SORBONNE UNIVERSITÉ

CHINA SCHOLARSHIP COUNCIL

Appel à projets
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Title of the research project :

Synergistic impacts of serotoninergic pharmacology and neurostimulation to restore spor

Thesis supervisor (HDR) :

Name : Vivodztev Surname : Isabelle

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(site, dresse, bulding, office…) Faculté de Médecine Pierre et Marie Curie - Site Pitié-Salpêtrièr

Research Unit

Name : Neurophysiologie Respiratoire Expérimentale et Clinique

Code *(ex. UMR xxxx)* :

UMR_S1158

Doctorate School

Thesis supervisor's doctorate school (candidate's futur doctoral school) : ED 394, Physiology, Physiopathology and Therapeutics (P2T)

PhD student currently supervised by the thesis supervisor (number, year of the first inscription) :

Isabelle Vivodtzev is a full-time researcher with no actual thesis supervision.

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Joint supervisor :

Name : Surname :

Title :

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Research Unit

Name :

Code *(ex. UMR xxxx)* :

École doctorale

Joint supervisor's doctorate school : Or, if non SU :

PhD student currently supervised by the joint supervisor (number, year of the first inscription) :

Joint supervisor :

Name : Surname :

Title :

email :

Professional adress :
(site, dresse, bulding, office…)

Research Unit

Name :

Code *(ex. UMR xxxx)* :

École doctorale

Joint supervisor's doctorate school : Or, if non SU :

PhD student currently supervised by the joint supervisor (number, year of the first inscription) :

Description of the research project

Position of the project as it relates to the state of the art

A majority of **spinal cord injury** (SCI) occur at a **cervical level** and results in locomotor impairments but also in a marked respiratory dysfunction[1]. When injury is high (C3 or above), survivors can be **rendered ventilator dependent**, a sequela that dramatically compromises quality of life[3] and increases mortality rate[4]. Furthermore, even in lower cervical injury (C4-C8), this reduction in respiratory capacity causes subsequent morbidity and mortality as it crucially limits the level of intensity during exercise-based rehabilitation, a key parameter of recovery and reduction in secondary health complications of SCI. The best therapeutic approaches available today mostly rely on assistive devices but not on permanent functional recovery. Restoration of respiratory muscle functions and locomotion is hence a top priority for these patients to reduce reliance on ventilation devices and also increase prevention to **secondary health complications** in those with cervical injury who kept ability to train.

SCI is characterized by a reduction in bidirectional communication between brainstem and muscles. One reason for this neural pathways' alteration is a depletion in serotonin (5-HT) at the below-lesion level (Chopek et al. 2015). Indeed, **serotoninergic influences are pivotal** in restauration of **both respiratory and locomotor** control after injury but intrinsic 5-HT is synthetized in brainstem raphe nuclei and not in the spinal cord, making this neuromodulation vulnerable to SCI. However, a growing number of studies show that 5-HT pathways can be activated by 5-HT agonists, and, among them, 5-HT_{1A} agonists may constitute a "fruitful strategy" to restore spinal networks after SCI (Pizzolato et al., 2021). Indeed, it could be of particular interest in cervical SCI since 5- HT_{1A} receptors are distributed both in ventral and dorsal horn, hence participating in the central pattern generator activity and afferent regulations. Moreover, **5-HT_{1A} agonists exert considerable facilitation** of both spinally-mediated respiratory and locomotor functions. For example, treatment with the systemic 5-HT_{1A} agonist Buspirone temporarily restore respiratory function in rats after C5 hemi-contusion (Choi et al. 2005) and it can acutely initiate locomotor function in T8 transected mice (Jeffrey-Gauthier et al. 2018). Furthermore, two very recent studies report that buspirone increases grasping function and grip strength after C4 injury in rats (Ahmed et al. 2021; Jin et al. 2021). And in fact, the combination of **Buspirone and muscle training could be even more promising**. Indeed, when combined with exercise, 5-HT_{1A} treatment enables long-lasting gains in locomotion in animals (Jeffrey-Gauthier et al. 2018) and human case report (Gad et al. 2017). Moreover, our own findings show that Buspirone (although prescribed for its generalized anxiolytic effect at a clinical safe dose) improves gain in aerobic capacity and respiratory function during whole-body row training (allowed by NMES) in patients with cSCI (Vivodtzev et al. 2021).

The mechanisms of action of Buspirone are still unknown, however. It may improve **neurotransmission in latent descending pathways** or excitability of **spared motoneurons** by restoring spinal dendritic density (Ganzer et al. 2018). It might also enhance transmission of proprioceptive sensory inputs (Teng et al. 2003). Of note, these effects could be **increased when combined** with muscle stimulation due to common cellular and molecular mechanisms. Of note, NMES, like Buspirone, increases plasmatic levels of brain-derived neurotrophic factor (Miyamoto et al. 2018), well-known to promote **neurogenesis, neuroplasticity, and synaptogenesis** (Muller et al. 2020). Importantly, NMES of locomotor and respiratory muscles can be performed in patients with SCI, even at chronic stage and it may also have unrevealed neuroplastic effect when combined with buspirone. If the mechanisms of action of Buspirone and NMES on neuroplasticity would be confirmed, it would be extremely relevant to use them simultaneously to boost respiratory and locomotor motoneurons regeneration in cSCI.

Hence, buspirone and NMES are safe in humans and their combination may be a promising solution to address respiratory and locomotor dysfunctions in cervical SCI. We developed a mouse model of hemi-contusion at C3 (C3HC) that provides both respiratory (18% lower tidal volume) and locomotor dysfunction (limbs paralysis with 30% loss in gastrocnemius mass). We also developed lower limbs and intercostal NMES training in mouse and found promising preliminary results (Lottri-koffi et al. 2019; Coustillet et al., European Respiratory Society meeting 2021).

Project's objectives and research hypotheses

This project aims to investigate the synergistic effects of Buspirone combined with NMES to improve neuroplasticity and functional recovery in cSCI via changes in 1/ **respiratory capacity** and **locomotor function -Aim #1**, 2/ **neuromuscular response -Aim #2**, 3/ **nerve sprouting and remodeling processes** of the bulbospinal and cortico-/mesencephalic-spinal fibers tracts as well as **motoneuron plasticity - Aim #3,** in our preclinical mouse model of C3HC.

Moreover, one of the most challenging part of medical research is to ensure that benefits found based on animal's data also work in humans. Our research unit is in fact a highly translational unit thanks to its close articulation with the hospital **La Pitié-Salpêtrière.** Clinicians (and among them Pr C. Morelot Panzini) regularly take care of patients who come for a phrenic stimulation implantation check-up. These patients are tetraplegics and dependent on mechanical ventilation (MV). Provided convincing results from the animal studies, we would investigate the same therapeutic approach (daily administration of Buspirone associated with NMES training) in n = 8 patients (SCI above C4 and relying on MV)– **Aim #4** (optional aim for student interested in a translational approach).

Methodology to reach the scientific objectives

Animals: OF-1 mice will be used, in accordance with the European Communities Council Directive (2010/63/UE) for care and experimental procedures and with the French regulations (permission #26668 already obtained). All appropriate means will be used to avoid animal suffering. C3 hemi-contusion (or laminectomy in control group) will be used to mimic traumatic cervical injury in human and to ensure impact on both respiratory and locomotor (hindlimb & forelimb) functions and on muscle physiology and phenotype[25].

Protocol: All animals will be homogeneously assigned to [buspirone + NMES], [buspirone + sham], [placebo + NMES] or [placebo + sham] (see Figure below). After treatment, evaluations will include functional tests, EMG measurements, anterograde/retrograde tracing and tissue histology (brainstem, spinal cord and muscle). In addition, plasmatic and cerebrospinal fluid that will be extracted for protein expression. Subgroups of animals will be used to determine the optimal "therapeutic window". One subgroup will start intervention at W2 (subacute) while the other will start W4 or W6 (chronic).

Intervention: A 2-week intervention will be used since both buspirone and NMES can induce significant changes in respiratory and muscle functions respectively[27,28]. Buspirone (1,5 mg/kg) will be injected intraperitoneally, as previously described[15,21]. In addition, strictly non-invasive NMES of respiratory and locomotor muscles will be performed through cutaneous electrodes under 1.5-2.5% isoflurane for <30 min/day[27]. Plethysmography and muscle strength recordings will be used for optimal intensity of NMES. *Respiratory muscle* (external intercostal and abdominal muscles) will be stimulated based on previous studies[27,29] and under control of thoracic and abdominal piezoelectric sensors.

Measurements and outcomes:

Aim #1: **Respiration and locomotor function**s will be assessed by plethysmography (during spontaneous and challenged ventilation), and 24h-actimetry for spontaneous physical activity in the three conditions: before surgery, after C3HC or laminectomy and after treatment.

Aim #2: **Neuromuscular response** will be assessed by electrophysiological recordings in all groups after treatment.

Aim #3: **Sprouting and remodeling processes** will be assessed via anterograde/retrograde tracing[36], and expression changes of TrkB and Akt in the spinal cord and the brainstem. Motoneuron plasticity will be assessed via expression changes of functional synaptic markers (synaptophysin and homer).

Humans (Optional human study): Tetraplegics patients with a level of injury at C4 or above and relying on 24h-mechanical ventilation (MV).

Aim #4: As a translational approach is anticipated for this project, a student *willing to participate*, could also be involved in this translational aim in which the **same therapeutic approach** (daily administration

of Buspirone associated with NMES training) **will be investigated in n = 8 patients** on 1/ spontaneous ventilation, 2/ diaphragm and intercostal muscle structure as well as 3/ respiratory and locomotor muscles evoked force. This approach would be performed with help (research engineer and Master students) to offer a complementary/translational experience in human research.

Adequation with the UMR_S1158 institute

The present project will be run by Dr Isabelle Vivodtzev (Inserm researcher), who works for 4 years with tetraplegic patients in Boston (Spaulding Rehabilitation Hospital, Haravrd Medical School, Cambridge, MA, US) from 2016-2019 and developed a dedicated division to novel therapeutic strategies in SCI in the research unit UMR_S1158 Inserm, Sorbonne University (Paris, France). She has in-depth expertise on NMES of locomotor muscles and neuroplasticity in tetraplegia and works closely with Prof. Laurence Bodineau and Dr Florence Cayetanot who have an internationally recognized expertise in locomotion and central control of breathing.

This project will be the first to investigate the mechanisms of action of Buspirone on nerve regeneration, excitability and plasticity in cervical SCI. The anticipated results will provide novel strategies of ventilatory recovery by developing approaches of inducible respiratory neuroplasticity in individuals with SCI-induced hypoventilation, in line with the UMR_S1158 unit main focus (https://medecine.sorbonne-universite.fr/research/research-unit/mrsu-1158-relations-between-the-nervous-system-and-the-respiratory-system/?lang=en).

Publications of the thesis supervisor in relation with the project

Neuromuscular electrical stimulation of the respiratory muscles based on breathing modelization in a mouse model of cervical spinal cord injury. Coustillet T**., Cayetanot F., Bodineau L.** and **Vivodtzev** I. ***Eur Respir Journal, 2021***, Abstract of the ERS meeting, Sept 2021

Serotonin 1A agonist and cardiopulmonary improvements with whole-body exercise in acute, high-level spinal cord injury: a retrospective analysis. **Vivodtzev** I, Picard G, O'Connor K, Taylor JA . ***Eur J Appl Physiol.*** **2021,** Feb;121(2):453-463. PMID: 33099664

Ventilatory support during whole-body row training improves oxygen uptake efficiency in patients with high-level spinal cord injury: A pilot study. **Vivodtzev** I, Napolitano A, Picard G, Taylor JA. ***Respir Med*****. 2020**, Sep;171:106104. PMID: 32795903

Mild to Moderate Sleep Apnea is Linked to Hypoxia-induced Motor Recovery After Spinal Cord Injury. **Vivodtzev** I, Tan AQ, Hermann M, Jayaraman A, Stahl V, Rymer WZ, Mitchell GS, Hayes HB, Trumbower RD. ***Am J Respir Crit Care Med*****. 2020**, Sep 15;202(6):887-890, PMID: 32369393

Acute Ventilatory Support During Whole-Body Hybrid Rowing in Patients With High-Level Spinal Cord Injury: A Randomized Controlled Crossover Trial. **Vivodtzev** I, Picard G, Cepeda FX, Taylor JA. ***Chest*****, 2020,** May;157(5):1230-1240, PMID: 31738927

Chronic neuromuscular electrical stimulation improves muscle mass and insulin sensitivity in a mouse model. Lotri-Koffi A, Pauly M, Lemarié E, Godin-Ribuot D, Tamisier R, Pépin JL, **Vivodtzev** I. ***Scientific Report*** **2019**, May 10;9(1):7252, PMID: 31076597

Expected skills of the student

The student will be expected to have or to develop:

- Basics in cell biology, physiology and/or neurophysiology
- Ability to use molecular and cellular biology technologies or eager to learn how to use them
- Ability to carry out scientific monitoring and to synthesize written documents
- Transversal skills: analytical skills, global vision, ethical sense
- Developing skills and qualities such as autonomy, rigor, resilience, patience, creativity, sense of observation, openness, precision and/or ability to come up with new ideas
- Interpersonal skills: curiosity, sense of responsibility, commitment to one's work, capacity of adaptation