Spikes matter for phase-locked bursting in inhibitory neurons

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(Dated: November 22, 2011)

We show that inhibitory networks composed of two endogenously bursting neurons can robustly display several coexistent phase locked states in addition to stable anti-phase and in-phase bursting. This work complements and enhances our recent result [S. Jalil, I. Belykh, A. Shilnikov, Phys. Rev. E, V. 81, 045201(R) (2010)] that fast reciprocal inhibition can synchronize bursting neurons due to spike interactions. We reveal the role of spikes in generating multiple phase-locked states and demonstrate that this multistability is generic by analyzing diverse models of bursting networks with various fast inhibitory synapses; the individual cell models include the reduced leech heart interneuron, the Sherman model for pancreatic beta cells, and the Purkinje neuron model.

PACS numbers: 05.45.Xt, 87.19.La
INTRODUCTION

Phase locking between oscillatory cells plays a significant role in functions of various regulatory and nervous systems such as vision, and memory [1]. These and many other systems are controlled by central pattern generators (CPGs) [2–5]. CPGs are small neuronal networks that generate various phase-locked bursting rhythms, including cardiac beating and locomotor behaviors [3]. The advancement of electrophysiological tools and methods has allowed biophysical properties of several CPGs and their component neurons to be identified and studied [3, 6, 7]. CPGs of many animals are composed of pairs of reciprocally inhibiting coupled neurons. These universal pairs, often referred to as half-center oscillators, typically produce alternating, or anti-phase bursting rhythms. They can also produce less robust in-phase bursting when driven externally or endogenously [11].

The original concept of the half-center oscillator was proposed by T. G. Brown in 1911 [5] while studying animal locomotor behaviors and their associated neuronal rhythms. Brown proposed that anti-phase bursting in the classical half-center oscillator (HCO) [5] is due to interactions between individually spiking (non-bursting) neurons. In this paper, the HCO is viewed more broadly as a reciprocally inhibitory, two-neuron configuration that can produce anti-phase bursting. There has been much work on mechanisms that generate bursting in individual and networked neurons, including transitions to bursting, tonic spiking or quiescence [8–10, 12–14].

Interactions between neurons in CPG networks are highly nonlinear and nonhomogeneous as the neurons receive uncorrelated driving inputs from each other at the same time. Nevertheless, the stunning feature of all CPGs is the robust and stable timing in their oscillatory rhythms [4]. There are several aspects to consider when seeking explanations for this generic and noticeable CPG phenomenon. These aspects include properties of individual neurons or neuronal models, types and time scales of synaptic coupling, as well as network architectures of CPGs, see [2, 11, 12, 15–36] and the references therein. The mechanisms that give rise to anti-phase bursting in the HCO have been under special scrutiny [2, 15]. Some of the mechanisms are synaptic release, post-inhibitory rebound, and synaptic escape mechanisms [2]. Reciprocal inhibition has been shown to facilitate anti-phase bursting in the HCOs [2, 15, 16, 25, 34] as long as the inhibition stays fast [16, 19, 22], meaning the synaptic decay is on the time scale of spike duration. Rubin and Terman demonstrated that synchronous oscillations are unstable in a HCO composed of fast inhibitory spiking (non-bursting) cells [19, 22], unless each cell has at least two slow intrinsic variables [21]. In a seminal paper [16], van Vreeswijk, Abbott and Ermentrout showed that the non-instantaneous “inhibition, not excitation, synchronizes” oscillatory neurons, resulting in simultaneous spike firing. Another
possible mechanism of the stable co-existence of in-phase and anti-phase bursting in the HCO is due to time-delayed synapses [15].

In our recent Rapid Communication [35], we demonstrated that the fast non-delayed reciprocal inhibition can stably synchronize endogenously bursting cells in the HCO due to spike interactions during the active phase. Fast inhibition is typical in many neural networks such as the leech heartbeat CPG [37] and the Tritonia swim CPG [38]. The bistability in HCO networks can make the CPG multistable with a bursting repertoire of two (anti-phase and in-phase) or more complex rhythms [36]. Such HCOs with bursting neurons contrast with the HCOs comprised of relaxation-type cells, which is only capable of generating a single anti-phase rhythm. Less robust compared to anti-phase bursting, in-phase bursting can be effectively established in the HCO after both cells have received an external inhibition from another bursting neuron [11, 12]. Our previous study [35] indicated the significance of spike interactions for establishing synchrony in fast inhibitory networks.

In this paper, we revisit the HCOs composed of square-wave bursters in order to examine a more general property of the coupled neurons - the coexistence of multiple phase-locked states. This coexistence results in the occurrence of specific phase lags or time delays between the nearest intervals of spiking or active phases in voltage traces. To the best of our knowledge, this phenomenon for realistically fast synapses has not been reported elsewhere. Multiple phase-locked states, however, were reported in several cases of slow inhibitory [15, 16] and fast excitatory synapses [17]. We assert that multistability is a common phenomenon for bursting neurons coupled reciprocally by fast inhibitory synapses. To justify this assertion, we analyze diverse models of bursting neurons such as leech heart interneurons [9, 10], Sherman pancreatic beta cells [18], Purkinje neurons [13], and consider a few models of fast non-delayed inhibitory synapses. This analysis reveals the general ability of such HCOs to produce multiple co-existent phase-locked bursting patterns. We demonstrate that the number of phase-locked states is basically determined by the number of spikes in the burst.

The layout of this paper is as follows. In the next section, Sec. 2, we introduce the HCO concept employed in this study, and describe the models of the neurons and synapses. In Sec. 3, we present weakly coupled HCOs, and the methods and materials used to explore phase-locked states; namely, the way the phase of a bursting cycle is defined for examinations of co-existent phase-locked states. Next in this section, we explain how inhibition and spike interactions induce stable phase-locked states. We measure these spike interactions and construct stability diagrams for phase lags between bursts produced by each coupled neuron. We introduce an effective potential to quantify the stability of phase-locked states through the depths of wells which correspond to stable states.
Finally, we analyze and compare HCOs, made of square-wave bursters, using Poincaré mappings for phase lags, whose fixed points correspond to phase-locked states. In Sec. 4, we study the stability of in-phase bursting in strongly coupled HCOs. The models employed in this study are presented in the Appendix.

NETWORK MODEL

We model a HCO network by means of Hodgkin-Huxley type equations

\[ CV_i' = F(V_i, h_i, m_i) - I_{syn}^{(ij)}, \quad \tau(V_i)h'_i = G(V_i, h_i), \quad i, j = 1, 2, \]

(1)

where \( V_i \) stands for the \( i \)-th neuron membrane potential, and \( h_i \) stands for the gating (in)activation variable(s) describing kinetics of specific ion current(s) with a characteristic time scale(s) \( \tau(V) \). Neurons composing the HCO are assumed to be identical. The individual neuron model we use are: (i) the 3D reduced model of the leech heart interneuron [9, 10]; (ii) Sherman \( \beta \)-cell model [18]; and (iii) the 5D single compartment Purkinje neuron model [13]. All three models (see Appendix) are referred to as square-wave bursters, named for the resemblance of the shape of the envelope of bursts in the voltage traces [8].

The inhibitory synaptic current \( I_{syn}^{(ij)} \) from the presynaptic cell \( j \), flowing into the postsynaptic cell \( i \), is recast as follows

\[ I_{syn}^{(ij)} = g_{syn}^{(ij)}(V_i - E_{syn})S(V_j), \]

(2)

where \( g_{syn}^{(ij)} \) is the maximal conductance of the synapse. In other words, the maximum possible strength of the synaptic coupling from pre-synaptic neuron \( j \) to post-synaptic neuron \( i \) is given by \( g_{syn}^{(ij)} \). Unless stated otherwise, the connections are assumed to be symmetric such that \( g_{syn}^{(ij)} = g_{syn}^{(ji)} = g_{syn} \). The reversal potential is set so that \( E_{syn} < V_i(t) \) at all times \( t \) to ensure the inhibitory nature of the current. Specifically, \( E_{syn} = -0.0625 \) V is fixed for the leech heart interneuron model, and \( E_{syn} = -0.08 \) V is set for the Sherman \( \beta \)-cell and Purkinje cell models. In this study, we employ four distinct models of the synaptic function \( S(V_j) \), representing fast non-delayed synapses. Multiple phase locked states turned out to be a common feature of HCO networks regardless of any particular choice of \( S(V_j) \) considered in this paper.

1. "Heaviside" synapse

This is the simplest representation of the synapses [17, 21]: \( S(V_j) = H(V_j - \Theta_{syn}) \) with \( H = \{0, 1\} \). The synapse activates instantaneously, \( S(V_j) = 1 \), as soon as the membrane
potential $V_j$ of the presynaptic neuron exceeds the synaptic threshold $\Theta_{\text{syn}}$, and deactivates instantaneously, $S(V_j) = 0$, after $V_j$ drops below $\Theta_{\text{syn}}$. The synaptic threshold $\Theta_{\text{syn}}$ is chosen to ensure that every spike of the bursting cell crosses the threshold (Fig. 1A). The actual value for $\Theta_{\text{syn}}$ is determined for each model individually. Unless specified otherwise, we fixed $\Theta_{\text{syn}} = -0.0225$ V for the leech heart interneuron model, $\Theta_{\text{syn}} = -0.03$ V for the Sherman model, and $\Theta_{\text{syn}} = -0.036$ V for the Purkinje cell model.

2. Fast threshold modulatory (FTM) synapse

The coupling function is modeled by the sigmoidal

$$S(V_j) = \frac{1}{1 + \exp\{-1000(V_j - \Theta_{\text{syn}})\}}.$$  

(3)

This coupling form was introduced and called the fast threshold modulation by Somers and Kopell [17]. It is a smooth version of the Heaviside coupling function with the same rise and decay times (compare panels A and B in Fig. 1). The FTM is a remarkable model of a realistic fast synapse [17, 21], such as that in the leech heart CPG [37], as it yields a nearly instantaneous response from the synapse on the post-synaptic neuron.
3. α-dynamical synapse

In this frequently used model of the synapse [15, 16] the coupling function \( S(V_j) \) is described by the following ODE:

\[
S'(V_j) = \alpha (1 - S)[1 + \exp(-1000(V_j - \Theta_{syn}))]^{-1} - \beta S. \tag{4}
\]

Here, \( \alpha = 1000 \) and \( \beta = 100 \) are set to match the rate of the synaptic onset, decay, and maximum efficacy \( (S \approx 1) \) similar to the FTM synapse (see Fig. 1C). Decreasing \( \beta \) makes the synaptic current last longer.

4. Leech heart dynamical synapse

The last model for fast synapses is from leech heart CPG, introduced in [37], where \( S(V_j) = Y M(V_j) \) is such that the fitted dynamics of the variables \( Y \) and \( M \) are governed by the auxiliary ODE system:

\[
\begin{align*}
\dot{X} &= \frac{[[1 + \exp(-1000(V_j - \Theta_{syn}))]^{-1} - X]}{0.002}, \\
\dot{Y} &= \frac{(X - Y)}{0.011}, \\
\dot{M} &= \frac{[0.1 + 0.9[1 + \exp(-1000(V_j + 0.04))]^{-1} - M]}{0.2}.
\end{align*} \tag{5}
\]

We conclude this section with the remark that despite quantitative disparities due to the renormalization, all four models of the fast synapses consistently demonstrate homogeneous outcomes for all three neuron models of bursters in the HCO network.

WEAKLY COUPLED HCO: MULTIPLE PHASE-LOCKED STATES

Phase definition and phase-locking

We begin with the HCO (1) composed of the leech heart interneurons (6) coupled by weak FTM inhibitory connections (3). We reported earlier [35] that this HCO bursts not only in antiphase as predicted and generally accepted, but it stably bursts in-phase as well. In the following, we will show that the given HCO possesses multiple, co-existent phase-locked states, in addition to in-phase and anti-phase bursting. To do so, we first introduce the phase of the periodic bursting orbit and demonstrate the co-existence of several phase locked states due to spike interactions in overlapping bursts.

The algorithm for identifying phase legs between the bursting neurons of the HCO is based on the observation that two solutions of the HCO on the synchronous bursting orbit are the same solution
FIG. 2. (color online). (A) Periodic bursting orbit in a 3D projection of the phase space of the leech heart HCO. Dark and lighter balls represent schematically the densely distributed initial phases, \( \phi_0 \), for (reference) neurons 1 (reference) and, \( \phi_0 + \Delta \phi^{(0)} \), for neuron 2 across the bursting orbit of a normalized 1-period. (B) The sequence, \( \{ \Delta \phi^{(n)} \} \), for every (out of 600) initial phase lag, \( \Delta \phi^{(0)} \), is identified from the traces at the instances when the ascending voltage \( V_{1,2} \) pass through an auxiliary threshold \( \Theta_{th} \) shown in (A).

passing through some initial point with some delay. By varying the delay one can produce a densely distributed set (we use 10,000 points per second) of initial points or phases across the bursting orbit. The phase of a burst cycle is initiated/reset every cycle after the voltage, \( V(t) \), reaches some auxiliary threshold \( \Theta_{th} = -0.0425 \, \text{V} \) (see Fig. 2A), set halfway between the spiking and quiescent voltage values. The phase lag, \( \Delta \phi^{(n)} \), between the cells is defined through the time delay, \( \tau_n \), between identical phase points on the \( n \)-th bursting cycle, \( V_1(t_n) = \Theta_{th} \) and \( V_2(t_n + \tau_n) = \Theta_{th} \) (see Fig. 2B). The delay, \( \tau_n \), normalized over the recurrence period, \( T_1^{(n)} = t^{(n)} - t^{(n-1)} \), given by the times of two successive identical phase points of reference neuron 1, defines the sequence of the phase lags \( \Delta \phi^{(n)} = \tau^{(n)}/T_1^{(n)} \). A detailed account for the computational routine is given in the Appendix and in [36].

We consider the case of the weak inhibitory coupling \( g_s = 0.005 \) between the neurons in the HCO. Such weak coupling does not change drastically the phase lags, \( \Delta \phi^{(n)} \), between the neurons over a bursting cycle thereby allowing us to follow “continuous” evolution of the phase lags, \( \Delta \phi^{(n)} \),
FIG. 3. (color online)(A) Exponential convergence of initial phase lags to four co-existent phase-locked states over 200 burst cycles of the leech heart HCO. Parameters are $\Theta_{syn} = -0.0225$, $V_{k2}^{shift} = -0.022$, and $g_s = 0.005$. $\Delta \phi^{(n)} = 0$ and $0.5$ correspond to stable in-phase and unstable anti-phase bursting, respectively. The right panel shows the established bursting cycles (dark and light/green colors for neurons 1 and 2, respectively) corresponding to the selected phase-locked states (thick lines in Panel A). Symbols $\times$ and $\blacksquare$ are same in Figs. 4C-D.

as number, $n$, of bursting cycle progresses. Word of caution: such continuous evolution may be hard to achieve when the individual neuron is defined by parameters close to a bifurcation such as the one underlying slow transition from bursting to tonic spiking or quiescence. Slow evolution of the phase lags, however, lets us systematically single out all co-existing stable phase-locked states and identify the separating thresholds - unstable states, as we evaluate the convergence rates given by $\Delta \phi^{(n+1)} - \Delta \phi^{(n)}$. Figure 3(A) represents the evolution of the phase lags, $\Delta \phi^{(n)}$, plotted against the number of burst cycles, $n$, for the leech heart HCO generating four-spikes per burst. By assessing convergent tendencies of $\Delta \phi^{(n)}$, as $n$ increases, in the figure one can clearly identify four stable phase locked states (non-linear thick curves), which include the synchronous state, $\Delta \phi^{(n)} = 0$. Unstable states are invisible, but they exist between every pair of stable states. We see four unstable states, which include the anti-phase state $\Delta \phi^{(n)} = 0.5$ (constant thick curve). Panel (B) of Fig. 3 depicts the voltage traces for bursting patterns corresponding to the states: anti-phase (B1) through in-phase (B5). In what follows, we give an explanation for causes of these multistable states.
The mechanism of multistability: two opposite roles of inhibition

In order to account for multistability, we notice that inhibition may cause phase point to speed up or slow down compared to that of the uncoupled trajectory, and these two opposite effects set up the system for multistability by allowing phase lag to vary. Since inhibition acts as a downward force, the phase point slows down in the upward stroke, whereas it speeds up in the downward stroke. Figure 4 shows instances of spike interactions that result in growth (arrows facing outward) or decay (arrows facing inward) of the phase lags due to the two opposite roles of inhibition. We label the neuron that spikes first, the leading neuron (black traces) and the later one, delayed neuron. When the phase point of the leading neuron (A1-closed circle) slows down but that of the delayed neuron (A2-closed circle) speeds up, the phase lag decays, as the phases essentially move towards each other. On the other hand, when the former (A3-open circle) speeds up and later (A2-open circle) slows down, phase lag grows for the opposite reason. We average these two effects over a burst cycle, and see that some burst pair configurations given by the initial phase delay may lead to overall growth or overall decay shown in Fig. 5(A). The vertical axis represents the amount of phase lag variation at the end of a burst, given a specific phase lag at the beginning of the burst. In other words, the amount of phase lag variation averaged over a burst is not constant with respect to phase lag and may even vanish.

The next question is what stops the phase lag from varying such that phase-locking occurs? To answer this question we point to the fact that synaptic threshold decouples the neurons when the spikes are aligned and the level of $\Theta_{syn}$ is high enough to cross all the spikes, as a result there are barriers that need to be overcome for certain configurations. In other words, if all the spikes are aligned in a given configuration, then the neurons are decoupled completely, inhibition is absent and the drive to vary phase lag is missing.

To conclude the explanation as to why spike interactions give rise to the phase locked states, we emphasize the ability of inhibition to speed up or slow down the phases numerous times due to spikes. In addition, neurons decouple periodically during spiking phases of both neurons due to synaptic threshold crossing the spikes. Moreover, when the spikes are aligned, the phases speed up and slow down at the same time, making it harder to have opposite effects of inhibition at the same time, leading to small variation in phase lag. As a result, with weak enough coupling and high enough synaptic threshold it is possible to lock these phases. This property is uniquely attributed to bursting cells with spikes as opposed to slow relaxation oscillator-type neurons without fast spikes that are capable of producing single anti-phase bursting.
FIG. 4. (color online) (A1-A3) Phase points on traces showing two opposite effects. Arrows (B) Transients of the averaged net synaptic current $\Delta <\text{IPSC}>$ converging to two non-zero equilibrium levels representing the (B3) and (B4) phase-locked states. Transients (red) converging to the zero level for the stable in-phase (solid) phase locked state, as well as unstable anti-phase state (dashed).

**Stability diagrams**

To analyze and quantify the stability of the several phase-locked states (Fig. 3) we employ 1D stability diagrams (shown in Fig. 5) representing snapshots of the $n$-th iterate of the difference between the current and preceding phase lags: i.e. $[\Delta \phi^{(n+1)} - \Delta \phi^{(n)}]$, plotted against the initial distribution $0 \leq \Delta \phi \leq 0.5$. For dense enough initial distribution, $n$ can be taken as small as 2, which would give a scalar number corresponding to every initial condition. Observe that $[\Delta \phi^{(n+1)} - \Delta \phi^{(n)}]$ can also be viewed as the change rate over a single burst cycle on the $n$-th step. If the change rate does not vary for some initial phase lag $\Delta \phi^*$, then the latter corresponds to a fixed point of the iterative process. A zero of the graph $[\Delta \phi^{(n+1)} - \Delta \phi^{(n)}]$ vs. $\Delta \phi$ is an fixed point. The stability of the point is determined by the derivative $d[\Delta \phi^{(n+1)} - \Delta \phi^{(n)}]/d\Delta \phi$ at $\Delta \phi^*$. The fixed...
FIG. 5. (color online) (A) Two graphs (black and blue/grey) of the 1D stability diagram: zeros of the stationary distribution of the phase lag difference $[\Delta \phi^{(n+1)} - \Delta \phi^{(n)}]$ over the range $\Delta \phi = [0, 0.5]$ are phase-locked states: four stable (solid dark circles) separated by repellers in the four spikes bursting HCO at $V_{K2}^{\text{shift}} = -0.022$ at $g_{\text{syn}} = 0.005$ and $g_{\text{syn}} = 0.01$, respectively. (B) Normalized effective potential (integral) for $g_{\text{syn}} = 0.005$: different wells implying uneven robustness of the stable phase-locked states whose basins are separated by the thresholds. Solid grey circles indicate intermediate (saddle-node) states. (C) Zeros indicated by solid circles corresponding to seven stable phase-locked states, in the eight spikes bursting HCO at $V_{K2}^{\text{shift}} = -0.024$, in the 1D stability diagram.
point is attracting if the derivative is negative, or repelling if the derivative is positive. The basins of the attractors (four total as in Fig. 3) of the HCO network are separated by the repellers in this 1D phase portrait. Panel (A) of Fig. 5 shows the two 1D phase portraits of the leech heart HCO with a weak $g_{syn} = 0.005$ (black graph), and a stronger $g_{syn} = 0.01$ (blue/grey graph) coupling. In both cases, the fixed points are located at the same zeros of graph $[\Delta \phi^{(n+1)} - \Delta \phi^{(n)}]$. However, local (in)stability of the fixed point becomes quantitatively stronger with an increased coupling strength.

In addition to local stability, the robustness of the stable phase-locked states can be characterized in terms of an effective potential being a normalized integral numerically evaluated from the stability diagram in Inset A. In Inset B, the normalized effective potential plotted against the phase lag distribution $\Delta \phi$, reveals the profile of the potential wells corresponding to the basin of the attractors, and the barriers corresponding to the separating repellers in the leech HCO network. This diagram allows us to identify the most robust phase-locked state by differentiating the depth and width of the wells. Observe from this figure that the steepness of the potential well, scaled between 0 and 1, yields the [relative] non-local rate of convergence to the phase-locked states. This figure also shows that fast convergence to the in-phase ($\Delta \phi^* = 0$) state does not make it the most robust, as its basin is not as deep as those of other stable phase-locked states.

The comparison of Figs. 5A and 5B with the corresponding four spikes bursting trace (Fig. 6A) suggests that there is a (direct) correlation between the number of spikes per burst and the number of stable phase-locked states. To support the hypothesis we present Fig.5C showing a similar 1D stability diagram for the eight spikes bursting trace (Fig. 6C): now the leech HCO possesses seven attractors corresponding to the stable phase-locked states. The relation between number of spikes and that of phase locking is still consistent because there is only six spikes that fall in the range $0 \leq \Delta \phi \leq 0.5$ and the anti-phase state, which is located at $\Delta \phi = 0.5$ has switched the stability to the opposite type.

The qualitative examination of the stability of the fixed points for the phase lags together with the quantitative observation lets us hypothesize that spikes do matter for the emergence of multiple phase-locked states. The number of spikes per burst does yield an estimate for the number of phase locked states. However, complexity of the spike interactions due to timing, and irregularities of the spike characteristics, slow convergence due to weak coupling and the sensitivity of the two-time scales bursting solutions may cause inaccuracies in some models. Moreover, multistability of weakly coupled HCO becomes harder to describe properly as the duty cycle becomes greater resulting in long burst train with a larger number of spikes (Fig. 6G-H). The attraction basins
of the phase locked states meanwhile become narrower and less clearly identifiable, which means that the accurate numerical simulations would require unrealistically high resolution. We have presented the most tractable cases so far, and next, will introduce an alternate way of thoroughly examining multistability in all the cases considered so far in this paper. This method reduces the problem of finding and characterizing stability of phase-locked states to studies of 1D Poincaré return mappings.

Phase return maps

Identifying multiple phase-locked states of the bursting HCO can effectively be reduced to that of finding stable fixed points in 1D Poincaré return mappings defined as: $\Delta \phi^{(n)} \rightarrow \Delta \phi^{(n+k)}$, where $k$ is the degree of mapping. Specific values of $k$ depend on the individual cell model in question as they have distinct rates of the convergence to the phase-locked states. So, $(k - 1)$ is the number of successive burst cycles skipped in the traces to generate the mappings. In fact, the case of the weak coupling, which results in slow and smooth dependence of $\Delta \phi^{(n)}$ on the burst cycle number $n$ (Fig. 3A), implies that the integer $k$ may be chosen relatively large for the basins of attractions to be well identified. By choosing the degree $k$ so the mapping would reveal robust phase-locked states that are represented by stable fixed points located at intersection points of the flat sections of the mapping graph with a 45-degree line. Due to the large values of $k$, the unstable fixed points corresponding to the threshold separating the attraction basins reside at the discontinuity points of the mapping graph.

Figure 6 presents four pairs of panels each representing bursting rhythms and the corresponding return mappings for the four HCOs under consideration. Panels (A-B) and (C-D) depict, respectively, the voltage traces and the mappings $\Delta \phi^{(n)} \rightarrow \Delta \phi^{(n+k)}$ of degrees $k = 345$ and $k = 40$ for the weakly coupled leech heart HCOs, which robustly produce four and eight spikes per bursting cycles. Panels (E-F) and (G-H) are for the HCOs made of the Sherman pancreatic \(\beta\)-cell models, and the Purkinje cell models, respectively. The frames overlaid on top of the bursting traces denote half-period windows, $0 \leq \phi \leq 0.5$, with the spikes determining the number of phase-locked states.

By construction symmetry, the phase lag, $\Delta \phi$, is symmetric about the half-period point such that the phase lags outside and inside of the half-period frame are equivalent. This implies that only the spikes within the frames are critical for spike interactions leading to phase-locked states.

Figure 6 suggests that the CPG models under consideration possess the same universal properties, which are due to spike interactions contributing to the emergence of multiple phase-locked
FIG. 6. Bursting cycles generated by the HCO composed of the leech heart interneuron models (panels (A) and C), and the Sherman models (E) and the Purkinje cell models (G). Overlaid boxes indicate the reference half-period frames defining the spikes that effectively determine the number of phase-locked states in the networks; the horizontal lines set the synaptic thresholds in the HCOs. Panels (B), (D), (F) and (H) show the corresponding 1D return mappings: \( \Delta \phi^{(n)} \rightarrow \Delta \phi^{(n+k)} \) of degree \( k \) (\( k = 345, 40, 80 \), and 35, reps.).

(B) and (D): Four and seven stable fixed points in the mapping imply the coexistence of the same number of phase-locked states in the bursting leech heart HCOs (\( g_s = 0.005 \)).

(E-F): The Sherman model HCO (\( g_s = 0.001 \)) generating six-spikes bursting possess the same number of stable fixed point in the mapping.

(E) Zoom of the mapping (H) for the the Purkinje cell HCO (\( g_s = 0.001 \)) generating 62-spikes burst trains reveals multiple phase locked states within \([0.4, 0.5]\) range accumulating to anti-phase bursting.

states. There are some distinctions as well. For example, wide asymmetric spikes produced non-homogeneously by the leech heart interneuron model can result in more subtle attraction basins and less robust phase-locked states, including meta-stable ones near saddle-node equilibria (Fig. 6) or tangent fixed points (Fig. 6). Those meta-states have vanished, and phase-locked states gain robustness, as the number of spikes per burst becomes larger. Furthermore, narrow symmetric spikes produced evenly by the bursting Sherman model HCO contribute to the occurrence of robust phase-locked states with well defined (separated) basins of attraction (Fig. 6E-F). Remarkably, the number of the spikes occurring within the half-period windows in the leech heart and Sherman \( \beta \)-cell HCOs determines accurately the number of coexisting stable phase-locked states.

The Purkinje model generates long bursts with multiple, nearly instantaneous spikes at the chosen parameter values. Because of that, it is hard to identify a large number of all phase-
locked states with rather narrow attraction basins in the weakly coupled ($g_{syn} = 0.001$) HCO case due to slow convergence. To take a fewer spikes into consideration, we lowered the synaptic threshold, $\Theta_{syn}$, so that few spikes occurring closer to the end of the burst cycle can actually cross it (Fig. 6G). As a result, the corresponding Poincaré mapping $\Delta \phi^{(n)} \rightarrow \Delta \phi^{(n+k)}$ (here $k = 80$) has an array of fixed points, within $[0.4, 0.5]$ range, near the phase-locked state corresponding to anti-phase bursting produced by the HCO. This demonstrates also the significance of the choice for the synaptic threshold in modeling studies of larger network models like specific central pattern generators that are often comprised of several HCOs.

To resume this section of the paper, we point out that we have demonstrated how various intrinsic properties of the HCOs, including correlations between the number of spikes and the temporal characteristics of bursting including the spike frequency, duration and duty cycles, as well as the level of the synaptic threshold, all combined my determine the number of co-existing phase-locked states. While the strength of the synaptic coupling modulates the amplitude of the synaptic current, and hence influences the spike interaction, our simulations suggest that variations of the coupling strength do not essentially influence the number of stable phase-locked states as long as the coupling remains weak, which in turn guarantees the relatively slow convergence to either phase locked state. A significant increase in the coupling strength makes most phase-locked states disappear so that anti-phase bursting will solely persist in the HCO, which is the general convention. In the next section, we show that in-phase bursting can also persist as stable, though comparably less robust, in strongly coupled HCOs.

**STRONGLY COUPLED NETWORKS: STABLE IN-PHASE BURSTING**

We define strong inhibition through coupling that is sufficiently strong to establish anti-phase bursting rapidly, via the hold-then-release mechanism (due to a saddle-node bifurcation) [11], which happened to be functionally similar to synaptic release mechanism, common for relaxation oscillator-type spiking neurons [2, 15, 34]. The hold-then-release mechanism implies that the active pre-synaptic neurons locks down temporarily the inactive post-synaptic cell at the hyperpolarized state during a single half-oscillator bursting cycle. Fast inhibition implies that as soon as the active neuron ceases firing and becomes inactive, the other cell is released from inhibition, so they switch roles to produce the second half-oscillator bursting cycle. This cyclic switching between active and inactive phases in the HCO gives rise to highly robust anti-phase bursting. The details on emergent anti-phase rhythms in HCOs made of bursting neurons can be found in [12] and the references
therein. When the coupling is strong and the initial conditions of bursting cells are set so that one cell is active (above the synaptic threshold) while the other is inactive, then fast non-delayed reciprocal inhibition leads ultimately to anti-phase bursting in any HCO, independent of the choice of model of individual bursters and fast synapses. Once achieved, anti-phase bursting remains highly resistant to external voltage perturbations, however not when long [periodic] inhibition is forwarded to both cells from an external source. As shown in [11], this external inhibition establishes in-phase synchronization in the HCO.

In the previous section, we have stressed that the coexistence of multiple phase-locked states is a peculiar paradigm of the weakly, reciprocally inhibitory coupled HCO made of (nearly) identical cells. Increasing the coupling strength makes most, but not all, phase-locked states disappear eventually. Nevertheless, both weakly and strongly coupled HCOs exhibit anti-phase bursting generally, and the emergent mechanisms are diversely different: a fragile balance between spike timing and IPSCs in the weak coupling case and the robust hold-then-release mechanism in the strong coupling case.

A peculiar feature of the strong coupling is the robustness of in-phase bursting, emerging through a wide range of disperse initial conditions chosen within the spiking phase [35]. Here, we demonstrate that in-phase bursting, co-existing with anti-phase bursting, is a generic property of the HCO, composed of endogenously bursting (nearly identical) neurons reciprocally coupled by fast non-delayed inhibitory synapses. In what follows, we reveal the stability and robustness of in-phase bursting with respect to transversal perturbations against the phase mismatch (off-set) between two neurons in the HCO. More specifically, we examine how the shape of the attraction basin of in-phase bursting varies along the in-phase bursting orbit. To do so, we first parameterize the bursting cycle with respect to a phase, defined on modulo 1, as described in the previous section. Next, the in-phase bursting cycle is discretized with a mesh comprised of reference phase values (see Fig. 7A). Each reference phase is employed to identify a local basin of attraction by gradually advancing, $\Delta \phi > 0$, or delaying, $\Delta \phi < 0$, the initial phase of the perturbed or the non-reference member of the HCO. In the remaining panels of Fig. 7, we plot the initial perturbations, $\Delta \phi$, that resulted in spike and/or burst synchrony, against phase, $\phi$, for the leech heart HCO with mutually inhibitory synapses described by the four models given in Sec. 2.

The vertical (dark) bars in the Fig. 7B-H panels, representing the largest deviation values of the phase perturbation, $\Delta \phi$, reveal that the width of the “synchronization band” varies with the phase: it is maximized during the active or spiking period, and shrinks during the quiescent period. For larger initial phase mismatches the cells of the HCO will settle in anti-phase bursting. Figure 7
FIG. 7. (A) Bursting cycle of the leech heart HCO at $g_s = 0.4$ is phase-parameterized on the interval $[0, 1]$; dots indicate some reference phases used for identifying the attraction basins of in-phase bursting. The horizontal line across the spikes sets the level of the synaptic threshold $\Theta_{syn} = -0.0225$. Attraction basins of the in-phase state plotted against the phase along the bursting cycle for four models of inhibitory synapses: (B) Heaviside, (C) FTM coupling; (D) heterogeneous FTM coupling with $g_s^{(12)} = 0.4$ and $g_s^{(21)} = 0.44$; (E) $\alpha$-dynamical synapse; (F) leech heart dynamical synapse. All cases reveal that the widest synchronization zone occurs during the tonic-spiking period of bursting, while quiescent period yields a narrow basin. In all panels the range of $\Delta\phi$ is scaled between $[-0.05, 0.05]$.

also demonstrates that all selected models of inhibitory synapses agree well, both quantitatively and qualitatively. Furthermore, as expected, longer lasting inhibitory inputs of the $\alpha$-dynamical (4) and leech heart dynamical synapses (5) (cf. Fig. 1) ensure some wider synchronization zones. Indeed, beyond the critical values after which the synapse is considered slow or slowly decaying in time, anti-phase bursting becomes non-observable thus leaving in-phase bursting as the only stable state; this is a classical result by [16]. On the contrary, when synapses are fast, anti-phase bursting largely dominates over much weaker in-phase bursting in the inhibitory HCOs (1). In-phase bursting necessarily requires close initial burst overlapping. Based on the analysis done in the previous section, we conclude that spike interactions bound the attraction basin of in-phase synchrony. In [35], we employed variational equations to demonstrate that stable in-phase bursting is caused
FIG. 8. Biparametric ($\Theta_{syn}$, $g_{syn}$)-diagrams depicting stability zones (dark) of in-phase bursting in the leech heart HCO with inhibitory coupling due to (A) the Heaviside function based synapse; (B) the FTM coupling; (C) the $\alpha$-dynamical synapse; (D) the leech heart dynamical synapse. Color bar showing the maximal difference in the voltage values between the cells: zero for in-phase bursting and 0.08 for anti-phase bursting. The parameters are $V_{shift K2} = 0.02$, $I_{app} = 0.006$, $\bar{g}_{Na} = 160$, $\tau_{K2} = 0.9$.

by the ability of fast inhibition to switch from fast desynchronizing spike to longer synchronizing impact after the spikes cross over the synaptic threshold. This synchronization property of spiking contrasts the HCO made of cells exhibiting spike-free relaxation-type bursting, such as plateau-bursting where the fast inhibition carrying only desynchronizing effects makes stable synchrony impossible.

In addition to variation of the level of synaptic threshold, $\Theta_{syn}$, that of the synaptic strength, $g_{syn}$, is used to examine the synchronization properties of in-phase bursting in strongly coupled HCOs. Figure 8 presents bi-parametric sweeping of ($\Theta_{syn}$, $g_{syn}$)-bifurcation diagram for in-phase bursting in the leech heart HCO with the four selected models of synapses. In the diagrams, shaded areas correspond to stability islands of in-phase bursting. For the given leech interneuron model, the synaptic coupling with $g_{syn}$ exceeding 0.02 is considered strong as it leads right away to robust anti-phase bursting via the hold-then-release mechanism [11]. Note that within the plausible range of values for the synaptic threshold, $[-0.015, -0.005]$, the HCO possesses the largest stability islands where it can exhibit in-phase bursting. In this range, the synaptic threshold crosses middle of all spikes, which ensures an optimal stabilizing balance for inhibiting synaptic currents to promote
in-phase bursting. Lowering or raising the synaptic threshold out of this range makes in-phase bursting less robust as the contribution of the spikes becomes less significant. After the synaptic threshold is lowered underneath the minimum voltage level of the spikes, the HCO cells begin bursting in antiphase generally, similar to pairs of relaxation oscillators [2], such as Morris-Lecar or FitzHugh-Nagumo spiking neurons, where the spike interactions play no functional role.

CONCLUSIONS

We have shown that fast non-delayed inhibitory HCOs composed of two endogenously bursting neurons can generate multiple co-existent phase-locked states, in addition to stable anti-phase and in-phase bursting. This is an extension of our previous result [35] that fast non-delayed reciprocal inhibition synchronizes HCOs, which contrasts with the customary view that reciprocal inhibition has to be slow or time-delayed to establish in-phase bursting. We have shown that the multistability of the HCOs is due to spike interactions and independent of specific choice of models for endogenous square-wave bursters and fast non-delayed synapses. Fast tonic spiking and fast inhibitions are the two necessary conditions for multistable bursting to exist in such HCOs. This is in contrast with plateau-like bursting where spike interaction is insignificant because of the slow frequency and smoothed spiking magnitude relative to the plausible range of the synaptic threshold levels. We have shown that the number and temporal characteristics of spikes determine the number of co-existent phase-locked states in weakly coupled HCOs. Besides, spikes are also attributed to be the necessary component for dynamically establishing the bi-stability in strongly coupled HCOs, where robust anti-phase bursting co-exists with less robust in-phase bursting. This study emphasizes the importance of detailed Hodgkin-Huxley models for credible modeling of larger central pattern generator (CPG) networks as opposed to employing relaxation oscillators, which might give rise to simplistic cooperative properties.

Our study of multiple phase locking in the HCOs and co-existing dynamical rhythms can help one better understand the origin of multistability and the nature of switching mechanisms between various neuronal rhythms that a multi-functional CPG can generate in response to changes in sensory inputs and external perturbation. Recent experimental studies [6] suggest that leech crawling and swimming can be generated by the same multifunctional CPG, capable of switching between the two locomotor patterns with no change in the types or strengths of connections among the coupled neurons. At the neuronal level, crawling is governed by the command neurons firing in synchrony, whereas the CPG switches to the swimming rhythm when the neurons switch to anti-
phase bursting. The duty cycle of in-phase bursting, generating the crawling rhythm, is 7-10 times longer than that of the swimming rhythm [6]. The duty cycle is conjectured to be the main control parameter that determines the rhythms and can trigger the switching between the rhythms [36]. Our study of the spike interactions, whose number and frequency are controlled by the duty cycle, together with previous studies of duty-cycle induced phase locking in larger inhibitory networks [11, 12, 35], promise to shed light on the genesis of switching mechanisms for emergent bursting patterns in real multifunctional CPGs and their realistic models.

ACKNOWLEDGMENT

This work was supported in part by the NFS grant DMS-1009744, RFFI grants No. 2100-065268 and No. 09-01-00498-a (to I.B.); NSF grant DMS-1009591, RFFI Grant No. 08-01-00083, the GSU Brains & Behavior Program, and project No. 14.740.11.0919 “Attracting leading scientists to Russian universities” (to A.S.). S.J. acknowledges the continuing PhD fellowship support through the GSU Brains & Behavior program. We would like to thank Dane Allen, Joe Youker, and Kristie Young for careful proofreading of the manuscript and valuable suggestions.

APPENDIX: INDIVIDUAL NEURON MODELS

Leech heart interneuron model

This model including the fast sodium current, $I_{Na}$, the slow potassium current, $I_{K2}$, and an ohmic leak current, $I_L$ is given by [10]:

\[
C \frac{dV}{dt} = -I_{Na}(V) - I_{K2}(V) - I_L(V) - I_{app},
\]

\[
I_{Na} = \bar{g}_{Na} n^3 h (V - E_{Na}), \quad n = n^\infty(V),
\]

\[
I_{K2} = \bar{g}_{K2} m^2 (V - E_K), \quad I_L = \bar{g}_L (V - E_L),
\]

\[
\tau_{Na} \frac{dh}{dt} = h^\infty(V) - h, \quad \tau_{K2} \frac{dm}{dt} = m^\infty(V) - m.
\]

Here, $V$ is the membrane potential, $n$ and $h$ are the gating variables for sodium channels, which activate and inactivate respectively as the membrane potential depolarizes; $m$ is the gating variable for potassium channels that activate slowly as the membrane potential hyperpolarizes. The sodium current activates instantaneously. The time constants for the gating variables, maximum conductances and reversal potentials for all the channels and leak current, and the membrane capacitance
are set as follows:
\[
\tau_{\text{Na}} = 0.0405 \text{ sec}, \quad \bar{g}_{\text{Na}} = 200 \text{ nS}, \quad E_{\text{Na}} = 0.045 \text{ V},
\]
\[
\tau_{K2} = 0.25 \text{ sec}, \quad \bar{g}_{K2} = 30 \text{ nS}, \quad E_{K} = -0.070 \text{ V},
\]
\[
C = 0.5 \text{ nF}, \quad \bar{g}_{L} = 8 \text{ nS}, \quad E_{L} = -0.046 \text{ V}.
\]
The steady state values of the gating variables are given by the following Boltzmann functions:
\[
n^\infty(V) = \left[1 + \exp(-150(V + 0.0305))\right]^{-1},
\]
\[
h^\infty(V) = \left[1 + \exp(500(V + 0.0333))\right]^{-1},
\]
\[
m^\infty(V) = \left[1 + \exp(-83(V + 0.018 + V_{\text{shift}}^{K2}))\right]^{-1}.
\]

An applied current, \(I_{\text{app}} = 0\) through the paper unless indicated otherwise (as in the strong coupling case, see Fig. 8). In this study, \(V_{\text{shift}}^{K2}\) is a primary bifurcation parameter that controls the number of spikes per burst.

**Sherman model of pancreatic beta cells**

This model [18] is based on two fast currents: calcium, \(I_{Ca}\), and persistent potassium, \(I_{K}\), and a slow potassium current, \(I_{s}\). \(V\) is the membrane potential and \(m\), \(n\) and \(s\) are the voltage dependent gating variables for these currents. The model is given by these ODEs:

\[
\tau \frac{dV}{dt} = -I_{Ca}(V) - I_{K}(V) - I_{s}(V),
\]
\[
I_{Ca} = \bar{g}_{Ca} m^\infty(V) (V - E_{Ca}),
\]
\[
I_{K} = \bar{g}_{K} n (V - E_{K}), \quad I_{s} = \bar{g}_{s} s (V - E_{K}),
\]
\[
\tau \frac{dn}{dt} = \lambda [n^\infty(V) - n], \quad \tau \frac{ds}{dt} = s^\infty(V) - s.
\]

The governing equations for the gating variables \(n\) and \(s\) are similar to those in (6), where the time constants, maximum conductances and values of reversal potentials are set as follows:

\[
\tau = 0.02 \text{ sec}, \quad \bar{g}_{Ca} = 3.6 \text{ nS}, \quad E_{Ca} = 0.025 \text{ V},
\]
\[
\tau_{s} = 5 \text{ sec}, \quad \bar{g}_{K} = 10 \text{ nS}, \quad E_{K} = -0.075 \text{ V},
\]
\[
\lambda = 1, \quad \bar{g}_{s} = 4 \text{ nS}.
\]

In the model, an additional scaling factor, \(\lambda\), controls the time scale of the persistent potassium channels. The steady state values of the gating variables are given by the Boltzmann functions:

\[
m^\infty(V) = \left[1 + \exp(-83.34(V + 0.02))\right]^{-1}
\]
\[
n^\infty(V) = \left[1 + \exp(-178.57(V + 0.016))\right]^{-1}
\]
\[
s^\infty(V) = \left[1 + \exp(-100(V + 0.035245))\right]^{-1}.
\]
Purkinje neuron model

This model [13] includes five currents: the sodium current, $I_{Na}$, with slow inactivation, $h$, and fast instantaneous activation, $m^\infty$; the delayed rectifier potassium current, $I_K$, with activation, $n$; the non-inactivating calcium current, $I_{Ca}$, with activation $c$; the muscarinic receptor suppressed potassium current, $I_M$, with activation $M$; $I_L$ is the leak current, and $I_{app}$ is an applied current. The individual cell model is given by

$$\frac{dV}{dt} = -I_{Na}(V) - I_K(V) - I_{Ca}(V) - I_M(V) - I_L(V) - I_{app},$$

$$I_{Na} = \bar{g}_{Na} m^3 h (V - E_{Na}), \quad m = m^\infty(V),$$

$$I_K = \bar{g}_K n^4 (V - E_K), \quad I_{Ca} = \bar{g}_{Ca} c^2 (V - E_{Ca}),$$

$$I_M = \bar{g}_M (V - E_M), \quad I_L = \bar{g}_L (V - E_L).$$

(8)

The governing equations for the gating variables $h$, $n$, $c$, and $M$ are similar to those in (6) where the values for maximum conductances and reversal potentials are set accordingly:

- $\bar{g}_{Na} = 152 \text{ nS}$, $E_{Na} = 50 \text{ mV}$,
- $\bar{g}_K = 10 \text{ nS}$, $E_K = -75 \text{ mV}$,
- $\bar{g}_{Ca} = 1 \text{ nS}$, $E_{Ca} = 125 \text{ mV}$,
- $\bar{g}_M = 0.75 \text{ nS}$, $E_M = -95 \text{ mV}$,
- $g_L = 2 \text{ nS}$, $E_L = -70 \text{ mV}$.

Voltage dependent time scales for the gating variables, measured in msec, and governed by the following functions:

$$\tau_n = 0.25 + 4.35 \exp(-0.1|V + 10|),$$

$$\tau_h = 0.15 + 1.15[1 + \exp(0.0667(V + 33.5))]^{-1},$$

$$\tau_c = [\alpha_{Ca} + \beta_{Ca}]^{-1}, \quad \tau_M = [\alpha_M + \beta_M]^{-1},$$

where

- $\alpha_{Ca} = 1.6/(1 + \exp(-0.072(V - 5)))$,
- $\beta_{Ca} = 0.02(V + 8.9)/(-1 + \exp(0.2(V + 8.9)))$,
- $\alpha_M = 0.02/(1 + \exp(-0.2(V + 20)))$,
- $\beta_M = 0.01 \exp(-0.0556(V + 43))$. 
The steady state values of the gating variables are given by the following Boltzmann functions:

\[
\begin{align*}
n^\infty(V) &= [1 + \exp(-0.1(V + 29.5))]^{-1} \\
m^\infty(V) &= [1 + \exp(-0.1(V + 34.5))]^{-1} \\
h^\infty(V) &= [1 + \exp(0.0935(V + 59.4))]^{-1} \\
c^\infty(V) &= \alpha_{Ca}\tau_c, \quad M^\infty(V) = \alpha_M\tau_M.
\end{align*}
\]

Here, the applied current is a bifurcation parameter set for the cell to be a long burster as \(I_{\text{app}} = -27\) nA.