M-type potassium conductance controls the emergence of neural phase codes: a combined experimental and neuron modelling study

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Rate and phase codes are believed to be important in neural information processing. Hippocampal place cells provide a good example where both coding schemes coexist during spatial information processing. Spike rate increases in the place field, whereas spike phase precesses relative to the ongoing theta oscillation. However, what intrinsic mechanism allows for a single neuron to generate spike output patterns that contain both neural codes is unknown. Using dynamic clamp, we simulate an in vivo-like subthreshold dynamics of place cells to in vitro CA1 pyramidal neurons to establish an in vitro model of spike phase precession. Using this in vitro model, we show that membrane potential oscillation (MPO) dynamics is important in the emergence of spike phase codes: blocking the slowly activating, non-inactivating K⁺ current (I_M), which is known to control subthreshold MPO, disrupts MPO and abolishes spike phase precession. We verify the importance of adaptive I_M in the generation of phase codes using both an adaptive integrate-and-fire and a Hodgkin–Huxley (HH) neuron model. Especially, using the HH model, we further show that it is the perisomatically located I_M with slow activation kinetics that is crucial for the generation of phase codes. These results suggest an important functional role of I_M in single neuron computation, where I_M serves as an intrinsic mechanism allowing for dual rate and phase coding in single neurons.

1. Introduction

It is believed that neural codes are used in neural information processing and broadly speaking, two different types of neural codes have been proposed: rate code, represented as firing rates, and temporal codes, represented as precise timing of spikes. Although the rate coding scheme has been widely accepted [1], accumulating evidence supports the existence and importance of temporal codes in neural information processing [2]. In temporal coding, a reference is required to determine the timing of spikes, and network oscillations serve as an ideal reference for spike activity, converting temporal codes to phase codes [3,4]. Hippocampal place cells provide an excellent example of rate and phase codes coexisting in the spike output patterns of single neurons: the spike rate increases when an animal is in a particular part of an environment, called a place field [5], whereas the spike phase progressively advances relative to the theta-frequency local field potential (LFP) oscillation, called phase precession, which codes the specific location of an animal in the place field [6]. Thus, understanding the mechanisms underlying place cell activity would not only shed light on the principles of neural computation using the two neural coding schemes, but also give clues as to how a single neuron can multiplex two different types of neural codes from its spike output.

Many models have suggested possible cellular input mechanisms that could explain the phase precession that is observed in vivo [6–14]. Recently, intracellular recordings of hippocampal place cells revealed a subthreshold signature of
individual place cells in vivo: an asymmetric ramp-like depolarization with increased somatic membrane potential oscillation (MPO) while in the place field, and phase precession of the peak MPO relative to the LFP [15]. That is, whatever the synaptic, intrinsic and network mechanisms are [6–14], the overall final input that a place cell receives at the soma after dendritic integration is a right-skewed asymmetric ramp-like input (R-ARI) during somatic theta oscillation. However, whether the R-ARI during theta oscillation alone is sufficient for the generation of both spike phase and rate codes has never been investigated in vitro. If R-ARI during oscillation can capture the characteristic features of theta-related place cell activity in vitro, it will serve as an ideal model to test the intrinsic biophysical mechanism explaining how a single neuron can translate an input into rate and phase output patterns that can be multiplexed into rate and phase codes. Whether a neuron can translate an input into rate and phase codes at a single-cell level will largely depend on the intrinsic membrane conductances that regulate the spike output.

In this study, dynamic clamp-simulated excitatory ramp-like input and inhibitory theta-frequency oscillation were injected to CA1 pyramidal neurons in vitro to show that an R-ARI during theta oscillation can generate spike phase precession and firing rate code. Using this in vitro model, we find that the subthreshold-activated adaptive $I_M$ is necessary for the emergence of phase codes, because blocking $I_M$ completely disrupted the MPO and consequently abolished phase code. Using an adaptive integrate-and-fire (aIF) and a full-morphology multi-compartment Hodgkin–Huxley (HH) neuron model, we confirmed the sufficiency of adaptive $I_M$ for the emergence of spike phase code from rate code.

2. Material and methods

2.1. Slice preparation

Horizontal hippocampal slices (350 μm) from Sprague–Dawley rats (postnatal days 21–25) were cut with a vibratome (VT1000S, Leica) after decapitation following anaesthesia, in accordance with the guidelines of Institutional Animal Care and Use Committee of Korea University (KLUAC/C2011-114). The brain was placed in an ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl; 3 KCl; 1.25 NaH$_2$PO$_4$; 2 MgSO$_4$; 2 CaCl$_2$; 25 NaHCO$_3$ and 10 glucose. The ACSF had a pH of 7.2–7.3 and was oxygenated with carbogen gas (95% O$_2$, 5% CO$_2$). Slices were incubated at room temperature (22–25°C) containing oxygenated ACSF for an hour before recording.

2.2. Electrophysiology

Whole-cell patch-clamp recordings of CA1 pyramidal neurons were performed using a MultiClamp 700B (Molecular Devices) in current clamp mode with borosilicate patch pipettes (6–8 MΩ) containing intracellular solution containing (in mM): 110 K-gluconate; 40 HEPES; 4 NaCl; 4 ATP-Mg; and 0.3 GTP (pH 7.2–7.3, 270–300 mOsmol). Experiments were first conducted in the control ACSF with 10 μM of XE991 was added to the ACSF to block $I_M$ intact (figures 1 and 2), and 10 μM of XE991 was added to the ACSF to block $I_M$ following the control experiments (figure 3). All recordings were performed at 31–34°C. Data were low-pass filtered at 2 kHz and acquired at 5 kHz using an ITC-18 AD board (HEKA). Igor Pro software (Wavemetrics) was used to generate command signals, to acquire data and to analyse the data.

2.3. Recording protocols

The ramp input and oscillatory theta-frequency oscillation were simulated as excitatory ($g_{exc}$) and inhibitory conductance ($g_{inh}$). 

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Figure 1. Right-skewed asymmetric ramp-like input is required for spike phase precession in CA1 pyramidal neuron in vitro. (a) (top) A dynamic clamp-simulated excitatory symmetric ramp-like depolarizing input (SRI, $g_{exc-SRI}$ green), a step current input ($I_{step}$) and inhibitory theta-frequency (5 Hz) oscillation ($g_{inh}$). (Bottom) Voltage response to $g_{exc} + I_{step} + g_{inh}$ is plotted below ($V_m$). (b–d) Same as (a), but with left-skewed asymmetric ramp-like depolarizing input (L-ARI, $g_{exc-L-ARI}$), right-skewed asymmetric ramp-like depolarizing input (R-ARI, $g_{exc-R-ARI}$, c) and with no ramp ($g_{inh} = 0, d$). (e) Average firing rate map in response to different ramp shapes ($n = 14$). (f–i), (top) Expanded voltage trace ($V_m$) of the dashed box in figure 1a–d. Spike times (black line) plotted with $g_{inh}$ (maximum inhibition upwards) where grey lines indicate the peak of $g_{inh}$ (0°/360°). (Bottom) A sample plot of spike phases relative to $g_{inh}$ against time plotted over two cycles with SRI (f), L-ARI (g), R-ARI (h) and no ramp (i). (j) Mean spike phases in each 1 s bin for each input condition. Data presented as mean ± s.e.m ($n = 14$).
Figure 2. Phase precession of membrane potential oscillation (MPO) with right-skewed asymmetric ramp-like input in CA1 pyramidal neurons in vitro. (a) (Left) A dynamic clamp-simulated excitatory right-skewed asymmetric ramp-like input (R-ARI, $g_{\text{exc_R-ARI}}$, top), a step current input ($I_{\text{step}}$, middle) and inhibitory theta-frequency (5 Hz) oscillation ($g_{\text{inh}}$, bottom). (Right) A sample voltage response ($V_m$) of a CA1 pyramidal neuron subjected to $g_{\text{exc_R-ARI}} + I_{\text{step}} + g_{\text{inh}}$ in the control condition. Grey lines indicate the peak of MPO (dashed line). (b) Expanded voltage trace ($V_m$, top) of the dashed area in figure 2a right, showing spike times (black bars) relative to $g_{\text{inh}}$ (maximum inhibition upwards). Grey lines indicate the peak of $g_{\text{inh}}$ (0°/360°). (c–e) A sample plot of spike phases relative to $g_{\text{inh}}$ (c), peak MPO phases relative to $g_{\text{inh}}$ (d) and spike phases relative to MPO (e) plotted against time over two cycles in control condition. (f) Mean spike phases in each 1 s bin for spike phases relative to $g_{\text{inh}}$ (filled circles), peak MPO phases relative to $g_{\text{inh}}$ (empty circles) and spike phases relative to MPO (empty triangles). (g) An example trace of $g_{\text{inh}}$ (top) and bandpass-filtered MPO (bottom). (h) Bar plot of $g_{\text{inh}}$ frequency and mean MPO frequency. Data presented as mean ± s.e.m. ($n = 12$). Inset: **$p < 0.01$. 

respectively, using a dynamic clamp [16]. The dynamic clamp is a combination of hardware and software implementation used to create artificial conductances in neurons. Here, ITC-18 AD board driven by custom-made functions written in Igor Pro software was used. To simulate a given conductance ($g(t)$) into neurons, dynamic clamp computes the difference between the membrane potential ($V(t)$) and the reversal potential ($E_{\text{rev}}$) for the conductance to be simulated, multiplies that with $g(t)$ to calculate the amount of current to be injected to the neuron ($I(t)$), which is given as the equation below [16,17].

$$I(t) = g(t) \cdot (V(t) - E_{\text{rev}}).$$

For inhibitory conductance, $E_{\text{rev}} = -70$ mV and, for excitatory conductance, $E_{\text{rev}} = 0$ mV. $g_{\text{exc}}(t)$ was simulated as

$$g_{\text{exc}}(t) = \frac{t - t_{\text{in}}}{t_{\text{peak}} - t_{\text{in}}} \cdot g_{\text{amp}} \cdot t, \quad t_{\text{in}} \leq t \leq t_{\text{peak}}$$

and

$$g_{\text{exc}}(t) = -\frac{t - t_{\text{out}}}{t_{\text{peak}} - t_{\text{out}}} \cdot g_{\text{amp}} \cdot t, \quad t_{\text{peak}} \leq t \leq t_{\text{out}},$$

where $t_{\text{in}}$ is the start of a ramp input, $t_{\text{out}}$ is the end of a ramp input, $t_{\text{peak}}$ is the peak of the ramp input and $g_{\text{amp}}$ is the excitatory ramp conductance. The overall $g_{\text{exc}} = 1–5$ nS. In all experiments, $t_{\text{in}} = 0$ s and $t_{\text{out}} = 5$ s, but $t_{\text{peak}}$ varied over 1, 2.5 and 4 s to simulate a left-skewed ARI (L-ARI), a symmetric ramp input (SRI) and a right-skewed ARI (R-ARI), respectively (figure 1). Only R-ARI was used in figures 2–7.

Theta-frequency oscillation (figures 1–7) was simulated as inhibitory oscillatory conductance ($g_{\text{inh}}(t)$) to reflect that CA1 pyramidal neurons receive perisomatic inhibition during theta oscillation [18,19]. Oscillation frequency was set to 5 Hz, as has been adopted in many in vitro studies on hippocampal phase advancements [3,11,20,21]. $g_{\text{inh}}(t)$ was modelled to be constant in amplitude to reflect the constant LFP recorded in vitro [22]. Theta oscillation is given as

$$g_{\text{inh}}(t) = g_{\text{inh_\text{amp}}} \times \sin(2\pi ft), \quad t_{\text{in}} \leq t \leq t_{\text{out}},$$

where $g_{\text{inh_\text{amp}}}$ is the peak-to-peak amplitude of $g_{\text{inh}}$ and frequency ($f$) = 5 Hz with $g_{\text{inh}} = 0.5–1$ nS. The tonic step current ($I_{\text{amp}}$) = 10–100 pA (figures 1–3) and Gaussian noise were superimposed as current (s.d. $\sigma = 10–50$ pA). All experiments were repeated five times.

2.4. Data analysis

For subthreshold MPO analysis, spikes were removed from the voltage traces by removing the values 2 ms before and 5 ms after the peak of a spike, and the removed values were linearly interpolated. The resulting voltage trace was bandpass-filtered between 4 and 10 Hz using a linear phase finite impulse response filter with a Hamming window of 10 ms width. To calculate the phase of a spike occurring at time $t$, we defined the time of peak...
Figure 3. $I_E$ controls spike phase precession by modulating membrane potential oscillation (MPO). (a) (Top) A dynamic clamp-simulated excitatory right-skewed asymmetric ramp-like input (R-ARI, $g_{exc-R-ARI}$), a step current input ($I_{step}$) and inhibitory theta-frequency (5 Hz) oscillation ($g_{inh}$). (Bottom) A sample voltage response of a CA1 pyramidal neuron subjected to $g_{exc-R-ARI} + I_{step} + g_{inh}$ in the presence of 10 μM XE991. (b) Average firing rate map in the presence of 10 μM XE991 (grey) and firing rate map of control condition (black) from figure 2. (c) Expanded voltage trace ($V_m$, top) of the dashed box in figure 3a bottom, bandpass-filtered MPO (middle) with spike times (black bars) relative to $g_{inh}$ (bottom, maximum inhibition upwards). Grey lines indicate the peak of $g_{inh}$ (0°/360°) and black circles indicate peak MPO times. (d–f) A sample plot of spike phases relative to $g_{inh}$ (d), peak MPO phases relative to $g_{inh}$ (e) and spike phases relative to MPO (f) plotted against time over two cycles in the presence of 10 μM XE991. (g–i) Mean spike phases relative to $g_{inh}$ (g), mean peak MPO phases relative to $g_{inh}$ (h) and mean spike phases relative to MPO (i) in each 1 s bin in the control condition (figure 2f, filled circles, $n = 12$) and in the presence of 10 μM XE991 (empty circles) in the same neuron in figure 2. (j) An example trace of $g_{inh}$ (top), the bandpass-filtered MPO in the control condition (middle) and in the presence of XE991 (bottom). (k) Bar plot of $g_{inh}$ frequency (white), mean MPO frequency in control condition (black) and in the presence of XE991 (grey). Data presented as mean ± s.e.m. ($n = 12$). Inset: $p < 0.01$. 
oscillation immediately preceding and following the spike as $t_1$ and $t_2$, respectively, and calculated the phase as $360 \times (t - t_1)/(t_2 - t_1)$. Peak oscillation was defined as the maximum $\Phi_{\text{peak}}$ and peak subthreshold MPO. The firing rate map was calculated by dividing the total number of spikes generated into 500 ms bins.

An ANOVA was used for multiple comparisons and if significant differences were found, post hoc t-tests were performed. For circular data, circular statistics were used, and statistical testing of the difference between mean phases was performed with the Watson–Williams test [23]. All average data are presented in the form of mean ± s.e.m.

2.5. Integrate-and-fire neuron model

A simple leaky IF neuron model [24] was simulated as

$$\tau_m \frac{dV_m(t)}{dt} = E_L - V_m(t) + R_m I_{\text{ext}}(t),$$

where the membrane time constant $\tau_m = 170$ ms, input resistance $R_m = 220$ MΩ, leak reversal potential $E_L = -60$ mV and $I_{\text{ext}}(t)$ is the external step current input. Spike threshold $V_{\text{th}} = -56$ mV and reset voltage $V_{\text{reset}} = -65$ mV. An adaptive IF (aIF) neuron was modelled following a previously described formalism [25,26],

$$\tau_m \frac{dV_m(t)}{dt} = E_L - V_m(t) - R_m I_{\text{exc}}(t) + R_m I_{\text{ext}}(t),$$

$$I_{\text{exc}}(t) = g_{\text{exc}}(t)(V_m(t) - E_{\text{exc}})$$

and

$$\tau_m \frac{dz(t)}{dt} = \frac{1}{1 + e^{-(V_m(t) - \Phi(z(t)))/\sigma}} - z(t),$$

where $z(t)$ is the gating variable of the M-channel, $E_{\text{exc}}$ is the reversal potential for $K^+$ current $-95$ mV. For M-channel simulation ($g_{\text{M}}$, high $g_{\text{M}}$ conductance ($g_{\text{M,high}}$) = 180 nS, low $g_{\text{M}}$ conductance ($g_{\text{M,low}}$) = 90 nS, $\tau_m = 550$ ms, $\beta = -45$ mV and $\gamma = 2.4$ mV. With the IF simulations, $g_{\text{inh}} = 1.0$ nS, $g_{\text{exc}} = 1.0$ nS, $I_{\text{ext}}(t) = 70$ pA and Gaussian noise (s.d. $\sigma = 50$ pA).

2.6. Hodgkin–Huxley neuron model

A full-morphology multi-compartment neuron model of hippocampal CA1 was constructed as an HH neuron model [27] using the NEURON program [28]. The morphology of the CA1 pyramidal neuron model (figure 5a) was obtained from the NeuronMorpho database (http://neuronmorpho.org), which contains 155 compartments. Passive membrane properties are shown in table 1. Leak ($I_{\text{leak}}$), fast sodium ($I_{\text{Na}}$), delayed-rectifier potassium ($I_{\text{KDR}}$), A-type potassium ($I_{\text{KATC}}$), M-type potassium ($I_{\text{M}}$) and hyperpolarization-activated ($I_{\text{h}}$) currents were included and were adopted from [29]. Maximal conductance for each channel is shown in table 2.

The activation kinetics of the M-type $K^+$ channel was modelled as

$$\frac{dm}{dt} = \frac{m_{\text{in}} - m}{\tau_m} - \frac{m - m_{\text{out}}}{\tau_m},$$

where $m$ is an activation probability, $m_{\text{in}}$ is a steady-state probability, $\tau_m$ is the slope of activation probability curve (figure 7a) and $\tau_m$ is the time constant. In the control HH model, the slope was 0.1 and to model slow activation and fast activation of the M-type $K^+$ channel, the slope was set to 0.025 and 0.4 (figure 7a).

Same as the in vitro experiments, the R-ARI was simulated as $g_{\text{exc}} = 0.3$ nS, and theta oscillation was simulated as $g_{\text{inh}} = 0.8$ nS. $I_{\text{ext}}(t) = 50$ pA and Gaussian noise (s.d. $\sigma = 50$ pA). All NEURON simulations were sampled at 40 kHz and the temperature was set to 32°C.
3. Results

3.1. Asymmetric ramp-like input during theta oscillation as an in vitro model of spike phase precession

To establish an in vitro model of hippocampal phase precession, different shapes of \( g_{\text{exc}} \) and a constant \( g_{\text{inh}} \) (5 Hz) were simulated to a whole-cell patch-clamped CA1 pyramidal neuron using the dynamic clamp (figure 1a–d). When SRI, L-ARI, R-ARI and no ramp inputs were superimposed, the firing rate increased until the peak of the ramp and then decreased (figure 1e–d bottom). The firing rate map revealed that ramp-like excitatory inputs could reliably produce an increased firing rate (figure 1e). Apart from the case with no ramp, which produced a constant firing rate (figure 1e, black), the location of peak firing coincided with the peak location of the ramp input (figure 1e, green, blue and red).

To investigate the spike phase relations with different ramp input shapes, spike phases relative to \( g_{\text{inh}} \) were calculated to assign a phase value for each spike (black bars, figure 1f–i top) where maximum inhibition was defined as 0°/360° (grey bars, figure 1f–i top). With the symmetric ramp input \( g_{\text{exc,SRI}} \), initial spike phase precession was followed by phase precession (figure 1f). When the mean spike phases were calculated in every 1 s bin, the mean phases of the first (202.7° ± 21.9°) and the fifth bins (220.2° ± 25.6°) were not significantly different (Watson–Williams test, \( p < 0.05 \), \( n = 14 \); figure 1g). For the left-shifted asymmetric ramp-like input \( g_{\text{exc,L-ARI}} \), the resulting spike phase precession (figure 1g) and the mean spike phase of the first (165.6° ± 10.5°) 1 s bin occurred significantly earlier than that of the fifth 1 s bin (251.9° ± 31.8°, Watson–Williams test, \( p < 0.01 \), \( n = 14 \); figure 1h black). For the right-shifted asymmetric ramp-like input \( g_{\text{exc,R-ARI}} \), which is the input similar to that observed in vivo [15], the resulting spike phases relative to \( g_{\text{inh}} \) (figure 1h top) showed a prominent spike phase precession (figure 1h). The mean spike phase of the first (275.2° ± 19.7°) 1 s bin occurred significantly later than that of the fifth 1 s bin (170.4° ± 12.6°, Watson–Williams test, \( p < 0.01 \), \( n = 14 \); figure 1j black), similar to the in vivo result [15]. Lastly, without any ramp-like input, the spike phases were phase-locked to 180° (figure 1j black) with the mean spike phase of the first (193.5° ± 21.9°) and the fifth 1 s bin not being significantly different (212.9° ± 21.2°, Watson–Williams test, \( p > 0.05 \), \( n = 14 \); figure 1j black). These results suggest that an overall R-ARI during theta-frequency oscillation is required for the generation of spike phase precession as observed in vivo and such input simulation can serve as an in vitro model for hippocampal place cells where both spike rate and phase codes coexist.
3.2 Phase precession of the subthreshold membrane potential oscillation

Peak MPO in vivo showed phase precession relative to the LFP, whereas spike phases were phase-locked to the MPO [15]. To test whether our in vitro model could also capture the MPO characteristics observed in vivo, we simulated R-ARI and $g_{inh}$ superimposed with $I_m$ (figure 2a, left) in addition to 12 CA1 pyramidal neurons and MPO (figure 2b) obtained from the resulting voltage response ($V_m$, figure 2a right) (see Material and Methods). Consistent with figure 1b, R-ARI during $g_{inh}$ showed robust spike phase precession (figure 2b, black bar and figure 2c) and the mean spike phase relative to $g_{inh}$ of the first 1 s bin (278.9 ± 13.1°) occurred significantly later than that of the fifth 1 s bin (171.7 ± 19.3°, Watson–Williams test, $p < 0.01$, $n = 12$, figure 2f filled circles). When the phase relationship between MPO and $g_{inh}$ was analysed, the peak MPO (figure 2b, black circles) relative to $g_{inh}$ also showed robust phase precession (figure 2b, black circles; figure 2f) with the mean peak MPO phase of the first 1 s bin (264.5 ± 13.4°) also occurring significantly later than that of the fifth 1 s bin (170.6 ± 11.4°, Watson–Williams test, $p < 0.01$, $n = 12$, figure 2f, empty circles). However, spike phases relative to MPO were phase-locked to MPO (figure 2b, black bar; figure 2f), with the mean spike phases of the first and the fifth 1 s bins showing no difference (Watson–Williams test, $p > 0.05$, $n = 12$, figure 2f, empty triangles).

Spike and peak MPO phase precession in place cells have been suggested to be owing to the increased MPO frequency relative to LFP theta oscillation frequency [15]. Thus, we directly compared the imposed $g_{inh}$ frequency (5 Hz, figure 2g top) and the mean MPO frequency (figure 2g, bottom) and found that indeed MPO frequency (6.57 ± 0.4 Hz) significantly increased compared to that of the $g_{inh}$ (paired t-test, $p < 0.01$, $n = 12$, figure 2h). These results suggest that the R-ARI during theta oscillation can closely capture not only the spikes, but also the subthreshold MPO characteristics of place cell in vitro and that the increase of the MPO frequency accompanies spike phase precession.
3.3. Adaptive conductance controls the emergence of spike phase code via controlling subthreshold membrane potential oscillation

Because MPO dynamics is directly related to phase precession, the intrinsic conductance that controls MPO may hold a key in the emergence of phase precession at a cellular level. Among many intrinsic conductances, the Kv7 channel that underlies the slowly activating, non-inactivating K⁺ current (I_{Ks}) is known to control spike rate adaptation [30], neural excitability [31,32] and subthreshold membrane dynamics [31,33]. Thus, the selective Kv7 blocker, XE991 (10 μM) was applied in the same neuron to figure 2 to test the effect of Kv7 on MPO and spike phase precession. When the same $g_{\text{exc-R-ARI}}$ during $g_{\text{inh}}$ with $I_{\text{step}}$ was simulated in the presence of XE991 (figure 3a top), the resulting voltage response showed rate code (figure 3a bottom, $V_m$) as reflected in the firing rate map (figure 3b, empty circles) but the overall firing rate in the presence of XE991 was increased compared with the control condition (figure 3b, filled circles, $n = 12$).

When the spike and peak MPO phases were analysed relative to $g_{\text{inh}}$ in the presence of XE991 and spike phases were analysed relative to MPO (figure 3c), the spike phases (figure 3d) and the peak MPO phases (figure 3c) both became scattered across phases, whereas spike phases relative to MPO showed phase locking (figure 3f). Directly comparing the control condition (figure 3g–i filled circles) with that in the presence of XE991 (figure 3g–i empty circles), the mean phase of the first 1 s bins of the control condition occurred significantly later than that in the presence of XE991 for both the spikes and the peak MPO phases relative to $g_{\text{inh}}$. By contrast, the spike phases relative to MPO were phase-locked to 360° even in the presence of XE991 (figure 3i empty circles), similar to the control condition (figure 3i, filled circles).

The disruption of spike and peak MPO phase precession relative to $g_{\text{inh}}$ is related to disruption of MPO, because the mean MPO frequency in the presence of XE991 (8.28 ± 0.42 Hz) was significantly increased compared with the mean MPO frequency in the control condition (6.57 ± 0.4 Hz; figure 3j–k, t-test, $p < 0.01, n = 12$). These results suggest that adaptive I_{M} is important and necessary for the decoupling of spike phase and spike rate codes, and that the emergence of phase code is tightly controlled by neural excitability and the MPO dynamics.

3.4. Adaptive conductance modulates the emergence of phase precession in an adaptive integrate-and-fire neuron model

Although the experiment with XE991 in figure 3 demonstrated the necessity of I_{M} for phase code, there is a possibility that XE991 could have interfered with K⁺ channels other than Kv7 channels [34], thus whether I_{M} is sufficient in phase coding needs further study. Hence, we constructed a simple aIF neuron model [25] with a conductance that mimics I_{M} ($g_{M}$, figure 4a) [26]. A simple IF neuron, by definition, has no $g_{M}$ ($g_{M} = 0$, figure 4a, black trace) and shows a constant interspike interval (ISI; figure 4b, black). However, in aIF neuron models with $g_{M_{\text{low}}}$ and $g_{M_{\text{high}}}$ (aIFM_{low} and aIFM_{high} respectively), spike rate adaptation occurred (figure 4b, left) and the increase in ISI was greater with $g_{M_{\text{high}}}$ than with $g_{M_{\text{low}}}$ (figure 4b, right). When the R-ARI during $g_{\text{inh}}$ was superimposed with $I_{\text{step}}$ (figure 4c), the analysis of the voltage response and MPO of the IF model ($V_m$ and MPO, respectively; figure 4d bottom) showed no spike nor MPO phase precession (figure 4d, bottom right). However, as $g_{M}$ increased from $g_{M_{\text{low}}}$ (figure 4e) to $g_{M_{\text{high}}}$ (figure 4f), spike and MPO phase precession relative to $g_{\text{inh}}$ gradually emerged, with the aIFM_{high} model showing a near 360° phase precession (figure 4f, bottom left and middle). In both cases, spike phases relative to MPO were phase-locked to 360° (figure 4f, bottom right).

Directly comparing the three different IF models, the spike rate map showed a progressive decrease in peak spike frequency with increasing $g_{M}$ to $g_{M_{\text{high}}}$ (figure 4g). Phase precession became more apparent when the mean phases in 1 s bins were directly compared for spike phases relative to $g_{\text{inh}}$ (figure 4h), for peak MPO phases relative to $g_{\text{inh}}$ (figure 4i) and spike phases relative to MPO (figure 4j). The emergence of phase code with $g_{M}$ accompanied an increase in MPO frequency.
(figure 4k, red, blue bars) compared with the imposed \( g_{inh} \) frequency (5 Hz, figure 4k, white bar) and in the IF model, the MPO frequency became even faster (figure 4k, black bar).

### 3.5. M-channel located in perisomatic region modulates phase precession in full-morphology multi-compartment Hodgkin–Huxley model of CA1 pyramidal neuron

Although the aIF model could demonstrate the sufficiency of \( I_m \) for phase precession, it is a highly simplified model with limitations in providing mechanistic insights. MPO frequency has been suggested to be modulated also by \( I_m \) [31,35,36]. In addition, different subcellular distribution of \( I_m \) [33,37,38] may also have an influence on phase precession. Therefore, we built a full-morphology multi-compartment HH model of CA1 pyramidal neuron (figure 5n).

When the same R-ARI input with \( g_{inh} \) superimposed to \( I_{dep} \) (figure 5b) was simulated to the HH model, the resulting \( V_m \) (figure 5c top) and MPO (figure 5c middle) showed robust spike and MPO phase precession relative to \( g_{inh} \) (figure 5c bottom, figure 5d left, middle and figure 5k,1, red) and spike phase locking to \( g_{inh} \) (figure 5d right and figure 5m, red). Without \( I_m \) in the HH model (figure 5e), both spike and MPO phase precession were completely abolished (figure 5f,k,l,m, black), consistent with in vitro (figure 3) and IF simulation results (figure 4d). Therefore, our HH model could closely recapitulate the spike and MPO phase precession phenomenon.

When the same simulation was repeated in the absence of \( I_h \) (figure 5g), still a robust spike and MPO phase precession were observed (figure 5g bottom and figure 5h left, middle and figure 5k,l, blue), whereas spike phases were phase-locked to \( g_{inh} \) (figure 5h right and figure 5m, blue). The spike rate maps and MPO frequencies of the control HH model and the HH model without \( I_h \) were similar (figure 5i,j, red and blue) but, the HH model without \( I_m \) showed substantial increase in firing rate and MPO frequency (figure 5i,j, black).

Next, we investigated the effect of subcellular distribution of M-channels on phase precession. When \( I_m \) was inserted only in the perisomatic region (figure 6a), \( V_m \) and MPO (figure 6b) showed robust spike and MPO phase precession (figure 6c), which are similar to the control HH model in figure 5c,d. However, when \( I_m \) was inserted only in the dendritic region (figure 6d), \( V_m \) and MPO (figure 6e) showed little spike and MPO precession (figure 6f). Peak firing rate of the spike rate map (figure 6g) and the MPO frequency (figure 6h) both were increased in the HH model with dendritic \( I_m \) than in the HH model with perisomatic \( I_m \). Directly comparing the mean phases of perisomatic \( I_m \) model and dendritic \( I_m \) model, it was clear that spike and MPO phase precession are greater with the perisomatic \( I_m \) (figure 6i,j). These HH simulation results all together suggest that subthreshold-activated \( I_m \) predominantly located in the perisomatic region holds the key in modulating the phase precession.

### 3.6. Slow activation kinetics of the M-channel is important in phase precession

HH model simulation results (figures 5 and 6) suggest that phase precession requires \( I_m \)-like subthreshold-activated current. To show the sufficiency of \( I_m \) in phase precession, we modulated the M-channel gating variable, \( m \) (figure 7a), and investigated its effect on phase precession. When \( m \) was made slower (figure 7a, blue) than that of the control \( m \) (figure 7a, red), near 360° spike and MPO phase precession were still observed (figure 7b left and right). However, when \( m \) was made faster (figure 7a, black), only up to 180° phase precession was observed for spike and MPO (figure 7c, left and right). Directly comparing the mean spike (figure 7d left) and MPO phases (figure 7d right) relative to \( g_{inh} \), it became clearer that the early part of the phase precession is greater with slower \( m \) (figure 7d, blue and red) than with faster \( m \) (figure 7d, black). These results indicate that it is the slow-activation characteristics of \( I_m \) that are important in the emergence of phase codes.

### 4. Discussion

Here, we report that a simple asymmetric ramp-like input during inhibitory theta oscillation can generate spike rate and phase codes simultaneously in CA1 hippocampal neuron’s spike output. The important results of the present in vitro and in silico study in relation to in vivo results are that the R-ARI during theta oscillation can: (i) generate spike phase precession in a single trial in an in vitro model (figures 1–7); (ii) generate phase precession of the peak MPO relative to theta oscillation (figures 2, 4–7); and (iii) induce phase locking of the spike phase relative to MPO (figures 2–7), which is similar to intracellular recordings of place cells in vivo [15]. To the best of our knowledge, this is the first in vitro demonstration that such simple input can capture not only the spikes, but also the MPO characteristics of in vivo phase precession [15]. In addition, using this in vitro phase precession model, we demonstrate that the subthreshold-activated adaptive mechanism provided by \( I_m \) is necessary for the neuron to generate spike phase codes (figure 3). Further, our aIF (figure 4) and the full-morphology multi-compartment HH models (figures 5–7) helped us demonstrate the sufficiency of perisomatic-distribution of slowly activated \( I_m \) in phase code generation, suggesting that \( I_m \) plays a key functional role in...
decoupling spike phase code and firing rate code in single neuronal spike output.

The shape of place cell's receptive field has been suggested to become highly negatively skewed with experience, changing from symmetric to asymmetric shape [39]. In our study, the symmetric ARI in vitro gives rise to spike precession followed by spike recession, whereas the negatively skewed R-ARI could generate robust 360° phase precession (figure 1). Therefore, based on our in vitro experiments, it seems that the change in ramp-input shape from symmetric to asymmetric shape makes the phase precession and consequently the phase coding more robust, similar to in vivo results [12].

Our in vitro model is novel in that it can generate near 360° phase precession in a single trial, whereas other in vitro models showed approximately 180° phase precession [11,21] or the phase precession was represented as a function of input strength [11]. Our result is also the first in vitro model to directly capture the MPO characteristics similar to in vivo results [15]: phase locking of spikes relative to MPO and the increase in MPO frequency relative to the inhibitory theta oscillation which resulted in phase precession of MPO (figure 2).

Phase precession with R-ARI in our study appears to occur in two distinct clusters (figures 1h and 2c). At the beginning of the R-ARI, the neuron received gradual increases in excitatory and oscillatory inhibitory drives which were, at first, just above spike threshold but not sufficient to elicit spikes near the minimum of perisomatic oscillatory inhibition (180°). Thus, spikes occurred with an onset-latency, which restricted the spikes to the first 360–180° compartment, showing a strong correlation between spike phase and time (figure 2c, first 3 s). As the amount of excitation increased, however, spikes occurred at the minimum of perisomatic oscillatory inhibition and further increases in excitation advanced the spikes forward, clustering most spikes in the second 0–180° compartment (figure 2c). Such bimodality of theta phase precession is similar to that observed in vivo [6,12,40] and in an analytical model [41]. However, owing to the increase in excitation towards the peak of R-ARI, spikes often occurred in bursts and doublets, causing a wider spread of spike phases also into the 180–360° compartment and this spread of spike phases makes the early part of the phase precession clearer than that of the later part of R-ARI. The discrepancy with in vivo data may arise, first, because we have used all spikes in analysing spike phases relative to theta-frequency g_\theta_{ab}, whereas in some in vivo studies, only the first spike within each cycle of theta LFP was used [42]. Second, the discrepancy may be due to our in vitro input model being too simplified to capture the dynamic interaction of excitatory and inhibitory inputs impinging upon CA1 pyramidial neurons in vivo [11,22]. We used constant g_\theta_{ab} to represent perisomatic inhibition received by CA1 pyramidial neurons during hippocampal theta-frequency oscillation [18,19], which has been an in vitro model for phase precession used in other studies, too [21,24,27,43]. Especially, appropriately timed activation of the perisomatic-targeting parvalbumin-expressing (PV) interneurons paired with phasic dendritic excitation to CA1 pyramidial neuron has been suggested as possible network mechanism for phase precession in vitro [11]. Moreover, in vitro, CA1 pyramidial neurons are found to receive inhibitions from not only from perisomatic-targeting PV cells, but also from dendritic-targeting somatostatin-expressing (SOM) interneurons, where PV cells are activated at the beginning of the place field while SOM cells are activated more towards the end of the place field [22]. Therefore, in addition to intrinsic neuronal mechanisms, network mechanisms should be taken into account in future studies to fully elucidate the mechanisms underlying phase precession in vivo.

Previously, hyperpolarization-activated cation channels (I_h), persistent sodium channels (I_{NaP}), and slowly activated potassium channels (I_{Ks}) have been hypothesized as possible intrinsic mechanisms for phase precession [8,9,21,44] but they could not completely block phase precession. In our study, we directly tested these hypotheses in in vitro and in silico to show for the first time that it is the subthreshold-activated adaptive current, I_M, that serves as an intrinsic mechanism underlying the generation of hippocampal spike rate and phase coding via modulating MPO. Blocking subthreshold-activated I_M disrupted the MPO characteristics and completely abolished spike and peak MPO phase precession relative to g_\theta_{ab} in in vitro (figure 3), in allF neuron model (figure 4d) and in full-morphology HH neuron model (figure 5e,f). Using our multi-compartment HH model, we were able to show that I_h, another subthreshold-activated conductance, which has been reported to modulate the frequency of subthreshold MPO [35,36], has little effect on phase coding (figure 5g,h). In addition, we found that perisomatically distributed I_h is required (figure 6) and that it is the slowly activating channel kinetics of I_M which is the key to the decoupling of spike rate and phase codes (figure 7). Thus, subthreshold-activated I_M located in the perisomatic region seems to have a functional role in modulating the neural codes. Our result is in line with other studies which reported that functionally important I_M is mostly located in the perisomatic region for controlling synaptic integration and excitability of CA1 pyramidial neurons [29,33]. From our results, we believe that I_M may serve as the intrinsic mechanism for phase coding at a cellular level, but we are aware that neural coding mechanism is very complex thus orchestrated activation of many other intrinsic mechanisms as well as network mechanisms needs consideration to fully uncover the secret behind the emergence of neural codes.

I_M was discovered several decades ago [30] and is known to underlie spike rate adaptation [45], subthreshold neuronal excitability [31,32], intrinsic subthreshold resonance [31] and spike after-hyperpolarization [32]. I_M has been previously implicated to be important for temporal coding in analytical models [26], but the role of I_M in phase precession has never been directly demonstrated with in vitro experiments. Here, we propose for the first time the functional role of I_M in neurocomputation where it serves as the intrinsic mechanism multiplexing spike outputs into rate and phase codes at the single-cell level. I_M is also known to increase markedly after the first postnatal week [46] and interestingly, the number of place cells and their stability increase with development [47], which suggests the possibility that developmental increase in I_M may contribute to the emergence of phase precession with age [47]. Ubiquitous in the brain [48] and strategically located mostly in the perisomatic region [33] to directly control the spike output, I_M has the potential to control the emergence of dual rate and phase coding, consequently serving as an intrinsic mechanism for neuronal computation.

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