Emergent elements of inspiratory rhythmogenesis: network synchronization and synchrony propagation

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Supplemental Information

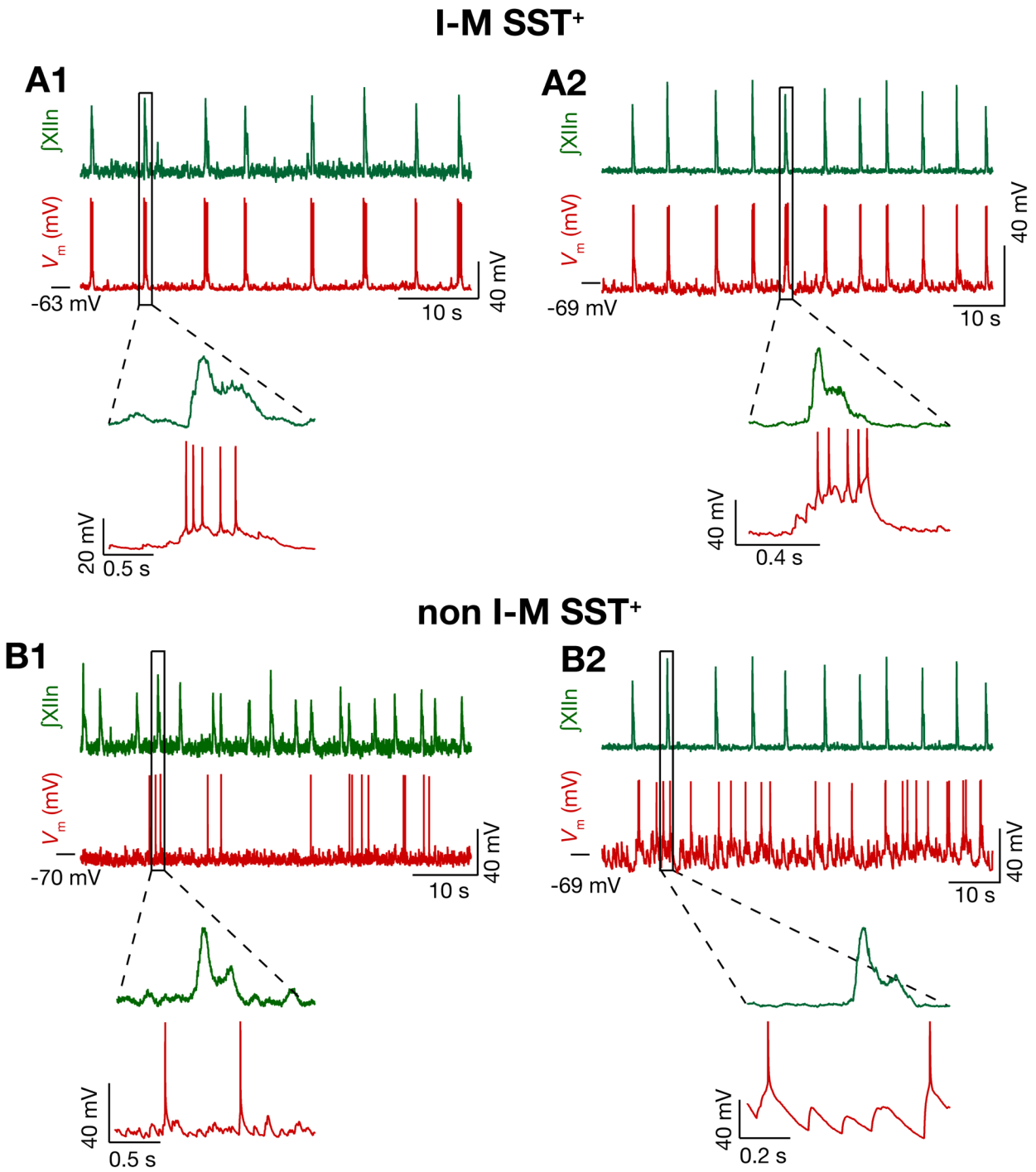

Figure S1. I-M SST$^+$ neurons fire in phase with XIIn I-burst. ***Related to Figure 2***

Firing profiles of inspiratory-modulated (I-M) SST$^+$ (A1-A2) and non I-M SST$^+$ (B1-B2) neurons in 9 mM $[K^+]_{ACSF}$. Traces in A1 are for same neuron as Fig. 2 (A1-A3). Neurons in (A2) and (B2) were recorded simultaneously.

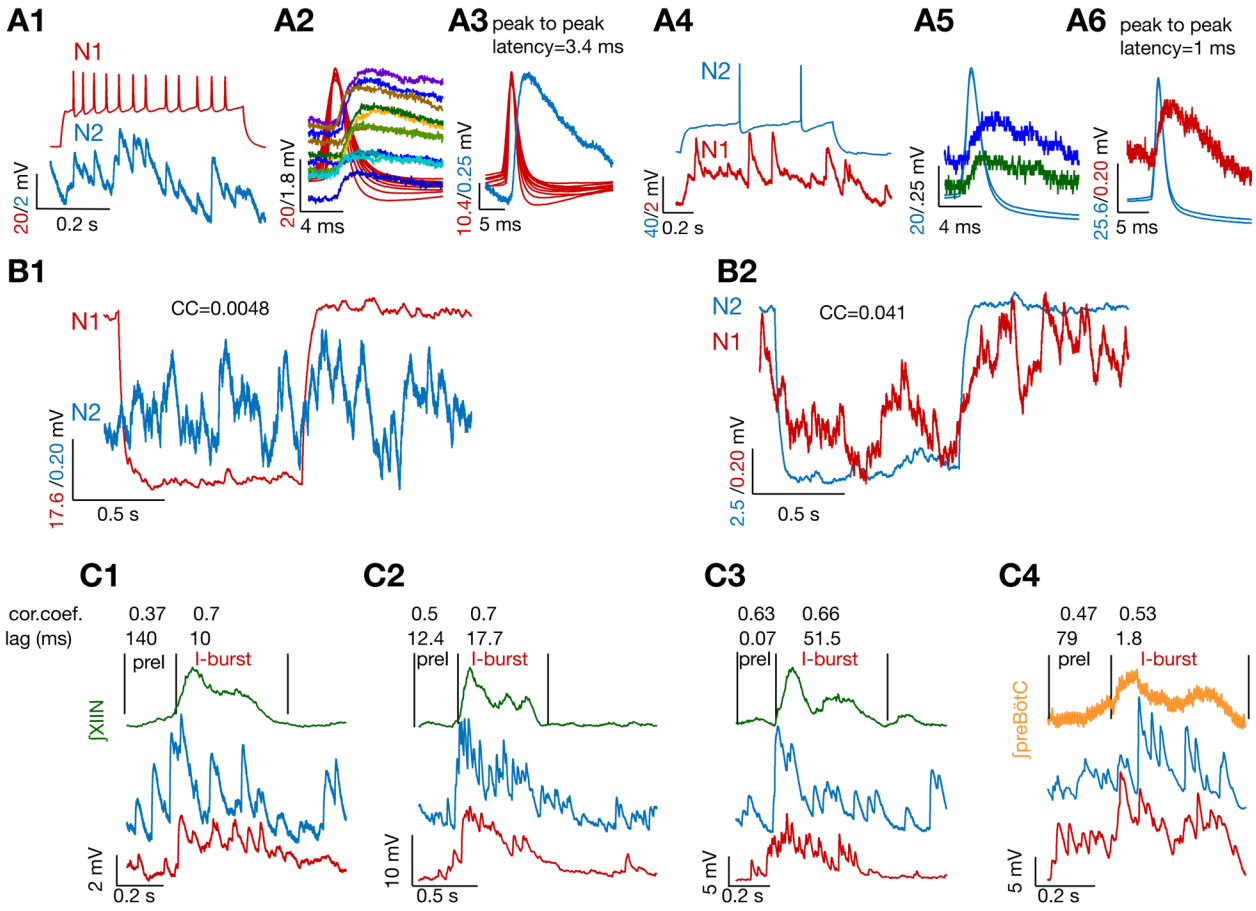

Figure S2. preBötC I-M SST$^+$ neurons have sparse synaptic connectivity. ***Related to Figure 3***

(A1-A6) V_m of the only connected pair of neurons (1/50 tested) (N1 and N2). Spike triggered individual (A2: N2 and A5: N1) and averaged postsynaptic potentials (A3: N2; mean EPSP amplitude = 1.7 mV, latency to peak = 3.4 ms. and A6: N1; mean PSP amplitude = 0.22 mV, latency to peak = 1 ms) PSP latency of <2 ms indicates electrical coupling; also, note that the onset of PSP in A5 is substantially earlier than the EPSPs in A2. This was the *only* synaptically connected pair among 50 pairs of SST$^+$ neurons tested. (B1-B2) Simultaneously recorded averaged V_m traces (from 16-20 trials) from N1 and N2 when a hyperpolarizing current pulse was injected in either to assess electrical coupling. CC: coupling coefficient; This was also the only synaptically- or electrically-coupled pair among 50 pairs of SST$^+$ neurons tested. Thus, N1 ⇒ N2 connection was excitatory synaptic, and N2 ⇒ N1 connection was electrotonic. (C1-C4) V_m of four different simultaneously recorded I-M SST$^+$ pairs (red, blue) with correlated EPSPs during preI and I-bursts. Traces in (C1) are from N1 and N2 shown above. Pairs (C2), (C3), (C4) were not synaptically connected.

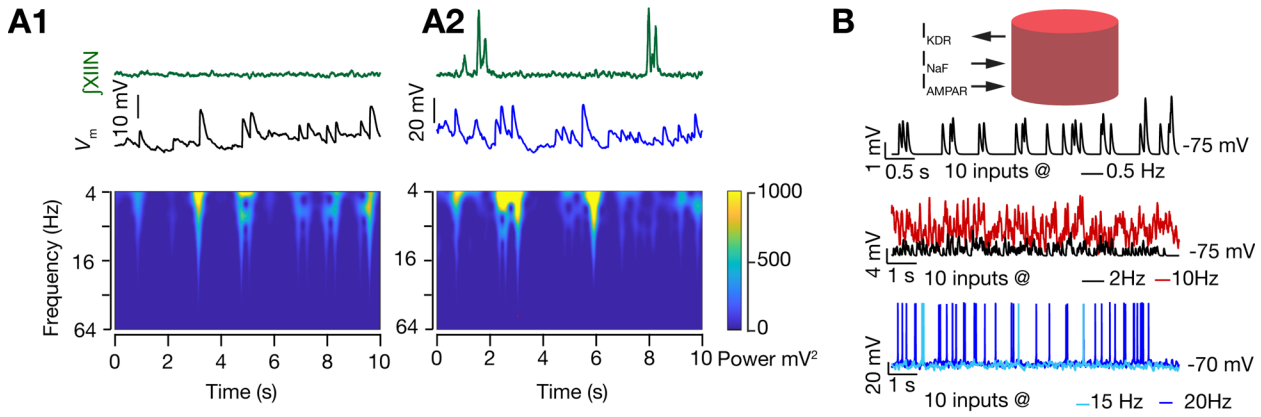

Figure S3. **Input synchrony was absent in non I-M SST$^+$ neurons during I-bursts**. ***Related to Figure 4***

(A1-A2) Frequency-time plot of non I-M SST$^+$ neuron; same as in Figure 4B1-B2 but when the neuron was not spiking, in 9 mM $[K^+]_{ACSF}$. (B**)** Representation of a single compartmental biophysical neuronal model with three currents (see STAR Methods), bottom: V_m under conditions where 10 synaptic inputs were randomly activated at various frequencies, as indicated.

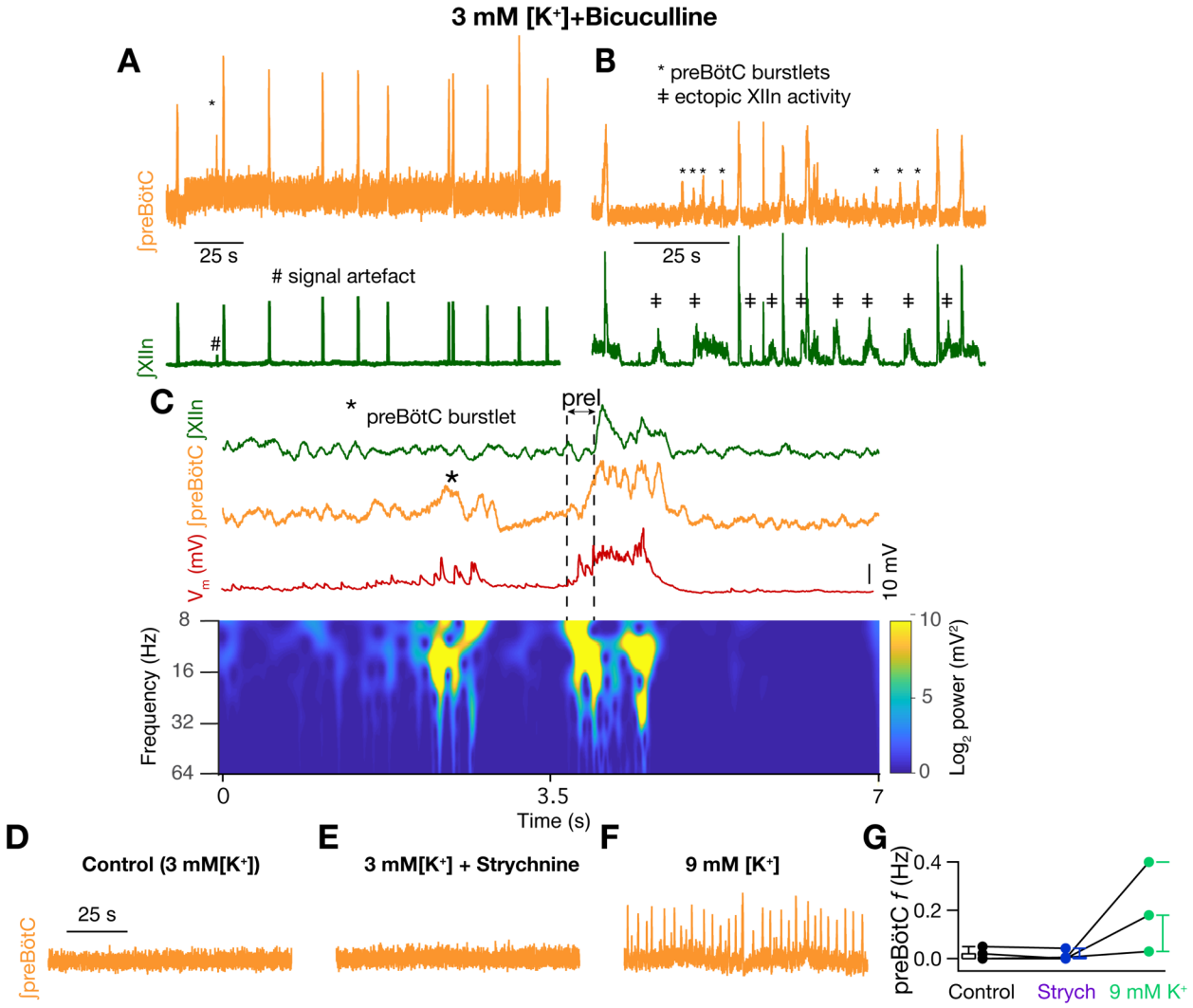

Figure S4. $GABA_A$ inhibition gates preBötC rhythmogenesis. ***Related to Figure 5***

(A-B) Simultaneously recorded preBötC (orange) and XIIn (green) activity in two brainstem slices showing preBötC burstlets (marked by *) that did not result in XIIn I-bursts under 10 µM Bicuculline (BIC). In a few slices recorded under this condition, ectopic XIIn bursts ((B); marked ǂ) were observed that were not concurrent with preBötC activity and, thus, were not considered for analysis. (C) preBötC and XIIn activity and V_m of an I-M SST^+ neuron under BIC-induced rhythmic (3 mM $[K^+]_{ACSF}$) conditions. corresponding frequency-time plot of V_m plotted at bottom. Note the evolution of input synchrony onto this I-M SST^+ neuron in preI period. (D-G**)** preBötC activity of representative slice recorded under control (3 mM $[K^+]_{ACSF}$; (D), 2 µM Strychnine (E) and in 9 mM $[K^+]_{ACSF}$ after strychnine washout from the same slice (F). (G) Activity of 5 slices (including that depicted in Figure 5C; 3 of these slices were also recorded in 9 mM $[K^+]_{ACSF}$ (green) after strychnine washout, e.g., as shown in D-F.

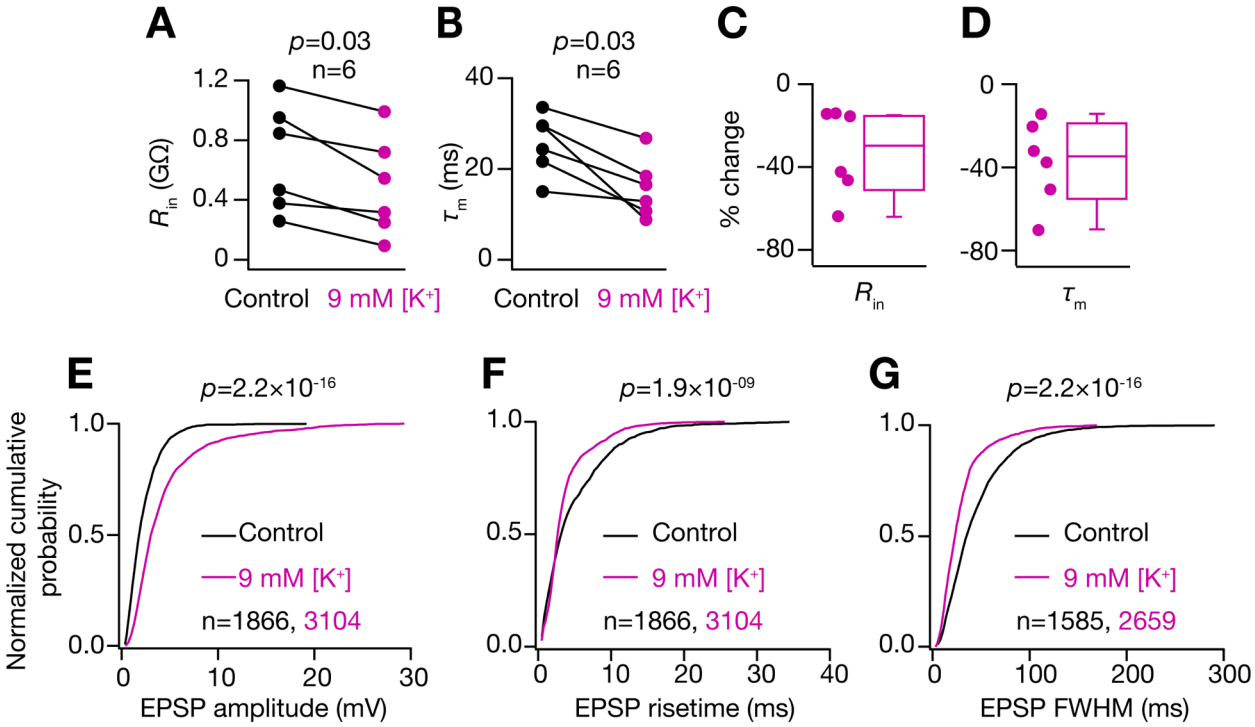

Figure S5. **I-M SST$^+$ neurons shift to higher conductance state in 9 mM $[K^+]_{ACSF}$.** ***Related to Figures 5 and 6***

(A) R_{in}, and (B) τ_m, of I-M SST$^+$ neurons recorded in control, i.e., 3 mM $[K]_{ACSF}$, and rhythmic 9 mM $[K^+]_{ACSF}$ conditions. (C-D) Percentage change in R_{in} (C) and τ_m (D) of these neurons under 9 mM $[K^+]_{ACSF}$. (E-G) normalized cumulative probability of spontaneous EPSP amplitude (E), 20%-80% rise time (F), and FWHM (G) of I-M SST$^+$ neurons in control (black) and rhythmic 9 mM $[K^+]_{ACSF}$ (pink) conditions. Note the increase in EPSP amplitude in (E) even with a decrease in R_{in} of these neurons with change of ACSF from 3 to 9 mM $[K^+]$, which could be attributed to an increase in the presynaptic release probability with steady-state depolarization of axon terminals under 9 mM $[K^+]_{ACSF}$. (A-B) *p* value for Wilcoxon signed rank test; (E-G) *p* value for Wilcoxon rank sum test. For (E-G) N = number of neurons=14