drug exposure (0-10 min). (E) Normalized peak frequencies of gamma oscillations during the entire 40 min. (F) Normalized peak frequencies of drug period (30-40 min shown in E) relative to peak frequencies of pre-drug period (0-10 min). * $p < 0.05$. The number of slices tested is indicated by “ n ”.

Fig. 6. Phenytoin reduces I_{NaP} in PVBCs.

(A) A representative reconstruction of an anatomically and neurochemically identified PVBC. The cell was filled with biocytin during whole-cell patch-clamp recordings and was imaged using a Zeiss confocal microscope. Axon arbors were restricted to the pyramidal cell layer of the CA1 region, whereas the dendrites covered most CA1 layers. The recorded PV+ cell showed tdTomato (TOM, thus PV+, right column). Ori., stratum oriens; Pyr., stratum pyramidale; Rad., stratum radiatum. (B) Under control conditions in the presence of synaptic blockers, a neurochemically identified PVBC manifested current responses to voltage ramps

(-80 mV to -20 mV, 50 mVs $^{-1}$) before (black trace) and during (red trace) bath application of TTX (1 μ M). The subtracted current was referred to as I_{NaP} (green trace). (**C, D**) In slices pretreated with phenytoin, similar voltage ramps produced negligible I_{NaP} (**C**) or reduced I_{NaP} (**D**) in neurochemically identified PVBCs. (**E**) Summary data of I_{NaP} peak current values. Numbers in bar graph indicate number of PVBCs. (**F, G**) The TTX-subtracted current responses (I_{NaP}) shown in **B** and **C** were converted to conductance in **F** and **G**, respectively. Note that there was negligible conductance in the PVBC, which was recorded from a hippocampal slice pretreated with phenytoin. $*p < 0.05$.

Fig. 7. Phenytoin reduces excitability of PVBCs.

(A) Examples of APs from PVBCs (1 s –long pulses, +300 pA or +700 pA from –60 mV). The data displayed in the left column were from a PVBC in a hippocampal slice pretreated with DMSO control ACSF, whereas the data shown in the right column were from a PVBC in a hippocampal slice pretreated with phenytoin. (B) Summary of the firing frequency of the recorded PVBCs. (C) Summary of the rheobase of the recorded PVBCs. The numbers in the bars represent numbers for PVBCs. * $p < 0.05$. The number of cells tested is indicated by “ n ”.

Fig. 8. Phenytoin reduces I_{NaP} in CA1 pyramidal cells.

(A) A representative reconstruction of an anatomically identified pyramidal cell that was filled with biocytin during whole-cell patch-clamp recordings (see the methods section for the details). Dendrites of the recorded pyramidal cell covered most CA1 layers. The soma was located in the pyramidal cell layer. Ori., stratum oriens; Pyr., stratum pyramidale; Rad., stratum radiatum; L.M., stratum lacunosum-moleculare. (B) Under control conditions in the presence of synaptic blockers, voltage ramps (-80 mV to -20 mV, 50 mV s $^{-1}$) were used to evoke I_{NaP} in a pyramidal cell. (C) In a hippocampal slice pretreated with phenytoin, similar

voltage ramps produced a decrease in I_{NaP} of a pyramidal cell. **(D)** Summary data of I_{NaP} peak current values. Numbers in the bars indicate number for pyramidal cells. **(E, F)** The TTX-subtracted current responses (I_{NaP}) shown in B and C were converted to conductance in E and F, respectively. Note that there was lower amplitude conductance in the pyramidal cell, which was recorded from a hippocampal slice pretreated with phenytoin, compared to that in the pyramidal cell, which was recorded from a hippocampal slice pretreated with DMSO control ACSF. $*p < 0.05$.

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Fig. 9. Phenytoin reduces excitability of CA1 pyramidal cells.

(A) Examples of APs from pyramidal cells (1 s-long pulses, +150 pA, 200 pA, or +500 pA from −65 mV). The voltage traces displayed in the left column were from a pyramidal cell in a hippocampal slice pretreated with DMSO control ACSF, whereas the voltage traces shown in the right column were from a pyramidal cell in a hippocampal slice pretreated with phenytoin. (B) Summary of the firing frequency of the recorded pyramidal cells. (C) Summary of the rheobase of the recorded pyramidal cells. Numbers in the bars represents number for pyramidal cells. * $p < 0.05$. The number of cells tested is indicated by “n”.

Fig. 10. CA3 pyramidal cells also express I_{NaP} .

(A) Under control conditions in the presence of synaptic blockers, voltage ramps (-80 mV to -20 mV, 50 mV s^{-1}) were used to evoke I_{NaP} in a CA3 pyramidal cell. (B) The TTX-subtracted current response (I_{NaP}) shown in A was converted to conductance. (C) Summary data of I_{NaP} peak current values. The Number in the bar indicates number for pyramidal cells.

Table 1

No effects of phenytoin on AP properties of PVBCs and pyramidal cells in the hippocampus.

PVBCs			
	Ctrl	Phenytoin	Statistics
AP threshold (mV)	-36.19 ± 2.51 ($n = 6$)	-33.87 ± 1.19 ($n = 6$)	Student t-test ($p = 0.43$)
AP amplitude (mV)	48.00 ± 3.44 ($n = 6$)	46.91 ± 1.86 ($n = 6$)	Student t-test ($p = 0.78$)
Half-width of AP (ms)	0.31 ± 0.02 ($n = 6$)	0.27 ± 0.01 ($n = 6$)	Student t-test ($p = 0.12$)
Pyramidal cells			
	Ctrl	Phenytoin	Statistics
AP threshold (mV)	-38.63 ± 1.16 ($n = 6$)	-38.47 ± 1.09 ($n = 9$)	Mann-Whitney test ($p = 0.44$)
AP amplitude (mV)	90.56 ± 1.37 ($n = 6$)	87.34 ± 1.16 ($n = 9$)	Student t-test ($p = 0.11$)
Half-width of AP (ms)	0.95 ± 0.03 ($n = 6$)	0.96 ± 0.02 ($n = 9$)	Mann-Whitney test ($p = 0.76$)