Asymmetry and modulation of spike timing in electrically coupled neurons

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Severson J, Haas JS. Asymmetry and modulation of spike timing in electrically coupled neurons. J Neurophysiol 113: 1743–1751, 2015. First published December 24, 2014; doi:10.1152/jn.00843.2014.—Electrical coupling mediates interactions between neurons of the thalamic reticular nucleus (TRN), which play a critical role in regulating thalamocortical and corticothalamic communication by inhibiting thalamic relay cells. Accumulating evidence has shown that asymmetry of electrical synapses is a fundamental and dynamic property, but the effect of asymmetry on coupled networks is unexplored. Recording from paired patches in rat brain slices, we investigate asymmetry in the subthreshold regime and show that electrical synapses can exert powerful effects on the spike times of coupled neighbors. Electrical synaptic signaling modulates spike timing by 10–20 ms, in an effect that also exhibits asymmetry. Furthermore, we show through modeling that coupling asymmetry expands the set of outputs for pairs of coupled neurons through enhanced regions of synchrony and reversals of spike order. These results highlight the power and specificity of signaling exerted by electrical synapses, which contribute to information flow across the brain.

A recent focus of interest in electrical synapses is their potential for plasticity, but whether the signals transmitted across electrical synapses are physiologically powerful and how the properties of these synapses impact spiking in gap junction-coupled neurons remain unclear. Among the more notable yet underappreciated of those properties is asymmetry.

Asymmetry is marked by differences in measurements of coupling for an electrical synapse when stimulated from one side or the other. That is, current flow is preferential in one direction across the synapse. Asymmetry, also known as rectification, is a well-known property of invertebrate electrical synapses; the definitive demonstration of electrical synapses in crayfish also showed the markedly asymmetric nature of that connection (Furshpan and Potter 1959). Substantial rectification has additionally been demonstrated at electrical synapses in cultured leech sensory neurons (Davis 1989; Kristan et al. 1990). Electrical synapses have also demonstrated interactions between neurons of the thalamic reticular nucleus (TRN), which play a critical role in regulating thalamocortical and corticothalamic communication by inhibiting thalamic relay cells. Accumulating evidence has shown that asymmetry of electrical synapses is a fundamental and dynamic property, but the effect of asymmetry on coupled networks is unexplored. From paired patches in rat brain slices, we investigate asymmetry in the subthreshold regime and show that electrical synapses can exert powerful effects on the spike times of coupled neighbors. Electrical synaptic signaling modulates spike timing by 10–20 ms, in an effect that also exhibits asymmetry. Furthermore, we show through modeling that coupling asymmetry expands the set of outputs for pairs of coupled neurons through enhanced regions of synchrony and reversals of spike order. These results highlight the power and specificity of signaling exerted by electrical synapses, which contribute to information flow across the brain.

Changes in asymmetry, indicating shifts in the preferential direction of electrical signaling within coupled networks, have been noted after long-term modification of electrical synapses in TRN following activity in coupled neurons (Haas et al. 2011) and during modulation of coupling strength by GABAergic input (Lefler et al. 2014) or NMDA application (Turecek et al. 2014) to neurons in the inferior olive. Thus it is increasingly evident that asymmetry is a dynamically regulated contributor to the topology of electrically coupled networks.

Electrical synapses are known to contribute to excitability (Logan et al. 1996) and synchronize spiking among coupled neurons (Bennett and Zuzkin 2004; Blatow et al. 2003; De Zeeuw et al. 1996; Devor and Yarom 2002; Galarreta and Hestrin 1999; Gibson et al. 1999; Hughes et al. 2011; Long et al. 2004; Turecek et al. 2014). Modeling studies have reiterated the roles that electrical synapses play in generating and stabilizing synchrony (Chow and Kopell 2000; Elson et al. 1998; Lewis and Rinzel 2003; Ostojic et al. 2009; Pfeuty et al. 2005; Saraga et al. 2006; Soto-Trevino et al. 2005), even as out-of-phase oscillations (Sherman and Rinzel 1992). Recent work has shown that complete rectification of electrical synapses enlarges the repertoire of network outcomes (Gutierrez and Marder 2013). However, the impact of asymmetric synapses on spike coordination in the mammalian brain has not been demonstrated or explored.

Neurons of the TRN receive collateral input from ascending thalamocortical fibers and descending corticothalamic fibers and provide an inhibitory signal to thalamocortical projection neurons. A central and understudied issue within the area of thalamocortical synchrony (Huguenard and McCormick 2007; Steriade et al. 1993) is the exact mechanism by which the inhibition provided by the TRN is coordinated. Intra-TRN coordination is almost certainly greatly impacted by the electrical synapses that densely interconnect TRN neurons (Landisman et al. 2002).

Here we evaluate the extent of electrical synapse asymmetry in coupled TRN neurons and extend the idea of asymmetry to include its impact on spike timing. We show that the effects of electrical synapses and asymmetry on spike times are often substantial, leading to alterations in spike onset by tens of milliseconds. We explore the effects of electrical synaptic asymmetry in a two-cell model and show that asymmetry expands the set of outputs exhibited by a pair of neurons in response to near-coincident inputs. Together, these results demonstrate that asymmetry of electrical synapses alters the dynamic range and the computational role that these synapses play within a neuronal network.

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METHODS

Electrophysiology. Horizontal slices 350–400 μm thick were obtained from Sprague-Dawley rats aged postnatal day (P)11–P14 of either sex. Rats were anesthetized with isoflurane and euthanized in accordance with federal animal welfare guidelines and protocols reviewed and approved by the Lehigh Institutional Animal Care and Use Committee (IACUC). Slices were cut and incubated in sucrose solution (in mM): 72 sucrose, 83 NaCl, 2.5 KCl, 1 NaPO4, 3.3 MgSO4, 26.2 NaHCO3, 22 dextrose, and 0.5 CaCl2. Slices were incubated at 36°C for 20 min and returned to room temperature until recording. The bath solution for recording contained (in mM) 126 NaCl, 3 KCl, 1.25 NaHPO4, 2 MgSO4, 26 NaHCO3, 10 dextrose, and 2 CaCl2 (300–305 mosM), saturated with 95% O2-5% CO2. The submersion recording chamber was held at 34°C (TC-324B, Warner Instruments). Micropipettes were filled with (in mM) 135 K-glucuronate, 2 KCl, 4 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, and 10 phosphocreatine-Tris (pH 7.25, 295 mosM). For voltage-clamp measurements, 135 mM CsMSO4 was substituted for K-gluconate. Either 1 M CsOH or 1 M KOH was used to adjust pH of the internal solution. The approximate bath flow rate was 2 m/min. Voltages are reported as corrected for the liquid junction potential and bridge balanced. The TRN synapse current was visualized under IR-DIC optics (SliceScope, Scientifica). Signals were amplified and low-pass filtered at 8 kHz (MultiClamp, Axon Instruments), digitized at 20 kHz (lab-written MATLAB routines controlling a National Instruments USB6221 DAQ board), and stored for off-line analysis in MATLAB (R2012a, MathWorks).

Modeling. For each TRN cell, a single-compartment Hodgkin-Huxley model was created from the ionic current activation equations previously published for a TRN cell (Haas and Landisman 2012; Traub et al. 2005). We used $I_{\text{Na}}, I_{\text{K}}, I_{\text{leak}}, I_{\text{Kg}}$, and $I_T$ with peak conductances of 60.5, 60, 5, 0.025, 0.1 and 0.75 μA/cm², respectively, and membrane capacitance of 1 μF/cm². Reversal potentials were 50 mV (sodium), −100 mV (potassium), 125 mV (calcium), −40 mV (AR) and −75 mV (leak). Chemical synaptic input was integrated as $dS/dt = \alpha \delta K(1 - S) - \beta S$ with $\alpha = 5 \text{ ms}^{-1}$ and $\beta = 25 \text{ ms}^{-1}$; input was activated at defined arrival times (e.g., $t_1, t_2$) by $K$, a 1-ms square pulse representing a presynaptic spike. The electrical synapse was modeled as an ohmic (static) conductance coupled applied to the voltage difference between the neurons, individually for each neuron. A steady applied current of 0.1 μA/cm² was used to bring neurons closer to spiking threshold. Simulations were performed in MATLAB (R2012a, MathWorks) by ode23, a second-order Runge-Kutta solution with maximal time step 0.01 ms. The same set of initial conditions was used for all simulations.

RESULTS

Subthreshold asymmetry. The strength of coupling between neurons is quantified by the coupling coefficient (cc), which is typically measured by injecting a long step of current into one neuron and measuring the resulting voltage deflections in both neurons (Fig. 1, A and B). To minimize distortion from the effects of voltage-activated conductances on the cc, the step of current should be only as large as necessary to produce a reliably measurable voltage in the coupled neuron. For TRN cells with input resistance ($R_{\text{in}}$) of 100–300 MΩ and a minimum cc of 0.02, we used −100-pA current injections while maintaining both cells at a baseline of −70 mV to produce 10–30 mV presynaptic deflections ($\Delta V$) in the injected cell, resulting in stable deflections ($\delta V$) equal to or larger than −0.02 mV in the postsynaptic cell. Steps were repeated 10 times and averaged. The resting potentials of both neurons should be kept constant during measurements, as at different resting potentials active conductances can amplify both presynaptic and postsynaptic measurements and coupling (Curti et al. 2012; Haas and Landisman 2012). For current injection $I_1$ into cell 1, the coupling measured in cell 2 is $cc_{12} = \delta V_2/\Delta V_1$ (Fig. 1A) and vice versa for $cc_{21}$ (Fig. 1B). The quantity $cc_{12}$ measures coupling in the direction of cell 2. We quantify asymmetry with the ratio of the two directional strength measurements—coupling or conductance—for the same synapse (e.g., $cc_{12}/cc_{21}$, which is 0.18/0.13 for the pair in Fig. 1). Asymmetry that is larger in the direction of cell 2 favors transmission into cell 2.

A likely contributor to observed asymmetry is the difference in $R_{\text{in}}$ between coupled cells. When current flows between nonidentical cells, one should expect to measure larger deflections and thus larger coupling coefficients from $\delta V$ in the cell
with higher $R_{in}$ and smaller $\delta V$ in the cell with lower $R_{in}$, as is evident for the pair in Fig. 1, A and B. Differences in apparent $R_{in}$ could also come from variations in the complement of ionic membrane conductances in the two cells and cell morphology. For current applied to cell 1, the coupling coefficient $c_1c_2$ is related to $R_2$ of cell 2 and the gap junction resistance $R_{12}$ by $c_1c_2 = R_2/(R_2 + R_{12})$ (Bennett 1966), and likewise for $c_2c_1$. Assuming a single, symmetric $R_{in} = R_{12} = R_{21}$, the measured ratio $c_1c_2/c_2c_1$ becomes, as a function of $R_{in}$, $(R_1R_2 + R_1R_3)/(R_2R_1 + R_1R_3)$. Then with $R_1 >> R_3$ and $R_2$, this ratio is dominated by the second product in the numerator and denominator and reduces to the approximation of $R_1/R_2$.

To explore the expected relationship between coupling coefficient asymmetry and $R_{in}$, we plotted the cc ratio against the $R_{in}$ ratio for a cohort of 240 pairs (Fig. 1C). To examine whether the cell with larger $R_{in}$ would determine the amount or direction of asymmetry, here we used the larger of the $R_{in}$s in each pair for the numerator of the $R_{in}$ ratio (i.e., as cell 2), so that the $R_{in}$ ratio $R_{in}/R_1$ was $>1$ for each pair. In this manner, coupling measured in the cell with larger $R_{in}$, $cc_{12}$, was always in the numerator of the cc ratio. These data show that coupling coefficient asymmetry generally increases with $R_{in}$ ratio $R_{in}/R_1$.

The linear fit to the data had a slope of 0.98 and $R^2 = 0.36$, indicating that $R_{in}$ ratio is overall a poor predictor of the value or direction of coupling coefficient asymmetry. Asymmetry in coupling coefficient varied widely: the mean value of asymmetry over all coupling coefficients (using cc ratios $\geq 1$ for each pair for this comparison) was 1.22.

Neurons with different $R_{in}$ can show matched coupling coefficients through an asymmetric synapse; neurons with mismatched $R_{in}$ could produce different coupling coefficients (and thus apparent asymmetry) through a perfectly symmetric synapse. Thus while coupling coefficient asymmetry may be an indication of asymmetry, it is the coupling conductance $G_c$, the inverse of the resistance $R_{12}$, that provides the best measure of the synapse and its symmetry. Thus $R_{in}$ above and $G_c$ were calculated from expressions that contributed to the conductance of the gap junction to each cell’s $R_{in}$ and allow for the possibility of asymmetry (Fortier 2010). For current injected into cell 1 the estimate of gap junction resistance is

$$R_{12} = \frac{(\Delta V_1 - \delta V_2)(\Delta V_1\Delta V_2 - \delta V_1 \delta V_2)}{\delta V_2(\Delta V_1I_2 - \delta V_1I_1)}$$

and

$$R_1 = \frac{\Delta V_1\Delta V_2 - \delta V_1 \delta V_2}{\Delta V_1I_1 - \delta V_1I_2}$$

and likewise for $R_{21}$ and $R_2$.

In our data set conductance asymmetry (the ratio $R_{21}/R_{12}$) was consistently less than coupling coefficient asymmetry (Fig. 1D), reiterating that the contribution of $R_{in}$ to coupling coefficient can be misleading when assessing asymmetry. As expected, conductance asymmetry was not related to $R_{in}$ ratio (Fig. 1E). Substantial asymmetry remained in the distribution of conductances, where the mean (using the ratio of conductances $\geq 1$ for each pair) was 1.16. Restricting analysis to pairs with well-matched $R_{in}$s, $R_{in}$ ratio $<1.1$, we found cc and $G_c$ asymmetries of $1.1 \pm 0.06$ (mean $\pm$ SE, $n = 60$ pairs). Additionally, despite great variability in intrinsic properties such as $R_{in}$ (Parker et al. 2009) that could amplify asymmetry over development, we found that coupling asymmetry was similar at P6 (cc ratio of 1.19 $\pm$ 0.07, mean $\pm$ SE, $n = 10$ pairs, $P < 0.05$). Similar to previous reports for coupled pairs in TRN, we saw no evidence of chemical synaptic transmission in our data.

For a more direct measure of coupling and asymmetry, we used slow voltage-clamp ramps ($\pm 20$–$30$ mV from baseline of $-70$ mV, over 20 s) in a set of pairs, which allowed us to clamp transjunctional voltage and record transjunctional current in the postsynaptic cell. We then directly calculated coupling conductance, using the current deflection in the coupled cell (held at $-70$ mV) during the ramp divided by the clamped transjunctional voltage. These data showed that while coupling is independent of transjunctional voltage for physiological ranges of voltage differences, as expected for connexin36-based synapses (Srinivas et al. 1999), transjunctional currents varied with the direction of the ramp across the synapse, resulting in asymmetric conductance measurements (Fig. 2, A–C). Over a set of 15 pairs, conductance asymmetry...
(as above, taken as the ratio > 1 for each pair) averaged 1.6 ± 0.3 (mean ± SE). Asymmetry measured in voltage clamp was moderately correlated to asymmetry measured in current-clamp mode (Fig. 2D; $R^2 = 0.48$). Together, these subthreshold measurements reinforce that asymmetry is a fundamental property of electrical synapses.

The power of electrical synapses: modulation of spike timing. Coupling coefficients and conductances indicate the strength of electrical synapses but do not relate their efficacy or their effect on a neighbor’s spike times, making comparisons to chemical synapses difficult. Electrical synapses are well known to enhance synchrony, and increases in correlation have been widely shown in recordings, but a direct demonstration of the impact of the synapse on neuronal integration and spike timing was lacking. Thus, to better understand the basis of how electrical synapses coordinate firing between coupled neurons, we sought to examine the effects of spiking activity in a coupled neighbor on spike times in TRN neurons.

In response to current injections of increasing amplitude from a baseline voltage of −70 mV, spike onset latencies in one neuron decrease (Fig. 3A). This basic, predictable response property was repeated as a baseline for comparison, with current injections of increasing amplitude separately delivered to both cells of a coupled pair.

To measure the modulation of latency by an electrical synapse, we repeated the same set of current injections to cell 1 while also driving spikes in the coupled neighbor cell 2 (Fig. 3B; each stimulus was repeated 5 times). Spikes in the coupled neighbor were driven with sufficiently strong stimuli that they always occurred before the onset of the spike in cell 1, i.e., such that the current across the electrical synapse would precede the spike in cell 1. The additional input from the spikes in the coupled neighbor cell 2 decreased the latencies of the spikes in the stimulated neuron 1 (Fig. 3C). The differences in latency between the silent-neighbor and active-neighbor conditions decreased as the strength of the stimulus increased. When this paradigm was repeated in a reversed fashion within the same cell pair—the increasing-amplitude stimuli were delivered to cell 2, while coupled neighbor cell 1 spiked first (Fig. 3, D and E)—again, latency in cell 2 decreased from previous values (Fig. 3F). For both cell 1 and cell 2, activity in the coupled neuron transformed a previously subthreshold current input into a spike-eliciting input, at 75 pA for cell 1 (Fig. 3C) and 50 pA for cell 2 (Fig. 3F).
The latency modulation was 8.6 ms (Fig. 4A), the mean within-synapse difference in perithreshold modulation by one cell was much larger than the perithreshold difference was 6.1 ms. Over all 18 pairs examined, we performed the same experiment in uncoupled pairs. We used a 20% increase in the amplitude of the pulses used to drive bursts as a substitute for the excitation provided by the electrical synapse. For these control experiments, we saw similar average decreases in onset latency (13.7 ± 1.5 ms, n = 9 pairs), indicating that the increases in excitation were similar to those provided by the electrical synapse. However, the mean difference in latency modulation was 4.8 ± 2.1 ms; differences in latency modulation were not significant across these random pairings or for any shufflings of those pairings. Thus the differences in latency modulation in coupled cells are due to the asymmetry of the signals transmitted across the electrical synapse.

We quantified the asymmetry of spike time modulation over the set of pairs stimulated with the paradigm as in Fig. 3. We assessed asymmetry of spike time modulation in two ways: for the perithreshold spike in each neuron and for the cumulative sum of modulations over the increasing-amplitude stimuli used for each neuron. For the pair shown in Fig. 3, the perithreshold asymmetry was 16.6/10.5, or 1.58. The cumulative asymmetry was taken as the sum of modulations (burst onset differences) in Fig. 3C divided by the sum of modulations in Fig. 3F; for the pair in Fig. 3, that was 1.65. These two measures of asymmetry were closely related (Fig. 4B), as they were dominated by the first term, the perithreshold asymmetry. Neither $R_{in}$ nor subthreshold asymmetry was well-correlated to spiking asymmetry (Fig. 4, C and D). Together, these results show that electrical synapses exert powerful influence on the timing of spikes in coupled neighbors, in a manner that is also asymmetric.

**Impact of asymmetry on coupled networks.** To explore the effects of asymmetry on spike coordination in a detailed manner, we modeled a pair of TRN cells and coupled them with an electrical synapse, which we varied in asymmetry (Fig. 5A; see METHODS). Briefly, the model cells were identical and composed of fast sodium, fast and delayed-rectifier potassium, and low-threshold calcium (T) conductances. Each cell received synaptic dual-exponential fast synaptic (AMPA type) inputs resulting in perithreshold excitatory postsynaptic potentials (EPSPs; 6 mV), which varied in arrival time by up to 20 ms, representing afferent input to TRN from thalamic or cortical synapses. The electrical synapse was an ohmic conductance applied to the instantaneous difference in voltage between the two neurons and was separately varied in each direction while maintaining a constant average coupling strength.

For simulations in which both the difference in timing of the synaptic input to the TRN cells $t_1 - t_2$, and the asymmetry of the electrical synapse between the TRN cells varied, there are four basic schemes represented in each square plot shown Fig. 5, B, D, and F, as quadrants of those fields: in region i (Fig. 5, D and F, bottom left), input arrives first to cell 2 and the synapse asymmetry favors transmission into cell 1; in region ii (Fig. 5, D and F, top left), input arrives first to cell 2 but the synapse favors cell 2; in region iii (Fig. 5, D and F, top right), input arrives first to cell 1 and the synapse is asymmetric toward cell 2; and in region iv (Fig. 5, D and F, bottom right), input arrives first to cell 1 but the synapse favors cell 1. To identify how asymmetry of electrical synapses in the TRN might affect the synchrony and timing of its output, we evaluated the difference in burst onset of the two TRN cells, in milliseconds. Physiologically, the difference in burst onset (or
synchrony) in TRN cells determines the subsequent timing of the GABAergic signals delivered to thalamus by the TRN.

We compare three simulations. The first set uses a weaker synapse, with average coupling strength maintained at $cc = 0.1$, and in these simulations TRN spike timing is overall dominated by the arrival times of the inputs (Fig. 5B). Here, the electrical synapse helped to decrease the differences in burst onset, which are overall smaller than the differences in input timing $t_2 - t_1$, with a trend toward enhanced synchrony when the synapse favors the cell whose input arrived second because of summation.

The second simulation used an electrical synapse with average $cc$ matched to the average $cc$ measured in TRN cells, 0.15 (Haas et al. 2011) (Fig. 5D). Here, two additional trends are notable. First, for the larger input time differences $t_2 - t_1$, asymmetry allows either neuron to lead in spiking. This reversal of burst onset order from baseline is shown in the traces in Fig. 5E: for these perithreshold inputs, the synapse can reverse the spiking order by varying the combination of input integration with summation of electrical synaptic signals. At $t_2 - t_1 = -20$ ms, with cell 2 receiving input first, the electrical synapse that favors cell 1 (Fig. 5E, region i) helps cell 1 to fire first in two ways. The initial input to cell 2 is favorably passed toward cell 1 and raises its excitation level. When the second input arrives to cell 1, it then spikes but then shares that excitation with its neighbor less favorably, increasing the delay between cell 1 and cell 2. In the alternative case, for the same input timing order but a synapse that favors cell 2 (Fig. 5E, region ii), cell 1 receives less excitation through the electrical synapse from the initial input to cell 2; when the second input arrives to cell 1, that input is more favorably shared with cell 2, which spikes almost immediately, while cell 1 has still not yet reached threshold. These examples illustrate the reverberatory dynamics of asymmetric synapses.
Second, the range of input time differences \( t_2 - t_1 \) that yields synchronous spiking is larger. For weaker synapses, perfect synchrony is possible for a small range of \( t_2 - t_1 \) near 0 and synchrony only depends on the overall strength of the synapse. With a stronger asymmetric synapse, almost all values of \( t_2 - t_1 \) allow synchrony for at least one value of asymmetry, where the spiking order reverses.

Figure 5F shows the results from a simulation with average electrical synapse \( cc \) of 0.2. Here, we note that the direction of the electrical synapse has grown to dominate the spiking order, a shift highlighted by the swap of colors on each side of the plots between Fig. 5, B and F. For \( t_2 - t_1 = -20 \) ms, the electrical synapse that favors cell 1 (Fig. 5G, region i) helps cell 1 to fire first again, through similar mechanisms as above. In the alternative case, for the same input timing order but a synapse that favors cell 2 (Fig. 5G, region ii), the favored spike in cell 2 reverberates to immediately initiate a synchronous spike in cell 1.

We tested our model’s predictions with further experiments. We delivered closely timed pulse inputs, with differences in onset timing \( t_2 - t_1 \) from \(-30\) to \(30 \) ms, at perithreshold amplitudes (determined as for Fig. 3, the smallest amplitude of an input that drove a burst from \(-70 \) mV) to pairs of coupled TRN neurons. As predicted, inputs that arrived first to cell 2 resulted in spikes first in cell 2 (Fig. 6, A1 and B1). For inputs that arrived first in cell 1, with a synapse that favored cell 2, we saw reversals of spiking order (Fig. 6, A3 and B3), in which cell 2 spiked first. These results correspond to Fig. 5D, which predicts that the cell favored by the synapse always spikes first. The reversal effect was most powerful for input time differences \(<30 \) ms, corresponding to the effective time window for combination of electrical synaptic signals with input activation; after \(30 \) ms, the inputs are too far apart for the electrical synapse to affect neighboring spike times.

Together, these results show that asymmetric electrical synapses are poised to potently influence the neuronal circuits and networks in which they are embedded, by determining how coupled cells pass on, synchronize, or reverse their spike timing to near-synchronous input.

DISCUSSION

Here we have demonstrated that asymmetry is a fundamental property of electrical synapses in the TRN and that these synapses and their asymmetry exert a powerful influence on the timing of spikes in coupled neighbors. Together, these properties of electrical synapses expand and predict the set of behaviors that a pair of coupled neurons, provided with near-coincident perithreshold inputs, exhibit.

These results show that the true power of electrical synapses has been underappreciated, perhaps because of the difficulties in accessing them. Our results clearly demonstrate that these

**Fig. 6. Reversal of spiking order in a pair of coupled neurons.** A1: spiking for a trial where input arrived first to cell 2 (green); spiking order followed the input. Mean \( cc 0.13 \), \( cc \) ratio 1.69, and \( G_c \) ratio 1.5 for this pair. A2: difference in latency plotted against difference in input times. For inputs that arrived first to cell 2 \( t_2 - t_1 < 0 \), spiking followed the input timing; for inputs that arrived first to cell 1 \( t_2 - t_1 > 0 \), synaptic asymmetry altered the spiking order. Black line: unity. A3: spiking for a trial where input arrived first to cell 1 (blue); the spiking order reversed the input order. B1–B3: for A1–A3, for a pair with \( cc 0.12 \), \( cc \) ratio 1.26, and \( G_c \) ratio 1.03.
synapses are powerful modulators of spike times at the perithreshold levels of activation, where activity in neighboring neurons can easily alter spike times by 10–20 ms. Thus our results show that electrical synapses are likely to be potent determinants of the timing of inhibition delivered to the thalamus by the TRN and rhythmogenesis in the thalamocortical system (Huguenard and McCormick 2007; Steriade et al. 1993). This influential role in spike timing may be recapitulated in activated, coupled GABAergic neurons within the awake cortex (Gentet et al. 2010) and thus participate in the detailed and timed cortical balance between excitation and inhibition (Haider and McCormick 2009; Renart et al. 2010; Wehr and Zador 2003).

Several possible physiological aspects of gap junction formation could produce asymmetry of the synapse. Differences in distance between the synapse and the soma of the two coupled cells can contribute to apparent asymmetry measured at the soma. It is notable that in the connexin36 knockout substantial coupling remains in the TRN (Lee et al. 2014), implying the contribution of a yet-unknown protein to coupling or asymmetry. In Drosophila giant fiber, rectifying channels result from heteromeric combinations of transcripts from the innexin gene shaking-B (Phelan et al. 2008). In goldfish, two closely related connexin36 homologs, connexin34.5 and connexin35 (O’Brien et al. 1998), form heterotypic and functionally asymmetric synapses at the Mauthner synapse (Rash et al. 2013). Expression of varied transcripts or posttranslational modifications of one or possibly more connexin genes brings the possibility that a single neuron could, in theory, deliver individually tailored sets of hemichannel subunits to a given gap junction, or to different synapses with different neighboring neurons, with the resulting asymmetry of an individual electrical synapse varying because of the contributions from both sides (Marder 2009). In mammalian systems, transjunctional asymmetry of free Mg$^{2+}$ ions results in asymmetric voltage gating of connexin36-based synapses (Palacios-Prado et al. 2013), implying a separate mechanism for asymmetry and its regulation. Finally, electrical synapses are modulated by other voltage-activated membrane conductances (Curti et al. 2012) such as the persistent sodium current (Haas and Landisman 2012); varying expression levels and subcellular localization of these conductances between coupled neurons could also contribute to effective asymmetry.

Within the TRN, and for similar electrically coupled networks across the brain, these results underline the effective heterogeneity of electrical coupling. That is, regardless of how asymmetry arises, both the strength and the direction of coupling are heterogeneous across coupled networks. In the dorsal cochlear nucleus, asymmetry and voltage-gated amplification work together to produce such strong signaling that EPSPs in fusiform cells relayed through gap junctions result in stellate cell spiking, with much weaker signaling in the opposite direction (Apostolides and Trussell 2013, 2014). Although unknown, asymmetric coupling in the TRN may be arranged in a deterministic, directional topology that complements existing topology in the TRN (Deleuze and Huguenard 2006; Lee et al. 2014). We suggest that the directionality of electrical synaptic signaling in the TRN could be a way to fine-tune the intra-TRN response to afferent sensory inputs and shape the inhibition provided to thalamus. Electrical synapses could be topographically arranged in strong to weak configurations based on anatomical or functional considerations, where one neuron or set of neurons could operate as the center of a hub within a sensory subnucleus of the TRN, with weaker communication between sensory subnuclei. Alternatively, subnuclei of the TRN could communicate with each other preferentially over within-nucleus signaling. As intra-TRN connectivity is yet understudied, the topography of its strong electrical connectivity may play a role in its function within thalamocortical networks.

Recent results indicate that asymmetry is a property open to modulation or modification during physiological activity (Haas et al. 2011; Lefler et al. 2014; Turecek et al. 2014; Wang et al. 2014). The implication of shifts in asymmetry is that the topography of coupled networks, however it is arranged, can be modified. Our modeling results demonstrate that relatively subtle shifts in coupling strength asymmetry can dramatically alter spiking order in coupled pairs. We expect that these shifts would extrapolate to a network—that shifts in asymmetry will fundamentally change the spiking orders, synchrony possibilities, and other characteristics of coupled networks of interneurons. Given the substantial challenges to investigation of these effects in direct recordings, further and expanded computational studies may provide a foundation for their investigation.

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