The ins and outs of deep breathing: mechanisms of respiratory motor plasticity

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THE RESPIRATORY CONTROL SYSTEM is not just reflexive; it is smart, it learns, and, in fact, it has a working memory. The respiratory system listens to and carefully remembers how previous respiratory stimuli affect breathing. Respiratory memory is laid down by regulating synaptic strength between respiratory neurons. Repeated hypoxic bouts trigger a form of respiratory memory that functions to strengthen the ability of respiratory motoneurons to trigger contraction of breathing muscles. This type of motor plasticity is known as long-term facilitation (LTF); it serves to deepen breathing and functions to improve effective lung ventilation.

In this issue of the Journal of Applied Physiology, Sandhu and co-workers (11) make an important contribution to our understanding of LTF mechanisms. They show that a simple and routine experimental manipulation, i.e., cutting the phrenic nerve (phrenicotomy), has marked affects on the strength of LTF. Remarkably, they show that phrenicotomy itself can evoke LTF-like activity. Their results are biologically significant, because they demonstrate that neural signals traveling in the phrenic nerve affect the fundamental processes underlying LTF.

LTF of breathing can be triggered by multiple brief episodes of hypoxia similar to those experienced in sleep-disordered breathing (7, 12). A single continuous hypoxic bout does not cause LTF. Episodic hypoxia can induce ventilatory LTF in a variety of animals, including awake and sleeping humans and rats (6, 8). LTF is typically characterized by an increase in the depth of each breath after a return to normoxia. In electrophysiological terms, it is manifested as an increase in respiratory motoneuron activity and heightened respiratory muscle tone. Episodic hypoxia triggers LTF of phrenic and hypoglossal motoneuron function, which acts to heighten respiratory drive onto diaphragm and genioglossus muscles.

Potentiation of respiratory motoneuron function underlies LTF. Mechanisms mediating LTF of motoneuron function have been extensively studied in vivo and in vitro (1, 2). Evidence to date suggests that episodic hypoxia activates raphe neurons, which in turn release serotonin onto motoneurons; this causes serotonin (5-HT2) receptor activation and increases brain-derived neurotrophic factor synthesis and tyrosine kinase (TrkB) receptor stimulation (1). This cascade of events ultimately strengthens inspiratory glutamatergic drives onto phrenic motoneurons, which increases diaphragmatic motor tone, i.e., LTF. Determining how episodic hypoxia affects respiratory motoneuron function is therefore at the heart of understanding LTF mechanisms.

Hypoxia-induced LTF is typically studied in ventilated, anesthetized rats in which chemical and mechanical feedback is tightly controlled (1, 7). Levels of arterial O2 and CO2 are carefully monitored and adjusted, and mechanical feedback from lung stretch receptors is removed by vagotomy. Such experimental manipulations ensure that respiratory feedback does not interfere with LTF mechanisms. Changes in phrenic (or hypoglossal) nerve activity are used to show that episodic hypoxia (typically three 5-min hypoxic episodes, each separated by 5 min of normoxia) triggers LTF of motoneuron function. Nerve recordings are generally made from the proximal end of cut nerves.

Sandhu et al. (11) asked a simple, but very clever, question: Does cutting the phrenic nerve, i.e., phrenicotomy, affect LTF expression? They asked this question for two reasons: 1) motoneuron excitability is sensitive to axon damage or axotomy, and 2) afferent signals running in the phrenic nerve impact motoneuron function. They hypothesized that removal of afferent activity and/or axotomy may impact LTF expression; therefore, they studied hypoxia-induced LTF in anesthetized rats with and without intact phrenic nerves.

Sandhu et al. (11) discovered that phrenicotomy itself triggers LTF-like phenomena. Specifically, they showed that cutting the phrenic nerves caused a persistent facilitation of inspiratory phrenic activity that peaked at maximal levels 30 min after the phrenic nerves were cut and lasted up to 60 min. They also found that phrenicotomy strengthened episodic hypoxia-induced LTF. When phrenic nerves were intact, episodic hypoxia only triggered a 26% in phrenic activity (i.e., LTF), but when nerves were cut, this same intervention potently increased inspiratory activity by 75% above baseline levels.

Sandhu et al. (11) propose that phrenic motoneuron axotomy and/or removal of phrenic afferent inputs increases motoneuron excitability and strengthens LTF. They are careful to point out that phrenicotomy itself is not necessary for activating hypoxia-induced LTF; rather, it sets up certain preconditions that strengthen it. Indeed, LTF can be induced in humans and rats with intact phrenic nerves (6, 8, 12), illustrating that phrenic axotomy is not a prerequisite for LTF activation.

Axotomy could interfere with LTF mechanisms by affecting normal motoneuron physiology. For example, axotomy rapidly influences motoneuron excitability by disturbing calcium regulation and cell morphology (9). It also disrupts the normal retrograde movement of neurotrophic factors (e.g., brain-derived neurotrophic factor) from axon terminals to cell bodies (3). Axotomy also has pronounced effects on gene regulation and expression (10). Because LTF requires new protein synthesis and recruits neurotrophic signaling pathways (1), axotomy could have side effects that alter motoneuron function and, hence, LTF expression.

Ascending feedback from phrenic afferents could also influence LTF mechanisms. Phrenic afferents relay a variety of signals (e.g., mechanoreceptors) directly to respiratory motoneurons and to the neurocircuits that drive breathing (5). Sandhu et al. (11) show that phrenic afferents discharge during
lungs deflation and that their removal affects motoneuron physiology. They also show that severing ascending inputs amplifies hypoxia-induced LTF phrenic activity. Their results therefore suggest that such afferents normally function to restrain motoneuron excitability and limit LTF expression.

This study is the first to document that retrograde signals traveling in the phrenic nerve impact LTF. Future work is required to determine exactly how these signals influence LTF mechanisms. Afferent feedback from mechanoreceptors has profound effects on breathing, yet its influence on LTF is largely unexplored. Recent work shows that feedback from vagal afferents affects hypoxia-induced LTF expression (4) and that repeated modulation of vagal afferents themselves can actually trigger respiratory LTF (12). Determining how respiratory afferents influence LTF mechanisms therefore requires further consideration.

Numerous diseases cause breathing abnormalities that can cripple normal everyday life. People with spinal cord injury or neurodegenerative diseases often require mechanical ventilation, because damage or disease prevents sufficient respiratory muscle activation. In 18 million North Americans, the normal suppression of upper airway muscle tone during sleep contributes to obstructive sleep apnea. Harnessing LTF mechanisms could therefore be used to strengthen respiratory motor tone and, thus, improve effective lung ventilation in such breathing disorders.

Sandhu et al. (11) make a substantial contribution to our understanding of LTF mechanisms. Their results indicate that damage (i.e., axotomy) to respiratory motoneurons influences hypoxia-induced breathing plasticity. The degree to which axotomy interferes with fundamental LTF mechanisms is unknown but warrants further investigation, given that motoneuron axotomy (e.g., phrenic and hypoglossal) is a routine procedure in studies that examine LTF physiology (1, 2). Their research also illustrates that respiratory afferent feedback impacts normal mechanisms of motoneuron LTF. Dissecting LTF pathways will not only deepen our understanding of respiratory physiology, it will also provide a more sophisticated view of the fundamental mechanisms governing brain plasticity.

**REFERENCES**