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LUMBAR MUSCLE INFLAMMATION ALTERS SPINALLY MEDIATED LOCOMOTOR RECOVERY INDUCED BY TRAINING IN A MOUSE MODEL OF COMPLETE SPINAL CORD INJURY

RENAUD JEFFREY-GAUTHIER, [a,b] **MATHIEU PICHÉ** [b,c] **AND HUGUES LEBLOND** [a,b]*

[a] *Department of Anatomy, Université du Québec à Trois-Rivières (UQTR), 3351 boul. des Forges, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada*

[b] *CogNAC Research Group, Université du Québec à Trois-Rivières, 3351 boul. des Forges, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada*

[c] *Department of Chiropractic, Université du Québec à Trois-Rivières, 3351 boul. des Forges, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada*

Abstract—Locomotor networks after spinal cord injury (SCI) are shaped by training-activated proprioceptive and cutaneous inputs. Nociception from injured tissues may alter these changes but has largely been overlooked. The objective of the present study was to ascertain whether lumbar muscle inflammation hinders locomotion recovery in a mouse model of complete SCI. Lower limb kinematics during treadmill training was assessed before and after complete SCI at T8 (2, 7, 14, 21 and 28 days post-injury). Locomotor recovery was compared in 4 groups of CD1 mice: control spinal mice; spinal mice with daily locomotor training; spinal mice with lumbar muscle inflammation (Complete Freund's Adjuvant (CFA) injection); and spinal mice with locomotor training and CFA. On day 28, H-reflex excitability and its inhibition at high-frequency stimulation (frequency-dependent depression: FDD) were compared between groups, all of which showed locomotor recovery. Recovery was enhanced by training, whereas lumbar muscle inflammation hindered these effects (knee angular excursion and paw drag: p's < 0.05). In addition, lumbar muscle inflammation impaired hind limb coupling during locomotion ($p < 0.05$) throughout recovery. Also, H-reflex disinhibition was prevented by training, with or without CFA injection (p's < 0.05). Altogether, these results indicate that back muscle inflammation modulates spinally mediated locomotor recovery in mice with complete SCI, in part, by reducing adaptive changes induced by training.

Key words: locomotion, nociception, chronic pain, training, H-reflex, central pattern generator.

INTRODUCTION

Neuroplasticity of the spinal cord underlies both adaptive and maladaptive processes after spinal cord injury (SCI). On the one hand, the plasticity of spinal circuits fosters improvement of motor function, including locomotion (Rossignol et al., 2014). On the other hand, it also supports the development of central sensitization (Carlton et al., 2009; Hulsebosch et al., 2009; Redondo-Castro et al., 2013), which may lead to neuropathic pain. By removing the influence of supraspinal structures on descending pathways that regulate locomotion, animal models of complete SCI have provided important knowledge on how spinal networks respond to injury to support locomotor recovery (Frigon and Rossignol, 2006; Rossignol et al., 2014). Based on these findings, recent studies have aimed to develop interventions that promote functional recovery to address extensive functional loss in SCI patients (Dietz and Fouad, 2014). For instance, locomotor training protocols currently included in multimodal interventions are based on experimental evidence from animal models, showing that daily treadmill training improves locomotor re-expression after partial (Barrière et al., 2008; Rossignol et al., 2009; Martinez et al., 2011) or complete SCI (Barbeau and Rossignol, 1987; Rossignol et al., 2014).

In mice, evaluation of hind limb kinematics indicates that treadmill training allows the re-expression of locomotion in 2–3 weeks after complete SCI at T8, in the absence of axonal regrowth through the lesion (Leblond et al., 2003). As demonstrated previously in cats, spinal locomotion in mice is generated by activation of central pattern generators (CPGs) (Meehan et al., 2012). This is consistent with neuroplasticity occurring in spinal locomotor networks, independently of supraspinal structures and descending regulatory pathways.

Acute and chronic pain are frequently associated with SCI. Because they also involve spinal processes and interactions with motor networks, they may alter spinal plasticity (Crown et al., 2002; Joynes et al., 2003; Grau et al., 2014) and hinder the beneficial effects of training (Ferguson et al., 2012). In experiments on spinally transected rats, spinal plasticity allowed withdrawal reflex conditioning, but this was severely altered by nociceptive electrical stimulation (Grau et al., 1998) or peripheral inflammation (Hook et al., 2008). Moreover, nociceptive electrical stimulation impaired functional recovery of locomotion after spinal cord contusion (Garraway et al.,

*Correspondence to, H. Leblond: Department of Anatomy, CogNAC Research Group, Université du Québec à Trois-Rivières, 3351 boul. des Forges, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada.
E-mail address: hugues.leblond@uqtr.ca (H. Leblond).

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2011), indicating that nociception-induced alteration of neuroplasticity may be clinically relevant. Since remnant descending pathways may influence both spinal nociceptive processing and motor control, it is still unclear how nociception affects locomotor spinal networks after complete SCI.

One objective of locomotor training is to prevent the development of spinal disinhibition, which induces spasticity and motor dysfunction (Thilmann et al., 1991). Accordingly, H-reflex frequency-dependent depression (FDD) is preserved in trained SCI rats but not in SCI controls (Côté et al., 2011; Singh et al., 2011; Côté et al., 2014). Moreover, preserved FDD in spinally transected mice is associated with better functional outcomes (Lee et al., 2009). Most relevant to the present study, central sensitization can decrease H-reflex inhibition by FDD due to interactions between dorsal and ventral horn neurons (Lee-Kubli and Calcutt, 2014). Since training and nociception compete to influence spinal plasticity and FDD, we suggest that they may interact and influence functional outcomes during locomotor recovery after complete SCI. To our knowledge, this has never been investigated although it is critical from a rehabilitation perspective.

The aim of the present work was to evaluate the interplay between training and peripheral inflammation on spinally mediated locomotor re-expression after complete SCI in mice. Inflammation was induced by injecting complete Freund's adjuvant (CFA) in sublesional lumbar muscles. Moreover, the impact of training and lumbar muscle inflammation on spinal activity was assessed by comparing H-reflex FDD 28 days after complete SCI. Results indicate opposing effects of training and lumbar muscle inflammation on locomotion. Moreover, training prevented the SCI-induced reflex disinhibition, with or without inflammation.

EXPERIMENTAL PROCEDURES

Animal care and ethics

This experiment was performed on 28 female CD1 mice (body weight 20 g; Charles River Laboratories, Saint-Constant, QC, Canada). Living conditions were strictly controlled by laboratory and facility staff, providing a 12-/12-h light–dark cycle and ambient temperature of 26 °C to temper the impact of immobility on body homeostasis. Upon arrival, the mice were allowed to habituate to the treadmill apparatus (Exer-3/6, Columbus Instruments, Columbus, OH, USA) with 10-min bouts of locomotion every day as well as to the afore-mentioned conditions for 1 week. The animals were housed 5 per cage before spinal cord section. After surgery, they were allowed to recover from anesthesia in individual cages and then returned to their previous housing. The animals were weighed prior to and every day after surgery to ensure comparable general health between groups. Care was taken to optimize animal comfort: the bladder was emptied twice a day as long as it was necessary, and food and water were accessible at all times. In addition to perioperative care, hydration was supported by injection of warm saline (1 ml s.c.) during the first 48 h. All manipulations and procedures were in accordance with Canadian Council on Animal Care guidelines, were previously approved by the UQTR Animal Care Committee, and adhered to directives of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

Surgical procedures

Spinal surgery on mice has been described previously by Leblond and colleagues. Briefly, surgical procedures were performed under isoflurane anesthesia (2% mixed with O_2 95% and CO_2 5%, 200 ml/min) and perioperative nonsteroidal anti-inflammatory drug (carprofen, 10 mg/kg s.c.) and opioid analgesic (buprenorphine, 0.1 mg/kg s.c.) treatment to minimize suffering. The mice were placed on a heating pad to prevent hypothermia. One cm of skin was excised in the rostral part of the bump on the back, the paraspinal muscles were scraped off the spine, and double laminectomy of T8 vertebrae exposed the spinal cord. Xylocaine was applied at the section site to avoid uncontrolled secondary neural damage. The spinal segment was completely lesioned together with the dural sac with micro-scissors. Such a transection produced a large gap with clear disconnection between rostral and caudal stumps. The space was then filled with absorbable hemostat (Surgicel) to avoid excessive bleeding. Muscular and dermal tissues were then sutured in layers, and anesthesia was discontinued. Post mortem inspection confirmed SCI completeness with a clear scar visible on the entire circumference of the spinal cord.

Experimental interventions

Lumbar muscle inflammation. Inflammation was induced in half of the mice (n = 14) by injecting CFA (Sigma F5881, 100 μl of 0.5 mg/ml heat-killed *Mycobacterium tuberculosis* diluted 1:1 in warm saline 0.9%) into the lumbar muscles, which produced chronic inflammatory changes (Chacur et al., 2009; Sandkühler, 2009). Injections were administered 4 days after complete SCI to avoid interactions with anti-inflammatory perioperative treatment. To target the lumbar muscles more efficiently, a small incision was made in the skin on the lower back to expose the muscles of isoflurane-anesthetized mice. Four CFA injections (each 25 μl) were given bilaterally at L1 and L5. The injection needles were secured in place for 10 min after the injection to allow CFA diffusion into the surrounding tissue. The skin was then sutured and anesthesia was discontinued.

Locomotor training. Locomotor training was conducted daily in 13 mice, 6 of which also received bilateral injection of CFA and 7 did not. Training began 2 days after spinal section and consisted of 10-min sessions of hind limb walking at 12 m/min on a motor-driven treadmill while the forelimbs were lying on a platform. The experimenter provided weight support and balance by holding the animal by the base of the tail,