Short Duty Cycle Destabilizes a Half-Center Oscillator, But Gap Junctions Can Restabilize the Anti-Phase Pattern

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Submitted 12 August 2003; accepted in final form 29 September 2003

Bem, Tiaza and John Rinzel. Short duty cycle destabilizes a half-center oscillator, but gap junctions can restabilize the anti-phase pattern. J Neurophysiol 91: 693–703, 2004. First published October 22, 2003; 10.1152/jn.00783.2003. Mutually inhibitory pacemaker neurons with duty cycle close to 50% operate as a half-center oscillator (anti-phase coordination, i.e., 180° out of phase), even in the presence of weak to modest gap junctional coupling. For electrical coupling strength above a critical value synchronization occurs. But, as shown here with modeling studies, the effects of electrical coupling depend critically on a cell’s duty cycle. Instead of oscillating either in-phase or anti-phase, model cells with short duty cycle express additional rhythmic patterns, and different transitions between them, depending on electrical coupling strength. For weak or no electrical coupling, cells do not oscillate in anti-phase but instead exhibit almost in-phase activity. Strengthening this weak coupling leads to stable anti-phase activity. With yet stronger electrical coupling stable in-phase (synchrony) emerges but it coexists with the anti-phase pattern. Thus the network shows bistability for an intermediate range of coupling strength. For sufficiently strong electrical coupling synchrony is the network’s only attracting rhythmic state. Our results, numerical and analytical (phase plane analysis), are based on a minimal but biophysically motivated pacemaker model for the slowly oscillating envelope of bursting neurons. However, illustrations for an Hodgkin–Huxley model suggest that some of our results for short duty cycle may extend to patterning of repetitive spikes. In particular, electrical coupling of intermediate strength may promote anti-phase activity and provide bistability of anti-phase and in-phase spiking.

INTRODUCTION

Networks of inhibitory neurons exist in various neural systems and have been extensively studied. Important insights into their underlying mechanisms have been typically obtained with reduced, idealized models—both theoretically and experimentally. For example, the neural drive for alternately activating cycles of antagonistic muscle groups in locomotion and respiration is provided by central pattern generator (CPG) networks (Cohen et al. 1988). In isolated CPGs and in models for them, the half-center oscillator, composed of reciprocally inhibitory pairs of pacemaker neurons, plays a critical role (Arshawski et al. 1993; Rowat and Selverston 1993; Selverston and Moulin 1985). Furthermore, the dynamics of inhibitory neurons in the reticular nucleus were shown to determine whether the thalamus displays spindle or delta sleep rhythms (Rubin and Terman 2000; Terman et al. 1996) and whether the inhibitory neurons burst synchronously (Golomb et al. 1994). Networks of inhibitory neurons studied in vitro and with computational models have been suggested to synchronize spiking cells in the frequency range (approximately 40 Hz) in hippocampal and neocortical circuits (Traub et al. 1997; Wang and Buzsáki 1996; Whittington et al. 1995). Whereas the classical view, largely shaped by the half-center oscillator model from the context of CPGs, holds that mutual inhibition provides anti-phase (AP) coordination between neurons, we appreciate from the recent studies that inhibitory neurons may also synchronize (Golomb et al. 1994; Traub et al. 1997; Van Vreeswijk et al. 1994; Wang and Buzsáki 1996; Wang and Rinzel 1992; Whittington et al. 1995). On the other hand, pure synchrony may be fragile, depending for example on synaptic time scales being not too fast or neurons being not too different from one another (Golomb et al. 1994; Van Vreeswijk et al. 1994; Wang and Buzsáki 1996; Wang and Rinzel 1992; White et al. 1998). Other, more recent, findings indicate that our understanding of temporal patterning in inhibitory networks needs to be yet further reevaluated. Namely, it has been shown that, in the CNS, synaptic inhibition often coexists with gap junctional coupling (Galaretta and Hestrin 1999; Gibson et al. 1999). Since electrical coupling typically is thought to synchronize cells’ activity (even the half-center oscillator synchronizes for very strong electrical coupling) (Bem et al. 2002), it becomes important to understand a functional role for the coexistence of these two types of coupling, which one may think have opposing effects on coordinating cells. In particular, it is important to determine various rhythmic states or patterns of the network and how transitions between them occur.

Since gap junction–mediated electrical interaction depends only on the membrane potential differences between coupled cells, it can occur throughout the cycle. In contrast synaptic inhibition requires sufficient presynaptic depolarization and interaction effects (for inhibition with fast kinetics) are therefore limited to specific phases during cell activity. In this context, a cell’s duty cycle, which is the fraction of the period during which the cell is depolarized above a threshold for spike generation, should play a role in determining the relative influence of these two types of coupling in the network. Here we demonstrate, using a simple two-cell network model and fast synaptic conductance kinetics, that the role of duty cycle can be quite dramatic. In the short duty cycle regime we find, in addition to AP and in-phase (IP) coordination, as for cells with large duty cycle, that new dynamic features emerge. If electrical coupling is absent or weak a pattern of almost-in-phase (AIP) activity occurs. In contrast, for stronger but not too...
strong gap junctions the stable AP and IP behaviors coexist over a wide range of electrical coupling strength, thus demonstrating a bistability. Our results apply directly to pacemaker cells, interacting through the envelope of membrane potential oscillations (not spikes per se), and also, in the very short duty cycle regime and fast synaptic kinetic, to spiking cells.

In RESULTS we first describe simulations that demonstrate the occurrence of different rhythmic states of the network, depending on electrical coupling strength and on a cell’s duty cycle. Next we focus on instability of the AP solution in the regime of weak electrical coupling, occurring for cells with short duty cycle. We present theoretical results, using phase plane analysis and singular perturbation theory. We first show that inhibition compresses a cell’s cycle duration in a way that is phase dependent. Then we explain how this effect produces instability of the AP solution and how it leads to the AIP activity pattern. We also show how sufficient electrical coupling stabilizes the AP pattern. Finally we suggest that our results may apply to spiking cells by providing an illustration with simulations of two mutually inhibitory, and gap junction–coupled, Hodgkin-Huxley cell models.

METHODS

The network model consists of two identical reciprocally inhibitory neuronal oscillators, which, in addition to chemical synapses, are also interconnected by electrical coupling. A single cell is modeled as a two-variable oscillator (Rowat and Selverston 1993). The equations are

\[
\begin{align*}
\tau_a \frac{dV}{dt} &= -I_{leak}(V) - I_{syn}(V, V_p) - I_{gap}(V, V_p) - w \\
\tau_s \frac{dw}{dt} &= w_s(V) - w
\end{align*}
\]

Equation 1 is a membrane current balance equation, \(V\) and \(V_p\) are the membrane potential deviations of a cell and its partner from a reference voltage (this reference value is midway between the depolarized and hyperpolarized voltage ranges during a pacemaker’s intrinsic cycle). The instantaneous current \(I_{fast} = -V + \tanh(g_{fast} V)\) combines the leak current and a fast \(V\)-dependent inward current; it is \(N\)-shaped to a degree determined by \(g_{fast}\). Synaptic transmission is instantaneous, with a synaptic current given by \(I_{syn} = g_{syn} S_v(V_p) (V - V_{syn})\), where \(g_{syn}\) is maximal synaptic conductance, \(V_{syn}\) is the synaptic reversal potential, and the sigmoid function \(S_v(V_p) = \frac{1}{1 + \exp(V_p - \theta_{syn})/k_{syn}}\) determines the fraction of transmitter released as a function of the membrane potential \(V_p\) of the presynaptic cell, and \(\theta_{syn}\) represents the midpoint for synaptic activation (we set \(\theta_{syn} = 0\)). The function can be tuned into a more graded or a more sharper threshold-like regime, depending on \(k_{syn}\), which is the steepness parameter at \(V_p = 0\). Gap junction coupling is represented by \(I_{gap} = g_{gap} (V - V_p)\). A slow variable \(w\) represents the slow recovery current, with voltage dependent activation function \(w_s(V) = g_{slow} V\).

The duty cycle is dependent on parameters \(\tau_1\) and \(\tau_2\), which determine the value of \(\tau_{act}\) in the active and silent phase of cell cycle and thereby the durations of the two phases. \(\tau_{act}(V)\) approaches \(\tau_1\) during the active phase \((V > 0)\) and \(\tau_2\) during the silent phase \((V < 0)\) of the cycle, as given by \(\tau_{act}(V) = \tau_1 + (\tau_2 - \tau_1)\omega(V)\) (here the sigmoid function \(\omega(V) = \frac{1}{1 + \exp(V/V_k)}\) has the slope \(1/(4k)\) at \(V = 0\)). Another pair of identical equations for \(V_p\) and \(w_p\) describes the behavior of the partner cell. In this idealized model all variables and parameters are dimensionless.

For Hodgkin-Huxley model cells we use the standard model equations (Koch 1999). Here the dynamics of synaptic transmission are described by

\[
\tau_s \frac{dS(t)}{dt} = S_v(V_p) (1 - S(t)) - S(t)
\]

where \(S(t)\) is the fraction of transmitter released at time \(t\), \(V_p\) is the potential of presynaptic cell, \(S_v(V_p)\) has the same meaning as in Eq. 1, and \(\tau_s\) is the time constant of the synaptic rise and decay (Note: \(S\) is driven by \(V_p\)).

Simulation were run using the software XPPAUT developed by B. Ermentrout (http://www.pitt.edu/~phase/). We used the Runge-Kutta (2nd order) integration method, with the integration time step \(dt = 0.01\) and \(\epsilon = 1 \times 10^{-3}\).

RESULTS

Description of coordination patterns and effects of duty cycle

We first demonstrate basic effects of the duty cycle on the dynamics of two oscillators interconnected by instantaneous synaptic inhibition and by electrical coupling in the case when oscillations are in the relaxation regime \((\tau_{syn} \ll \tau_{act}, i.e.,\text{, voltage changes due to current imbalance are very fast compared to activation/speed of slow recovery current})\). We treat the gap junction strength and duty cycle as control parameters, imagining that both might be affected by neuromodulators.

If the duty cycles of neurons are close to 0.5, i.e., the active phase occupies approximately one-half of the intrinsic cycle (Fig. 1A, left), the behavior of the network follows our intuition. With weak electrical coupling synaptic inhibition dominates and cells express AP oscillations whereas for strong electrical coupling they oscillate IP (Fig. 1A, right). A transition between AP and IP activity occurs at some critical electrical coupling strength, which increases with increasing strength of inhibitory synapses (not shown).

In contrast, cells with a short duty cycle, i.e., with the active phase considerably shorter than one-half of the cycle (Fig. 1B, left), when coupled by synaptic inhibition and gap junctions can express different rhythmic behaviors and transitions. First, depending on the value of \(g_{gap}\), three types of rhythmic pattern can be generated (Fig. 1B, right), instead of just two types (Fig. 1A, right). In addition to the AP and IP oscillations, as in the case of duty cycle 0.5, a new activity pattern arises. We call it the AIP activity, since the active phases of the two cells are occurring here very close to each other (Fig. 1B, right, top trace).

Second, transitions between rhythmic states of the network are different from in the case when the intrinsic duty cycle is about 0.5. These transitions are illustrated on a response diagram that shows, for a wide range of reciprocal inhibition (cf. black circles correspond to \(g_{gap} = 0.05\)), the occurrence of stable patterns of activity, depending on electrical coupling strength (Fig. 1C). If electrical coupling is absent or weak, both AP and IP patterns are unstable (not shown), instead, an AIP pattern is the stable state (dashed oblique lines, Fig. 1C). Stronger electrical coupling stabilizes the AP pattern (horizontal lines, Fig. 1C) whereas the AIP pattern disappears. With further increase of electrical coupling there is an overlap of the stable AP (horizontal lines) and IP (vertical lines) behaviors in a wide range of moderate electrical coupling strength. Finally, for electrical coupling sufficiently strong the IP pattern be-
comes the only stable oscillatory state. The critical values of electrical coupling at which transitions between patterns occur increase with the strength of inhibitory synapses: the stronger is the inhibition, the larger is the electrical coupling that is necessary to stabilize AP and then IP patterns (see borders between different areas, Fig. 1C). However, if inhibition and electrical coupling are too strong synchronous oscillations become completely damped and both the IP and AP states disappear (Fig. 1C, white area). We supplement the response diagram by quantifying features of the patterns, amplitude, and period of oscillations over a range of $g_{\text{gap}}$ for two different values of $g_{\text{syn}}$ (Fig. 1D). Most noticeable is the decrease of amplitude of the AP pattern with increasing electrical coupling, due mostly to a decrease of $V_{\text{max}}$ in the active phase (with little change in period). By contrast, the amplitude of the IP pattern is independent of $g_{\text{gap}}$ (because the electrical coupling current is equal to 0). Counterintuitively, the period of the IP pattern decreases with increasing inhibition; this can be explained by the lowering of the $V$-nullcline’s right knee which reduces the $w$ range traversed by the cells in both active and silent phases (not shown).

Our results demonstrate a bistability of two rhythmic states of the network of inhibitory neurons with short duty cycles, occurring at moderate electrical coupling strength (Fig. 1, C and D). This is further illustrated in Fig. 2, where a brief stimulus (triangle) applied to one of the cells produces an abrupt transition between AP and IP oscillations (top) whereas a second application of the stimulus restores the initial pattern of activity (bottom).

Next we offer an explanation for such effects of the cells’ intrinsic duty cycle on the network’s dynamics. We focus only on networks dominated by inhibition, in which electrical coupling is weak or totally absent. As suggested by the computations of Fig. 1, B and C, in such a network cells with very short duty cycle cannot oscillate in AP since the AP solution is unstable in the absence of sufficiently strong electrical coupling. Instead, they will generate the AIP pattern of activity (Fig. 1, B–D). This was further tested in simulations, in which two cells with 0.5 duty cycles, coupled only by synaptic inhibition, were initially oscillating in AP. In this experiment we were testing how decreasing a cell’s duty cycle $dT$, where $d$ is the duration of the active phase and $T$ is the cycle duration, affects cells’ coordination, measured as a phase shift $\Phi$ between two cells (Fig. 3, top).

As illustrated in Fig. 3 (bottom), decreasing duty cycle $dT$ changes the phase relationships between the oscillators. For duty cycle not much less then 0.5 ($dT = 0.5$ to 0.3) cells generate purely AP activity ($\Phi = 0.5$). With further decrease of
the duty cycle \((dT = 0.3 \text{ to } 0.1)\) the AP coordination is lost and the phase shift between cells is decreasing almost linearly \((\Phi = 0.5 \text{ to } 0.1)\). This part of the curve corresponds to the AIP solution (cf. Fig. 1B, right).

**AP and AIP behaviors for short duty cycle**

Decreasing the duty cycle of cells oscillating in AP leads to the AIP pattern (Fig. 3). However, in the short duty cycle regime, the AP solution still exists (not shown). We want to understand now why this state is unstable. To analyse it we apply singular perturbation theory. We are going to show on the phase plane how the perturbation of the AP solution leads to a difference in the cycle durations of the two cells, which produces instability. Moreover we will show that the only attracting state of the network is the AIP solution, i.e., that any phase difference between cells with a small perturbation applied will evolve toward this pattern. First, we explain how oscillatory behavior of a single rhythmic cell is represented on the phase plane.

**Single oscillatory cell’s trajectory on the phase plane**

Figure 4A shows the voltage trace of an uncoupled cell \((\text{top})\) and the corresponding trajectory on the phase plane \((V, w)\) \((\text{bottom})\). In the relaxation regime \((w \text{ very slow compared with } V)\) that we consider here, the trajectory consists of four pieces. Two of these pieces lie along the left and right branches of the cubic-shaped curve, called V-nullcline [thin line, obtained as the relationship between \(V\) and \(w\) by setting \(dV/dt = 0\) in Eq. (1)]. They correspond to the cell’s silent and active phases, respectively. The other two pieces, which begin at the “knees” of the V-nullcline, correspond to jumping between these two phases (horizontal lines with double arrows). In the relaxation regime the transition between branches of the V-nullcline is very fast compared with evolution along these branches.

An important feature of oscillatory behavior is that the speed of evolution along branches is not constant but is equal to the product of \(1/\tau_w (V)\) times \((w(V) - w)\). This latter factor is the \((\text{vertical})\) distance between the cell’s actual position on the phase plane and the other curve, called the \(w\)-nullcline [sigmoid-shaped thin line, obtained as the relationship between \(V\) and \(w\) by setting \(dw/dt = 0\) in Eq. (2), Fig. 4A, bottom]. Just after a jump down from the right to left branch of the V-nullcline the speed of evolution is highest whereas it reaches its minimal value when the cell is at the knee (cf. \(x\) and \(y\), Fig. 4A, bottom). Thus depending on the \(w\)-nullcline’s shape and the function for \(\tau_w (V)\), the cell can spend different amounts of time on the left and on the right branch of the V-nullcline. (Actually in our model \(\tau_w (V)\) is practically constant on the left branch and on the right branch.) This difference of traverse times determines the cell’s duty cycle but does not affect the trajectory, as viewed in the phase plane, which we will call “the free cell trajectory.” However, if two identical oscillatory cells are reciprocally coupled by synaptic inhibition, their trajectories are modified according to a cell’s duty cycle.

**Inhibition alters the speed of an oscillator’s evolution in a phase-dependent way**

Figure 4B illustrates voltage traces \((\text{top})\) and phase plane trajectories \((\text{bottom})\) of two such cells with short duty cycle generating AP oscillations when coupled by inhibition. When one of the cells jumps up from the silent to active phase (see open and filled circles, respectively) it crosses the voltage threshold for transmitter release (thin vertical line, Fig. 4B, right). Therefore, due to instantaneous inhibitory influence,
the other cell is immediately removed from the free cell trajectory (see dashed circles, open and filled, respectively) and then evolves downward along a shifted $V$-nullcline (see cubic curve, shifted down), as long as its partner is active. After the active cell completes its active phase and passes to the silent phase, the partner cell is released from inhibition and returns to its free cell trajectory. The trajectories of both cells are identical. The phase of inhibition occurs in the two cells at the same position on the phase plane (see $w_{\text{ON}}$ and $w_{\text{OFF}}$, Fig. 4B, bottom), exactly midway (in time) of the silent phase of the cycle. Importantly, when the cells evolve on the shifted nullcline, the rate $dw/dt$ at which $w$ falls is larger than on the free cell trajectory for the same $w$ range. This faster rate is produced by increased distance from the $w$-nullcline (cf. $x$ and $y$, Fig. 4B, middle, right) and it leads to a reduction or compression of the cycle duration. This compression can be estimated by comparing the silent phase trajectories of a coupled and isolated cell. These trajectories are identical everywhere except in the interval between $w_{\text{ON}}$ and $w_{\text{OFF}}$, where the phase of inhibition occurs (Fig. 4C, left and right, respectively).

For this comparison imagine that both cells start their cycles at the same position $w_{\text{OFF}}$ on the left branch of the $V$-nullcline. As long as the trajectories are identical the two cells will evolve identically. Thus the cells evolve counterclockwise until they both reach simultaneously the position $w_{\text{ON}}$ on the left branch of the $V$-nullcline (Fig. 4C, left and right, open circles). The inhibited cell now moves to the shifted trajectory whereas the isolated cell continues evolving along the free cell trajectory. After time $\Delta t_{\text{inh}}$, where is the duration of the phase of inhibition, the inhibited cell returns to the free cell trajectory at the position $w_{\text{OFF}}$ and thereby completes the cycle (Fig. 4C, left, filled circle). At this moment, however, the isolated cell, which has been evolving with a lower speed, is further from the knee, reaching only the position $w_{\text{OFF}} - \Delta w$ (note: $\Delta w < 0$) (Fig. 4C, right, filled circle). This $\Delta w$ satisfies

$$\Delta w = (\Delta (dw/dt))t_{\text{inh}}$$

Here $\langle \Delta (dw/dt) \rangle = (\langle (dw/dt) \rangle - \langle dw/dt \rangle)$ is a difference between the speed of evolution of the coupled $(dw/dt)_c$ and isolated cell $(dw/dt)_i$, averaged over time $\Delta t_{\text{inh}}$. The cycle of the isolated cell will therefore be completed with some delay $\Delta t$, equal to the time required by that cell to pass through $\Delta w$ and reach the position $w_{\text{OFF}}$. Thus the cycle duration of the coupled cell $T_i$ is compressed by amount $\Delta t$, comparing it to the isolated cell’s cycle duration $T_i - \Delta t$

$$T_i = T_i - \Delta t$$

Importantly, the amount of compression (Eq. 5) depends on when the inhibition occurs in the cycle. Suppose for example that our cell (Fig. 4C) is inhibited later in the silent phase. This will correspond to some $\Delta w'$, situated closer to the knee than $\Delta w$ (see Fig. 4C). It turns out that $\Delta w'$ will be larger than $\Delta w$ (the argument developed by Kopell and Somers 1995 for the case of mutual excitation can be easily adapted to our case). Therefore this leads to a larger difference $\Delta t'$ between the cycle duration of the isolated and coupled cell (the isolated cell spends more time passing through a larger distance with a lower speed). Similarly, if the inhibition occurs earlier in the silent phase (is advanced) then the compression is smaller. Thus a phase of inhibition which is delayed, i.e., occurs closer
to the knee of the $V$-nullcline, producing a larger compression of the cycle $T_i$ than an advanced phase of inhibition. This phase dependence of compression underlies the instability of the AP solution, which we describe next. We note that the effects of compression were previously exploited in (Kopell and Sommers 1995) to demonstrate that mutually excitatory, short duty cycle, relaxation oscillators can oscillate in stable AP—the counter situation to that we are considering here.

**Small perturbation of AP leads to AIP behavior**

Consider two coupled inhibitory cells oscillating in AP. Their phase plane trajectories are shown in Fig. 5A (top, solid and dashed lines). We will refer to them as cell A (left) and cell B (right). Now we will consider the effect of a small perturbation in $w$ delivered to cell B. We perturb cell B at the end of cell A’s silent phase (solid circle, top left). Our initial perturbation moves cell B down instantaneously by $\delta w^0$ (dashed circle, top right). We will show that this deviation of cell B’s position from $w^0_{ON}$ (at the time of cell A’s up-jump) grows from cycle to cycle, demonstrating instability of the AP solution.

Let’s look at the positions of both cells at the end of the next cycle, when cell A again reaches the knee (solid circle, middle left). The trajectories in the middle describe the cells’ histories in this first cycle (solid and dashed curves, middle). Cell B started the cycle at the perturbed position $w^0_{ON} - \delta w^0$ on the free cell trajectory, just when it begins to receive inhibition. Thus, when released from inhibition, it is closer to the knee than in the previous cycle (see $w^0_{OFF}$ and $w^0_{OFF}$, middle and top, respectively). Therefore the time $t'$ required by cell B to reach $w^0_{knee}$ and to start the active phase of the cycle is shorter than in the previous cycle (see $t'$ and $t'$, right, middle and top, respectively). This in turn advances the onset of the left cell’s inhibited phase (see $t'$ and $t'$, left, middle and top, respectively). Thus, as the result of the perturbation, the phases of inhibition of two cells occur at different positions on their trajectories; whereas the inhibition of cell A is advanced, the inhibition of cell B is delayed compared with the AP solution. This leads to, as we said above, a difference in the cells’ cycle durations. Cell B will have, due to a stronger compression effect, a shorter cycle than cell A.

Therefore in time when cell A completes one cycle (i.e., reaches $w^0_{knee}$, see solid circle, middle), cell B completes more than one cycle (i.e., evolves below $w^0_{ON} - \delta w^0$ and reaches $w^0_{ON} - \delta w^1$, see dashed circle, middle). Thus the initial perturbation increases (cf. $\delta w^0$ and $\delta w^1$, right, top and middle). At successive cycles cell B is closer to the knee when cell A jumps up while cell A is further from the knee when cell B jumps up. Thus the difference in the cycle durations in the two cells will successively increase. This will last as long as cell B is above the knee when it is released from inhibition (i.e., as long as $w^0_{OFF}$ is above $w^0_{knee}$, cf. Fig. 5A, middle right). When the phase of inhibition of cell B is so delayed that its offset occurs at the level of the knee (not shown), the compression produced by

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**Fig. 5.** AP oscillations of cells with short duty cycle are unstable for electrical coupling absent (A) or weak (B). A: in a theoretical experiment reciprocally inhibitory cells oscillate in perfect AP, each receiving inhibition midway through its silent phase (equal time $t'$ above and below the phase of inhibition). The cycle durations ($T'$) are equal and the cells’ trajectories are identical (solid and dashed lines) (top). At time $t = 0$, a perturbation is applied to the right cell (cell B) that decreases instantaneously its $w$ value by the amount $\delta w_0$ and thereby its phase of receiving inhibition is now delayed until later in the cycle (top, right). The immediately next cycles have different durations ($T'$), due to different cycle compression produced by an advanced (left) and delayed (right) phase of inhibition (middle). Therefore when one cell completes a cycle (cell A, left), the other cell evolves below a position where it started the cycle, which increases the initial delay for the phase when it receives inhibition (see $\delta w_1$) (right, middle). After $k$ cycles, the cells’ cycle durations ($T'$) become equalized due to an extension of cell B’s trajectory, for which the offset of the phase of inhibition $w^0_{OFF}$ occurs now below $w^0_{knee}$ (bottom, right). B: in an analogous simulation experiment cells are additionally coupled by weak electrical synapses. After the perturbation is applied (triangle) to cell B (open circles) its offset from inhibition occurs with decreasing distance from the knee (see $w^0_{OFF} - w^0_{knee}$) (bottom) whereas the difference between the cells’ cycle durations increases (see $T'/T'$) (top). Starting from the moment when $w^0_{OFF}$ reaches $w^0_{knee}$, the cycle duration difference gradually diminishes to 0. Parameters: $g_{OFF}$ = 0.03, other parameters as in the legend to Fig. 1B.
inhibition is maximal and the cell has its shortest cycle. However, with a further delay in successive cycles the cell will eventually evolve, during the phase of inhibition, to below $w_{\text{knee}}$. From here it will jump directly to the right branch of the $V$-nullcline when released from inhibition (see $w_{\text{OFF}}$ below $w_{\text{knee}}$, Fig. 5A, bottom right). Starting from this moment both the silent and the active phase durations of cell B will be increased in successive cycles until the cycle durations of both cells become equal. This is the AIP solution, with cell B’s up-jump occurring simultaneously with cell A’s down-jump.

In the AIP state, the cycle of each cell consists of three segments of unequal duration, which give the same total result. First, the active phase is longer in the “follower” (B) than in the “leader” (A) cell since the distance that the follower cell has to pass on the right branch of $V$-nullcline after its up-jump is now increased (Fig. 5A, bottom right and left). Second, the phase of inhibition, which lasts as long as the partner’s active phase, is correspondingly longer in the leader than in the follower cell. Third, the duration of the remaining part of the silent phase (in which no inhibition is received) is the same in both cells. Indeed, this is the time interval between the follower cell’s down-jump and the onset of its phase of inhibition that corresponds exactly to the time during which the leader cell evolves after being released from inhibition until its up-jump occurs (cf. $\tau$, Fig. 5A, bottom right and left).

Figure 5B demonstrates the results of simulations in which, as described above, a perturbation (triangle) was applied to one of two reciprocally inhibitory cells that are oscillating in AP (for a few cycles because their initial conditions were set exactly on the AP trajectory). In addition to synaptic coupling the cells were also coupled by a small gap junctional conductance ($g_{\text{gap}} = 0.03$, a value below the range of electrical coupling that stabilizes the AP; cf. Fig. 1B, bottom). The electrical coupling reduces the difference in cycle durations produced by inhibition and thereby slows down (for illustration purposes) the transition from the unstable AP to the stable AIP solution. As seen, an imposed initial delay (at $n = 0$) of the phase of inhibition to cell B (cf. $\delta w_{\text{i}}$, Fig. 5A, top), produces in successive cycles ($n = 0$ to 14) a shortening of its cycle accompanied by an elongation of cell A’s cycle (open and filled circles, respectively, Fig. 5B, top) until the offset of the inhibited phase ($w_{\text{OFF}}$) occurs at the knee ($w_{\text{knee}}$) ($n = 14$) (Fig. 5B, bottom). As predicted by the theory, starting from this moment, the cycle duration of the perturbed cell gradually increases as the two cycle durations become equal ($n = 30$) (Fig. 5B, top).

The above mechanism of instability of the AP pattern is based on the difference between cycle durations produced by an advanced versus delayed inhibition. The instability occurs because the more the phase of inhibition is delayed, the shorter the cycle. When there are also gap junction connections between cells, these relationships change. The electrical coupling modifies cells’ trajectories and therefore affects the speed of evolution, not only during the phase of inhibition, as does solely synaptic coupling, but all over the cycle.

### AIP to AP transition due to electrical coupling

We now offer a mechanistic and intuitive explanation for how electrical coupling can induce a transition from the AIP to the AP state. Our explanation is supported by the numerically simulated response showing the evolution from the AIP to the AP state after electrical coupling is introduced.

Multiple and competing factors contribute to the transition. Synaptic inhibition and compression favor the AIP state. But, when one cell is active and the other is not, electrical coupling works against synaptic inhibition, tending to bring cells together across the two branches in the phase plane. Moreover, gap junction coupling also acts to bring cells’ voltages together when they are on the same branch. As a consequence this force contributes to pulling the follower and leader together during the long time interval between the follower’s active phase and leader’s next active phase, primarily by inducing the follower to drop behind and thereby to destabilize the AIP state. If the gap junction coupling is strong enough but not too strong the system reaches a compromise of AP instead of AIP.

We now formalize this argument a bit more. Imagine the two cells oscillating in the AIP pattern (Fig. 6A, solid lines). We consider a moment of the cycle when the leader cell (A) is at the end of its active phase and the follower cell (B) is ready to jump up due to release from inhibition. We now turn on electrical coupling and look at the two cells’ trajectories (thick dotted lines) after cell A jumps down. The up-jump of B will be now delayed since B now receives a negative (hyperpolarizing) current due to electrical coupling with its network partner (i.e., $-i_{\text{gap}} = -g_{\text{gap}}(V_{\text{B}} - V_{\text{A}})$ and $V_{\text{B}} > V_{\text{A}}$). Cell B will therefore move to its nullcline (thick dotted line) that is shifted down with respect to the free nullcline (solid line) along which it will evolve until it reaches the lowered knee. The transition of this follower cell to the active phase will no longer occur due to release from inhibition but rather it will “escape,” as we know occurs during the AP state. On the other hand, cell A will receive during this time a positive coupling current and therefore will evolve (thick dotted line) along its nullcline that is shifted above the free nullcline (thin dotted line). This nullcline for A will shift leftward when cell B jumps up and crosses the threshold for sending inhibition. Therefore, during this phase when B is active, A receives both depolarizing and hyperpolarizing coupling currents, electrically and synaptically mediated, respectively. (For the particular case shown in Fig. 6 the inhibitory current is dominant, therefore the inhibited cell displacement is leftward from the free nullcline.)

The significant delay in B’s firing just after activation of the gap junctions means that B begins to fall behind A. This delay opens a brief gap between the down-jump of A and up-jump of B. During this short gap between the cells’ active phases (A followed by B) the coupling current acts to bring them closer together. On the other hand, during the long gap from B’s down-jump to A’s up-jump (inherited from the AIP pattern), the coupling current has more time to pull the cells together. This current underlies B’s continued fall behind A until the AP state is reached when the cells’ trajectories are identical. Notice the evolution of cycle duration in the top of Fig. 6B: B’s cycle duration is decreasing and A’s cycle duration is increasing (except for one cycle, see figure legend). During this transient evolution we see in the phase plane that the position at which the leader A is released from inhibition, $w_{\text{OFF}}$, drifts downward while the corresponding position for B drifts upward (Fig. 6B, bottom).

The steady-state AP trajectory (thick dotted line) is shown in Fig. 6C. After one of the cells jumps down, which occurs at a specific $w$-level, $w_{\text{ID}}$, the cell (solid white circle) evolves above
the latter cell reaches the knee of its nullcline at \( w_{\text{HU}} \) (dotted shaded circle), it jumps up toward the right branch of the free nullcline but does not quite reach this branch since it still receives negative electrical coupling current. This current is now even more negative because the voltage difference between the cell and its partner (solid shaded circle) has increased. Therefore during its entire active phase the cell is evolving below the free nullcline, whereas the partner cell, receiving a combined inhibitory/electrical coupling, is evolving between \( w_{\text{ON}} \) and \( w_{\text{OFF}} \). Here, at \( w_{\text{OFF}} \) it is released from inhibition when the other cell jumps down and a new, identical half-cycle begins.

The arguments we presented here are valid only for some range of electrical coupling strength. Above some critical value of \( g_{\text{gap}} \), the coupling force that tries to pull cells together is so strong that \( w \) values become equalized when both cells are in the silent phase and the cells jump up together—this is the IP state.

### Fig. 6. Transition from AIP to AP pattern occurs with sufficient electrical coupling

A: initial AIP oscillations (solid line trajectories) are perturbed by introducing electrical coupling at the moment when the leading cell is at the end of its active phase (left, solid white circle) whereas the follower cell is ready to jump up (right, dashed white circle). Therefore cells’ evolution (dashed lines) occurs along the nullclines, which are, due to the opposite coupling currents, shifted below and above (see dotted lines, left and right, respectively) their AIP trajectories (see shaded circles, solid and dashed, respectively) until the follower cell’s upstroke occurs (see dashed line with arrows, right). B: evolution of cycle duration (top) and \( w_{\text{OFF}} \) (bottom) induced by the timed introduction of electrical coupling. After this parametric perturbation was applied (triangle, cycle \( n = 5 \)) the follower cell cycles slower (white circles), abruptly at first then less slow in successive cycles, whereas the leader cell increases its cycle duration (black circles) until eventually they reach the same cycle duration (\( n = 27 \)). Notice that the perturbation initially increases the cycle duration of both follower and leader cells (\( n = 6 \)). The latter cell’s cycle duration is here suddenly increased because the cell jumps down in this first cycle following perturbation from the knee of the free nullcline (see A, left) and not from the knee of downward shifted nullcline (see C). In parallel, the position at which the leader cell is released from inhibition (see \( w_{\text{OFF}} \), black circles, bottom) evolves downward, toward the knee, whereas the analogous position of the follower cell evolves upward (see white circles, bottom) until the cells finally reach the same level of \( w_{\text{OFF}} \). C: the trajectory of the AP oscillation (dashed line) is identical for both cells. After one of the cells jumps down (see \( w_{\text{JF}} \), solid white circle) and the other cell is released from inhibition (see \( w_{\text{OFF}} \), dashed white circle), the two cells evolve in a nonactive phase until the latter cell jumps up (see \( w_{\text{HU}} \), solid shaded circle) and the former cell starts to receive inhibition (see \( w_{\text{ON}} \), dashed shaded circle).

### Hodgkin–Huxley neurons with fast inhibitory synapses need electrical coupling to generate AP spikes

Next we explore whether the results that we obtained for short duty cycle relaxation oscillators and instantaneous synaptic interactions may be to applicable to conductance-based models of spiking cells with synaptic conductance kinetics. Here we used a pair of mutually inhibitory Hodgkin–Huxley cell models (with time and \( V \)-dependent recovery variables and finite decay-time synapses).

For these simulations, the synaptic conductance rises and falls exponentially (Eq. 3) with a time constant \( \tau_s \) (\( \tau_r = 0 \) corresponds to instantaneous inhibition). We find that the AP pattern is unstable and an AIP-like state is attracting when electrical coupling is absent or weak and \( \tau_s \) is very fast, say \( \tau_s = 1 \) ms. As for our relaxation oscillator model, the AP pattern is stabilized for less weak gap junctions, and there is bistability of the AP and IP states for moderate coupling strength (for instance, for \( g_{\text{gap}} = 0.06 \) ms/cm² when \( \tau_s = 1 \) ms). Of course, the IP behavior is the only stable mode for strong electrical connections (not shown; cf. Fig. 1C).

Figure 7A shows the time course of the phase difference between the two inhibitory model cells when the weak electrical coupling is turned off and then on again. The cells, initially oscillating in AP, i.e., with a phase shift \( \Phi \) equal to 0.5, switch reversibly to a reduced phase difference when electrical coupling is set to zero (see triangles), thereby demonstrating the stabilizing influence of gap junctions on the AP pattern.

In this model, the pattern seen in the absence of electrical coupling is not exactly AIP but rather is something between AIP and AP; the phase difference between the cells is not exactly equal to the spike’s duration (divided by the period) but rather greater (cf. Fig. 7A, top left, and Fig. 1B, right). Factors that contribute to deviations from a pure AIP pattern include noninstantaneous synapses and nonrelaxation intrinsic dynamics. As illustrated, the phase difference is smaller when the synaptic kinetics are made faster. This observation is consistent with a previous finding for inhibitory-coupled Hodgkin–Huxley model neurons that speeding up the synaptic conductance (from moderately fast to very fast) decreases the phase difference from 180° between the oscillators (see Fig. 8 of Van Vreeswijk et al. 1994).
synapses, the smaller the phase difference between neurons in the absence of electrical coupling (more AIP-like) and a larger electrical coupling strength is needed to reach the AP pattern. To confirm that deviation from our relaxation oscillator idealization also contributes to preventing the Hodgkin–Huxley cells from oscillating at pure AIP, we carried out two manipulations (Fig. 7B).

First, we assumed instantaneous activation of the sodium current and then we decreased artificially the membrane time constant by reducing the membrane capacitance, with the limiting case of zero capacitance being the relaxation limit. These successive accelerations of the intrinsic regenerative dynamics produce a shift in $\Phi$ toward more AIP-like behavior. Altogether these simulations suggest that our results obtained using model networks comprised of relaxation oscillators can be applicable to spiking cells that interact through fast inhibitory synapses.

**DISCUSSION**

Summarizing, we see that networks comprised of rhythmic inhibitory cells with short duty cycle express several dynamic behaviors that are counterintuitive. First, despite a traditional view, two identical neurons mutually interconnected by synaptic inhibition do not oscillate in AP but exhibit AIP activity (Figs. 1, B and C, and 2). Second, although electrical coupling is thought to be a synchronizing agent, adding modestly such coupling to the network does not produce synchrony but paradoxically changes the coordination from the AIP to AP pattern, which becomes a stable solution (Fig. 1, B–D). Third, a further increase of gap junctional–coupling strength provides bistability of the AP and IP solutions (Fig. 1C). We are currently developing rigorous proofs for the case of combined electrical and synaptic coupling, but they involve advanced mathematical tools that are not suited for this presentation.

We have shown here that a key phenomenon responsible for instability of the AP oscillations of cells with short duty cycle is a compression of the cycle duration, produced by inhibition (Fig. 4C). As this effect is phase dependent, it appears that only cells that are inhibited exactly at the same phases of their cycles will have equal cycle durations. During AP activity each cell is inhibited exactly midway through its silent phase. The cells’ cycle durations are equal and their time courses are identical (modulo a shift by 0.5 cycle). However, any perturbation leads to inhibition occurring at different moments of the cells’ silent phases. Therefore, due to differential compression effects, these cycle duration differences evolve through successive cycles until a new, stable solution is reached (Fig. 5B)–the AIP pattern. The cycle durations have again become equalized but now the cells’ time courses are not identical—they do not superimpose by time shifting them. The cells cycle with the same period but they have different active and silent phase durations. This is because the two cells are subject to different mechanisms in the transition between the silent and active phase. In one cell the transition occurs through the escape mechanism. The cell jumps up to its active phase by reaching a threshold at the end of its recovery, as if it were uncoupled, i.e., it rounds the “knee” on the hyperpolarized portion of its limit cycle. (Escaping like this also occurs during AP activity.) The other cell jumps to its active phase through release from inhibition (which occurs below the knee). This increases its

**FIG. 7.** Reciprocally inhibitory Hodgkin–Huxley neurons express pure AP behavior only in the presence of electrical coupling (A) but slowing down the synapses also promotes AP coordination (B). A: phase-shift ($\Phi$) versus cycle number ($n$) for two Hodgkin–Huxley neurons shows AP oscillations ($\Phi = 0.5$) when cells are electrically coupled, for $n < 9$. When $g_{gap}$ is set equal to zero for a number of cycles ($n = 9$ until 43, see arrows), they reversibly switch coordination to a smaller value of $\Phi$; this $\Phi$ value decreases with increased synaptic speed [cf. open circles ($\tau_e = 1.43$ ms) and black circles ($\tau_e = 1$ ms)]. B: increasing the electrical coupling strength between neurons leads to a gradual increasing of the phase shift (of the steady-state oscillations) until the AP state ($\Phi = 0.5$) is reached at a particular value of $g_{gap}$ (solid curves). This critical value of $g_{gap}$ is larger when the synapses are faster. For synapses sufficiently slow (say, $\tau_e = 2.5$ ms), the neurons express stable AP behavior even without electrical coupling. When intrinsic kinetics are speeded up (nonsolid curves) $\Phi$ is reduced toward the limit of AIP behavior (here, $\tau_e = 0.2$ ms). Accelerating the kinetics was done by assuming instantaneous activation of the Na current ($m(V) = m_s(V)$) and then effectually decreasing the membrane time constant $\tau_m$ by reducing the membrane capacitance, by a factor of 2 and then to 0 (the single white square, at $g_{gap} = 0$). Values of $\tau_e$ and $\tau_m$ are indicated along side of the curves (in ms). Parameters: standard parameters of the Hodgkin–Huxley model (with temperature correction factor, $\phi = 0.56$), synaptic current: $g_{syn} = -12$ mV, $g_{syn} = 50$ mV, $k_{syn} = 0.05$ mV, $\xi_{syn} = 1.0$ mS/cm$^2$ (in A and B); $g_{gap} = 0.05$ mS/cm$^2$ (in A).
active and silent phase durations and thereby compensates for the different cycle duration effects produced by inhibition. During the evolution toward the AIP pattern the “follower” cell is racing toward the “leader” and decreasing the time interval between them, then slowing as it approaches the “leader” from behind where the cycle durations are again equal.

Interestingly, increasing the cells’ duty cycle (a reverse experiment to that illustrated in Fig. 3) stabilizes the AP solution; both cells leave the silent phase through release from inhibition (not shown) (cf. Fig. 2), as is in the case of duty cycle 0.5. In other words, if cells’ duty cycle is of moderate length, as in many of CPG networks, cells can express the AP pattern without electrical coupling, solely due to reciprocal inhibition (not shown). For this, however, inhibition must be strong enough to speed up sufficiently a cell’s evolution during the phase of inhibition (i.e., to increase compression). The cell therefore after the jump down receives inhibition over its entire silent phase trajectory until below the knee from where it is released. (i.e., the silent phase is compressed so that it consists only of the phase of inhibition.). This AP pattern is therefore quite different from the AP pattern for short duty cycle in which both cells jump up to the active phase by escaping from the knee (Fig. 5).

Reiterating, the AP pattern in the case of short duty cycle shows no activity during substantial portions of its temporal profile. Although we have been motivated by the two-cell half-center oscillator, this type of pattern (with long periods of neither cell firing) has not been reported to our knowledge in the literature of two-cell CPG. The typical illustrations have one cell active for half of the period and the other cell active for the other half. What can we conclude about a half-center oscillator with such a fully active time course? As discussed in the previous paragraph, this could involve short duty cycle pacemakers only if $g_{syn}$ is quite strong. More likely, we expect that pacemakers of not small duty cycle are involved, operating in classical release mode. Alternatively, the two cells might be excitatory units (i.e., not autonomous pacemakers) and operating in escape mode (Wang and Rinzel 1992).

Our pacemaker models originated from the CPG context where the time course of $V$ represents the envelope wave forms of rhythmic bursting neurons; see also Golomb et al. 1994, Kopell and Somers 1995, and Rubin and Terman 2000 for other examples of synthetically coupled bursters being modeled with only envelope waveforms, assuming that spikes have been averaged out. In the short duty cycle regime our model’s $V$ time course looks spike-like and so it’s fair to ask if we can apply our results to gain insight into the coordination patterns of mutually inhibitory spiking neurons. Recent modeling studies (e.g., Van Vreeswijk et al. 1994; Lewis and Rinzel 2003) have exploited the integrate-and-fire idealization to characterize the locking patterns for inhibitory ($\alpha$-function) coupling. For fast synaptic conductances the AP state is stable, and realized for most initial conditions. This result seems inconsistent with our finding that the AIP pattern, rather than the AP pattern, is the attracting state when electrical coupling is very weak. A major difference between these idealizations, integrate-and-fire units and relaxation oscillators, is that the latter has a legitimate voltage-gated recovery process ($w$ in our model) that is time dependent (slow compared with the membrane time constant). This feature of our model underlies the phase-dependent compression that is produced by inhibition and that is responsible for the AP state’s instability when electrical coupling is very weak. This in turn suggests that network models in which the spiking cells’ intrinsic oscillatory dynamics are based on slow internal negative feedback (e.g., repolarizing potassium currents that are slow relative to the fast activating inward currents) should also exhibit instability of the AP behavior for fast synaptic inhibition.

Another factor that underlies the compression effect is the shape of $w(V)$—this function cannot be flat in the voltage range of the silent phase. This assumption is not really constraining. Indeed, any cell capable of postinhibitory rebound should not have a flat $w(V)$ below the rest potential. Otherwise the resting level of $w$ cannot be reduced by preceding hyperpolarization, a condition necessary for rebound in this class of idealized models. Compression effects can also arise in another class of models, in which the pacemaker’s slow negative feedback is based on inactivation of an inward current (e.g., as in a low threshold transient calcium current) instead of slow activation of an outward current. The addition of other currents may enhance or reduce the pattern-generating effects of compression. For example, consider the consequences of a transient potassium current that can be activated at hyperpolarized levels. Inhibition that removes inactivation could then lead to an anticompression effect. In this case we would expect the AIP pattern to be precluded and AP behavior more likely.

Also, when we extrapolate our conclusions for short duty cycle to the case of spiking neurons, we should reconsider our assumption that for us a “fast” synaptic time scale means very fast, i.e., instantaneous; the postsynaptic conductance in our model is taken as an instantaneous function of presynaptic membrane potential. In fairness, the effect of inhibition’s time scale, which we have neglected, was described in the integrate-and-fire framework (Lewis and Rinzel 2003; Van Vreeswijk et al. 1994).

These issues motivated us to illustrate, for a spiking model, some of the solution behaviors that we found for our relaxation oscillator model by using a pair of mutually inhibitory Hodgkin–Huxley cell models (with time and $V$-dependent recovery variables and finite decay-time synapses). Indeed, the simulations confirmed that our results obtained using model networks comprised of relaxation oscillators may be applicable to spiking cells that interact through fast inhibitory synapses (Fig. 7).

Wrapping up, we have shown here by using both computational and theoretical approaches that temporal patterning in networks of inhibitory cells can be dramatically altered by electrical coupling if the intrinsic cellular duty cycle is short and synapses are fast. Despite the traditional view, AP activity does not necessarily arise as a consequence of reciprocal inhibition: both spiking cells and pacemakers of short duty cycle, interacting through the envelope of membrane potential, express AIP and not AP activity unless gap junctional coupling is present. Once the AP pattern is established by gap junctions, adequate increase of electrical coupling promotes IP activity, although over a wide range of moderate electrical coupling strength both states coexist. While it is tempting to think (according to tradition) of electrical and inhibitory coupling as opposite forces, accumulating evidence (Lewis and Rinzel 2003; Traub et al. 2001; this study) suggests that this view is oversimplified when consideration is given to synaptic time scales, intrinsic dynamics, and relative strength of these cou-
plings. These forces, when acting together, do not necessarily annihilate each other but instead introduce a kind of a “tension” in the network that can, say with neuromodulation, favor one or another synchronization pattern, or even, in the case of bistability, enable immediate switching between two opposite behaviors (i.e., IP and AP states) under the influence of a precisely timed, brief stimulus. However, for the class of systems that we consider here, such tension-induced bistability arises only if there is an asymmetry in how two forces act throughout the cycle, i.e., if inhibition acts only for a small part of the cycle due to a short intrinsic duty cycle.

ACKNOWLEDGMENTS

We acknowledge the hospitality of the Mathematical Biosciences Institute at Ohio State University where both authors were extended-stay visitors in 2002. We are especially thankful to Dr. David Terman for helpful discussions on mathematical aspects of our results.

GRANTS

This work was supported by Kosciuszko Foundation, Center for Neuroscience (visitor grant), North Atlantic Treaty Organization Collaborative Linkage Grant 978877 and Committee for Scientific Research status grant 18/5t. This material is based on work partly supported by National Science Foundation Grant 0112050.

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