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Détermination des facteurs bénéfiques et néfastes à la récupération locomotrice à la suite d'une section spinale complète chez la souris.

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Résumé

Différents modèles animaux ont permis de déterminer le rôle des réseaux locomoteurs spinaux dans la production de la locomotion à la suite d'une lésion de la moelle épinière (LM pour lésion médullaire). De plus, il a été démontré que des interventions ciblant leur activation améliorent la récupération en favorisant leur adaptation fonctionnelle et structurale par des mécanismes plastiques tels que ceux impliqués dans l'apprentissage et la mémoire. L'entraînement locomoteur permet ainsi d'améliorer progressivement la récupération locomotrice médiée par les réseaux locomoteurs spinaux, incluant lors de section complète de la moelle épinière. En plus de l'entraînement, une multitude de facteurs cliniques, des processus associés à la blessure elle-même ou à son traitement, sont susceptibles d'influencer l'activation des réseaux spinaux ou leur potentiel d'adaptation plastique. Afin d'optimiser la récupération fonctionnelle, l'impact de ces facteurs et les mécanismes impliqués doivent être clarifiés. L'objectif de cette thèse est d'évaluer l'influence de l'entraînement sur la récupération fonctionnelle dans deux conditions de réadaptation spécifiques : 1) lors de présence d'inflammation musculo-squelettique lié à l'étiologie traumatique de la LM et 2) lors de traitement combinant entraînement et buspirone, un agoniste sérotoninergique en étude préclinique pour ses effets pro-locomoteurs.

La première condition à l'étude, la présence d'inflammation musculo-squelettique, est une comorbidité fréquente de la LM et est associée à un pire pronostic qu'en absence d'inflammation. Considérant le rôle des réseaux spinaux dans le traitement de l'information sensorielle et nociceptive et de leur contribution à la régulation de la locomotion générée par les circuits spinaux, leur influence sur la récupération locomotrice devait être définit. En utilisant un modèle de souris avec une section mi-thoracique complète, nous avons évalué l'impact d'une réaction inflammatoire persistante par injection d'adjuvant de Freund (CFA) dans les muscles lombaires sous-lésionnels sur le rétablissement du rythme et patron locomoteur, en présence ou non d'entraînement (étude 1). Nos résultats montrent que l'inflammation des muscles lombaires perturbe la récupération locomotrice. Les afférences sensorielles jouent un rôle important dans le rétablissement de la locomotion en modulant l'activité du CPG et en influençant l'excitabilité de circuits réflexes impliqués dans la locomotion. En évaluant les changements d'excitabilité

du réflexe de Hoffmann associés à l'injection de CFA à la suite d'une section, nous avons observé que l'inflammation des muscles lombaires induit un état de désinhibition spinale dans les premiers jours post-injection (étude 2). Toutefois, cet effet ne perdurait pas pour toute la durée de la réponse neuroinflammatoire tel qu'évaluée par l'activation de la microglie dans la moelle épinière lombaire (étude 3) ce qui suggère que les déficits locomoteurs associés au CFA s'expliquent au moins partiellement par un mécanisme indépendant de la désinhibition spinale.

La deuxième condition à l'étude, la combinaison d'un traitement à la buspirone au protocole d'entraînement, présente un réel potentiel thérapeutique selon un essai préclinique récent. Toutefois, son influence sur les réseaux locomoteurs spinaux est inconnue, de même que l'influence de l'utilisation de la buspirone sur l'adaptation des réseaux spinaux par l'entraînement. En utilisant notre modèle de souris ayant une section spinale complète, nous avons montré que la buspirone facilite l'activation des réseaux locomoteurs spinaux (étude 4). Cependant, cette facilitation était associée à des changements limités de la récupération médiée par l'entraînement. En comparant l'impact de la buspirone chez des animaux aux statuts fonctionnels différents (récupération symétrique partielle et récupération asymétrique) causés par un paradigme de lésion distinct (section complète et hémisection), des changements dans la récupération médiée par l'entraînement ont été observés, ce qui suggère que l'impact positif de la buspirone sur la récupération locomotrice est causé, au moins en partie, par la favorisation d'adaptations plastiques.

En conclusion, cette thèse décrit deux conditions qui modifient de façon opposée la récupération locomotrice régulée par les réseaux locomoteurs spinaux. Ces découvertes renforcent le concept que les réseaux locomoteurs spinaux sont plastiques et jouent un rôle primordial dans la récupération locomotrice et ajoutent de nouvelles connaissances sur les facteurs qui en influencent la réadaptation.

Mots-clés : moelle épinière, section, hémisection, locomotion, plasticité, générateur de patron central, sérotonine, inflammation, réflexe de Hoffmann, dépression post-activation, désinhibition, afférences sensorielles, nociception

Abstract

Locomotor spinal networks contribution to locomotor recovery has been described in various animal models of spinal cord injury (SCI). It is now assumed that these networks are plastic and will re-express, to some extent, locomotion by hosting activity-dependent adaptation similar to those observed in motor learning and memory. Thus, locomotor training promotes behavioral recovery mediated by locomotor spinal networks after a SCI, including after a complete transection and disconnection from the brain. In addition to training, various factors can exert beneficial or detrimental influence on locomotor spinal networks activity and plasticity, including factors associated with the traumatic origin of spinal injury itself or its pharmacological treatment. In order to direct plasticity toward adaptation and promote recovery, the influence of such factors must be clarified. The objectives of the present thesis is to determine the contribution of training to recovery in two specific conditions of rehabilitation: 1) in presence of musculoskeletal inflammation related to the traumatic etiology of SCI and 2) in combination with a pharmacological intervention, administration of the 5HT agonist buspirone, used in preclinical studies to facilitate locomotor activity.

The first condition, the presence of musculoskeletal inflammation, is frequently observed in patients after a SCI and is associated with poor functional recovery. Considering the role of spinal networks in sensory processing and the selective recruitment by non-nociceptive and nociceptive afferents of reflex pathways implicated in locomotion, the impact of inflammation on locomotor recovery must be determined. In a model of complete transection in mice, we evaluate the impact of lumbar muscle inflammation induced by intramuscular complete Freund adjuvant (CFA) injection on locomotor recovery mediated by spinal networks, with or without locomotor training. Our results show that lumbar muscle inflammation hindered locomotor recovery (study 1). In a second experiment, we examined if CFA injection changed sensory transmission to spinal cord by measuring the evolution of Hoffmann reflex frequency-dependent depression, which is progressively attenuated after spinal transection. We found that lumbar muscle inflammation induced a short term suppression of the Hoffmann reflex frequency-dependent depression, thereby altering spinal excitability (study 2). However, this short term change in excitability did not match the temporal course of locomotor deficits and

central neuroinflammatory response as measured with the enhanced presence of microglia in spinal cord, suggesting that inflammation-induced locomotor deficits rely at least partially on mechanism independent of spinal excitability (study 3).

The second condition under investigation, the use of buspirone to enhance locomotor recovery after a SCI, shows great therapeutic potential. Results from a preclinical trial on SCI patients show that it can enhance locomotor activity, but it is not known if it does so by reactivating dormant descending pathways or locomotor spinal networks. Using our model of complete transection in mice, we showed that buspirone strongly activates locomotor spinal networks and allow near full expression of locomotion on day 2 post-transection (study 4). However, this facilitation was associated with limited long term effect when combined with training. Improved long term effect of buspirone depending on residual function after transection (partial vs absent function) caused by different lesion paradigms (hemisection preceding transection vs transection only) suggests that buspirone promotes recovery by enhancing use-dependent plastic changes.

In conclusion, this thesis describes two conditions that influence locomotor recovery mediated by spinal networks oppositely. These findings give new insights on the role of locomotor spinal networks plasticity in recovery after a SCI including spinal transection and provide evidence both beneficial and detrimental factors contributes to functional rehabilitation.

Keywords : spinal cord, transection, hemisection, locomotion, plasticity, central pattern generator, serotonin, inflammation, neuromodulation, Hoffmann reflex, frequency-dependent depression, disinhibition, sensory afferents, nociception

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IaIN	Interneurone Ia
5-HT	Sérotonine
8-OH-DPAT	*8-hydroxy-2 (di-n-propylamino) tetralin*
ANOVA	Analyse de variance
ATP	Adénosine triphosphate
BDNF	*Brain-derived neurotrophic factor*
BK	Bradykinine
BSA	*Bovine serum albumin*
CCL-21	*CC chemokine ligand-21*
CFA	*Complete Freund's adjuvant*
ChAT	*Choline acetyl transferase*
CGRP	*Calcitonin gene-related peptide*
CLR	*Cerebellar locomotor region*
CRSNG	Conseil canadien de recherche en sciences naturelles et génie
DA	Dopamine
DPH	*Days post-hemisection*
DPS	*Days post-spinalization*
EMG	Électromyogramme
EPSC	*Excitatory post-synaptic current*
FCQ	Fondation chiropratique du Québec
FDD	*Frequency-dependent depression*
FITC	*Fluorescein isothiocyanate*
FRA	*Flexion reflex afferent*
FREQ	Fréquence
FRQS	Fonds de recherche du Québec - Santé
GABA	*Gamma-aminobutyrique acid*
Hmax	Amplitude maximale du réflexe de Hoffmann
HS	*Hemisected spinal mice*
HSB	*Hemisected spinal mice with buspirone*

Hslp	Pente de recrutement du réflexe de Hoffmann
KCl	Chlorure de potassium
IASP	*International association for the study of pain*
IL-1β	Interleukine type 1β
IL-6	Interleukine type 6
I.M.	Intramusculaire
I.P.	Intrapéritonéal
KCC2	*Potassium cation cotransporter type 2*
L-DOPA	L-3,4-dihydroxyphénylalanine
LFL	*Left fore limb*
LHL	*Left hind limb*
LM	Lésion médullaire
m-CPP	*m*-chlorophenylpiperazine
MLR	*Mesencephalic locomotor region*
Mmax	Amplitude maximale de l'onde M
Mslp	Pente de recrutement de l'onde M
MN	Motoneurone
MT	*Motor threshold*
MTP	Articulation métatarsophalangienne
NA	Noradrénaline
$Na_v1.8$	*Voltage-gated sodium channel subtype 1.8*
NGF	*Nerve growth factor*
NMDA	Acide N-méthyl-D-aspartique
NS	Nociceptif spécifique
NSERC	*Natural science and engineering research council of Canada*
PAD	*Primary afferent depolarization*
PBS	*Phosphate-buffered saline*
PCA	*Principal component analysis*
PF	*Pattern formation*
PFA	Paraformaldéhyde
PGE_2	Prostaglandine E_2

PPR	*Parapyramidal region*
Réflexe-H	Réflexe de Hoffmann
RFL	*Right fore limb*
RG	*Rhythm generator*
RHL	*Right hind limb*
ROI	*Region of interest*
RT	*Room temperature*
S	*Spinal mice*
SB	*Spinal mice with buspirone*
SCI	*Spinal cord injury*
SCL	*Spinal cord lesion*
SEM	*Standard error of mean*
SLR	*Subthalamic locomotor region*
SNC	Système nerveux central
SNP	Système nerveux périphérique
TNF-α	*Tumor necrosis factor α*
TRPA1	*Transient receptor potential ankyrin type 1*
TRPV1	*Transient receptor potential vanilloid type 1*
TTX	Tétrodotoxine
UQTR	Université du Québec à Trois-Rivières
VIP	*Vasoactive intestinal peptide*
WDR	*Wide-dynamic range*
AHP	*Afterhyperpolarization*

Liste des abréviations

g	Gramme
mm	Millimètre
ms	Milliseconde
uL	Microlitre

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Chapitre I : Introduction et contexte théorique

1.1 Introduction générale aux lésions de la moelle épinière

Au Canada chaque année, 1389 nouvelles personnes (41/million) subiront une lésion de la moelle épinière (LM pour lésion médullaire) d'étiologie traumatique (Sekhon and Fehlings, 2001, Noonan et al., 2012). Les déficits fonctionnels comprennent des troubles moteurs (paraplégie/tétraplégie), sensoriels (dysesthésie/douleur neuropathique) et autonomiques (incontinence/perte de fonction sexuelle/dysréflexie autonomique) résultant de la perte de connectivité entre les différents réseaux neuronaux supra- et sous-lésionnels. Parmi les différents objectifs de la réadaptation, la récupération de la locomotion est l'une des priorités chez les patients afin de regagner leur autonomie (Anderson, 2004).

La pathophysiologie de la LM comprend un mécanisme de blessure primaire qui endommage directement les axones de neurones composant la moelle épinière (Rowland et al., 2008). De plus, des dommages à la microvasculature et l'altération de l'intégrité de la barrière hématoencéphalique perturbent la perfusion des tissus et causent des débalancements énergétiques, ioniques et glutamatergiques (Kwon et al., 2004). Cette première lésion est suivie par une réaction neurotoxique secondaire. Celle-ci amplifie les dommages tissulaires, notamment en aggravant la démyélinisation d'axones voisins, et interrompt davantage la connectivité entre les structures supraspinales, spinales et le système nerveux périphérique (SNP). La formation d'une cicatrice gliale et sa consolidation durant les 2 premières années réduisent le potentiel de récupération (Figley et al., 2011).

La réadaptation des patients ayant une LM vise à favoriser la survenue de changements plastiques adaptatifs sous-tendant la récupération fonctionnelle tout en minimisant la survenue de changements plastiques mésadaptatifs associés à la perte de connectivité caractéristique de la LM. En ce sens, beaucoup d'attention scientifique a été portée sur l'importance de l'expérience sensorimotrice, notamment par la thérapie basée sur l'activité (entraînement), dans le but de diriger la plasticité vers la récupération fonctionnelle. Bien que les changements plastiques associés à la LM soient largement distribués, les réseaux spinaux constituent un système idéal afin d'étudier l'impact de changements plastiques sur la fonction locomotrice par :

1) leur capacité à générer la locomotion de façon autonome et 2) leur susceptibilité à être modulé par diverses formes d'expériences sensorimotrices dont l'entraînement.

Plusieurs facteurs bénéfiques et néfastes influencent la capacité des réseaux locomoteurs spinaux de générer la locomotion. Certains sont bien connus, notamment l'influence du système neuromodulateur monoaminergique (sérotonine, noradrénaline, dopamine) sur la régulation de l'activité locomotrice. Des avancées dans les 50 dernières années sur l'utilisation de pharmacothérapie neuromodulatrice suggèrent que cette intervention amène les réseaux locomoteurs spinaux dans un état permissif à la génération de la locomotion (Barbeau and Rossignol, 1994). Récemment, l'agoniste des récepteurs 5-HT_{1A} buspirone a été utilisé lors d'essai clinique (Gerasimenko et al., 2015, Freyvert et al., 2018). Malgré le rôle de ces récepteurs dans la régulation de la locomotion, la capacité de la buspirone de déclencher à elle seule la locomotion ou d'améliorer sa récupération à la suite d'une LM reste à être démontrée.

D'autres facteurs sont moins compris, notamment l'influence des comorbidités qui compliquent fréquemment les cas cliniques de LM, comme les lésions musculosquelettiques (Johnson et al., 1998). Il a été suggéré que la présence de lésions musculosquelettiques due au mécanisme de blessure (trauma) ou à ses conséquences (utilisation d'une chaise roulante) réduisait le potentiel de récupération fonctionnelle (Kalpakjian et al., 2007, Dvorak et al., 2017). Les changements centraux associés à l'activité nociceptive et à l'inflammation pourraient expliquer un tel résultat. De récentes évidences expérimentales suggèrent qu'ils diminuent le potentiel de changements plastiques des réseaux de la moelle épinière (Grau et al., 2014). Leur impact dans la régulation d'activité locomotrice spinale n'a toutefois pas été démontré. Considérant que les changements plastiques sont essentiels à la récupération locomotrice à la suite d'une LM, l'impact d'inflammation musculosquelettique doit être clarifié.

En étudiant la réexpression de la locomotion dans un modèle de section spinale complète chez la souris, l'objectif général de cette thèse est de déterminer des facteurs bénéfiques (étude 4) et néfastes (études 1-3) à la récupération de la marche à la suite d'une LM.

1.2 Rôle des réseaux locomoteurs spinaux dans la locomotion

1.2.1 Générateur de patron central

À la suite d'une lésion spinale, la perte de connectivité entre les centres supraspinaux responsables de la locomotion et les réseaux locomoteurs spinaux engendre une perturbation de l'activité neuronale sous-lésionnelle. Celle-ci est observable par une paralysie flaccide et l'absence totale de mouvement dans les premiers jours suivants la LM. La récupération de la locomotion est cependant possible, et ce même dans les cas de section spinale complète survenant à l'âge adulte dans différents modèles animaux comme le chat (Barbeau and Rossignol, 1987), le rat (Alluin et al., 2015) et la souris (Leblond et al., 2003). À la condition que certains éléments soient fournis (ex. : maintien de l'équilibre), le comportement locomoteur de l'animal spinal est composé des mêmes phases de support et de balancement en alternance réciproque avec le membre controlatéral que lors de locomotion chez l'animal intact. Cette capacité locomotrice résiduelle démontre le rôle central joué par les réseaux locomoteurs spinaux, composés d'un générateur de patron central (CPG pour *central pattern generator*), d'interneurones de voies réflexes et de motoneurones, dans la génération de la locomotion.

Chez tous les vertébrés, des poissons aux primates, le réseau neuronal responsable de la locomotion de base est le CPG, situé dans la moelle épinière. Les CPGs sont des réseaux dont la fonction est de générer les signaux de délais, de phase et d'intensité aux motoneurones impliqués dans la production d'activité rythmique comme la locomotion, la mastication et la respiration (Marder and Bucher, 2001, Harris-Warrick, 2011).

> Dans le présent ouvrage, le terme CPG réfère exclusivement au CPG locomoteur.

La capacité intrinsèque du CPG à s'activer en absence d'influence des centres supra-spinaux et des inputs sensoriels a été suggérée dès 1912 par Thomas Graham Brown à la suite d'un enregistrement d'activité spinale rythmique chez des chats décérébrés, c.-à-d. dont la portion du système nerveux central (SNC) rostrale au plan passant par les corps mamillaires et collicules supérieurs a été retirée, et désafférentés (Stuart and Hultborn, 2008). Il élabora sa théorie du demi-centre (*half-center theory*) selon laquelle l'activité rythmique de la locomotion est générée en alternance par un mécanisme médié par l'inhibition réciproque de deux groupes

de neurones aux actions opposées (Jones et al., 2011). Le modèle de Brown n'exclut toutefois pas l'influence de certaines structures supra-spinales. Il a été observé plus récemment que l'intégrité de certaines régions du tronc cérébral, dont la région locomotrice mésencéphalique (MLR; Shik et al., 1969), la région locomotrice sous-thalamique (SLR), la région locomotrice pontine (PLR) et la région locomotrice cérébelleuse (CLR; Mori et al., 1999) est nécessaire pour observer des épisodes spontanés de locomotion chez le chat décérébré. Il en est de même chez le rat (Skinner and Garcia-Rill, 1984) et la souris (Stecina, 2017). Ces régions sont nécessaires, entre autres, à l'initiation de la locomotion par l'activation de la voie réticulo-spinale, par l'action de neuromodulateurs sur le CPG (Schmidt and Jordan, 2000) et à l'intégration d'information sensorielle pour le maintien de l'équilibre (Morton and Bastian, 2004). Ils ne sont toutefois pas essentiels à la génération du patron locomoteur basal.

En plus de préserver la majorité des circuits impliqués dans la locomotion, la décérébration mamillo-colliculaire permet d'évaluer l'activité spinale sans influence d'agents anesthésiques. Pour cette raison, toutes les mesures d'excitabilité de réflexes spinaux effectuées dans le cours de cette thèse ont été colligées chez l'animal décérébré non anesthésié.

En absence totale de commande supra-spinale comme lors de section spinale complète, le CPG peut générer de façon autonome le patron locomoteur, spécialement chez les nouveau-nés. Chez le chaton (1-2 semaine post-naissance) ayant une section complète à T12 et placé sur un tapis roulant, ce patron possède globalement les caractéristiques de la locomotion décérébrée (Grillner, 1973). Notamment, les coordinations intra-membre et inter-membre sont préservées, de même que l'adaptation de la durée de la phase de support en fonction de la vitesse du tapis roulant (Forssberg et al., 1980a, Forssberg et al., 1980b). Des résultats similaires ont été observés chez le rat (Stelzner et al., 1975) et l'opossum (Saunders et al., 1998, Wheaton et al., 2011) opérés durant la période néonatale. La découverte de la conservation de la fonction locomotrice chez des animaux ayant une section spinale complète quelques jours après la naissance, soit avant l'apprentissage de la marche, démontre que la locomotion résulte de l'expression de circuits spinaux génétiquement déterminés.

1.2.2 Composition du CPG

Malgré plus de cent ans de recherche sur le CPG, l'organisation neuronale de celui-ci reste en grande partie incomprise. Portés par le développement d'outils génétiques chez la souris et de modèles mathématiques, de récents travaux expérimentaux et computationnels suggèrent une organisation hiérarchique des modules responsables des caractéristiques du patron locomoteur. Les évidences récentes provenant de plusieurs laboratoires convergent vers un modèle comprenant des modules dépendant de la vitesse de locomotion (McLean and Dougherty, 2015) et organisés hiérarchiquement en deux niveaux : le premier niveau comprend un réseau de neurones largement distribué sur plusieurs segments spinaux et qui fournit le rythme basal aux neurones de deuxième niveau qui, eux, raffinent le patron d'activation avant de transmettre la commande finale aux motoneurones (Rybak et al., 2006, McCrea and Rybak, 2008, Kiehn, 2011). Les neurones de premier niveau constituent le réseau RG (pour *rhythm generator*) : ils agissent comme l'« horloge » du CPG et détermine l'output rythmique du système. Ceux-ci répondent à certains critères parmi les suivants (Brownstone and Wilson, 2008, Brownstone and Bui, 2010), ils sont : 1) principalement glutamatergiques (Lundberg, 1981), 2) distribués dans la région ventromédiale (Kjaerulff and Kiehn, 1996) de la moelle épinière thoracolombaire (Cazalets et al., 1995, Cowley and Schmidt, 1997, Marcoux and Rossignol, 2000), 3) reçoivent des inputs réticulo-spinaux (Noga et al., 2003) et sérotoninergiques descendants (Liu and Jordan, 2005), 4) reçoivent des afférences Ib (Conway et al., 1987) et II (Lundberg et al., 1987, Perreault et al., 1995) mis en évidence par la capacité de ces afférences à réinitialiser le rythme, 5) capable d'excitation mutuelle ou récurrente (Roberts and Tunstall, 1990, Rowat and Selverston, 1997), 6) possèdent des propriétés intrinsèques (ex : potentiels de plateau) qui contribuent à la génération de patron d'activité rythmique (Russell and Hartline, 1978, Kiehn et al., 1996) et 7) présentent une telle activité rythmique lors d'épisodes locomoteurs spontanés ou déclenchés, incluant lors de locomotion fictive (Jiang et al., 1999, Kwan et al., 2009). Les neurones de deuxième niveau constituent le réseau PF (pour *pattern formation*) : ils reçoivent la commande rythmique des RG et coordonnent les délais de phase et l'intensité de l'activité des différentes populations de motoneurones, façonnant ainsi l'alternance de flexion et d'extension et de mouvements droit et gauche. Ces neurones sont distribués dans toute la moelle épinière lombaire, mais plus

abondamment au segment L4 chez la souris avec des groupes distincts situés dans les laminae V-VII (projettent principalement vers les motoneurones ipsilatéraux) et VIII (projettent également vers les motoneurones controlatéraux; Coulon et al., 2011).

Différentes classes d'interneurones contribuant aux réseaux RG et PF ont été identifiées électrophysiologiquement chez le chat ou, plus récemment, par une approche génétique chez la souris. Ces interneurones, dont une majorité sont ipsilatéraux, sont distribués dans tout le renflement lombaire. Quatre groupes d'interneurones spinaux (V0, V1, V2, V3) et certains interneurones non classifiés, mais exprimant les facteurs de transcription Hb9 (Caldeira et al., 2017) ou Shox2 (Dougherty et al., 2013) répondent présentement à ces critères. En étudiant les caractéristiques de délétions spontanées dans l'activité motoneuronale durant un épisode de locomotion fictive (c-a-d, une absence d'une ou plusieurs bouffées d'activité EMG dans un cycle), il a été observé qu'elles peuvent être causées par un manquement dans l'activité du réseau RG ou PF, selon qu'elles réinitialisent (*resetting deletions*) ou non (*non-resetting deletions*) l'activité locomotrice (Lafreniere-Roula and McCrea, 2005). Deux classes d'interneurones du type V2a, tous deux projetant ipsilatéralement et impliqués dans la locomotion (Dougherty and Kiehn, 2010, Zhong et al., 2010), se distinguent par leur comportement respectivement oscillatoire et silencieux durant ces délétions (Zhong et al., 2012). L'ablation génétique de ces interneurones V2a a mené à une perte d'adaptation du rythme locomoteur en fonction de la vitesse chez des souris $V2a^{-/-}$(Crone et al., 2009). Une portion des interneurones V2a expriment également le facteur de transcription Shox2 ou Chx10 et projettent vers des interneurones commissuraux (Dougherty et al., 2013), suggérant qu'ils contribuent à uniformiser le rythme entre les modules contrôlant les pattes droite et gauche (Crone et al., 2008). Ces résultats suggèrent que chacune des classes d'interneurones V2a contribue spécifiquement soit au réseau RG ou au réseau PF. Les interneurones V1 constituent un autre groupe d'interneurones projetant ipsilatéralement dont l'ablation diminue la fréquence de bouffée d'activité locomotrice (Gosgnach et al., 2006). L'ablation des V1 combinée à celle d'interneurones V2b altère également la coordination entre fléchisseurs et extenseurs (Zhang et al., 2014), suggérant qu'ils participent également à la formation du patron d'activité locomotrice.

Des interneurones commissuraux (V0 et V3) contribuant à la locomotion ont été identifiés expérimentalement. Les interneurones V0 se divisent en 2 populations organisées ventro-dorsalement, les V0v (ventral, excitateur) et V0d (dorsal, inhibiteur). Ces interneurones sont respectivement activés à moyenne et basse fréquence d'activité rythmique locomotrice (Zagoraiou et al., 2009) et contribuent à la coordination droite-gauche à ces vitesses (Talpalar et al., 2013). Les interneurones V3 présentent également une activité rythmique et une organisation en deux sous-populations aux propriétés distinctes (Borowska et al., 2013), mais leur rôle dans la locomotion demeure peu connu. Des résultats non publiés suggèrent qu'ils sont impliqués dans le contrôle de la vitesse (Bellardita et al., 2018). Ces évidences renforcent la théorie de l'organisation modulaire du CPG chez les mammifères. De plus, elles suggèrent que le rythme et les différences d'ajustement des phases d'extension et de flexion et l'alternance droite/gauche, bien qu'influencés par le feedback sensoriel, sont médiés centralement (Shevtsova et al., 2015).

En somme, le CPG possède, par un réseau hiérarchique d'interneurones activés de façon rythmique et dont l'inhibition réciproque et les propriétés intrinsèques façonnent l'alternance de phases de flexion et d'extension ainsi que l'alternance droite-gauche, l'organisation nécessaire pour réexprimer la locomotion à la suite d'une LM.

Cette réexpression médiée par le CPG en contexte de section complète constitue un modèle expérimental unique pour déterminer le rôle de différentes interventions pour favoriser la récupération locomotrice. Tel que décrit dans la prochaine section, ce modèle a permis d'étudier l'influence de l'entraînement sur tapis roulant sur la récupération ainsi que le rôle des afférences sensorielles dans celle-ci. Dans le cadre de cette thèse, il nous a permis de déterminer la contribution de facteurs contextuels, bénéfiques ou néfastes, à la récupération locomotrice.

1.2.3 Contribution des afférences sensorielles à l'activité du CPG

La section précédente a permis de décrire comment le CPG peut générer l'activité locomotrice en absence d'inputs supraspinaux et sensoriels. Évidemment, cela ne signifie pas que les afférences sensorielles ne contribuent pas considérablement à réguler la commande finale transmise aux motoneurones lors de la locomotion. Les afférences situées dans les fuseaux

musculaires (Ia, II), dans les organes tendineux de Golgi (Ib), dans les articulations et dans la peau peuvent tous influencer l'output locomoteur en modulant l'activité 1) du CPG, 2) d'interneurones de voies réflexes impliqués dans la locomotion, 3) des motoneurones ou 4) d'autres afférences. Dépendamment des circuits communs utilisés par ces afférences et le CPG, leur activation peut entraîner l'activité rythmique du CPG et ajuster les délais de phase (ex : stimulation des afférences articulaires et musculaire du groupe Ib et II; Gossard et al., 1994, Schomburg et al., 1998) alors que d'autres permettent d'ajuster la fluidité et coordination entre flexion et extension (ex : stimulation des afférences Ia et II; Edgley et al., 1988, Hiebert et al., 1996, Akay et al., 2014). Des études récentes sur l'origine embryologique des interneurones des voies réflexes les associent aux interneurones V0-V3 dont la présence dans le CPG a été décrite dans la section précédente, suggérant qu'ils font part de l'organisation des réseaux locomoteurs spinaux. Alors qu'une majorité d'interneurones impliqués dans la locomotion est localisée dans la zone intermédiaire et ventro-médiale de la moelle épinière, peu d'évidence expérimentale supporte la contribution d'interneurones de la corne dorsale dans l'activité basale du CPG. Cependant, un groupe d'interneurones GABAergiques situé dans les laminae V-VI médiales et possédant une activité rythmique lors de locomotion fictive a récemment été identifié (Wilson et al., 2010). Leur profil de réponse électrophysiologique et en imagerie calcique à la suite d'une stimulation électrique des racines dorsales suggère qu'ils agissent comme intermédiaire unique dans une voie disynaptique entre les afférences sensorielles et les motoneurones et participent aux mécanismes d'inhibition réciproque impliqués dans la locomotion. De nombreux autres interneurones assurant une transmission disynaptique de l'information sensorielle vers les motoneurones impliquée dans le contrôle moteur ont récemment été identifiés (Bui et al., 2015). Ces résultats soutiennent le rôle important que jouent les afférences sensorielles dans la régulation de l'activité locomotrice.

La démonstration la plus claire du rôle des afférences sensorielles sur l'activité du CPG est l'ajustement de la durée des phases de flexion et d'extension et de mouvements droit et gauche et des bouffées EMGs des muscles dans leur phase d'activation respective en fonction de la vitesse du tapis roulant chez l'animal avec une section spinale complète (Forssberg et al., 1980a, Forssberg et al., 1980b, Barbeau and Rossignol, 1987). Ce phénomène est dû à l'activation d'afférences sensorielles lors du mouvement généré par la locomotion. Il est

également possible de révéler le rôle des afférences sensorielles en modifiant leur apport au patron locomoteur. En appliquant de l'assistance ou de la résistance à la flexion de la hanche lors de la phase de balancement lors de locomotion sur tapis roulant chez le chat décérébré, il a été observé que l'activité EMG des muscles fléchisseurs ilio-psoas et sartorius était diminuée et augmentée, respectivement (Lam and Pearson, 2001). Ce résultat indique que l'activité de muscles fléchisseurs est modulée par les afférences proprioceptives.

1.2.3.1 Afférences Ia

Une des populations inteneuronales les mieux décrites chez les mammifères consiste en un interneurone responsable de l'inhibition réciproque entre l'activité des motoneurones fléchisseurs et extenseurs (Figure 1.1A). Ces interneurones glycinergiques, identifiés interneurones Ia, sont situés dans la laminae VII et dérivés embryologiquement des interneurones V1 (Pratt and Jordan, 1987, Alvarez et al., 2005). Ils sont directement activés par les afférences Ia et projettent leurs axones vers les motoneurones (Eccles and Lundberg, 1958) et interneurone Ia (Hultborn et al., 1976a) antagonistes et inhibent leurs activités. La suppression de leur activité par l'antagoniste glycinergique strychnine ou par ablation génétique mène à la coactivation des motoneurones fléchisseurs et extenseurs (Cowley and Schmidt, 1995, Akay et al., 2014), ce qui suggère qu'ils contribuent à la coordination intra-membre. En plus des afférences primaires Ia, les IaINs reçoivent des inputs excitateurs provenant des afférences cutanées de bas seuil ipsilatérales (Rossi and Mazzocchio, 1988) ainsi que des afférences cutanées, articulaires et musculaires de haut seuil bilatérales (Jankowska et al., 1967) et des inputs inhibiteurs des cellules de Renshaw ipsilatérales (Hultborn et al., 1971).

Les afférences du groupe Ia contribuent au patron locomoteur en transmettant centralement l'information sur la variation de longueur du muscle par l'activation de récepteurs contenus dans le fuseau musculaire. Les fibres Ia projettent directement aux motoneurones et génèrent le réflexe d'étirement ou son analogue recruté par stimulation électrique, le réflexe de Hoffmann (réflexe-H, voir Section Considérations méthodologiques pour plus d'informations). Lors d'activité locomotrice, l'amplitude du réflexe-H du muscle soléaire est fortement modulée en fonction des phases locomotrices (Akazawa et al., 1982, Duenas and Rudomin, 1988, Stein

and Capaday, 1988). L'amplitude du réflexe-H est augmentée en phase d'extension, puis réduite en phase de flexion (Bennett et al., 1996). Bien que des mécanismes supra-spinaux (Chan et al., 1986) et périphériques (Bennett et al., 1996) soient impliqués, des mécanismes spinaux, incluant la modulation de l'inhibition présynaptique des fibres Ia (Duenas and Rudomin, 1988, Ménard et al., 1999), jouent un rôle primordial. L'inhibition présynaptique est un phénomène répandu dans le SNC et on l'observe également au niveau des afférences primaires. Dans ce cas précis, l'inhibition est causée par la dépolarisation de l'afférence primaire (PAD pour *primary afferent depolarization*) par l'activation d'une voie GABAergique disynaptique qui diminue la transmission du potentiel d'action et la relâche de neurotransmetteurs à la synapse Ia-motoneurone (Eccles et al., 1961, Andersen et al., 1962, Willis, 2006). L'information sensorielle est donc modifiée avant d'atteindre sa cible neuronale (ex.: le motoneurone) afin d'optimiser la discrimination sensorielle et la performance motrice (Fink et al., 2014). L'activité de cette voie GABAergique disynaptique est modulée lors de la locomotion par l'activation des afférences sensorielles produit par le mouvement (Gossard, 1996) et par l'activation directe des neurones du CPG (Gossard and Rossignol, 1990, Ménard et al., 1999). La dégradation de la coordination motrice après l'ablation génétique des interneurones de cette voie disynaptique chez la souris suggère que l'inhibition présynaptique est impliquée dans la fluidité des mouvements, notamment en diminuant le gain du feedback proprioceptif (Fink et al., 2014). L'ensemble de ces mécanismes impliquant les fibres Ia, inhibition présynaptique et inhibition par les IaINs, permettrait de stabiliser les segments durant la phase d'extension, tout en facilitant le mouvement locomoteur en phase de flexion.

1.2.3.2 Autres afférences

Une autre population d'interneurones spinaux modulée par les afférences Ib et II a également été abondamment étudiée. Ces interneurones, identifiés interneurones I/II, sont influencés par les deux types d'afférences, indiquant que ceux-ci renforcent mutuellement leur influence sur les motoneurones (Jankowska et al., 2009, Jankowska and Edgley, 2010). Au repos, ces interneurones divisés en deux classes modulent l'excitabilité des motoneurones antagonistes et agonistes par une transmission soit excitatrice ou inhibitrice non-réciproque,

respectivement (Figure 1.1B) (Edgley and Jankowska, 1987, Cabaj et al., 2006, Jankowska and Edgley, 2010). Durant la locomotion, ces interneurones sont activés en phase avec le rythme et le patron locomoteur (Shefchyk et al., 1990, Gossard et al., 1994, Angel et al., 2005), mais leur influence sur l'excitabilité des motoneurones est renversée (Pearson and Collins, 1993, Whelan et al., 1995). Ils excitent les motoneurones des muscles extenseurs spécifiquement lors de locomotion (Pearson and Collins, 1993) et inhibent l'activité des motoneurones fléchisseurs ipsilatéraux (Duysens and Pearson, 1980), prévenant ainsi la génération d'une phase de flexion. Chez les quadrupèdes, la diminution de la charge des muscles extenseurs lorsque la patte se trouve postérieure à la hanche permet de réinitialiser la phase locomotrice (Andersson and Grillner, 1981). Ce phénomène est dû à la levée des inputs excitateurs des afférences Ib vers les interneurones I/II (Whelan et al., 1995). Chez le chat, ces interneurones sont modulés par l'activation de la MLR (Edgley et al., 1988) et sont activés da façon rythmique lors de locomotion fictive suggérant qu'ils sont impliqués dans la locomotion.

Les afférences articulaires, notamment celles transmettant l'information de positionnement de la hanche, influencent également les délais de phase du patron locomoteur et, lorsqu'un certain angle est atteint, entraînent l'initiation de la phase de balancement (Grillner and Rossignol, 1978).

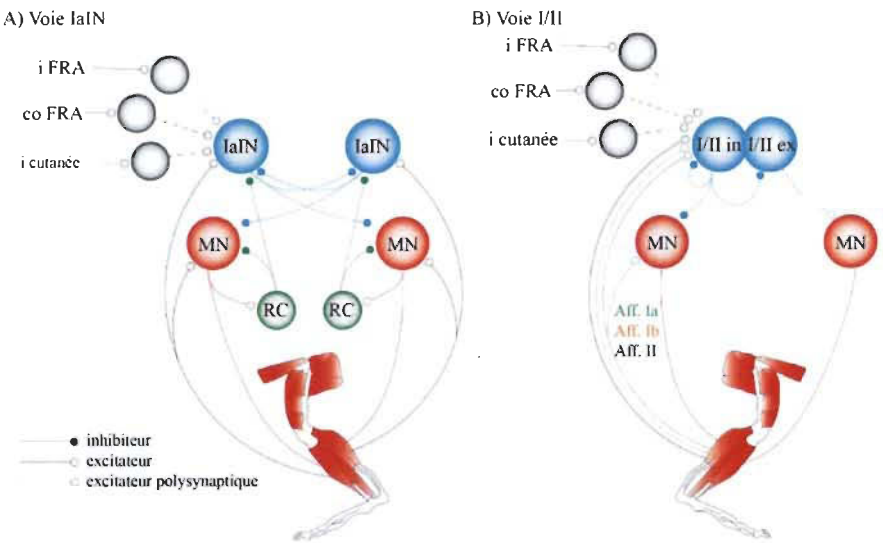

Figure 1.1 Schéma des voies de modulation des interneurones spinaux par les afférences sensorielles.

Figure 1.1 (légende) : (A) Inhibition réciproque et (B) non-réciproque. Lors de locomotion, l'inhibition non-réciproque est inversée en excitation (adapté de Jankowska E. (2013). Spinal Interneurons. In: Pfaff D.W. (eds) *Neuroscience in the 21st Century*, Springer, New York, NY). IaIN : interneurone Ia, I/II : interneurone I/II, MN : motoneurone, RC : cellule de Renshaw, FRA : afférences du réflexe de flexion, i : ipsilatéral, co : controlatéral

Les afférences cutanées, principalement ceux de la cheville et de la patte (Duysens and Loeb, 1980), sont également impliquées dans la régulation de l'activité locomotrice au niveau spinal. Il a été démontré que l'activation d'afférences cutanées par le recrutement des mécanorécepteurs de bas seuil (fibres Aβ) produit des patrons d'activité stéréotypique permettant d'éviter des obstacles (Forssberg, 1979, Drew and Rossignol, 1987, Quevedo et al., 2005) et d'ajuster les délais de phase et niveau d'activité musculaire en fonction de la phase locomotrice (Duysens and Pearson, 1976, Duysens and Loeb, 1980), notamment par leur action sur les interneurones IaIN et I/II. Le rôle des afférences cutanées a été précisé par l'identification génétique d'interneurones localisés dans une voie disynaptique transmettant des inputs depuis les mécanorécepteurs de bas seuil de la patte vers les motoneurones. L'inhibition sélective de ces interneurones, identifiés dI3, abolie complètement l'activation réflexe des motoneurones par les afférences cutanées et diminue les performances locomotrices des souris au test de marche sur échelle (Bui et al., 2013). Ce résultat démontre le rôle des afférences cutanées dans le contrôle locomoteur et indique qu'elles sont importantes pour certains aspects qualitatifs plus spécifiquement évalués dans de telles tâches de locomotion complexifiées.

Jusqu'à présent, l'influence des afférences proprioceptives, articulaires et cutanées sur l'activité du CPG a été décrite. Qu'en est-il des afférences nociceptives ? Des évidences indiquent qu'une interaction bidirectionnelle existe au niveau spinal. À l'instar du réflexe-H, l'activation stéréotypique des muscles fléchisseurs accompagnée d'une inhibition des muscles extenseurs par un stimulus électrique de haute intensité (c.-à-d. le réflexe de flexion) est modulée en fonction de la phase locomotrice dans plusieurs modèles animaux et même chez l'humain (Crenna and Frigo, 1984). La réponse de flexion est facilitée en fin de phase de support et en fin de phase de balancement. Des mécanismes de contrôle de la stabilité sont possiblement responsables de cette modulation en fonction de la phase locomotrice, ce qui suggère une interaction entre le CPG et le circuit de ce réflexe nociceptif. Dans la direction opposée, les

afférences nociceptives semblent également moduler l'activité du CPG. Ceci a été suggéré dès les années 60 à la suite de travaux qui mirent en lumière l'influence des afférences du réflexe de flexion (FRA pour *flexion reflex afferents*) sur les délais de phase locomotrice (Eccles, 1959). Cependant, les FRAs ne sont pas que nociceptifs. Ils comprennent des afférences articulaires, cutanées et musculaires de haut seuil, incluant des afférences du groupe III. Comme leur nom l'indique, ces afférences excitent les motoneurones des muscles fléchisseurs et inhibent ceux des muscles extenseurs; Eccles, 1959). En plus de leurs actions communes sur les motoneurones, ils ont été classés dans un même système dû à leur influence sur les mêmes cibles spinales, incluant les interneurones IaIN et I/II (Lindstrom, 1974, Hultborn et al., 1976b, Lundberg, 1979) et supraspinales (formation réticulaire, cervelet; Lundberg and Oscarsson, 1962). Plusieurs observations suggèrent un rôle fonctionnel des FRAs dans la régulation de la commande locomotrice. Lors de locomotion fictive induite par l'application spinale de L-DOPA chez le chat spinal, leur recrutement module le rythme locomoteur selon le patron du réflexe de flexion. En phase d'extension, le recrutement des FRAs interrompt l'activité des extenseurs et active les fléchisseurs en phase d'extension (réinitialisation du rythme) alors qu'en phase de flexion, cela cause un prolongement de la phase de flexion (Schomburg et al., 1998). Leur recrutement mène également à une réponse réflexe stéréotypique en extension ou en flexion du membre controlatéral selon que la patte stimulée est en flexion ou en extension, respectivement (Rossignol and Gauthier, 1980). Les mécanismes impliqués comprennent la modulation de l'inhibition réciproque d'interneurones impliqués dans la locomotion (Jankowska et al., 1967b) et l'ajustement du gain des afférences Ia de façon présynaptique (Andén et al., 1966, Jankowska et al., 1967a). Bien que les afférences nociceptives empruntent, au moins partiellement, les voies des FRAs (Steffens and Schomburg, 1993), il est important de préciser que le rôle fonctionnel des afférences nociceptives dans les effets des FRAs mentionnés ci-dessus est toujours débattu.

1.3 Récupération locomotrice à la suite d'une lésion spinale

1.3.1 Rôle des afférences sensorielles dans l'adaptation des réseaux locomoteurs spinaux à la suite d'une lésion

Alors que le patron locomoteur est généré par le CPG et influencé par les afférences sensorielles, il importe de souligner qu'un animal spinalisé à l'âge adulte montre peu de signes d'activité locomotrice de façon autonome et paraîtra complètement paralysé dans sa cage. En plus de devoir maintenir l'équilibre de l'animal, l'application d'un pincement de la base de la queue, près de la région périnéale, semble mettre le CPG dans un état permissif à générer l'activité locomotrice et suffit à la production de mouvements locomoteurs primitifs, et ce, dès les premiers jours suivants une section spinale mi-thoracique (Barbeau and Rossignol, 1987).

Un pincement de la queue a été effectuée pour déclencher la locomotion dans les études présentées dans cette thèse afin de permettre la description de la locomotion générée par le CPG chez la souris adulte.

Bien que son utilisation soit décrite dans de nombreux ouvrages (Meisel and Rakerd, 1982, Barbeau and Rossignol, 1987), on connait peu les mécanismes responsables de l'activation du CPG par le pincement de la queue. L'hypothèse la plus courante suggère qu'elle active les voies des afférences sacro-caudales (Sławińska et al., 2014) dont la stimulation électrique facilite l'activation du CPG (Lev-Tov et al., 2010) en absence de contrôle supra-spinal.

Les travaux sur la récupération locomotrice des pattes postérieures après une lésion complète, ou partielle, et l'influence des afférences sensorielles sur celle-ci a été l'objet de plusieurs revues de la littérature (Bouyer and Rossignol, 2001, Rossignol et al., 2006, Rossignol et al., 2008). Ces études montrent l'habilité remarquable des réseaux locomoteurs spinaux d'optimiser la récupération en fonction des déficits spécifiques de chaque type de lésion. Dans différents modèles animaux, il a été montré qu'il est possible de récupérer une locomotion quadrupède sur tapis roulant similaire à celle précédant la lésion partielle (Barrière et al., 2008, Alluin et al., 2011; pour revue voir Rossignol et al., 2009) et cette récupération est améliorée par l'entraînement sur tapis roulant (Barrière et al., 2008). La contribution de changements

plastiques au niveau spinal à cette récupération a été révélée chez le chat en performant une deuxième lésion, complète, à un segment spinal inférieur à la première (Barrière et al., 2008). Les animaux ayant préalablement récupérés d'une hémilésion latérale montrent une capacité locomotrice résiduelle 24 h après la section (Barrière et al., 2008, Barrière et al., 2010), et ce principalement ipsilatéralement à l'hémilésion. Le même type de paradigme de double lésion a aussi été repris afin de révéler des changements plastiques spinaux induits par des lésions de nerfs périphériques (Bouyer et al., 2001, pour revue voir Bouyer and Rossignol, 2001). À la suite de la lésion du nerf innervant les muscles fléchisseurs de la cheville tibial antérieur et long extenseur des orteils, un chat autrement intact montre un patron cinématique normal excepté une légère diminution de la flexion dorsale de la cheville compensée par une augmentation de la flexion de la hanche et du genou (Carrier et al., 1997). Une section spinale effectuée après la récupération post-neurectomie sur le même animal permet d'observer une augmentation importante du mouvement en flexion de la hanche et du genou de même que des bouffées EMGs des muscles fléchisseurs amplifiés (Carrier et al., 1997). Cette amplification du mouvement et de l'activité des muscles fléchisseurs n'est pas observée chez des chats spinalisés avant la neurectomie (Rossignol and Bouyer, 2004), indiquant que les adaptations plastiques ont principalement eu lieu au niveau spinal. Il est également possible d'influencer les changements plastiques au niveau spinal après une section en contrôlant l'activation d'afférences sensorielles. Chez le lapin, qui peut alterner entre patron en phase (bond) et hors phase, il est possible, par l'installation de l'animal dans une contention mécanique permettant soit la locomotion en phase ou hors phase, d'influencer les réseaux locomoteurs spinaux à favoriser un patron plutôt qu'un autre (Viala et al., 1986). Cette habileté reflète la plasticité fonctionnelle des réseaux locomoteurs spinaux et la contribution importante des afférences sensorielles à leur réexpression.

1.3.2 Plasticité des réseaux locomoteurs spinaux et entraînement

La thérapie basée sur l'activité, incluant l'entraînement locomoteur, favorise l'activation des réseaux locomoteurs spinaux par les afférences sensorielles et contribue, par des mécanismes Hebbiens, à stabiliser les voies impliquées dans la locomotion et faciliter leur

recrutement. L'entraînement locomoteur accélère la récupération locomotrice à la suite d'une section spinale complète chez le chat adulte (Smith et al., 1982, Lovely et al., 1986, de Leon et al., 1998). Bien que les premières études sur les modèles de section chez des rongeurs (de Leon et al., 2002, Fong et al., 2005) aient rapporté un potentiel de récupération moindre que pour le chat (Chau et al., 1998a, de Leon et al., 2002, Fong et al., 2005, pour revue voir Battistuzzo et al., 2012), de plus en plus d'évidences suggèrent un excellent potentiel de récupération et une réexpression locomotrice similaire à la locomotion de l'animal intact en 3 semaines chez le rat (Alluin et al., 2015) et la souris (Leblond et al., 2003, Lapointe et al., 2006). Puisque la récupération locomotrice associée à l'entraînement ne peut être expliquée par des changements au niveau musculaire (Roy et al., 1998) ou par la régénérescence des voies descendantes (Tillakaratne et al., 2010), cette amélioration doit provenir de changements plastiques affectant les réseaux locomoteurs spinaux et leur activation par les afférences sensorielles (Grillner, 2002, Rossignol et al., 2014).

Pour les rongeurs mais également pour d'autres espèces, la récupération est modulée par le type (de Leon et al., 2002, Cai et al., 2006, Edgerton and Roy, 2009) et le volume d'entraînement (Cha et al., 2007) et semble être optimale lorsque la tâche recrute spécifiquement les réseaux locomoteurs (García-Alías et al., 2009) et ce avec le moins d'assistance possible (Cai et al., 2006). Ces résultats suggèrent que la récupération n'est pas simplement spontanée et que celle-ci partage des caractéristiques avec d'autres formes d'apprentissage. Par exemple, il a été démontré chez le chat que l'effet de l'entraînement perdure pendant 6 semaines d'absence d'entraînement (De Leon et al., 1999). Passé ce délai, d'importants déficits locomoteurs peuvent être observés. Cependant, le rétablissement du protocole d'entraînement chez ces animaux mène à une récupération locomotrice en 1 semaine (De Leon et al., 1999), suggérant que le comportement moteur « appris » est maintenu par l'entraînement et détérioré en absence d'entraînement.

Afin d'optimiser la récupération de la locomotion chez la souris à la suite d'une section spinale complète, la méthodologie employée dans cette thèse consiste à fournir à la souris l'équilibre nécessaire pour assurer un positionnement adéquat des pattes postérieures par rapport au tapis roulant et un pincement de la queue de la plus faible intensité capable de générer la

locomotion. Les séances d'entraînement débutent dès le jour 2 post- section puisque l'initiation tardive de l'entraînement diminue la performance locomotrice (Norrie et al., 2005).

En plus d'augmenter le nombre de pas que l'animal (chat, rat ou souris) peut performer sur une période de temps donnée, celui-ci récupère progressivement le patron cinématique de la locomotion normale dans chaque articulation, permettant ainsi l'alternance entre flexion et extension. La régularisation de la durée de phase de support et de balancement et l'amélioration de la longueur du pas qui en découlent est suivi du rétablissement de l'alternance réciproque des phases locomotrices entre les pattes droite et gauche, aussi identifiée couplage homologue (Rossignol et al., 2014). En parallèle, l'animal récupère également la capacité de placer adéquatement sa patte sur la plante, pourvu que les afférences cutanées soient intactes (Bouyer and Rossignol, 1998). Un contact sur l'aspect dorsal de la patte empêche la mise en charge de la patte postérieure, mène à la traînée de la patte sur le tapis roulant et diminue l'efficacité de l'entraînement pour rétablir la locomotion.

L'évaluation de chacun des paramètres locomoteurs susmentionnés (nombre de pas, cinématique articulaire, durée de phases, longueur de pas, couplage homologue, placement plantaire et traînée de la patte) a été effectuée dans les expériences rapportées dans cette thèse afin de mesurer avec sensibilité la récupération locomotrice.

Un ensemble de facteurs électrophysiologiques et neuroanatomiques expliquent, au moins partiellement, la récupération locomotrice associée à l'entraînement. Des évidences expérimentales récentes décrivent des changements des propriétés des motoneurones (Petruska et al., 2007, D'Amico et al., 2014) et du profil de réponse des motoneurones à une stimulation répétée des afférences proprioceptives Ia (Côté et al., 2011). Une dépression progressive de l'amplitude du réflexe-H lors de stimulation à des fréquences supérieures à 0,3 Hz (FDD pour *frequency-dependent depression*) est normalement observée chez l'animal intact (Thompson et al., 1992) dû à un mécanisme inconnu dont les hypothèses principales sont l'inhibition présynaptique d'origine différente de la transmission par les PADs (Crone and Nielsen, 1989) et la facilitation des motoneurones associée à la fréquence de recrutement d'interneurone inhibiteur (Boulenguez et al., 2010). Cette FDD est progressivement atténuée durant le premier mois suivant une lésion médullaire partielle (Thompson et al., 1992, Thompson et al., 1998) et

complète (Lee et al., 2005, Yates et al., 2008a, Liu et al., 2010). L'altération de la FDD est associée à une diminution de l'expression membranaire du cotransporteur potassium-chlore 2 (KCC2) dans les motoneurones qui transportent le chlore à l'extérieur de la cellule et contribue ainsi au bas niveau intracellulaire de cet ion. L'homéostasie du chlore est intimement liée au tonus inhibiteur GABAergique et glycinergique : une diminution de KCC2 peut causer une atténuation de l'inhibition ou même une facilitation par GABA et glycine. Ce mécanisme, dont les évidences proposent un dérèglement médié par BDNF (Tashiro et al., 2015), est impliqué dans différentes conditions neurologiques impliquant une hyperactivité neuronale, notamment l'épilepsie (Huberfeld et al., 2007), la douleur neuropathique (Hasbargen et al., 2010) et la spasticité associée aux LM (Boulenguez et al., 2010). En outre, l'entraînement prévient l'atténuation de FDD du réflexe-H associée à la LM ou section (Reese et al., 2005, Yates et al., 2008b, Côté et al., 2011, Côté et al., 2014) en rétablissant l'expression de BDNF (Gómez-Pinilla et al., 2001, Boyce et al., 2007), l'homéostasie du chlore et l'expression motoneuronale de KCC2 (Côté et al., 2014). Toutefois, l'entraînement ne peut rétablir la FDD après le développement d'hyperréflexie (Yates et al., 2008b) suggérant qu'une initiation précoce est nécessaire afin d'observer l'impact positif de l'entraînement sur ce mécanisme.

Puisque la modulation efficace du réflexe-H à haute fréquence et le rétablissement de la FDD médié par KCC2 sont associés à une meilleure récupération de la locomotion après une LM chez la souris (Lee et al., 2009), nous avons évalué l'impact de facteurs contextuels à la réadaptation sur ceux-ci (étude 2-3).

1.4 Facteurs influençant la récupération

Comme discuté dans les sections précédentes, la récupération locomotrice associée à l'entraînement repose sur des changements neuroplastiques largement distribués (CPG, afférences sensorielles, motoneurones) qui sont adaptatifs, c.-a.-d. qu'ils favorisent la fonction. D'autres phénomènes associés à la LM causent des changements mésadaptatifs, incluant le développement de douleur et de sensibilisation centrale, qui sont eux aussi largement distribués. Des évidences récentes chez l'animal (Grau et al., 2014) suggèrent qu'ils concurrencent pour influencer des circuits spinaux communs. Un des objectifs de cette thèse est d'évaluer si

l'interaction entre l'entraînement et des processus reliés à la douleur influence la récupération locomotrice.

1.4.1 Influence des comorbidités sur la récupération

Chez l'humain, des comorbidités sont fréquemment présentes lors de LM et semblent influencer le pronostic (Dvorak et al., 2017). Les plus communes sont la douleur chronique et la spasticité (Johnson et al., 1998, Barrett et al., 2003, Siddall et al., 2003, Finnerup et al., 2014). Globalement, la plupart des études rapportent une prévalence de douleur chronique après une LM d'environ 65 %, et ce peu importe le niveau et l'importance de la lésion de même que la durée de la condition (Rintala et al., 1998, Siddall et al., 1999, Jensen et al., 2005). Parmi les différents types de douleur, la douleur musculosquelettique est la plus fréquemment rencontrée, avec 60 % des cas, suivie par la douleur neuropathique (34 %) et la douleur viscérale (6 %). On ne connait pas l'étendue de leur influence sur la récupération fonctionnelle et les mécanismes impliqués. Considérant que ces comorbidités représentent un facteur limitant la récupération fonctionnelle chez les patients, la caractérisation du phénomène ainsi que les mécanismes impliqués doivent être investigués.

En utilisant notre modèle de souris avec une section spinale complète, nous avons évalué l'impact d'une inflammation musculaire sous-lésionnelle par l'injection d'adjuvant de Freund (CFA) sur la récupération locomotrice (étude 1) et le développement d'hyperréflexie (étude 2). Enfin, nous avons caractérisé la contribution de deux mécanismes, l'activation de microglie et la régulation de KCC2, aux résultats comportementaux observés (étude 3).

1.4.1.1 Activité nociceptive et inflammation

Comme la locomotion, l'expérience de la douleur nécessite l'activation de réseaux neuronaux largement distribués, et ce en réponse ou non à un stimulus nociceptif. Mais qu'est-ce que la douleur et comment diffère-t-elle de la nociception ? Selon l'*International Association for the Study of Pain* (IASP), la douleur se définit comme une expérience sensorielle et émotionnelle désagréable associée avec un dommage tissulaire actuel ou potentiel, ou décrite

dans ces termes[1]. Elle peut être le résultat de changements plastiques affectant n'importe quel réseau neuronal de la « matrice » de la douleur et ne nécessite pas de stimulation nociceptive. Le phénomène de douleur du membre fantôme, pour lequel aucune activité nociceptive n'est impliquée, illustre cette évidence. La nociception, quant à elle, réfère à toutes formes de traitement d'informations déclenchées par un stimulus nociceptif, c'est-à-dire un stimulus qui est dommageable pour un tissu normal. Elle peut, ou non, engendrer de la douleur. L'absence de douleur ressentie par un soldat blessé au combat exemplifie cette caractéristique.

La nociception est due à l'activation de récepteurs spécialisés retrouvés sur les branches terminales de fibres sensorielles spécifiques. Les nocicepteurs cutanés (fibres Aδ et C) ont été plus abondamment décrits, mais ils existent également dans les muscles, viscères et autres tissus du corps. Dans le tissu musculaire, les fibres transmettant les informations nociceptives sont morphologiquement et physiologiquement similaires aux nocicepteurs cutanés, quoique la nomenclature organise celles-ci selon leur diamètre et non leur vitesse de conduction (Lloyd, 1943). Les fibres afférentes musculaires du groupe III (myélinisées) et IV (non myélinisées), d'environ 3-4 μm de diamètre, correspondent généralement aux fibres cutanées Aδ et C. Leur vitesse de conduction, respectivement 3,1-13,5 ms et 0,6-1,2 ms, correspond également à celles observées dans les fibres cutanées (Simone et al., 1994). Plus de 60 % de ces fibres possèdent des terminaisons nerveuses sensibles aux stimulations mécaniques de haute intensité (Marchettini et al., 1996) et correspondent à la définition de nocicepteurs. Comme pour les fibres cutanées, les fibres nociceptives musculaires déchargent également en présence d'agents chimiques comme la capsaïcine (Marchettini et al., 1996) ou du salin hypertonique. La capacité de signaler la présence d'agents chimiques est notamment responsable de la sensation de douleur accompagnant une réaction inflammation musculaire. Les substances présentes dans le tissu musculaire inflammé qui activent les fibres nociceptives comprennent, mais ne se limitent pas à la bradykinine (BK), la 5-HT, l'histamine, les ions H^+, l'adénosine triphosphate (ATP), la prostaglandine PGE2 ainsi que le facteur neurotrophique NGF (Fock and Mense, 1976, Dray, 1995, McMahon, 1996, Reinert et al., 1998, Mense, 2008, Mense, 2009). La dose d'agents algésiques nécessaire à l'activation des fibres nociceptives est similaire à celle du seuil de

[1] http://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698#Pain

douleur (Schmidt et al., 1995). En plus d'activer directement les fibres nociceptives, la présence d'agents algésiques augmente la sensibilité des nocicepteurs aux stimuli en abaissant leur seuil d'excitabilité (Woolf and Salter, 2000, Mense, 2008). En résumé, la réponse inflammatoire mène à la sensibilisation périphérique des nocicepteurs responsable d'une diminution du seuil d'activation des fibres nociceptives et d'une augmentation de l'intensité de douleur perçue en réponse à d'un stimulus externe.

1.4.1.2 Modèle expérimental d'inflammation musculaire

L'administration de CFA est un modèle expérimental de douleur chronique récemment appliqué à l'étude de la lombalgie chez l'animal (Taguchi et al., 2008, Touj et al., 2017). Il implique à la fois des mécanismes neurogènes et non neurogènes. La conjoncture de ces mécanismes mène à l'activation et la sensibilisation de la voie nociceptive tant au niveau périphérique, spinale que supraspinale et contribue au développement d'hypersensibilité (Larson et al., 1986).

Bien que certains mécanismes supraspinaux comme la désinhibition de la facilitation descendante jouent un rôle significatif dans le développement de douleur chronique (Zhang et al., 2011), ceux-ci n'ont pas d'influence dans le modèle de section médullaire à l'étude dans cette thèse et ne seront donc pas abordés. Par contre, la sensibilisation des nocicepteurs (Schuelert et al., 2015), des afférences primaires (Hutchins et al., 2000, Ambalavanar et al., 2006a, Asgar et al., 2015, Schuelert et al., 2015) et des neurones de deuxième ordre dans la corne dorsale (Hoheisel et al., 1998, Hutchins et al., 2000, Taguchi et al., 2008, Xu et al., 2008, Zhang et al., 2008b) qui caractérise le modèle d'administration intramusculaire de CFA est susceptible d'influencer la récupération locomotrice dans l'expérience menée dans cette thèse.

Le CFA est composé de *Mycobacterium tuberculosis* tué par la chaleur et mis en suspension dans de l'huile de paraffine. En présence d'antigène, l'ajout de CFA stimule fortement le système immunitaire. Au niveau périphérique, la présence de CFA dans les tissus active et sensibilise les fibres nociceptives, notamment par l'action du récepteur TRPA1 (Asgar et al., 2015). La relâche de NGF causée par l'activation des fibres nociceptives est associée à des changements de propriétés de ces mêmes fibres et diminue la durée des potentiels d'action

(PA) et augmente la fréquence de décharge évoquée et spontanée (Djouhri et al., 2001). L'AHP (pour *afterhyperpolarization*) est également diminuée dans les fibres nociceptives de type C à la suite d'administration de CFA (Weng et al., 2012, Hatch et al., 2013, Djouhri et al., 2015), mais le mécanisme n'est pas connu. De plus, les champs récepteurs des neurones de la corne dorsale superficielle couvrent une superficie en moyenne 2,5 fois plus grande que les champs récepteurs contrôles 6 h à 5 jours à la suite d'injection de CFA (Hylden et al., 1989). L'administration de CFA change également les propriétés des fibres sensorielles de gros calibre Aβ et diminue leur seuil d'excitabilité par un mécanisme impliquant les canaux sodiques $Na_v1.8$ (Belkouch et al., 2014), ce qui suggère une contribution des fibres non nociceptives au développement d'hypersensibilité mécanique.

Le contenu des fibres nociceptives en substance P, CGRP (pour *calcitonin gene-related peptide*) et VIP (pour *vasoactive intestinal peptide*) est augmenté dès les premières heures à la suite d'un stimulus inflammatoire (Reinert et al., 1998, Ambalavanar et al., 2006b). Lors d'inflammation chronique, le nombre de fibres afférentes exprimant CGRP est également augmenté (Ambalavanar et al., 2006a). Cette augmentation d'expression concorde avec la diminution de seuil de sensibilité mécanique et thermique.

Au niveau spinal, l'administration de CFA intramusculaire augmente l'amplitude des EPSCs (pour *excitatory post-synaptic currents*) médiés par les récepteurs NMDA in vitro. Ceux-ci sont fortement atténués par l'application spinale de clonidine (agoniste adrénergique α2). Ces changements concordent avec l'atténuation induite par l'injection intra-thécale de clonidine sur la diminution du seuil de sensibilité mécanique associée au CFA in vivo (Fan et al., 2014). Ces résultats suggèrent que le phénomène de sommation temporelle contribue à la sensibilisation centrale induite par l'administration de CFA.

L'administration de CFA dans le muscle altère le profil de la microglie spinale, notamment en augmentant leur quantité et leur morphologie due à leur activation médiée par le facteur de transcription p38 (Kiyomoto et al., 2015). Ces changements ont été observés aussi bien dans les laminae profondes que superficielles de la corne dorsale et sont associées à la diminution du seuil de sensibilité mécanique par la relâche microgliale de médiateurs inflammatoires comme IL-1β, IL-6 et TNF-α (Raghavendra et al., 2004, Chacur et al., 2009).

Ces modifications perdurent au moins 12 jours post-injection et concordent avec les changements de seuil de retrait à la suite d'une stimulation au filament de Von Frey.

Comme la plupart des modèles inflammatoires, l'administration de CFA pourrait sensibiliser la voie nociceptive en induisant une désinhibition médiée par la perte de tonus inhibiteur GABAergique et glycinergique (Crowley et al., 2016). Il a été rapporté que le CFA inverse l'influence d'agoniste et d'antagoniste GABA sur le seuil de sensibilité mécanique au filament de Von Frey évalué 72 h après l'injection (Anseloni and Gold, 2008). Par son action sur la PGE_2, l'administration de CFA réduit également l'inhibition glycinergique dans la corne dorsale superficielle jusqu'à 2 semaines post-injection (Ahmadi et al., 2002, Müller et al., 2003, Harvey et al., 2004). De plus, l'administration de CFA réduit durablement l'expression de KCC2 dans la corne dorsale lorsqu'évaluée dans les 2 premières semaines post-injection (Zhang et al., 2008a, Lin et al., 2017). Cet effet contribue à amplifier encore davantage la désinhibition du circuit nociceptif et concorde avec le développement d'hypersensibilité mécanique (Zhu et al., 2014). La diminution de KCC2 est potentiellement médiée par l'augmentation d'expression de BDNF associée à l'administration de CFA (Obata et al., 2003).

Le modèle d'inflammation expérimentale utilisé dans cette thèse prévoit d'administrer le CFA dans les muscles lombaires sous-lésionnels. L'objectif est de reproduire les changements plastiques centraux associés à l'inflammation musculosquelettique chronique et d'évaluer l'influence de l'entraînement locomoteur sur ceux-ci (étude 3) en utilisant un modèle qui ne modifie pas la locomotion chez l'animal intact (données non présentées).

1.4.1.3 Interaction entre l'inflammation musculosquelettique et l'entraînement

Au niveau spinal, des évidences font état d'interaction entre les changements mésadaptatifs liés à l'activité nociceptive et les changements adaptatifs associés à différents protocoles induisant de la plasticité activité-dépendante comme le conditionnement de réflexe ou l'entraînement locomoteur. Par exemple, il a été observé que l'activité nociceptive, sous forme de choc électrique de forte intensité (Grau et al., 1998, Crown and Grau, 2001, Ferguson et al., 2006) ou d'inflammation périphérique (Hook et al., 2008, Baumbauer et al., 2009), diminue la capacité de conditionnement du réflexe de flexion tout en induisant un état

d'hypersensibilité mécanique (Crown et al., 2002a). Le déficit de conditionnement perdure pour une période d'environ 48 h, puis le réflexe peut être conditionné à nouveau (Crown et al., 2002b). Le développement d'hypersensibilité mécanique et de déficits d'apprentissage représenteraient des formes de plasticité qui s'opposent au conditionnement et à l'entraînement locomoteur par un mécanisme impliquant les récepteurs NMDA (Ferguson et al., 2012). Récemment, il a été observé que l'activité nociceptive sous forme de choc électrique atténue la récupération locomotrice à la suite d'une LM partielle (Garraway et al., 2011). Toutefois, on ne sait pas si ce phénomène est médié par des changements ciblant les circuits spinaux ou supraspinaux. Considérant l'influence majeure des processus liés à la récupération locomotrice ainsi qu'à la nociception et sa modulation sur les circuits spinaux, leur contribution à l'interaction entre douleur et motricité doit être clarifiée. Comme ces mécanismes sont cliniquement importants et permettraient d'optimiser la récupération fonctionnelle des patients, notre objectif est de décrire, à l'aide d'un modèle de souris spinale, l'implication des réseaux spinaux dans l'impact de l'activité nociceptive sur la récupération locomotrice.

Hypothèse 1 : L'inflammation des muscles lombaires atténue la récupération locomotrice à la suite d'une section spinale complète.

Les processus reliés à la douleur et la récupération semblent concurrencer pour influencer des circuits spinaux communs. Comme décrit au chapitre 3.2, la FDD du réflexe-H est un prédicteur électrophysiologique de la récupération fonctionnelle à la suite d'une LM. Des évidences récentes suggèrent également que cette mesure peut être altérée par des changements spinaux impliqués dans les processus liés à la douleur inflammatoire et neuropathique (Lee-Kubli and Calcutt, 2014). Considérant l'influence opposée de l'entraînement et des processus inflammatoires sur la FDD lorsqu'évaluée séparément, notre objectif secondaire est de décrire les changements affectant la FDD lors de la présence concomitante d'entraînement et d'inflammation lombaire.

Hypothèse 1a : L'inflammation des muscles lombaires perturbe la dépression du réflexe-H dépendante de la fréquence durant la récupération post-lésion.

Afin de déterminer les changements neuroanatomiques responsables des déficits locomoteurs associés à l'inflammation lombaire, notre troisième objectif est d'évaluer

l'expression de molécules modulée de façon opposée par l'inflammation et l'entraînement locomoteur à la suite d'une section spinale complète. L'expression de la microglie est augmentée dans la moelle épinière lors d'administration de CFA (Chacur et al., 2009). À l'inverse, l'expression de KCC2 est augmentée lors d'entraînement (Côté et al., 2014) et diminuée lors de douleur (Hasbargen et al., 2010) lorsque ceux-ci sont présentés séparément.

Hypothèse 1 b : Considérant que l'inflammation et l'entraînement concurrencent pour influencer l'expression de KCC2 et de la microglie, ceux-ci seront moins modulés lorsque les deux sont présentés de façon simultanée dans la période de récupération de la marche. Leur expression sera associée à la capacité locomotrice résiduelle des souris.

1.4.2 Influence d'une approche pharmacologique et d'entraînement combinée

Des facteurs bénéfiques influencent également la récupération locomotrice à la suite d'une LM. Les systèmes monoaminergiques contribuent fortement à la récupération locomotrice à la suite d'une lésion partielle, notamment par des changements neuroanatomiques ciblant la voie réticulo-spinale (Ballermann and Fouad, 2006), les voies propriospinales (Zaporozhets et al., 2011, van den Brand et al., 2012, Cowley et al., 2015) et les réseaux locomoteurs spinaux (Saruhashi et al., 1996). La présence de nombreux récepteurs monoaminergiques dans les niveaux spinaux où se situe le CPG (Giroux et al., 1999, Otoshi et al., 2009) et l'influence de l'entraînement locomoteur sur leur expression (Chopek et al., 2015) suggère un rôle de ce système dans la réexpression de la locomotion à la suite de section spinale complète et qu'il contribue à l'effet positif de l'entraînement.

1.4.2.1 Rôle du système sérotoninergique dans la locomotion

La démonstration de l'effet marqué de la DOPA, intermédiaire métabolique des catécholamines, sur l'activité des voies réflexes impliquées dans la locomotion (Jankowska et al., 1967b) ou encore de la clonidine, agoniste noradrénergique α_2, sur la facilitation de la

récupération de la marche chez le chat spinalisé à l'âge adulte (Chau et al., 1998b, a) ont encouragé la réalisation d'études sur l'utilisation d'outils pharmacologiques pour améliorer la récupération locomotrice à la suite d'une LM. Parmi les différentes interventions qui influencent l'activité locomotrice chez les rongeurs, l'administration de sérotonine (5-HT) est celle qui active les réseaux locomoteurs spinaux de la façon la plus robuste (Cazalets et al., 1992, Cowley and Schmidt, 1995, Schmidt and Jordan, 2000, Zaporozhets et al., 2011) et possiblement celle qui possède le meilleur potentiel thérapeutique. En outre, contrairement à la transmission synaptique permettant à un neurone présynaptique d'influencer un neurone postsynaptique, les monoamines, incluant la 5-HT, peuvent influencer des cibles situées loin du site de relâche et agissent donc sur un ensemble de neurones, ce qui les définit comme neuromodulateurs (Zoli et al., 1999; Descarries and Mechawar, 2000).

Il a été démontré que la 5-HT promeut la locomotion (Jordan et al., 2008) et favorise son activité stéréotypique par la régulation de réflexes spinaux (Jankowska et al., 2000, Schmidt and Jordan, 2000). La 5-HT est relâchée dans la moelle épinière par l'action de neurones de la région mésencéphalique locomotrice (MLR) sur leurs cibles réticulo-spinales (Jordan, 1998). Celles-ci comprennent des neurones sérotoninergiques situés dans la région parapyramidale (PPR) récemment identifiée comme médiateur de l'activité rythmique du CPG (Liu and Jordan, 2005, Jordan et al., 2008) et médiés par les récepteurs 5-HT_{2A} et 5-HT_7 (Liu and Jordan, 2005) (Jordan et al., 2008). L'impact fonctionnel de l'administration d'antagonistes et de l'ablation génétique de ces sous-types de récepteurs a également été démontré par l'altération du rythme et la perte d'alternation droite-gauche du patron cinématique et EMG chez la souris in vivo (Liu et al., 2009), supportant les découvertes des modèles in vitro. En plus des récepteurs 5-HT_{2A} et 5-HT_7, d'autres récepteurs 5-HT participent à la régulation de la locomotion, incluant les récepteurs 5-HT_1 (Beato and Nistri, 1998, voir section 1.4.2.2).

Les neurones 5-HT projettent vers les cornes ventrales et dorsales par le funicule ventro-latéral (Carlsson et al., 1964). Ceux-ci agissent en activant des récepteurs abondamment distribués dans tout le SNC, incluant les interneurones et motoneurones des réseaux locomoteurs spinaux (Giroux et al., 1999, Otoshi et al., 2009). L'effet de la relâche de 5-HT dépend de sa concentration. À des concentrations basses, la 5-HT augmente le rythme et la coordination entre les bouffées électroneurographiques (ENGs) enregistrées aux racines ventrales de L2

(fléchisseurs) et L5 (extenseurs) de l'activité locomotrice induite par administration de NMDA ou DOPA (Jiang et al., 1999, Madriaga et al., 2004, Pearlstein et al., 2005). À de hautes concentrations, la 5-HT seule permet de générer une activité rythmique et coordonnée (Nishimaru et al., 2000), potentiellement dû à l'activation de récepteurs non sérotoninergiques (ex. : dopaminergiques; Madriaga et al., 2004) et à la modulation directe de l'excitabilité des motoneurones par la plus grande diffusion de la 5-HT dans l'espace extracellulaire (Perrier et al., 2013).

1.4.2.2 Facilitation de la récupération

En présence d'une lésion complète de la moelle épinière, la contribution des voies réticulo- et propriospinales à la récupération locomotrice est supprimée. Cependant, l'activation des récepteurs 5-HT peut influencer des mécanismes spinaux pour favoriser la récupération. Au jour 3 post- section, la concentration de 5-HT sous-lésionnelle est drastiquement abaissée à environ 5-10 % de sa quantité d'origine (Andén et al., 1964, Carlsson et al., 1973, Magnusson, 1973), laissant les interneurones spinaux sérotoninergiques la seule source de 5-HT sous-lésionnelle (Newton and Hamill, 1988). L'expression de différents sous-types de récepteurs 5-HT, incluant les récepteurs 5-HT_{1A} (Otoshi et al., 2009), 5-$HT_{2A/C}$ (Kim et al., 1999, Chopek et al., 2015), 5-HT_7 (Chopek et al., 2015) est augmentée dans la moelle épinière lombaire sous-lésionnelle et ciblent les réseaux locomoteurs spinaux (Otoshi et al., 2009, Chopek et al., 2018). De plus, l'activité constitutive de certains récepteurs est augmentée, notamment les 5-HT_{2C} (Fouad et al., 2010, Murray et al., 2010). Encore plus important, l'entraînement locomoteur amplifie ces changements d'expression et d'activité constitutive (Ballermann and Fouad, 2006, Engesser-Cesar et al., 2007). En absence de transmission supra-spinale, ces changements associés à l'entraînement pourraient être médiés par l'activité des afférences sensorielles. C'est le cas pour la régulation de l'expression du récepteur 5-HT_{1A} au niveau des motoneurones des extenseurs, pour lesquels l'intégrité des afférences sensorielles est requise pour observer une augmentation de leur expression associée à l'entraînement post-section (Otoshi et al., 2009).

Plusieurs études des modèles de section complète ont rapporté une amélioration à court et long terme des capacités locomotrices par l'administration de différents agonistes 5-HT chez

le rat et la souris (tableau 1-1, page 28). Globalement, l'administration d'agoniste des récepteurs 5-HT améliorent la coordination droite-gauche, la variabilité du patron de marche et facilite la flexion en phase de balancement (médié par 5-$HT_{1A/7}$) et augmentent l'extension et le support de poids en phase de support (médié par 5-$HT_{2A/C/3}$). D'autres évidences suggèrent que ceux-ci agissent en favorisant l'impact positif de l'entraînement par un mécanisme activité-dépendant (Fong et al., 2005, Gerasimenko et al., 2007, Ichiyama et al., 2008). Ces découvertes ont un potentiel thérapeutique évident pour améliorer la récupération fonctionnelle des patients. Toutefois, beaucoup de ces agonistes présentent des effets neurotropes indésirables chez l'humain incluant des hallucinations (Nelson et al., 1999), ce qui les rend incompatibles avec une utilisation en clinique.

Tableau 1-1. Effets de différents agonistes 5-HT sur la locomotion

RÉFÉRENCE	AGONISTE	MODÈLE (LÉSION)	INTERVENTION	EFFETS PROLOCOMOTEURS	LIMITES
ANTRI, MOUFFLE, ORSAL & BARTHE. (2003)	5-$HT_{1A/7}$	Rat (section)	8-OH-DPAT (0,25 mg/kg)	Amélioration du score moteur (effet aigu et chronique) ; augmentation et diminution de la durée de bouffée EMG du m. vastus medialis et tibialis anterior, respectivement	Échelle non validée
LANDRY ET AL. (2006)	5-HT_{1A} et/ou 5-HT_7	Souris (section)	8-OH-DPAT (1 mg/kg) + antagoniste 5-HT_7 (SB269970) ou antagoniste 5-HT_{1A} (WAY100,135/635)	Augmentation de la fréquence et amplitude des mouvements « locomotor-like » et de l'excursion angulaire de la hanche, du genou et de la cheville médiées par 5-HT_{1A} et 5-HT_7	Dose minimale effective pour déclencher un mouvement « locomotor-like » ; Mesure de mouvement « locomotor-like »
KIM, MURRAY, & SIMANSKY. (2001)	5-HT_{2C}	Rat néonatal (section)	*m*-CPP (0,15 mg//kg)	Augmentation du support de poids	Modèle néonatal
ANTRI, ORSAL & BARTHE. (2002)	5-$HT_{2A/C}$	Rat (section)	Quipazine (0,1 mg/kg)	Amélioration du score moteur ; diminution de la durée de bouffée EMG du m. tibialis anterior	Échelle non validée

FONG ET AL. (2005)	5-$HT_{2A/C}$	Souris (section)	Quipazine (0.2-2 mg/kg) et/ou entraînement	Effets synergétiques de la quipazine et de l'entraînement sur l'augmentation du nombre de pas et le rythme locomoteur	Dose variable ; Contention de l'animal dans un harnais et placé en position debout
GERASIMENKO ET AL. (2007)	5-$HT_{2A/C}$	Rat (section)	Quipazine (0,3 mg/kg) et/ou entraînement	Augmentation de la durée de bouffée EMG des mm. tibialis anterior et gastroc. Medialis lors de traitement combinant quipazine entraînement	N = 9 ; Contention de l'animal dans un harnais et placé en position debout
ANTRI, BARTHE, MOUFFLE & ORSAL. (2005)	5-$HT_{1A/7}$ + 5-$HT_{2A/C}$	Rat (section)	8-OH-DPAT (0.125mg/kg) + Quipazine (0.125mg/kg)	Amélioration du score moteur qui perdure 45 jours après la dernière injection	Échelle non validée ; dose variable dépendamment des groupes
COURTINE ET AL. (2009)	5-$HT_{1A/7}$ et/ou 5-$HT_{2A/C}$	Rat (section)	8-OH-DPAT (0.1-0.3mg/kg) + Quipazine (0.3mg/kg) + stimulation électrique épidurale (L2 et S1)	Effet complémentaire de 5-$HT_{1A/7}$ et 5-HT_2 pour améliorer la coordination droite-gauche, la capacité de support de poids et la cinématique	Contention de l'animal dans un harnais et placé en position debout
MUSIENKO ET AL. (2011)	5-$HT_{1A/7}$ et/ou 5-$HT_{2A/C}$ et/ou 5-HT_3	Rat (section)	8-OH-DPAT (0.1-0.05mg/kg) et/ou Quipazine (0,2 mg/kg) et/ou SR57227A (1,5 mg/kg) + stimulation électrique épidurale (L2 et S1)	5-HT_{1A} améliore la coordination droite-gauche, réduit la variabilité cinématique, augmente le support de poids, facilite la flexion et diminue la traînée de la patte. 5-$HT_{2A/C}$ facilite l'extension et améliore le support de poids. 5-HT_3 facilite l'extension, améliore le support de poids et diminue la traînée de la patte et la variabilité du couplage homologue. 5-HT_7 améliore de façon limitée le rythme et coordination. 5-HT_{1A}, 5-$HT_{2A/C}$ et 5-HT_7 ont des effets complémentaires	Contention de l'animal dans un harnais et placé en position debout

1.4.2.3 Utilisation de la buspirone pour améliorer la récupération locomotrice

Récemment, l'effet prolocomoteur de la buspirone, un composé pharmacologique approuvé par la Food and Drug Administration (FDA) et agissant principalement comme agoniste des récepteurs 5-HT_{1A} (voir section 5.1), a été évalué dans une étude préclinique chez des patients ayant une lésion complète de la moelle épinière (Gerasimenko et al., 2015). Les auteurs de cette étude ont conclu que la buspirone facilite la transmission de commande supraspinale par des voies sérotoninergiques inactives mais anatomiquement préservées par la lésion spinale. Chez la souris, deux études soulèvent que la buspirone combinée à la carbidopa et L-DOPA facilite la récupération de la locomotion malgré une section spinale complète (Guertin et al., 2011, Ung et al., 2012), suggérant que l'effet prolocomoteur pourrait être médié par la modulation des réseaux locomoteurs spinaux. Considérant le rôle central joué par ceux-ci dans la récupération locomotrice, le mécanisme impliqué dans l'effet prolocomoteur de la buspirone doit être clarifié. Notre objectif est de vérifier l'impact à court et long terme de la buspirone combinée à l'entraînement locomoteur chez des souris ayant une section spinale complète.

Hypothèse 2a : La buspirone facilite la récupération locomotrice des souris ayant une section spinale complète.

Puisque les évidences suggèrent que la modulation d'expression des récepteurs 5-HT_{1A} dépend de l'influence des afférences sensorielles dans la récupération (Otoshi et al., 2009), un deuxième objectif était d'évaluer si l'amélioration médiée par la buspirone différait en fonction du statut fonctionnel des réseaux locomoteurs spinaux. Le paradigme de double lésion, dans lequel l'animal récupère d'une première hémilésion avant de subir une section complète (voir section 5.2), permet d'étudier l'influence d'intervention pharmacologique ou d'entraînement sur des animaux dont l'activité locomotrice médiée par le CPG est partiellement rétablie.

Hypothèse 2 b : La facilitation médiée par la buspirone sera plus importante chez des souris ayant une section spinale complète lorsque celle-ci est intégrée dans un paradigme de double lésion.

1.5 Considérations méthodologiques

1.5.1 Le réflexe monosynaptique

La contraction de courte latence d'un muscle en réponse à un étirement de ses fuseaux est la conséquence fonctionnelle de l'activation d'une boucle réflexe monosynaptique entre les afférences Ia et le motoneurone identifié comme le réflexe d'étirement. Il a été décrit pour la première fois par Sherrington chez le chat décérébré (Sherrington, 1892) et permet de réguler l'activité des motoneurones afin d'ajuster la contraction du muscle lors d'un changement de longueur de ses fibres (Liddell and Sherrington, 1924). Lorsqu'un muscle est étiré, le changement de longueur du fuseau musculaire active les fibres afférentes Ia qui transmettent un potentiel d'action vers les motoneurones localisés dans la corne ventrale. Son excitabilité est modulée par des voies descendantes (Feldman and Orlovsky, 1972; pour revue, voir Schieppati, 1987), par des circuits spinaux (Crone et al., 1987) et par l'activité d'autres afférences (Hultborn et al., 1987). La relation entre la charge des muscles (load) et l'amplitude du réflexe-H en contexte statique et dynamique exemplifie la modulation du réflexe-H. En posture statique, l'amplitude du réflexe-H est inversement proportionnelle à la charge du membre inférieur (Nakazawa et al., 2004). Durant la locomotion, ce même réflexe est augmenté en phase de support (charge) et inhibé en phase de balancement (décharge) (Capaday and Stein, 1986). Ce résultat démontre l'importante intégration sensorimotrice survenant dans cette voie monosynaptique. Il est, par conséquent, abondamment utilisé comme outil pour étudier l'intégration de ces différents mécanismes de contrôle.

Le réflexe de Hoffmann, ou réflexe-H, est l'analogue électrique du réflexe d'étirement et active donc les mêmes fibres Ia et motoneurones (figure 1.2). Le réflexe du muscle soléaire a été le premier étudié par Paul Hoffmann en 1918 qui a montré qu'une réponse réflexe est fiablement évoquée par la stimulation électrique du nerf tibial dans la fosse poplitée. L'enregistrement électrophysiologique montre une réponse motrice directe (onde M, 5-10 ms de latence) qui est suivie par le réflexe-H (25-35 ms de latence). Le réflexe-H est généralement évoqué à des intensités de stimulations inférieures à l'onde M due au plus gros diamètre des

fibres Ia comparativement aux motoneurones. Chez l'humain, la différence de vitesse de propagation entre les fibres Ia et les motoneurones et la plus grande distance entre le site de stimulation et la moelle épinière induit un délai entre l'arrivée au soma motoneuronal des inputs Ia et des inputs antidromiques du motoneurone, à la faveur des Ia. À la suite de l'activation du motoneurone par les fibres Ia, le potentiel d'action se propageant orthodromiquement dans le motoneurone peut entrer en collision avec le potentiel antidromique, ce qui a pour effet de diminuer l'amplitude du réflexe-H de façon proportionnelle à l'amplitude de l'onde F. Ce phénomène est révélé par la diminution de l'amplitude du réflexe-H à des intensités de stimulation supramaximales au recrutement du réflexe-H.

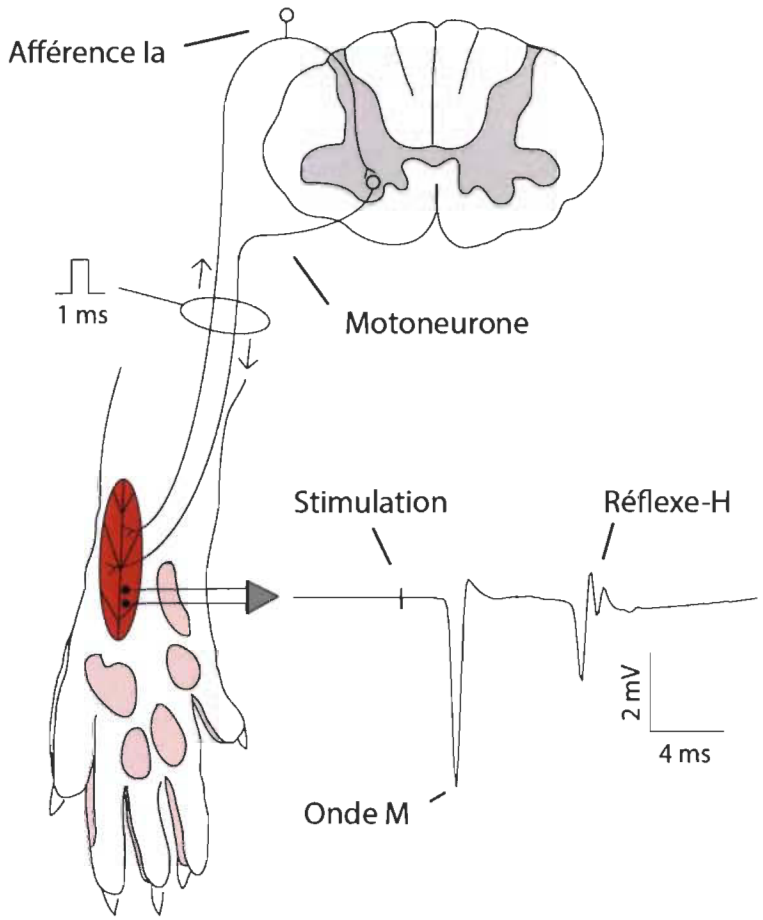

Figure 1.2 Circuit schématique du réflexe de Hoffmann

Chez la souris, une réponse similaire composée d'une onde M (1,8-2 ms de latence) et d'un réflexe-H (5-6,5 ms) est évoquée en réponse à une stimulation électrique des muscles interosseux de la patte. Contrairement à ce qui est observé chez l'humain, plusieurs études,

incluant la nôtre, ont rapporté un seuil de recrutement similaire entre l'onde M et le réflexe-H (figure 3.2). Cette différence entre les modèles animaux et humain n'est pas comprise pour le moment. Il est normalement considéré que la réponse recrutée en même temps ou légèrement après l'onde M est de nature monosynaptique due à sa courte latence et à son inhibition lors de stimulations répétées. Alternativement, dans certains écrits, les auteurs n'ont pas voulu présumer du recrutement monosynaptique des motoneurones par les fibres Ia et identifie la réponse EMG mesurée comme une onde F/H. Cette appellation suggère que le réflexe-H est contaminé par la propagation d'un potentiel d'action antidromique induit par la stimulation du nerf et qui recrute directement le motoneurone sans passer par les fibres Ia (onde F). Ceci concorde avec l'absence de diminution du réflexe à des intensités supramaximales, suggérant que les potentiels orthodromiques et antidromiques se propageant dans le motoneurone n'entrent pas en collision.

1.5.2 Action pharmacologique de la buspirone

L'effet caractéristique de la buspirone serait induit par son rôle d'agoniste partiel des récepteurs 5-HT_{1A} (Loane and Politis, 2012). De façon simplifiée, un agoniste partiel est une drogue se liant au récepteur et l'active mais avec une efficacité moindre qu'un agoniste complet (Calvey and Williams, 2008). L'agoniste partiel possède des caractéristiques qui lui sont propres dépendamment de la quantité d'agoniste complet présent ainsi que la densité des récepteurs (Zhu, 2005).

Les récepteurs 5-HT_{1A} sont retrouvés en grande quantité de L3-L5 dans les laminae II-IV, VII et IX (Otoshi et al., 2009). Malgré la présence de récepteur 5-HT_{1A} au niveau des motoneurones, peu d'études ont évalué l'influence de la buspirone sur l'excitabilité des ceux-ci. Les résultats d'une étude suggèrent que la buspirone ne dépolarise pas les motoneurones mais atténuerait l'inhibition du réflexe-H d'origine supra-spinale (Yomono et al., 1992). La buspirone interagit également avec le système dopaminergique. La buspirone atténue l'effet dyskinésique de la L-DOPA de façon dose-dépendante par un mécanisme médié par les récepteurs 5-HT_{1A} (Eskow et al., 2007). Elle est sans effet sur la transmission glutamatergique et GABAergique (Deyn and Macdonald, 1988).

La buspirone possède une demi-vie entre 30-90 minutes lors d'administration i.v. (5 mg/kg) et environ 60 minutes lors d'administration orale (20 mg/kg) chez le rat (Caccia et al., 1983, Kim et al., 2016). Aucune étude n'a spécifiquement évalué la demi-vie de la buspirone administrée en i.p., mais il est considéré qu'elle agit pendant au moins 20 minutes à la suite d'une injection i.p., couvrant ainsi la période de temps lors de laquelle la souris était entraînée dans notre protocole.

1.5.3 Paradigme de double lésion

Afin d'évaluer l'effet de la buspirone sur l'adaptation des réseaux locomoteurs spinaux, nous avons caractérisé l'effet de la buspirone sur la récupération chez des souris à la suite d'un paradigme de double lésion. Ce modèle expérimental développé chez le chat (Barrière et al., 2008, Martinez et al., 2011) permet à l'animal de récupérer d'une hémilésion latérale avant de complètement sectionner la moelle épinière au même niveau ou un niveau à proximité. Chez le chat, ce type de modèle permet d'observer un inversement des déficits moteurs médiés par la section : les déficits sont plus importants du côté opposé à l'hémilésion dû à la préservation partielle des fonctions locomotrices du côté de l'hémilésion. Ce résultat ne peut être expliqué par des changements supraspinaux ou propriospinaux, démontrant ainsi que des changements plastiques ciblant les réseaux locomoteurs spinaux sous l'hémilésion ont déjà eu lieu. Nous avons adapté ce modèle pour la souris afin de répondre à notre deuxième objectif pour cette étude. Brièvement, une hémilésion latérale gauche au niveau de T7 était effectuée. L'animal était entraîné quotidiennement sur tapis roulant pendant 3 semaines, avec de l'aide fournie par l'expérimentateur au besoin et le patron locomoteur évalué une fois par semaine. Avant la section, peu ou pas de déficit n'était observé dans le patron locomoteur. À la suite de la section, l'entraînement était réinitialisé, de même que l'évaluation du patron locomoteur une fois par semaine.

Chapitre II: Article 1 - Lumbar muscle inflammation alters spinally-mediated locomotor recovery induced by training in a mouse model of complete spinal cord injury

Jeffrey-Gauthier R, Piché M, Leblond H (2017). Lumbar muscle inflammation alters spinally-mediated locomotor recovery induced by training in a mouse model of complete spinal cord injury. Neuroscience. 359 :69–81.

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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.

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 to 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., 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 sub-lesional 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 transection. 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 transection 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 transection 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, with perineal pinching triggering locomotion. If no locomotion was triggered, passive hind limb cycling movements were supplied manually by the experimenter.

Assessment of locomotor recovery

Locomotion of each mouse was measured at different times prior to (baseline acquisition) and after complete SCI (on days 2, 7, 14, 21 and 28). A high-speed camera (Proselica GC, Allied Vision Technologies, Irwin, PA, USA; 90 frames/s), placed perpendicularly to the treadmill, recorded (StreamPix 5 software, NorPix, Montreal, QC, Canada) the longest bout of consecutive step cycles that the mice could complete during 5-min sessions. Intra-limb coordination was assessed with kinematics evaluation of left hind limb joint movement during locomotion. Anatomical landmarks (iliac crest, greater trochanter, knee, ankle, 5^{th} metatarsophalangeal (MTP) joint and tip of the 5^{th} toe) were flagged in black for later conversion of x-y coordinates to measure angular excursion of the hip, knee, ankle and MTP joints on the left hind limb throughout the step cycles. Hindpaw contact and lift events were labeled manually for the left and right hind limbs.

Locomotor cycles were divided into stance and swing subphases, stance lasting from one contact to the next ipsilateral lift, and swing lasting from one lift to subsequent ipsilateral paw contact on the treadmill. Paw drag frequently substituted paw elevation during swing after SCI, in which case, stance would be defined as backward hindpaw movement and swing as forward movement. Stance and swing durations were measured together, with the proportion of drag during swing being reported separately. Step length was measured at each locomotor cycle along with the ability of the mice to achieve proper paw placement at contact in front of the hip joint. Paw placement is reported as the horizontal distance between the hip joint and the contact point between the paw and the treadmill belt. Positive values represent paw contact in front of the hip joint while negative values represent paw contact behind the hip joint. Hind limb coupling was assessed as a measure of inter-limb coordination as it represents the phase relation between left and right step cycles. Coupling was calculated at each step cycle as the timing at which the left hind limb begins a cycle in relation to the ongoing right limb cycle and expressed in radians. Thus, for perfectly-alternated steps, coupling value would be π, while in hopping it would be 0 or 2π.

Terminal experiment

H-reflex was recorded in each mouse 28 days after complete SCI. This terminal experiment was performed on decerebrated unanesthetized mice to avoid anesthesia-induced spinal hypoactivity. H-reflex was successfully recorded in more than 80% of the mice (23/28) with near equal distribution across groups: control spinal mice (5/8), trained spinal mice (7/7), spinal mice with CFA (6/7), and trained spinal mice with CFA (4/6).

Decerebration procedure

Decerebration, a commonly-used procedure assessing spinal activity in many animal models, has recently been adapted for mice (Meehan et al., 2017, Meehan et al., 2012, Nakanishi and Whelan, 2012). Briefly, the mice were prepped under isoflurane anesthesia (2% mixed with O_2 95% and CO_2 5%, 200 ml/min). They were tracheotomized to allow artificial ventilation (SAR-830/P Ventilator; CWE, Inc., Ardmore, PA, USA). Body temperature was monitored by rectal probe and maintained at 37±0.5 °C with a heating pad. Both carotid arteries were ligated to decrease cerebral perfusion. The mice were secured in a stereotaxic frame, and their skulls were opened bilaterally from 2 mm anterior to the bregma to 1 mm anterior to occipital suture and from temporalis muscle attachment on both sides to as close as possible to midline sagittal suture. Brain tissue was then aspirated by micro-vacuum following a 45° line between the caudal cortex and the optic chiasm (precollicular, premamillary decerebration). The intracerebral void was then filled with absorbable hemostat (Surgicel) and gauze, and the scalp was sutured.

H-reflex recording protocol

After decerebration, an opening was made in the left leg, and the lateral gastrocnemius muscle was separated gently from the medial gastrocnemius to expose the tibial nerve. A paraffin oil pool was set to avoid desiccation of the nerve while it was mounted on a bipolar hook electrode for stimulation. Bipolar wire electrodes (Cooner Wire Company, Chatsworth, CA, USA) were inserted in the interosseous muscle of the hindpaw for EMG recording and a ground electrode was inserted in the skin between the stimulating and recording electrodes. Only then was isoflurane anesthesia discontinued and followed by 30-min rest to avoid anesthesia-induced H-reflex alteration. One-ms single-pulsed stimulations were delivered by a constant-

current stimulator (Model DS4, Digitimer Ltd., Welwyn Garden City, UK) and triggered by a computer-controlled sequencer (Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK).

First, graded stimulations at an inter-stimulus interval of 5 s (0.2 Hz) were delivered to generate H-reflex stimulus-response curves. A stimulus intensity that produced maximal H-reflex with stable M-wave closest to initiation of the M-wave plateau (≈1.8 x motor threshold: MT) was then selected to preferentially recruit Ia sensory fibers. This stimulus intensity was used in another set of stimulations where frequency was varied between 4 blocks of 25 stimulations (0.2, 5, 10 and 0.2 Hz) to assess FDD, mediated by spinal inhibitory processes. A 60-s inter-block interval was set. The first 5 responses of each block were discarded to allow H-reflex stabilization. Further analyses comprised only recordings with stable M-wave throughout the protocol (<15% variation between blocks), sufficient H-reflex responses at baseline (>10% of M-wave) and return of the H/M ratio to baseline in recovery block. Electromyography (EMG) signals were amplified (1,000X), bandpass-filtered (3–3,000 Hz, Grass Instruments, Quincy, MA, USA), digitized (sampling rate: 10 KHz) and recorded for offline analyses (Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK).

Histology and confirmation of pathological changes

Isoflurane exposure was resumed (4%, 300 ml/min) at the end of the terminal experiment, and these deeply-anesthetized animals were perfused through the heart with physiological saline (0.9%) and formalin (10% in 0.1 M phosphate buffer). Fixed lumbar paraspinal muscles were then resected bilaterally and kept overnight in formalin. Muscle tissues were dehydrated, embedded in paraffin wax, cut longitudinally into 8-μm slices and stained with hematoxylin, eosin and safran to confirm the CFA-induced inflammatory response.

Data analysis and statistics

Locomotor parameters were assessed by custom-made locomotion analysis software (Expresso, courtesy of Prof. Serge Rossignol). Joint movement is reported as the mean

excursion between maximal flexion (low angular value) and maximal extension (high anglular value) measured for each step cycle. Hind limb coupling values were plotted for each mouse at each time point during recovery and represented by vector. The direction of this vector expresses mean coupling values (C values), while its magnitude (R values) is a measure of concentration around the mean. R values are the opposite of dispersion, where values closer to 1 indicate lesser variance in the data (Zar, 1999). The data were pooled by CircStat toolbox (Berens, 2009) in MATLAB (Mathworks, Natick, MA, USA).

In the terminal experiment, H-reflex and M-wave recordings were analyzed with Spike2 software (version 8.02; Cambridge Electronic Design). To quantify M-wave and H-reflex, peak-to-peak amplitude of the EMG signals was calculated in distinct post-stimulation time windows (1–3 ms and 5–8 ms, respectively). MT and H-reflex threshold were determined as the lowest intensity producing M-wave and H-reflex, respectively. The M response and H-reflex of greatest amplitude (Mmax and Hmax, respectively) served to calculate the Hmax/Mmax ratio. The intensities at which the H threshold and Hmax were observed were expressed as multiples of MT. In the FDD protocol, mean H/M ratio was averaged from 20 successive responses for each block.

All results are expressed as mean ± SD. Statistical analyses were conducted with Statistica (version 13, Statsoft Inc., Tulsa, OK, USA), and the significance threshold was set at $p \leq 0.05$. Data distribution was confirmed with the Kolmogorov-Smirnov test to perform parametric tests. Non parametric tests were used for data that was not normally distributed.

Body weight values and improvement of locomotor parameters (number of steps achieved, joint angular excursions, stance and swing duration, paw drag, step length and paw placement) over time (days 7, 14, 21 and 28) and between groups (control spinal mice, trained spinal mice, spinal mice with CFA and trained spinal mice with CFA) were evaluated by repeated-measures ANOVA. To assess inter-limb coordination, hind limb coupling was compared between groups by the Kruskal-Wallis test for circular data (Fisher, 1995). This test was repeated for each time point to gauge inter-limb coordination recovery over time. Locomotor parameters were measured on day 2 but were excluded from statistical analyses since locomotion was not achieved at this time. Therefore, only descriptive statistics are reported for that time point.

Hmax/Mmax values from the terminal experiment were compared between groups by 1-way ANOVA, while H/M ratios calculated in the FDD protocol were compared between groups and frequencies by mixed ANOVA. For ANOVAs, planned comparisons were used to decompose significant effects and test *a priori* hypotheses. For the significant interaction between GROUP and TIME, this allowed the comparison 1) between control spinal mice and trained spinal mice to evaluate the impact of training, 2) between control spinal mice and spinal mice with CFA to evaluate the impact of CFA and 3) between trained spinal mice and trained spinal mice with CFA to evaluate the impact of CFA on training. Pearson's correlations were used to assess the associations between angular excursions, paw drag and FDD, with reporting of correlation coefficients.

Results

Chronic inflammatory changes in muscles after CFA injection

Chronic inflammatory changes in response to CFA injection were confirmed by lumbar muscle histology. As expected, CFA induced chronic inflammation in the muscles of all injected mice. As illustrated by individual examples in Figure 2.1, muscle tissues from mice injected with CFA manifested leukocytic infiltration (Figure 2.1B), which was not observed in any mice from the control groups (Figure 2.1A). This result confirms that inflammatory changes persisted until the terminal experiment.

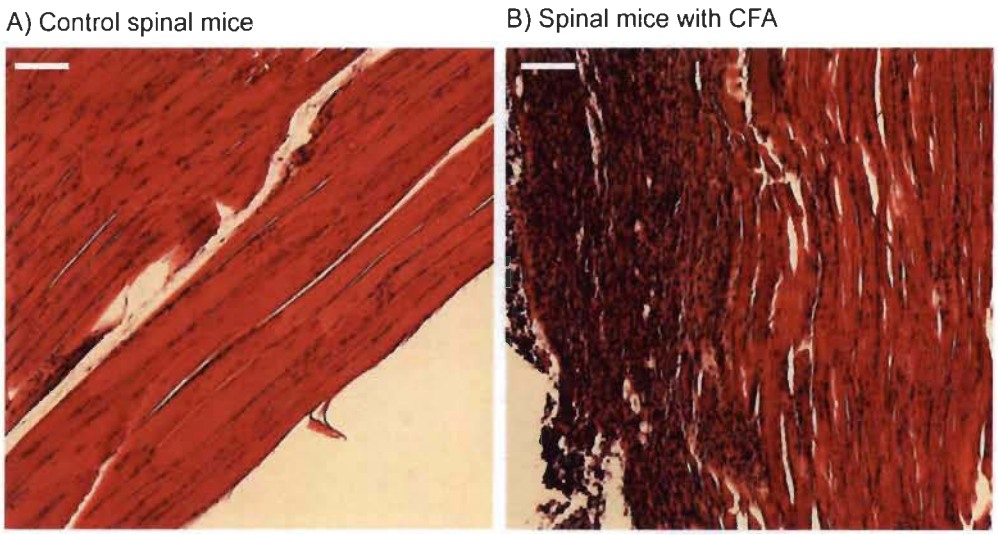

Figure 2.1. CFA-induced chronic inflammatory changes in lumbar muscles.

Histological inspection of lumbar muscles in control spinal mice (A) and spinal mice injected with CFA (B) revealed CFA-specific leukocytic infiltration in lumbar muscle at day 28 after complete SCI. Scale bar = 100 mm.

General health condition

Body weight was monitored to ensure that animals maintained good general health during recovery. Changes in body weight were comparable between groups (Table 2-1). Apart from the expected body weight loss on day 2 following spinal transection, body weight increased significantly over time from day 7 to day 28 (main effect: $F_{3, 72} = 10.7$, $p < 0.001$, $\eta_p^2 = 0.31$). These changes were also comparable between groups (interaction: $F_{9, 72} = 1.0$, $p = 0.49$, $\eta_p^2 = 0.11$).

Table 2-1. Body weight values, number of steps, stance and swing duration, step length and paw placement improvement over time.

	Day post-SCI	Control spinal mice		Trained spinal mice		Spinal mice with CFA		Trained spinal mice with CFA	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight values	7	27.9	1.7	24.3	1.3	26.4	1.8	25.2	1.9
	14	28.3	2	23.9	1.4	26.5	2	26	1.5
	21	28.6	2.1	24.4	1.3	26.7	1.6	26.4	2.5
	28	28.8	2	25.1	1.5	27.6	1.8	26.5	2.4
Number of steps	7	6.4	3.3	10.4	5.5	4.4	3	7.5	3.4
	14	7.7	2.1	12	2	9	2.2	10	4.5
	21	11.7	4.1	13.1	3.5	10.1	4.9	8.8	1.8
	28	13.3	5.4	16	8.6	8.3	3.8	10.2	1.9
Stance duration	7	356.2	270.5	196.9	77.6	332	180.7	363.8	241.8
	14	284.4	175.7	173.1	64.3	335.4	177.3	315.1	180.6
	21	242.1	57.5	165.1	21.8	319.9	91.5	275.4	80.4
	28	261.5	97.6	185	39.7	332.2	64.5	270.1	40.3
Swing duration	7	120.7	49.4	93.2	18.8	152.3	51	144	35.4
	14	108.2	21.8	82.2	14.9	128.2	33.8	119.7	26.6
	21	106.1	21.3	86.4	14.3	115.9	23.9	109.1	27.2
	28	111.6	34.6	90.2	20.5	104.4	14.7	109.1	22.5
Step length	7	11.1	6	16.1	4.8	9.1	7.7	11.8	5.2
	14	20.2	4.8	21.1	6.1	22.4	8.1	21.6	9
	21	26.8	6.7	27.5	4.3	24.1	12.8	28.4	7.8
	28	29.7	5.1	32	9.9	26	10.1	26.4	6.3
Paw placement	7	-17	6.1	-17.3	6	-15.6	9.1	-15.1	4.9
	14	-8.6	6.9	-10.3	6.8	-1.9	9.3	-5	9.6
	21	-3.7	10.8	-4.6	5.9	-3.2	10.2	-0.7	8.1
	28	-1.6	4.8	0.3	7.9	-1.6	9.2	-0.8	7.8

General health and locomotion parameters were not different between groups during recovery (no significant interaction). See Results section for additional details on main effects of TIME and GROUP.

Improvement of maximal number of steps achieved after complete SCI

As expected, stepping was completely abolished on day 2 after complete SCI in all mice. However, the number of consecutive steps increased significantly over time (main effect: $F_{3, 72} = 8.4$, $p < 0.001$, $\eta_p^2 = 0.26$; Table 2-1). Moreover, the number of steps taken was significantly different between groups (main effect: $F_{3, 72} = 4.8$, $p < 0.01$, $\eta_p^2 = 0.37$). Nevertheless, improvement in the number of steps over time was not different between groups (interaction: $F_{9, 72} = 1.2$, $p = 0.32$, $\eta_p^2 = 0.13$). Planned comparisons for the main effect of time revealed that the number of steps was significantly increased between successive time points (p's <0.01) until day 14, after which no subsequent improvement was seen (no difference between days 21 and 14, $p = 0.16$, and between days 28 and 21, $p = 0.42$). In addition, planned contrast for the main effect of group disclosed that stepping improved in trained spinal mice compared to control spinal mice ($p < 0.05$) and trained spinal mice with CFA ($p < 0.05$).

Restoration of intra-limb coordination during the recovery period

Kinematics evaluation of the left hind limb reveal intra-limb coordination recovery after complete SCI. Figure 2.2A illustrates how angular excursion was measured during locomotion. Figure 2.2B and 2.2C respectively show knee and MTP angular excursion at days 7, 14, 21 and 28 with dashed lines expressing baseline mean joint excursion measured prior to complete SCI. As expected, hind limb kinematics during locomotion was markedly altered on day 2 after complete SCI as almost no movement could be observed except for small hip and knee flexions without involvement of the ankle and MTP joints. Recovery was characterized by progressive improvement of angular excursion over time, and was apparent in all joints and in each group. While this effect did not reach significance in hip and ankle angular excursion recovery, knee angular excursion improved significantly over time (main time effect: $F_{4, 72} = 11.2$, $p < 0.001$, $\eta_p^2 = 0.38$) with significantly different angular excursions between groups (main effect: $F_{3, 72} = 11.7$, $p < 0.001$, $\eta_p^2 = 0.66$). Moreover, knee angular excursion recovery over time was different between groups (interaction: $F_{12, 72} = 1.9$, $p = 0.05$, $\eta_p^2 = 0.24$).

Planned comparisons revealed that training significantly improved knee angular excursion, as knee excursion was increased in trained spinal mice versus control spinal mice at

days 14 ($p < 0.05$), 21 ($p < 0.05$) and 28 ($p < 0.01$). Comparison between spinal mice with CFA and control spinal mice showed that CFA injection decreased knee angular excursion, but the difference reached significance only on day 7 ($p < 0.05$). Knee angular excursion dwindled in trained spinal mice with CFA compared to trained spinal mice on days 7 ($p < 0.001$) and 14 ($p < 0.01$) and marginally on day 28 ($p = 0.06$; Figure 2.2B).

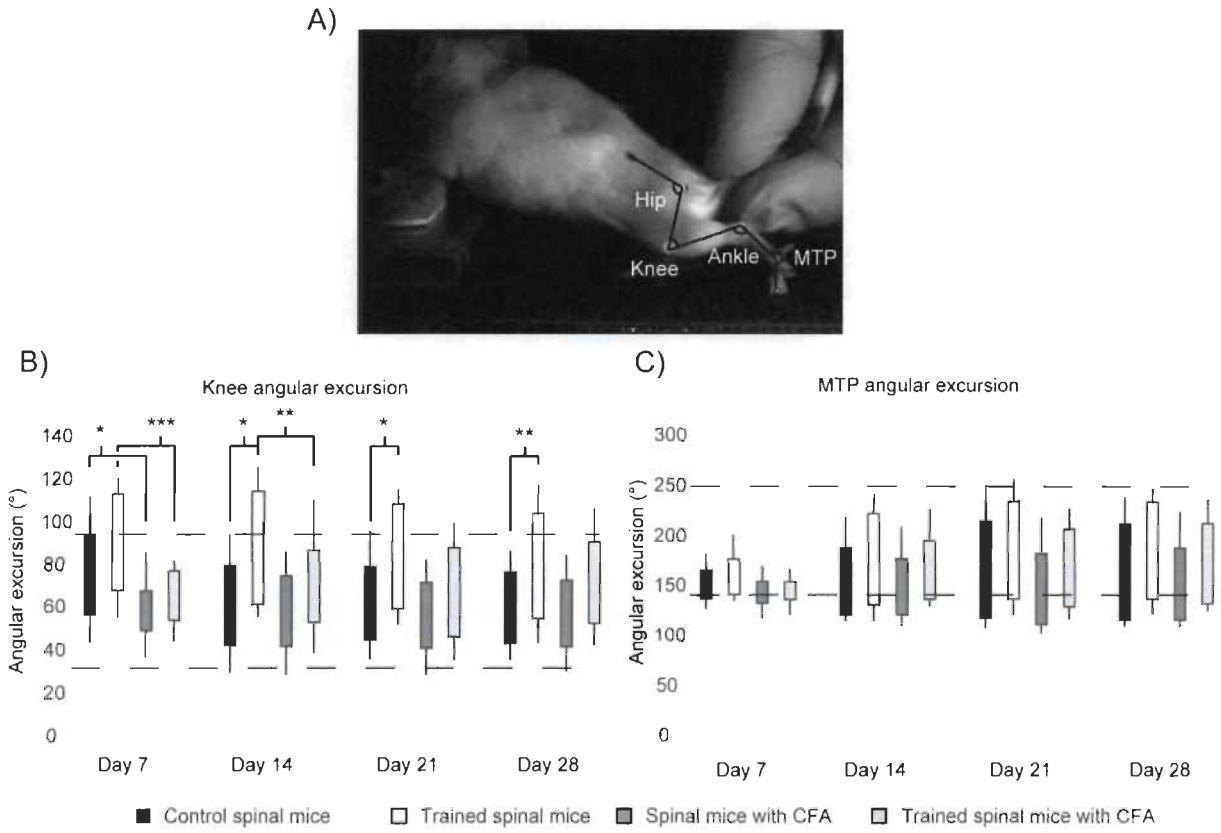

Figure 2.2. Mean knee and MTP joints angular excursion.

Angular excursion measured between maximal flexion (low angular value) and maximal extension (high angular value). Angular excursion (A) was altered at the knee and MTP joints over recovery period. Knee excursion was increased in trained spinal mice compared to control spinal mice and trained spinal mice with CFA (B). Maximal MTP angulation (top half of histogram bars) was decreased significantly in spinal mice with CFA and trained spinal mice with CFA compared to trained spinal mice throughout recovery (C). Superior and inferior dashed lines represent maximal extension and flexion angulation during locomotion, respectively, averaged from excursion values from all mice in intact conditions. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Figure 2.2C illustrates MTP joint kinematics over time in comparison to pre-SCI (dashed lines). Since MTP angular excursion is increased by inadequate placement of the dorsum of the paw on the treadmill (hyperflexion) and maximal angulation is influenced by adequate placement of the sole of the paw on the treadmill, the latter was assessed to quantify MTP involvement in recovery (see upper ends of histogram bars in Figure 2.2C). For all groups combined, maximal MTP angulation increased significantly over time (main effect: $F_{3, 72} = 39.0$, $p < 0.001$, $\eta_p^2 = 0.63$). Moreover, maximal MTP angulation was significantly different between groups (main effect: $F_{3, 72} = 4.1$, $p < 0.05$, $\eta_p^2 = 0.35$). However, maximal MTP angulation increases over time were not significantly different between groups (interaction: $F_{9, 72} = 0.7$, $p = 0.72$, $\eta_p^2 = 0.08$). Planned comparisons for the main effect of group revealed that maximal MTP angulation was marginally augmented in trained spinal mice versus control spinal mice and trained spinal mice with CFA ($p = 0.065$ and $p = 0.04$, respectively; Figure 2.2C).

Stance and swing duration alterations after complete SCI

Stance and swing durations were increased on day 2 after complete SCI. Changes in stance were significantly different between groups (main effect: $F_{3, 72} = 3.1$, $p < 0.05$, $\eta_p^2 = 0.28$), but with no difference observed over time (main effect: $F_{3, 72} = 1.7$, $p = 0.17$, $\eta_p^2 = 0.07$). Also, differences in stance between groups did not vary over time (interaction: $F_{9, 72} = 0.4$, $p = 0.95$, $\eta_p^2 = 0.04$). Planned comparisons for the main effect of group revealed that compared to trained spinal mice, stance duration was marginally decreased in control spinal mice ($p = 0.06$) and significantly declined in trained spinal mice with CFA ($p < 0.05$; see Table 2-1).

Alteration of swing duration was significantly different between groups (main effect: $F_{3, 72} = 5.4$, $p < 0.01$, $\eta_p^2 = 0.40$), and over time (main effect: $F_{3, 72} = 5.2$, $p < 0.01$, $\eta_p^2 = 0.18$). However, improvement over time was not significantly different between groups (interaction: $F_{3, 72} = 1.0$, $p < 0.48$, $\eta_p^2 = 0.11$). Planned comparisons for the group effect showed that compared to trained spinal mice, swing duration was significantly shorter in control spinal mice ($p < 0.03$) and in trained spinal mice with CFA ($p < 0.01$; see Table 2-1).

Improvement of hindpaw drag during recovery

As expected, 2 days after complete SCI, hindpaw elevation during swing was completely abolished in all groups, leading to hindpaw drag on the treadmill during the entire swing phase (Figure 2.3). Partial recovery of paw elevation during swing was achieved within 28 days after complete SCI. Accordingly, for all groups combined, paw drag decreased significantly over time post-SCI (main effect: $F_{3, 72} = 24.6$, $p < 0.001$, $\eta_p^2 = 0.51$). Moreover, hindpaw drag was different between groups (main effect: $F_{3, 72} = 9.2$, $p < 0.001$, $\eta_p^2 = 0.54$). In addition, the decrease in hindpaw drag over time was different between groups (interaction: $F_{9, 72} = 2.1$, $p < 0.05$, $\eta_p^2 = 0.21$). Planned contrasts revealed that hindpaw drag was reduced in trained spinal mice compared to control spinal mice (Figure 2.3), and this difference reached significance on days 14 ($p < 0.001$), 21 ($p < 0.01$) and 28 ($p < 0.01$). Moreover, hindpaw drag was significantly decreased in trained spinal mice compared to trained spinal mice with CFA on days 7, 14, 21 and 28 (p's < 0.05).

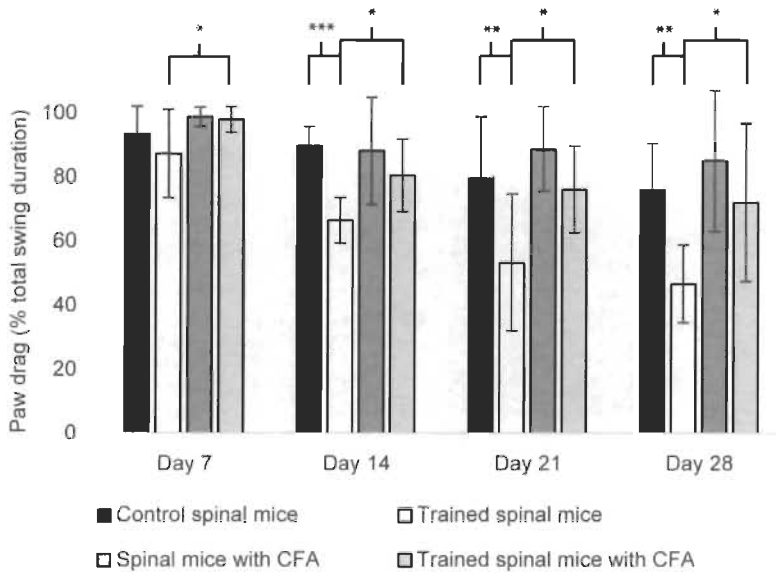

Figure 2.3. Mean paw drag during swing.

Paw drag on the treadmill during swing phase was decreased significantly in spinal mice compared to control spinal mice for days 14, 21 and 28. Similar discrepancies were observed between trained spinal mice compared to trained spinal mice with CFA for days 7, 14, 21 and 28. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Step length increases during recovery attributed to improved paw placement at contact

Consistent with the kinematics alterations, step length was severely reduced after complete SCI (see Table 2-1). Although no significant difference was evident between groups (main effect: $F_{3, 72} = 0.4$, $p < 0.76$, $\eta_p^2 = 0.05$), step length increased significantly over time (main effect: $F_{3, 72} = 81.8$, $p < 0.001$, $\eta_p^2 = 0.77$). Similarly, no difference was observed between groups for paw placement (main effect: $F_{3, 72} = 0.3$, $p < 0.83$, $\eta_p^2 = 0.03$), although it improved significantly over time in all groups combined (main effect: $F_{3, 72} = 50.2$, $p < 0.001$, $\eta_p^2 = 0.68$). Variation over time did not differ between groups regarding both step length (interaction: $F_{3, 72} = 1.4$, $p = 0.19$, $\eta_p^2 = 0.15$) and paw placement (interaction: $F_{3, 72} = 1.1$, $p = 0.38$, $\eta_p^2 = 0.12$).

Recovery of inter-limb coordination

Hind limb coupling recovery was assessed to compare inter-limb coordination improvement over time between groups. Hind limb coupling is illustrated in Figure 2.4 with vector plots, in which downward vectors indicate perfect alternation between left and right hind limb movement. Generally, all mice retrieved an alternated locomotor pattern within 28 days after complete SCI. However, hind limb coupling was significantly different between groups (Kruskal-Wallis: $p < 0.05$). Planned contrasts for the effect of group revealed marginally poorer coupling in spinal mice with CFA compared to control spinal mice ($p = 0.07$) and significantly poorer coupling in trained spinal mice with CFA compared to trained spinal mice ($p < 0.05$). No difference was observed between control spinal mice and trained spinal mice ($p = 0.59$). Also, coupling in trained spinal mice with CFA compared to trained spinal mice was not significantly different on days 7 ($p = 0.39$), 14 ($p = 0.39$), 21 ($p = 0.17$) and 28 ($p = 0.39$).

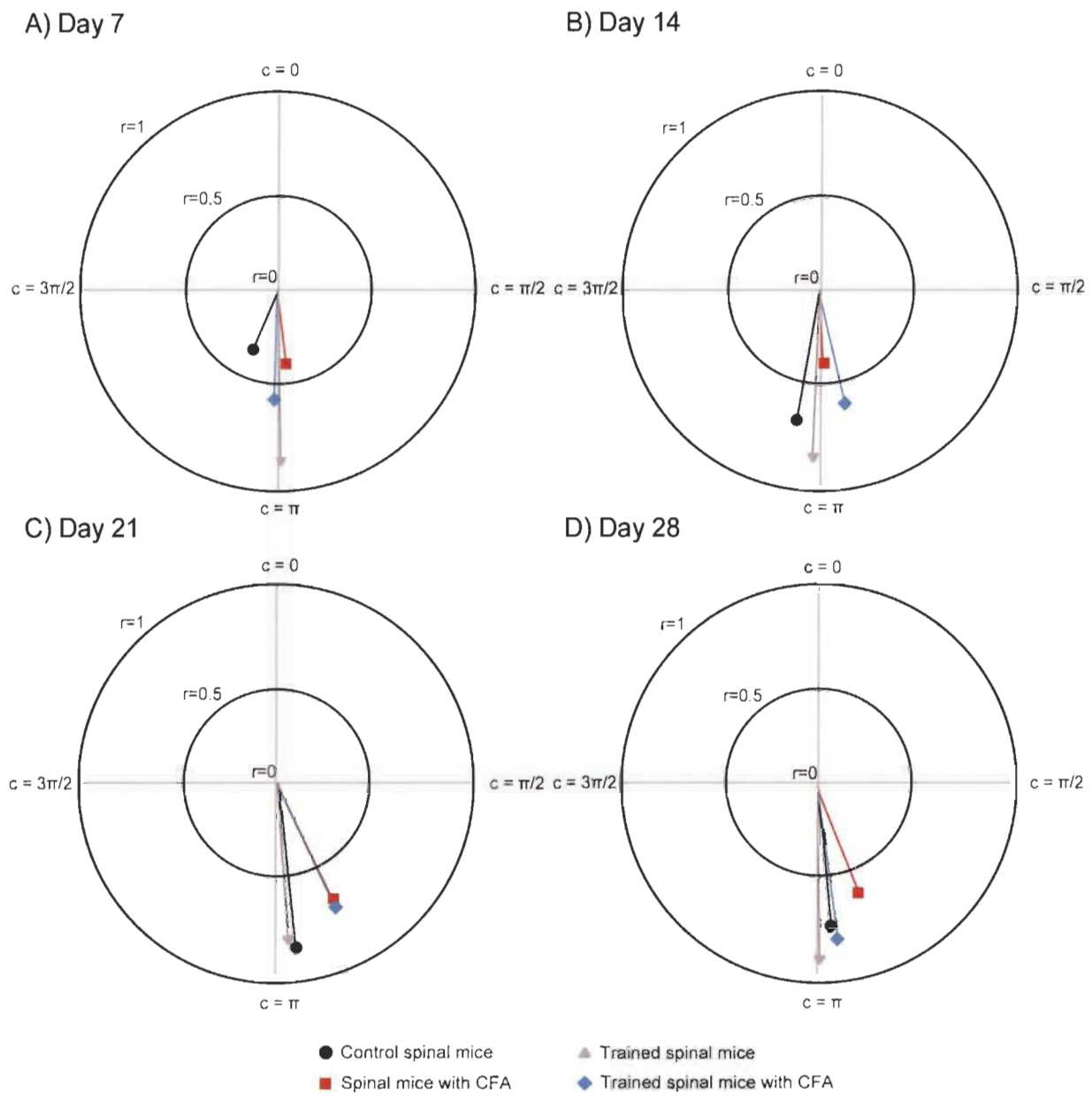

Figure 2.4. Hind limb coupling recovery.

Mean hind limb coupling phase relation (C values) and concentration around mean (R values) are shown for each group over time. Coupling was improved in trained spinal mice on day 7 (A) and depressed in spinal mice with CFA on days 14 (B), 21 (C) and 28 (D) compared to control spinal mice, but these effects did not reach significance.

State of Hoffmann-reflex activity 28 days after complete SCI

No difference between groups was apparent in H-reflex recruitment properties on day 28 after complete SCI. For all groups combined, M-response and H-reflex latencies were 1.77 ± 0.22 ms and 5.44 ± 0.43 ms, respectively. Stimulus intensities for H-reflex threshold and H-reflex maximal amplitude were 0.92 ± 0.20 x MT and 1.59 ± 0.59 x MT, respectively) while Hmax/Mmax was 0.33 ± 0.15 arbitrary units (Table 2-2).

Table 2-2. M-wave and H-reflex recruitment properties

	Control spinal mice		Trained spinal mice		Spinal mice with CFA		Trained spinal mice with CFA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
M latency (ms)	1.68	0.24	1.76	0.06	1.72	0.26	1.91	0.33
H latency (ms)	5.77	0.79	5.28	0.29	5.22	0.20	5.47	0.42
H threshold (x MT)	0.92	0.18	0.95	0.19	0.88	0.15	0.94	0.26
Hmax (xMT)	1,22	0,23	1,63	0.56	2.13	1.27	1.36	0.30
Hmax/Mmax	0.42	0.21	0.28	0.16	0.30	0.14	0.30	0.09

M- and H-response latencies were similar between groups. Stimulus intensities required to evoke M- and H-response thresholds as well as M- and H-response maximal amplitudes showed no difference between groups.

As for H-reflex FDD, increasing tibial nerve stimulation frequency resulted in decreased H/M ratio in all groups (see individual example in Figure 2.5A). However, the H/M ratio was significantly different between groups (main effect: $F_{3, 38} = 4.6$, $p < 0.05$, $\eta_p^2 = 0.42$), and the expected frequency effect occurred (main effect: $F_{2, 38} = 79.7$, $p < 0.001$, $\eta_p^2 = 0.81$). In addition, frequency-dependent changes in H/M ratio were significantly different between groups (interaction: $F_{6, 38} = 2.7$, $p < 0.05$, $\eta_p^2 = 0.30$). Planned contrasts revealed that H/M ratio was less depressed in control spinal mice at both 5 and 10 Hz (p's <0.05; Figure 2.5B) than in trained spinal mice. Moreover, H/M ratio was less depressed in spinal mice with CFA compared to trained spinal mice with CFA, but this difference reached significance only at 5 Hz ($p < 0.05$). Also, less depressed H/M ratio at 5 Hz and 10 Hz was associated with more paw drag ($r = -0.47$,

p <0.05 and r = -0.50, p <0.05, respectively; Figure 2.5C). Moreover, less depressed H/M ratio was associated with decreased maximal knee angulation, but reached significance only at 5 Hz (r = -0.43, p < 0.05; Figure 2.5D).

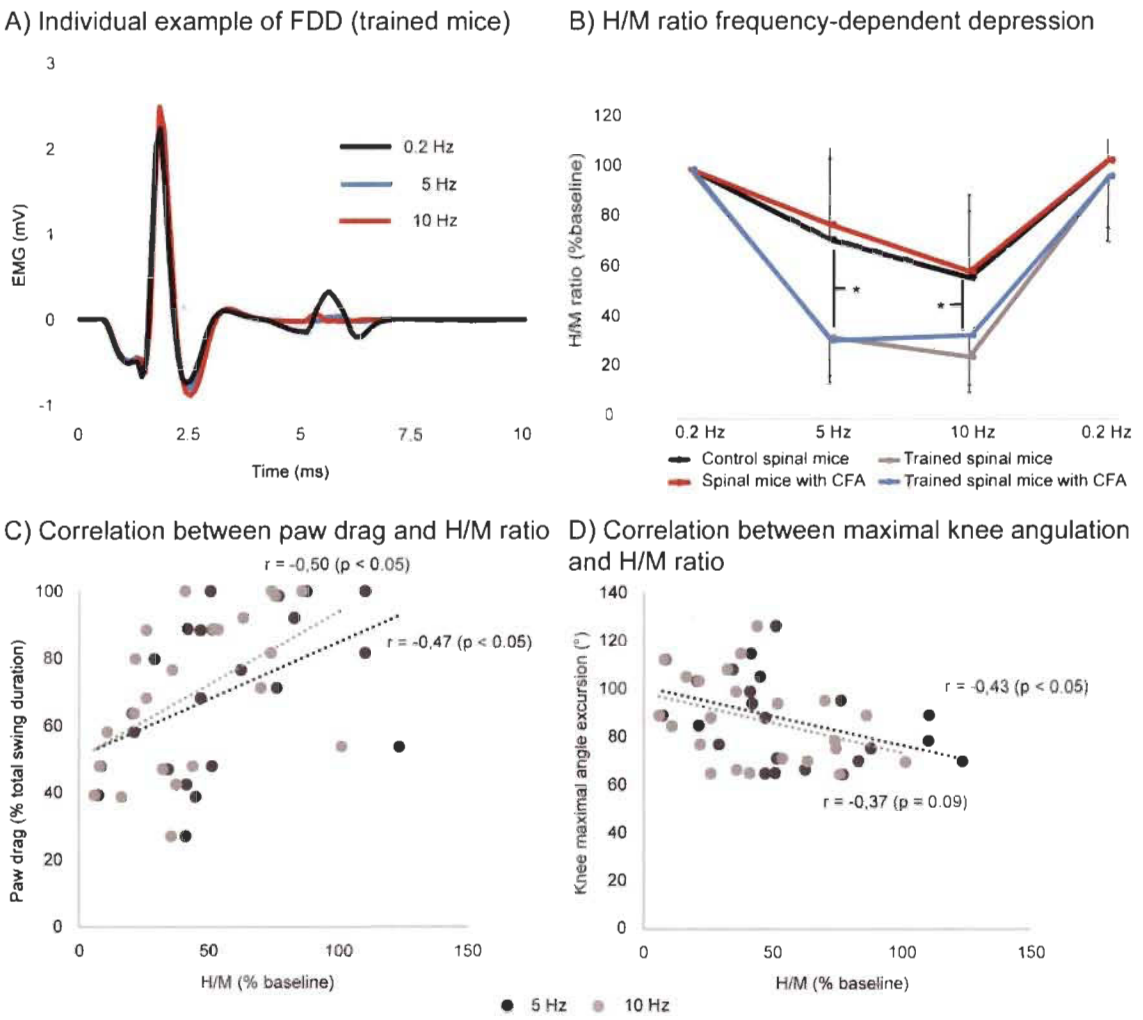

Figure 2.5. Frequency-dependent depression of the H-reflex.

Peak-to-peak amplitude of H- and M-responses was compared using the H/M ratio between frequency blocks. It was nearly abolished in trained spinal mice and spinal mice with CFA (individual example in A, pooled data in B) but not in control spinal mice and spinal mice with CFA (B). Less depressed H/M ratio was associated with increased paw drag (C), and decreased knee maximal angle excursion (D). *p <0.05.

Discussion

A novel finding of the present study is that lumbar muscle inflammation below the lesion impacts the beneficial effects of training on locomotor recovery. Unlike previous experiments, the current model of complete SCI excluded the contribution of supraspinal centers. Thus, the effects of inflammation on locomotor spinal networks and locomotor recovery are of purely spinal origin. In addition, the electrophysiological data showed that preservation of spinal inhibition (FDD of H-reflex) is a good marker of the positive impact of training on recovery, allowing the effects of inflammation to be tested.

Lumbar muscle inflammation alters locomotor re-expression

In various animal models, locomotion resulting from locomotor spinal networks activation occurs spontaneously when animals are stimulated appropriately after complete SCI. In the present study, the hind limbs moved in alternation as early as day 7, locomotor movements being mainly restricted to small hip flexions and extensions with paw dragging on the treadmill. Over time, control spinal mice recovered treadmill locomotion with partial paw elevation and movement involving the mobilization of all joints, including the ankle and MTP joints. Training enhanced locomotor re-expression, mostly by improving knee angular excursion and reducing paw drag on days 14, 21 and 28. In addition, training reduced swing duration and increased stepping as well as maximal MTP angulation. These results support previous findings showing that training allows locomotor recovery in 2 to 3 weeks after complete SCI in mice (Leblond et al., 2003) and other animal models (Lovely et al., 1990, Chau et al., 1998, de Leon et al., 1998, Martinez et al., 2012, Alluin et al., 2015) by inducing activity-dependent plastic changes in locomotor spinal networks.

The main finding of this study is that lumbar muscle inflammation abolishes training-induced locomotion improvement after complete SCI. Indeed, all training-induced locomotor improvements (i.e., number of steps taken, knee and MTP angular excursion, swing durations and paw drag) were altered in CFA mice. These results demonstrate that activity-dependent plasticity after complete SCI is influenced by peripheral inflammation. Similarly, previous studies showed that peripheral nociception (Hook et al., 2008) and randomly-delivered electrical

stimulation (Grau et al., 1998) prevent adaptive conditioning of the withdrawal reflex (Grau et al., 1998) by modulating spinal plasticity (Grau et al., 2014). This metaplasticity is suspected to be responsible for nociception-induced locomotor deficits after spinal contusion injury (Garraway et al., 2011). The abolition of most training-induced locomotion improvements after complete SCI in the present study is consistent with and extends these findings, showing that neuroplasticity occurs in spinal locomotor networks, independently of supraspinal structures and descending regulatory pathways. Moreover, inter-limb coordination conveyed by locomotor spinal networks was altered by inflammation, with or without training. The impact of CFA injection into lumbar muscles on locomotor spinal networks activity shows that heterotopic nociceptive inputs affect hind limb motor control. This suggests that nociceptive muscle afferents from the back project to dorsal horn neurons with spinal networks being partly common to locomotor spinal networks. It is not known whether CPG neurons in adult mice are affected by nociceptive inputs. However, it has been shown previously that the chemical activation of Aδ- and C-fibers by intrathecal application of capsaicin on isolated neonatal mouse spinal cords completely abolishes CPG activity (Mandadi et al., 2009). Future studies could clarify whether the effects observed in the present work depend on CPGs by demonstrating the impact of nociceptive back afferents on fictive locomotion in decerebrated adult spinal mice. Additionally, the CFA pain model has been shown to induce a chronic activation of spinal microglia associated with TNF-α signaling in dorsal horn that receive nociceptive inputs elicited by the injection (Chacur et al., 2009). It is reasonable to suggest that increased TNF-α overexpression in dorsal horn at locomotor network levels and associated central sensitization are implicated in the present results (Huie et al., 2012). Previous work also showed that sensitization induced by TNF-α was associated with increased cell death after spinal cord injury and enhanced excitotoxicity to glutamate (Beattie et al., 2002, Ferguson et al., 2008, Garraway et al., 2014). While an increased rostro-caudal volume of the lesion cannot be completely ruled out in the present study, the completeness of the lesion and its location several levels rostral to lumbar CPGs suggest that increased cell death is not responsible for the enhanced locomotor deficits observed in spinal mice with CFA.

Training prevents the development of H-reflex disinhibition

The interactive outcomes of nociception and training are bilateral. In a previous investigation, it was shown that training could protect spinal networks from the deleterious effect of nociception on withdrawal reflex conditioning and could even reverse it when training followed nociceptive inputs (Baumbauer and Grau, 2011). In the present study, training partially reversed the influence of peripheral inflammation, as it prevented knee angular excursion deficits induced by CFA on day 7. Locomotor training may act via a mechanism other than nociception to induce adaptive changes in locomotor spinal networks. For instance, the present results showed that training prevented the development of H-reflex disinhibition. In rats, H-reflex disinhibition and associated attenuation of FDD occur around days 15 to 30 after SCI (Yates et al., 2008). The present data indicate similar alterations in mice since FDD was significantly decreased in control spinal mice compared to trained spinal mice on day 28. Moreover, H-reflex disinhibition was associated with poor locomotor recovery, as illustrated in Figure 2.5. These results support previously-reported associations between FDD preservation and functional recovery after partial SCI in rats (Lee et al., 2005) and mice (Lee et al., 2009). Moreover, the present findings uphold the protective impact of training on FDD after complete SCI as seen in rats (Reese et al., 2005, Yates et al., 2008, Côté et al., 2011, Côté et al., 2014). Interestingly, CFA did not alter positive training effects on FDD since no difference was observed between trained spinal mice and trained spinal mice with CFA, which suggests that H-reflex disinhibition and alteration of training-induced locomotor improvements by inflammation rely on distinct mechanisms.

The unanesthethized decerebrated preparation and its recent application to small mammals, including the mouse (Meehan et al., 2012, Nakanishi and Whelan, 2012, Meehan et al., 2017), has opened new ways to investigate spinal sensorimotor responses to pain and the related nociceptive processes. This was not possible in animals under anesthesia. Using such preparation, it was reported that the soleus H-reflex was altered by homonymous muscle inflammation, ischemia and fatigue in decerebrated spinal cats (Kalezic et al., 2004, Schomburg et al., 2007) and rats (Della Torre, 2002). In the present work, nociceptive inputs from the back muscles did not affect the hind limb monosynaptic reflex, which indicates that nociceptive inputs projecting to spinal segments receiving Ia afferent inputs do not modulate monosynaptic

reflexes under the present experimental conditions. On the one hand, while no FDD difference was seen between trained spinal mice and spinal mice with CFA on day 28, it is possible that differences could be detected earlier. On the other hand, lumbar muscle nociceptive inputs could have altered crossed facilitatory and crossed inhibitory reflex activity, as reported recently (Schomburg et al., 2015), which could be implicated in the alteration of locomotion by inflammation observed in this study. Moreover, an alternative rationale is that sensitization could have changed the input activity induced by tail holding and perineal stimulation that triggers CPG-induced locomotion. This could be implicated in previously reported acute inhibition of spinal cat locomotion by back pinching (Frigon et al., 2012). While this possibility could not be examined thoroughly, locomotion performed 4 hours post-injection suggests that no acute change occurs in locomotor-like activity triggered by perineal pinching. Further investigations are needed to pinpoint mechanistic origin and electrophysiological markers associated with inflammation-induced locomotor deficits.

Limitations and future directions

CFA has been employed extensively in several animal models to study chronic pain mechanisms. While some authors previously observed that CFA-induced sensitization might recover during the first week post-injection (Eliav et al., 1999, Djouhri et al., 2006), most experimental evidences report a sensitization lasting up to 3 weeks or more (Ambalavanar et al., 2006, Tétreault et al., 2011). Importantly, discrepancy in temporal effects of CFA seem to be highly dependent on experimental methods and outcome measures (Eliav et al., 2009, Djouhri et al., 2015). In a previous study in mice, intradermal CFA injection into the lumbar area reduced hot plate reaction time and tail flick latency for at least 20 days (Larson et al., 1986) without inflammatory changes in hindpaws, indicating that CFA injection induced central sensitization of neurons receiving inputs from the back, the tail and lower limb structures. This could be explained by the wide distribution of nociceptive inputs from lower back muscles as it was shown that nociceptive inputs for multifidus muscle just lateral to L5 vertebrae project to L1-L5 dorsal horns, with maximal projection to L3 spinal level (Taguchi et al., 2007). Moreover, CFA was shown to induce changes in the extent of receptive fields of lower back neurons in dorsal horn (Taguchi et al., 2008). Thus, central sensitization evoked by CFA may elicit

maladaptive spinal plasticity that interacts with spinal locomotor networks, which could underlie results from the present study.

The present experiment tested a novel model to assess the impact of heterotopic CFA injection on locomotor spinal networks activity. Spinally-mediated locomotion was recorded, with the experimenter providing weight support and balance to minimize the implication of back muscles on locomotor activity. Yet, the impact of back muscle tissue damage on locomotion cannot be completely ruled out. While CFA injection in hindpaw or gastrocnemius were previously shown to alter exploration (Chacur et al., 2009) and locomotion (Tétreault et al., 2011) in spinally intact rats, observational evidence from our laboratory rather showed no locomotor alteration up to 24 days following CFA injection in lumbar muscles in spinally intact mice. Additionally, the experimenter was not blinded to group composition due to the presence of a scar in CFA-injected mice. While it could introduce an unconscious bias during training session, kinematic data showed no difference in animal positioning on treadmill, suggesting a minimal involvement of such bias.

Alternatively, the induction of systemic inflammation by CFA injection cannot be completely ruled out. However, there was no evidence of such systemic effect of CFA in the present study. For instance, the impact of CFA on weight loss after SCI, which we measured daily after SCI for the entire time of the experiment but did not report it in Results Section, was similar between groups. Moreover, there was no behavioral evidence of pain or stress elicited by CFA injection. Therefore, we propose that the effects of lumbar muscle inflammation on locomotion recovery after SCI are mediated by central and not peripheral changes, through alteration of spinal plasticity.

Conclusion

The present results show that lumbar muscle inflammation alter use-dependent neuronal plasticity responsible for locomotor re-expression after complete SCI. This has significant implications for the field of rehabilitation in SCI patients since musculoskeletal tissue damage as well as acute and chronic pain are frequently associated with SCI. In addition, the present

study provides further evidence of the role of spinal disinhibition in recovery deficits after SCI as well as the importance of training to preserve normal spinal reflex inhibition.

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Conflict of Interest

No competing financial interests exist.

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Chapitre III: Article 2 - H-reflex disinhibition by lumbar muscle inflammation in a mouse model of spinal cord injury

Jeffrey-Gauthier R, Piché M, Leblond H. (2018). H-reflex disinhibition by lumbar muscle inflammation in a mouse model of spinal cord injury. Neuroscience Letters, 690:36–41.

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Renaud Jeffrey-Gauthier: planification, collecte de données, analyse et interprétation, rédaction, révision

Mathieu Piché : planification, révision

Hugues Leblond : planification, révision

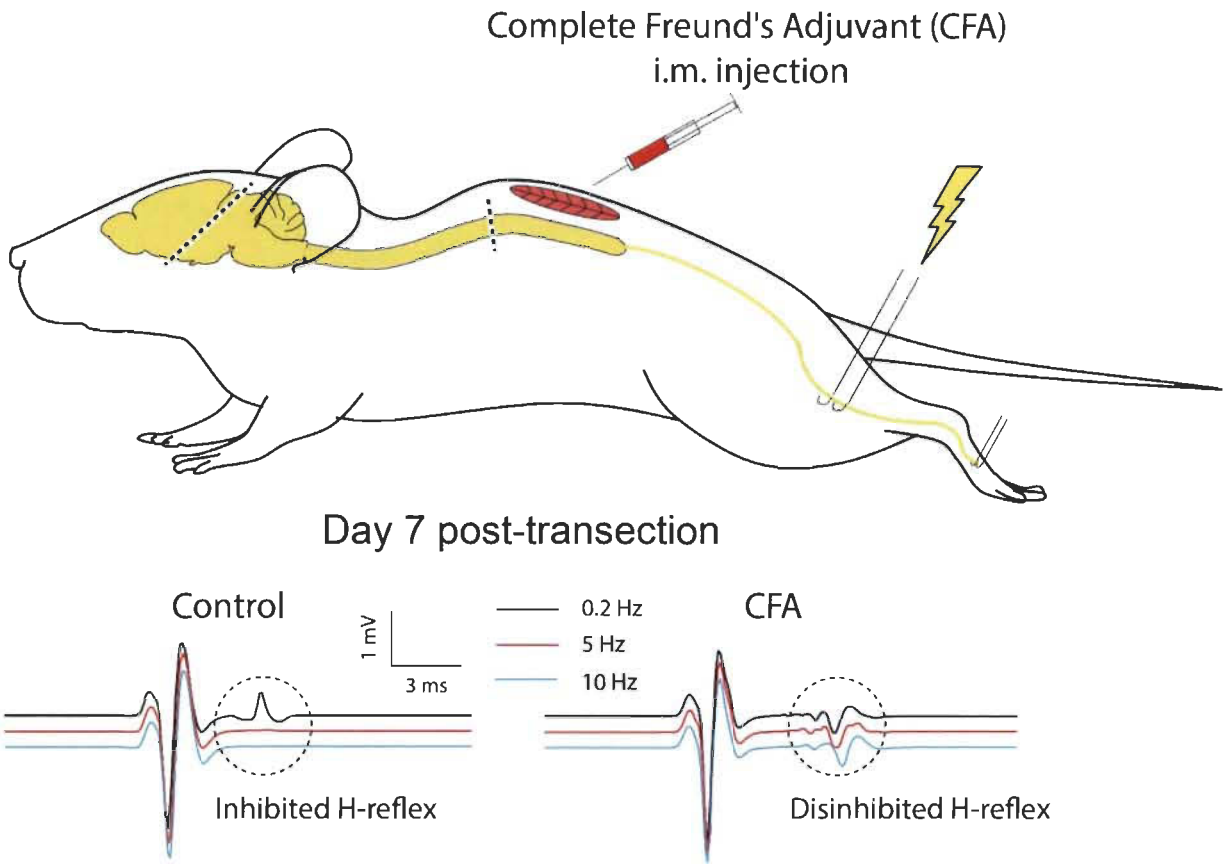

Figure 3.1. Graphical abstract

Abstract

Inflammation is a common comorbidity in patients with traumatic spinal cord injury (SCI). Recent reports indicate that inflammation hinders functional recovery in animal models of SCI. However, the spinal mechanisms underlying this alteration are currently unknown. Considering that spinal plasticity is a therapeutic target in patients and animal models of SCI, these mechanisms remain to be clarified. Using injections of complete Freund's adjuvant (CFA) in lumbar muscles as a model of persistent inflammation, the objective of this study was to assess the impact of inflammation on spinal reflex excitability after a complete midthoracic spinal transection in mice. To this end, the excitability of spinal reflexes was examined by measuring H-reflex frequency-dependent depression (FDD) on days 7, 14 and 28 following a complete spinal transection. H-reflex parameters were compared between spinal mice with CFA and control spinal mice. On day 7, lumbar muscle inflammation disinhibited the H-reflex, reflected by an attenuation of H-reflex FDD ($p<0.01$), although this effect did not persist later

on, either on day 14 or day 28. These results indicate that lumbar muscle inflammation alters spinal reflex excitability transiently in spinal mice. Considering that changes in spinal reflex excitability are associated with poor functional recovery after SCI, this implies that inflammation should be treated effectively to promote optimal recovery following SCI.

Introduction

Spinal cord injury (SCI) and chronic inflammation induce cellular, synaptic and spinal circuitry alterations. These alterations enhance neuronal excitability and cause hyperreflexia, which limits functional recovery. In addition to neuropathic pain caused by SCI itself (central pain), a considerable proportion of patients with SCI present concomitant inflammation and pain of somatic origin (Johnson et al., 1998, Barrett et al., 2003, Siddall et al., 2003, Finnerup et al., 2014). However, the impact of this peripheral source of nociceptive inputs on excitability of spinal reflexes and on functional recovery after SCI has been largely overlooked. It is essential to examine this issue in order to prevent negative clinical outcomes due to the interference of pain-related processes.

The assessment of frequency-dependent depression (FDD) of the Hoffmann reflex (H-reflex) is a useful method to assess changes in spinal reflex excitability occurring after SCI. In animal models of SCI, H-reflex FDD measured with graded stimulation frequencies is attenuated (Thompson et al., 1992, Thompson et al., 1998, Yates et al., 2008). However, recovery and reversal of this attenuation may occur with training (Reese et al., 2005, Côté et al., 2014), which also improves functional outcomes (Lee et al., 2009). In addition, H-reflex FDD attenuation was reported in animal models of neuropathic pain (Lee-Kubli and Calcutt, 2014, Jolivalt et al., 2015). Thus, the magnitude of H-reflex FDD is a useful index of spinal reflex excitability to assess changes induced by both SCI and chronic pain.

Recently, inflammation in lumbar paraspinal muscles was shown to alter the recovery of locomotion elicited by perineal stimulation in spinally transected mice (Jeffrey-Gauthier et al., 2017). Moreover, capsaicin applied directly on L4-S4 segments of the spinal cord was shown to disrupt the locomotor rhythm induced by electrical stimulation of sacral peripheral afferents (Mandadi et al., 2013). Such functional alterations may rely on changes in the activation of

spinal reflexes induced by peripheral afferent inputs. However, the impact of lumbar muscle inflammation on the activation of these spinal circuits and reflexes is currently unknown. Considering the clinical impact of this sensorimotor interaction, this interaction should be clarified.

The objective of this study is to examine the impact of lumbar muscle inflammation on H-reflex FDD and excitability in spinally transected mice. Based on the studies mentioned above, we hypothesized that lumbar muscle inflammation would attenuate H-reflex FDD in mice after transection.

Material and methods

Animal care and ethics

All experimental procedures were conducted in accordance with the Canadian Council on Animal Care guidelines were approved by the UQTR Animal Care Committee, and adhered to ethical standards set by the Committee for Research and Ethical Issues of the International Association for the Study of Pain. Experiments were performed on 61 adult female CD1 mice (body weight 25 g; Charles River Laboratories, Saint-Constant, QC, Canada). Housing conditions included a 12-12-h light-dark cycle at an ambient temperature of 26 °C. Each animal received the same perioperative care comprising the administration of anti-inflammatory drug (carprofen, 10mg/kg s.c.) once a day, starting the day before the surgery and ending the day after. Additional injections of analgesic drug (buprenorphine, 0.1 mg/kg s.c.) and sterile physiological saline (0.9 %, 1 ml, s.c.) were administered before and after the surgery, respectively. Bladders were expressed manually, as needed. Animals were weighted every day until the terminal experiment to confirm good health and to ensure comparable health conditions between groups.

Experimental Interventions

Spinal transection was performed under isoflurane anesthesia (2 %) with medical O_2 at a flow rate of 100 ml/min. Perioperative nonsteroidal anti-inflammatory (carprofen, 10 mg/kg s.c.) and opioid (buprenorphine, 0.1 mg/kg s.c.) therapy was administered to minimize pain and

discomfort. During anesthesia induction, animals were placed on a heating pad and the skin overlying T5-T9 vertebrae was shaved. A 1-cm long skin incision was made to separate paraspinal muscles from the spine and a bilateral laminectomy of T7 and T8 vertebrae was performed to expose the spinal cord. Lidocaine was applied on the spinal cord just before transection with micro-scissors. The dural sac was dissected and a clear disconnection between the rostral and caudal stumps of the spinal cord was confirmed visually. The gap was then filled with absorbable hemostats (Surgicel, Ethicon, Somerville, NJ, USA) to promote hemostasis and prevent excessive bleeding. Muscles and skin were then sutured in layers after which anesthesia was discontinued. Complete spinal cord transection was further confirmed post-mortem with the observation of a wide scar on the entire circumference of the T8 segment.

Spinally transected mice were randomly assigned to the CFA (n=31) or control (n=33) groups and distributed randomly into subgroups, for which the terminal experiment was performed on different days following spinal transection: day 7 (CFA group: n =7; control group: n = 10), day 14 (CFA group: n = 9; control group: n = 9), or day 28 (CFA group: n = 14; control group: n = 12). To avoid potential interactions with perioperative anti-inflammatory therapy, CFA was injected in lumbar paraspinal muscles 4 days following spinal transection. Under isoflurane anesthesia (2 %) with medical O_2 at a flow rate of 100 ml/min, animals were placed on a heating pad and a midline 1-cm skin incision was done over the lumbar spine to expose lumbar muscles bilaterally. CFA (Sigma F5881, 100 µl of 0.5 mg/ml heat-killed *Mycobacterium tuberculosis* diluted 1:1 in warm saline 0.9%) was injected at L1 and L5 segments on both sides of the spine in doses of 25 µl (i.m.) per injection site. After injections, the skin was sutured and anesthesia was discontinued. This procedure is known to produce persistent inflammation (Chacur et al., 2009, Sandkühler, 2009).

Decerebration procedure

In order to assess the impact of pain processes on H-reflex during the terminal experiment, mice were decerebrated as described previously (Nakanishi and Whelan, 2012, Meehan et al., 2017). Briefly, the mice were anesthetized with isoflurane (2 %) with medical O_2 at a flow rate of 60 ml/min. Tracheotomy was performed to provide adequate ventilation (≈90 resp/min, SAR-830/P Ventilator; CWE, Inc., Ardmore, PA, USA) and end-tidal CO_2 level was

continuously monitored (CAP-STAR-100 carbon dioxide analyser; CWE Inc) and kept constant between 2.5 and 3.5% by adjusting respiratory rate and tidal volume. Body temperature was monitored with a rectal probe and maintained at 37±0.5 °C with a heating pad. Both carotid arteries were ligated to decrease cerebral perfusion and the head of the animal was stabilized in a stereotaxic frame. Craniotomy was performed to remove the skull between the nasal and occipital bones. Cerebral hemispheres were aspirated using a fine-tipped glass pipette following a 45° line between the caudal cortex and the optic chiasm (precollicular, premamillary decerebration). The intracerebral void was filled with absorbable hemostats and gauze and the scalp was then sutured.

H-reflex recordings

After decerebration, the left tibial nerve was exposed and was kept in a mineral oil pool made with skin flaps to prevent desiccation. The nerve was hooked on a bipolar electrode for stimulation. A bipolar wire electrode (Cooner Wire Company, Chatsworth, CA, USA) was also inserted in the interosseous muscles of the left hindpaw for recording. A subcutaneous electrode was inserted between the stimulating and recording electrodes to ground the animal. Anesthesia was then discontinued and a 30-min rest was allowed for the effect of anesthesia to wear off.

A constant-current stimulator (Model DS4, Digitimer Ltd., Welwyn Garden City, UK) was triggered by a computer-controlled sequencer (Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK) to deliver 1-ms pulses. First, stimuli of graded intensity were delivered at a constant frequency (0.2 Hz), ranging between the intensity just below the motor threshold (MT) and the intensity at which the M-wave clearly plateaued. Using a stimulus intensity that evoked stable M-wave and near-maximal H-reflex amplitude (approximately 1.8 x MT), three blocks of 25 stimuli at constant intensity and graded frequency (0.2, 5, 10 Hz) were delivered with an inter-block interval of 60 s. This allowed measuring H-reflex FDD. Finally, a recovery block of 25 stimuli was delivered at 0.2 Hz to confirm that responses were comparable before and after the FDD protocol and to rule out nonspecific temporal effects. The first 5 responses of each block were discarded to allow reflex stabilization. Recordings were made with stable M-wave (<15% variation) and robust H-reflex responses at baseline (>10% of M-wave). Stability was confirmed with return of the H/M ratio to baseline in the recovery block.

Electromyography (EMG) signal was amplified (1000X), band pass-filtered (3–3000 Hz, Grass Instruments, Quincy, MA, USA), digitized (sampling rate: 10 KHz) and recorded for offline analyses (Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK). H-reflexes were elicited during quiescent EMG background.

Data acquisition and statistical analyses

Data were analyzed with Spike2 software (version 8.02; Cambridge Electronic Design). To quantify M-waves and H-reflexes, peak-to-peak amplitude of the EMG signal was calculated in post-stimulus time windows (1–3 ms and 5–8 ms, respectively). MT and H-reflex threshold were determined as the lowest intensity producing the M-wave and the H-reflex, respectively. Responses of greatest amplitude (Mmax and Hmax) were used to calculate the Hmax/Mmax ratio. Intensities at which the H-reflex threshold and Hmax were observed were expressed as multiples of the MT. H-reflex latency was also measured. The slopes of the M and H recruitment curve were determined by fitting their respective curve to a sigmoidal function. M slope (Mslp) and H slope (Hslp) were then used to calculate a recruitment ratio (Hslp/Mslp). In the FDD protocol, the mean H/M ratio was averaged from 20 successive responses for each block and transformed into Z scores.

Statistical analyses were conducted using Statistica v13 (Dell Inc., Tulsa, OK, USA). All results are presented as mean ± SEM and significance threshold was set at $p \leq 0.05$. Data distribution was inspected visually and normality was confirmed with the Kolmogorov-Smirnov test. Body weight values, H latency, H threshold, Hmax, Hmax/Mmax and Hslp/Mslp were compared by a two-way ANOVA with GROUP and TIME as between-subject factors. H/M ratios during FDD were compared by a mixed ANOVA with GROUP and TIME as between-subject factors and frequency (FREQ) as within-subject factor. Planned comparisons were used to decompose significant effects and test *a priori* hypotheses. Effect sizes are reported based on partial eta-squared (η_p^2).

Results

General health condition

Body weight was monitored to ensure that animals maintained good general health and presented no systemic symptoms (data not shown). Changes in body weight were significantly affected by TIME ($F_{2, 58} = 4.2$, $p = 0.02$, $\eta_p^2 = 0.13$) but were comparable between groups ($F_{1, 58} = 0.3$, $p = 0.59$, $\eta_p^2 = 0.01$). Planned contrasts revealed that body weight measured at the time of terminal experiment was lower on day 14 compared to day 7 ($p = 0.19$) and reached significance on day 28 ($p = 0.005$).

H-reflex excitability

H-reflex recruitment curves were compared between CFA and control groups over time to examine how inflammation may affect H-reflex excitability. Individual examples are shown in Figure 3.2, where M-wave and H-reflex recruitment curves are similar between groups. As for H-reflex parameters (see Table 3-1), the H latency was not significantly different between groups (main effect: $F_{1, 58} = 1.8$, $p = 0.18$, $\eta_p^2 = 0.04$), but it was significantly decreased over time (main effect: $F_{2, 58} = 5.2$, $p = 0.01$, $\eta_p^2 = 0.18$) with no interaction between group and time (interaction: $F_{2, 58} = 1.2$, $p = 0.29$, $\eta_p^2 < 0.08$).

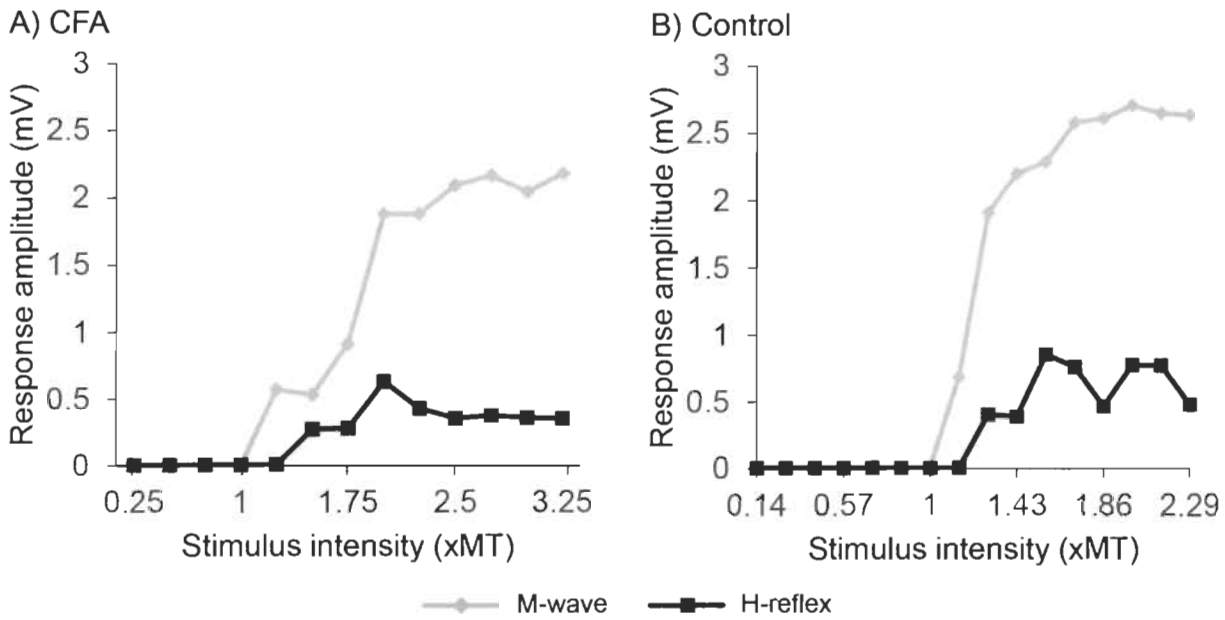

Figure 3.2. M-wave and H-reflex recruitment curves

Figure 3.2 (legend): Individual examples of peak-to-peak amplitude response to single stimulus measured for graded stimulus intensity for CFA and control spinal groups.

Moreover, H-reflex threshold was not significantly different between groups (main effect: $F_{1, 58} = 0.5$, $p = 0.5$, $\eta_p^2 < 0.01$), but it was significantly decreased over time (main effect: $F_{2, 58} = 3.4$, $p = 0.04$, $\eta_p^2 = 0.12$), with no interaction between group and time (interaction: $F_{2, 58} = 1.3$, $p = 0.3$, $\eta_p^2 = 0.04$). In contrast, Hmax, Hmax/Mmax and Hslp/Mslp were not significantly different between groups (main effect: $F_{1, 58} = 3.4$, $p = 0.07$, $\eta_p^2 = 0.06$; $F_{1, 58} = 0.1$, $p = 0.8$, $\eta_p^2 < 0.01$; and $F_{1, 58} = 1.9$, $p = 0.17$, $\eta_p^2 < 0.04$, respectively) or over time (main effect: $F_{2, 58} = 0.7$, $p = 0.48$, $\eta_p^2 = 0.02$; $F_{2, 58} = 0.2$, $p = 0.8$, $\eta_p^2 < 0.01$ and $F_{2, 58} = 0.7$, $p = 0.52$, $\eta_p^2 = 0.03$, respectively) with no interaction between group and time (interaction: $F_{2, 58} = 0.5$, $p = 0.6$, $\eta_p^2 = 0.02$; $F_{2, 58} = 0.2$, $p = 0.8$, $\eta_p^2 = 0.01$ and $F_{2, 58} < 0.1$, $p = 0.97$, $\eta_p^2 < 0.01$, respectively).

Table 3-1. H-reflex recruitment parameters in CFA and control spinal groups

Time	Group	H latency (ms)	H threshold (xMT)	H max (xMT)	Hmax/Mmax	Hslp/Mslp
Day 7	CFA	6.38 ± 0.19	1.06 ± 0.05	1.86 ± 0.17	0.29 ± 0.06	1.17 ± 0.40
	Control	5.76 ± 0.22	1.13 ± 0.08	1.77 ± 0.15	0.29 ± 0.03	1.48 ± 0.19
Day 14	CFA	5.49 ± 0.15	1.07 ± 0.03	2.19 ± 0.49	0.31 ± 0.03	1.53 ± 0.29
	Control	5.52 ± 0.24	0.99 ± 0.07	1.57 ± 0.16	0.29 ± 0.05	1.96 ± 0.45
Day 28	CFA	5.51 ± 0.20	0.87 ± 0.07	1.75 ± 0.26	0.28 ± 0.04	1.26 ± 0.20
	Control	5.52 ± 0.22	0.99 ± 0.06	1.37 ± 0.07	0.34 ± 0.02	1.75 ± 0.56

H latency, H threshold, Hmax, Hmax/Mmax and Hslp/Mslp were not significantly different between groups.

H-reflex frequency-dependent depression

Individual measures from CFA mice and control mice shown in Figure 3.3A and 3.3B illustrate the disparity in FDD between groups on day 7. As for group analyses, H/M ratio was significantly affected by stimulation frequency (main effect: $F_{2, 116} = 137.8$, $p < 0.001$, $\eta_p^2 = 0.70$) and between CFA and control groups (main effect: $F_{1, 116} = 7.7$, $p = 0.007$, $\eta_p^2 = 0.12$), but not

over time (main effect: $F_{2, 116} = 1.2$, $p = 0.3$, $\eta_p^2 = 0.04$) (see Figure 3.3C). Moreover, the effect of frequency was significantly different between groups (interaction: $F_{2, 116} = 5.4$, $p = 0.006$, $\eta_p^2 = 0.09$) and over time (interaction: $F_{4, 116} = 2.5$, $p = 0.04$, $\eta_p^2 = 0.08$). Planned contrasts revealed that H-reflex FDD was significantly weaker at 5 and 10 Hz in CFA mice compared to controls on day 7 (5 Hz, $p = 0.05$; 10 Hz, $p < 0.001$), but not on day 14 (5 Hz, $p = 0.5$; 10 Hz, $p = 0.6$) or day 28 (5 Hz, $p = 0.8$; 10 Hz, $p = 0.6$). In CFA mice, FDD was improved on days 14 and 28 compared to day 7, with significantly enhanced depression at 10 Hz (p's <0.001) but not at 5 Hz (p's> 0.1). In control mice, the depression at both 5 and 10 Hz was comparable on day 14 ($p = 0.9$ and 0.6, respectively) and 28 ($p = 0.19$ and 0.17, respectively) compared to day 7.

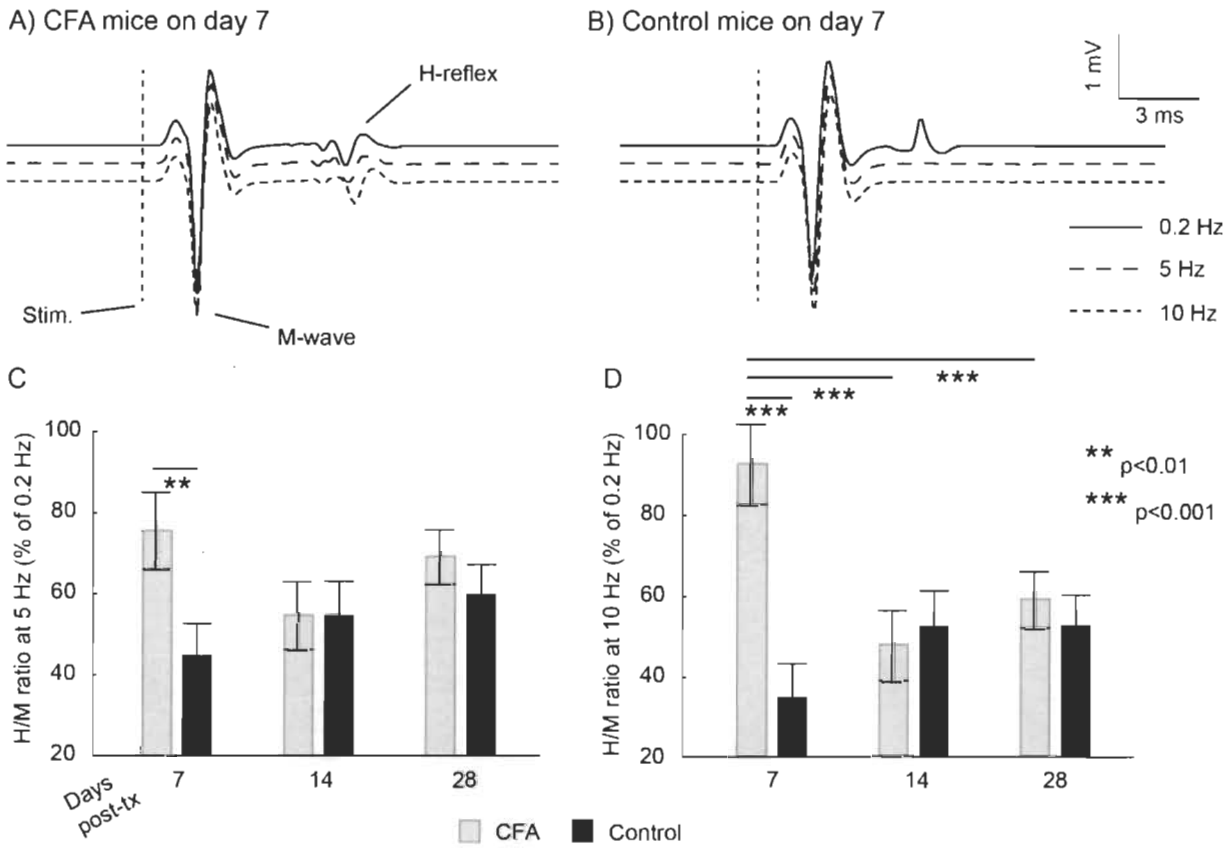

Figure 3.3. Frequency-dependent depression attenuation after spinal transection

FDD was affected by the CFA injection. (A, B) Individual M-wave and H-reflex elicited electrical stimulation of graded frequencies. The H-reflex and M-wave were constant across frequencies in CFA spinal mice, while the H-reflex was depressed at 5 and 10 Hz in control

spinal mice (B). Mean H-reflex depression was significantly attenuated at 5 Hz (C) and 10 Hz (D) for CFA mice compared to control mice on day 7 post-transection, but recovered on day 14 and 28. ** $p \leq 0.01$, *** $p \leq 0.001$.

Discussion

The novel finding of the present study is that lumbar inflammation induced by CFA injection attenuates H-reflex FDD in the early phase following spinal transection, while this effect is abolished later on. Consistent with previous findings showing that lumbar inflammation alters the recovery of locomotion in the early phase following spinal transection (Jeffrey-Gauthier et al., 2017), these results suggest that inflammation also alters H-reflex inhibition.

Also termed rate-dependent depression, post-activation depression, or homosynaptic depression, frequency-dependent depression (FDD) of the H-reflex has been used in previous studies to examine spinal reflex disinhibition induced by neurological conditions. These include spinal cord injury (Thompson et al., 1992, Kakinohana et al., 2006, Yates et al., 2008, Tan et al., 2012, Bandaru et al., 2015), multiple sclerosis (Nielsen et al., 1995), stroke (Lee et al., 2014, Toda et al., 2014) as well as pain conditions (Jolivalt et al., 2008, Lee-Kubli and Calcutt, 2014, Jolivalt et al., 2015). Most relevant to the present study, H-reflex FDD is attenuated in animal models of SCI (Thompson et al., 1992). Previous investigations have shown that FDD attenuates progressively after SCI, with maximal reduction occurring around day 28 post-contusion, in rats (Thompson et al., 1992) and mice (Lee et al., 2009). Because the H-reflex is strongly modulated by supraspinal control (Lundberg, 1964), the present results suggest that complete spinal transection alters FDD earlier than incomplete spinal injury (Lee et al., 2005). Accordingly, the progressive attenuation of FDD in the control group is consistent with this idea, although the effect did not reach statistical significance. More importantly, the near complete FDD suppression in CFA mice on day 7 shows that inflammation, at least during its subacute phase, greatly alters spinal excitability in mice with complete spinal transection. This may contribute to previous findings showing that mice with back muscle inflammation showed locomotor deficits after complete spinal transection compared to control spinal mice (Jeffrey-Gauthier et al., 2017). Although these deficits were observed from day 7 to day 28 post-transection, it is not clear why FDD attenuation was observed on day 7 only, considering that FDD attenuation is

associated with poor functional recovery (Lee et al., 2009). Future studies are needed to examine the mechanism by which inflammation causes spinal excitability changes, locomotor deficits and the relationship between spinal excitability changes and locomotor deficits.

It is difficult to attribute the effect of CFA to changes targeting either Ia afferents, motoneurons or both. A previous report on FDD shows no concurrent alteration in motoneuron excitability and rather suggested that it reflects a decreased probability of neurotransmitter release by Ia afferents due to repeated activation (Hultborn et al., 1996). It would involve mechanisms occurring in the presynaptic component of the reflex that differ, at least in part, from «classical» presynaptic inhibition (Curtis and Eccles, 1960, Hultborn et al., 1996). In the present study, we cannot rule out excitability changes targeting the lumbar motoneuron pool. In the rodent, the smaller conduction velocity differences between Ia afferents and motoneurons and the longer central delay presumably cause orthodromic and antidromic volleys to merge instead of collide (Meinck, 1976). It would thus induce a contamination of the H-reflex by antidromic activation of the motoneuron (i.e. F-wave) and prevent its inhibition by supramaximal stimulations. The absence of H-reflex depression at high intensity in our recruitment curves validates these assumptions and is consistent with prior investigations (Côté et al., 2011). Since the F-wave is not affected by stimulation frequency, an enhanced H-reflex contamination targeting specifically CFA mice on day 7 could contribute to the present results.

Inflammatory changes in response to CFA injection have been extensively studied by our group (Jeffrey-Gauthier et al., 2017, Touj et al., 2017) and others (Chacur et al., 2009). The evidence demonstrates that primary sensory (Obata et al., 2003) and dorsal horn neurons (Hylden et al., 1989, Hoheisel and Mense, 2015) are activated and sensitized by the release of inflammatory mediators in response to peripheral inflammation. These effects (activation and sensitization) are segment- and side-specific (Dubner and Ruda, 1992). As in limb muscles, nociceptive inputs from back muscles diverge. For instance a nociceptive stimulation at L5 will lead to the activation of dorsal horn neurons from L1 to L5, with maximal activation at L3 (Taguchi et al., 2007). Thus, it is possible, although not evaluated in the present experiment, that nociceptive inputs activated by inflammatory mediators directly modulate the Ia-motoneuron circuit of the hindpaw's extensors muscles. This remains to be investigated. Systemic inflammatory response to CFA administration could also influence spinal activity.

However, the comparable body weight values of CFA and control mice suggests comparable health condition across groups and does not support a contribution of this mechanism.

Recently, H-reflex FDD was used to characterize changes in H-reflex excitability associated with the development of neuropathic pain (Lee-Kubli and Calcutt, 2014, Jolivalt et al., 2015). It was established that spinal disinhibition contributes to brain derived neurotrophic factor (BDNF)-dependent neuropathic pain states. The spinal mechanism is still unknown, but might involve decreased spinal expression of the potassium chloride co-transporter 2 (KCC2) (Jolivalt et al., 2008). Inflammation induced by CFA was also shown to decrease the expression of KCC2 in the dorsal horn up to 7 days following CFA injection (Lin et al., 2016). Interestingly, this mechanism is thought to be critical in the development of hyperreflexia and spasticity following SCI (Lu et al., 2008, Stil et al., 2011, Tashiro et al., 2015). The present results indicate that inflammation causes hyperreflexia in the early phase of SCI. It remains to be determined whether inflammation potentiates SCI-induced reflex disinhibition or whether its effects are produced through mechanisms independent of SCI.

In a previous investigation in mice, nociceptive muscle stimuli increased Hmax/Mmax while nociceptive cutaneous stimuli from the foot sole produced the opposite effect (Schomburg et al., 2013). In the present study, no difference was observed between CFA and control groups for the intensity producing Hmax and for the Hmax/Mmax ratio, despite the significant decrease in FDD on day 7. This contrasts with several studies that reported decreased FDD along with increased Hmax/Mmax ratio (Lee et al., 2005, Côté et al., 2014, Escobar-Corona et al., 2017). However, conflicting results were reported when comparing experimental effects on the Hmax/Mmax ratio and other measures, including H-reflex FDD or H-reflex operant conditioning (Chen et al., 2001). This suggests that the Hmax/Mmax ratio may not be sensitive to changes in H-reflex excitability following SCI. Alternatively, H-reflex disinhibition observed in the present study may have been confounded by other changes at the cellular, synaptic or circuit level.

It should be emphasized that the present results were obtained in unanesthetized decerebrated mice. This preparation was established recently (Meehan et al., 2012, Nakanishi and Whelan, 2012) and was shown to provide reliable measures of motoneurons' electrophysiological properties (Meehan et al., 2017). Moreover, it was used recently to evaluate

the impact of pain-related processes on the soleus H-reflex in decerebrated spinal cats (Schomburg et al., 2007) and rats (Della Torre, 2002). In these studies, homonymous H-reflex was facilitated by muscle inflammation and muscle ischemia, respectively. Our findings are consistent and extend these results by showing that muscle inflammation causes H-reflex disinhibition.

Conclusion

In conclusion, the findings of the present study indicate that inflammation in lumbar muscles can cause H-reflex disinhibition, especially in the subacute stage. Moreover, the results provide a temporal profile of the impact of inflammation on H-reflex excitability and suggest that inflammation may influence excitability changes related to SCI.

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Chapitre IV: Article 3 - Locomotor deficits induced by inflammation involve spinal microglia and are independent of KCC2 expression in a mouse model of complete spinal transection.

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Abstract

Spinal cord injury (SCI) is associated with damage to musculoskeletal tissues of the spine. Recent findings show that pain and inflammatory processes caused by musculoskeletal injury mediate plastic changes in the spinal cord that could impede with adaptive plastic changes responsible for functional recovery by influencing a common mechanism oppositely. Despite the importance of promoting adaptive plasticity and reducing pain for SCI patient rehabilitation, the underlying mechanism is still unknown. In the present study, we assessed the influence of step-training and lumbar muscle inflammation induced by complete Freund's adjuvant (CFA) on locomotor recovery in a mouse model of complete spinal transection. Behavioral outcomes of each of these interventions alone or in combination were assessed together with histological changes to the well described microglia-BDNF-KCC2 pathway in both ventral and dorsal horns in the sublesional spinal cord. This pathway is critically implicated in the dorsal horn sensitization observed in neuropathic pain and mediates the regulation of spinal excitability by

step-training. Results show that central pattern generator (CPG)-mediated hind limb movements at the hip, knee, ankle and metatarsophalangeal joint during locomotion were influenced oppositely by step-training and CFA injection. Moreover, CFA injection enhanced the expression of the microglial marker Iba1 in both ventral and dorsal horn, with or without step-training. However, this change was not associated with a modulation of KCC2 expression, suggesting that locomotor deficits induced by inflammmation are independent of KCC2 expression in the sublesional spinal cord. These results indicate that musculoskeletal injury hinders locomotor recovery after SCI by a microglia-dependent process.

Introduction

Pain affects about 80% of spinal cord injured (SCI) patients (Siddall et al., 2003) and is present during rehabilitation, including during locomotor training. While interacting maladaptive and adaptive plasticity could cause pain to hinder functional recovery, clinical researchers identified a knowledge gap on the subject in the literature (Dvorak et al., 2017). Such experiments have considerable implications and could promote recovery and patients' quality of life.

In animal models of SCI, there is accumulating evidence showing that experimental nociception and inflammation can impair locomotor recovery (Garraway et al., 2011, Jeffrey-Gauthier et al., 2017) by acting directly or indirectly (Jeffrey-Gauthier et al., 2018b) on locomotor spinal networks. Importantly, step-training contributes to recovery by inducing plastic changes targeting those same networks (de Leon et al., 1998, Ichiyama et al., 2008, Alluin et al., 2015) suggesting that pain-related processes and step-training compete to influence the same spinal pathways. Using an instrumental learning paradigm of flexion reflex conditioning in spinal rats, it was demonstrated that electrical stimulations at the nociceptive range could block task-related training effects on reflex conditioning (Ferguson et al., 2006) by a glia-dependent mechanism (Huie et al., 2012a, Grau et al., 2014). Conversely, training could prevent the disruptive effect of nociceptive inputs on reflex conditioning (Crown and Grau, 2001) by a brain-derived neurotrophic factor (BDNF)-dependent mechanism (Gómez-Pinilla et al., 2007, Huie et al., 2012b).

In addition to the contribution of glia and BDNF, recent findings show that expression of the cation-chloride cotransporter type 2 (KCC2) is pivotal in chloride homeostasis responsible for GABA inhibitory transmission and is reduced after SCI (Boulenguez et al., 2010). Importantly, KCC2 expression is modulated by both step-training and pain-related processes oppositely. On one hand, step-training increases KCC2 expression in lumbar motoneurons' membrane and attenuates H-reflex disinhibition after SCI in rats (Côté et al., 2014) by a BDNF-dependent mechanism (Côté et al., 2011). On the other hand, persistent inflammation induced by CFA injection in the hind paw of rats enhances pain sensitivity by decreasing KCC2 expression in lumbar dorsal horns (Zhang et al., 2008). However, no study has investigated KCC2 expression when step-training and inflammation occur concurrently. Considering the high prevalence of pain in SCI patients, the interaction of pain and training and the contribution of glia and KCC2 should be clarified.

The objective of the present study was to describe the impact of concurrent step-training and lumbar muscle inflammation on locomotor recovery and microglia/KCC2 expression in lumbar spinal cord after a complete mid-thoracic spinal transection in mice. We hypothesize that inflammation would hinder locomotor recovery by increasing microglial activity and downregulating KCC2 while step-training would rescue recovery by influencing microglia and KCC2 oppositely.

Methods

Animal care and ethics

This experiment was performed on 25 female CD1 mice (body weight: 25 g; Charles River Laboratories, Saint-Constant, QC, Canada). Animals arrived two weeks prior to testing to habituate to their environment, ambient temperature (26 °C) and inmates (5 mice/cage). Living conditions were provided by the laboratory and facility staff in a 12-12 h light-dark cycle with constant access to food and water. All manipulations and procedures were in accordance with Canadian Council on Animal Care guidelines, were approved by the UQTR Animal Care Committee and adhered to guidelines from the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

Surgical procedures

Surgical procedures were performed under anesthesia with isoflurane (2% mixed with medical O_2, flow rate: 100 ml/min), preceded by the administration of the analgesic drug buprenorphine (0.1 mg/kg, s.c.) and followed by the administration of sterile physiological saline (0.9 %, 1 ml, s.c.). Moreover, perioperative care initiated the day before surgery and terminated the day after surgery was provided by administering the anti-inflammatory drug carprofen (10 mg/kg, s.c., q.d.) and the analgesic drug buprenorphine (0.1 mg/kg, s.c., p.r.n.). After surgery, bladders were manually expressed twice a day and hydration was closely monitored. Access to water was facilitated and a bolus of warm saline (1 ml, s.c.) was provided when required. Spinal transections were performed as described previously (Leblond et al., 2003). Briefly, animals were placed on a heating pad while anesthetized. The skin overlying the spine from T5 to T9 was shaved and incised. The paraspinal muscles were separated from the spine on each side. Then, a double laminectomy of T7 and T8 vertebrae was performed to expose the spinal cord. Lidocaine was applied on the spinal cord to avoid secondary damage just before complete transection with micro-scissors. The dural sac was also completely transected, causing a retraction of the rostral and caudal stumps and a clear discontinuation of the spinal cord. The void was filled with absorbable hemostats (Surgicel, Ethicon, Somerville, NJ, USA) to promote hemostasis and prevent excessive bleeding. Muscular and cutaneous tissues were then sutured back in layers after which anesthesia was discontinued. Complete spinal cord transection was further confirmed post-mortem with the visualization of a wide scar on the entire T8 spinal segment.

Experimental interventions

Locomotion baseline was assessed with a motor-driven treadmill apparatus (Exer-3/6, Columbus Instruments, Columbus, OH, USA) with an implemented speed of 12 m/min and recorded for each mouse prior to spinal transection. After surgery, spinally transected mice were randomly assigned to one of the following groups: untrained (n=6), trained (n=6), untrained with CFA (n=7) and trained with CFA (n=6). Subsequent locomotor evaluations of the spinally transected mice or training sessions were performed while animals were provided with 1) a platform overhanging the treadmill on which they can rest their forelimbs, 2) balance from tail

holding by the experimenter and 3) tail pinching. The latter has been experimentally recognized as a non-invasive stimulation that allows stepping generation in animals transected as adults (Meisel and Rakerd, 1982) and used to study locomotor recovery after spinal transection by our group (Leblond et al., 2003, Jeffrey-Gauthier et al., 2018a) and others (Sławińska et al., 2014).

Trained mice and trained mice with CFA were provided with daily locomotor training (6 days/week) and consisting of 10 min session of tail pinching-triggered locomotion on the treadmill. This training regimen was initiated on day 2 post-transection and terminated at the end of the experiment on day 28 post-transection.

Untrained mice with CFA and trained mice with CFA were administered 4 bolus of CFA (Sigma F5881, 4X25 µl of 0.5 mg/ml heat-killed *Mycobacterium tuberculosis* diluted 1:1 in warm saline 0.9%) in left and right lumbar muscles at L1 and L5 segments. These injections were performed on isoflurane anesthetized animals on day 4 post-transection to avoid interaction with the anti-inflammatory drug carprofen given perioperative. 1 cm of skin overlying the L1-L5 spine was incised and injections were performed directly in exposed lumbar muscles. Injecting needles were secured in place for 10 min to ensure proper CFA diffusion in the muscles. Thereafter, skin was sutured and anesthesia discontinued. This procedure has been shown to cause an inflammatory response that persists over a month (Ambalavanar et al., 2006).

Assessment of locomotor recovery

Locomotor function was assessed for each animal at different times after complete spinal transection (on day 2, 7, 14, 21 and 28 post-transection). A high-speed camera (Proselica GC, Allied Vision Technologies, Irwin, PA, USA; 90 frames/s) facing the left side of the animal on the treadmill was used to record (StreamPix 5 software, NorPix, Montreal, QC, Canada) the longest bout of consecutive step cycles that mice could complete in 5 min sessions. Intra-limb coordination was assessed with kinematics evaluation of the hind limb joint movement during locomotion. Anatomical landmarks (iliac crest, great trochanter, knee ankle, 5^{th} MTP and the tip of the 5^{th} toe) were marked and digitalized into XY coordinates to document angular excursions of the hip, knee, ankle and MTP throughout the locomotor bout. Step length was measured for right and left hind limbs at each cycle. Duration of swing phase and stance phase were noted between consecutive lift and contact and between consecutive contact and lift,

respectively. In the absence of clear paws lifts, swing and stance were identified as forward and backward paw movements, respectively. Dragging during swing was calculated as the proportion of the swing phase being achieved with the paw in contact with the treadmill belt. Coordination between hind limbs was assessed with the evaluation of the homologous coupling, i.e. the phase relation between left and right step cycles. Homologous coupling value was scored between 0 and 1 with 0.5 consisting of out-of-phase left and right step cycles. Homologous coupling constancy over locomotor bout was also scored between 0 and 1, both extremes representing a random phase coupling or a constantly out-of-phase coupling, respectively.

Immunohistochemistry and microscopy

Anesthetized mice were perfused through the heart with PBS then paraformaldehyde (4% in PBS). The perfused lumbar spinal cord was removed and immersed in sucrose (30 % in PBS) for cryoprotection. After 24 h, the spinal cord was removed from sucrose, put in OCT, then stored at -80 °C.

Frozen perfused tissue samples were cut in transversal sections of 25 µm from L2-L4 with a cryostat (Leica CM3050 S, Leica Biosystems, Concord, ON, Canada) and disposed on two sets of microscope slides for each mouse. Immunofluorostaining was achieved directly on these slides. They were covered with PBS-based incubation buffer (5 % donkey serum, 3 % bovine serum albumin – BSA, 0.1 % Triton X) with 0.9% lysine for 1 h at room temperature (RT) to suppress non-specific binding. Slides from set #1 were incubated overnight at RT in incubation buffer with rabbit anti-KCC2 antibody (1:100, Millipore Catalog No. ab07-432) and goat anti-ChAT antibody (1:50, Millipore Catalog No. AB144P). Slides from set #2 were incubated overnight at RT in incubation buffer with rabbit anti-Iba1 (1:1000, Wako Catalog No. 019–19741), a marker specifically found on microglia. On the next day, the slides were washed 3X10 min in PBS, incubated in a dark chamber at RT for 2 h with the FITC-conjugated donkey anti-rabbit (1:400, Jackson Laboratories Catalog No. 711-095-152) and rhodamine red-conjugated donkey anti-goat (1:400, Jackson Laboratories Catalog No. 705-295-003) antibodies for set #1 and AF647-conjugated donkey anti-rabbit antibody (1:400, Jackson Laboratories Catalog No. 711-605-152) for set #2. Then, the slides were washed 3X10 min in PBS, dipped

in distilled water and dried at RT in a dark chamber. At the end, the slides were covered with DAPI fluoromount (Southern Biotech, Birmingham, AL, USA) and protected with a cover slip.

For KCC2 visualization on motoneurons, images were acquired at 40X using a confocal laser scanning microscope (Leica TCS SP8, Leica Microsystems, Concord, ON, Canada). Motoneurons were identified as ChAT-expressing cells in the ventral horn. KCC2 expression measurement was achieved on every motoneuron for which the soma was clearly visualized. Signal intensity was averaged from 3 regions of interest (ROI) evenly distributed on the plasma membrane and normalized to the signal intensity in the cytosol. Motoneurons selection and measurement were performed using LAS X software by a blind experimenter. A minimum of 13 motoneurons were examined per animal. For KCC2 evaluation in dorsal horn and Iba1 evaluation in both ventral and dorsal horns, images were acquired at 20X using a fluorescent photonic microscope (Olympus BX51WI, Olympus Lifescience, Richmond Hill, ON, Canada). ROIs for KCC2 mean signal intensity and Iba1 area fraction evaluation were determined based on the mouse spinal cord anatomy described in the Allen Mouse Spinal Cord Atlas (© 2018 Allen Institute for Brain Science. Allen Mouse Spinal Cord Atlas. Available from: mousespinal.brain-map.org) and measured using ImageJ software (NIH, Bethesda, MD, USA) by a blind experimenter. A minimum of 3 sections were averaged per animal.

Data analysis and statistics

All results are expressed as mean ± SEM. Significant differences in locomotor parameters (number of steps, angular excursions of hind limb joints, step length, stance and swing durations, drag, homologous coupling value and homologous coupling constancy) over time and between groups groups were assessed with a mixed ANOVA using Statistica (TIBCO Software, Palo Alto, CA, USA). Significance threshold was set at 0.05. In order to illustrate the overall differences in recovery between groups, locomotor parameters were examined using a principal component analysis comprising all parameters significantly different between groups. Significant differences in the principal component between groups were assessed with a one-way ANOVA. Significant group differences were decomposed with planned comparisons to test a priori hypotheses. Training and CFA influence on Iba1 area fraction and KCC2 signal intensity were also assessed with a one-way ANOVA to assess the influence of group on KCC2 or of

CFA on microglia involvement, respectively. Significant effects were decomposed with planned comparisons to test a priori hypotheses.

Results

Number of steps performed after a complete spinal transection

The number of consecutive steps mice could achieve decreased greatly after spinal transection compared to baseline. During recovery, the number of steps varied over time ($F_{4, 84} = 7.6$, $p < 0.001$) but not between groups ($F_{3, 84} = 1.0$, $p = 0.40$, table 4-1). Planned contrasts for the main effect of time revealed that the number of steps increases between consecutive time points reached significance on days 7 ($p = 0.002$) and 14 ($p = 0.04$), but not on days 21 ($p = 0.15$) and 28 ($p = 0.69$).

Table 4-1. Locomotor parameters after transection

	DAY	UNTRAINED		TRAINED		UNTRAINED CFA		TRAINED CFA	
		mean	sem	mean	sem	mean	sem	mean	sem
NBR OF STEPS	2	8.83	0.87	7.25	2.20	6.57	1.07	6.00	1.64
	7	11.75	1.62	14.00	2.49	8.36	2.03	10.42	1.93
	14	13.92	2.72	13.67	2.77	15.21	1.73	13.17	1.75
	21	12.50	1.56	16.58	2.82	11.00	1.61	9.75	0.68
	28	12.83	1.72	13.00	1.49	12.21	3.49	14.17	1.55
STANCE (MS)	2	109.82	52.83	423.22	217.26	153.13	49.52	450.53	348.66
	7	135.93	51.63	161.98	40.68	209.72	86.72	102.82	37.43
	14	140.48	50.18	163.45	38.93	146.56	38.82	200.51	50.98
	21	140.43	54.61	170.41	38.60	222.55	64.75	172.08	40.09
	28	133.63	54.05	191.66	41.49	164.97	45.96	170.50	40.96
SWING (MS)	2	61.96	21.57	109.61	27.54	91.03	28.24	110.14	27.17
	7	80.01	26.68	78.15	14.63	77.62	16.33	165.06	44.94
	14	54.98	15.53	84.65	15.88	64.84	13.30	81.44	15.72
	21	60.83	17.06	80.20	14.69	62.08	12.00	79.68	14.38
	28	64.14	17.80	74.27	12.99	60.07	11.16	85.10	15.44
DRAG (% SWING)	2	99.75	0.12	99.04	0.36	99.09	0.57	95.88	1.65
	7	95.23	1.63	92.95	1.82	98.81	0.48	94.37	2.19
	14	79.74	5.43	78.34	4.44	88.66	2.61	85.09	2.49
	21	71.00	3.30	63.29	3.77	79.46	3.07	58.74	5.85

STEP LENGTH (MM)	28	58.41	6.21	55.26	2.72	63.08	2.85	64.23	3.86
	2	6.03	0.51	7.51	0.64	4.69	0.26	8.10	1.29
	7	17.64	1.48	19.81	0.77	11.72	0.87	17.24	1.90
	14	22.55	1.82	21.56	1.41	**17.16***	1.18	**29.60***	3.81
	21	**27.07***	1.05	26.06	0.83	**21.33***	1.17	26.54	0.79
	28	30.07	0.93	27.74	0.92	25.82	0.55	26.81	0.79
HOMOLOGOUS COUPLING	2	0.43	0.02	0.35	0.02	0.32	0.03	0.34	0.03
	7	0.41	0.01	0.43	0.01	0.42	0.01	0.44	0.01
	14	0.46	0.01	0.41	0.00	0.41	0.02	0.41	0.01
	21	0.41	0.01	0.45	0.01	0.41	0.01	0.45	0.01
	28	0.45	0.01	0.44	0.01	0.41	0.01	0.43	0.01
COUPLING CONSTANCY	2	0.65	0.04	0.61	0.04	0.63	0.05	0.69	0.04
	7	0.75	0.03	0.72	0.03	0.65	0.04	0.65	0.04
	14	0.70	0.03	0.70	0.04	0.71	0.02	0.75	0.02
	21	0.88	0.01	0.71	0.03	0.77	0.02	0.82	0.02
	28	0.80	0.03	0.87	0.02	0.82	0.03	0.88	0.01

Table 4-1 legend: Step length was significantly decreased in untrained CFA mice compared to trained CFA mice on day 14 ($p = 0.05$) and compared to untrained mice on day 21 ($p = 0.04$). Other variables of interest (number of steps, stance and swing durations, paw drag, homologous coupling and coupling constancy) did not differ between groups ($p's > 0.05$, see in text for more details).

Joint kinematics

Hind limb joint angles were evaluated throughout locomotor bout over time for each animal (see Figure 4.1A for individual examples of 750 ms sequence of locomotion). Intra-limb coordination was visualized for each animal by plotting hip, knee and ankle angle changes across step cycles in a relative joint angle plot (right; Figure 4.1A-E). Individual examples of the hind limb joints variations over a locomotor sample of 750 ms are displayed in stick diagrams for each group (left; Figure 4.1A-D). A relative joint angle plot and stick diagram are depicted from an intact mouse to provide an appreciation of normal locomotion (Figure 4.1E). Relative joint angle variations during normal locomotion involve a multi-joint biphasic pattern of alternation between flexion and extension that is lost in untrained mice, untrained CFA mice and trained CFA mice. Trained mice show a partial recovery of the multi-joint biphasic pattern of flexion and extension.

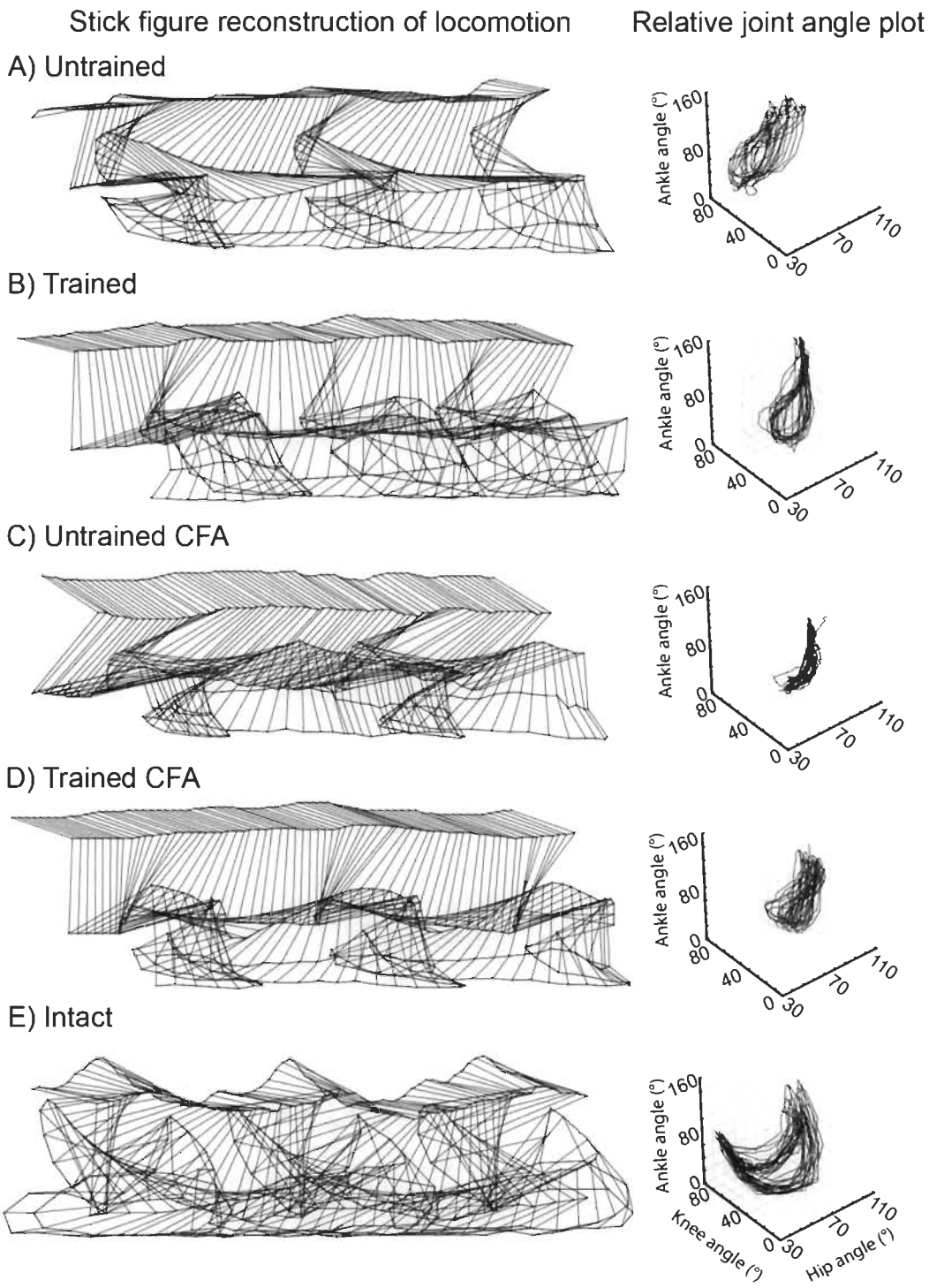

Figure 4.1 Locomotor kinematics overview

(A) Stick figure displays individual examples of angular excursions of hind limb joints during a 750-ms recording of treadmill locomotion for each group on day 28 post-transection and in an intact mouse. (B) Relative joint angle plots of ankle excursion in function of hip and knee

excursions during locomotion show decreased hind limb coordination and an abnormal locomotor kinematic pattern on day 28 compared to the intact state. These plots illustrate the decreased angular excursions in untrained CFA mice compared to untrained and trained mice and compared to trained CFA mice.

Hip joint

Hip angular excursion was measured between maximal flexion and extension angles for each step cycle and averaged over locomotor bout. It decreased after spinal transection compared to baseline (see Figure 4.1). During recovery, hip angular excursion increased over time ($F_{4, 84} = 14.5$, $p < 0.001$) and across groups ($F_{3, 84} = 4.1$, $p = 0.02$), but hip angular excursion improvement over time did not differ across groups ($F_{12, 84} = 0.6$, $p = 0.81$). Planned contrasts for the main effect of time revealed that hip angular excursion increases between consecutive time points reached significance on day 14 ($p = 0.01$), but not on days 7 ($p = 0.09$), 21 ($p = 0.51$) and 28 ($p = 0.23$). Planned contrasts for the main effect of group revealed that hip angular excursion was impaired in untrained CFA mice compared to untrained mice ($p = 0.009$) and trained CFA mice ($p = 0.03$). Hip angular excursion was comparable in untrained and trained mice ($p = 0.89$).

Knee joint

Knee angular excursion was measured between maximal flexion and extension angles for each step cycle and averaged over locomotor bout. It decreased after spinal transection compared to baseline (see Figure 4.1). During recovery, knee angular excursion increased over time ($F_{4, 84} = 4.7$, $p = 0.002$) and across groups ($F_{3, 84} = 3.7$, $p = 0.03$), but knee angular excursion improvement over time did not differ across groups ($F_{12, 84} = 1.4$, $p = 0.18$). Planned contrasts for the main effect of time revealed that knee angular excursion increases between consecutive time points reached significance on day 7 ($p = 0.008$), but not on days 14 ($p = 0.72$), 21 ($p = 0.09$) and 28 ($p = 0.25$). Planned contrasts for the main effect of group revealed that improvements in knee angular excursion in trained mice compared to untrained mice did not reach significance ($p = 0.14$). Moreover, impairments in knee angular excursion in untrained

CFA mice compared to untrained mice and trained CFA mice did not reach significance ($p = 0.10$ and $p = 0.07$, respectively).

Ankle joint

Ankle angular excursion was measured between maximal flexion and extension angles for each step cycle and averaged over locomotor bout. It decreased after spinal transection compared to baseline (see Figure 4.1). During recovery, ankle angular excursion increased over time ($F_{4, 84} = 25.9$, $p < 0.001$) and across groups ($F_{3, 84} = 4.6$, $p = 0.01$), but ankle angular excursion improvement over time did not differ across groups ($F_{12, 84} = 0.9$, $p = 0.53$). Planned contrasts for the main effect of time revealed that ankle angular excursion increases between consecutive time points reached significance on day 7 ($p < 0.001$), but not on days 14 ($p = 0.08$), 21 ($p = 0.12$) and 28 ($p = 0.63$). Planned contrasts for the main effect of group revealed that ankle angular excursion was impaired in untrained CFA mice compared to untrained mice ($p = 0.02$) and trained CFA mice ($p = 0.01$). Ankle angular excursion was comparable in untrained and trained mice ($p = 0.48$).

MTP joint

MTP maximal extension angle was measured for each step and averaged over locomotor bout as an index of adequate contact of the paw on its plantar aspect. Figure 4.1C shows an example of MTP extension impairment, which prevents proper plantar paw placement in this animal of the untrained CFA group. During recovery, MTP extension increased over time ($F_{4, 84} = 39.1$, $p < 0.001$) but not across groups ($F_{3, 84} = 2.5$, $p = 0.09$) and MTP extension improvement over time did not differ across groups ($F_{12, 84} = 1.4$, $p = 0.20$). Planned contrasts for the main effect of time revealed that MTP extension increases between consecutive time points reached significance on days 7 ($p = 0.003$) and 14 ($p = 0.003$), but not on days 21 ($p = 0.09$) and 28 ($p = 0.06$).

Phase durations

Stance and swing duration were altered by spinal transection. For stance phase, it decreased over time during recovery, but this effect did not reach significance ($F_{4, 84} = 1.3$, $p =$

0.28). Stance duration was also comparable across groups ($F_{3, 84} = 0.3$, $p = 0.79$, table 4-1). Swing duration increased after spinal transection. During recovery, swing duration decreased over time ($F_{4, 84} = 3.3$, $p = 0.01$) but not across groups ($F_{3, 84} = 0.5$, $p = 0.70$, table 4-1). Planned contrasts for the main effect of time revealed that swing duration decreases between consecutive time points reached significance on day 14 ($p = 0.03$), but not on days 7 ($p = 0.71$), 21 ($p = 0.72$) and 28 ($p = 0.95$). Paw dragging on the treadmill belt during swing phase is typically not observed in normal locomotion but very common after SCI, including transection. Various amounts of paw dragging can be deciphered from stick diagram of Figure 4.1. During recovery, drag decreased over time ($F_{4, 84} = 58.8$, $p < 0.001$) similarly across groups ($F_{3, 84} = 0.8$, $p = 0.50$, table 4-1). Planned contrasts for the main effect of time revealed that drag increases between consecutive time points reached significance on days 7 ($p = 0.04$), 14 ($p < 0.001$), 21 ($p < 0.001$) or 28 ($p = 0.04$).

Step length

Step length was measured between consecutive paw contact and lift for each step and averaged over locomotor bout. It decreased after spinal transection compared to baseline. During recovery, step length increased over time ($F_{4, 84} = 49.6$, $p < 0.001$) and across groups ($F_{3, 84} = 3.1$, $p = 0.05$, table 4-1), but step length improvement over time did not differ across groups ($F_{12, 84} = 0.9$, $p = 0.55$). Planned contrasts for the main effect of time revealed that step length increases between consecutive time points reached significance on days 7 ($p < 0.001$), 14 ($p = 0.007$) and 28 ($p = 0.01$), but not on day 21 ($p = 0.26$). Planned contrasts for the main effect of group revealed that step length was impaired in untrained CFA mice compared to untrained mice ($p = 0.04$) and trained CFA mice ($p = 0.01$). Step length was comparable in untrained and trained mice ($p = 0.95$).

Coordination between hind limbs

Homologous coupling value provides the phase relation between left and right step cycles, with 0 consisting of in-phase movement and 0.5 consisting of out-of-phase alternation. It was decreased after spinal transection compared to baseline. During recovery, homologous

coupling value increased over time ($F_{4, 84} = 4.1$, $p = 0.004$) but did not differ across groups ($F_{3, 84} = 1.1$, $p = 0.36$, table 4-1). Planned contrasts for the main effect of time revealed that homologous coupling increases between consecutive time points reached significance on day 7 ($p = 0.02$), but not on days 14 ($p = 0.88$), 21 ($p = 0.47$) and 28 ($p = 0.83$). Homologous coupling constancy throughout locomotor bout is greatly reduced after spinal transection. During recovery, it increased over time ($F_{4, 84} = 4.9$, $p = 0.001$) but not across groups ($F_{3, 84} = 0.6$, $p = 0.60$, Table 4-1). Planned contrasts for the main effect of time revealed that coupling constancy increases between consecutive time points reached significance on day 21 ($p = 0.03$), but not on days 7 ($p = 0.48$), 14 ($p = 0.66$) and 28 ($p = 0.19$).

Principal component analysis of locomotor recovery

To illustrate the overall impact of inflammation and step-training on locomotor recovery, a principal component analysis was performed to evaluate the difference between groups, regardless of time, on a reduced set of latent variables (2) that influenced the locomotor parameters (measured variables). The contribution of each measured variables to the statistically derived components is displayed in a factorial plan in Figure 4.2A. The first component explains 31.3 % of the variance and mostly displays *locomotor recovery* as defined by enhanced angular excursion of the hip, knee, ankle and MTP and increased step length. The second component explains 13% of the variance and mostly displays random differences observed on day 2. Thus, it was not considered for further analysis. Locomotor recovery (component 1) was influenced by training and CFA oppositely (Figure 4.2A). The animals' component score differed significantly depending on group ($F_{3, 21} = 4.3$, $p = 0.02$, Figure 4.2B). Planned contrasts revealed that untrained CFA mice had a lower score compared to untrained mice and trained CFA mice ($p = 0.02$ and $p = 0.03$, respectively).

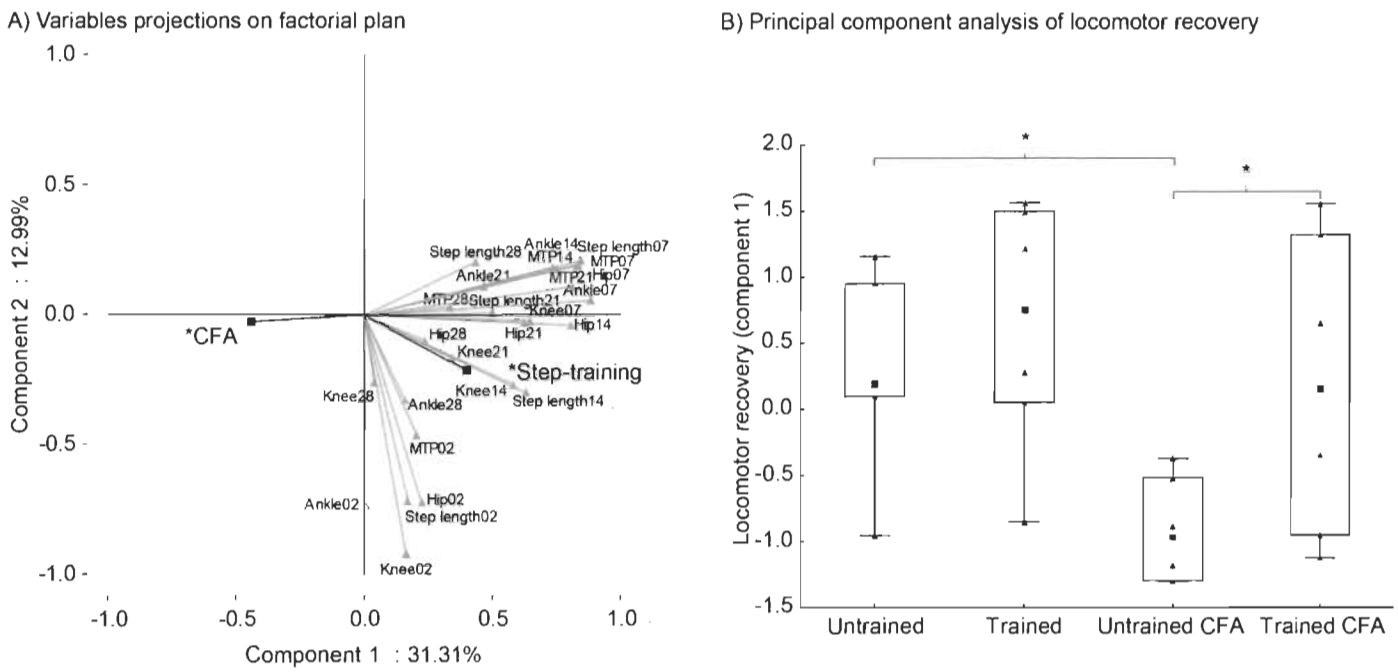

Figure 4.2. Principal component analysis of locomotor recovery

(A) Values of locomotor variables for which recovery differed between groups (angular excursions of hip, knee, ankle and MTP and step length) were evaluated on each time points with a principal component analysis to reveal an underlying latent variable of locomotor recovery (component 1). Step-training and CFA administration influence on the amplitude of angular excursion is also shown. (B) The component score median (black squares), 25–75% centiles (boxes) and individual data (grey triangles) are shown. Untrained CFA mice show impaired locomotor recovery compared to untrained mice and trained CFA mice ($p = 0.02$ and $p = 0.03$, respectively).

IHC analyses

Expression of Iba1 (specific to microglia) and KCC2 were assessed in both dorsal and ventral horns in lumbar spinal cord (L2-L4, individual examples in Figure 4.3A) respectively to obtain histological evidence of the central neuroinflammatory response to CFA injection and to evaluate whether step-training exerts its effect by upregulating KCC2. Iba1 expression was compared between CFA mice (untrained CFA and trained CFA mice) and non-CFA mice (untrained and trained mice). It was significantly increased in CFA mice compared to non-CFA

mice in both dorsal and ventral horns (p's = 0.02, Figure 4.3B). For KCC2, signal intensity was assessed in motoneurones' membrane in addition to dorsal horn. The membrane content was normalized to the cytosol content. KCC2 expression was similar between groups in both dorsal horns ($F_{3, 20} = 1.1$, p = 0.14, Figure 4.4B) and motoneurons ($F_{3, 20} = 1.9$, p = 0.16, Figure 4.5B).

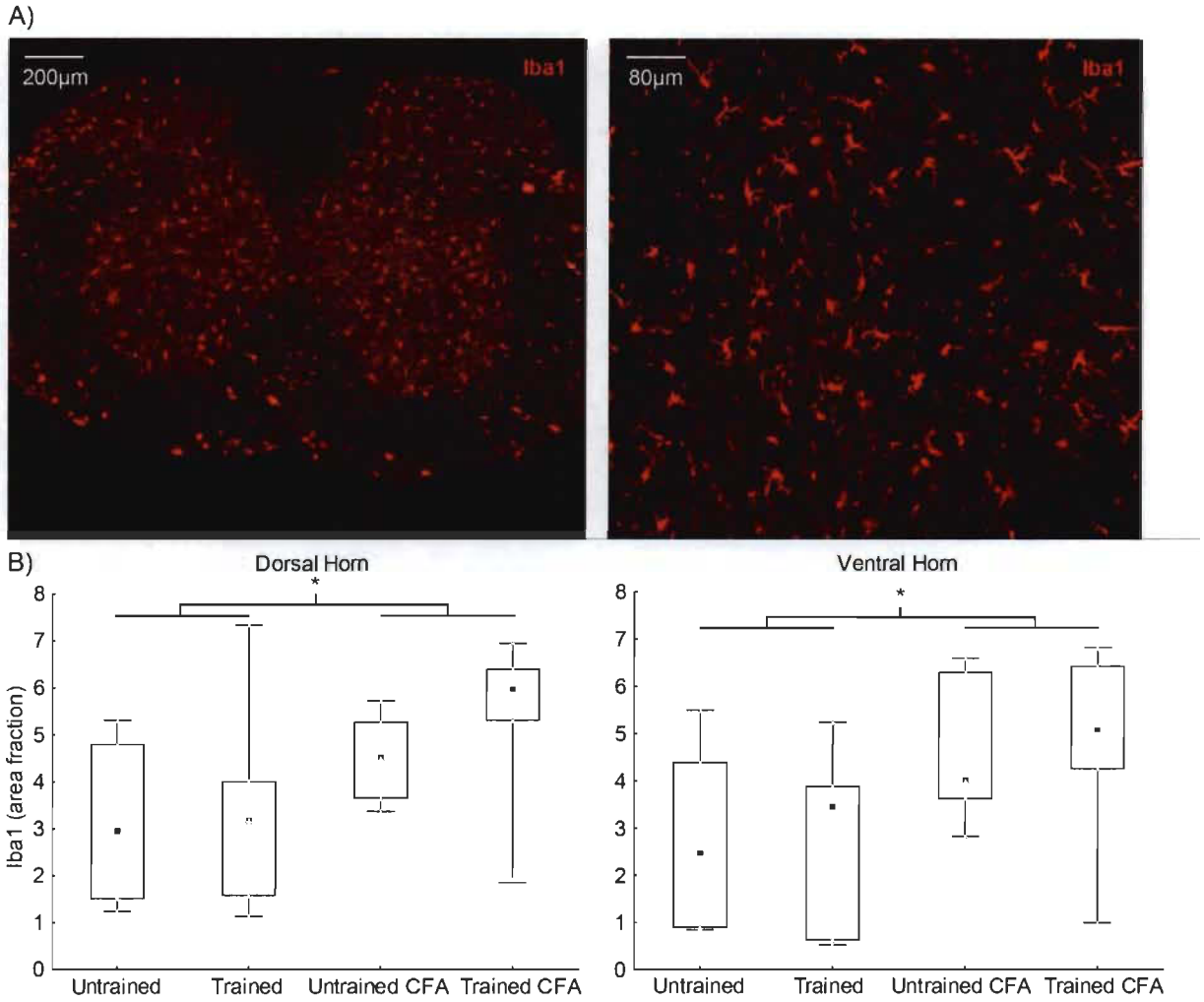

Figure 4.3. Microglial expression in the L2-L4 spinal cord

Iba1+ cells were labeled for microglia visualization in the L1-L2 spinal cord (A). Area fraction of stained tissue in dorsal and ventral horns was evaluated (B). The area fraction median (black squares), 25–75% centiles (boxes) and individual data (grey triangles) are shown. Regardless of training, Iba1 area fraction was enhanced in CFA-injected mice (both untrained CFA and trained

CFA) compared to controls (both untrained and trained mice) in dorsal and ventral horns (p's = 0.02).

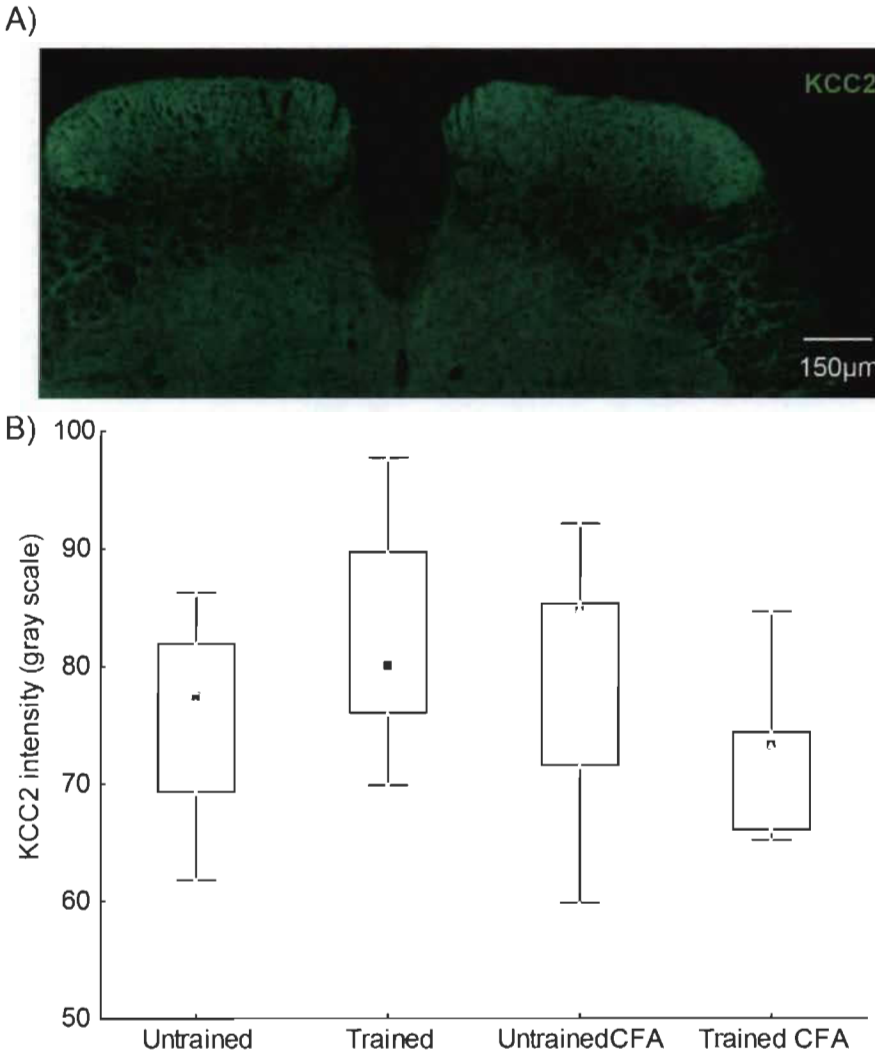

Figure 4.4. KCC2 expression in the L2-L4 dorsal horn

(A) Individual example of KCC2 expression in the dorsal horn. (B) The signal intensity median (black squares), 25–75% centiles (boxes) and individual data (grey triangles) are shown. It did not differ between groups (p's> 0.05).

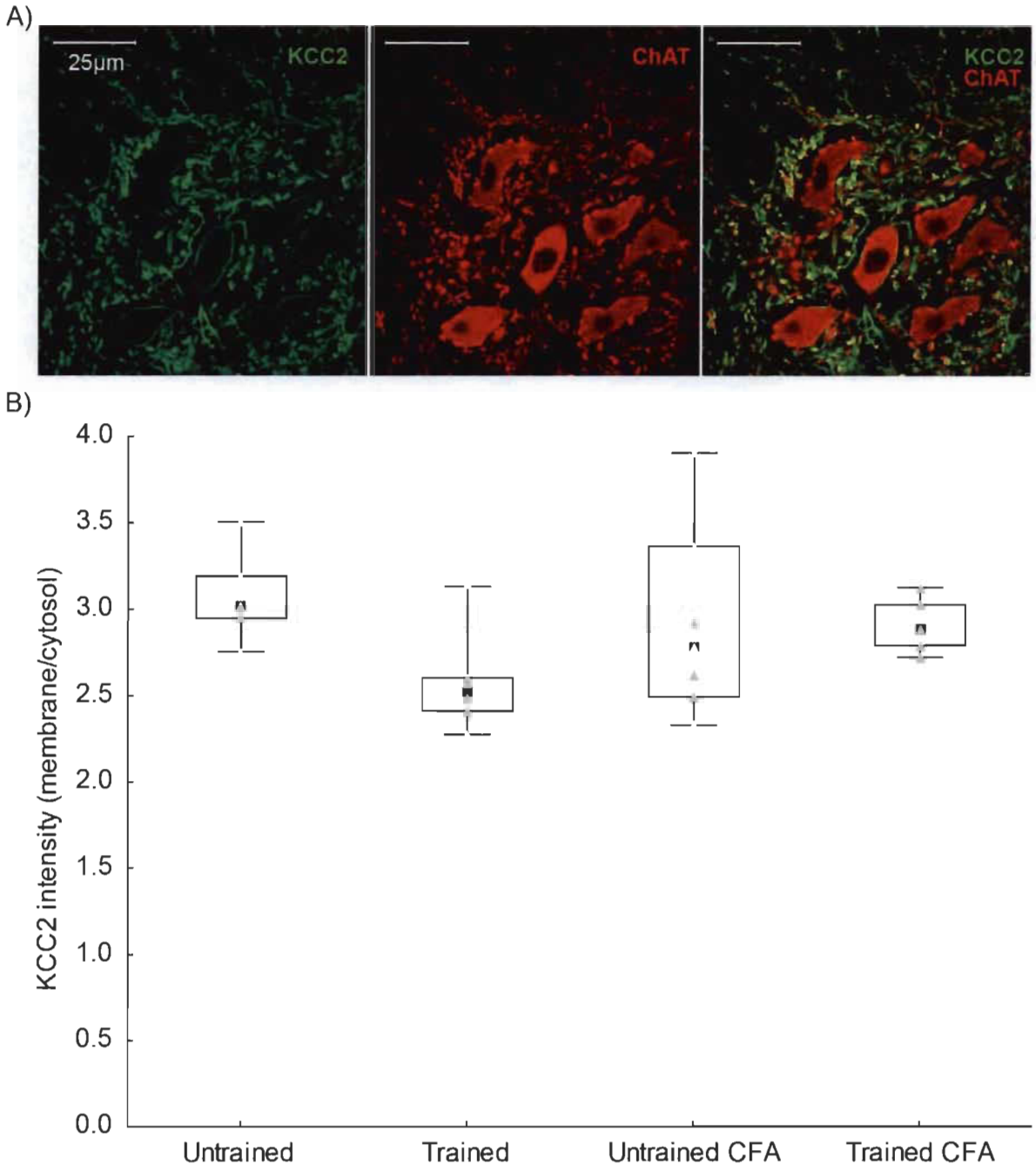

Figure 4.5. KCC2 expression in the L2-L4 ventral horn

(A) Individual example of KCC2 expression in the ventral horn. ChAT+ cells (red) were labeled for motoneurons visualization. The KCC2 signal intensity in the membrane was normalized to the cytosol signal intensity. (B) The signal intensity median (black squares), 25–75% centiles (boxes) and individual data (grey triangles) are shown. It did not differ between groups (p's> 0.05).

Discussion

Pain and inflammation processes are frequent after a neurological trauma, including SCI, and may be present during rehabilitation. Recent evidences from animal models suggests that activity-based therapy and pain-related processes oppositely influence spinal plasticity. The results presented in this study on a mouse model of complete spinal cord transection are consistent with these evidences and add information on behavioral outcomes and mechanisms that contribute or not to this phenomenon. The main findings are 1) step-training and lumbar muscle inflammation oppositely influence hind limb joints kinematics and step length recovery, 2) lumbar muscle inflammation increases microglial expression in both dorsal and ventral horns and 3) KCC2 expression is not modulated by step-training or inflammation.

Lumbar muscle inflammation and training have opposing effects on locomotor recovery after a complete spinal transection

Recent findings indicate that peripheral inflammation and nociception could impair locomotor recovery (Garraway et al., 2011, Jeffrey-Gauthier et al., 2017). Moreover, it has been shown that passive stretchings of the hindlimb of lesioned rats hinder locomotor recovery by activating nociceptive afferents (Keller, 2017). The present study is consistent with these observations and shows that step-training can attenuate the inflammation-induced locomotor deficits. Importantly, the PCA approach revealed that step-training and lumbar muscle inflammation oppositely influence hind limb kinematic recovery and step length. In a series of experiments on spinal rats, Grau and colleagues demonstrated how nociceptive stimuli administered to the hind paw hindered adaptive plasticity in a task of flexion reflex conditioning (Grau et al., 1998, Grau et al., 2006). More importantly, they showed that previous task-related training attenuates the negative influence of nociceptive inputs on reflex conditioning (Crown and Grau, 2001). They suggest that training protect against disruptive effect of nociceptive inputs by facilitating adaptive plasticity by a BDNF-dependent mechanism (Huie et al., 2012b). Using a model of complete spinal transection, our findings are consistent with their interpretation that training and inflammation act on a common mechanism of spinal origin.

Lumbar muscle inflammation modulates microglia expression, with or without training

CFA administered in lumbar muscle of T8-transected mice enhanced microglial expression in L2-L4 spinal cord, both in dorsal and ventral horns, several weeks after injection. In rats, such increase has been reported two weeks after CFA administration in the triceps surae (Chacur et al., 2009) or in the plantar aspect of the hind paw (Raghavendra et al., 2004). In the study of Chacur and colleagues, microglial expression changes were evaluated in superficial and deep laminae of the L5 dorsal horn. They showed that microglial expression was increased in both regions and was associated with a decrease in the withdrawal threshold in response to Von Frey filament stimulation (i.e. development of hypersensitivity). These changes were attenuated by minocycline by inhibiting microglial activation or by anti-TNFα antibody by suppressing its secretion by microglia. However, the importance of microglia contribution to inflammation-related spinal processes including dorsal horn hypersensitivity development is debated (Clark et al., 2007, Lin et al., 2007) and could be influenced by stimulus-dependent factors such as dosage and injection site (Clark et al., 2007).

Our behavioral data suggest that step-training could allow recovery to a similar level than mice that were not administered CFA. However, step-training did not attenuate CFA-induced increase in microglial expression. This result supports the previous demonstration that training was not associated with decreased presence of Iba1+ cells despite decreased inflammatory gene expression (Shin et al., 2014). It also suggests that step-training attenuation of CFA-induced locomotor deficits most probably rely on a distinct mechanism. Non-linear relationship between microglial and spinal activity may still influence training-based plasticity underlying locomotor recovery. Indeed, previous evidences demonstrated that sublesional microglial activity could be downmodulated by early step-training (Detloff et al., 2014). In the contusion model, microglia is implicated in various outcomes contributing to functional recovery (for review see Donnelly and Popovich, 2008, David and Kroner, 2011) including spared tissue volume (Watanabe et al., 1999) and dorsal horn activity both above-level (Carlton et al., 2009) and below-level of injury (Hains and Waxman, 2006). While the role of microglia in maladaptive plasticity leading to neuropathic pain has been extensively described (Hains and Waxman, 2006, Zhao et al., 2007, Detloff et al., 2008, Gwak and Hulsebosch, 2009, Gwak et al., 2009; for review see Hulsebosch et al., 2009), its impact on other behavioral outcomes that

rely on plasticity, including training-mediated locomotor recovery, is not known. Considering that spared tissue does not contribute to below-level spinal activity in models of complete spinal transection, such models are valuable and should be used to determine training influence on microglial activity and its role in SCI outcomes.

Behavioral and histological changes mediated by inflammation and training are independent of KCC2 expression in both ventral and dorsal horns

KCC2-dependent chloride homeostasis is essential for adequate GABAergic and glycinergic inhibitory transmission (Rivera et al., 2005, Lu et al., 2008) and is disrupted by a KCC2 reduction after SCI (Boulenguez et al., 2010). Its decrease in ventral and dorsal horns causes disinhibition leading to motor (spasticity, hyperreflexia; Boulenguez et al., 2010, Modol et al., 2014) and sensory impairments (neuropathic pain) (Lu et al., 2008, Hasbargen et al., 2010), respectively. Importantly, it is also reduced by inflammation by a microglia-dependent mechanism (Zhang et al., 2008, Wu et al., 2009, Lin et al., 2017), suggesting that inflammation during rehabilitation after SCI could attenuate recovery by reducing KCC2 expression. Moreover, previous evidences showed that KCC2 expression in lumbar spinal cord is upregulated by early step-training in motoneurons' membrane after an SCI (Côté et al., 2014) and in dorsal horns after a peripheral nerve injury (Mòdol et al., 2014, López-Álvarez et al., 2015, Sánchez-Brualla et al., 2017). In both cases, KCC2 upregulation was associated with decreased hyperreflexia and dorsal horn sensitization. Altogether, these findings suggest that KCC2 expression is influenced by inflammation and training.

In contrast to our hypothesis, we did not observe any changes in KCC2 expression in dorsal horn and motoneurons between our groups. We assume that 1) CFA was inefficient in causing more changes to KCC2 expression than the concurrent SCI-induced KCC2 downregulation and that 2) training was inefficient in causing more changes to KCC2 expression than the concurrent KCC2 upregulation associated with spontaneous recovery. Moreover, we observed that many mice could perform spontaneous locomotor movements in their cage. This capacity provided uncontrolled self-training to animals regardless of their group. This is

consistent with previous report showing limited effect of training on locomotor recovery following SCI in mice model (Battistuzzo et al., 2016; for review see Battistuzzo et al., 2012).

This should not be interpreted as like mice cannot be trained to foster adaptive plasticity. It was evidenced recently that mice that recovered from a left hemisection preserved some locomotor function on the side of the hemisection after a subsequent complete transection (Jeffrey-Gauthier et al., 2018), indicating that locomotor spinal networks of mice can be modified by training similar to what was described in cats (Barrière et al., 2008). However, differences in recovery between strains of mice suggest that unknown genetic factors influence recovery importantly (Basso et al., 2006). Such discrepancies between species and between strains among specie indicate that findings from study using a mouse model of spinal cord injury should be interpreted with caution when compared with rats, cats and humans.

Conclusion

The present results show that lumbar muscle inflammation impaired locomotor recovery after a complete spinal transection in mice and is associated with enhanced microglial expression in sublesional spinal cord. In addition, the present study provides further evidence of the beneficial impact of training by counteracting inflammation effect on recovery. This has significant implications for the field of rehabilitation in SCI patients since musculoskeletal tissue damage as well as acute and chronic pain are frequently associated with SCI.

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Authors disclose statement

No competing financial interests exist.

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Chapitre V: Article 4 - Facilitation of locomotor spinal networks activity by buspirone after a complete spinal lesion in mice

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Abstract

Despite efforts to potentiate spinal cord lesioned (SCL) patients' functional recovery with multi-targeted therapy combining pharmacological treatment and training, consistent improvements in locomotor control by descending transmission or spinal network facilitation are still eluding clinicians and researchers. Lately, US Food and Drug Administration-approved buspirone has shown promise and promoted locomotor-like movement occurrence in SCL patients, but evidence on how and where it exerts its effects is lacking. The objective of the present study was, first, to verify buspirone effect on locomotor spinal network and to evaluate if it promoted functional recovery when combined with training. Also, we evaluated buspirone impact on locomotion in mice that recovered from a previous hemisection before sustaining the spinal transection. This dual lesion paradigm has allowed to confirm spinal network involvement in recovery after an incomplete SCL. Buspirone acutely increased the number of

steps taken, the coupling strength between hindlimbs, angular excursion of the hip joint during locomotion and improved paw positioning at contact and paw drag (p's <0.05). Moreover, it induced long-lasting improvements of paw positioning at contact and paw drag when combined with training in mice after a dual lesion paradigm. Altogether, the results indicate that buspirone exerts considerable acute facilitation of spinally-mediated locomotion and could be used in combination with training to promote functional recovery after SCL.

Introduction

Pharmacological facilitation of spinal networks concomitant with activity-based therapy including treadmill exercise can foster functional recovery of locomotion after spinal cord lesion (SCL; Brustein and Rossignol 1999; Chau, et al. 1998a; Courtine, et al. 2009 ; Guertin, et al. 2011; Roy, et al. 2012). Notably, the Food and Drug Administration (FDA)-approved buspirone, a serotonergic (5-HT) receptor agonist acting on receptor subtype 1A, has recently been investigated in a preclinical study of human SCL patients for potency in locomotor rhythm generation. Gerasimenko et al. (2015) concluded that buspirone facilitates neurotransmission in dormant descending pathways (Gerasimenko, et al. 2015). Alternatively, buspirone could exert its pro-locomotor effects by promoting spinal central pattern generator (CPG) activity, but this mechanism of action has not been investigated. Indeed, 5-HT_{1A} receptors are numerous in lumbar enlargement of the spinal cord of mammals (Giroux, et al. 1999 ; Otoshi, et al. 2009), and their activation with the selective 5-$HT_{1A/7}$ agonist 8-OH-DPAT enhances locomotor-like movement in spinal cord lesioned mice (Lapointe, et al. 2008). However, it is not clear whether $5HT_{1A}$ receptors contributes to locomotion. Thus, it is critical to have a better understanding of buspirone effect on spinal circuitry if we want to extent its use to promote locomotor recovery in SCL patients.

It has been demonstrated that 5-HT elicits locomotion (Jordan, et al. 2008; Sławińska, et al. 2014) and promotes its stereotypic pattern by regulating reflex pathways (Jankowska, et al. 2000; Schmidt and Jordan 2000). Accordingly, the disruption of serotonergic pathways by SCL results in severe loss of locomotor function that is associated with decreased activation and expression of spinal 5-HT receptors, including 5-HT_{1A} (Chopek, et al. 2015). Previous work has

revealed that 5-HT-related functional and neuroanatomical changes occur in spinal networks (e.g. increased receptor expression, Chopek, et al. 2015, and constitutive activity, Fouad, et al. 2010) and in remnant descending serotonergic pathways in cases of partial lesion (Leszczyńska, et al. 2015), especially with training (Ballermann and Fouad 2006). It has been determined that SCL-induced depression of 5-HT_{1A} receptor expression recovers progressively in weeks after transection in spinal rats, but such revival is not observed in deafferented spinal rats (Otoshi, et al. 2009), indicating potential interaction between training and 5-HT_{1A} activity and supporting further research on buspirone as a pharmacological facilitator of spinal locomotor networks.

The main objective of the present study is to determine the efficiency of buspirone to promote functional locomotor recovery in a spinally-transected mouse model (Leblond, et al. 2003). We hypothesize that buspirone would acutely facilitate locomotion and promote long-lasting functional recovery.

Because evidence suggests that 5-HT_{1A} activity is relying on afferent input to promote recovery (Otoshi, et al. 2009), we examined whether buspirone would exert a different effect on recovery from a dual lesion paradigm in which mice were allowed to recover from a left hemisection for 3 weeks before sustaining a transection. This second goal aimed at characterizing buspirone effect on locomotor recovery depending on locomotor function and spinal networks status.

Methods

Animal care and ethics

This experiment was performed on 19 female CD1 mice (body weight: 25±5 g; Charles River Laboratories, Saint-Constant, QC, Canada). Living conditions were implemented by laboratory and facility staff in a 12-12 h light-dark cycle. Ambient temperature was kept constant at 26 °C. Upon arrival, the mice were housed 5 per cage, with access to food and water *ad libitum*. They were allowed a 1-week habituation period to familiarize themselves with the facility environment and staff as well as with the treadmill (Exer-3/6, Columbus Instruments, Columbus, OH, USA). Thereafter, spinal cord surgeries were performed under anesthesia. All mice were allowed time to recover from anesthesia in separate cages before returning them to

their prior housing and mates. All animals were weighed before and every day after surgery to ensure comparable general health conditions between groups. As part of peri-operative care, hydration was closely monitored after surgery and, when required, warm saline (1 ml s.c.) was administered during the first 48 h. Bladders were voided manually twice a day. All manipulations and procedures were in accordance with Canadian Council on Animal Care guidelines, were approved previously 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

Surgeries were undertaken under general anesthesia with isoflurane (2% mixed with O_2 95% and CO_2 5%, 200 ml/min) combined with the perioperative anti-inflammatory drug carprofen (10 mg/kg s.c.) and the opioid analgesic buprenorphine (0.1 mg/kg s.c.) to minimize suffering. Spinal surgeries were performed as described previously. (Leblond, et al. 2003) Briefly, the animals were placed on heating pads and 1 cm of skin was excised in the rostral part of the thoracic spine kyphosis. Then, the paraspinal muscles were scraped off each side of the spine, and the spinal cord was exposed by double laminectomy at T7 and T8 vertebrae. Before sectioning the spinal cord, xylocaine droplets were applied at the lesion site to prevent uncontrolled secondary neural damage. The T8 spinal segment was completely severed by micro-scissors. Concurrent sectioning of the dural sac during transection clearly separated the rostral and caudal stumps with clear visualization of the spinal canal anterior and lateral walls. This large gap was then filled with absorbable hemostats (Surgicel, Ethicon, Somerville, NJ, USA) to promote hemostasis. Muscles and skin were sutured in layers and anesthesia was discontinued. Post-mortem visualization of the spinal cord confirmed transection completeness with a wide scar still present on the entire circumference of the T8 segment.

Experimental interventions

Left hemisection and recovery prior to transection

Prior to T8 transection, partial T7 sectioning targeted the left hemicord in 9 mice. Upon exposure of the spinal cord following the same procedure as for transection, the left half of T7 segment was severed by micro-scissors. Three weeks of daily treadmill training session allowed the animals to recover locomotor function prior to spinal transection. We refer to these animals as "hemisected then spinalized" or HS (n=5) in the present paper. Mice that underwent T8 transection without previous hemisection are referred to as "spinalized" or S (n=5; Table 5-1).

Pharmacological intervention

Upon day 2 after complete transection, buspirone (8 mg/kg i.p.) was injected daily in 9 transected mice (4 HSB, 5 SB ; Table 5-1) prior to training sessions on the treadmill until the end of the recovery period. During recording day, locomotor function was assessed prior to, and after, injection. Post-injection locomotion was evaluated 20 min after injection. Each test lasted approximately 5 min. The experimenter strictly followed this procedure in order to ensure maximal buspirone absorption and minimal metabolization (Kim, et al. 2016). We discriminate buspirone effects between acute (pre vs post injection from the same recording day) and chronic (pre-injection, across recording days).

Table 5-1. Group distribution

Group	Label	Recordings (DPH)	Recordings (DPS)	
Spinalized (n=5)	S		2, 8, 15, 21	
Spinalized, treated with buspirone (n=5)	SB		Pre-injection 2, 8, 15, 21	Post-injection 2, 8, 15, 21
Hemisected then spinalized (n=5)	HS	2, 8, 15	2, 8, 15, 21	
Hemisected then spinalized, treated with buspirone (n=4)	HSB	2, 8, 15	Pre-injection 2, 8, 15, 21	Post-injection 2, 8, 15, 21

DPH = days post-hemisection, DPS = days post-spinalization

Assessment of locomotor recovery

Locomotion on the treadmill was assessed prior to (baseline) and on several days post-hemisection (DPH 2, DPH 8, DPH 15) and spinalization (DPS 2, DPS 8, DPS 15, DPS 21). The belt speed was fixed at 12 m/min to optimize locomotor movements' observation as described elsewhere (Leblond, et al. 2003). Black signs were made on left hindlimb anatomical landmarks (iliac crest, femoral trochanter, ankle, metatarsophalangeal joint (MTP) and distal end of the 5th toe) which were later translated into x-y coordinates with a customized software (Expresso, courtesy of Professor Serge Rossignol) and used to monitor angular excursions of joints during locomotion. Hindlimb step pattern and kinematics were evaluated during quadrupedal locomotion before transection. After transection, the forelimbs lay on a platform and, thus, the

same analyses were conducted during bipedal locomotion. Weight support and balance were afforded by holding the animal by the tail and additional perineal pinching was provided to elicit locomotion as necessary. The animals were placed with their left side facing a high-speed camera (Proselica GC, Allied Vision Technologies, Irwin, PA, USA; 90 frames/s), and the longest locomotor bouts that the animals could perform were recorded (StreamPix 5 software, NorPix, Montréal, QC, Canada). The number of steps the animals took during the bouts were noted. Phase-coupling between the ipsilateral forelimb and hindlimb, also referred to as homolateral coupling, was measured by the timing of forelimb cycle initiation in relation to the ongoing ipsilateral hindlimb cycle and reported as a ratio prior to and after hemisection (DPH 2, DPH 8, DPH 15) but not after transection since forelimbs stepping was not evaluated. Phase-coupling between hindlimbs, also referred to as homologous coupling, was assessed at baseline, after hemisection (DPH 2, DPH 8, DPH 15) and after spinalization (DPS 2, DPS 8, DPS 15, DPS 21). Left and right hindlimb step cycles were divided into phases, with the paw contacting the treadmill belt at stance and being lifted at swing. For time points where paw drag substitutes for paw elevation (e.g. DPS 2), swing was defined as forward paw movement and stance as backward paw movement. Stance and swing duration were measured together with drag duration during swing. The capacity for proper paw placement in front of the hip at stance initiation was measured as the horizontal distance between the distal end of the 5^{th} toe and hip joint for both left and right paw.

Histology

The animals were sacrificed 3 weeks after transection with a lethal dose of pentobarbital followed by intra-cardiac perfusion of paraformaldehyde (PFA, 4% mixed with 0.01M phosphate-buffered saline, PBS). One cm of spinal cord centered around the T7 hemisection was extracted and incubated overnight in PFA and later transferred to sucrose (30% mixed with 0.01M PBS) at 4 °C for 3 days to provide cryoprotection. Spinal cords were then embedded in optimum cutting temperature (OCT) compound and stored in a -80 °C freezer. The frozen spinal cords were cut in 30 μm tick sections, and mounted on a slide. Images were digitally captured with an Axio Examiner A1 microscope (Zeiss, Germany), a digital camera (Axiocam Mrm, Germany), and Zen software (Zeiss, Germany). Digital images were then processed in

Photoshop CS6.0 (Adobe Systems, San Jose, CA). The lesion size was quantified as a percentage of severed white matter (Shah, et al. 2013).

Data analysis and statistics

Locomotor parameters (step occurrence, phase-coupling, phase duration, angular excursions of joints, paw position at contact and drag duration) were gauged by a customized software (Expresso) and pooled in MATLAB (Mathworks, Natick, MA, USA). Coupling ratio in each step cycle was plotted for each locomotor bout recorded and reported as vectors in circular plots. The mean of individual coupling ratio, measured at each step, determined the direction of the vector (phase ratio), and the concentration of values around mean, its magnitude (R-value). Phase ratio spans from 0 to 1, with strictly alternated coupling lending a value of 0.5. The R-value also span from 0 to 1. Concentration being the opposite of dispersion, an R-value closer to 1 indicates less variance and thus a more constant synchronization of movements between limbs (Zar 1999). Hindlimb coupling data were pooled in MATLAB with the CircStat toolbox (Berens 2009).

Angular excursions of left hindlimb joints were measured as the averaged angular span of joints between maximally-flexed and maximally-extended positions in each step cycle. Drag duration is reported as proportions of the entire swing duration after transection (DPS 2, 8, 15 and 21). For mice that had a previous hemisection, the left versus right drag asymmetry index (AI) was calculated on the same time points as described previously (Martinez, et al. 2012) according to the following formula:

$$AI = average\ (right\ drag - left\ drag) / average\ (right\ drag + left\ drag)$$

All results are expressed as mean ± SEM. Statistical analyses were conducted with Statistica (version 13, Statsoft Inc., Tulsa, OK, USA) with threshold of significance set at $p \leq 0.05$. Data distribution normality was assessed by the Kolmogorov-Smirnov test.

Significance of effects was assessed by repeated-measures ANOVAs. Acute effect of buspirone on locomotor function was assessed (main BUSPIRONE effect). Locomotion was

also compared over time (main TIME effect) and between groups (main GROUP effect). Lastly, effect of previous hemisection on locomotion symmetry was evaluated by comparing the left versus right hindlimb function (main SIDE effect). Planned comparisons served to decompose significant main and interaction effects and verify *a priori* hypotheses.

Results

Spared white matter after left hemisection

The value of spared white matter was calculated both on the targeted side of hemisection (left) and on the contralateral side (right). Similar hemisection size and shape were confirmed by lesion histology. For all hemisected animals comprised, 27±14% of white matter was spared ipsilateral to hemisection. The sectioning mostly preserved the right hemicord, as we measured 73±11% of spared white matter contralateral to hemisection. One-way ANOVA discerned no difference in spared white matter between groups (no main GROUP effect: $F_{1,7} = 1.0$, $p = 0.35$, $\eta_p^2 = 0.13$). Altogether, these results showed that the partial sectioning successfully severed left hemicord and preserved right hemicord.

Recovery from previous hemisection and buspirone improve step occurrence

The number of steps observed during the longest locomotor bout the animals could performed was greatly reduced after transection, but not after hemisection. Moreover, step occurrence was acutely influenced by buspirone (main BUSPIRONE effect : $F_{1,21} = 34.3$, $p < 0.001$, $\eta_p^2 = 0.83$; see individual examples in Figure 5.1A), as the number of steps performed was increased in both SB and HSB post-injection (gray bars in Figure 5.1B-D) compared to pre-injection (black bars in Figure 5.1B-D). This acute effect of buspirone was significantly influenced by time (BUSPIRONE*TIME interaction : $F_{3,21} = 5.3$, $p = 0.007$, $\eta_p^2 = 0.43$), as post-injection improvement is more profound on early time points after transection in SB (Figure 5.1B) and HSB (Figure 5.1C-D). Buspirone also differently affected step occurrence depending on limb and group evaluated (SIDE*BUSPIRONE*TIME*GROUP interaction: $F_{3,21} = 4.3$, $p = 0.02$, $\eta_p^2 = 0.38$). In SB, buspirone acutely enhanced step occurrence on DPS 2 (p

<0.001), 8 ($p = 0.04$), 15 ($p = 0.04$) and 21 ($p = 0.02$). In HSB, buspirone improved step occurrence on DPS 2 ($p = 0.002$) and DPS 8 ($p = 0.03$), but not on DPS 15 ($p = 0.11$) and DPS 21 ($p = 0.87$) for both left and right hindlimb.

In addition to this acute effect of buspirone, the number of consecutive steps improved significantly over time in all groups (main TIME effect: $F_{4, 60} = 37.0$, $p < 0.001$, $\eta_p^2 = 0.71$). The increased number of steps taken differed significantly between limbs (SIDE*TIME interaction: $F_{4, 60} = 5.5$, $p < 0.001$, $\eta_p^2 = 0.27$) and marginally between groups (TIME*GROUP interaction: $F_{12, 60} = 1.7$, $p = 0.09$, $\eta_p^2 = 0.25$) with significant difference between limbs across groups (SIDE*GROUP interaction: $F_{12, 60} = 2.3$, $p = 0.02$, $\eta_p^2 = 0.31$). In HS, planned comparisons revealed that left hindlimb (white bars in Figure 5.1C) took significantly more steps than right hindlimb (white bars in Figure 5.1D) on DPS 2 ($p = 0.001$), 8 ($p = 0.008$), 15 ($p = 0.01$) and marginally on DPS 21 ($p = 0.07$). Moreover, left and right hindlimb step occurrence increased significantly in HS (white and black bars of 5.1C-D) compared to S (white and black bars of 5.1B) on DPS 2 ($p_{left} = 0.001$; $p_{right} = 0.01$) and 8 ($p_{left} = 0.02$; $p_{right} = 0.05$), but not on DPS 15 ($p_{left} = 0.41$; $p_{right} = 0.28$) and 21 ($p_{left} = 0.09$; $p_{right} = 0.1$).

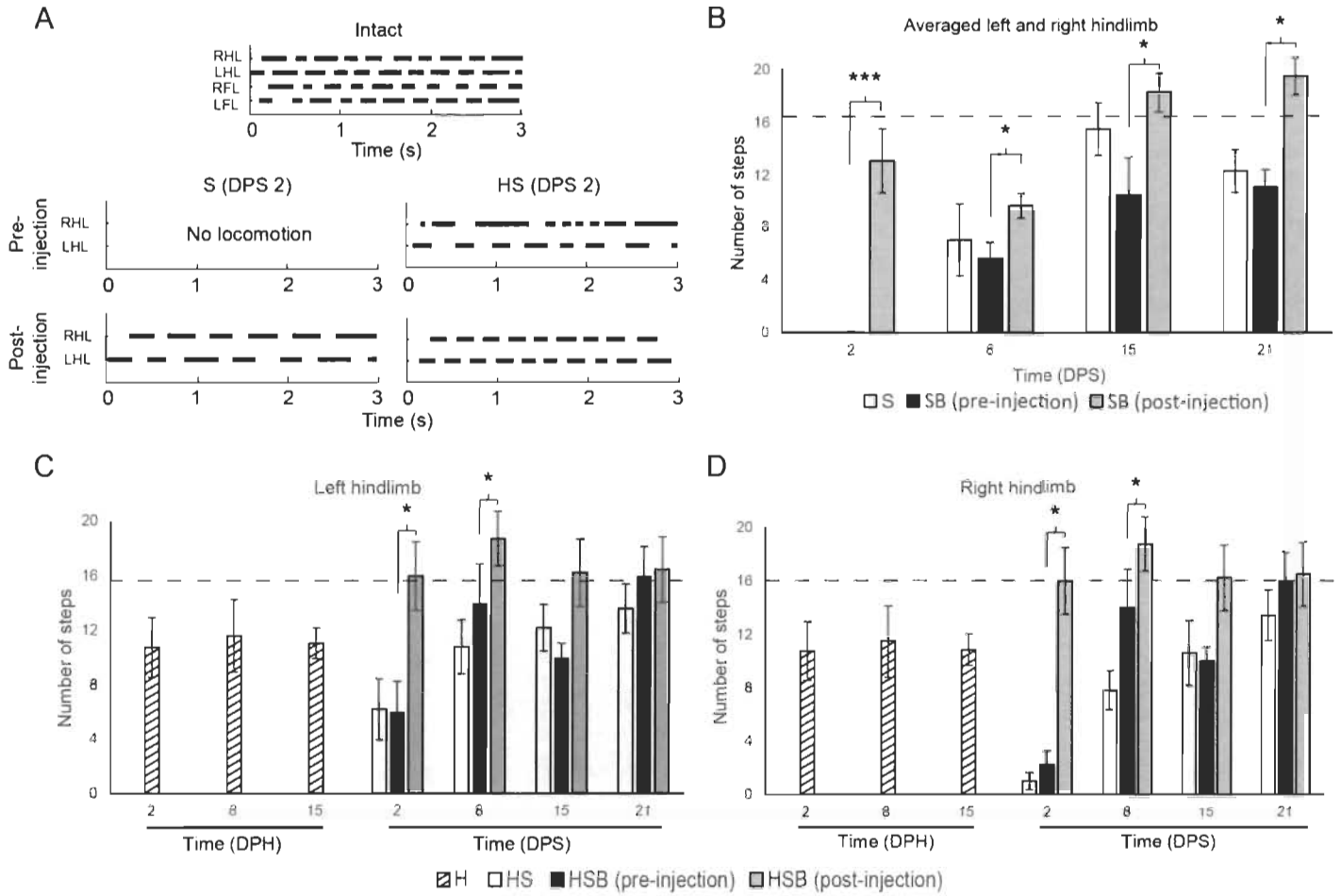

Figure 5.1. Number of steps performed before and after hemisection and transection

(A) Step cycle diagrams display number of steps and alternation between stance (dark bands) and swing (spaces between dark bands) before complete transection and on DPS 2 in SB and HSB both before and after buspirone injection. (B) The number of steps performed was averaged from left and right steps. The dashed line represents the averaged baseline value. (C-D) The number of left and right steps performed by HS and HSB. The dashed bars (white and black) represents the average number of steps hemisected (H) mice could performed before undergoing the transection and the buspirone injections. The numbers of steps performed was severely decreased after transection and restored acutely by buspirone in both SB (B) and HSB (C-D). RHL: right hindlimb, LHL: left hindlimb, RFL: right forelimb, LFL: left forelimb. * $p \leq 0.05$, *** $p \leq 0.001$

Homolateral coupling alteration after left hemisection

During locomotion, homolateral coupling (i.e. forelimb versus ipsilateral hindlimb) has a phase ratio around 0.35 on right and left side, as observed at baseline (Table 5-2). After hemisection, phase ratio shifted between the left forelimb (LFL) and hindlimb (LHL) on DPH 2 and DPH 8, and recovered on DPH 15. This shift was not observed between right forelimb (RFL) and right hindlimb (RHL). However, no significant difference in phase were observed over time (no main TIME effect: $F_{3, 21} = 1.1$, $p = 0.36$, $\eta_p^2 = 0.14$) or between left versus right pair of limbs (no main SIDE effect: $F_{1, 21} = 1.9$, $p = 0.21$, $\eta_p^2 = 0.22$).

Left hemisection decreased homolateral coupling strength, as R values decreased significantly over time (main TIME effect: $F_{3, 21} = 11.5$, $p < 0.001$, $\eta_p^2 = 0.62$) and this effect was not different between left versus right pairs of limbs (no SIDE*TIME interaction: $F_{3, 21} = 0.4$, $p = 0.74$, $\eta_p^2 = 0.06$). Planned comparisons of the main effect of time revealed that for both left and right pairs of limbs, coupling strength was significantly decreased at DPH 2 ($p = 0.002$), DPH 8 ($p < 0.001$) and DPH 15 ($p = 0.02$) compared to baseline. Overall, this shows that left hemisection desynchronized both left and right homolateral coupling up to 15 days after the lesion.

Table 5-2. Mean phase coupling after hemisection

	Days (DPH)	C-value	R-value
LFL vs LHL	Intact	0.37 ± 0.03	0.78 ± 0.04
	2	0.48 ± 0.16	0.35 ± 0.10
	8	0.58 ± 0.12	0.29 ± 0.06
	15	0.32 ± 0.11	0.42 ± 0.16
RFL vs RHL	Intact	0.38 ± 0.03	0.78 ± 0.05
	2	0.36 ± 0.10	0.39 ± 0.14
	8	0.38 ± 0.07	0.31 ± 0.10
	15	0.38 ± 0.04	0.53 ± 0.12

C values and R values from H mice locomotion were averaged. Homolateral coupling on the left side, measured between left forelimb (LFL) and left hindlimb (LHL), and on the right side

between right forelimb (RFL) and right hindlimb (RHL) are more variable as shown by the decreased R value on DPH 2, DPH 8 and DPH 15 compared to baseline.

Buspirone improves the hindlimb coupling after transection

The coupling between left and right hindlimbs (homologous coupling) was impaired following a complete transection in all groups. As illustrated by the gait diagram in Figure 5.1A, buspirone improved the stepping and coupling between left and right hindlimbs in both SB and HSB. The acute enhancement by buspirone did not reach significance for all time points combined (no main BUSPIRONE effect: $F_{1, 21} = 3.0$, $p = 0.13$, $\eta_p^2 = 0.30$). We then verified homologous coupling recovery after complete transection in all groups. In all groups combined, phase ratio spread from the baseline ratio of 0.5 and recovered over time (main TIME effect: $F_{4, 60} = 2.9$, $p = 0.03$, $\eta_p^2 = 0.16$, Table 5-3), but this recovery over time did not differ between groups (no TIME*GROUP interaction: $F_{12, 60} = 0.3$, $p = 0.99$, $\eta_p^2 = 0.05$). In all groups combined, planned contrasts of the main TIME effect disclosed that phase coupling was altered in comparison to baseline on DPS 2 ($p = 0.02$) and marginally on DPS 15 ($p = 0.07$) but not on DPS 8 ($p = 0.42$) and 21 ($p = 0.32$).

Table 5-3. Mean phase coupling after transection

	Days (DPS)	S	SB		HS	HSB	
			Pre	Post		Pre	Post
C value	Intact	0.48 ± 0.02	0.50 ± 0.04		0.51 ± 0.02	0.49 ± 0.02	
	2	0.23 ± 0.15	0.35 ± 0.16	0.45 ± 0.03	0.30 ± 0.08	0.44 ± 0.10	0.48 ± 0.04
	8	0.38 ± 0.10	0.47 ± 0.10	0.52 ± 0.03	0.51 ± 0.04	0.50 ± 0.02	0.52 ± 0.02
	15	0.36 ± 0.06	0.45 ± 0.06	0.58 ± 0.03	0.43 ± 0.11	0.49 ± 0.02	0.46 ± 0.02
	21	0.45 ± 0.07	0.50 ± 0.03	0.54 ± 0.02	0.50 ± 0.02	0.53 ± 0.03	0.47 ± 0.02
R value	Intact	0.97 ± 0.01	0.90 ± 0.04		0.89 ± 0.03	0.96 ± 0.01	
	2	0.27 ± 0.17	0.45 ± 0.21	0.73 ± 0.08	0.45 ± 0.19	0.88 ± 0.06	0.91 ± 0.02
	8	0.72 ± 0.18	0.86 ± 0.08	0.87 ± 0.05	0.87 ± 0.05	0.95 ± 0.02	0.94 ± 0.02
	15	0.92 ± 0.02	0.88 ± 0.06	0.95 ± 0.01	0.81 ± 0.10	0.88 ± 0.03	0.89 ± 0.04
	21	0.90 ± 0.03	0.93 ± 0.02	0.93 ± 0.02	0.85 ± 0.07	0.94 ± 0.02	0.90 ± 0.03

C values and R values were averaged for each group. Left versus right hindlimb coupling is altered and variability is increased on DPS 2 compared to baseline.

Lesioning the spinal cord similarly decreased the strength of the coupling, as the R values decreased after transection (Table 5-3). As illustrated in Figure 5.1A, buspirone improved the coupling regularity on DPS 2. However, no significant acute effect of buspirone was noted (no main BUSPIRONE effect: $F_{1, 21} = 1.3$, $p = 0.29$, $\eta_p^2 = 0.16$). R values recovered over time (main TIME effect: $F_{4, 60} = 12.0$, $p < 0.001$, $\eta_p^2 = 0.44$), and recovery over time was similar between groups (no TIME*GROUP interaction, $F_{12, 60} = 1.3$, $p = 0.24$, $\eta_p^2 = 0.21$). Planned comparisons of the main TIME effect showed that in all groups combined, the strength of the coupling decreased significantly from baseline on DPS 2 ($p < 0.001$) and marginally on DPS 15 ($p = 0.10$) but not on DPS 8 ($p = 0.20$) and 21 ($p = 0.41$).

Recovery from a previous hemisection and buspirone improve hip excursion

Averaged excursion of the hip joint, calculated between maximal flexion and maximal extension in each step cycle (Figure 5.2A-B), was measured during locomotion at baseline, after

hemisection and after transection up until DPS 21. In S mice, excursion of the hip joint during locomotion was severely decreased on DPS 2 (Figure 5.2C, green line) after transection compared to baseline (Figure 5.2C, red line). However, the hip excursion was only minimally modified at the same time point in HS (Figure 5.2C, blue line). In the latter, flexion was initiated earlier in the step cycle compared to baseline, occurring well before the lift.

Buspirone injection acutely increased hip excursion in SB and HSB (Figure 5.2D-E; main BUSPIRONE effect : $F_{1, 21} = 27.4$, $p = 0.001$, $\eta_p^2 = 0.80$) with marginally different excursion improvement post-injection (gray bars vs black bars) over time (BUSPIRONE*TIME interaction : $F_{3, 21} = 2.9$, $p = 0.06$, $\eta_p^2 = 0.29$) and between groups (BUSPIRONE*GROUP interaction : $F_{1, 21} = 3.7$, $p = 0.1$, $\eta_p^2 = 0.34$). In SB, buspirone acutely enhanced hip excursion on DPS 2 ($p = 0.03$), DPS 8 ($p = 0.05$) and DPS 15 ($p = 0.02$), but not DPS 21 ($p = 0.17$). In HSB, buspirone acutely enhanced hip excursion on DPS 2 ($p = 0.02$), but not on DPS 8 ($p = 0.28$), 15 ($p = 0.90$) and 21 ($p = 0.55$). Moreover, hip excursion was greater after buspirone injection in HSB (gray bars in Figure 5.2E) compared to SB (gray bars of Figure 5.2D) on DPS 2 ($p = 0.002$) but not on DPS 8 ($p = 0.46$), 15 ($p = 0.17$) and 21 ($p = 0.58$).

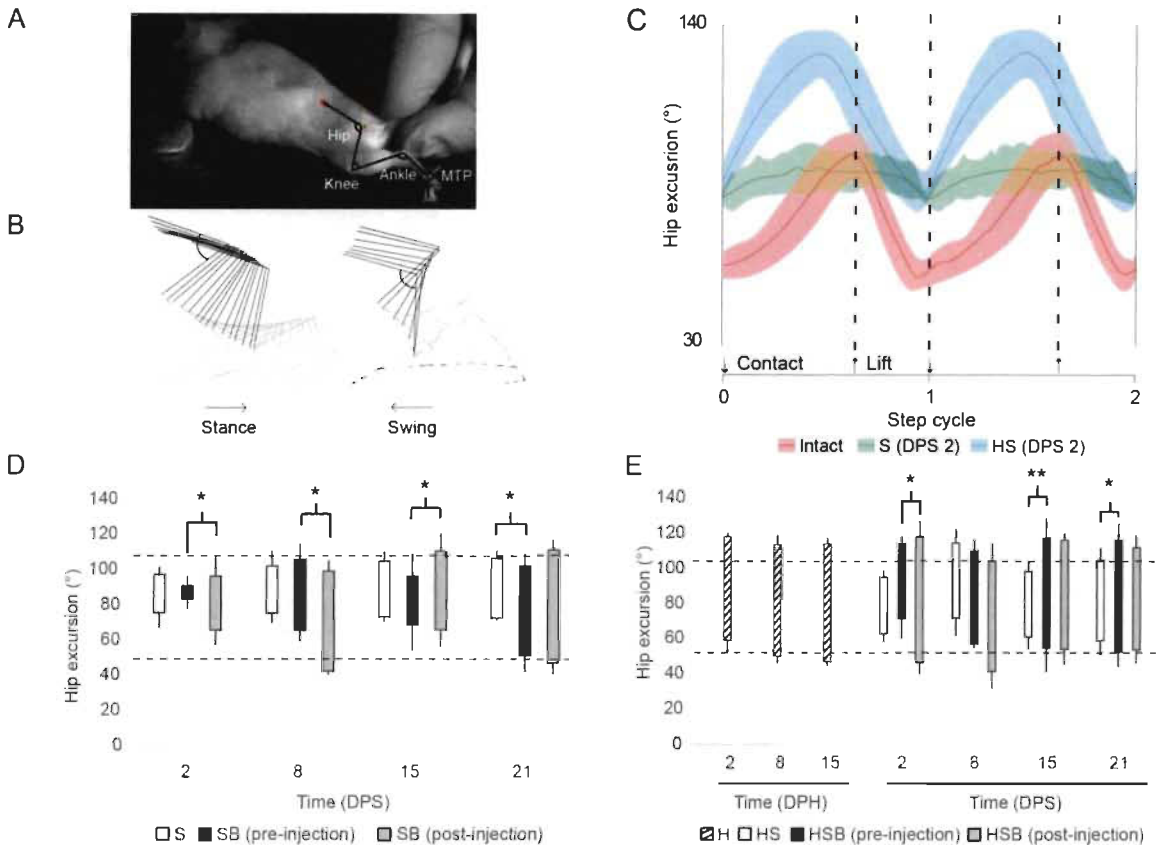

Figure 5.2. Hip excursion recovery after transection

(A) Individual example of hindlimb positioning at the end of the stance phase during locomotor bouts on DPS 8 with superposed hindlimb sticks representation used to calculate the angular excursions of joints. (B) Typical stick figure displaying hindlimb movement during 1 stance (left panel) and swing (right panel) cycle. This step was taken from intact state locomotion to facilitate comprehension of how joints excursion was calculated between maximally-flexed and maximally-extended positions. (C) Mean and standard deviation (dark and light lines, respectively) of hip excursion were measured from the longest locomotor bouts the animal could perform, 2 step cycles are displayed to facilitate comprehension. Hip excursion is severely decreased on DPS 2 in S (green line) compared to HS (blue line) or baseline (red line). (D, E) Averaged hip excursion throughout the recovery period after hemisection and transection. Buspirone chronic effect was evaluated by comparing white and black bars, while its acute effect was assessed by comparing black and gray bars. The impact of hemisection was examined by comparing white, black or gray bars from (D) to the concordant bars in (E). * $p \leq 0.05$, ** $p \leq 0.01$

In addition to the acute effect of buspirone, hip excursion increased over time in all groups combined (main TIME effect: $F_{4, 56} = 15.7$, $p < 0.001$, $\eta_p^2 = 0.53$). Moreover, it was significantly different between groups (main GROUP effect: $F_{3, 56} = 6.4$, $p = 0.006$, $\eta_p^2 = 0.58$) and improvement over time differed significantly between groups (TIME*GROUP interaction: $F_{12, 56} = 4.0$, $p < 0.001$, $\eta_p^2 = 0.46$). Planned contrasts showed that hip excursion of SB (pre-injection) was increased significantly compared to S (black versus white bars in Figure 5.2D) on DPS 21 ($p = 0.05$), but not on DPS 2 ($p = 0.24$), DPS 8 (0.11) and DPS 15 ($p = 0.50$). It was also increased in HSB (pre-injection) compared to HS on all time points during recovery (black versus white bars in Figure 5.2E) and reached significance on DPS 15 ($p = 0.01$) and DPS 21 ($p = 0.04$) but not on DPS 2 ($p = 0.36$) and DPS 8 ($p = 0.24$).

Planned comparisons also disclosed that recovery from a previous hemisection improved hip excursion in HSB (pre-injection) compared to SB (pre-injection) on all time points (black bars of Figure 5.2D-E) and reached significance on DPS 2 ($p = 0.009$) and DPS 15 ($p = 0.002$), but not on DPS 8 ($p = 0.18$) and DPS 21 ($p = 0.15$). In contrast, recovery from a previous hemisection did not significantly improved hip excursion in HS (white bars in Figure 5.2E) compared to S (white bars in Figure 5.2D) on any time points during recovery after transection (p's> 0.08).

Previous hemisection and buspirone improve knee excursion after transection

Averaged excursion of the knee joint was measured from maximal flexion to maximal extension in each step cycle (Figure 5.3A) at baseline, after hemisection and after transection up until DPS 21. On DPS 2, knee excursion was severely altered in S (Figure 5.3B, green line) while considerable knee excursion capacity was preserved in HS (Figure 5.3B, blue line). However, the pattern was greatly impaired, with knee progressively flexing throughout the swing phase instead of a swift flexion followed by progressive extension as observed at baseline (Figure 5.3B, red line).

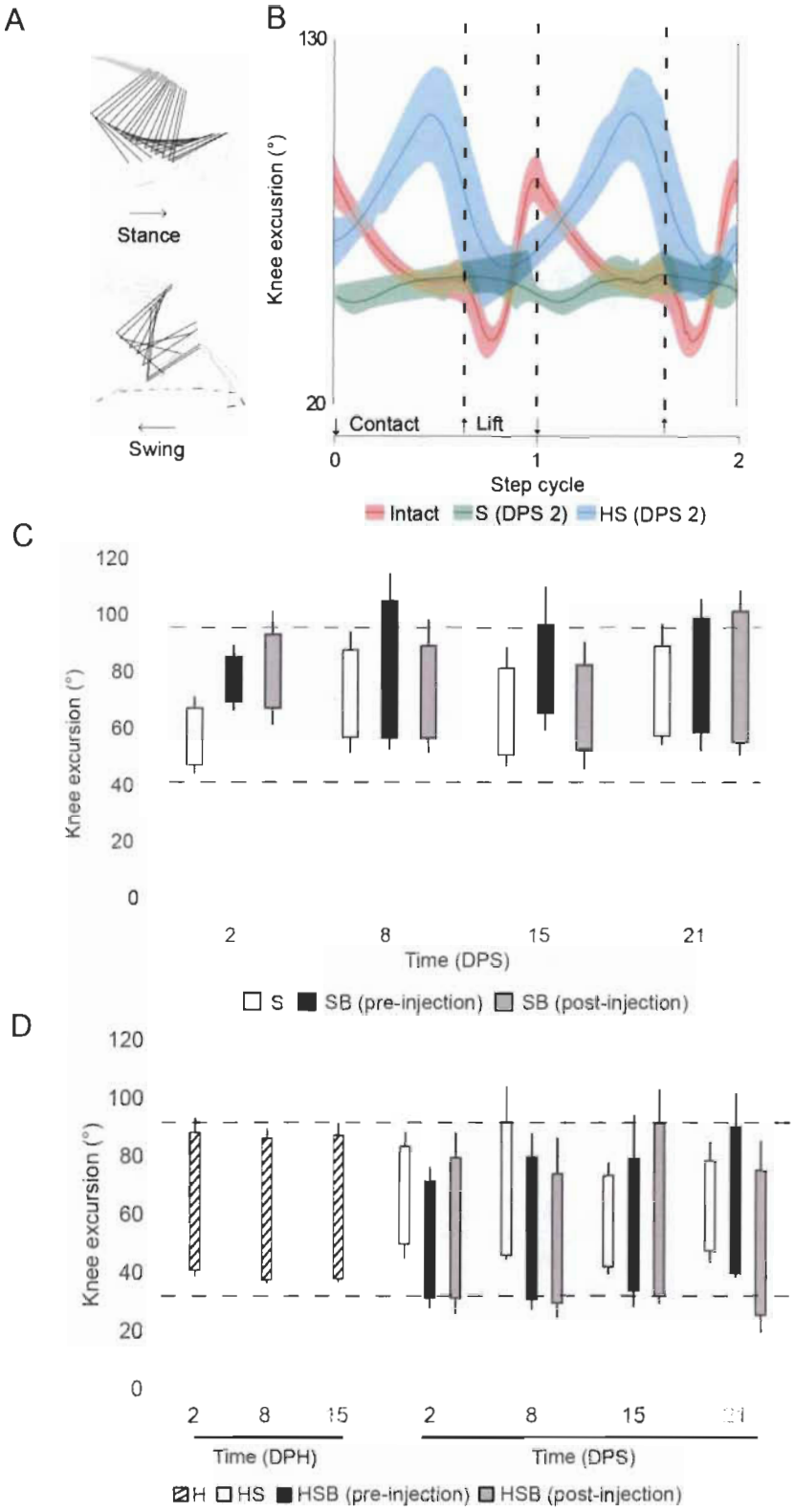

Figure 5.3. Knee excursion before and after transection

Figure 5.3 legend: (A) Stick figure depicting an example of 1 step performed at baseline. (B) Mean and standard deviation (dark and light lines, respectively) of knee excursion, averaged and displayed for 2 consecutive steps is abolished on DPS 2 in S (green line), while it is present in HS (blue line) but altered compared to baseline (red line). Knee excursion recovered progressively in S and SB (C) as well as in HS and HSB (D).

Buspirone improved knee excursion marginally (main BUSPIRONE effect : $F_{1, 21} = 5.2$, $p = 0.056$, $\eta_p^2 = 0.43$) but without difference over time (no BUSPIRONE*TIME interaction : $F_{3, 21} = 0.9$, $p = 0.46$, $\eta_p^2 = 0.11$) or between groups (no BUSPIRONE*GROUP interaction : $F_{1, 21} = 0.0$, $p = 0.96$, $\eta_p^2 = 0.00$). In all groups, knee excursion improved over time (main TIME effect: $F_{4, 56} = 7.9$, $p < 0.001$, $\eta_p^2 = 0.36$; Figure 5.3C-D). Moreover, knee excursion differed between groups (main GROUP effect: $F_{3, 56} = 25.8$, $p < 0.001$, $\eta_p^2 = 0.85$) but the improvement over time was not significantly different between groups (no TIME*GROUP interaction: $F_{12, 56} = 1.4$, $p = 0.19$, $\eta_p^2 = 0.23$). Planned contrasts of the main GROUP effect disclosed that knee excursion was enhanced in HSB (pre-injection; black bars of Figure 5.3D) compared to HS (white bars in Figure 5.3D, $p = 0.003$) and SB (pre-injection; black bars in Figure 5.3C, $p < 0.001$).

Previous hemisection and buspirone improve ankle flexion

Ankle flexion calculated as the minimal angle in each step cycle averaged over the locomotor bout was assessed (Figure 5.4A) at baseline and throughout recovery period. After transection, the ability of S mice to flex their ankles during locomotion was severely compromised (Figure 5.4B, green line) while it was preserved, albeit impaired, in HS (Figure 5.4B, blue line). Moreover, ankle flexion was acutely enhanced by buspirone (main BUSPIRONE effect : $F_{1, 21} = 12.6$, $p = 0.009$, $\eta_p^2 = 0.64$). This improvement was different over time (BUSPIRONE*TIME interaction : $F_{3, 21} = 22.9$, $p = 0.009$, $\eta_p^2 = 0.77$). In SB, buspirone acutely improved ankle flexion on all time points (gray bars vs black bars in Figure 5.4C) and reached significance on DPS 2 ($p < 0.001$) but not on DPS 8 ($p = 0.64$), DPS 15 ($p = 0.12$) or DPS 21 ($p = 0.07$). Similarly, in HSB (gray bars vs black bars in Figure 5.4D), buspirone acutely improved ankle flexion on DPS 2 ($p = 0.01$) but not on DPS 8 ($p = 0.15$), DPS 15 ($p = 0.61$) and DPS 21 ($p = 0.83$).

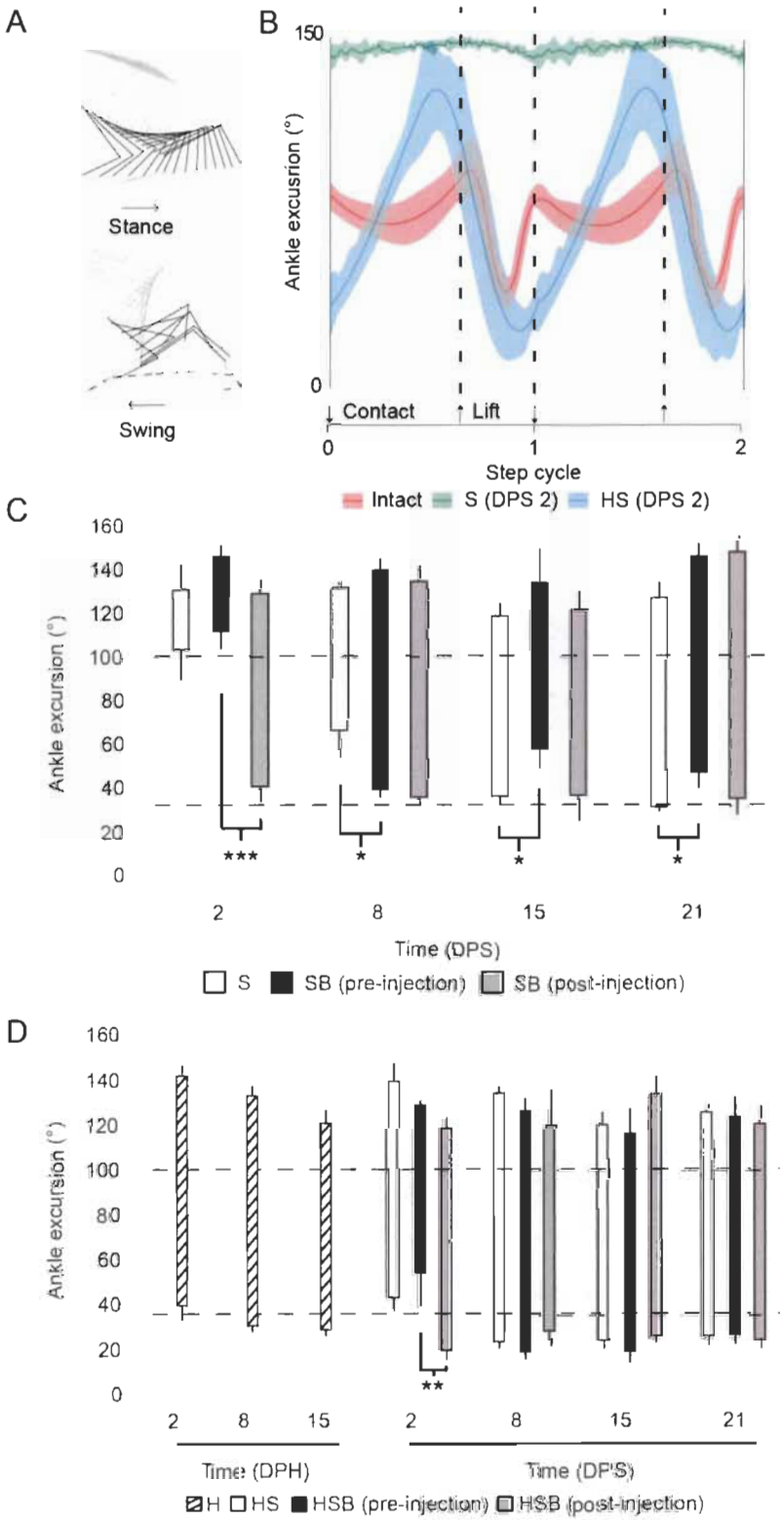

Figure 5.4. Ankle flexion recovery after transection

Figure 5.4 legend: (A) Stick figure displaying an example of 1 step in intact state locomotion. (B) Mean and standard deviation (dark and light lines, respectively) of ankle excursion before transection (red line), on DPS 2 in S (green line) and HS (blue line). Ankle flexion is calculated as the minimal angle at each step cycle averaged over the locomotor bout, i.e. lower ends of bars in (C) and (D). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Ankle flexion improved over time in all groups (main TIME effect: $F_{4, 56} = 37.4$, $p < 0.001$, $\eta_p^2 = 0.73$, Figure 5.4C-D). Ankle flexion also differed between groups (main GROUP effect: $F_{3, 56} = 13.1$, $p < 0.001$, $\eta_p^2 = 0.74$). Moreover, its improvement over time was significantly different between groups (TIME*GROUP interaction: $F_{12, 56} = 5.1$, $p < 0.001$, $\eta_p^2 = 0.52$). Planned comparisons revealed reduced ankle flexion in S (white bars in Figure 5.4C) compared to HS (white bars in Figure 5.4D) on DPS 2 ($p = 0.003$) and DPS 8 ($p < 0.001$) but not DPS 15 ($p = 0.25$) and DPS 21 ($p = 0.67$) as well as compared to SB (pre-injection, black bars in Figure 5.4C) on DPS 8 ($p = 0.02$). However, SB had reduced ankle flexion compared to S on DPS 15 ($p = 0.03$) and DPS 21 ($p = 0.05$). Moreover, ankle flexion of HSB (pre-injection, black bars in Figure 5.4D) was increased significantly compared to SB (pre-injection, black bars of Figure 5.4C) on DPS 2 ($p = 0.005$), DPS 8 ($p = 0.004$), DPS 15 ($p = 0.001$) and DPS 21 ($p = 0.03$).

Improved MTP extension associated with plantar paw placement

MTP extension was calculated as the maximal angle in each step cycle averaged over the locomotor bout examined. After transection, improper stance positioning of the paw on its dorsum rather than on the plantar surface greatly reduced MTP extension in S (Figure 5.5B, green line) but not in HS (Figure 5.5B, blue line). Alike other joints, the MTP excursion pattern was altered in HS as the flexion was initiated earlier than lift. Buspirone injection acutely enhanced MTP extension (main BUSPIRONE effect : $F_{1, 21} = 12.7$, $p = 0.009$, $\eta_p^2 = 0.64$) and this improvement differed over time (BUSPIRONE*TIME interaction : $F_{3, 21} = 7.2$, $p = 0.002$, $\eta_p^2 = 0.51$). Planned contrasts disclosed that in both SB and HSB (Figure 5.5C-D), buspirone

improved MTP extension on DPS 2 ($p = 0.005$) but not on DPS 8 ($p = 0.76$), DPS 15 ($p = 0.18$) and DPS 21 ($p = 0.13$).

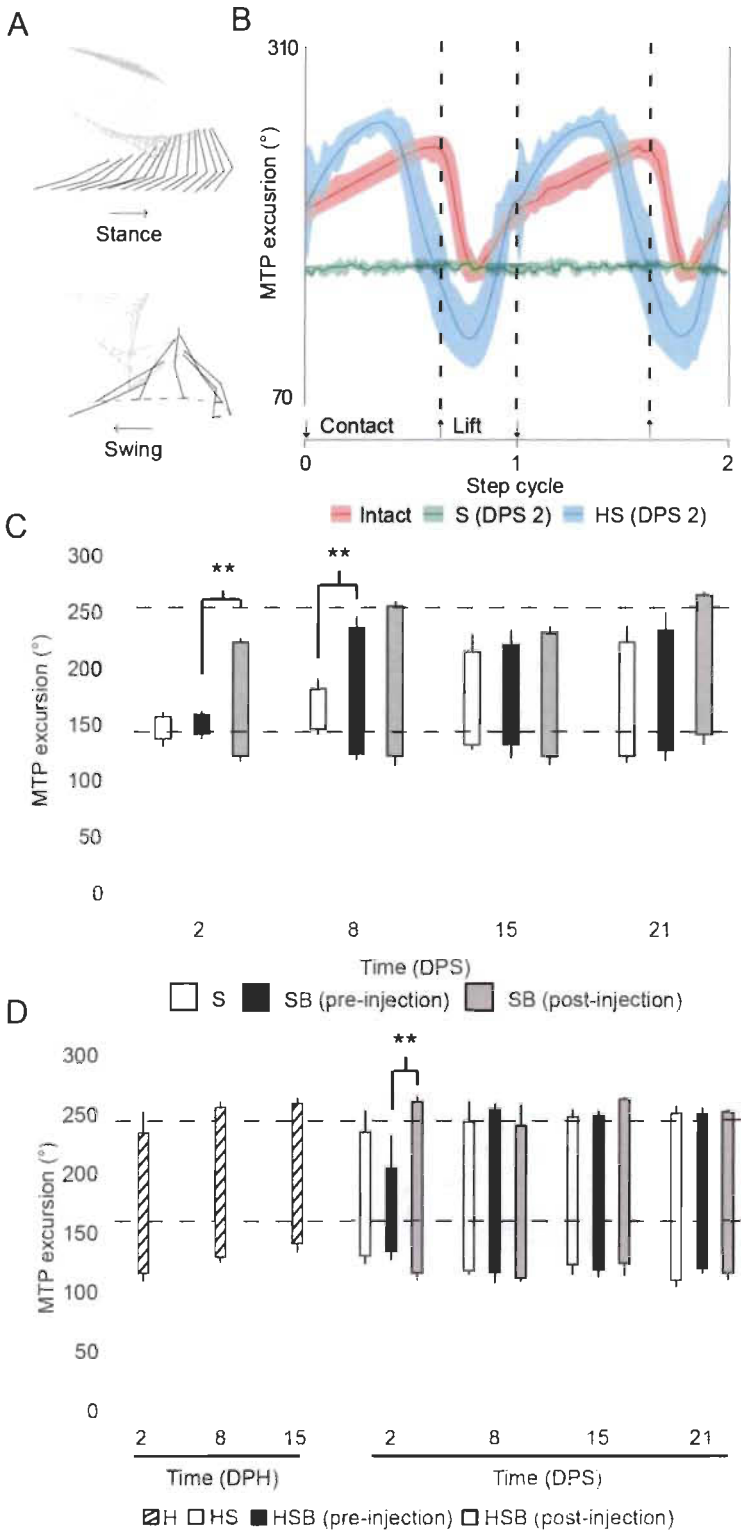

Figure 5.5. MTP extension is a measure of plantar placement during recovery after transection

Figure 5.5 legend: (A) Stick figure with highlighted MTP joint movement at baseline. (B) Mean and standard deviation (dark and light lines, respectively) of MTP excursion shows severe attenuation of MTP involvement on DPS 2 in S (green line) compared to baseline (red line). MTP extension displays an altered pattern in HS on DPS 2 (blue line) with a precocious flexion phase during stance. (C-D) Averaged MTP extension, i.e. upper ends of bars, in each group at each time point. MTP extension recovery over time in S and SB (C) as well as in HS and HSB (D). ** $p \leq 0.01$

Recovery occurred in all groups. As a result, MTP extension increased significantly over time (main TIME effect: $F_{4, 56} = 15.2$, $p < 0.001$, $\eta_p^2 = 0.52$). Moreover, MTP extension differed between groups (main GROUP effect: $F_{3, 56} = 15.6$, $p < 0.001$, $\eta_p^2 = 0.77$). MTP extension recovery over time also differed between groups (TIME*GROUP interaction: $F_{12, 56} = 2.4$, $p = 0.02$, $\eta_p^2 = 0.33$). Planned comparisons revealed that MTP extension was reduced in S (white bars in Figure 5.5C) compared to HS (white bars in Figure 5.5D) on all time points during recovery and reached significance on DPS 2 ($p = 0.004$) and DPS 8 ($p = 0.006$) but not on DPS 15 ($p = 0.11$) or DPS 21 ($p = 0.32$). MTP extension was also reduced in S compared to SB (pre-injection; black bars of Figure 5.5C) on DPS 8 ($p = 0.02$) but not on DPS 2 ($p = 0.92$), DPS 15 ($p = 0.98$) and DPS 21 ($p = 0.78$).

Paw positioning in front of hip at contact is restored by buspirone

The capacity of mice to achieve proper paw contact in front of the hip joint was severely altered after transection in all groups (see individual examples at baseline and on DPS 2 in Figure 5.6A). Using the hip joint as the reference point, we measured the position of the 5th toe at contact on all time points. A positive value was assigned for paw positioning at contact in front of hip joint and a negative value was assign for paw positioning at contact behind the hip. Buspirone acutely improved paw positioning at contact (main BUSPIRONE effect : $F_{1, 21} = 18.3$, $p = 0.004$, $\eta_p^2 = 0.72$). Moreover, acute enhancement of paw positioning by buspirone varied over time (BUSPIRONE*TIME interaction : $F_{3, 21} = 5.8$, $p = 0.005$, $\eta_p^2 = 0.45$). Planned contrasts showed that, in both S (Figure 5.6B) and HS (Figure 5.6C-D), paw positioning was

improved bilaterally post-injection (gray bars) compared to pre-injection (black bars) on all time points during recovery and reached significance on DPS 2 ($p = 0.005$) and DPS 15 ($p = 0.005$), but not on DPS 8 ($p = 0.31$) and DPS 21 ($p = 0.21$).

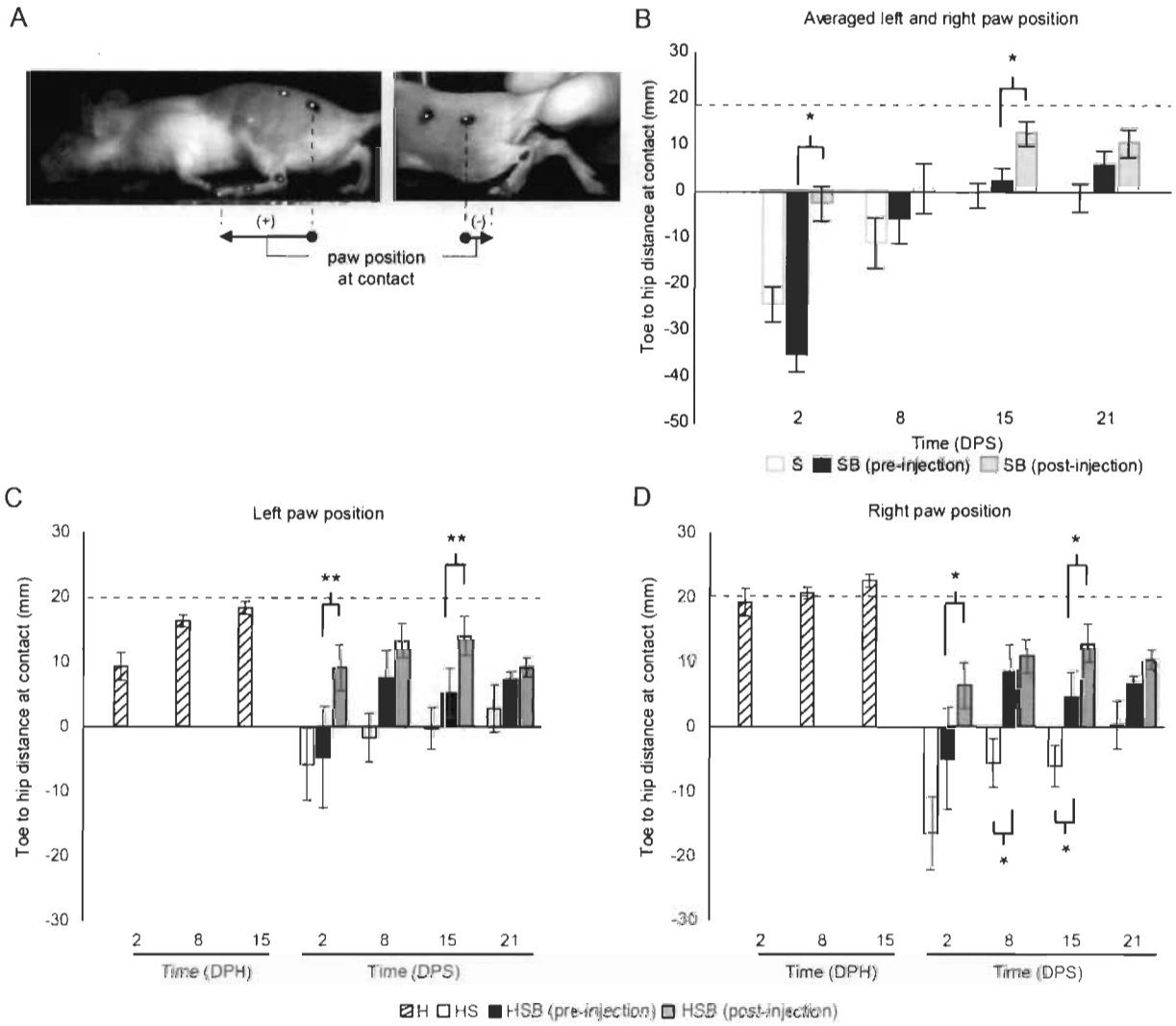

Figure 5.6. Paw positioning at contact in relation to hip joint

(A) Individual examples of a normal positioning at contact in front of hip joint observed at baseline (left) and a deficient positioning at contact behind hip joint observed on DPS 2 after transection (right). (B) Toe to hip distance averaged for left and right paws in S and SB. Left (C) and right (D) paw positioning at contact in HS and HSB. In both naive and hemisected, toe to hip distance was increased post-injection (gray lines). Right paw positioning (D) improved

in hemisected mice with buspirone (pre-injection; black line) compared to untreated hemisected mice (dashed line). * $p \leq 0.05$

Paw positioning recovered over time in all groups (main TIME effect: $F_{4, 60} = 73.1$, $p < 0.001$, $\eta_p^2 = 0.83$). Paw positioning differed between groups (main GROUP effect: $F_{3, 60} = 6.3$, $p = 0.006$, $\eta_p^2 = 0.56$). Moreover, its recovery over time differed between groups (TIME*GROUP interaction: $F_{12, 60} = 4.0$, $p < 0.001$, $\eta_p^2 = 0.45$). In addition to the aforementioned acute effect, buspirone also provided long-lasting improvement of paw positioning. Left and right paw positioning was enhanced in SB (pre-injection; black bars in Figure 5.6B) compared to S (white bars in Figure 5.6B) at DPS 8, DPS 15 and DPS 21 but this effect never reached significance (p's> 0.10). Similarly, left paw positioning was enhanced on all time points in HSB (pre-injection; black bars in Figure 5.6C) compared to HS (white bars in Figure 5.6C), but failed to reach significance (p's> 0.1). However, right paw positioning improved significantly in HSB (pre-injection; black bars in figure 5.6D) compared to HS (white bars in Figure 5.6D) on DPS 8 ($p = 0.03$) and DPS 15 ($p = 0.01$) but not on DPS 21 ($p = 0.15$).

Previous hemisection also influenced paw positioning recovery, as it differed depending on the limb evaluated (SIDE*TIME interaction: $F_{12, 60} = 2.3$, $p = 0.02$, $\eta_p^2 = 0.32$). In HS, planned comparisons revealed that paw positioning was significantly better in left hindlimb (Figure 5.6C) than right hindlimb (Figure 6D) on DPS 2 ($p = 0.001$) and DPS 21 ($p = 0.03$) but not on DPS 8 ($p = 0.29$) and DPS 15 ($p = 0.08$). Moreover, left paw positioning was improved in HS (white bars in Figure 5.6C) compared to S (white bars in Figure 5.6B) on DPS 2 ($p = 0.005$) but not on DPS 8 ($p = 0.16$), DPS 15 ($p = 0.90$) and DPS 21 ($p = 0.39$). In contrast, right paw positioning was similar in HS (white bars in Figure 5.6D) and S (white bars of Figure 5.6B) on every time points after transection (p's> 0.2) except for significantly-hindered right paw positioning in HS on DPS 15 ($p = 0.02$).

Stance and swing duration changes after transection and buspirone injection

Stance duration increased on DPS 2 after transection (Figure 5.7A) due to the inability of the mice to initiate the next swing phase. During recovery, stance duration decreased over

time (main TIME effect: $F_{4, 60} = 3.3$, $p = 0.02$, $\eta_p^2 = 0.18$) but its restoration did not differ between groups (no TIME*GROUP interaction: $F_{12, 60} = 1.7$, $p = 0.10$, $\eta_p^2 = 0.25$). Planned contrasts for the main TIME effect disclosed that stance duration increased marginally in all groups compared to baseline on DPS 2 ($p = 0.066$) but then returned to baseline on DPS 8 ($p = 0.33$), DPS 15 ($p = 0.35$) and DPS 21 ($p = 0.12$). Buspirone didn't modify stance duration significantly (no BUSPIRONE effect: $F_{1, 21} = 1.5$, $p = 0.26$, $\eta_p^2 = 0.18$), but the injection noticeably decreased stance duration at DPS 2 (Figure 5.7B).

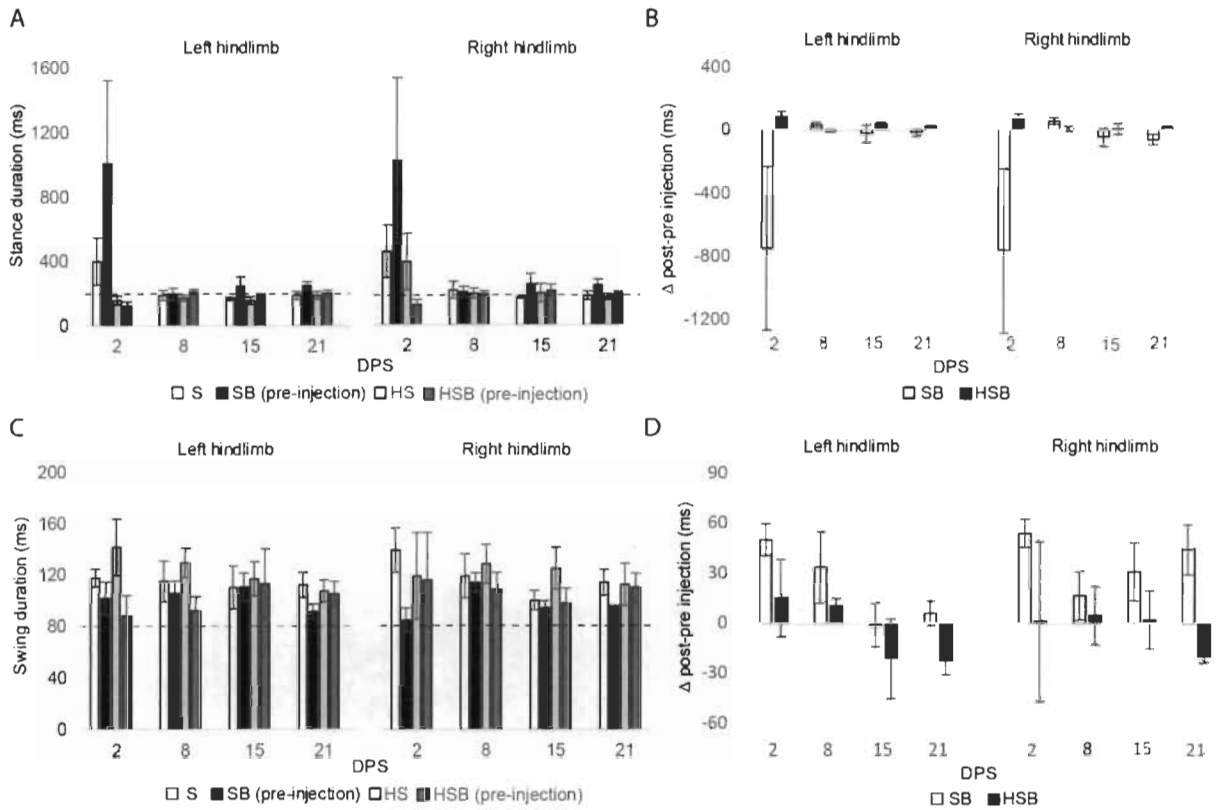

Figure 5.7. Stance and swing duration after transection

(A) Stance duration is increased on DPS 2 but return to baseline (dotted line) on DPS 8 for both left and right hindlimb. (B) Stance duration differs after buspirone injection compared to pre-injection on DPS 2 only, for both hindlimb. (C) Swing duration is increased after transection compared to baseline (dotted line) but does not recover. (D) Buspirone had no clear effect on swing duration, as the difference between pre-injection and post-injection did not reached significance.

Similarly, swing phase duration increased in all groups after transection (main TIME effect: $F_{4, 60} = 6.2$, $p < 0.001$, $\eta_p^2 = 0.29$, Figure 5.7C) without difference between groups (no TIME*GROUP interaction: $F_{12, 60} = 0.5$, $p = 0.89$, $\eta_p^2 = 0.09$). In contrast with stance, increased swing duration did not recover and remained significantly increased compared to baseline on DPS 2 ($p = 0.004$), DPS 8, DPS 15 and DPS 21 (p's <0.001). No significant changes were induced by buspirone (no BUSPIRONE effect: $F_{1, 21} = 4.0$, $p = 0.08$, $\eta_p^2 = 0.37$, Figure 5.7D).

Drag duration changes after transection and buspirone injection

The proportion of swing phase being achieved with paw dragging on the treadmill, which was not observed at baseline, increased after transection (see individual examples of the 5th toe trajectory recovery in Figure 5.8A). Drag decreased over time during recovery (main TIME effect: $F_{4, 60} = 137.8$, $p < 0.001$, $\eta_p^2 = 0.90$) but drag recovery over time did not differ between groups (no TIME*GROUP interaction: $F_{12, 60} = 1.2$, $p = 0.34$, $\eta_p^2 = 0.19$). Moreover, buspirone acutely hindered paw drag (main BUSPIRONE effect : $F_{1, 21} = 34.3$, $p < 0.001$, $\eta_p^2 = 0.83$). In SB (Figure 5.8B), short-term reduction of paw drag post-injection (gray bars) compared to pre-injection (black bars) reached significance on DPS 2 ($p = 0.02$) and DPS 21 ($p = 0.02$) but not on DPS 8 ($p = 0.08$) and DPS 15 ($p = 0.14$).

Drag presented differently in mice that underwent a previous hemisection, depending on the side of the limb evaluated (main SIDE effect: $F_{1, 60} = 9.2$, $p = 0.008$, $\eta_p^2 = 0.38$) and left versus right drag difference varied over time and between groups (SIDE*TIME*GROUP interaction: $F_{12, 60} = 2.5$, $p = 0.01$, $\eta_p^2 = 0.33$). Figure 5.8C illustrates left versus right paw drag asymmetry recovery over time in H (dashed bars), HS (white bars), HSB (pre-injection; black bars) and HSB (post-injection; gray bars). After left hemisection, left hindpaw was dragging considerably more than right hindpaw, yielding a negative AI (see AI formula in Methods section). Left paw drag recovered partially before complete transection but the asymmetry persisted. After transection, right paw was dragging more than left paw, yielding a shift of AI towards positive values. Planned comparisons revealed that drag asymmetry was hindered in HSB (pre-injection) compared to HS and the difference reached significance on DPS 15 ($p = 0.04$) but not on DPS 2 ($p = 0.58$), DPS 8 ($p = 0.13$) and DPS 21 (0.34). However, buspirone

showed no acute effect on drag asymmetry (no main BUSPIRONE effect: $F_{1,9} = 0.9$, $p = 0.41$, $\eta_p^2 = 0.24$) as post-injection values (gray bars in Figure 5.8C) were similar to pre-injection values.

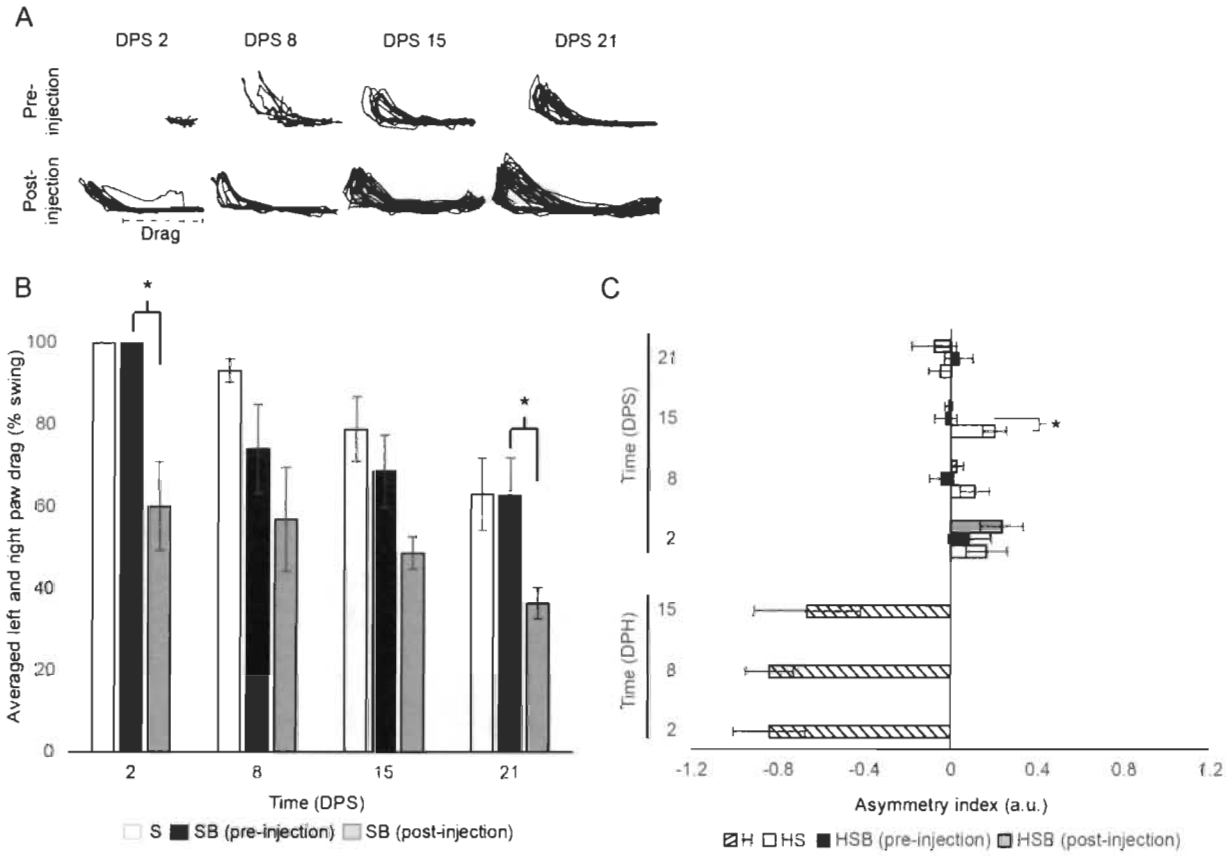

Figure 5.8. Paw drag during the swing phase

(A) Trajectory of the left 5th toe during locomotor bouts on DPS 2, DPS 8, DPS 15 and DPS 21 illustrates the late onset of paw elevation during the swing phase which results in paw drag. Paw drag decreased during recovery in naive mice and naive mice with buspirone (B) with acute, but not chronic, effect of buspirone injection on paw drag. (C) On DPS 2 after hemisection, paw drag was significantly worse ipsilateral to the hemisection, leading to major paw drag asymmetry. After transection, this asymmetry shifted contralaterally. There was no evident acute effect of buspirone, but paw drag asymmetry was significantly improved in hemisected mice with buspirone (preinjection; black bars), compared to untreated hemisected mice (white bars). $p \leq 0.05$

Discussion

This study took a combined behavioral and pharmacological approach and obtained 3 novel findings. First, buspirone, a 5-HT_{1A} partial agonist, exerted powerful acute facilitation of locomotor function in completely transected mice. Second, it acutely re-established symmetry in previously-hemisected spinal mice that showed an asymmetrical stepping pattern. Third, buspirone treatment combined with a training regimen induced some long-lasting improvements of locomotor functions in transected mice that had previously recovered from a partial section. The latter finding suggests that buspirone might potentiate residual functionality. Finally, partial preservation of left hindlimb locomotor function after transection in previously-hemisected mice confirmed that, as previously shown in cats (Barrière, et al. 2008 ; Martinez, et al. 2011), locomotor spinal network plasticity is implicated in functional restoration in mice. Altogether, these results show that buspirone, albeit a promising drug for rehabilitation therapies, must be used in combination with other interventions to promote long-lasting recovery.

Buspirone strongly facilitates spinal networks activity implied in locomotion

Buspirone had a significant acute impact on locomotion function. It reliably enhanced stepping, paw placement and hip, ankle and MTP excursions during the step cycle. Also, it decreased paw drag 20 min after injection. This novel finding complements our understanding of buspirone's impact on locomotion by showing that it alone can promote locomotion as early as 2 days after complete transection. Previous work has disclosed that combinatorial therapy with buspirone, apomorphine, benserazide and l-dopa induces treadmill locomotion in previously-untrained and paraplegic mice (Guertin, et al. 2011). Combination of buspirone, carbidopa and l-dopa also improved locomotor re-expression in mice after transection (Ung, et al. 2012). In these studies, buspirone, along with other pharmacological agents, also showed promising effects on locomotion after transection. However, the use of a harness to hold the animals on the treadmill, the absence of perineal stimulation and the buspirone dosage prevented the major acute effect of buspirone detailed herein. In the present study, the impact of buspirone alone or combined with training on locomotor recovery in the transected mouse model confirms its value in multi-targeted rehabilitation therapy. It is noteworthy that FDA-approved buspirone

was tested recently in a preclinical investigation in complete SCL patients (Gerasimenko, et al. 2015). It was shown to be a potent enhancer of epidural electrical stimulation-evoked locomotor-like activity.

Our mechanistic understanding of buspirone's impact on locomotion is still unsatisfactory. In addition to its effect on 5-HT_{1A} receptors, it has low affinity as an antagonist of D_2 dopaminergic receptors and weak affinity as an agonist for 5-HT_2 receptors (Hoyer, et al. 1994 ; Loane and Politis 2012). Evidence from studies on functions other than locomotion (e.g., anxiety) showed that its main pharmacological action occurs via activation of 5-HT_{1A} receptors (Loane and Politis 2012). Besides, previous work on the impact of 8-OH-DPAT, a selective 5-$HT_{1A/7}$ agonist, on locomotor recovery gave results similar to ours (Antri, et al. 2005; Antri, et al. 2003; Courtine, et al. 2009 ; Landry, et al. 2006; Lapointe, et al. 2008 ; Musienko, et al. 2011), suggesting that similar mechanisms are involved. 5- HT_{1A} receptors are abundant in the spinal cord and, most importantly, in locomotor-activated neurons of lumbar enlargement (Giroux, et al. 1999 ; Noga, et al. 2009). However, their role in locomotion has not been clearly determined. Both excitatory and inhibitory transmission can occur upon 5-HT_{1A} receptor activation, depending on the target (e.g., motoneurons versus muscle afferents or interneurons) or its location (e.g. perisomal versus dendritic). For instance, 5-HT_{1A} was found to increase motoneuron excitability by modulating K^+ conductance (Perrier, et al. 2003; Takahashi and Berger 1990), yet it has been demonstrated to suppress plateau potentials (Perrier and Cotel 2008). In addition, large amount of 5-HT_{1A} released in the ventral horn can lead to spillover and activation of receptors located on the initial segment of motoneuron axons (Perrier and Cotel 2015). This mechanism was recently implicated in decreased motoneuronal excitability during fatiguing exercise in humans (D'Amico, et al. 2017).

5-HT_{1A} receptors seem to have a coherent impact on spinal reflex pathways. In spinalized rats, 8-OH-DPAT decreased motoneuron responses to Ia afferent input (i.e., the Hoffmann (H)-reflex; Hasegawa and Ono 1996; Hedo, et al. 2002) and downmodulated its enhancement by 5-HT_{2C} (Gajendiran 2008). Also, 5-HT_{1A} activation restored group II muscle afferent cross inhibition that was disrupted by spinal transection (Aggelopoulos, et al. 1996). Inhibition of these reflex pathways was observed in fictive locomotion in cats (Perreault, et al. 1999), indicating that attenuation of these reflexes is important in generating stereotypic movements

such as locomotion. In the context of SCL, locomotor recovery is paralleled by an enhancement of spinal reflexes (Lee, et al. 2009). However, recent studies have provided evidence that increased reflex activity caused by dysregulation in inhibitory mechanisms lead to spasticity and functional deficits (Bose, et al. 2012). Buspirone might facilitate locomotion by preventing such hyperreflexia.

Buspirone alone has limited long-lasting effect on locomotor recovery

In contrast with its acute effects, buspirone had limited long-lasting influence on locomotor recovery. On the one hand, hip excursion was enhanced in SB on DPS 21 and in HSB on DPS 15 and DPS 21 compared to their respective control groups. Also, drag symmetry was attained by DPS 15 in HSB and only by DPS 21 in HS. On the other hand, it induced no change on step occurrence, homologous coupling, knee, ankle and MTP excursions, and paw positioning at contact. These results suggest that locomotor facilitation by buspirone might not potentiate the effect of training, but rather have an additive effect that rely, at least partly, on different mechanism than training.

Alternatively, these results could be explained by changes in 5-HT_{1A} receptors density and sensitivity induced by training, chronic buspirone treatment or the lesion itself. After hemisection or complete thoracic transection, lumbar spinal cord levels of serotonin decrease rapidly (Hains, et al. 2002; Saruhashi, et al. 1996). In response to denervation, 5-HT_{1A} receptors has been documented to become hypersensitized and their density progressively upregulated without normalisation of their sensitivity (Giroux, et al. 1999 ; Laporte, et al. 1995; Otoshi, et al. 2009). This upregulation depends on afferent inputs (Otoshi, et al. 2009) and might therefore be influenced by training. Chronic buspirone treatment might also influence 5-HT_{1A} receptor density, however, it has been shown to be without effect after an olfactory bulbectomy (Sato, et al. 2008). Overall, transection and training-induced 5-HT_{1A} density upregulation could explain, at least partly, the change in buspirone effect over time and across groups. In turn, these density changes most likely modify its effect since partial agonist as buspirone are highly affected by their targeted effector's density (Zhu 2005).

Locomotor recovery after partial section in the mouse model rely on spinal networks

In the present experiment, partial section targeting the left half of the T7 spinal cord produced moderate left hindlimb locomotor deficits on DPH 2 (increased homolateral and homologous coupling variability, hindered paw placement at contact and increased paw drag), some of which recovered completely (homologous coupling variability and paw placement) and some partially (homolateral coupling variability and paw drag) during the 3-week recovery period. No right hindlimb locomotor deficit was observed apart from increased homolateral coupling variability which was partly restored during the recovery period. Overall, these results are in agreement with previous work that demonstrated locomotor function recovery in the first 2–3 weeks after hemisection in other animal models, such as cats (Barrière, et al. 2010 ; Barrière, et al. 2008 ; Martinez, et al. 2011; Rossignol, et al. 2009) and rats (Arvanian, et al. 2009; Leszczyńska, et al. 2015). In the mouse model, previous experiments showed severe ipsilateral locomotor deficits during the first 4–8 weeks after T12 hemisection that recovered with documented supraspinal (Boido, et al. 2009) and propriospinal axonal outgrowth (Courtine, et al. 2008 ; Goldshmit, et al. 2008). Here, we validated the compensatory changes that occurred in the spinal network after T7 left hemisection by a transection-evoked right-to-left shift in stepping capacity. After complete transection, mice that had undergone previous left section showed improved stepping, better paw positioning and decreased paw drag on their left hindlimb compared to their right hindlimb. Some locomotor parameters (stepping and paw positioning) were also improved in the right hindlimb in previously HS compared to S. Thus, complete transection revealed plastic changes induced by partial sectioning. Such effect has also been described in cats in which this dual lesion paradigm has been developed first (Barrière, et al. 2010 ; Martinez, et al. 2011). Several mechanisms could be involved, including enhanced intraspinal excitatory drive (Rank, et al. 2015) and constitutive activity of 5-HT_2 receptors (Fouad, et al. 2010), but such demonstration was out of the scope of this study. Others have implicated serotonergic pathways in recovery after partial section (Gerin, et al. 2010; Leszczyńska, et al. 2015; Saruhashi, et al. 1996). This could explain why several insignificant long-lasting effect of buspirone in SB (e.g. paw positioning in front of hip, drag recovery) reached significance in HSB mice. Alternatively, it could suggest that buspirone potentiates

residual functionality. In this context, it encourages its investigation in clinical studies since most SCL patients present some level of function.

Limitations and future directions

Because of its potential clinical application in locomotor recovery after SCI, evaluation of buspirone's effects on spinal networks should be prioritized. The behavioral effect of buspirone on locomotion observed in this study is highly similar to that of the selective 5-$HT_{1A/7}$ agonist 8-OH-DPAT reported in earlier work which supports the hypothesis that both rely on 5-HT_{1A} activation, but the complete mechanism of action is still unknown. In another experiment from our laboratory, buspirone emulated 8-OH-DPAT effect on H-reflex and inhibited it in acutely spinalized, decerebrated mice (Develle and Leblond, in preparation). Altogether, these experiments underline that buspirone, like 8-OH-DPAT, could be facilitating locomotion by modulating the gain of Ia reflex pathways. Additional work on buspirone impact on reflex pathways such as group II muscle afferent crossed inhibition would improve our understanding of buspirone effect on locomotion and encourage its use to promote functional recovery in SCL patients.

It is important to keep in mind that an exteroceptive stimulation like perineal pinching is essential to elicit locomotion after complete transection in adult mice, even if preceded by a hemisection. The amount of stimulation needed to elicit stepping movements varied from one animal to the other. Pressure was progressively adjusted by the experimenter to ensure that the CPG was provided with the right amount of sensory input that elicit locomotion. For technical reason, experimenter that provided perineal stimulation was not in a blind situation so he knew which treatment mice had, but all range of stimulation from light touch to strong pinching of perineal simulation was always made and recorded. It should be noted that bout of locomotion chosen for analysis was made by another experimenter that was not aware of the treatment or the perineal pressure used. The latter viewed all the recorded sequences offline and selected the longest bout of regular stepping that occurs and did the analysis on that sequence. The amount of support was consistent over time in the same animal, but improvements occurred as they required less support later in recovery compared to early after transection. This is consistent

with the methodology used in prior studies evaluating spinal locomotion in rodents transected as adults, which would be otherwise impossible (Meisel and Rakerd 1982). The rationale behind the use of such stimulation has been clearly stated elsewhere (Sławińska, et al. 2014) and is not developed further here.

In the present experiment, we focused on the period where most of the locomotor recovery is occurring in mice as established previously (Leblond, et al. 2003). However, it is not known whether these improvements will be useful in the long term and translate into better functional outcomes during the chronic phase of recovery. Such evaluation should be prioritized, including investigations on other animal models which have a locomotor recovery rate similar to humans like non-human primates.

Conclusion

The present study shows that buspirone acutely elicited locomotion as early as 2 days after transection. Moreover, buspirone and training had additive effects on recovery and promoted functional recovery, mostly in mice that had residual functionality. Moreover, we provided evidence that locomotor recovery after partial spinal cord section in mice relies on spinal networks. This encourages the use of mice as a research model of partial SCL while providing further support for buspirone use in multi-targeted therapy of SCL patients.

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Chapitre VI : Discussion

Les travaux inclus dans cette thèse ont permis de déterminer un facteur néfaste (inflammation des muscles lombaires) et un facteur bénéfique (injection i.p. de buspirone) à la récupération locomotrice dans un modèle de section spinale complète chez la souris, écartant *a priori* toute contribution supra-spinale. Ces découvertes renforcent le concept que celle-ci est un phénomène plastique qui peut être modulé par l'expérience sensorimotrice. Considérant l'importance de l'entraînement dans la phase de réadaptation et la présence concurrente de ces facteurs dans un cadre thérapeutique écologique, l'influence de ces facteurs sur l'effet positif de l'entraînement locomoteur sur la récupération a été évaluée. Globalement, l'inflammation était associée à une moins bonne récupération locomotrice (Jeffrey-Gauthier et al., 2017). Plus spécifiquement, les souris ayant reçu une injection de CFA avaient une excursion angulaire des articulations de la patte postérieure (hanche, genou, cheville et MTP) diminuée par rapport aux souris sans CFA (article en préparation, étude 3). Cette perturbation de la cinématique était également associée à une atténuation de la longueur de pas et de l'élévation de la patte en phase de balancement menant ainsi à la traînée de la patte sur le tapis roulant (Jeffrey-Gauthier et al., 2017). Malgré ces déficits intra-membres, la coordination inter-membre (alternation droite-gauche) était peu affectée par le CFA. Ces effets sur la réexpression du patron locomoteur par les réseaux locomoteurs spinaux à la suite d'une section complète ont été observés sur un modèle d'inflammation qui ne cause pas de déficits locomoteurs chez l'animal intact (données non publiées).

Des changements à la fois périphériques et centraux peuvent contribuer à l'impact de l'injection de CFA dans la récupération locomotrice. Certains mécanismes ont été partiellement décrits dans cette thèse tandis que d'autres demeurent spéculatifs et nécessitent d'être étudiés. Les mécanismes principaux présentés dans cette discussion sont : 1) l'interaction entre l'activité afférente nociceptive et non nociceptive dans l'activation des réseaux locomoteurs spinaux et voies réflexes communes (section 6.1), 2) l'interaction entre les changements centraux induits par l'entraînement et l'inflammation des muscles lombaires (section 6.2) et 3) le rôle du contrôle moteur du tronc par les centres supraspinaux et spinaux dans la récupération de la locomotion avec support de poids après une section spinale complète (section 6.3).

La buspirone quant à elle pouvait déclencher la locomotion, tout en ayant un effet limité sur la récupération lorsque combiné à l'entraînement (Jeffrey-Gauthier et al., 2018). Toutefois, elle a amélioré la récupération d'une locomotion symétrique lors d'un paradigme de double lésion, indiquant que la buspirone peut faciliter la génération d'adaptations plastiques médiées par l'entraînement. Ces différents mécanismes sont détaillés dans cette discussion et illustrés à la figure 6.1.

6.1 Interaction entre l'activité non nociceptive et nociceptive dans le recrutement de voies réflexes communes aux réseaux locomoteurs spinaux

Le résultat principal de cette thèse est que l'inflammation des muscles lombaires induite par l'injection de CFA diminue la récupération locomotrice chez la souris ayant une section spinale complète. Cette découverte a été supportée par des évidences récentes chez le rat. En tentant de valider la contribution d'étirements passifs des muscles des pattes postérieures à la réadaptation post-LM, Keller et al. ont observé que l'étirement nuisait à la récupération locomotrice (Keller et al., 2017) et que cet effet était médié par les fibres nociceptives (Keller, 2017). En réduisant le nombre d'afférences nociceptives par injection intrapéritonéale de capsaïcine durant la période néonatale (Nagy et al., 1981, Nagy et al., 1983), la perturbation de la récupération locomotrice induite par l'étirement des muscles des pattes postérieures était fortement atténuée (Keller, 2017), ce qui confirme l'implication des fibres nociceptives dans les déficits de récupération locomotrice observés.

Il a été démontré chez le rat (Touj et al., 2017), puis confirmé dans notre modèle de souris (Jeffrey-Gauthier et al., 2017), que l'injection de CFA dans les muscles lombaires induit une myosite avec infiltration leucocytaire qui persiste pendant au moins 4 semaines. Une augmentation de l'expression de microglies dans les segments L2-L4 a également été mesurée au jour 24 post-injection (article en préparation), supportant un rôle de ces cellules dans les processus spinaux reliés à l'inflammation médiée par le CFA, tel que rapporté chez le rat (Chacur et al., 2009). En plus de la contribution de la microglie au développement de

sensibilisation de la corne dorsale (voir section 6.2), l'administration intramusculaire de CFA active/sensibilise les afférences primaires et neurones nociceptifs-spécifiques spinaux (Schuelert et al., 2015), en augmentant l'expression de bradykinine, des récepteurs TRPA1 (pour *transient receptor potential cation channel subfamily A, number 1*), des neuropeptides CGRP et substance P dans les fibres afférentes et la corne dorsale (Hutchins et al., 2000, Ambalavanar et al., 2006a, Asgar et al., 2015, Schuelert et al., 2015). L'activation/sensibilisation des afférences primaires transmettant vers les cornes dorsales de la moelle épinière lombaire induite par le CFA, principalement à T12 pour l'injection à L1 et L3 pour l'injection à L5 (Taguchi et al., 2008), peut possiblement perturber l'activation de voies spinales communes aux réseaux locomoteurs. Notamment, il a été observé en locomotion fictive chez le lapin (Viala et al., 1978) et en locomotion spinale chez le chat (Frigon et al., 2012) que l'activation d'afférences non nociceptives cutanées du dos inhibe l'activité locomotrice, suggérant une transmission des afférences du dos vers le CPG. Cependant, l'influence de l'activation/sensibilisation des afférences nociceptives musculaires du dos sur l'activité du CPG n'est pas connue.

Plusieurs évidences suggèrent que les afférences nociceptives transmettent leurs inputs au CPG et peuvent à la fois faciliter (Kniffki et al., 1981, Mandadi et al., 2009) et nuire à son expression (Kniffki et al., 1981, Keller et al., 2017). En étudiant l'activité locomotrice déclenchée par la L-DOPA chez le chat spinal, Kniffi et al. ont montré que l'activation des afférences musculaires nociceptives (groupes III-IV) par l'administration de bradykinine triacetate ou de KCl dans le muscle triceps surale pouvait modulé l'activité locomotrice de différentes façons. À la suite d'application de L-DOPA, la stimulation des fibres des groupes III-IV déclenchait une activité rythmique dans les pools motoneuronaux qui ne montraient pas d'activité rythmique préalable, et causait une augmentation du rythme dans les pools motoneuronaux déjà rythmiquement actifs (Kniffki et al., 1981). Toutefois, l'activité rythmique en cours était occasionnellement remplacée par une activité tonique lors de l'activation des mêmes afférences. Cette découverte suggère une interaction complexe entre l'activité nociceptive et l'activité locomotrice en cours.

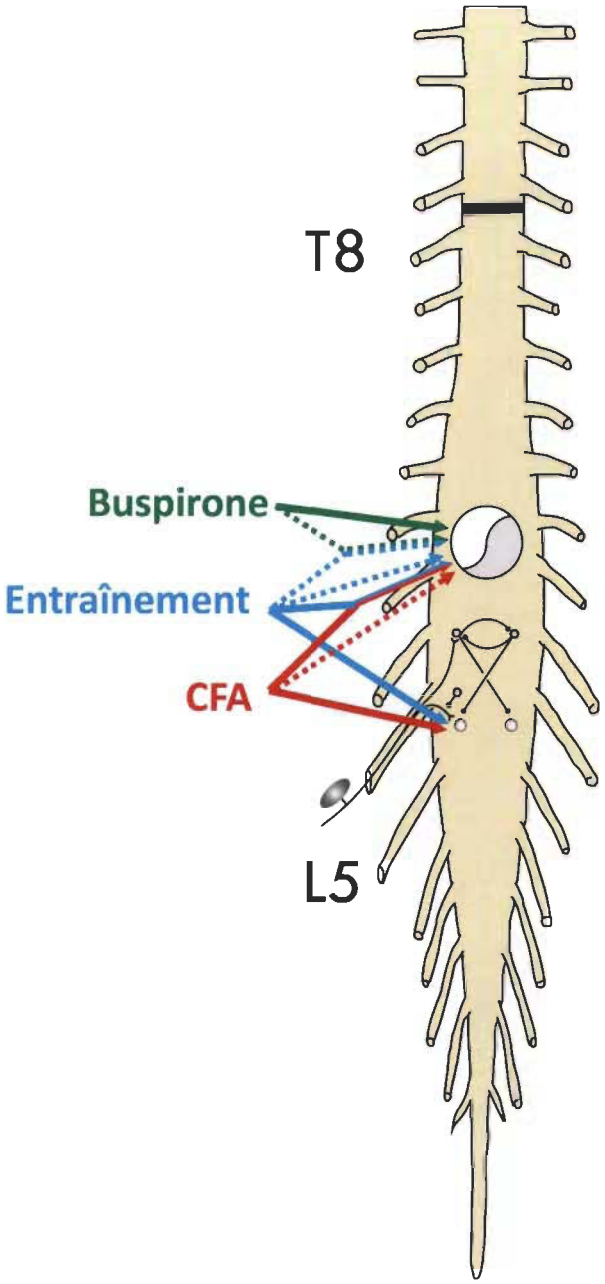

Figure 6.1. Cibles possibles de la buspirone et du CFA et interactions avec l'entraînement

Sites possibles d'adaptations contribuant aux effets du CFA (flèches rouges), de la buspirone (flèches vertes) et de l'entraînement (flèches bleues) sur la locomotion après une section (ligne noire). Le circuit réflexe schématisé est un exemple de circuit impliqué dans la locomotion (inhibition réciproque d'interneurone Ia) mais n'est pas illustré dans sa position anatomique réelle. Symbole taoïste = CPG, interneurones = sphères foncées, motoneurones = sphères claires, afférence = ellipse, synapse inhibitrice = cercle noir.

Des évidences récentes démontrent le rôle des afférences nociceptives sur l'activité locomotrice médiée par le CPG. Dans une préparation de moelle épinière isolée de rat *in vitro*, il a été démontré que l'activation du CPG par la stimulation d'afférences sacro-caudales, l'analogue électrique du pincement de la queue, dépend en partie de l'activation de fibres exprimant le récepteur TRPV1 (Mandadi et al., 2009, Mandadi et al., 2013b) et retrouvé exclusivement sur les fibres nociceptives (Julius and Basbaum, 2001, Cavanaugh et al., 2011). La désensibilisation de ces fibres nociceptives par l'application de capsaïcine bloque l'activité locomotrice du CPG déclenchée par la stimulation électrique des afférences sacro-caudales (Mandadi et al., 2013a, Mandadi et al., 2013b), démontrant le rôle important des afférences nociceptives dans le recrutement du CPG par celles-ci.

Dans notre modèle, il est peu probable que l'injection de CFA empêche le déclenchement de la locomotion par le pincement de la queue en désensibilisant les fibres nociceptives impliquées.

Contrairement à la capsaïcine, l'administration de CFA ne désensibilise pas les récepteurs TRPV1 et augmente plutôt leur expression dans les terminaisons des fibres nociceptives et non nociceptives dans la corne dorsale (Yu et al., 2008), suggérant que le CFA faciliterait le recrutement du CPG par l'activation d'afférences sacro-caudales ou par le pincement de la queue et favoriserait le déclenchement de la locomotion, ce qui n'a pas été observé. De plus, l'activation de fibres nociceptives n'est pas le seul mécanisme impliqué dans l'impact des afférences sacro-caudales sur l'activité du CPG. L'activation d'interneurones-relais par des afférences sacro-caudales non nociceptive (Strauss and Lev-Tov, 2003, Etlin et al., 2010, Etlin et al., 2013, Finkel et al., 2014) représente une deuxième voie permettant à ceux-ci de moduler l'activité du CPG de façon indépendante des fibres nociceptives.

L'impact des afférences nociceptives est mieux compris dans les voies réflexes communes aux réseaux locomoteurs, notamment les voies réflexes Ia, Ib, II et FRA (voir section 1.3.1 pour description). Chez le chat spinal, l'application d'un stimulus thermique nociceptif sur l'aspect dorsal de la patte postérieure facilite le recrutement d'interneurones Ia et II lombaires par une stimulation électrique (Schomburg et al., 2000). L'effet s'inverse et devient inhibiteur pour ces mêmes interneurones au niveau sacral. Cette interaction entre nocicepteurs et recrutement d'interneurones I/II est au moins partiellement médié par les fibres C puisque

l'effet persiste après l'inactivation des fibres Aδ par la tétrodotoxine (TTX; Schomburg et al., 2000). L'application d'une stimulation thermique nociceptive facilite également les réflexes cutanés homosegmentaires recrutés par stimulation de mécanorécepteurs de bas seuil et enregistrés dans des motoneurones lombaires (Behrends et al., 1983). Considérant que les afférences cutanées de la patte contribuent à la récupération locomotrice (Bouyer and Rossignol, 1998), il est possible que l'activation hétérosegmentaire d'afférences nociceptives module la transmission d'inputs cutanés vers les réseaux locomoteurs spinaux et perturbe ainsi l'activité locomotrice.

Récemment, il a été observé que l'activation réflexe des motoneurones par les afférences Ia (réflexe-H) étaient désinhibé (c.-à-d. diminution de la dépression dépendante de la fréquence (FDD) de recrutement du réflexe-H) dans des modèles de douleur médiée par la relâche spinale de BDNF (Jolivalt et al., 2008, Lee-Kubli and Calcutt, 2014). En évaluant la modulation fréquence-dépendante du réflexe-H à différents temps post-section, nous avons observé que l'inflammation des muscles lombaires est associée à une désinhibition du réflexe-H dans les premiers jours de la réponse inflammatoire (Jeffrey-Gauthier et al., 2019). Considérant que le gain des afférences Ia est normalement atténué pour faciliter le mouvement de balancement (voir section 1.3.1), il est possible que cette désinhibition du réflexe-H post-section médiée par l'inflammation perturbe la récupération locomotrice, mais la contribution de ce mécanisme à l'impact comportemental de l'inflammation reste à être démontrée. De plus, cette désinhibition était de courte durée puisqu'elle n'était plus observée après une semaine post-injection. Le rétablissement chez les souris ayant reçu l'injection de CFA d'une FDD similaire aux souris contrôles aux jours 14 et 28 post-section, et ce en dépit des différences de récupération locomotrice entre les groupes, indique que ce mécanisme n'explique au mieux que partiellement les déficits locomoteurs observés.

6.2 Changements centraux impliqués dans l'influence opposée de l'entraînement et de l'inflammation

En plus de l'interaction entre l'activité nociceptive et non nociceptive au niveau périphérique, un nombre croissant de modèles expérimentaux fait maintenant état d'interactions multiples entre les processus centraux adaptatifs sous-tendant la récupération motrice et les processus mésadaptatifs liés à l'activité nociceptive et à la sensibilisation centrale.

Dans cette thèse, le développement de sensibilisation centrale (ex. : hyperréactivité mécanique et thermique) n'a pas été évalué dû au manque de résolution de ce type de mesure à la suite d'une section spinale dans le modèle murin.

Ce phénomène est toutefois abondamment décrit dans les modèles animaux de LM (Hains and Waxman, 2006, Detloff et al., 2008, Carlton et al., 2009; pour revue voir Hulsebosch et al., 2009). La sensibilisation centrale affecte l'intégration dans la moelle épinière d'informations sensorielles nociceptive (Meisner et al., 2010, Walters, 2012) et non nociceptive (Gwak and Hulsebosch, 2011) et est influencée par l'expérience sensorimotrice, incluant l'entraînement locomoteur sur tapis roulant chez le rat (Hutchinson et al., 2004, Detloff et al., 2014) et la souris (Nees et al., 2016, Sliwinski et al., 2018).

Des évidences récentes indiquent que les processus reliés à la douleur, incluant le développement de sensibilisation centrale et l'apprentissage moteur, compétitionnent pour influencer des mécanismes communs dans la moelle épinière. Dans une série d'expériences, Grau et al. (1998) ont décrit partiellement les mécanismes impliqués en utilisant un modèle basique d'apprentissage ; le conditionnement du réflexe de flexion chez le rat spinal. Dans ce modèle, un animal reçoit des stimulations électriques de fortes intensités à la patte à chaque fois que celle-ci est en extension (dépendant de la position). Pourvu que l'émission de stimulations respecte ce critère de position, le rat spinal « apprenait » à augmenter progressivement la durée de la contraction réflexe des muscles fléchisseurs caractéristiques du réflexe de flexion, maintenant ainsi sa patte en flexion plus longtemps ce qui diminuait la fréquence des stimulations (Grau et al., 1998). À l'opposé, un animal recevant des stimulations indépendamment de la position de la patte « n'apprenait » pas à maintenir sa patte en flexion et recevant plus de chocs (Crown et al., 2002b). À la suite de ce protocole de stimulations, le même

animal n'apprenait pas à maintenir sa patte en flexion lors de stimulations qui répondaient au critère de position, possiblement dû à la préséance des processus mésadaptatifs reliés à la stimulation nociceptive sur les processus adaptatifs.

Cette démonstration a été faite par plusieurs études subséquentes. L'application préalable de capsaïcine (Hook et al., 2008) ou de carrageenan (Ferguson et al., 2006) à une des pattes de l'animal (ipsilatéralement ou controlatéralement aux stimulations) bloquait l'effet de l'apprentissage et l'animal n'apprenait pas à maintenir sa patte en flexion. À l'opposé, l'application de ces substances algogènes après le conditionnement du réflexe par des stimulations répondant au critère de position, n'annulait pas le comportement acquis. Étonnamment, l'hyperréactivité mécanique associée à l'application de capsaïcine ou de carrageenan et évaluée par des stimulations aux filaments de Von Frey, n'était observée que chez les animaux qui ne démontraient pas d'apprentissage (Hook et al., 2008, Baumbauer et al., 2012), suggérant que l'apprentissage prévenait le développement de sensibilisation centrale.

L'apprentissage ou déficit d'apprentissage chez l'animal spinal tel que décrit lors du conditionnement du réflexe de flexion semble également influencer la récupération de comportement moteur complexe comme la locomotion. À la suite d'une contusion spinale, les animaux recevant des stimulations électriques indépendamment de la position de leur patte démontraient une capacité atténuée de récupérer la locomotion (Grau et al., 2004, Garraway et al., 2011, Grau et al., 2016). Ces déficits locomoteurs étaient associés à une accentuation des processus apoptotiques médiés par la relâche amplifiée de TNF-α (Garraway et al., 2014), à un plus haut taux de mortalité et, chez les survivants, à une plus importante perte de poids (Grau et al., 2004). Bien que l'accentuation de l'apoptose puisse expliquer les déficits locomoteurs, des études subséquentes ont également démontré que les stimulations électriques induisaient des changements importants sous le niveau de la lésion qui pouvait directement perturber les réseaux locomoteurs spinaux ou leur capacité d'adaptation plastique. En effet, une augmentation du recrutement spinal des cellules gliales (Huie et al., 2012), incluant la microglie (Grau et al., 2014) et une modulation de la relâche de BDNF (Gómez-Pinilla et al., 2007, Garraway et al., 2011) peuvent influer sur les réseaux locomoteurs spinaux et justifiait d'évaluer l'impact de stimulations nociceptives sur la récupération locomotrice dans un modèle de section spinale complète. Les résultats présentés dans cette thèse supportent l'hypothèse que les déficits

locomoteurs associés aux stimulations nociceptives détaillées dans les études de Grau et al. sont causés par des changements fonctionnels de l'activité des réseaux sous-lésionnels, incluant le CPG (Jeffrey-Gauthier et al., 2017).

6.2.1 Implications de la voie microglie-BDNF-KCC2

Les études mentionnées dans la section précédente font état d'interaction entre les processus liés à la douleur, le développement de sensibilisation centrale et la récupération locomotrice. Afin de tester l'hypothèse que ces deux phénomènes compétitionnent pour influencer des mécanismes communs (Mercier et al., 2017), des changements neuroanatomiques associés à la fois au développement de sensibilisation centrale et à la récupération locomotrice ont été évalués lors de présence concurrente d'entraînement locomoteur et d'inflammation des muscles lombaires chez des souris après une section spinale. Parmi les différents mécanismes impliqués dans les processus liés à la douleur et la sensibilisation, de même que la récupération locomotrice (voir sections 1.3.2 et 1.4.1.2 pour détails), il est possible de constater qu'un même mécanisme pourrait influencer les deux phénomènes de façon opposée. Ce mécanisme contrôle l'expression du transporteur de chlore KCC2 qui régule l'homéostasie du chlore et détermine le tonus inhibiteur médié par le neurotransmetteur GABA (Lu et al., 2008, Lee et al., 2011), lui-même impliqué à différents niveaux de voies réflexes et dans les mécanismes d'inhibition présynaptique impliqués dans la locomotion (section 1.3.1).

La modulation d'expression de KCC2 dans la corne dorsale est abondamment décrite concernant le développement de sensibilisation centrale associé à une lésion nerveuse périphérique (Miletic and Miletic, 2008, Mòdol et al., 2014, Kitayama et al., 2016; pour revue voir Kitayama, 2018) et centrale (Lu et al., 2008), ainsi que dans d'autres modèles de douleur incluant la douleur inflammatoire (Zhang et al., 2008b, Wu et al., 2009). Le modèle de lésion nerveuse périphérique, quoique substantiellement différent du modèle de lésion centrale, a permis de déterminer comment les microglies, activées à distance du site lésionnel (Biber et al., 2011), contribuent à la diminution d'expression de KCC2 (Morgado et al., 2011) par la relâche de BDNF (Rivera et al., 2002, Coull et al., 2005, Biggs et al., 2010). Étonnamment, cette hyperactivité microgliale peut être diminuée par l'entraînement sur tapis roulant, restituant ainsi

la relâche de BDNF (Almeida et al., 2015) et l'expression de KCC2 (López-Álvarez et al., 2015) à des niveaux basaux et atténuant l'hyperréactivité sensorielle (Detloff et al., 2014, Almeida et al., 2015). Dans le cas des LM, la microglie est augmentée dans la corne dorsale mais également dans la zone intermédiaire et la corne ventrale (Carlson et al., 1998) principalement près de la lésion mais également recrutée rostralement et caudalement par l'activité de CCL21 (Zhao et al., 2007).

Dans notre modèle expérimental d'inflammation des muscles lombaires, une activation microgliale causée par l'inflammation (Chacur et al., 2009) se surimpose à celle causée par la section spinale.

Conformément à cette hypothèse, nos résultats montrent que l'inflammation des muscles lombaires est associée à une présence accrue de microglie sous-lésionnelle (article en préparation), et ce autant dans la corne dorsale que ventrale.

Bien que les changements d'expression de KCC2 et de microglie soient spatialement corrélés après une LM, aucune évidence ne démontre de lien d'association à la suite de ce type de lésion. Les évidences montrent que l'expression de KCC2 est diminuée 2) dans la corne dorsale et liée au développement de douleur neuropathique (Lu et al., 2008), 2) dans la corne ventrale et associée au développement de spasticité (Boulenguez et al., 2010),et 3) dans la zone intermédiaire et associée à une faible transmission d'inputs propriospinaux (Chen et al., 2018). Notre hypothèse était que l'augmentation de microglies associée à l'inflammation des muscles lombaires serait associée à une diminution d'expression de KCC2 et aux déficits locomoteurs. De plus, puisqu'il a été démontré que l'entraînement à un effet opposé sur l'expression de la microglie (diminution) (Detloff et al., 2014) et KCC2 (augmentation) (Côté et al., 2014), nous nous attendions à ce que l'entraînement améliore la récupération locomotrice chez des souris CFA en rétablissant l'expression de ceux-ci. Contrairement à nos hypothèses, les déficits locomoteurs et changements d'expression microgliale n'ont pas causé de modulation d'expression de KCC2 dans la moelle aux segments L2 à L4, aussi bien dans la corne dorsale, ventrale que la zone intermédiaire (article en préparation). De plus, l'entraînement n'a pas atténué l'augmentation d'expression de la microglie par le CFA. Ces résultats indiquent que l'entraînement et l'inflammation ont un effet opposé sur la récupération locomotrice en utilisant des voies au moins partiellement différentes et indépendantes de la voie microglie-BDNF-

KCC2. Cela suggère également que la microglie joue un rôle plus complexe dans le cas des LM que lors de lésions nerveuses périphériques et pourrait ne pas contribuer principalement à la régulation d'expression de KCC2.

6.3 Contribution supraspinale et spinale au contrôle moteur du tronc impliqué dans la récupération locomotrice

En plus des interactions entre les afférences sensorielles non nociceptives et nociceptives et des changements centraux détaillés ci-dessus, l'inflammation des muscles lombaires peut perturber l'activation des muscles du tronc lors de la locomotion et nuire à la récupération en influençant des mécanismes de contrôle supraspinaux et spinaux.

Bien qu'il soit envisageable d'attribuer un rôle important au contrôle postural dans les déficits locomoteurs induit par le CFA, il est important de souligner que l'inflammation des muscles lombaires ne modifie pas le patron locomoteur d'un animal ayant un système nerveux intact (données non présentées).

Le rôle des muscles axiaux pour générer la locomotion est abondamment décrit dans les modèles animaux dont le déplacement est ondulatoire (Cohen, 1987, Matsushima and Grillner, 1992, Roberts et al., 1998). Plus récemment, des études chez le chat (Koehler et al., 1984, Zomlefer et al., 1984), le rat (Falgairolle and Cazalets, 2007, Beliez et al., 2015) et l'humain (de Sèze et al., 2008, Ceccato et al., 2009) ont également établi l'implication des muscles du tronc à la locomotion quadrupède et bipède, notamment par l'observation d'activité rythmique en phase avec le patron locomoteur. À l'instar des pattes postérieures, l'enregistrement d'activité efférente rythmique dans les nerfs innervant les muscles dorsaux et abdominaux lors de locomotion fictive chez le chat spinal paralysé au curare ou dans la moelle épinière e du rat *in vitro* a confirmé que l'activité locomotrice du tronc est régulée dans la moelle épinière en absence d'influence supraspinale et périphérique (Koehler et al., 1984, Falgairolle and Cazalets, 2007). Contrairement aux animaux au déplacement ondulatoire, aucune évidence ne suggère l'existence d'un CPG thoracique. Des enregistrements électrophysiologiques dans la moelle épinière isolée du rat démontrent plutôt que le CPG lombaire transmet la commande d'activité

locomotrice vers les interneurones puis motoneurones thoraciques selon un gradient caudo-rostral par des voies propriospinales ascendantes (Beliez et al., 2015). Des afférences sensorielles modulent également l'activité des interneurones et motoneurones thoraciques impliqués dans la locomotion, notamment des afférences cutanées provenant des pattes postérieures (Wada et al., 1999), ce qui indique que le tronc et les pattes possèdent des mécanismes de contrôle locomoteur similaires. Ces mécanismes assurent un couplage entre l'activation des muscles fléchisseurs des pattes avec les muscles thoraciques ipsilatéraux en alternance avec l'activation d'efférences vers les muscles controlatéraux (Koehler et al., 1984).

En plus de contribuer directement au patron locomoteur, des évidences chez le rat indiquent que l'amélioration des contrôles stabilisateurs du tronc renforçait le couplage mécanique entre le tronc et les pattes postérieures lors de la marche, incluant lors de locomotion médiée par les réseaux locomoteurs spinaux (Pearson, 2001). Chez des rats spinalisés néonatalement, il a été observé qu'environ 20 % retrouvaient spontanément le support de poids de l'arrière-train et que la réorganisation de la représentation du tronc du cortex moteur est nécessaire à cette récupération du support de poids (Giszter et al., 2008). La réorganisation des cartes motrices du tronc associée à la section spinale comprend une expansion de la représentation du tronc vers le cortex désafférenté des régions sous-lésionnelles (Oza and Giszter, 2015). Ce phénomène est également observé chez le rat spinalisé à l'âge adulte (Ganzer et al., 2016) et l'entraînement locomoteur sur tapis roulant accentue ce changement plastique (Oza and Giszter, 2014, Ganzer et al., 2016). De plus, la lésion subséquente de la représentation corticale du tronc chez des rats ayant récupéré une locomotion avec support de poids supprimait cette amélioration (Manohar et al., 2017). Dans notre étude, il est possible que l'inflammation des muscles lombaires, impliqués dans le contrôle postural, perturbe la capacité des souris spinales à supporter leur poids et que ce déficit diminue à son tour la qualité de l'entraînement locomoteur.

L'importance du contrôle postural est incertaine lorsque la portion rostrale du corps repose sur une plateforme et que seulement le mouvement des pattes postérieures est entraîné et évalué, tel qu'effectué dans les études présentées.

Cependant, la perturbation des contrôles stabilisateurs du tronc peut influencer l'efficacité du couplage mécanique entre le tronc et les pattes postérieures lors du déplacement

autonome de l'animal dans sa cage (auto-entraînement, voir section 6.6). Considérant la contribution clinique évidente de ces découvertes et le manque de connaissance des mécanismes sous-jacents, il serait intéressant d'évaluer l'impact d'inflammation des muscles lombaires sur l'auto-entraînement, la capacité de support de poids et les changements plastiques ciblant la représentation du tronc dans le cortex moteur.

6.4 La locomotion médiée par les réseaux locomoteurs spinaux est déclenchée par la buspirone

Une découverte importante réalisée dans cette thèse est que la buspirone, un agoniste partiel des récepteurs 5-HT_{1A}, permet de déclencher un patron locomoteur des pattes postérieures chez une souris paraplégique, au jour 2 après une section spinale complète (Jeffrey-Gauthier et al., 2018). Les souris recevant la buspirone effectuaient plus de pas et augmentaient l'excursion angulaire de leurs hanches et de leurs chevilles, amélioraient le placement de leurs pattes sur l'aspect plantaire en avant de la hanche et diminuaient la traînée de leurs pattes lorsque comparé à un groupe contrôle. Lorsque l'animal avait récupéré d'une hémilésion gauche avant la section (paradigme de double lésion, voir section 5.3), l'administration de buspirone avait permis de corriger les asymétries du patron locomoteur dès le jour 2 suivant une section. Des études préalables ont montré que des thérapies combinant buspirone, apomorphine, benserazide et L-LOPA (Guertin et al., 2011) ou buspirone, carbidopa et L-DOPA (Ung et al., 2012) permettaient à des souris spinales paraplégiques non entraînées de réexprimer la locomotion (Guertin et al., 2011). Dans ces études, la buspirone montrait un potentiel thérapeutique intéressant. Toutefois, l'utilisation d'un harnais pour tenir l'animal sur le tapis roulant, l'absence de pincement de la queue et la dose de buspirone administrée empêchait d'observer l'effet aigu important de la buspirone tel que rapporté dans notre étude. En évaluant l'effet seul de la buspirone, nos résultats indiquent que la buspirone recrute les réseaux locomoteurs spinaux dans leur ensemble et permet d'engendrer un patron locomoteur symétrique indépendamment des adaptations préalables.

Le mécanisme d'action de la buspirone est encore incompris. Elle a une faible affinité comme antagoniste des récepteurs dopaminergiques D_2 et comme agoniste des récepteurs 5-HT_2 (Hoyer et al., 1994). Cependant, les auteurs d'études sur d'autres fonctions que la

locomotion (c.-à-d. l'anxiété) concluent que l'action pharmacologique principale de la buspirone est médiée par les récepteurs 5-HT_{1A} (Loane and Politis, 2012). De plus, des études sur l'effet de 8-OH-DPAT, un agoniste sélectif des récepteurs 5-$HT_{1A/7}$, sur la récupération locomotrice ont rapporté des effets similaires aux nôtres (Antri et al., 2003, Antri et al., 2005, Landry et al., 2006, Lapointe et al., 2008, Courtine et al., 2009, Musienko et al., 2011), suggérant que des mécanismes similaires sont impliqués.

Les récepteurs 5-HT_{1A} sont abondants dans la moelle épinière, incluant au renflement lombaire où est situé le CPG (Giroux et al., 1999, Noga et al., 2009), mais leur rôle dans la locomotion est peu connu. Ils sont impliqués à la fois dans la transmission excitatrice et inhibitrice dépendamment de leur cible (ex : motoneurones vs afférences musculaires ou interneurones) ou de leur localisation (somatique vs dendritique). Par exemple, les récepteurs 5-HT_{1A} peuvent à la fois augmenter et diminuer l'excitabilité des motoneurones en modulant la conductance à l'ion K^+ (Takahashi and Berger, 1990, Perrier et al., 2003) et en bloquant la génération de potentiel de plateau (Perrier and Cotel, 2008), respectivement. De plus, l'activation de récepteurs situés au niveau du soma dû au débordement de sérotonine hors de la synapse lors de fatigue centrale diminue l'excitabilité des motoneurones (Cotel et al., 2013). Ce mécanisme a récemment été impliqué dans la diminution de l'excitabilité des motoneurones par l'administration de buspirone chez l'humain (D'Amico et al., 2017), suggérant que l'action de la buspirone au niveau spinal est médiée par les récepteurs 5-HT_{1A}.

6.5 La buspirone facilite l'adaptation plastique des réseaux locomoteurs spinaux

Le rétablissement rapide de chats spinaux en combinant entraînement et administration de clonidine (Chau et al., 1998a) suggère que l'adaptation plastique des réseaux locomoteurs spinaux associée à l'entraînement peut être facilitée. Considérant l'impact aigu robuste de la buspirone sur le patron locomoteur chez la souris tel que détaillé précédemment, nous avions émis l'hypothèse que la combinaison de buspirone et d'entraînement transposerait l'effet aigu en amélioration à long terme de la récupération locomotrice par une facilitation des adaptations

plastiques. Toutefois, nous avons été étonnés de l'amélioration limitée associée à l'administration quotidienne de buspirone sur la récupération locomotrice associée à l'entraînement. La buspirone n'a pas eu d'effet significatif à long terme sur l'ensemble des paramètres locomoteurs et n'a pas permis une récupération plus rapide (Jeffrey-Gauthier et al., 2018).

Dans le modèle de récupération locomotrice des pattes postérieures après une section chez la souris, il est difficile de bien observer l'impact de l'entraînement sur la fonction (voir section 6.6). Il est donc normalement ardu de mesurer l'effet de la buspirone sur les adaptations plastiques associées à l'entraînement. Afin d'évaluer si la buspirone facilite l'adaptation plastique des réseaux locomoteurs spinaux, nous avons examiné l'effet à long terme de la buspirone combiné à l'entraînement à la suite d'un paradigme de double lésion. Comme mentionné à la section 5.3, ce paradigme permet de visualiser les changements au niveau spinal causés par une hémilésion par une section subséquente. Les animaux montrent une capacité résiduelle asymétrique, principalement dans le mouvement de la patte ipsilatérale à l'hémilésion, qui progressivement devient symétrique lorsque suffisamment d'entraînement a permis aux adaptations plastiques de transférer vers le côté controlatéral à l'hémilésion. La buspirone a accéléré la récupération d'un patron locomoteur symétrique, notamment en accélérant de façon significative l'amélioration de la traînée de la patte (Jeffrey-Gauthier et al., 2018). Ce résultat indique que la buspirone facilite l'adaptation plastique impliquée dans la récupération locomotrice à la suite d'une lésion spinale et suggère qu'elle pourrait améliorer la récupération fonctionnelle des patients ayant une LM.

6.6 Effets limités de l'entraînement seul sur la récupération locomotrice

Considérant la contribution d'adaptations plastiques à la récupération locomotrice, l'objectif de cette thèse était d'évaluer l'influence de différents facteurs afin d'optimiser le rétablissement de la fonction. L'expérience sensorimotrice a été manipulée de différentes façons : nous avons évalué la récupération locomotrice post-section lors d'inflammation des

muscles lombaires avec ou sans entraînement sur tapis roulant (Jeffrey-Gauthier et al., 2017) et lors de traitement à la buspirone avec ou sans fonction locomotrice résiduelle causée par la récupération à une lésion partielle préalable (Jeffrey-Gauthier et al., 2018). L'inflammation des muscles lombaires et l'administration de buspirone combinée à l'entraînement ont permis d'observer des changements dans la récupération médiée en partie par des changements plastiques. Toutefois, l'entraînement locomoteur n'a pas causé de changements robustes dans la récupération locomotrice chez la souris à la suite d'une section spinale complète. L'interprétation simple de ce constat est que la récupération est dans une certaine proportion un phénomène spontané. Cette interprétation sous-entend que les réseaux locomoteurs spinaux sont peu plastiques, ce qui était la conception la plus répandue parmi les chercheurs du domaine jusqu'aux années 80. Depuis, les découvertes effectuées chez le chat (Lovely et al., 1986, Barbeau and Rossignol, 1987, Lovely et al., 1990, de Leon et al., 1998) et le rat (Cai et al., 2006, Cha et al., 2007, Alluin et al., 2015; pour revue voir Edgerton and Roy, 2009, Roy et al., 2012, Serge Rossignol et al., 2014) ont démontré que l'adaptabilité plastique des réseaux locomoteurs spinaux contribue à la récupération locomotrice (Pearson, 2001) et que l'exposition répétée à l'activité neuronale sous-tendant les mouvements locomoteurs en accentue le rendement. Ce phénomène est à la base de l'amélioration de la récupération par l'entraînement tel que montré dans différents modèles animaux. Par exemple, des lapins spinaux peuvent être entraînés avec un appareil mécanique pour favoriser un patron de marche alterné ou en phase (Viala et al., 1986), ce qui indique qu'une exposition répétée à un mode de couplage inter-membre permet aux réseaux locomoteurs spinaux de développer une « préférence » qui est observable au niveau fonctionnel. Cependant, des évidences montrent que la récupération locomotrice de la souris semble moins influencée par l'entraînement comparativement au chat ou au rat (Battistuzzo et al., 2012, Battistuzzo et al., 2016). Une variation importante de la récupération locomotrice est même observée dépendamment de la souche de souris (Basso et al., 2006, Lapointe et al., 2006). Ces différences sont potentiellement causées par la difficulté d'entraîner adéquatement les souris dues à des facteurs biomécaniques comme le faible poids de l'animal. Notamment, des évidences associent l'amélioration de la marche par l'entraînement à une exposition répétée à la mise en charge des muscles des pattes postérieures associée au support de poids qu'exige une locomotion autonome (Cha et al., 2007). Considérant le faible poids de le souris et l'apport important de l'expérimentateur au support du poids lors du pincement de la queue nécessaire

pour générer la locomotion à la suite d'une section, il est possible que la souris ne soit pas exposée suffisamment aux afférences proprioceptives nécessaires pour améliorer la récupération au-delà de la récupération de souris non entraînées.

Les souris récupèrent rapidement la locomotion des pattes postérieures à la suite d'une section complète (Leblond et al., 2003). Si la réexpression locomotrice n'est pas améliorée par l'entraînement sur tapis roulant, quelle est la cause de cette récupération ? Il a été démontré qu'une part importante des changements plastiques médiés par l'expérience sensorimotrice et qui ciblent les réseaux spinaux provient de l'entraînement autonome (aussi appelé auto-entraînement) de l'animal dans sa cage (Meeteren et al., 2003). En observant le déplacement des souris spinales dans leur cage, il est possible de constater qu'elles se déplacent abondamment, principalement en traînant leur arrière-train au sol. L'activation d'afférences sensorielles par le mouvement du sol sous leur corps est comparable au défilement du tapis-roulant, aussi est-il possible d'observer par moment la génération de mouvements locomoteurs des pattes postérieures. Ce phénomène a récemment été impliqué dans la récupération locomotrice chez des rats ayant une lésion partielle de la moelle épinière (Fouad et al., 2000, Alluin et al., 2011). En restreignant l'activation des afférences sensorielles par le port d'une contention de l'arrière-train 10 h chaque jour, il a été observé que la récupération locomotrice à la suite d'une contusion spinale chez le rat était fortement atténuée (Caudle et al., 2011). Dans cette thèse, l'absence de mesure de cette activité d'auto-entraînement empêche d'apprécier sa contribution à l'amélioration de la récupération locomotrice. Considérant la contribution importante d'autostimulation dans la récupération chez le rat à la suite de contusion spinale, il serait intéressant d'évaluer dans une étude future son apport à la récupération locomotrice chez la souris à la suite d'une section complète.

Chapitre VII : Conclusion

Les lésions de la moelle épinière (LM) sont une cause importante de déficits moteurs et sensoriels, incluant des difficultés à la marche et le développement de douleur. Chez l'humain, une lésion incomplète résulte fréquemment en une incapacité totale de générer des mouvements volontaires. Les conséquences de la perte d'autonomie liée aux difficultés à la marche sont majeures tant pour l'individu que son entourage. Ce constant a conduit au développement d'un pôle thérapeutique et de recherche qui s'intéresse à l'optimisation de la fonction par l'entraînement. Toutefois, les résultats cliniques ne sont pas satisfaisants.

En contraste avec ce qui est observé chez les patients ayant une LM, l'entraînement locomoteur sur tapis roulant engendre d'importantes améliorations fonctionnelles dans plusieurs modèles animaux de LM. Il a d'abord été démontré chez le chat, et plus récemment chez le rat et la souris, que l'animal peut générer le patron locomoteur avec ses pattes postérieures comprenant des phases de support et de balancement adéquatement coordonnées en alternance inter-membre, et ce, même après une section spinale complète. Ce phénomène surprenant est médié par la réexpression de réseaux locomoteurs comprenant un générateur de patron central (CPG) et différentes voies réflexes situés dans la moelle épinière lombaire. De plus, l'entraînement sur tapis roulant accélère cette réexpression locomotrice par des mécanismes d'adaptations plastiques induits par l'activation des réseaux locomoteurs spinaux par les afférences sensorielles.

Les résultats présentés dans cette thèse ajoutent des connaissances sur le potentiel de récupération locomoteur à la suite de différents types de lésions (hémilésion, section) chez la souris. La transposition des découvertes concernant l'adaptation plastique des réseaux locomoteurs spinaux en utilisant le paradigme de double lésion depuis le chat et le rat à la souris est une découverte importante dans le domaine. En montrant qu'une souris ayant récupéré un patron de marche normal après une hémilésion conservait une capacité locomotrice résiduelle du côté de l'hémilésion après une section complète subséquente, nous avons démontré que la souris représente un bon modèle pour étudier la contribution d'adaptations plastiques à la récupération locomotrice médiée par les réseaux locomoteurs spinaux. Cela justifie l'utilisation

de ce modèle dans des études visant à déterminer les composants cellulaires du CPG par une approche génétique et permet d'appliquer les découvertes à d'autres modèles animaux.

Les études de cette thèse ont permis d'identifier deux facteurs qui influencent la récupération locomotrice à la suite d'une section spinale. En premier lieu, nous avons observé dans une première étude, puis confirmé dans une seconde étude, que l'inflammation des muscles lombaires atténue la récupération locomotrice médiée par les réseaux locomoteurs spinaux et que l'entraînement peut prévenir cet effet. En mesurant l'excitabilité du réflexe-H par de stimulations électriques répétées, nous avons montré que l'inflammation des muscles lombaires altère temporairement le recrutement des motoneurones par les afférences Ia en désinhibant la voie réflexe. Ce mécanisme est possiblement impliqué dans l'effet observé au début de la réaction inflammatoire. Toutefois, le déficit locomoteur est probablement causé au moins partiellement par un autre phénomène, incluant l'activation persistante de microglie dans le renflement lombaire par un mécanisme indépendant de KCC2. Ces découvertes ont des implications translationnelles évidentes. Des observations chez les patients LM suggèrent que différents facteurs associés à la lésion ou à son traitement peuvent influencer la récupération fonctionnelle (Dvorak et al., 2017). Notamment, les blessures musculosquelettiques associées à l'étiologie principalement traumatique des LM pourraient diminuer le pronostic de récupération (Noonan et al., 2012). Nos résultats supportent ces observations et proposent que les réseaux locomoteurs spinaux soient influencés par l'inflammation et que l'entraînement locomoteur prévienne cet effet. En deuxième lieu, nous avons démontré que la buspirone, un médicament approuvé par le FDA pour le traitement de l'anxiété, active fortement les réseaux locomoteurs spinaux et permet d'engendrer la locomotion dès le jour 2 après une section spinale chez des souris auparavant complètement paraplégiques. De plus, la buspirone améliore la récupération à long terme associée à l'entraînement en facilitant les adaptations plastiques. Considérant les résultats encourageants d'études précliniques sur la buspirone pour améliorer la locomotion chez les patients ayant une LM, ces découvertes ont des retombées importantes en proposant une contribution des adaptations plastiques spinales à la récupération locomotrice.

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