fMRI/fcMRI investigation showing cortical and subcortical pathways involving in phrenic nerve activation and respiration control in rats under 9.4Tesla.

Rupeng Li[1], Nicholas Flugstad[2], Xiping Liu[3], Christopher Pawela[1,2], Ji-Geng Yan[2], Hani S Matloub[2], and James S Hyde[1]
[1]*Biophysics, Medical College of Wisconsin, Milwaukee, WI, United States,* [2]*Plastic Surgery, Medical College of Wisconsin, Milwaukee, WI, United States,*
[3]*Anesthesiology, Medical College of Wisconsin, Milwaukee, WI, United States*

Introduction

The phrenic nerve has been extensively studied in the past few decades. In animals and humans it carries information for maintenance of respiration and the blood oxygenation level. It has been used clinically as a superior donor to repair upper extremity nerve injury due to the fact that this nerve fires constantly and spontaneously and yields good surgical outcomes. However, the underling mechanism of this nerve-transfer procedure remains unknown. Current medical knowledge shows the CNS center for the phrenic nerve is in the medulla. Davenport et al (10) have reported afferent sensory activation in the S1 area when the phrenic nerve is stimulated (1). However, this does not resolve the mystery that patients can regain sensory and voluntary motor function after this nerve is connected to an injured major nerve of the upper extremity. The phrenic nerve originates from No.3-5 cervical nerve roots (C3-C5). **Materials and Methods**

Animal preparation: Three Sprague-Dawley rats (weighting 400g) were used. The right side phrenic nerve was exposed on all animals and a microelectrode was placed onto the nerve trunk. In order to avoid possible interference from other nerves, all other nerves from C3 to T1 were transected and removed, leaving the right phrenic nerve as the only nerve in the area. Femoral vein catheter insertion and tracheal incubation was performed in to all animals. A mixture of Dexdomitor (0.1mg/kg/hr) and Pancuronium Bromide (20mg/kg/hr) was infused through the venous line. The animal was placed on a mechanical ventilator and scanned with a Bruker 9.4Tesla 30cm animal scanner. An electrical stimulation of 10Hz, lmA, 1ms was applied to stimulate the phrenic nerve and a block design was used. Forepaw electrode was placed on the left forepaw for reference scans with an electrical stimulation of 10Hz, 3ms, 2mA. All physiological parameters were monitored and maintained within normal range. **fMRI/fcMRI parameters and data analysis:** MRI data was acquired using a gradient-echo EPI sequence. FOV=3.5cm, TR=2S, TE=19 ms, matrix size 96 x 96, slice thickness 1mm. Each rat was scanned three times for resting-state data and twice for phrenic nerve and forepaw stimulation data. Due to the highly refined BOLD fMRI activation of the phrenic nerve, the data cannot be averaged and will be presented for individual animals. fcMRI was done using the seed-voxel technique. For each animal, the seed was chosen from the BOLD fMRI activation produced by phrenic nerve stimulation. A band-pass filter of 0.01Hz-0.1Hz was used. The fcMRI data was smoothed with a FWHM of 0.5mm. Multiple comparison was done to determine the threshold. The Fisher Z transform was applied to all fcMRI results.

Results

Figure 1 shows the BOLD fMRI result of left forepaw stimulation and right phrenic nerve stimulation of rat No.1. At a p value of 0.005, clear S1FL activation can be seen in the contralateral side of cortex in (A). This result is comparable to our previous forepaw stimulation results as well as to results from other research groups. Figure 1(B) demonstrates the example of phrenic nerve stimulation. Note the spatially close relationship of the phrenic activation with the S1FL area. The BOLD signal is highly localized and varies across animals. This activation occupies 1-2 slices overall (1 slice in rat1 and rat 3, 2 slices in rat2). Figure 2 shows the fcMRI result using a seed from phrenic nerve BOLD fMRI stimulation. Figures 2(A) and 2(B) were done before any electrical stimulation. 2(C) was acquired after all stimulations. All three fcMRI results are highly comparable with sensory cortex, motor cortex and thalamus involved in the network. Figure 3 shows the fcMRI result using left side motor cortex acquired from Figure 3A as seed. Interestingly, the sensory motor network was emphasized with distinct foci on each side of the cortex. The thalamus network, on the contrary, disappeared.

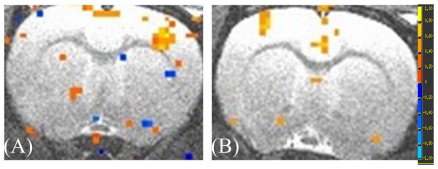

Fig.1. BOLD fMRI activation of (A) Left forepaw stimulation. (B) Right phrenic nerve stimulation.

Conclusion and Discussion

This study, for the first time, clearly demonstrates a higher center for the phrenic nerve in the CNS using fMRI techniques. We also revealed the sensory motor network related to the phrenic nerve using fcMRI. We found that the cortical center of the phrenic nerve locates closely to the S1FL and M1/M2 area. This could account for the great functional shift that occurs after the surgery when the phrenic nerve is used as donor. The thalamus also plays a role in this network, which needs to be further investigated. Clinically, it is known that phrenic nerve can be controlled on demand and have sensory function. Neither of these functions can be achieved by the brain stem and pons. Our finding shows that there are extra pathways in the cortical and subcortical regions that control the function of this important nerve. This result not only contributes to medical knowledge of the respiratory system, but also leads to further investigations of clinical applications.

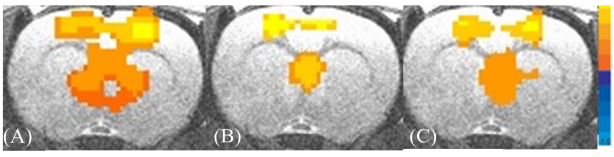

Fig.2. fcMRI showing sensory motor network with thalamus involved. (A) and (B) pre-stimulation fcMRI. (C) post-stimulation fcMRI

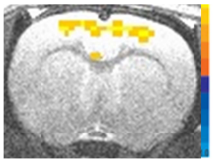

Fig.3. fcMRI showing sensory motor network using motor area from Fig2A as seed.

Reference 1. Davenport PW, Reep RL, Thompson FJ. *Phrenic nerve afferent activation of neurons in he cat SI cerebral cortex*. J Physiol. 2010 Mar 1;588(Pt 5):873-86. Epub 2010 Jan 11.