Enhancing Diaphragm Muscle Reinnervation Through Therapeutic Electrical Stimulation Following Spinal Accessory to Phrenic Nerve Transfer, Contrasting with Phrenic Direct Repair in a Rat Model

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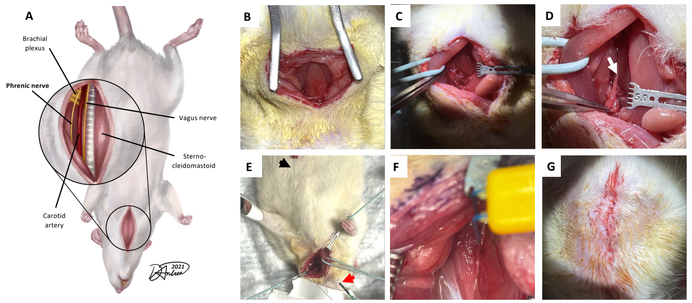
INTRODUCTION

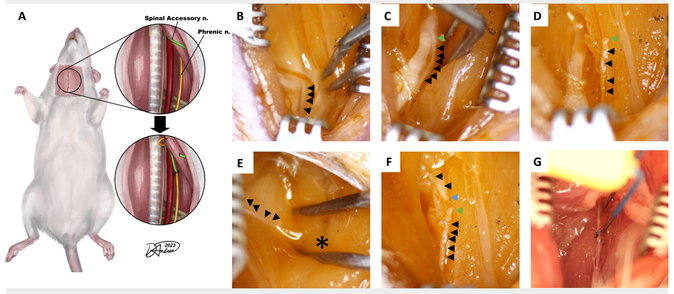
Diaphragm, functions as a breathing muscle, is the most used skeletal muscle though the entire human life, working periodically and nerve fall asleep. Diaphragmatic paralysis, causing shortness of breath, recurrent pneumonia, anxiety, insomnia, morning headache, excessive daytime somnolence, orthopnea, and fatigue. Diaphragmatic paralysis can either due to phrenic nerve palsy, impairing the axons, or high spinal cord injury (C3-C5), impairing the neuronal soma body. Natural recovery of phrenic nerve injury not only takes a year but still leaves two thirds of patients with unsatisfactory diaphragmatic function. Let alone, high spinal cord injury impairing the soma body mostly like stays as permanent.

Our study aims to restore diaphragm muscle function through spinal accessory nerve to phrenic nerve transfer. The spinal accessory nuclei rely from C1 to C4, less likely injured than the phrenic nuclei. Additionally, we investigate the potential of therapeutic electrical stimulation (20 Hz, 1-hour) to expedite diaphragm muscle reinnervation and functional recovery.

METHODS AND MATERIALS

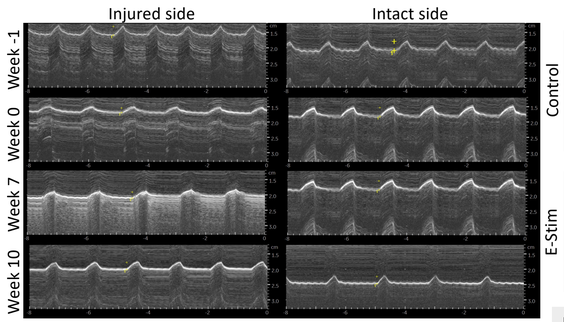


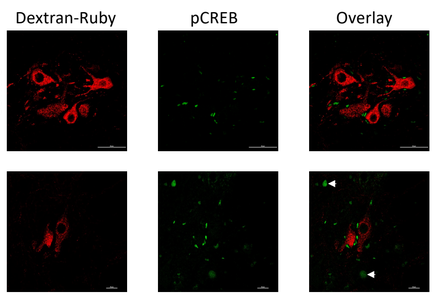
Phrenic nerve transection model followed by surgical repair and electrical stimulation. (A) Ventral view of the neck in the supine position. **(B)** Incision to expose sternohyoid muscle. **(C)** Dissecting through the omohyoid and the sternocleidomastoid muscles. **(D)** Phrenic nerve (arrow). **(E)** Diaphragmatic electromyographic. **(F)** Direct repair followed by 20 Hz 1 hour wired electrical stimulation. **(G)** Closure of incision with buried sutures.



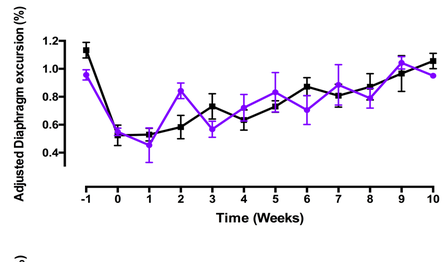
Spinal accessory to phrenic nerve transfer and electrical stimulation. (A) image showing the ventral view of the neck in the supine position. **(B, C)** Dissecting through the potential space between scalene muscles. Black arrows, phrenic nerve. Green arrow, distal end of phrenic nerve. **(D)** transected phrenic nerve. **(E)** Dissecting spinal accessory nerve and transecting at muscle innervation point. Black arrows, spinal accessory nerve. Star*, sternocleidomastoid. **(F)** rerouting spinal accessory nerve beneath omohyoid to reach phrenic nerve. Blue arrow, proximal end of the proximal end of the spinal accessory nerve. **(G)** electrical stimulation on the spinal accessory nerve.

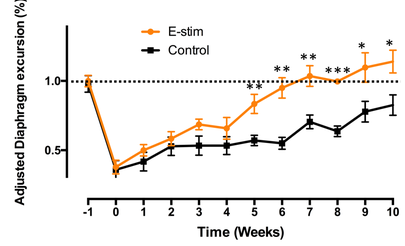
RESULTS



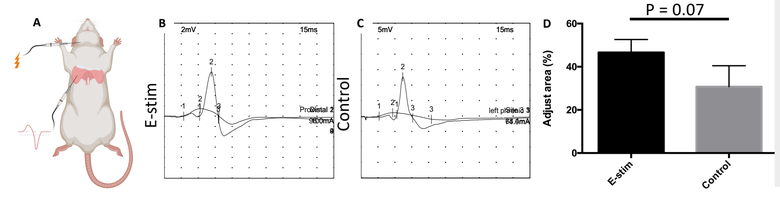


Electrical stimulation on phrenic nerve did not facilitate diaphragm functional recovery. Bilateral diaphragm excursion was measured weekly. Week -1 was pre-injury measurement. **Upper Left**: M mode ultrasound. Representative traces of diaphragm excursion. **Lower Left**: quantification of diaphragm excursion. Purple: phrenic nerve transection followed by direct repair and subsequent one hour 20 Hz electrical stimulation. Black: phrenic nerve transection followed by direct repair only. N = 6. Mean ± SEM. **Right**: electrical stimulation did not induce upregulation of phosphorylated CREB in rat phrenic neuronal nuclei.

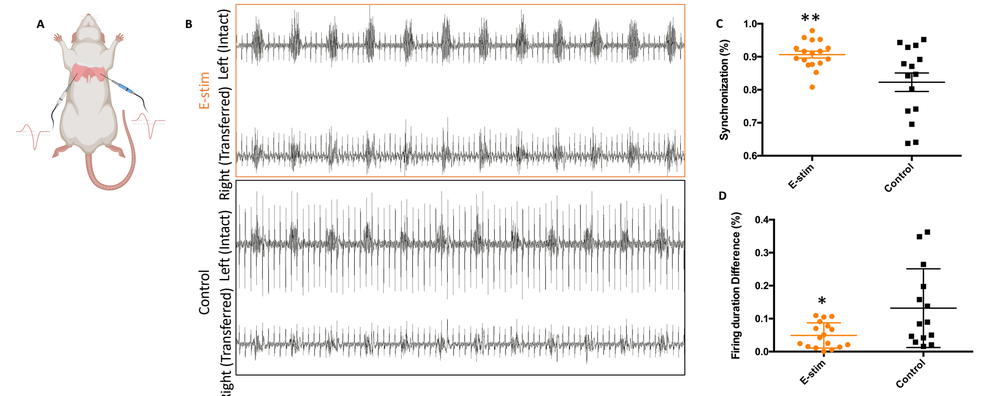


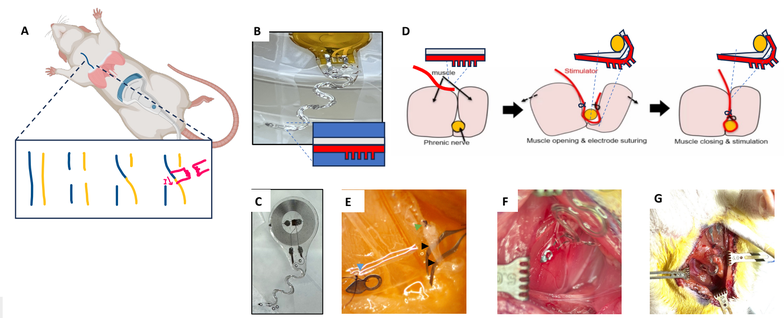


Electrical stimulation on spinal accessory nerve following nerve transfer facilitate diaphragm functional recovery. Quantification of diaphragm excursion. Adjusted diaphragm excursion is calculated by the excursion on the injured side/intact side. Orange: group of rats with SAN to PhN transfer and subsequent one hour 20 Hz electrical stimulation. Black: group of rats with SAN to PhN transfer only. N = 5. Mean ± SEM.

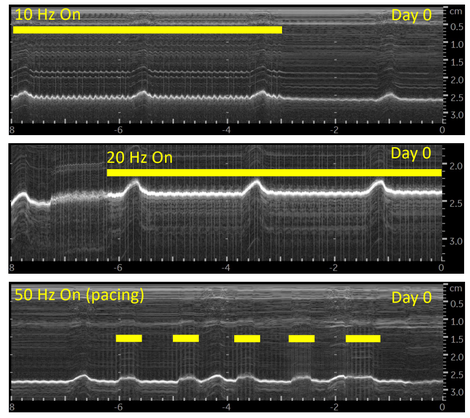


(A) Diagram showing the experiment design of the evoked CMAP study 10 weeks after nerve transfer. **(B, C)** representative CMAP traces. **(D)** quantification of CMAP.





Repetitive electrical stimulation on spinal accessory nerve following transferring to phrenic nerve by a transient bioresorbable wireless battery-free implantable device. (A) diagram of nerve interface implantation following nerve transfer. **(B, C)** device photo on z axis and x-y axis. **(D)** diagram of implantation strategy: electrodes go beneath the spinal accessory nerve with one suture to proximal muscles on each side. Natural force of proximal muscles closing up the space and make a full-round wrapping of the electrodes to the nerve. **(E)** a firm node of the device end to a proximal muscle. **(F)** an air node of the device laterally to the nerve. **(G)** an air node of the receptive coil to a proximal muscle subcutaneously at ipsilateral chest area.



The device implanted on an intact phrenic nerve generated ipsilateral diaphragmatic excursion. Upper: Diaphragmatic excursion at 10 Hz by 10 Hz electrical stimulation. **Middle:** Diaphragmatic excursion at 20 Hz by 20 Hz electrical stimulation. **Lower:** full range diaphragmatic excursion by 50 Hz electrical stimulation. Images were taken right after device implantation surgery. Yellow line: electrical stimulation was on. Rats had intact involuntary diaphragmatic excursion. Recording time was 8 seconds in total under M mode ultrasound.

Electrical stimulation made spinal accessory reinnervated hemidiaphragm firing more similar and synchronized to intact hemidiaphragm. (**A**) diagram of multichannel recording of involuntary respiratory EMG on both sides of the hemidiaphragm (**B**) representative EMG traces. Recording time 18 seconds. (**C**) quantitative synchronization rate between left and right hemidiaphragm. N = 17/5 or 15/5. (D) quantitative firing duration difference between left and right hemidiaphragm. N = 17/5 or 15/5.

CONCLUSION

Spinal accessory nerve transfer is a promising option to re-innervate diaphragm and restore spontaneous synchronized respiratory movement. Brief therapeutic electrical stimulation selectively improves regeneration of spinal accessory axons, but not phrenic axons, for functional restoration of the paralyzed diaphragm.

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