PERSPECTIVE

Novel role for transcranial magnetic stimulation to study post-traumatic respiratory neuroplasticity

Transcranial magnetic stimulation-a tool used in humans: Transcranial magnetic stimulation (TMS) is a non-invasive widespread clinical tool used to stimulate cortical areas in human subjects. This technique utilizes a brief, highly intense magnetic field applied to cortical areas, which locally depolarized interneurons (Weber and Eisen, 2002). When applied to primary motor cortex areas, TMS induces volleys of action potentials which propagate along corticospinal pathways from supraspinal levels to their muscular targets where motor evoked potentials (MEP) can be recorded. Accordingly, MEP amplitude and latency values provide good indexes of corticospinal pathway integrity or alterations.

In humans, TMS has proven to be effective to evoke diaphragmatic MEP (MEPdia) by recording the costal part of diaphragmatic muscle fibres supporting the effectiveness of a corticospinal pathway (Gandevia and Rothwell, 1987; Maskill et al., 1991). Although the precise anatomical and functional relationship between cortical and bulbar neurons remains unclear, there is some physiological evidence for a descending corticospinal pathway innervating the phrenic motoneuron pool in cat and rat (Lipski et al., 1986; Vinit et al., 2014). Cortical influence over respiratory neuronal control has been demonstrated in several animal preparations and clinically in humans, by electrically stimulating primary motor cortex and recording motor evoked responses with diaphragmatic electromyograms and also by the use of cortical neuromodulation techniques such as transcranial direct-current stimulation (tDCS) (Azabou et al., 2013) and/or repetitive TMS (rTMS) (Raux et al., 2010; Laviolette et al., 2013). Evaluation of the precise cellular and molecular events underlying the physiological modulations induced by such techniques remains difficult to study in humans, and the need of having a reliable preclinical model is then necessary.

Validation in a murine preclinical model: A first proof-of-principle study was to determine whether a preclinical respiratory impairment model could be used to develop, adapt and apply a human designed TMS procedure to a rat diaphragmatic neuronal circuitry. A single pulse of TMS was performed on adult male Sprague-Dawley rats using a human figure-of-eight coil (**Figure 1**) centered over precise rat cortical areas (Vinit et al., 2014). The pulse induced action potential volleys from the cortical and sub-cortical sites, descending through the main respiratory central pathways (rostral ventral respiratory group (rVRG) to phrenic motoneurons), and reaching the diaphragmatic muscle fibers *via* the phrenic nerve. The largest MEPdia were recorded when the center of the coil was positioned 6 mm caudal from Bregma, involving a specific stimulation of respiratory supraspinal pathways. Magnetic shielding of the coil with mu metal reduced magnetic field intensities and improved focality with increased motor threshold and lower amplitude recruitment curve. Moreover, transynaptic neuroanatomical tracing with pseudorabies virus (applied to the diaphragm) suggested that connections existed between the motor cortex, the periaqueductal grey cell regions, several brainstem neurons and spinal phrenic motoneurons (distributed in the C_{3-6} spinal cord). These results revealed the anatomical substrate through which supraspinal stimulation could convey descending action potential volleys to the spinal motoneurons (directly or indirectly). Altogether, this pilot study (Vinit et al., 2014) demonstrated the feasibility of applying such technique to specifically elicit a MEPdia following a single pulse TMS over rat brain structures and could be used in the assessment of respiratory supraspinal neuroplasticity.

Evaluation of respiratory neuroplasticity with TMS in a rat preclinical model of spinal cord injury: Clinical studies in respiratory deficiency patients caused by high spinal cord injury (SCI) are extremely limited. Patients living with such injuries develop respiratory insufficiencies and require ventilatory assistance. A series of studies already demonstrated that intermittent hypoxia could be also a type of non-invasive approach to improve respiratory function (Gonzalez-Rothi et al., 2015). However, to date no non-invasive therapeutics inducing spinal rewiring with full functional respiratory restoration is available. Having a viable, reliable and quantifiable preclinical respiratory model is badly needed to further understand the consequences of supraspinal neuromodulation as therapeutics. Given that the majority of SCIs are anatomically incomplete, another strategy for activating spared neural substrates is the stimulation of supraspinal neurons. In this context, we propose a novel application of the TMS to non-invasively target these spared pathways and reveal the post-lesional reorganization of such respiratory descending spinal pathways (Vinit et al., 2016).

The common preclinical model of respiratory insufficiency in adult rats is the partial C_2 spinal cord injury (C_2 SCI) which caused unilateral diaphragm paralysis (Keomani et al., 2014). This model allowed the investigation of a particular population of respiratory bulbospinal axons which crossed the midline at C_{3-6} spinal segment, namely the crossed phrenic pathway. We decided to use TMS to study the post-lesional supraspinal descending respiratory pathways in the rat. Interestingly, a lateral C_2 injury did not affect the amplitude and latency of the largest motor-evoked potential recorded from the diaphragm ipsilateral to the injury in response to a single pulse TMS, compared

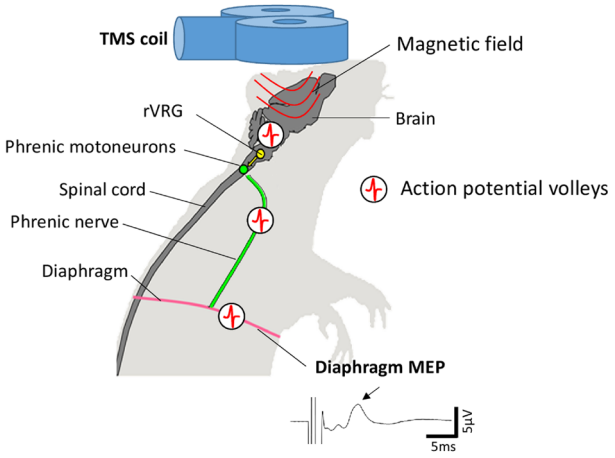

Figure 1 Schematic representation of transcranial magnetic stimulation (TMS) application to the rat neural control of breathing.
A TMS pulse (red semicircles) is induced above cortical and subcortical areas from a rat brain with a TMS coil (blue). It elicits action potential volleys which travel through the respiratory brainstem structures (yellow structure, rVRG, rostral Ventral Respiratory Group), and propagate to the phrenic motoneurons (green spinal structure, located from C_3 to C_6 spinal cord segments). Then, the signal transits through the phrenic nerve (green line) and elicits a specific diaphragm motor evoked potential (Diaphragm MEP).

to a sham animal. Although the rhythmic respiratory activity on the intact diaphragm was preserved at 7 days post-injury, no diaphragm activity was recorded on the injured side. However, a profound reorganization of the MEPdia evoked by TMS was also observed. The MEPdia was reduced on the non-injured side rather than the injured one. This suggested an increase in ipsilateral phrenic motoneurons excitability. Moreover, correlations between MEPdia amplitude and spontaneous contralateral diaphragmatic activity were observed. The larger diaphragm activity correlated with a larger MEPdia on the injured side, and a smaller MEPdia on the non-injured side. This study (Vinit et al., 2016) suggested, for the first time, the occurrence of a functional neuroplasticity process involving changes in motoneuron excitability balance between the injured and non-injured sides at a short post-lesional delay.

Perspectives: From new diagnostic tool to novel therapeutic strategy: Although preliminary, the results of the experiments obtained in our laboratory lay the foundations for exploring the therapeutic potential of this unique and non-invasive technique in preclinical animal models of respiratory injury or disease. There is a widespread appreciation for developing new and powerful strategies, particularly enhancing neural activity and neuroplasticity with the therapeutic potential of TMS. This painless clinical tool is suited for the study of respiratory supraspinal pathway excitability and plasticity in humans. Recent clinical studies in healthy patients (Raux et al., 2010; Azabou et al., 2013; Laviolette et al., 2013) have demonstrated that rTMS and tDCS could increase or decrease diaphragmatic MEP amplitude, respectively, in response to a single pulse TMS. In case of respiratory neuronal disordered breathing patients suffering from partial tetraplegia, the ability of supraspinal neuromodulation techniques, such as rTMS, are likely to potentiate diaphragmatic output. This hypothesis is of importance since a mild increase in phrenic motoneurons functioning would allow reducing the burden of respiratory care needed by these patients. Most of them display hypoventilation at night that requires nocturnal ventilation assistance. Thus, alleviating the heavy home care of these patients would be of great relevance.

Keeping in mind this clinical challenge and issues, preclinical studies are needed to test the beneficial effects of rTMS. Few studies led in rat or mice have reported various effects of rTMS on different cortical and brainstem structures. High-frequency rTMS protocols increase the production of brain derived neurotrophic factor (BDNF), a molecule involved in neuroplasticity, in whole cortex and hippocampus (Gersner et al., 2011; Wang et al., 2011). This factor also plays a key role in control of breathing especially during post lesional period following SCI (Lovett-Barr et al., 2012). In this view, we may hypothesize that BDNF, as many other neuroplastic molecules, could contribute to molecular and cellular mechanisms able to facilitate phrenic motoneuron activity. In our preclinical model of respiratory insufficiency (C_2 SCI), rTMS protocols would reactivate the deafferented phrenic motoneurons from the injured side.

Because our study has established that TMS could be used to show in rat the intrinsic reorganization of the respiratory spared pathways that arises after SCI, we may speculate that rTMS would enhance this neuroplastic effect and would allow significant respiratory functional recovery. More work needs to be done to demonstrate the therapeutical effect of rTMS on the molecular and cellular mechanisms underlying the functional recovery, and to determine the most efficient rTMS procedure prior to elaborating clinical trials.

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