

# Spike synchronization is population specific in the respiratory pattern generator: experimental evidence and network model

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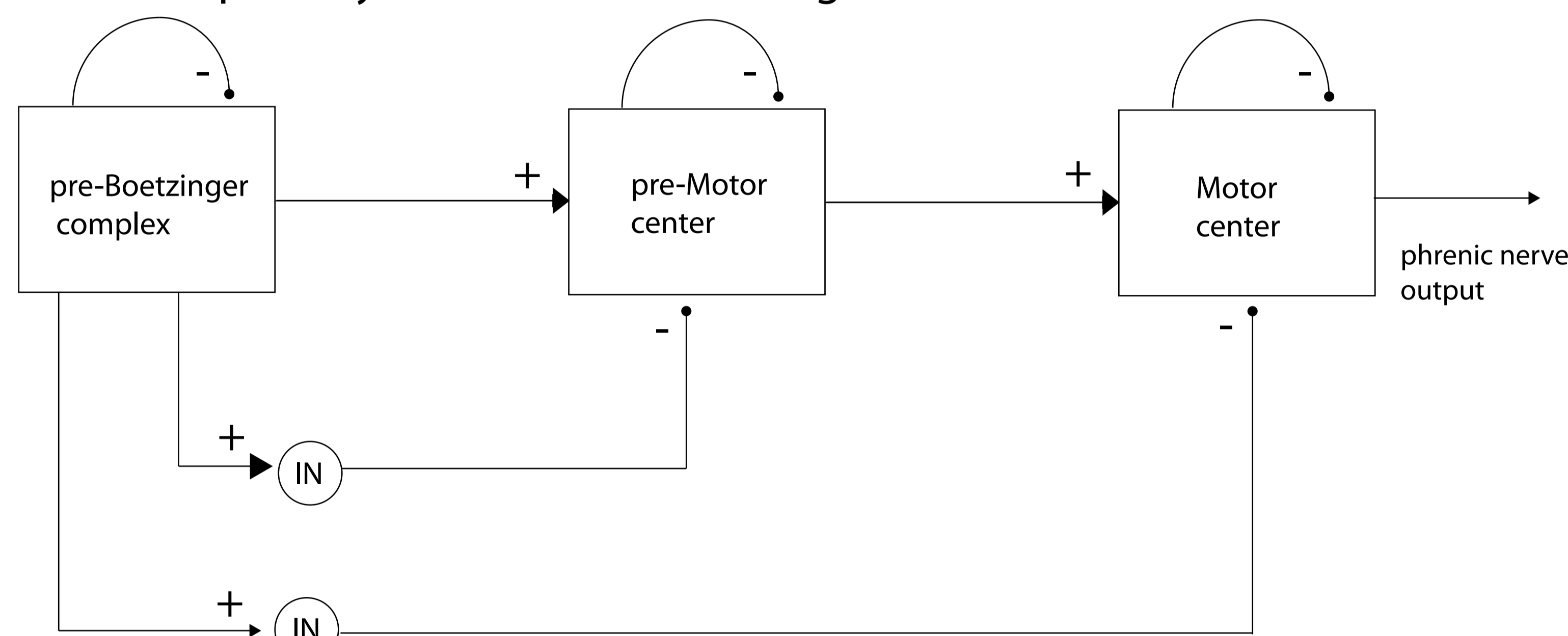
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## 1. Introduction

Breathing requires minimal effort. Arthur B. Otis in his seminal review stated that the work of breathing accounts for a small fraction of basal metabolism and that inspiration, not expiration, consumes energy [1]. While this view is widely accepted, no one understands how this is achieved, whether this is a malleable property and whether it can be utilized to attenuate respiratory failure as well as to facilitate weaning from artificial ventilation.

Several factors influence muscle force, like the force required for diaphragm contraction. The firing rate of motor neurons is a classical example in neuroscience textbooks. Another factor, which has attracted less attention, is the synchronization of spikes from motor neurons. Indeed, synchronization of (pre-)motor unit activity increases muscle force in a very energy efficient manner, preserving rather than increasing the firing frequency of active motor units.

In line with Otis' findings, **our general working hypothesis is that the respiratory control network uses synchrony to generate an efficient inspiratory pattern; one that minimizes the work of diaphragm contraction, chest-wall expansion and lung inflation.** Our ultimate objective is to understand how to modulate synchronization in this motor pattern in order to optimize, and even rescue, muscle function in respiratory failure and in weaning from the ventilator.

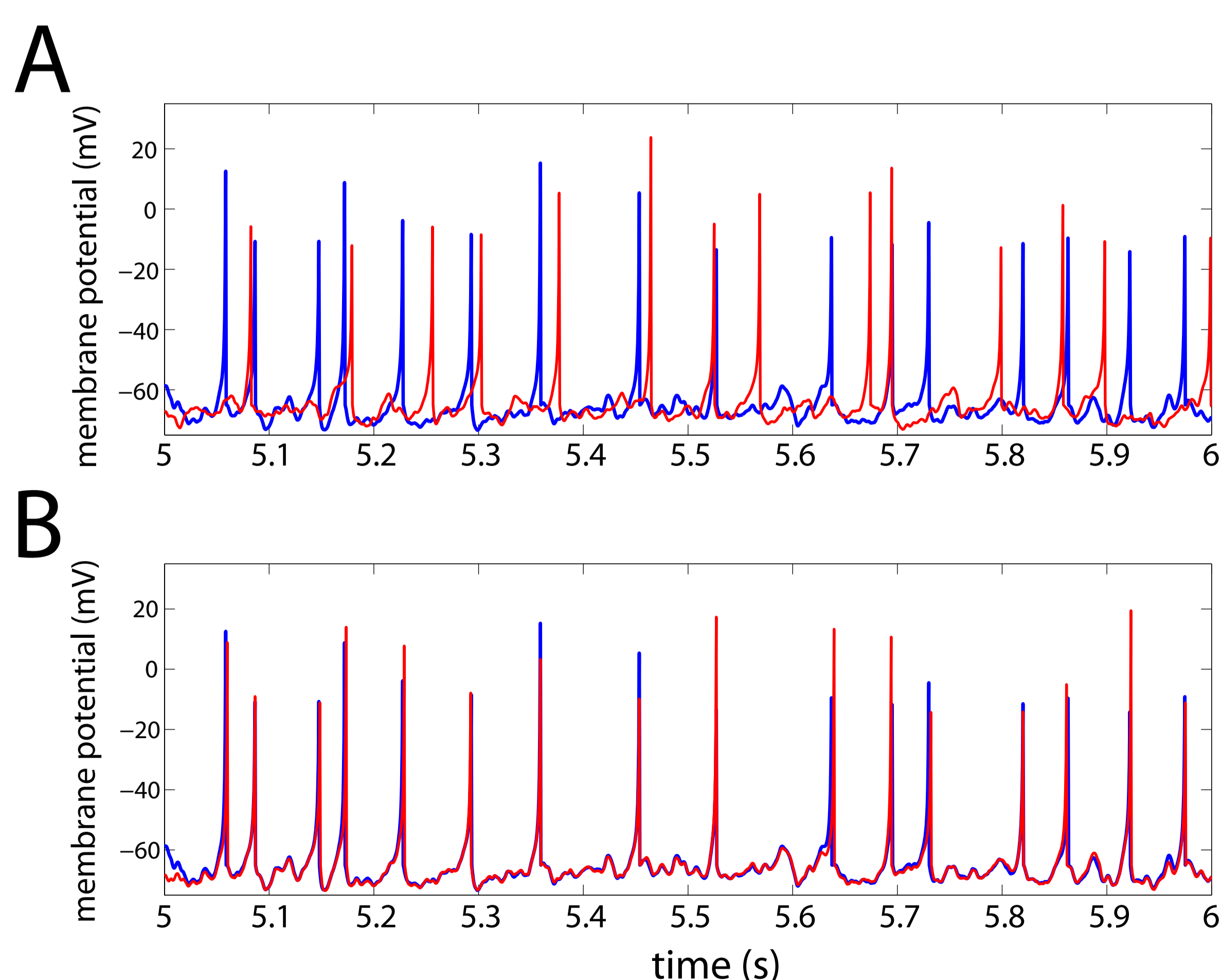


**Figure 1:** Sketch of the circuit modules in the ventral respiratory column. There are three major modules arranged in series: the pre-Bötzing complex, the pre-motor center and the motor center, whose projection neurons (motor neurons) bundle their axons forming the phrenic nerve. The last two modules contain recurrent inhibitory connections and feed forward excitation.

## 3. Computer Simulations

We have modeled a population of pre-motor neurons including an inspiratory and an expiratory population. The inspiratory neurons receive common feed-forward excitatory and inhibitory inputs from the pre-Boetzing complex, which leads to the synchronization of their spikes. The expiratory neurons also receive a mixture of inhibitory and expiratory inputs, but these inputs are not shared by different neurons.

**Figure 3:** Spike Synchronization is population specific. **A)** Two expiratory neurons (red and blue) receiving different barrages of synaptic inputs from the pre-Boetzing complex do not synchronize their spikes. **B)** Two inspiratory neurons (red and blue) receiving partially common inputs from the pre-Boetzing complex synchronize their spikes efficiently.

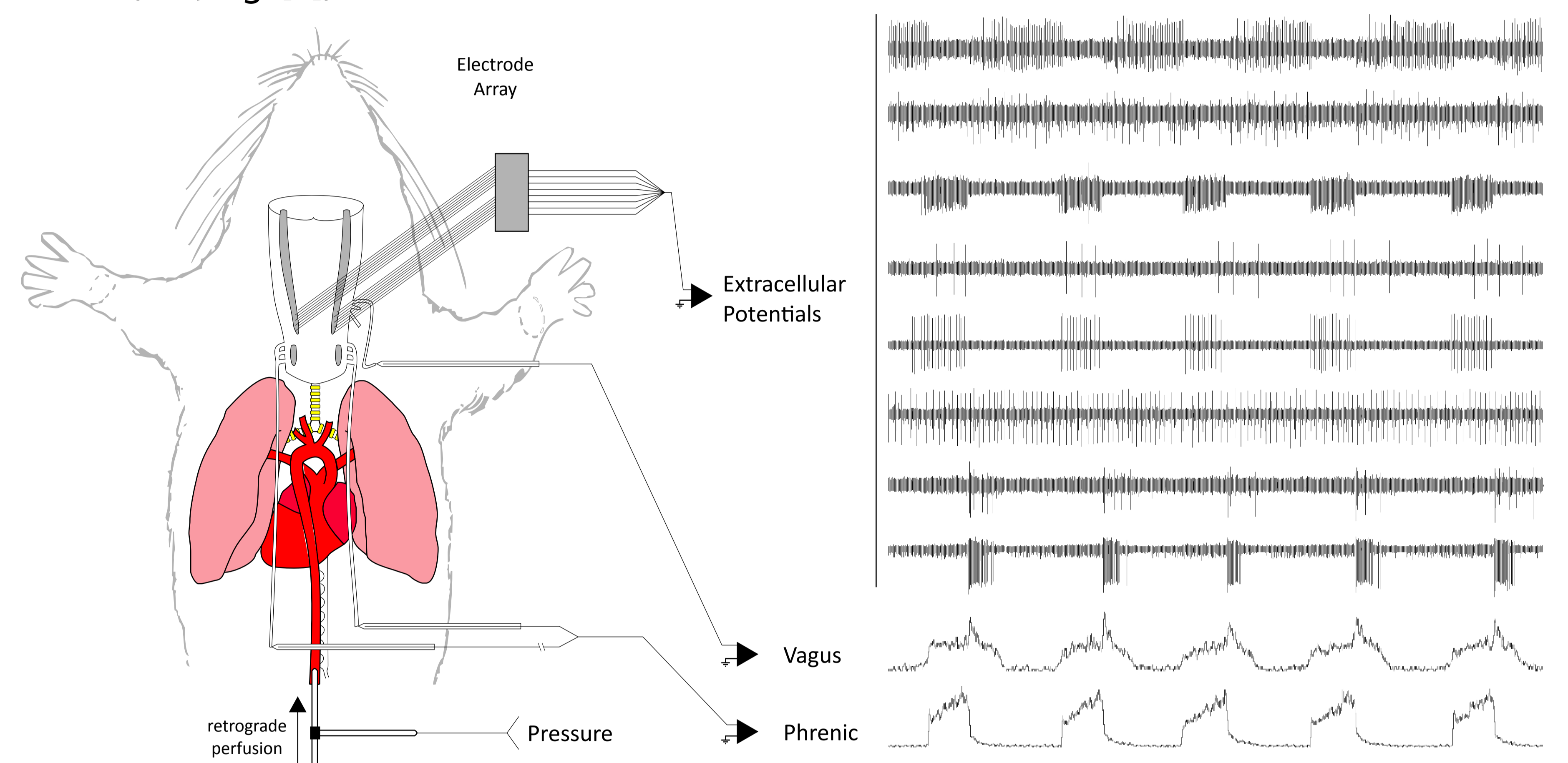


## References

- [1] AB Otis: The work of breathing. *Physiol Rev.* 1954, 34: 449-458
- [2] DM Baekey, TE Dick, JF Paton: Pontomedullary transection attenuates central respiratory modulation of sympathetic discharge, heart rate and the baroreceptor reflex in the in situ rat preparation. *Exp Physiol.* 2008, 93: 803-816.
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## 2. Experimental Methods

To test our hypothesis, we recorded ensembles of respiratory neurons in perfused in situ preparations (Sprague-Dawley/Harlan rats) that produces a ramping breathing pattern spontaneously (for details on preparation see [2]). A 16-channel microelectrode array placed 8 tungsten electrodes (10-12 MΩ) on either side of the spinal cord or brainstem. The design allowed for simultaneous bilateral recording of brainstem pre-motor neurons, and those of the pre-Bötzing complex. The independent depth adjustment of each electrode optimized the yield of isolated high signal-to-noise activity. In a typical experiment, we are able to record an ensemble of 6 to 16 neurons. Spike-synchrony between neuronal pairs was then calculated as reported in previous publications (see, e.g. [3]).

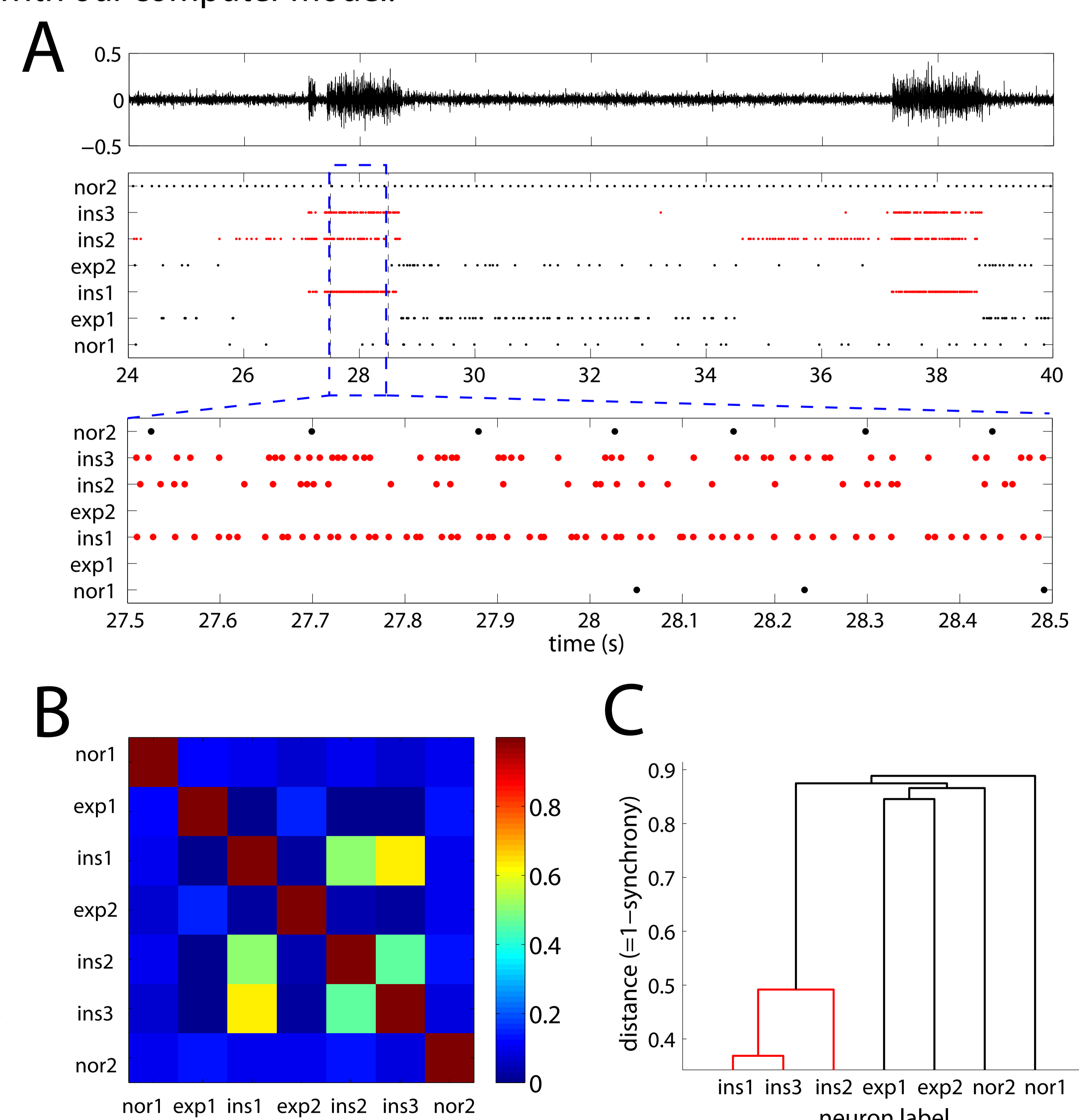


**Figure 2:** The in situ perfused rat preparation. A P21-28 rat is anesthetized with isoflurane, bisected, decerebrated, and retrogradely perfused through the descending aorta with a modified Ringer's solution bubbled with 95% O<sub>2</sub> / 5% CO<sub>2</sub>. Extracellular potentials of brainstem neurons are monitored with an adjustable array of high-impedance tungsten microelectrodes while efferent nerves (phrenic and vagus) are monitored using bipolar suction electrodes. Perfusion pressure is measured at the site of cannulation.

## 4. Experimental Results

The experiments confirm our hypothesis that inspiratory pre-motor neurons are selectively synchronized, in agreement with our computer model.

**Figure 4:** Pairwise synchrony is specific to pre-motor inspiratory neurons. **A)** Simultaneous recordings of phrenic nerve activity showing inspiratory bursts (top) and of pre-motor neurons (raster plots), including inspiratory (ins), expiratory (exp) and neither inspiratory nor expiratory (nor) neurons. **B)** Spike synchrony between neuronal pairs (normalized between 0 and 1) is only significant among inspiratory neurons. **C)** The clustering analysis clearly identifies this synchronized assembly of inspiratory neurons.



## Acknowledgments

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