Université de Montréal

Assessing locomotion in cats trained on a flat treadmill and on a ladder treadmill before and after spinal cord injury (SCI)

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Ce mémoire intitulé:

Assessing locomotion in cats trained on a flat treadmill and on a ladder treadmill before and after spinal cord injury (SCI)

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Résumé

Les effets des lésions de la moelle épinière sur la locomotion sont souvent évalués sur un tapis roulant avec une surface plane, ce qui demande peu d'implication active des structures supraspinales. L'objectif du présent travail est d'évaluer si un type d'entraînement nécessitant une plus grande part de contrôle volontaire (c.-à-d. supraspinal) pourrait améliorer la récupération de la marche chez le chat après une hémilésion unilatérale spinale au niveau thoracique (T10). Pour ce faire, pendant 6 semaines les chats ont été entrainés sur un tapis roulant conventionnel ou sur un tapis-échelle roulante, tâche requérant un placement des pattes plus précis. Les paramètres de la marche ont été évalués par cinématique et électromyographie (EMG) avant et une fois par semaine pendant 6 semaines après lésion.

Nos résultats comparant la marche sur tapis conventionnel à celle sur échelle roulante montrent des différences dans les excursions angulaires et les couplages entre les membres. On observe aussi des différences dans l'amplitude des EMG notamment une augmentation de la deuxième bouffée du muscle Semitendineux (St) sur l'échelle roulante. Après l'hémilésion spinale cette bouffée disparait du côté de la lésion tandis qu'elle est maintenue du côté intact. Après l'entrainement sur échelle roulante, on observe des changements de trajectoire de la patte et une disparition du pied tombant (foot drag) qui suggèrent une amélioration du contrôle de la musculature distale.

Nos résultats montrent que le patron locomoteur observé sur tapis conventionnel est influencé par le type d'entraînement procuré. De plus, certains paramètres de la locomotion suggèrent que l'entraînement sur échelle roulante, qui requiert plus de contrôle supraspinal, favorise une meilleure récupération de la marche après lésion spinale.

Mots-clés : Entraînement locomoteur, inputs supraspinal, hémilésion spinale, échelle roulante, tapis roulant, cinématique, électromyographie.

Abstract

The effects of spinal cord lesions on locomotion in animals is often assessed on a flat surface which requires minimal supraspinal demands. Here we investigated whether locomotor training requiring more voluntary commands could improve recovery in cats after a unilateral hemisection on the left side (Thoracic 10). Cats were trained for 6 weeks on a conventional flat treadmill (FTM) or on a 'ladder treadmill' (LTM) requiring precise foot positioning. Locomotor parameters such as kinematics and electromyographic activity (EMG) were evaluated before and once a week for 6 weeks after spinal hemisection.

There were differences in angular excursions and limb couplings when comparing walking on FTM and LTM and important changes occurred in the amplitude of EMGs such as an increased second burst in Semitendinosus (St) during LTM stepping. After the left hemisection, this burst disappeared on the left side while it was maintained on the right (non lesioned) side. With LTM training, we observed changes in limb trajectories and disappearance of foot drag of the left foot during FTM stepping suggesting a better control of distal musculature.

Our results show that locomotor patterns observed during FTM stepping are influenced by different training modalities and also that training on the LTM after a spinal hemisection, demanding more supraspinal control, leads to a better stepping pattern.

Keywords: locomotor training, supraspinal inputs, spinal hemisection, ladder treadmill, flat treadmill, kinematics, electromyography.

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List of abbreviations

CPG: central pattern generator.

EMG: electromyography

CNS: central nervous system

FTM: flat treadmill

LTM: ladder treadmill

SCI: spinal cord injury

MC: motor cortex

F: flexion phase

E1: first extension phase

E2: second extension phase

E3: third extension phase

MTP: metatarso-phalangeal articulation

Anatomy

St: *semitendinosus*

VL: *vastus lateralis*

Srt: *sartorious*

GM: *gastrocnemius medialis*

TA: *tibialis anterior*

EDB: *extensor digitorum brevis*

T: thoracique segment

L: lumbar segment

S: sacral segment

To my parents, Manuel and Maritza, and my sisters July, Mary and Anto who always encouraged me to go on every adventure, including this one.

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Chapter 1: Introduction

1.1 Background

Locomotor behaviors such as walking or swimming are fundamental motor acts giving animals and humans the ability to move in order to satisfy their needs and to survive.

There are in the spinal cord of all vertebrates, including humans, neural networks capable of generating much of the timing and pattern of complex, coordinated muscle activities such as walking. For locomotion one usually refers to the term "central pattern generator" or CPG to indicate a set of neurons whose properties and connectivity may give rise to rhythmic motor patterns. However, locomotor commands originating from the brain stem and cortex as well as sensory afferents can influence or modulate the CPG to meet environmental demands.

A better knowledge of the CPG and its interactions would allow developing strategies to reactivate or maintain locomotion when an insult has been produced in the Central Nervous System (CNS). For instance, after a complete spinal cord injury, locomotor training on a treadmill makes use of movement-related sensory information to reactivate and modulate the CPG activity. However, following a partial spinal cord injury, there is substantial evidence that residual supraspinal tracts also contribute to the recovery of locomotion. It is believed that activity in residual supraspinal pathways from cortical, subcortical, and brainstem motor areas as well as other chemically-defined nuclei (such Locus Coeruleus for norepinephrine and Raphe Nuclei for serotonin) induce plasticity at all levels of the central nervous system. Therefore, rehabilitative techniques, to be successful in re-establishing goal-directed locomotion, must activate supraspinal tracts to optimize plastic changes in spinal locomotor circuits.

The main objective of the present study was to investigate whether a locomotor training method that demands more supraspinal contribution will result in a better recovery of

locomotion in hemisected cats. The need for this is that often, after CNS lesions, locomotion in cats is assessed on an ordinary treadmill with little voluntary demands. We developed a training method based on a voluntary locomotor task that requires continuous adaptation of the steps through various feedbacks including vision. We modified a conventional flat treadmill by adding regularly spaced quadrangular rungs requiring the animal to voluntarily position the paws on the top of rungs. Thus, cats could walk and be trained on either a conventional treadmill with a flat belt (FTM) or on a moving horizontal ladder treadmill (LTM) fixed to the treadmill within the same treadmill enclosure.

Before presenting our results, the introduction will first summarize how animal models helped our comprehension of the CPG for locomotion and its organization, how locomotion is assessed and how certain locomotor tasks allow evaluating the adaptations necessary to voluntarily walk in highly demanding environments. Secondly, the interactions of the CPG with supraspinal and sensory inputs to regulate locomotion will be summarized. Finally, there will be a brief section on how animal models of complete and incomplete SCI have contributed to establish the importance of locomotor training.

1.2 Animal models to study locomotion

1.2.1 The Central pattern Generator

More than 100 years ago, in 1874, experiments in dogs with a spinal cord transection in the lower thoracic region reported locomotor rhythms that appeared in response to various non-rhythmic stimuli and also spontaneously if the animal was held in the air with the limbs extended (Eichhorst and Naunyn, 1874;Goltz and Freusberg, 1874). Years later, Sherrington attributed this rhythm (alternations between flexor- extensors) to reflex inputs of peripheral origin and their integration with the posture of the animal. He also brought forward the idea that these aspects were produced by cells centrally i.e. within the spinal cord, but that the peripheral input from sensory afferents must be provided (Sherrington, 1910a).

Brown later demonstrated that the cat spinal cord can generate a locomotor rhythm in the absence of such inputs from higher centers and afferent feedback. He proved that cats with a transected spinal cord and dorsal rhizotomy still showed rhythmic alternating contractions in ankle flexors and extensors. This was the basis of the concept of a spinal locomotor center which Brown termed the 'half-center' model in which one group of spinal interneurons induced activity in flexor motoneurons, and another group of interneurons in extensor motoneurons for each limb and individual signature discharge patterns of individuals muscles were molded by the afferent inputs (Brown, 1911;Brown, 1914).

Alternating activities between flexors and extensors can also be recorded in muscle nerves after immobilization of the limbs with curarization, therefore removing all movement-related sensory feedback. This preparation is called 'fictive locomotion' because there is no movement (Grillner, 1981). Fictive locomotion preparations not only provide evidence that the spinal cord, isolated from sensory feedback and descending inputs, can produce the same regular alternation in the activity of flexor and extensor at all joints in the cat hindlimb as observed during normal treadmill or overground locomotion, but also provide further insight into the organization of these circuits.

These and later investigations on animal models led to the concept of central pattern generators (CPGs), defined as a functional network which resides within the spinal cord of invertebrates and vertebrates that generates the rhythm and shapes the pattern of various rhythmic movements (Grillner, 1981). The CPG represents a central concept over which we construct and assess models of plasticity, for example after a spinal lesion.

1.2.2 Organization of the CPG

In mammals, the spinal CPGs are composed of populations of spinal interneurons (Grillner, 1981). The spinal locomotor network is regulated by intrinsic excitatory and inhibitory connections. During locomotion, motoneurons receive rhythmic alternating discharge patterns of glutamatergic excitation and glycinergic inhibition during the active and inactive phases, respectively (Cazalets, Borde et al., 1996;Grillner, 2003;Shefchyk and Jordan, 1985). Other synchronous gaits, such as galloping and hopping probably involve

reconfiguring inhibitory and excitatory connections within the spinal locomotor CPG (Cowley and Schmidt, 1995). The production of different complex activity patterns led Grillner to conclude that the locomotor CPG "... does not simply generate an alternate activation of flexors and extensors but a more delicate pattern that will sequentially start and terminate the activity in the appropriate muscles at the correct instance" (Grillner and Zangger, 1975). In other words, according to Grillner, the CPG produces both the rhythmic commands and the pattern of individual muscle activities ('signature' discharges).

Brown's theory of half centers assumes that there is one CPG for every limb. This assumption could explain the coordination of the limbs in various gaits. But walking backwards changes the coupling between hip and knee, while that between knee and ankle remains unchanged (Edgerton, Grillner et al., 1976). Rhythmic alternating activity can also occur in one group of flexors (for example in the knee) with a discharge in other flexors (for example those of the ankle) (Grillner and Zangger, 1979). Because of these results, Grillner proposed in 1981 that the mammalian locomotor CPG is composed of interconnected modules that coordinate activity around specific joints (Grillner, 1981), the so called 'unit' CPG.

More recent studies have shown that this unit of modules may or may not be dissociated from the rhythm-generating circuitry, especially during fictive locomotion. Therefore, a multilayered spinal locomotor CPG, in which rhythm-generation and pattern formation are functionally separated has been proposed (McCrea and Rybak, 2008). In this two-level CPG, the rhythm generator controls features of the rhythm (i.e., cycle period, phase durations/transitions) and projects to the pattern-formation level, which coordinates and distributes activity to individual motor pools (Fig. 1). Inputs from peripheral mechanoreceptors or supraspinal structures can regulate activity at each of two levels, including spinal motoneurons (Rossignol and Frigon, 2011).

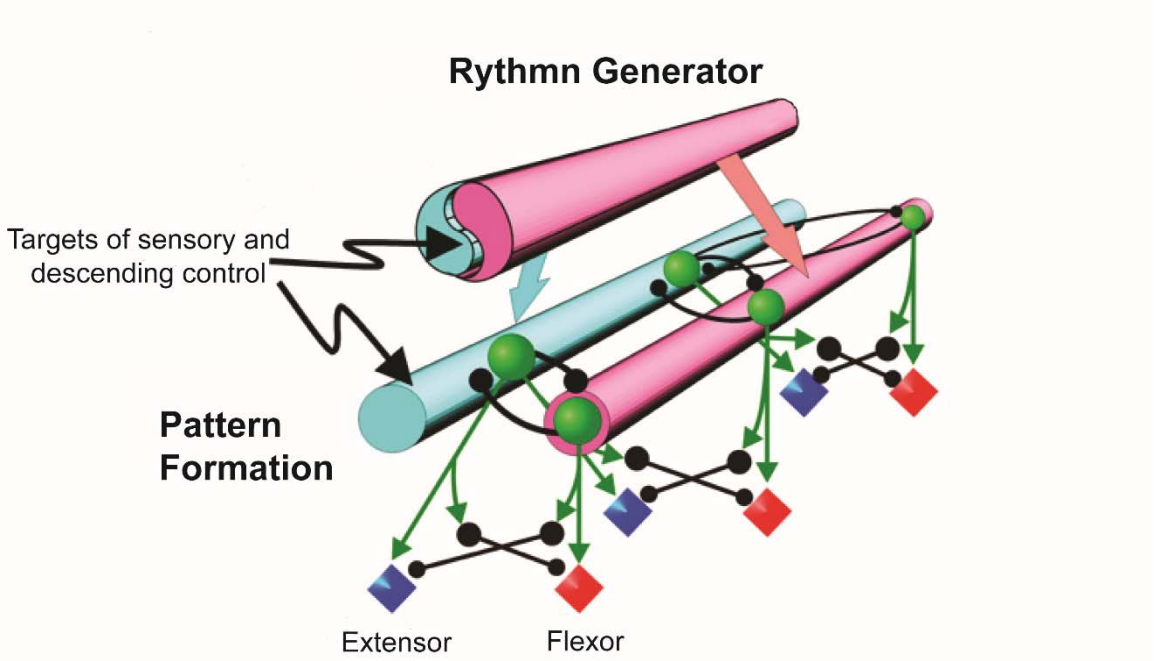

Figure 1. Schematic representation of the two-level hierarchical architecture of the CPG. In this model a single rhythm generator and multiple unit pattern formation modules are separated and would be replicated in all four limbs. Both levels could be influenced by supraspinal and sensory information. Figure modified from McCrea and Rybak, 2008.

1.2.3 Localization of the CPG

Transversal sections of the cat spinal cord have been used to determine the rostro-caudal localization of the CPG. By isolating the L6-S1 segment of the spinal cord it was found that they could generate alternating activity between flexors and extensors in the cat (Grillner and Zangger, 1979). There are also different gradients of rhythmogenesis throughout the spinal cord. For instance, the rostral and caudal parts of the spinal cord present different gradients of excitation (Kiehn, 2006). In the cat the L3-L4 segments have greater rhythmogenic capacity than other spinal segments. The importance of the L3-L4 segments have been shown by localized topical application of clonidine (an α-2 noradrenergic agonist) into these specific regions which was sufficient to induce walking movements. On the contrary, injection of the α-2 NA antagonist yohimbine into these

segments could block locomotion (Marcoux and Rossignol, 2000). Moreover, after a chronic spinal lesion at T13 on cats having recovered treadmill locomotion, a spinal transection performed on the L3-L4 segment abolished all locomotor activity demonstrating that the integrity of these segments is crucial for the expression of locomotion in spinal cats (Langlet, Leblond et al., 2005).

Several others experiments on the hen (Ho and O'Donovan, 1993), turtles (Mortin and Stein, 1989), rats (Cazalets, Borde et al., 1995) and in the cat (Deliagina, Orlovsky et al., 1983) have shown these specificity on the localization of this rhythmogenic areas in the spinal cord.

The transversal localization of these circuits have been revealed by several activity-labeling studies (Cina and Hochman, 2000;Dai, Noga et al., 2005;Kjaerulff, Barajon et al., 1994) and electrophysiological evidence (Tresch and Kiehn, 1999) showing that locomotor-related neurons are concentrated in a ventral location (laminae VII, VIII, and X). This finding have been confirmed in microlesion studies in the rodent (Bracci, Ballerini et al., 1996).

1.3 Locomotion and evaluation methods

Interactions between spinal locomotor CPG along with sensory afferents and descending inputs form a tripartite organization which allows gait modifications and adaptations to external conditions by generating various patterns of locomotion. This organization is central to our current understanding of how rhythmic patterns are continuously adapted to internal and external demands.

This chapter will first describe the kinematics and electromyography (EMG) activity recorded in intact animals during locomotion. This information is needed to specify the normal parameters generated by the nervous system for locomotion. Knowledge of limb kinematics has led to insights about the requirements for modifiable motor patterns (Stein and Smith, 1997).

1.3.1 Treadmill

The analysis of locomotion is usually done on a flat treadmill because the speed can be adjusted to different walking conditions of the cats while the sensory information from auditory, vestibular and visual systems remains approximately constant. Therefore, one of the main advantages of using treadmill over ground walking to study locomotion is that the velocity of locomotion can be controlled better and a large number of steps can be recorded and averaged allowing a statistical analysis of the different gait parameters. Also, the speed of the treadmill belt can be changed to cover the range used by the animal. This procedure allows collection of reliable data of continuous step cycles and the analysis of movement (Kinematics) and EMG activity.

However, in more natural surroundings the locomotor pattern is constantly adapted to the terrain and to the goals of the animal and the visual information varies continually (Halbertsma, 1983). This could introduce a variable in which adaptation through sensory feedback and voluntary corrections are more important for over ground walking than on an ordinary treadmill.

Variables such as speed or confinement to a constant environment are key to assess the efficacy of training methods and allows comparisons between them. Such variables could then be maintained on a treadmill and represent an important advantage of the treadmill compared to over ground locomotion especially in experimental locomotor training in which that same task (or steps) have to be repeated several times as will be used in this study.

1.3.2 Locomotor cycle and kinematics

A step cycle is defined as two successive contacts of the same foot on the treadmill. When any limb is in contact with the ground, it extends, and thus serves to propel the animal forward (stance phase). At the end of this phase, the limb is lifted from the ground by a movement of flexion, carried forward (swing phase), and finally is again placed upon the ground to repeat the cycle.

Other subdivisions of the swing and stance phases of the step cycle are commonly used.

These patterns were refined by describing the movements of different joints (hip, knee, ankle and metatarsophalangeal or MTP joints) in each phase (Phillipson, 1905).

According to this subdivision, swing starts by a flexion (F) of all joints; while the hip continues its flexion, the ankle and knee start extending (El) until the paw touches the ground. At paw contact, the knee and ankle are passively flexed during weight acceptance (E2 or yield phase), the MTP joint continues the extension initiated in El. During the third extension phase (E3 or push-off), all joints extend to propel the body forward. This phase ends when the foot is lifted off the ground and a new cycle starts (Fig. 2). Thus the swing phase is subdivided in F and E1 while stance is subdivided in E2 and E3.

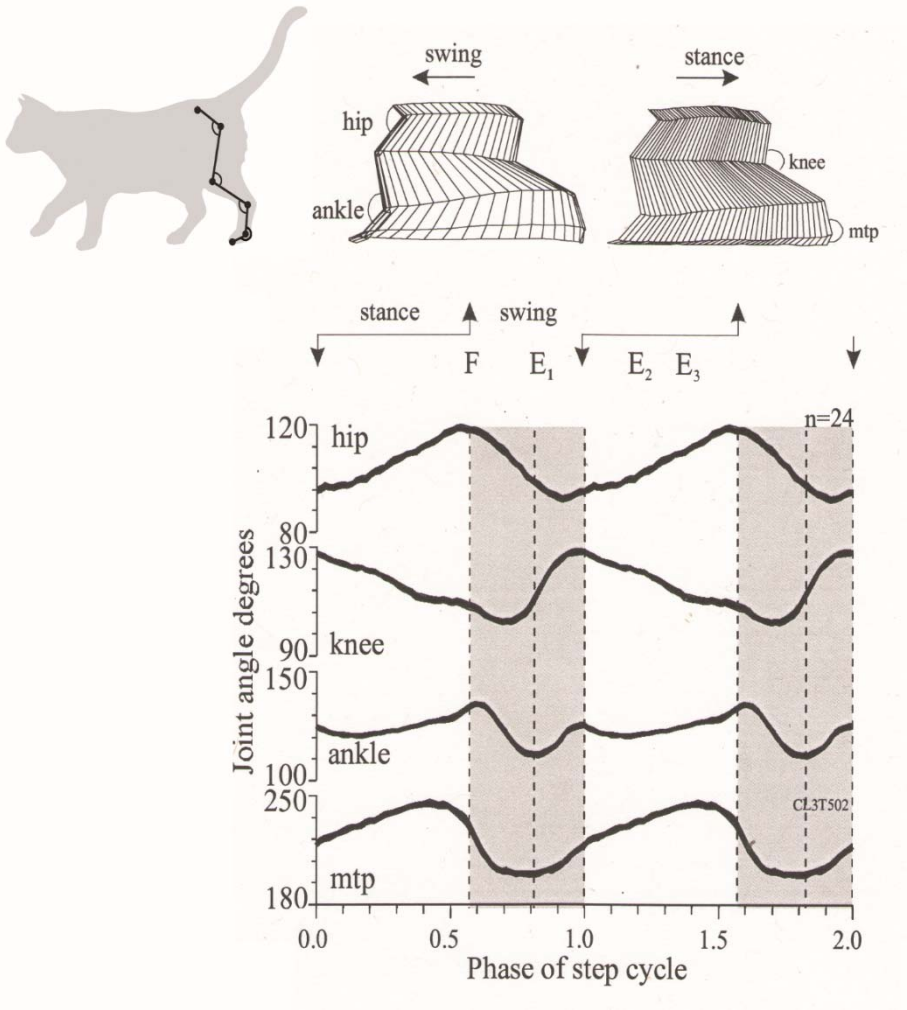

Figure 2. Representation of limb joint measurements used to extract limb kinematics (stick diagrams) displaying separately the swing and stance phases and angular excursions. For angular excursions, flexion is always represented by a downward deflection of the traces. Phillipson (1905) subdivisions are shown: F and E1 constitute swing while E2 and E3 constitute stance. Figure modified from (Rossignol and Bouyer, 2004).

1.3.3 Interlimb coordination

Accurate coordination between the limbs is essential in locomotion. Appropriate coordination ensures the dynamic stability of head and trunk, prevents stepping of limbs on one another, allows a precise and smooth contact against ground for each limb, and reduces oscillatory movements of head and trunk, thus decreasing the energy needed for locomotion.

Interlimb stepping is linked by both rigid neural programs and modifiable programs that give the animals the flexibility necessary to meet changing postural demands imposed by a dynamic environment (English, 1979). This suggests that limb coordination somehow results from an interaction between independent neuronal centres (CPGs) controlling each extremity (see (Halbertsma, 1983).

For walking gaits, the principal criterion for gait selection is stability. The body is always supported by two, three, or four paws at a time, so stability is increased by maximizing support by four feet, minimizing support by two feet, and, by selecting the combinations of two and three feet that are favorable to balance (Hildebrand, 1980). There seems to be a tendency to use more frequently a parallel coupling (same side of the body) between the forelimb and the hindlimb on the treadmill (Blaszczyk and Loeb, 1993) whereas during trot the forelimb and hindlimb are coupled to the contralateral hindlimb (diagonal coupling) (Hildebrand, 1980;Rossignol, 1996).

In symmetrical gaits, both hindlimbs and both forelimbs are coupled out of phase. During walking, the right hindpaw contacts the ground at 50% of the left cycle. Thus, a typical sequence of normal walking starting from the left hindlimb contact with the ground will pursue as follows: Left hindlimb – left forelimb – right hindlimb – right forelimb. Many neural mechanisms may participate in the control of interlimb coupling through different crossed pathways (Jankowska and Edgley, 1993;Jankowska and Noga, 1990) and other interlimb reflexes (Schomburg, 1990), and also through descending inputs to adapt to various external conditions (Blaszczyk and Loeb, 1993).

Interneurons may be responsible for the coordination between the CPGs, and they have been identified in invertebrate motor systems, such as the one controlling walking in the cockroach (Pearson and Iles, 1973). These interneurons appear to provide the next ganglion with an efferent copy, which might be used to produce intersegmental coordination. In cats a part of the ascending neuronal activity to the cerebellum provides efferent copy signals (Arshavsky, Berkinblit et al., 1972). Such signals could also be utilized to coordinate the CPGs (Grillner, 1981). Also, propriospinal system (see below) could also play an important role in relaying locomotor information between cervical and lumbar enlargements and therefore, the coupling of motor rhythms between the forelimbs and the hindlimbs (Cowley and Schmidt, 2000).

We studied limb coordination because its preservation or changes during the task we evaluate could be an expression of similar or different neural programs controlling locomotion within two different tasks (see chapter 2) presented in this project.

1.3.4 General muscle activity in cats

Functional classification on flexor and extensor muscles was first stablished based on experiments on decerebrate cats and dogs where flexion responses where produce in response to cutaneous stimulation on the limb or foot (Sherrington, 1910a). Muscles excited during the flexion reflex were generally classified as flexors and those muscles which responded by inhibition were classified as extensors. Flexor muscles generally close the joint angle whereas extensor do the opposite. For small toe muscles such as Extensor Digitorum Brevis, the dorsiflexion closes the metatarsophalangeal joint although the name suggests rather an extension.

Describing in detail the normal pattern of flexors and extensors EMGs reveals the complexity of the mechanisms generating locomotion. Groups of agonist and antagonist muscles, in both the forelimbs and the hindlimb, are activated sequentially during locomotion. The sequential activation of muscle gives the flexibility of movements by which descending systems can regulate and modify limb activity (Krouchev, Kalaska et al., 2006) as seen in Fig.3.

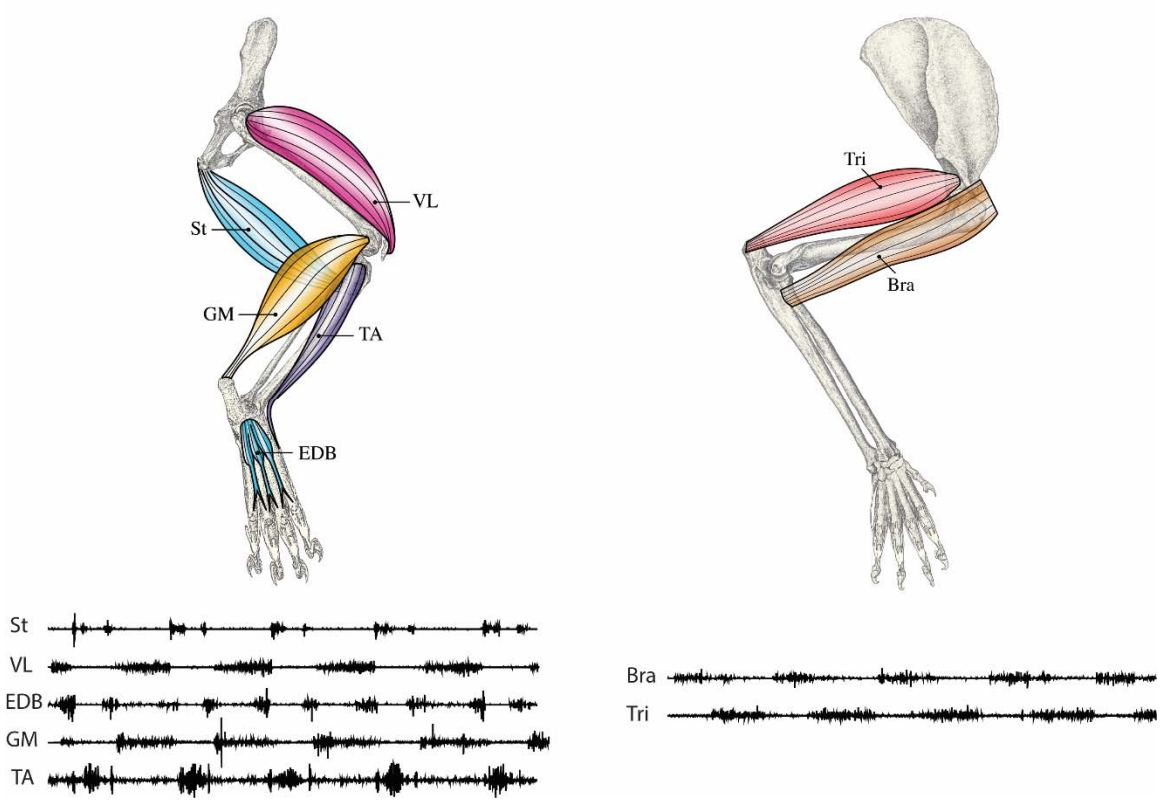

Figure 3. Schematic representation of chronically implated muscles used in this study showing origins and point of insertion and their respective raw EMG signals. Flexors and extensor are activated in an alternate and rythmic manner. Each muscle presents particular EMG "signatures" or shapes. St: Semitendinosus, VL: Vastus lateralis, EDB: Extensor digitorum brevis, GM: gastrocnemius medialis, TA: Tibialis anterior, Bra: biceps brachii, Tri: Triceps brachii.

Cats with chronically implanted EMG electrodes allow to assess how various muscles are utilized in such behaviors and how the nervous system specifically selects various anatomical patterns and combinations in appropriate phases.

Differences in EMG descriptions found in the literature might depend on the animal's inherent variability, strategies, anatomical variability, on what part of the muscle was recorded or what kind of electrodes was used (Loeb, 1993). However, some muscles have a more or less consistent pattern under various experimental conditions and are described below.

The knowledge of muscular dynamics, its specific EMG shapes or "signatures" and their normal activation during the different phases of the step cycle helps us identify changes when altering external conditions or intrinsic variables within the animal. Thus, it allows

us to understand how the locomotor system works, how it is modified and, depending on the study approach, hypothesize about the structures responsible for these changes.

1.3.5 Activity of flexor and extensor muscles

Extensors

During forward walking, extensor muscles across the hindlimb are co-active during most of the stance phase to counteract ground-reaction forces that tend to flex the joints (Perell, Gregor et al., 1993;Smith, Carlson-Kuhta et al., 1998;Smith, Edgerton et al., 1977).

Extensor muscles have very similar patterns of activity. They are activated in cats some 20-80 ms before the paw contact so that their discharge is not normally triggered by sensory events associated with contact. Except for Vastus Lateralis muscle (see below), most of the extensor muscles present a large main burst beginning just before ground contact and terminating just before the end of the stance phase. Although the activity in these muscles generally shows an on-off behavior, characteristic differences exist between extensor muscles (Grillner, 1981).

Flexors

Muscles related to the swing phase may have a more complex and versatile discharge pattern. These muscles usually have either one or two bursts of activity during swing. During all forms of walking, the cat's hip and ankle flexor muscles exhibit one burst of activity, and the burst is associated with a flexor muscle torque at each joint (Stein and Smith, 1997).

Flexor muscles are not all activated simultaneously. Instead, they show variations in the initiation, duration and termination of their activity during the step cycle. For instance, the initial period of activation in the most distal muscles, occurs just before the cat lifts the paw from the treadmill. Subsequently there is activation of the knee flexor muscles which serves to raise the paw from the support surface. Flexion of the ankle and transport of the

limb is followed by the braking action of bi-functional muscles (Wisleder, Zernicke et al., 1990) such as Semitendinosus (see below) and by activation of the distal flexors in preparation for landing (Krouchev, Kalaska et al., 2006).

Flexor-Extensor coordination

The main flexors (hip, knee, ankle and foot) reciprocate with the extensors in a complex way. The coordination between flexors and extensors can be illustrated on the dynamics of a few main muscles of the hindlimb (Fig.3). For example, one of the periods of activity in the semitendinous (St) occurred at the same time as a brief period of activity in an extensor muscle, the gastrocnemius lateralis (GL), whereas the other period of activity in the St occurred at the same time as a period of activity in the extensor digitorum brevis (EDB). The major periods of activity in the lateral (GL) and medial (GM) heads of the gastrocnemius also occurred at the same time during stance (Krouchev, Kalaska et al., 2006).

1.3.6 Individual muscular activity of relevance to this study

Flexors:

Semitendinosus (St)

Semitendinosus (St) has its origin on the tuberosity of the ischium and passes to the medial side of the tibia to insert into the crest of the tibia about 1 cm from its proximal end. St is a bifunctional muscle: it acts as a knee flexor and a hip extensor. This muscle has a particular discharge pattern: it has two EMG bursts during the swing phase of forward walking. One larger burst (first) with a sudden onset occurs around paw lift-off and a shorter burst (second), which is not always present at very low speeds of locomotion, precedes paw contact. Changes in the duration and amplitude of both bursts are closely tied to changes in the magnitude and duration of the flexor muscle torque at the knee joint (Smith, Chung et al., 1993).

The first St burst involved in the earliest part of swing when the paw is lifted off the ground is active before and during the F phase of the knee, indicating a knee flexor and not a hip extensor action (Halbertsma, 1983). The second St burst coincides with the first part of F phase of the hip and the first part of the E1 phase of the hip. This could be a combined hip extensor action. This second burst is more consistent at higher speeds and may be related to the need for a greater torque in flexion to decelerate both the hip and knee at the end of swing in preparation for foot contact (Smith, Chung et al., 1993;Wisleder, Zernicke et al., 1990).

Extensor digitorum brevis (EDB)

Ankle and digits extensor muscles are interesting in many aspects since they have an important role in positioning and stabilizing the foot, as well as flexing (plantar-flexion) the digits.

The extensor digitorum brevis (EDB) has its origin from the distal border of the calcaneal ligament and from the dorsal surfaces of the proximal ends of the three lateral metatarsals. It ends in three flat tendons which run in the interspaces between the four tendons of the extensor digitorum longus. This muscle prepares the paw for placement on the support surface. It has a large burst of activity at the beginning of the E1 phase, which declines when the ankle and foot extensors start their main activity and exhibits some residual activity throughout the support phase. The main extensor activity, evident by a second burst in the E2 phase, often has a rapid onset in connexion with the placing of the foot (Engberg, 1964). This muscle in particular is maximally active during the placing of the foot and differs from all other flexors in being relaxed in the flexion phase and the third extension phase (Engberg and Lundberg, 1969).

Tibialis anterior (TA)

Tibialis anterior muscle functions as an ankle dorsiflexor following the regular flexor pattern activity. It originates is from the proximal lateral side of the tibia and it ends distally in a strong tendon that insets into the outer surface of the first metatarsal. TA is strongly activated for a short period at the end of the third extension phase but this is followed by

another strong activation in the flexion phase and the muscle is only relaxing for a short period before a very strong burst of activity at the end of the first extension phase. The very striking increase in flexor activity late in the first extension phase may be needed for a deceleration of the more rapid forward swing of the leg in the trot (Engberg and Lundberg, 1969).

Biceps Brachii (Bra)

Biceps Brachii is an elbow flexor of the forelimb. It originates from the supracondyloid foramen and the humerus and it ends by a flat tendon which joins the tendon of the cleidobrachialis on the lateral surface of the ulna. This muscle starts its discharge before the F phase (paw-off), maintaining its activity during the whole swing phase and terminates before or at the paw contact.

Extensors:

Vastus lateralis (VL)

M. vastus lateralis is a part of the large quadriceps femoris muscle which covers the cranial, lateral and medial part of the femur or thigh. It is a knee extensor whose activity gradually starts before the onset of the stance phase, and usually is most active in the later part of the E3 phase. The VL activity terminates abruptly and almost always before the onset of the swing phase. (Engberg and Lundberg, 1969).

VL is thus active just before and during the major part of the support phase. This corroborates its supporting function and it could propel the animal during the last part of its activity.

Gastrocnemius medialis (GM)

Gastrocnemius medialis is an ankle extensor and knee flexor. It originates from the medial sesamoid bones of the femur and it inserts along with the gastrocnemius lateralis by the calcaneal tendon into the proximal end of the calcaneus. It has an intense burst of activity at the onset of the E1 phase of the ankle and 60 to 80 ms before the onset of the

stance phase. The activity drops gradually and terminates before the VL activity around 200 ms before the onset of the swing phase. This large activity occurring before foot contact has played an important role in defining the preprograming of muscle activity by a central generator in advance of the actual foot contact (Gorassini, Prochazka et al., 1994).

GM is active during the first part of the stance phase, which suggests a support function of the body after touch-down. GM is less active during the last part of the stance phase, the propulsive phase, where the knee extensors are still active. The delay between the EMG activity and the force development in the muscle could be responsible for the substantial ankle torque, which is needed during this phase (Manter, 1938).

Triceps Brachii (Tri)

Triceps Brachii is an elbow extensor of the forelimb. This muscle has three heads (lateral, long and medial) which originate from various areas on the scapula or on the humerus and ends at various points of the ulna. The three heads start their discharge prior to paw contact. This precedence increases with speed, from practically null during walking into several milliseconds during trotting and galloping in dogs. The long head of triceps that attaches to the scapula often has a short distinct burst just prior to swing (Drew and Rossignol, 1987;Rossignol, 1996).

1.4 Control and modulation of Locomotion

Locomotion results from intricate dynamic interactions between a central program and feedback mechanisms. The central program relies fundamentally on a genetically determined spinal circuitry (CPG) capable of generating the basic locomotor pattern and on various descending pathways that can trigger, stop, and steer locomotion. The feedback originates from muscles and skin afferents as well as from special senses (vision, audition, vestibular) and adapts the locomotor pattern to the requirements of the environment.

Afferent inputs from muscles or the skin reach the spinal cord, project to motoneurons directly or through interneurons, or through the CPG itself. Afferents also send collaterals through ascending pathways (directly or via relay interneurons) reaching supraspinal structures (telencephalon, brain stem, cerebellum), which in turn via cerebral cortex or brain stem, project down to the spinal cord on neurons that may or may not also be contacted by the same primary afferent. At the spinal cord level, sets of interneurons will be selected to modulate transmission during a given task or during a particular phase of a task. In this way it is ensured that reflex activations of given muscles occur only at the appropriate times in the step cycle (phase-dependent modulation) and generate the right locomotor pattern (Rossignol, Dubuc et al., 2006). See Fig. 4.

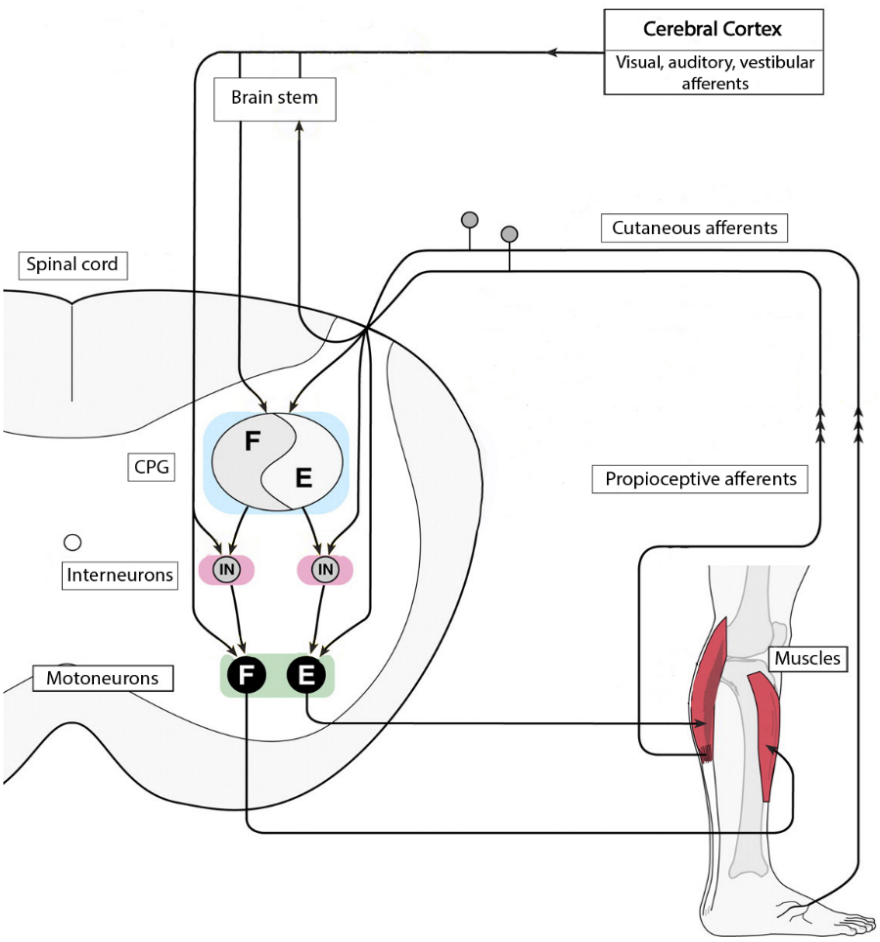

Figure 4. The control of locomotion is tripartite. Spinal CPG is at the core of the system and it can be altered or modulated by sensory inputs (propiospinal and/or cutaneous) and descending signals from supraspinal structures (cortical or subcortical). These 3 strucutres are in constant interaction to drive the appropiate commands to specific muscles to adapt locomotion to external situations. Interneurons in the spinal cord could participate in this modulation before reaching their respective motoneurons. Figure modified from Rossignol et al,. 2006.

The study of the tripartite system reveals at the same time the autonomy and the interdependence of the various control parts as it will be described in the next sections.

1.4.1 Sensory inputs

Sensory inputs include proprioceptive and cutaneous receptors. The implication of the sensory inputs to locomotion has been studied with various approaches such as recording the activity of afferents, inactivating the afferents through neurectomy or by stimulating the afferents throughout locomotion or in discrete phases of the cycle (as reviewed in (Rossignol, 1996). Results from fictive locomotion have shown that sensory inputs are not necessary to produce the basic locomotor pattern. What are then the roles of sensory information during locomotion?

The hindlimbs of a spinal cat, when walking on split-belt treadmill, can separately adapt their speed to that of their corresponding belt. This is the result of the sensory activity generated by foot contact with the belt (Forssberg, Grillner et al., 1980b). Obstruction of the hip movement (Grillner and Rossignol, 1978) or loading of extensor muscles (Duysens and Pearson, 1980) can completely suppress rhythmicity in a leg while the other legs continue to be rhythmically active.

This and many other experiments have proven that sensory inputs are crucial in adapting and modulating the operation of the CPG in the real environment. Sensory inputs can have global influences in allowing, preventing, or selecting motor patterns. Through dynamic interactions, sensory inputs can participate in the correct positioning of the paw in uneven terrain and they can also modify the frequency of the pattern, its intrinsic structure, or the amplitude of muscle discharges within locomotor phases (Rossignol, Dubuc et al., 2006). Afferent inputs most relevant to walking primarily arise from stretch- and load-sensitive mechanoreceptors located in muscles and skin.

1.4.1.1 Proprioceptive inputs

Proprioceptors (muscle, joint and deep fascia receptors) as well as cutaneous receptors have general roles such as providing signals acting as on-off switch to set the range of joint angular excursion within which locomotion can take place. However, an important role of muscle afferent feedback appears to be in setting the overall timing of the step cycle by

adjusting the duration of the various phases of the locomotor cycle and facilitating the switch between phases. Another important role is to regulate the output amplitude of muscles in various phases (as reviewed in Rossignol, Dubuc et al., 2006).

Experiments suggest that hip proprioceptors exert a powerful control over the initiation of the locomotor rhythm because lifting a spinal cat or dog by the thorax, thus extending passively the hindlimbs, triggers air stepping. This is largely due to stimulation of proprioceptors (especially at the hip), since removal of the skin does not abolish the response (Sherrington, 1910b). Contrarily, flexing the hip in the same preparation can prevent air stepping. Similarly, in chronic spinal cats, flexion of the hip joint on one side can abolish treadmill stepping on that side, while the other side continues to walk (Grillner and Rossignol, 1978).

As mentioned above, spinal cats can modify the structure of the step cycle of individual limbs when walking on a split treadmill moving at different speeds. Although cutaneous receptors of the foot pads cannot be discarded as an important source of inputs for such speed adaptation, the experimental evidence suggests that proprioceptive inputs from muscles and/or joints are the main contributors (Forssberg, Grillner et al., 1980b).

1.4.1.2 Cutaneous inputs

Early work of Sherrington showed that removing cutaneous inputs from the hindlimbs did not prevent locomotion even after spinalization (Sherrington, 1910a). This was largely supported by others who reported little deficits when cutting cutaneous nerves in intact cats (Duysens and Stein, 1978) or infiltrating the central foot pad with a local anesthetic (Engberg, 1964). In these experiments on cats with an intact spinal cord and with extensive denervation on the hindlimb, stepping was almost normal on a regular treadmill after compensation of the deficits through locomotor training.

Stimulation of the perineal region (scrotum, vulva and base of the tail, inguinal fold) (Krawitz, Fedirchuk et al., 2001), is most effective in triggering locomotion. The activation of unspecific afferents from the perineal region presumably underlies some important survival function (such as escape from a predator), but the mechanisms of interactions of these perineal afferents with the CPG are not known. Other types of tonic stimuli can

completely inhibit these locomotor movements in both normal and fictive locomotion. For example, pressure of the skin of the lumbar region (Viala and Buser, 1974) or controlled electrical stimulation (Viala, Orsal et al., 1978) will abolish locomotion in the rabbit.

Although cutaneous inputs may have some general roles to play in locomotion such as triggering walking (perineal stimulation) or inhibiting walking (inhibitory inputs from the skin of the back), skin afferents participate predominantly in the correction of limb, foot placement during stance on uneven terrain and also on the expression of locomotion after spinal lesions. For example, after a complete spinal lesion denervated cats never recovered locomotion even when submitted to training showing the importance of cutaneous inputs on the compensatory mechanisms to regain stepping (Bouyer and Rossignol, 2001).

1.4.2 Propriospinal system

Spinal segments are interconnected by short and long intraspinal pathways (i.e., propriospinal) that run close to the gray matter bilaterally.

It was demonstrated that after corticospinal tract lesions in rats, new connections could be established with the lumbosacral cord through cervical propriospinal pathways (Bareyre, Kerschensteiner et al., 2004). Propriospinal pathways appear to be of considerable importance for volitional aspects of locomotor recovery after an incomplete SCI in adult mice (Courtine, Song et al., 2008) and cats (Kato, Murakami et al., 1984) through the formation of new functional circuits.

Moreover, high spinal preparations are capable of developing coordinated activity involving both the fore- and the hindlimbs (Miller and Van der Meche, 1976). This is apparently achieved via propriospinal pathways which reciprocally interconnect the cervical and lumbar enlargements of the cord (Halbertsma, Miller et al., 1976;Miller, Van der Burg et al., 1975). Indeed, in experiments where synaptic transmission in the thoracic region was supressed in neonatal rats, a disruption in the motor rhythms between cervical and lumbar regions resulted (Cowley and Schmidt, 2000).

1.4.3 Supraspinal inputs and pathways

Supraspinal control of locomotion can be viewed from many aspects: initiation of locomotion, control of posture and propulsion, and corrections to adapt one or several steps to the environment. Several structures can be implicated in the initiation and can play a role in posture and correction and they will be described in this section.

1.4.3.1 Initiation of locomotion

Experiments applying transections and electrical stimulation at different levels of the neural axis have shown that the regions for initiation of locomotion are located in the brain stem, at supraspinal level (Shik, Severin et al., 1966). Electrical stimulation of a region of the midbrain can evoke locomotion in decerebrate cats and other mammals (Gelfand, Orlovsky et al., 1988;Shik and Orlovsky, 1976). The structure implicated on this initiation is the mesencephalic locomotor region (MLR).

The MLR region receives projections from several brain structures including the basal ganglia, the sensorimotor cortex and the limbic system and projects toward the middle part of the reticular formation (Garcia-Rill, Skinner et al., 1983;Shik, 1983;Steeves and Jordan, 1984). This last projection to the reticular formation would be one important connection implicated in the initiation of locomotion (Steeves and Jordan, 1980). The MLR does not project directly to the spinal cord. Instead it initiates and controls locomotion through monosynaptic connections to the reticular formation of the brain stem which, through the ventrolateral funiculi, activate the spinal locomotor networks to initiate locomotion (Garcia-Rill and Skinner, 1987;Shik, 1983).

1.4.3.2 Posture and corrections of steps during locomotion

In addition to a role in the initiation and termination of locomotion, the brain stem contains centers which are important for the modulation of locomotor activity. Reticulospinal, rubrospinal and vestibulospinal pathways are capable of influencing locomotor neural circuits in the spinal cord (Drew, Jiang et al., 2002a).

Descending pathways that influence motor activity, including locomotion, can be divided into two principal systems (Lawrence and Kuypers, 1968):

A medial system that includes the reticulospinal and vestibulospinal pathways.

The reticulospinal pathway arise from regions of the reticular formation in the brainstem projecting through the anterior funiculus of the spinal cord to the motoneurons of the axial musculature (Kuypers, 1981). Commands that initiate and modulate locomotor circuits in the spinal cord are transmitted through the reticulospinal tract. Moreover, the reticulospinal pathways are involved in the modifications of posture that anticipate and accompany voluntary movements and which ensure stability of the body during locomotion (Drew, Dubuc et al., 1986;Rossignol and Frigon, 2011).

The vestibulospinal pathway can be divided in a lateral vestibulospinal tract and medial vestibulospinal tract, both originate from 2 of the 4 vestibular nuclei in the brainstem. They course through the anterior funiculus of the spinal cord on the ipsilateral side. Because of its origin from the vestibular nucleus (lateral or medial), this pathway is implicated in the control over postural changes necessary to compensate for sloping ground by acting specially over the extensor muscles during the stance phase (Matsuyama and Drew, 2000). The destruction of the lateral vestibular nucleus decreases the activity of the extensor muscles in a decerebrate cat (Yu and Eidelberg, 1981).

Overall, the medial system has a relatively diffuse action on flexor and extensor muscles of the more proximal limb and the axial musculature and serves to adjust the posture of the animal. Results from lesion on the ventrolateral quadrant (Brustein and Rossignol, 1998;Gorska, Bem et al., 1990;Kuypers, 1963) of the spinal cord (which contains these pathways) and also microstimulation (Degtyarenko, Zavadskaya et al., 1993;Drew, 1991a;Drew and Rossignol, 1984) suggest that this system is responsible for producing the requisite muscle tonus necessary to support the body, for ensuring the lateral stability of the animal, and for producing step by step regulation of the level of muscle activity during locomotion.

Another important structure related to postural adaptations to external conditions is the cerebellum. Cerebellar contributions to locomotion has been hypothesised to help provide

adaptability to motor patterns but its specific role is still not clear. One theory suggest that the cerebellum might act as "real-time sensory processing device" (Bower, 1997), by processing sensory inputs and making alterations of the ongoing movement patterns. However other theory suggest that the cerebellum might alter ongoing movement patterns in a predictive manner based on a stored internal representation of a specific movement (Maschke et al., 2004; Smith and Shadmehr, 2005).

A lateral system, includes the corticospinal and rubrospinal pathways.

The rubrospinal pathway originates in the red nucleus of the midbrain. The axons immediately cross to the contralateral side of the midbrain, and course through the lateral funiculus reaching motoneurons in the spinal cord. The motor cortex and premotor cortex both project to the red nucleus from which the rubrospinal tracts arise (in the cat) resulting in an alternative way by witch voluntary commands can be sent to the spinal cord (Schieber, 2007).

The corticospinal pathway provides the most direct pathway over which the cerebral cortex controls movement. It originates mainly in the motor cortex from which its axons courses through the internal capsule and then to the cerebral peduncle on the brainstem. The axons then form the medullary pyramids on the ventral surface of the brainstem splitting in two from there. Most of the axons (90%) cross over to the contralateral side at the pyramidal decussation forming the lateral corticospinal tract that courses through the lateral funiculus of the spinal cord. The 10% of remaining axons form the anterior corticospinal tract that courses ipsilaterally through the anterior funiculus (Kuypers, 1981). Once they reach the spinal segment in which they end the axons cross to the contralateral side to innervate motoneurons of the ventral horn. Even though the corticospinal tract is not indispensable for the production of the basic locomotor rhythm in cats, it contributes to the regulation of locomotion, especially when, in order to adapt to external conditions, a requirement for precise control over paw placement is needed (Drew, Jiang et al., 2002b).

Altogether, the lateral system is responsible for voluntary and goal-directed aspects of locomotion, as well as fine control of the distal musculature. It also plays a critical role in more difficult locomotor tasks, such as obstacle avoidance and ladder walking (Drew, Prentice et al., 2004;Liddell and Phillips, 1944).

Lesions of the dorsolateral funiculi at the low thoracic level that completely interrupted both the corticospinal and rubrospinal pathways produced long-term deficits in locomotion on a level surface. These deficits included a foot drag that was probably caused by both a loss of cortico- and rubrospinal input to motoneurones controlling distal muscles as well as by a change in the relative timing of muscles acting around the hip and knee. Smaller lesions produced similar deficits from which the cats recovered relatively quickly. Cats with the largest lesions of the dorsolateral funiculi were unable to modify their gait sufficiently to step over obstacles attached to the treadmill belt even 3–5 months after the lesion suggesting that the corticospinal tract provides an important contribution to the modification of the basic locomotor rhythm to take into account the variations of the walking environment (Drew, Jiang et al., 2002a). These results also imply that the medial pathways, the reticulo- and vestibulospinal pathways, are unable to fully compensate for damage to the lateral pathways.

The knowledge of supraspinal pathways and of their functions in normal locomotion allows us to infer which supraspinal pathway is affected based on observed motor deficits. Also, based on the reduction of specific deficits, for example after locomotor training, we can infer which pathway is being activated and then compare advantages of a specific locomotor training method over another.

1.5 Skilled locomotion

Even though decerebrate cat preparations allow studying the organization of the CPG and its interaction with sensory information, these preparations do not throw any light on the roles of more rostral structures, such as, for example, the motor cortex (MC). For this, other neurophysiological investigations in conditions that require dynamic voluntary adaptations to the environment such as skilled locomotion are necessary (Armstrong, 1986a).

It has been hypothesized that the single most important event in the step cycle is the placement of the foot at touch-down. A precise control over this event is important to avoid

obstacles and holes in the terrain and to ensure a stable support (Halbertsma, 1983). So it is logical to think that many of the voluntary modifications are done to improve this aspect of skilled locomotion.

The contribution of the MC to the control of voluntary movement has been demonstrated by a large number of studies using single neuron recording techniques to probe the relationship between neuronal activity and behavior (Drew, 1988). Many authors have shown for example that, in cats, the discharge of identified projection neurons from motor cortex (pyramidal tract neuros, PTNs) are increased in locomotor tasks in which animals are required to modify their gait or to accurately control the position at which the foot is placed. These tasks include stepping over moving obstacles (Drew, 1988), peg walking (Donelan, McVea et al., 2009) and walking on a horizontal fixed ladder (Amos, Armstrong et al., 1990;Armstrong, 1986a;Beloozerova and Sirota, 1993).

These experiments suggested that the motor cortex is involved in the transformation of a visual signal (obstacle, barrier, or ladder rung) into a motor act (the modification of the basic locomotor rhythm) (Kalaska and Drew, 1993).

Experiments involving destruction of the MC or interruptions of the pyramidal tract have demonstrated the inability of lesioned cats to walk on wire mesh or on elevated horizontal bars (Chambers and Liu, 1957;Trendelenburg W, 1911). Without the MC, dogs standing stationary lost the ability to place the paw in the appropriate space zone (Grillner, 1981). The pattern of impulse activity in motor cortex cells changes during unexpected perturbations of the stepping of the cat, as well as during voluntary modification during walking (Amos, Armstrong et al., 1989;Drew, 1988). Patterns of activity on the MC can also be seen at different times of the step cycle. For example, recordings have shown that the mean activity of motor cortical neurons was elevated in late stance and early swing during accurate stepping, suggesting that there is an important cortical regulation at the moment of the paw contact with the ground (Beloozerova, Farrell et al., 2010).

Descending drives certainly contribute to the production of locomotion in normal conditions by refining the basic synergy and improving the level of inter-limb coordination which are otherwise limited at the spinal level only (Armstrong, 1986a).

The importance of these skilled locomotor tasks is that they challenge the animal to walk in more demanding conditions. This allows us to investigate deficits after lesions of the nervous system that otherwise might be undetected during level treadmill walking. For example the inactivation of the motor cortex by TTX produces several deficits on locomotion over a horizontal fixed ladder while it does not affect locomotion over a conventional treadmill (Beloozerova, Farrell et al., 2010). Also, after extensive cutaneous denervation of the hindlimb on cats with an intact spinal cord almost normal locomotion was achievable on a regular treadmill but locomotion on a horizontal ladder was not possible (Bouyer and Rossignol, 2001).

1.6 Animal models of SCI and the importance of locomotor training.

Spinal cord injury (SCI) in animal models provides an opportunity to study not only the functions of various CNS structures but how they adapt to different external conditions to maintain or recover locomotion.

1.6.1 Complete lesions

After complete section of the spinal cord, descending inputs to the spinal locomotor circuitry are altered because of the severance of pathways controlling the circuits for initiation and modification of locomotion. Remaining peripheral sensory afferents to spinal circuits as well as intrinsic changes of spinal circuits are then responsible of the plastic changes that allow locomotor recovery.

Even though it was first showed that kittens could recover locomotion after a complete spinal lesion (Forssberg, Grillner et al., 1980a;Forssberg, Grillner et al., 1980b;Grillner, 1973) subsequent studies showed that in adult cats recovery was also possible (Barbeau and Rossignol, 1987;Rossignol, Bélanger et al., 2000;Rossignol, Chau et al., 2002). Few days after spinalization, perineal stimulation can elicit rhythmic, alternating stepping in the hindlimbs, although plantar foot placement is absent and an important foot drag is seen.

Locomotor training on a treadmill during 3 weeks to 3 months (depending on the animal) allows such cats to recover alternate hindlimb movements, hindquarter support and plantar foot contact (Barbeau and Rossignol, 1987). These findings showed that locomotor recovery after a complete spinal lesion is achieved by interactions between the CPG with sensory feedback from the hindlimbs.

The importance of regular daily training (starting even after 1 month after transection) has been proved by many authors (Edgerton, de Guzman et al., 1991;Lovely, Gregor et al., 1986;Lovely, Gregor et al., 1990;Rossignol, Martinez et al., 2015). This is of great interest in the clinical situation, where training may be started late after the spinal injury (Barbeau, Dannakas et al., 1992;Wernig and Muller, 1992).

1.6.2 Incomplete lesions

Although models of complete SCI are important in determining intrinsic spinal mechanisms involved in locomotor recovery, most SCIs in humans are incomplete, and spared descending pathways can still access the spinal circuitry.

Lateral hemisection models, where several pathways are severed simultaneously on one side only, are relevant because they mimic the clinical situation in which spinal lesions due to accidents or falls damages several pathways producing characteristic deficits and directly influencing locomotor recovery (Rossignol and Frigon, 2011).

Although substantial recovery of hindlimb locomotion is observed following a lateral hemisection of the spinal cord, some deficits, mostly observed on the side of the lesion, can persist. Deficits observed after a lateral hemisection resemble those associated with a ventrolateral (transiently impaired body equilibrium, interlimb coupling) together with a dorsolateral (impaired skilled locomotion) lesions (Brustein and Rossignol, 1998;Gorska, Bem et al., 1990;Kuypers, 1963).

After incomplete SCI, spared pathways originating from supraspinal and propriospinal structures can play an active role in the recovery process, and also in restoring some voluntary control (Rossignol, Dubuc et al., 2006). However, intrinsic spinal circuits and peripheral afferents still remain to initiate and organize hindlimb locomotion. The

compensation by descending pathways may take different forms. Damaged pathways may regenerate while undamaged pathways may sprout or may change the efficacy of their transmission. In doing so, new circuits could result from new anatomical connections or from enhanced connectivity (enhancing existing circuits) (Rossignol and Frigon, 2011).

How important is the plasticity of the spinal CPG for the recovery of hindlimb locomotion after partial spinal lesions, considering that spared descending pathways still have access to the spinal cord? The essential role of the intrinsic spinal circuitry was clearly demonstrated by a dual spinal lesion paradigm, in which cats with a lateral hemisection at T10-T11 and then trained on a treadmill was followed several weeks later by a complete spinalization at T13 (Barrière, Cohen-Adad et al., 2008;Barrière, Frigon et al., 2010). Hindlimb walking was observed in all trained cats within hours following spinalization, a process that usually takes 2 to 3 weeks of treadmill training to appear in spinalized cats (Barbeau and Rossignol, 1987). This is a demonstration that important intrinsic changes had already occurred within the locomotor spinal circuitry following the partial spinal lesion. The first major difference between untrained cats compared to trained cats was an important asymmetry in stepping capacity between both hindlimbs observed during the first 10 days after complete spinalization. For instance, kinematic analysis in these cats showed that the right hindlimb remained most of the time in a flexed position while rhythmic activity was seen in the left lesioned side. However, in cats that were trained after the initial hemisection, the walking pattern after a complete spinalization was similar to what is observed in cats with an intact spinal cord, corroborating earlier evidence that the intrinsic spinal network can be improved by training (Barrière, Cohen-Adad et al., 2008;Barrière, Frigon et al., 2010;Martinez, Delivet-Mongrain et al., 2011). Plastic changes during the hemisected period might be the result of compensation by remnant descending inputs that imprint into the spinal cord new modes of functioning (Chen and Wolpaw, 2002).

Further experiments using the dual-lesion paradigm showed that minimizing the period of recovery between a spinal hemisection and the second complete lesion to 3 weeks and without locomotor training, asymmetric changes seen after the hemisection could be maintained after spinalization (Martinez, Delivet-Mongrain et al., 2011;Martinez, Delivet-

Mongrain et al., 2012b). Results from these experiments proved that even though locomotor recovery is possible without locomotor training, the way the spinal circuits could be reorganized depends on the conditions during the hemisected period. For example, cats that re-expressed the best bilateral hindlimb locomotion after spinalization were the ones with the most prominent locomotor deficits and had the larger initial hemisection. This asymmetrical reorganization of the spinal circuits in absence of locomotor training could only lead to the conclusion that "treadmill training leads to a more symmetrical spinal reorganization and a bilateral stepping pattern after spinalization by forcing the two hindlimbs to walk after hemisection" (Martinez, Delivet-Mongrain et al., 2011).

If locomotor training after partial lesions can induce such changes in the spinal circuitry for locomotion, could a training requiring more supraspinal commands improve locomotor recovery differently than a regular training on FTM after an incomplete spinal lesion? This question is the main issue investigated in this project. We try to answer this question by using a new method that increases the influence from supraspinal structures to the locomotor training.

1.7 Problem statement

As we have seen in this introduction, locomotion is controlled at multiple levels of the CNS and more complex locomotor tasks such as skilled locomotion require complex interactions in order to take place. After spinal lesions, the recovery of function involves optimizing these interactions between remaining structures resulting in the development of compensatory mechanism to optimize the basic locomotor function.

Plasticity within intrinsic spinal circuits is a critical component of hindlimb locomotor recovery. Locomotor control, seen as a tripartite system where CPG, sensory feedback and supraspinal structures are in constant interaction, is changed or interrupted somehow after a spinal lesion. **Thus, after SCI, targeting intrinsic spinal circuits by stimulating or engaging remaining pathways and sensory afferents should be a focus for rehabilitative strategies** (Barbeau and Rossignol, 1994;Harkema, 2008).

Previous work in our laboratory (Barrière, Frigon et al., 2010;Barrière, Leblond H. et al., 2008;Martinez, Delivet-Mongrain et al., 2011;Martinez, Delivet-Mongrain et al., 2012b) has shown that spinal circuits could be reorganized differently i.e. in a more symmetrical or asymmetrical way, depending on whether or not daily locomotor training was provided. During the double lesion paradigm, hemisection period was found to be crucial to induce changes within the spinal circuits. Indeed, during this period remnant connection with supraspinal structures have the direct possibility to reorganize differently and imprint durable functional changes within the spinal circuitry.

From this work and taking advantage of locomotor training during this critical hemisected period, we have created a training method that allows us to observe kinematics and EMG changes on a moving ladder, that we called ladder treadmill (LTM). The innovation of a moving ladder treadmill for EMG recordings and kinematic analyses offers the possibility of acquiring and averaging several steps that can be directly compared with flat treadmill in the same condition, at the same treadmill speed and confined to the same area of the treadmill. The LTM permits to train cats with a consistent repetitive walking in demanding conditions. This will require voluntary adaptation to a continuous movement environment and visual guidance, therefore requiring active participation of corticospinal pathways.

Will cats be able to walk on such LTM? Will the LTM change the kinematics and EMG characteristics in cats with an intact spinal cord? Could this training method that requires more supraspinal inputs (while maintaining the sensory inputs provided by a treadmill) improve the recovery after incomplete SCI?

Though the role of the motor cortex and other supraspinal structures for skilled locomotion is well established (Armstrong, 1986a;Beloozerova, Farrell et al., 2010;Beloozerova and Sirota, 1993;Krouchev, Kalaska et al., 2006), their contribution to the recovery of the locomotor pattern after partial SCI remains unclear.

As central commands are expressions of the individual's intentions to adapt to a particular environment, we expect to find kinematic changes induced by a task that continuously demands voluntary adaptations and changes on muscles activation patterns.

The work to be presented addresses different issues whose bases were presented in the introduction.

First, a device (ladder treadmill or LTM) was built to allow a comparison of the kinematics and EMGs with ordinary treadmill locomotion (FTM). We expected that even with an intact spinal cord, cats would have to adapt some aspects of the locomotor kinematics to accommodate walking on the rungs of the LTM and that corresponding muscle discharges in critical muscles especially related to precise positioning of the foot on the rungs would be changed. Second, LTM was used to train cats before and/or after a spinal hemisection to see how the removal of critical descending pathways would affect walking on the LTM and the effects that daily training could have in improving locomotion after hemisection.

Chapter 2: Article

Comparing Kinematics and Electromyographic activity in cats walking on a horizontal ladder treadmill or on a flat treadmill

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Abstract

Skilled locomotion studies have contributed to the understanding of the nervous structures implicated in walking with precise foot placement to negotiate obstacles or walk on specific targets. After CNS lesions, such precise locomotion may be impaired but could possibly be improved by locomotor training. There is however a need to train animals to walk repeatedly in a locomotor task requiring such continuous and repeated precision walking. Here we introduce a locomotor task utilising precision walking on a moving horizontal ladder made of quadrangular rungs attached to a treadmill belt. Cats walked regularly either on the surface of the rungs or on the surface of the flat treadmill at the same speed and in the same enclosure box and environment. Since cats walked at a fixed position relative to the video camera, we could record several consecutive steps with the associated electromyographic (EMG) activity recorded through chronically implanted electrodes. Thus we could average, on the same day, kinematic and EMG characteristics for the two different tasks demanding more or less precision walking. We found changes in peak amplitude excursion of different joints with corresponding changes in EMG characteristics. The preferred fore-hindlimb coupling was more diagonal on the ladder treadmill allowing cats to maintain greater stability in this condition. This initial work provides the basis for further studies on evaluating the ability to further train cats daily to walk after CNS lesions using 2 different locomotor tasks requiring more (ladder treadmill) or less (flat treadmill) voluntary control.

Introduction

In humans with Spinal Cord Injury (SCI), locomotor training paradigms used for rehabilitation (Barbeau, Ladouceur et al., 1999;Behrman and Harkema, 2000;Harkema, 2001;Somers, 1992) are largely based on extensive research in the control of locomotion in animal models. Some proofs of principle continue to be of value since the parameters of locomotor training are far from being all understood in humans. Locomotor training can promote reorganization of the spinal locomotor circuitry by providing locomotor movement-related sensory feedback to the intrinsic circuitry spontaneously evolving in the spinal cord after a complete spinal section (Barbeau and Rossignol, 1987;de Leon, Hodgson et al., 1998;Lovely, Gregor et al., 1986;Rossignol, Dubuc et al., 2006;Rossignol and Frigon, 2012;Rossignol, Martinez et al., 2015;Rossignol, Schmidt et al., 2014).

Beneficial effects of locomotor training have also been seen after incomplete SCI such as unilateral spinal hemisection (Barrière, Leblond H. et al., 2008;Barrière, Frigon et al., 2010;Martinez, Delivet-Mongrain et al., 2012a;Martinez, Delivet-Mongrain et al., 2012b;Martinez, Delivet-Mongrain et al., 2013), after bilateral dorsal (Jiang and Drew, 1996) or after bilateral ventral hemisection (Brustein and Rossignol, 1998). After unilateral hemisection, repetitive locomotor training led to a recovery of a mostly symmetrical walking pattern despite the massive imbalance of supraspinal commands (Martinez, Delivet-Mongrain et al., 2013). An important finding was that training not only improved locomotor performance in the hemispinal state but that some of the benefits could be carried over after a further complete spinalization two segments below the initial hemisection. In locomotor-trained cats, symmetrical hindlimb locomotion could be re-expressed 24 hours after this second complete spinal section, whereas asymmetrical gait persisted in untrained cats. Such a dual spinal lesion paradigm (hemisection followed by a complete section) emphasizes that hemisection may promote durable intrinsic changes in the sublesional spinal cord. This has also been recently demonstrated during “fictive” locomotion (Gossard, Delivet-Mongrain et al., 2015) in which the ipsilesional spinal cord below the lesion is much more rhythmically active than the other side. Globally then, the evidence is that the lesion modifies the spinal cord and also that locomotor training

contributes further to these spinal changes. We can thus ask if the spinal changes can be influenced by various locomotor training regimens having different requirements. The ability to perform precision walking in hemisected cats has not been assessed. Therefore we sought a paradigm in which cats could be trained to walk under 2 conditions: 1 requiring more voluntary demand (LTM) than the other (FTM).

We thus developed a paradigm in which cats could be trained to walk on an ordinary flat treadmill (FTM) or on a moving horizontal ladder treadmill (LTM) made of rungs fixed to the same flat treadmill belt (FTM) so that the cat had to step precisely on the rungs with all 4 limbs, a task requiring obviously more supraspinal controls. While studies on cats walking on a horizontal fixed ladder (Amos, Armstrong et al., 1990;Armstrong, 1986a;Beloozerova and Sirota, 1993), have concentrated their efforts in showing the involvement of supraspinal centers in such tasks, their aims were different from ours which were to submit groups of cats to a daily familiar routine using either precision walking (on the LTM) or ordinary walking (on the FTM) for several minutes every day for several weeks.

This innovative development permits therefore the acquisition of consecutive step cycles (training) performed within the same enclosure, in conditions requiring more (LTM) or less precision walking (FTM), at the same constrained speed in cats chronically implanted with electromyographic recordings and a fixed video recordings. In other words, the cats do not have to be transferred to a different setting and thus allows a more direct comparison of the two tasks. This initial study was deemed essential to establish precisely the electromyographic (EMG) and kinematics patterns in normal cats in the two conditions. This was considered as a first essential step for further work in cats with various partial spinal cord lesions in which we will seek to assess the potential of daily locomotor training in more or less demanding locomotor tasks.

Methods

Animal care. All procedures followed a protocol approved by the Ethics Committee at the Université de Montréal, according to the Canadian Guide for the Care and Use of Experimental Animals. The well-being of the cats was monitored daily and verified regularly by a veterinarian.

Ladder treadmill (LTM). Quadrangular hard foam rungs (35cm length, 5cm width, and 5cm height) were attached by Velcro bands glued on both sides of the belt and placed parallel to it. Rungs were spaced 6 to 8 centimeters apart to match the most regular sequences of step length i.e. the distance in which the cat regularly placed the paw at every two rungs with the 4 paws was the distance chosen for that particular cat (Fig. 1A). Recordings on the LTM were performed at 0.4 to 0.6 m/s. However, analyses were performed at 0.5 m/s to allow the comparisons of several regular steps typically seen at this speed.

Experimental paradigm. Adult female (n=4) and male (n=2) cats, weighing 3.3 to 4 kg, were first selected for their ability to walk regularly and continuously for several minutes (15-30 min) on a motor-driven flat treadmill (FTM) at speeds ranging from 0.4 to 0.8 m/s. Then, these cats were habituated to walk on the LTM task. The total period of habituation was 4 weeks. Once we observed regular stepping in both tasks and we confirmed the stabilization and reproducibility of these conditions in several sessions (7 to 10 sessions), we proceeded to the EMG implantation. 5 days after the surgery, 2 testing sessions were made to corroborate the function of the EMG signals and the regularity of the kinematic sequences. Then, in order to obtain corresponding kinematic and EMG values for locomotion in the 2 conditions (FTM and LTM), recording sessions were made on the same day for each cat and at the same speed (0,5m/s) for the two tasks.

Surgical procedures and EMG implantations. All surgical procedures for electrode implantation were done under general anesthesia and aseptic conditions as described in previous papers (Martinez, Delivet-Mongrain et al., 2013). Briefly, animals were first pre-medicated with Atravet (0.1 mg/Kg), glycopyrrolate (0.01 mg/Kg), and ketamine (10 mg/Kg). An endotracheal tube was then inserted for gaseous anesthesia (mixture of O_2 and isofluorane 2%). Cats were chronically implanted with intramuscular electrodes to record

EMG activity from flexor and extensor muscles in the hindlimb and forelimb on both sides. The implanted muscles were Semitendinosus (St; knee flexor and hip extensor), Sartorius (Srt; hip flexion and knee extension), Vastus Lateralis (VL; knee extensor), Gastrocnemius Medialis (GM; ankle extensor and knee flexor), Tibialis Anterior (TA; ankle flexor), Extensor Digitorum Brevis (EDB; dorsiflexor of the hindpaw digits), Triceps Brachii (Tri; elbow extensor) and Biceps Brachii (Bra; elbow flexor). Electrodes were led subcutaneously to two 15-pinhead connectors secured to the cranium using acrylic cement. Heart rate and respiration were monitored throughout the surgeries. 24 hours before the surgery an antibiotic (Convenia, 8mg/Kg) was administrated subcutaneously. Before the end of the surgeries, an analgesic (Buprenorphine, 0.01mg/Kg) was also administered subcutaneously. Additionally, a fentanyl patch (25 µg/Kg) was sutured to the skin to alleviate pain for about 5 days.

Kinematic and EMG recordings. During episodes of locomotion, cats were recorded on video from the left side with a digital camera and the data stored on a hard disk. Video images were deinterlaced to yield a resolution of 60 fields/s or 16.6 ms between fields. Reflective markers were placed over the iliac crest, greater trochanter, lateral malleolus, metatarsophalangeal (MTP) joint and one at the tip of the toes of the hindlimbs. This last marker was used to visually tag foot contact and lift off on video images and therefore determine the swing and stance periods. It also allowed us to determine the left paw trajectory during the swing phase.

The amplified (Lynx-8 amplifiers, Neuralynx) and filtered (bandwidth 100 Hz to 3 kHz) EMG signals were digitized at 1 kHz (NI-6071E, National Instruments) and stored in a computer. Kinematic and EMG recordings were synchronized using a SMPTE (Society of Motion Picture and Television Engineers) time code generator.

Kinematic and EMG Analyses.

Step cycle duration represents the time between two successive contacts of the same foot on the treadmill, whereas the stance duration refers to the time between foot contact and toe off which initiates the swing phase of a single limb. To avoid bias from subtle changes in the walking speed or unintentional displacements of the camera, the toe point was

normalized in relation to the hip marker fixed to the iliac crest. This insured that all angular measurements were referred to the same point in space in the X axis.

Step length was calculated using stance onset defined as the foot contact (foot contact was tagged manually on the first frame when the foot contacted the treadmill surface or the rungs). The distance travelled by the toe between two successive paw contacts of the same limb was defined as the step length, i.e., distance travelled during the stance and swing phase of a complete step cycle at a given speed.

Angular excursion. For each session, joints angles (hip, knee, ankle and metatarsophalangeal (MTP) joints) (Fig. 1A) and foot lifts/contacts were reconstructed from the deinterlaced video images. Then, kinematics were reconstructed from the X-Y coordinates of each marker using custom–made software allowing the calculation of the joint angular displacement and the display of the joint excursions. The kinematic model used (direction of flexion/extension for each joint) is illustrated in Fig 1 A. The model is such that (Fig.3A) flexion is represented by a downward deflection of the traces for each joint. Fig 3B illustrates the same data connecting the markers of the limbs in every single frame to yield a stick figure.

Paw trajectory. In order to assess more precisely the toe sagittal trajectory throughout the swing from toe off to toe contact, the XY coordinates of the toe reflective marker were extracted from the video data to obtain a normalized (with respect to the hip point) average trajectory of the toes in two-dimensional planes (2D).This normalization thus allowed to directly compare trajectories in both conditions.

The *coordination between fore- and hindlimbs* (i.e. homolateral coordination) and between both hindlimbs or forelimbs (i.e. homologous coordination), was calculated in the following manner: the period of stance, normalized to 1, was measured for individual limbs, 0 representing the left hindlimb foot contact (LHc). The beginning and end of the stance period were displayed as a polar plot with curved colored rectangles corresponding to the stance period of each coloured limb (Fig. 5). A coupling value of 0.5 means that the paw contact of a limb occurs at 50 % of the step cycle of the other limb also defined by its paw contact.

"Walking gaits" were identified by the periods of the duty cycles, in 15 to 20 steps, where 2 or more paws were in contact on the ground or on the rungs. The bipod gait (defined as a gait with 2 paws in contact with the ground) was divided according to the relation between paws as in Hildebrand (1980). A homolateral couplet is defined as the period where both forepaw and hindpaw on the same side of the body contact the treadmill at the same time, whereas a diagonal couplet is one in which the paws contacting the treadmill are on opposite sides of the body. Tripod gait (periods where 3 paws were in contact with the treadmill or rungs) were divided into: tripod anterior or TPa (both forepaws and 1 hindpaw) and tripod posterior or TPp (both hindpaws and one forepaw in contact with the ground). The two combinations were amalgamated in tripod gaits respective of the side of the paws.

EMG burst duration was calculated as the time between onset and offset of a single muscle discharge in several cycles. The EMG burst onset and offset were determined using custom-made software that detects the level of EMG signals with precision and average values over several (15-20) cycles and were visually verified. Some muscles such as St or EDB often have two distinct bursts per cycle and each could be detected and analyzed separately. The EMG signals were also rectified and integrated allowing to measure the area under the curve to determine amplitude.

Statistics. Individual kinematic data (step cycle characteristics, angular excursions, foot trajectories, walking gaits) from each animal was averaged from a minimum of 14 consecutive locomotor cycles. Analyses of kinematics were performed using SigmaPlot software (Systat software Inc., San Jose, USA). A Student t-test was first used within each animal to determine whether behavior of analyzed variables within kinematics, comparing FTM and LTM conditions were consistent among cats. For step length and cycle duration cats were grouped using individual mean values. For EMG duration and amplitude, mean values for each cat were taken and normalized according to the FTM values for the same cat. Then the mean values were used to group the cats. LTM values are presented as a percentage of the FTM values (which represents a fixed value of 100%) to reduce bias in voltage values on EMG discharges due to differences in electrodes functioning or intrinsic differences between cats. Circular statistical analyses were performed using Oriana (3.13; KCS, isle of Anglesey, UK) software in the six cats for kinematic parameters expressed in

polar plots. In all figures, statistical significance between conditions is indicated by an asterisk. To compare the performances on the FTM and LTM stepping a p value of < 0.05 was considered statistically significant. Results are presented as means ± standard error.

Results

Experiments were conducted in six adult cats with an intact spinal cord and which could maintain several consecutive and regular steps at 0,5 m/s. Cats were habituated to walk for 4 weeks either on the FTM and LTM so that the patterns, previously tested in several sessions, fully recorded in a single session, represent well learned and stable behaviors although there was no regular locomotor training as such of walking on the rungs or on the flat treadmill. For the present paper, we quantified more specifically the kinematics and EMG characteristics obtained from 15-20 consecutive steps on the FTM and LTM (both at 0,5 m/s) and compared the mean values obtained in the same cat on the same day, once a week.

Step cycle characteristics

Table 1 shows individual mean values of step length and duration for each limb, showing separate values for swing and stance periods of the step cycle. The average step length and step duration in all cats was similar (paired t-test, $p>0.05$), for FTM and LTM walking. This could be explained by the fact that rungs on the moving LTM were placed to accommodate the most comfortable step length of each cat with different sizes (see Methods). These average values were pooled for the six cats and displayed in fig. 2. There is no significant difference of these gait parameters between the FTM and the LTM tasks.

Angular excursions and limb trajectory

The limb kinematics and angular excursions of the left hindlimb (because markers were placed on the left hindlimb and video recordings were taken from the left side) during normal stepping on a flat treadmill (black lines) and on the ladder treadmill (red lines) are

shown in Fig. 3. Angular excursions of the hip, knee, ankle and MTP (fig. 3A) were superimposed and synchronized on the left hindpaw contact to visually extract differences or similitudes between these two tasks. Although angular excursions on the LTM followed and preserved the same shape as on the FTM (which was unexpected given the different demands of both tasks) there were significant differences in the total amplitude for all angles in all cats (Table 2). The most important changes when comparing both tasks consisted in an increase in knee and ankle peak flexion on the LTM. These occurred in the middle and later part of swing, before contacting the rungs and right after the beginning of the stance period where a yield at the ankle is often seen (see for instance Cat 3 in Fig 3A). Some cats also showed a greater yield during the whole stance period while walking on the LTM. Stick figures derived from sequences on FTM and LTM were quite similar (fig. 3B) although the foot trajectory, which in a way represents the additive actions of changes occurring at each joint, was somewhat different.

We analysed more specifically the paw trajectories (Fig. 4) and found that the beginning and end of the swing were remarkably similar in most cases but in several cases the upward displacement of the foot was higher when cats walked on the LTM, especially in mid-swing. In both FTM and LTM stepping, we observed a "hook" shape terminal trajectory when the paw contacted the treadmill or the rungs (see arrows in Fig 4) suggesting a small dorsiflexion before the plantar flexion leading to the contact with the surface of the LTM or FTM.

Interlimb coupling and walking gaits

Fig. 5 shows a polar representation of contacts and lifts for each limb and the relation between them. Each curved coloured rectangles represents the stance period of a given limb. The color code is given by the color code of the paws in the middle drawing. The sequence of foot contact is LHc, LFc, RHc, RFc as is usual in locomotion and this applies to both FTM and LTM walking. There was a strict alternation between homologous limbs (fore and hindlimbs). For instance, the Right Hindlimb contact (RHc) occurs very close to 0.5 phase of the Left Hindlimb contact. Similarly, the Right Hindlimb lift is out of phase by 0.5 phase with the left Hindlimb left (LHl). The left right coupling of homologous limbs

was maintained in walking on LTM but the hindlimb-forelimb coupling was changed so that the forelimb contacts the rungs later than it would on the FTM. This shift in homolateral coupling led to a difference in the number of paws in contact with the rungs concurrently. For example, periods between LFl (left forelimb lift) and LHc are larger on the FTM than on the LTM. This period corresponds to RF and RH (homologous limbs) contacts with the treadmill. Even though both forelimbs lift and contact preserve the same relation there was a significant change (Whatson-Williams F tests; $p<0.05$) and a shift in terms of time of arrival of these events in the step cycle when comparing FTM and LTM stepping.

The gait diagrams shown in Fig. 6 summarize these gait modifications. Here, the duty cycle of each limb is illustrated as colored horizontal bars using the same color code as in Fig. 5. The vertical bars (Violet and brown color) correspond to periods where two limbs are in contact with the rungs or the treadmill surface whereas periods between these vertical rectangles represent periods where 3 feet are in contact with ground. Fig 6B shows that bipod periods were different in FTM and LTM conditions. Cats on the FTM spend more time in lateral couplets (31.5% of the whole duty cycle) while on the LTM diagonal couplets last longer (24.2%) (t-test, $p<0.05$). Tripod and bipod gait periods were significantly different when compared within cats. However, when comparing averages of cats, there was not significant difference when comparing tripod gait periods between both LTM and FTM conditions (Fig. 6B Dark and light grey histogram bars).

Changes of EMG activity

There were some consistent changes (in amplitude or time profile) in the EMG activity of some of the muscles in all cats. Rectified and superimposed EMGs on the FTM (black lines) and on the LTM (red lines) synchronized on the contact of the respective limb are shown in fig. 7. Mean amplitude and duration EMG burst for each implanted muscle are shown in Fig. 8.

Hindlimb flexor muscles

The main changes were seen bilaterally in the bifunctional St muscle. Although the duration of the first burst related to swing onset showed no significant differences between the two tasks, the overall amplitude of the integrated burst was larger on the LTM (paired t-test; $p<0.05$). More remarkably, the amplitude and duration of the second St burst appearing just before foot contact was almost doubled on the LTM compared to the FTM (paired t-test; $p<0.05$) (Fig. 7 and 8). TA and Srt muscles showed no changes in shape, duration and amplitude on the LTM in 3 of the cats in which these two muscles were implanted.

EDB muscle has a special activity in locomotion. It is activated during the first extension phase but it does not relax immediately after the foot contact as the rest of the flexors (Engberg, 1964). However it will be included here in the flexor group. EDB presents 2 bursts of activity synchronized in time with those of the St. We found that EDB's first burst showed a more variable discharge pattern during FTM locomotion, i.e. there were cycles where this first burst did not appear. This made some of the step cycles' EDB activity to be tagged as 0, increasing both the variability and the SE and, in consequence, no statistical difference was seen when compared to the LTM (Fig 7 and 8). By contrast, on the LTM, EDB activity was always present and showed the characteristic double burst, much as far as timing is concerned. The second EDB burst which was always present in both conditions, showed on the LTM a significant increase in its amplitude (paired t-test, $p<0.05$) in comparison to the FTM but it showed no changes in duration.

Hindlimb extensor muscles

VL muscle showed a significant increase in amplitude on the LTM compared to the FTM (paired t-test, $p<0.05$) presumably to increase support during the stance phase but it did not show changes in the duration and profile of its activity. Of great interest is also the fact that this increase in VL activity affecting the knee mainly occurs during a period where there is a yield at the ankle, as seen in Figure 3A. GM muscle showed no significantly

changes in the amplitude and duration of the burst activity compared to the FTM. Contrary to what might have been predicted, the pre-contact activity of GM was not increased in the LTM condition.

Forelimb flexor and extensor muscles

Bra muscle's burst amplitude and duration was significantly increased in LTM stepping (paired t-test; $p<0.05$). Bra muscle also showed an early discharge activity only on the LTM probably due to changes in interlimb coupling and stance-limb combination as discussed above. Tri muscle's burst amplitude and duration showed no significant changes on the LTM compared to the FTM.

EMG phase shifts

The LTM task leads to changes in timing (but not duration) of stance and swing periods in order to precisely adapt to the task and presumably increase stability. These changes are reflected in the muscular discharges not only in amplitude and duration, but also by some phase shifting (Fig. 7, gray arrows). For instance, the first St burst on the LTM showed that the beginning of the discharge was phase-shifted but ended at the same time as on the FTM. The average VL burst on the LTM started at the same time as on the FTM but ended a few milliseconds later. This probably prolonged the support for the precise foot placement of the contralateral paw on the rungs. When synchronizing forelimb events on the left forepaw contact, we did not find phase shifts changes except for the early discharge in Bra muscle that was not seen on the FTM. This early discharge (that might be due to differences in walking gaits combinations or an early elbow flexion to reach further in front to catch the next rung and therefore adapt to the task) later overlaps with the EMG feature on the FTM. All these phase shifts on the EMG correspond to the changes observed in the kinematic parameters, specifically changes in walking gaits for successful adaptation to the LTM task.

Discussion

Locomotor abilities of animals are often evaluated on a treadmill (Edgerton, Courtine et al., 2008;Rossignol, Martinez et al., 2015). Locomotor training is similarly performed on a treadmill but a simple treadmill that requires not much voluntary control. While animals can walk with severe CNS lesions, their ability to perform precision walking is often neglected. We wanted to develop a treadmill condition which would provide all the benefits of recording in a treadmill environment while requiring more supraspinal control. A treadmill environment allows recordings on videos of multiple consecutive steps in a fixed frame of reference allowing also synchronized chronic EMG recordings that can be averaged over several steps. Here, chronically implanted cats with an intact spinal cord were habituated (not trained) to walk on a FTM and on a LTM made by fixing detachable rungs on the belt. Cats walked at a constant speed on the belt or on the rungs which obviously imposed a greater constraint since all the paws had to be placed on the rungs during stepping. We evaluated the changes in the EMG and kinematics in both conditions obtained on the same day. This constitutes an important baseline when foreseeing the effects that central lesions may have in locomotor tasks requiring more or less supraspinal demands.

Surprisingly, we found that the kinematic profile (step cycles characteristics, foot trajectory) of the left hindlimb facing the camera was hardly changed. However, the few changes in kinematics were accomplished by significant changes in muscle discharge especially of flexor muscles. On the other hand, interlimb coupling between fore and hindlimbs were considerably altered to increase stability.

Kinematics of walking on the FTM and LTM

No significant changes in step length, step duration were seen when comparing LTM with FTM locomotion (Figs. 2 and 3) at the same speed (0,5m/s). Previous studies reported similar findings on trajectory of limb movements, mean duration of the step cycle, swing and stance phases when comparing stepping on a horizontal ladder with normal flat stepping (Armstrong, 1991;Beloozerova, Farrell et al., 2010;Beloozerova and Sirota,

1993). Similitudes in our study can be explained by the fact that distance between rungs was determined in accordance with the comfortable step length of each cat as described in previous work (Beloozerova, Farrell et al., 2010;Beloozerova and Sirota, 1993). Rungs were placed so that continuous and regular step-lengths were obtained on the LTM walking and that cats would actually stride over the same number of rungs at each step. This however does not mean that cats did not voluntarily adapt to the LTM since a temporary blockage of vision hiding the incoming rungs drove the cats back a few steps so they could adapt their vision of incoming rungs.

Significant differences were found in the individual angular excursions in all cats. Beloozerova (2010) found an increase in the flexion of distal joints (MTP) that allows the cat to assume a more bent-forward posture and no changes in the values of proximal joint angles (hip, knee) when walking on a horizontal ladder. We found that changes in angular excursion appear to be related mainly to foot placement. In all cats walking on the LTM important increments in knee and ankle flexion that consistently followed the shape of joint excursions on the FTM stepping were found. Similar paw trajectories were observed in the two conditions. The foot trajectory tends to be higher (in the Y axis) during mid swing on the LTM. This is the result of small cumulative changes occurring at the knee and ankle which results in the increase of the foot trajectory. Of interest is the detailed trajectory of the foot just before contact. The trajectory makes a "hook" before contact (Fig.4) suggesting a precise and pre-planned dorsiflexion just before foot landing. The fact that cats were habituated (not trained) in either FTM or LTM do not seem to be a factor on the apparition of these "hooks" since a group of cats habituated to walk only on the FTM (not shown in this study) presented similar trajectories. This preplanned landing is similar to that described in cats during downward walking (Smith, Carlson-Kuhta et al., 1998).

Changes in homologous coupling were not seen as limbs of the same girdle kept a perfect alternation in both tasks (fig.5). No changes in the coupling between limbs when walking through different rung shapes compared with treadmill walking were mentioned in other studies (Hancock, 1985). However, changes in homolateral coupling were significantly different in our study. The choice of walking gaits and the selection of paw combinations are usually intended for stability. In quadruped animals, the body is always

supported by two, three, or four feet at a time, so stability is increased by maximizing support by four feet, minimizing support by two feet, and, in particular, by selecting the combinations of two and three feet so that balance is always kept (Hildebrand, 1980). We found that support dynamics were different between LTM and FTM tasks by modifying interlimb combination in order to successfully adapt to a specific task (Fig. 6). The period of time occupied by lateral couplets was larger in the FTM, contrary to the LTM, where diagonal couplets prevailed. We hypothesized that this diagonal combination gives an increased support and balance needed to successfully walk on the rungs while maintaining the same or the closest postural and locomotor dynamics as in the FTM stepping. Support by the two feet on opposite sides of the body (diagonal couplets) is much more stable because, as Hildebrand stated "the line of support then passes diagonally under the body in such a way as to come approximately below the center of gravity" (Hildebrand, 1980).

The similarities and differences in kinematic patterns on the LTM and the FTM might follow a kinematic plan for cats to adapt to the environment in the most efficient way. Indeed, limb kinematics are defined by a combination of joint angles and segment lengths. In our study parameters such as step length, duration and trajectories (Figs. 2 and 4) are maintained while the amplitude of angular excursion are different between tasks. This corresponds to the idea that joint variations are selected to achieve consistent or minimal changes in limb kinematics, i.e. the whole limb kinematics is preserved over individual joint kinematics (Chang, Auyang et al., 2009). In humans, research from episodic goal directed arm movements (Georgopoulos, 1991;Kalaska and Drew, 1993) has suggested that the level of cortical activity is correlated with a specific movement kinematic plan. These findings have led to the idea that movements are planned at a kinematic level based on either intrinsic or extrinsic reference frames and that the nervous system transforms this information into the appropriate motor commands for individual muscles. There is some evidence that skilled locomotion requiring precise foot placement may be controlled in a similar fashion (Georgopoulos and Grillner, 1989).

EMG changes in LTM and FTM walking

In the preceding section, we discussed the importance of foot landing during stepping. Single neuron recordings of the motor cortex have indeed shown an increase in discharge activity in many cortical cells occurred in the latter part of the swing phase, just prior to paw contact (Armstrong, 1986b;Beloozerova and Sirota, 1993). This suggests that the major requirement in the control of accuracy or end-point control is the regulation of muscles active just before the paw is placed on the contact surface. The only distal muscle recorded in our study was EDB. There was an important variability of the first EDB burst on the FTM but there was a consistent increase in amplitude during LTM locomotion and also the two bursts of activity which are variable during FTM became very regular during LTM.

The hip-extensor/knee-flexor St muscle might also be involved in accurate foot placement. The amplitude of the St first burst was increased which could account for a tendency to increase the height of the trajectory of the foot during swing. However, of greater interest is also a significant increase of the second burst occurring just before foot contact on the rungs. This second burst may participate in flexing the knee, decelerate the limb and bring the foot to an accurate landing on the rung. During stepping over obstacles the activity of muscles is greater than in normal locomotion to achieve more accurate limb trajectory (Beloozerova and Sirota, 1986;Beloozerova and Sirota, 1993;Drew, 1988).

Studies have also shown that the activity of ankle extensor muscles reach their maximum activity around the foot contact but that their onset occurs *before* contact. The onset of EMG occurring even before sensory feedback from contact has led to the view that this extensor activity is essentially centrally programmed (Enberg, 1969). An increased control around foot contact during stepping on the LTM may also involve a modified activity in extensor muscles as well. Indeed, we found an increase in muscular discharge in the extensor muscle VL that might probably be cortically driven to assure stabilization during stance. This is also a period where the ankle has a significant yield and the increase of VL activity might counterbalance this yield at the ankle.

The present work showed that voluntary adaptations are needed to achieve consistency or preservation of functional stepping close to normal during the LTM task. The supraspinal structures were certainly partly responsible for setting specific changes in kinematics and muscular activity to adapt to more complex external conditions. Previous studies have concentrated their efforts in understanding the adaptability of the locomotor system through detailed kinematics and EMG changes (e.g., during upslope or downslope walking) (Carlson-Kuhta, Trank et al., 1998;Smith, Carlson-Kuhta et al., 1998) or by investigating the structures implicated in voluntary commands during skilled locomotion (Armstrong, 1986a;Beloozerova, Farrell et al., 2010;Beloozerova and Sirota, 1993;Krouchev, Kalaska et al., 2006). From our point of view, we now see the use of the LTM task as a new method to investigate the implications of repetitive voluntary commands during locomotor training after SCI.

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Figures

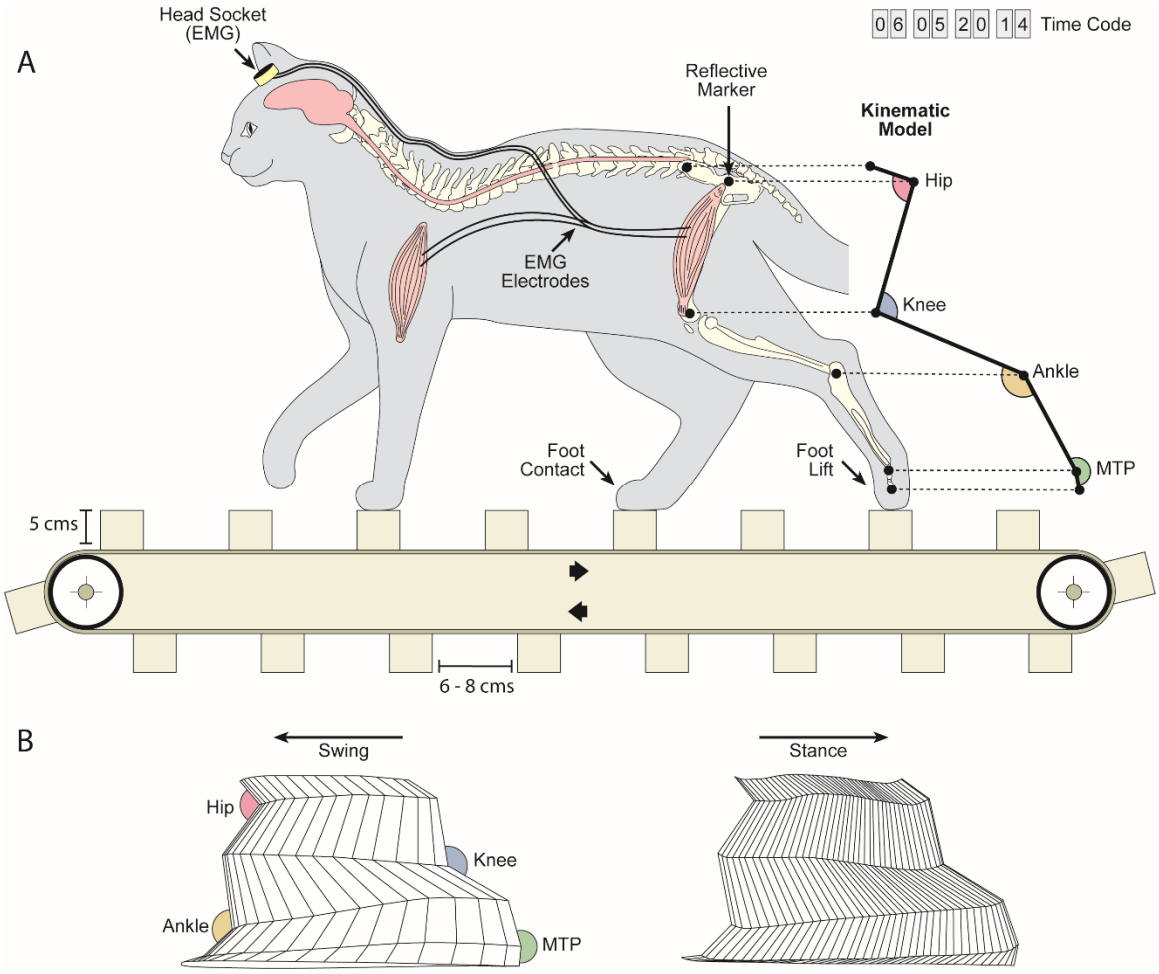

Fig.1 Schematic overview of the experimental protocol and ladder treadmill setup. **A**. Schematic representation of the left side view of a cat walking on the ladder treadmill belt (arrows within the belt indicate the direction of movement) where measurements and spacing between the rungs are shown. Pairs of bipolar EMG wires are implanted into various muscles (only one pair is represented here) and soldered to a multipin connector cemented to the skull. A digital time code (SMPTE) is used to synchronize video and EMG recordings. Reflective markers are placed at various points on the limb, and the angle measurements are taken in the indicated orientations. **B**. From this kinematic data the swing and stance phases of each cycle can be reconstructed as shown in stick figures and duty cycles. The foot contact and lift are also measured to determine cycle length and duration and also to synchronize EMG events when needed.

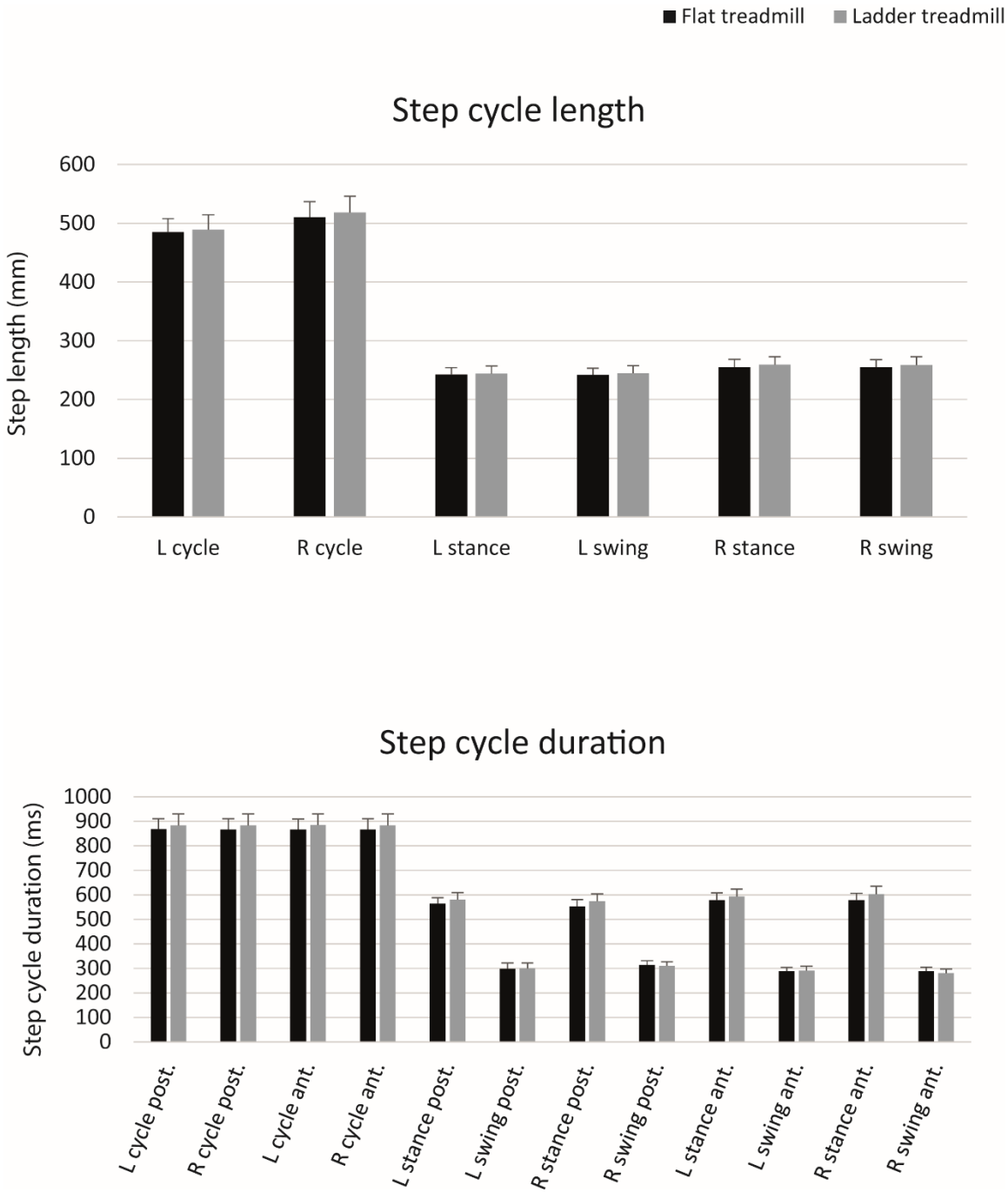

Fig. 2 Comparisons of step cycle length and duration on the FTM and on the LTM for all 6 cats. No significant changes in cycle and sub phases (stance and swing) were seen between the two tasks. Values are means; error bars represents standard error. L= left, R= right, ant= anterior, post= posterior.

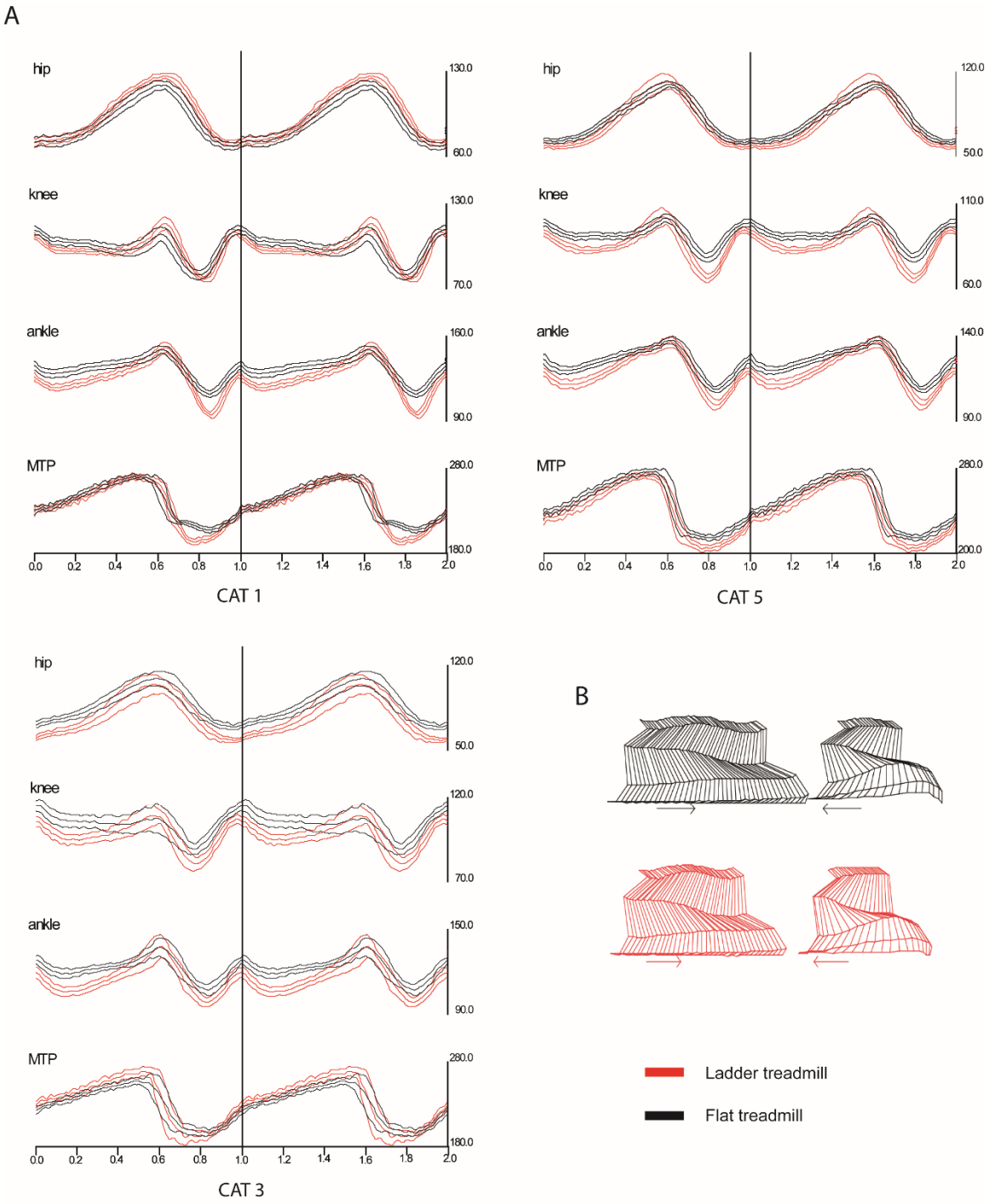

Fig. 3. A. Averaged angular excursion of the hip, knee, ankle and MTP joints of the left hindlimb synchronized on the left paw contact for three cats on the FTM (black lines) and on the LTM (red lines). **B**. Stick figures of swing and stance phase from one step cycle of the left hindlimb of cat 1 at 0.5 m/s on the FTM and on the LTM.

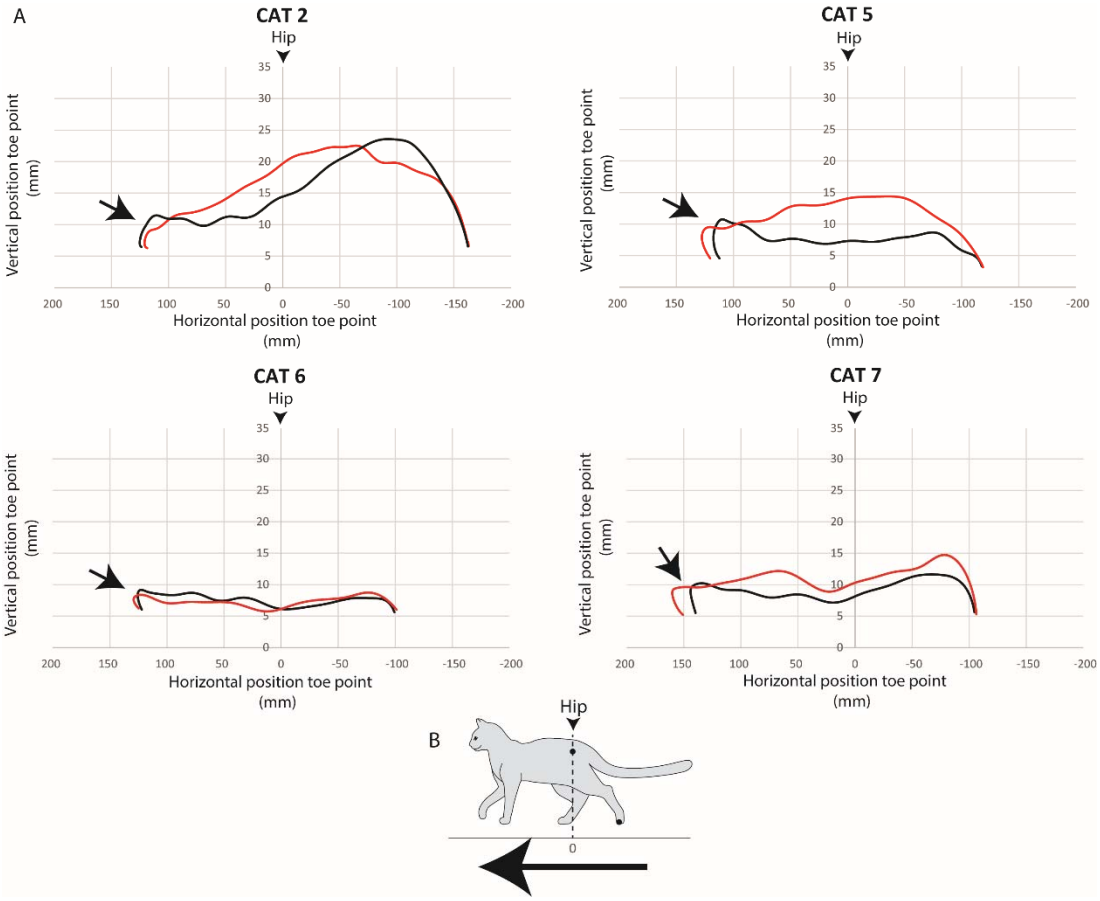

Fig. 4. A. Graphical 2d representations of left toe trajectories during FTM (black lines) and LTM (red lines) stepping in cat 2, 5, 6 and 7. A "hook" shape (black arrow) was seen before the foot contact in both FTM and LTM tasks. **B.** Representation of the walking direction and localization of the reflective marker on the left toe used as reference to calculate its trajectory. Point 0 represents the intersection of a vertical line projection starting from the reflective marker placed at the hip joint with the ground.

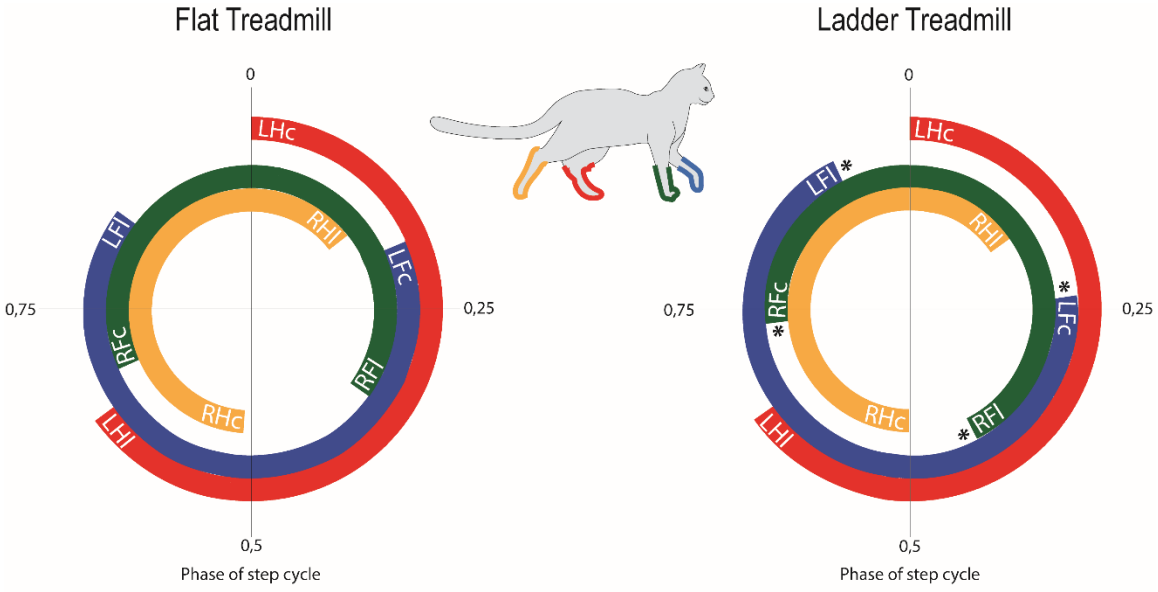

Fig. 5 Polar representation of interlimb coupling during flat treadmill (FTM) locomotion and ladder treadmill (LTM) locomotion. Colored lines represents the stance period during the step cycle for each limb while blank spaces between lines represents swing phase. Events are marked on the figure at the beginning (contact) or at the end (lift) of the stance period for each limb Time 0 represents the left hindfoot contact from which all other events where synchronized. LHc= left hindlimb contact, LHl= left hindlimb lift, RHc= right hindlimb contact, RHl= right hindlimb lift, LFc= left forelimb contact, LFl= left forelimb limb, RFc= right forelimb contact, RFl= right forelimb lift. Statistical differences between the same event on the FTM and on the LTM are indicated by the symbol *.

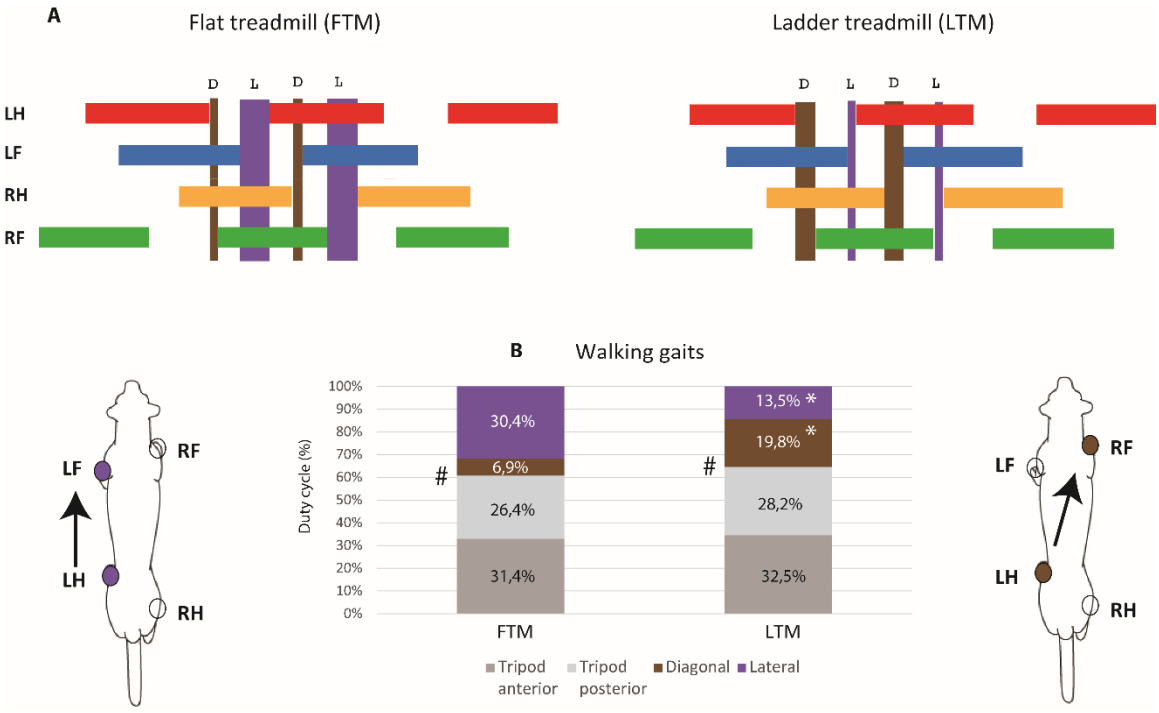

Fig. 6 A. Is an example of duty cycle of cat 1 walking on the FTM (left) and LTM (right). Colored horizontal bars illustrate the stance periods of each limb from which different combinations of limb contacts were extracted. Violet and brown vertical bars represent periods where 2 limbs were in contact with the ground (bipod gait) and spaces between them represents periods where 3 limbs are on the ground (tripod gait). Notice that diagonal couplets (D) combination last longer on the LTM whereas lateral couplets (L) predominate in the FTM condition. **B**. Pooled data of all six cats showing changes in interlimb coordination during FTM and LTM stepping. Values are presented as percentage of the duty cycle (100%). Values within each portion of bars represent the amount of time that the cat spent in a particular combination of limbs in contact with the ground. Violet and brown represents the periods of 2 limbs in contact with the ground in lateral or diagonal combination respectively. Dark and light grey are periods of 3 limbs in contact with the ground in tripod anterior and tripod posterior combination respectively. Figures on the sides are examples of lateral couplets (left) and diagonal couplet (right). Statistical differences ($p < 0.05$) between FTM and LTM condition are indicated by the symbol * while differences within the same condition are indicated by #.

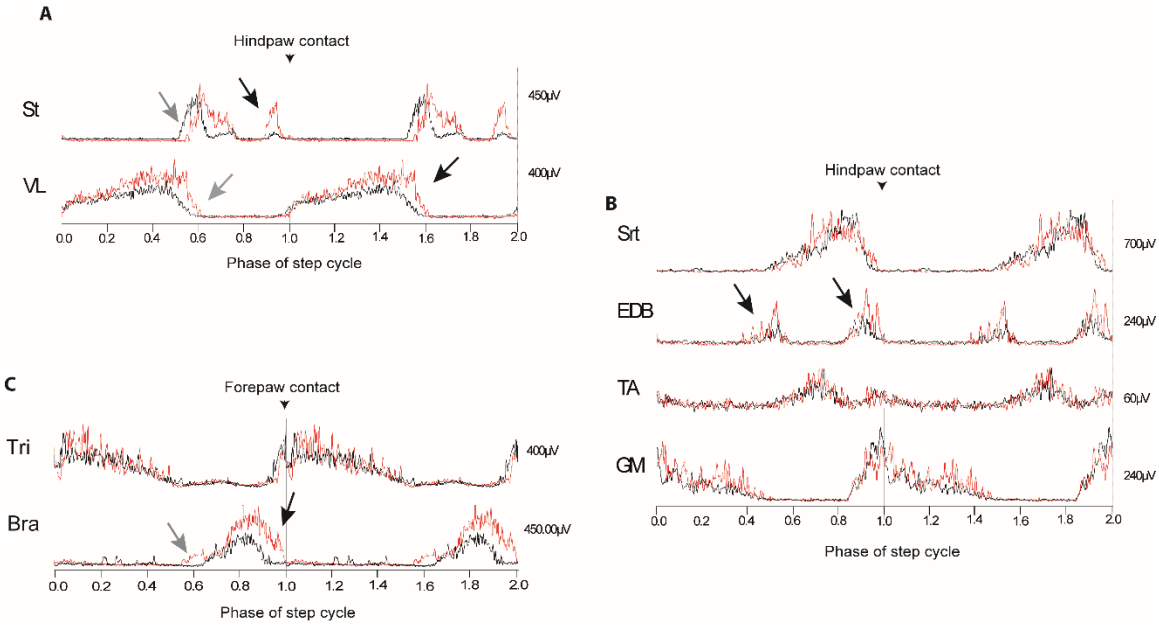

Fig. 7 Examples of rectified and averaged activity in implanted muscles electromyogram (EMG) profile of 15 steps on the FTM (black lines) superimposed on 20 steps on the LTM (red lines) from cat 1 (panel A and C) and cat 3 (panel B). Averages were synchronized on the contact of the forepaw or the hindpaw that corresponds to the muscles of the same limb, as indicated by the continuous vertical line at phase 1. The main differences in the EMG profiles between FTM and LTM are highlighted by black arrows. Phase shifts in the LTM task compared to the FTM are indicated by gray arrows. St= semitendinosus, VL= vastus lateralis, Srt= sartorius, EDB= extensor digitorum brevis, TA= Tibialis anterior, GM= gastrocnemius medialis, Tri= triceps brachii, Bra= biceps brachii.

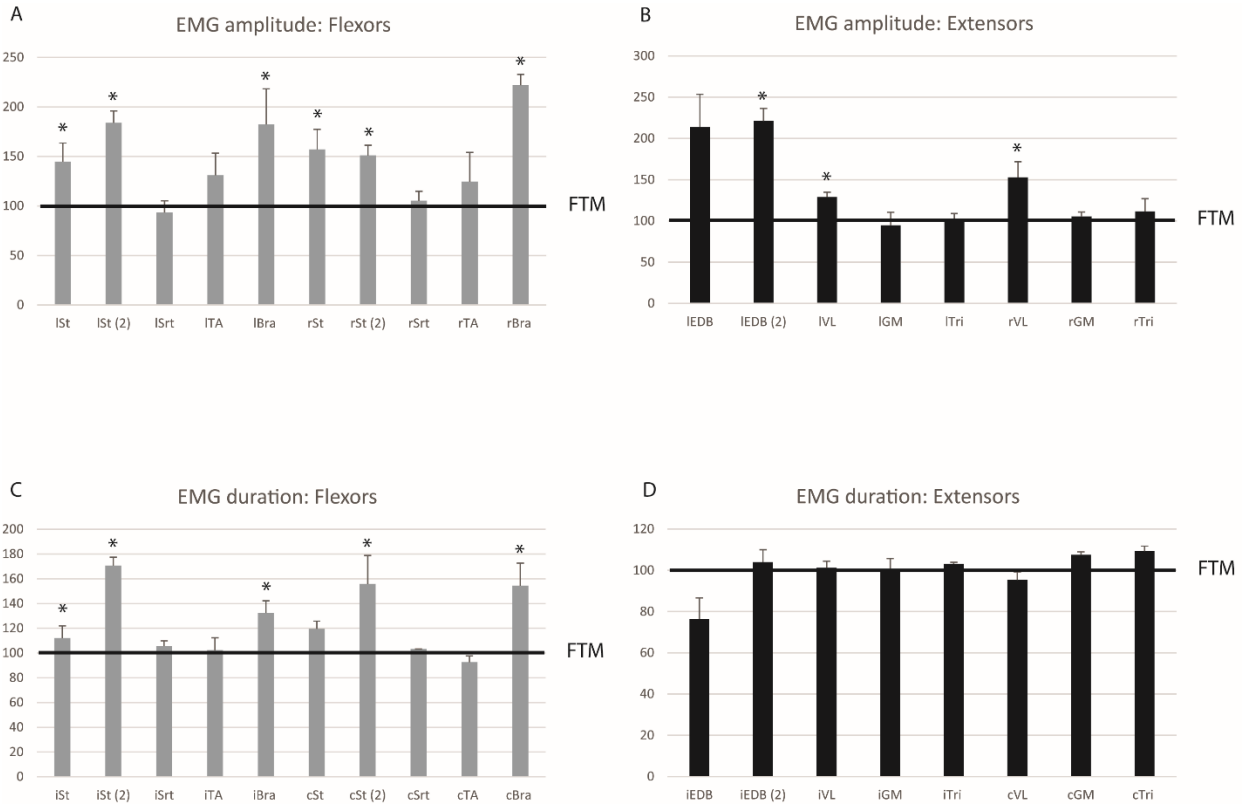

Fig. 8 EMG amplitude and duration on the LTM task for implanted flexors (panel A and C) and extensors (panel B and D) muscles for all cats. LTM values are presented as a percentage of the FTM values (which represents a fixed value of 100% shown as thick black line). l= left, r=right. St= semitendinosus, St (2)= semitendinosus second burst, Srt= sartorius, EDB= extensor digitorum brevis, EDB (2)= extensor digitorum brevis second burst, VL= vastus lateralis, GM= gastrocnemius medialis, TA= Tibialis anterior, Tri= triceps brachii, Bra= biceps brachii. Statistical differences between FTM and LTM condition for each muscle are indicated by the symbol *.

		Cat 1		Cat 2		Cat 3		Cat 4		Cat 5		Cat 6	
		FTM	LTM	FTM	LTM	FTM	LTM	FTM	LTM	FTM	LTM	FTM	LTM
Cycle duration (ms)	Posterior left cycle	996 ± 13	1009 ± 15	1009 ± 23	1045 ± 15	777 ± 12	841 ± 23	838 ± 9	800 ± 10	783 ± 8	824 ± 11	800 ± 6	775 ± 12
	Posterior right cycle	995 ± 17	1009 ± 16	1008 ± 17	1046 ± 18	774 ± 16	846 ± 11	837 ± 13	801 ± 12	781 ± 8	825 ± 12	801 ± 9	776 ± 12
	Anterior left cycle	998 ± 14	1008 ± 15	1004 ± 17	1045 ± 17	771 ± 14	847 ± 14	840 ± 10	805 ± 10	781 ± 8	824 ± 12	800 ± 7	775 ± 11
	Anterior right cycle	996 ± 12	1009 ± 17	1008 ± 17	1043 ± 15	771 ± 14	847 ± 8	840 ± 12	801 ± 12	780 ± 9	824 ± 12	802 ± 7	776 ± 12
	Posterior left stance	645 ± 11	662 ± 13	629 ± 17	677 ± 14	491 ± 12	527 ± 13	549 ± 8	521 ± 10	523 ± 6	561 ± 8	542 ± 5	532 ± 9
	Posterior left swing	351 ± 5	347 ± 4	380 ± 9	368 ± 7	285 ± 7	314 ± 14	288 ± 5	279 ± 5	259 ± 3	262 ± 5	224 ± 2	225 ± 1
	Posterior right stance	653 ± 13	662 ± 11	629 ± 11	669 ± 13	483 ± 13	544 ± 13	507 ± 8	506 ± 8	514 ± 7	551 ± 8	527 ± 7	508 ± 7
	Posterior right swing	342 ± 7	346 ± 7	379 ± 12	376 ± 8	291 ± 6	301 ± 14	329 ± 5	295 ± 6	266 ± 3	274 ± 5	274 ± 5	267 ± 6
	Anterior left stance	680 ± 12	686 ± 10	657 ± 13	690 ± 11	501 ± 10	553 ± 11	549 ± 7	533 ± 7	528 ± 7	548 ± 9	548 ± 5	548 ± 9
	Anterior left swing	317 ± 4	322 ± 7	346 ± 12	354 ± 8	270 ± 5	293 ± 11	290 ± 5	271 ± 7	253 ± 3	276 ± 5	252 ± 4	227 ± 3
	Anterior right stance	648 ± 12	670 ± 14	679 ± 18	735 ± 14	508 ± 11	552 ± 8	557 ± 9	550 ± 10	521 ± 7	553 ± 8	553 ± 5	553 ± 11
	Anterior right swing	347 ± 7	338 ± 5	329 ± 7	308 ± 6	262,2 ± 6	294 ± 6	282 ± 5	251 ± 4	259 ± 3	271 ± 6	249 ± 5	222 ± 3
Posterior step length (mm)	Left cycle	533 ± 4	588 ± 4	568 ± 5	544 ± 2	412 ± 6	424 ± 8	465 ± 2	453 ± 4	478 ± 4	472 ± 4	448 ± 5	451 ± 2
	Right cycle	575 ± 5	627 ± 3	604 ± 5	574 ± 2	438 ± 6	455 ± 9	486 ± 2	487 ± 4	490 ± 5	499 ± 5	464 ± 6	464 ± 3
	Left stance	267 ± 2	293 ± 2	285 ± 6	272 ± 2	206 ± 3	212 ± 2	233 ± 1	226 ± 2	239 ± 2	236 ± 3	224 ± 2	225 ± 1
	Left swing	266 ± 2	294 ± 2	283 ± 5	271 ± 2	205 ± 3	212 ± 6	232 ± 2	227 ± 2	238 ± 2	236 ± 2	224 ± 2	225 ± 1
	Right stance	287 ± 2	313 ± 1	302 ± 7	286 ± 2	219 ± 3	229 ± 4	243 ± 1	243 ± 2	245 ± 2	250 ± 2	232 ± 3	232 ± 2
	Right swing	288 ± 3	313 ± 2	301 ± 3	287 ± 3	219 ± 3	226 ± 8	243 ± 1	244 ± 2	245 ± 2	249 ± 4	232 ± 3	232 ± 1

Table 1. The table gives the values of kinematic parameters of averaged step length (mm) and averaged cycle duration (ms) for each individual cat limb in different phases (stance, swing and complete cycle) on the FTM and on the LTM for all 6 cats. Notice that most of these kinematic parameters have similar values within the tasks for each individual cat. Values are presented as means ± SE.

Amplitude of angular excursions (°)

CAT 1		CAT 2		CAT 3	
FTM	LTM	FTM	LTM	FTM	LTM
		HIP JOINT			
53 ± 2	59 ± 2***	54 ± 3	69 ± 3***	43 ± 3	48 ± 7*
		KNEE JOINT			
36 ± 3	42 ± 3***	31 ± 5	44 ± 3***	26 ± 4	33 ± 5***
		ANKLE JOINT			
40 ± 3	58 ± 2***	48 ± 7	66 ± 4***	35 ± 5	44 ± 6***
		MTP JOINT			
68 ± 2	82 ± 4***	79 ± 3	87 ± 5***	69 ± 5	86 ± 11***

CAT 5		CAT 6		CAT 7	
FTM	LTM	FTM	LTM	FTM	LTM
		HIP JOINT			
50 ± 2	47 ± 2***	38 ± 2	43 ± 1***	47 ± 2	58 ± 3***
		KNEE JOINT			
25 ± 2	37 ± 3***	27 ± 2	28 ± 2	31 ± 1	34 ± 2***
		ANKLE JOINT			
31 ± 1	33 ± 2*	44 ± 4	44 ± 3	31 ± 2	46 ± 4***
		MTP JOINT			
68 ± 4	75 ± 4***	86 ± 6	87 ± 5	79 ± 5	84 ± 4***

Table 2. Total amplitude of angular excursions of the hindlimb joints in degrees. Values are means ± SD. Significant differences between FTM and LTM are expressed by asterisks. * $p < 0.05$; *** $p < 0.001$.

Chapter 3: Spinal hemisection and training paradigms

The main objective of this section of the project is to compare daily locomotor training on the LTM or on a regular FTM after a unilateral spinal hemisection. The methodology is briefly described and some of the main results presented. More detailed results after spinal lesion will be presented soon in a second publication.

3.1 Methods

The general methodology is as described in the preceding chapter. All procedures followed a protocol approved by the Ethics Committee at the Université de Montréal, according to the Canadian Guide for the Care and Use of Experimental Animals. The wellbeing of the cats was monitored daily and verified regularly by a veterinarian. After EMG implantation and spinal lesions (see below), cats were housed in individual cages (104 x 76 x 94 cm) with food and water, as in previous studies (Barrière, Frigon et al., 2010;Barrière, Leblond H. et al., 2008;Martinez, Delivet-Mongrain et al., 2011). Such housing limits sensorimotor experience and self-training.

3.1.1 Training paradigm

Adult cats (n: 9, 2 males, 7 females) were first selected for their ability to walk regularly for several minutes (15-30 min) on a motor-driven treadmill and on the LTM. Fig. H1 illustrates the sequence of events of this training paradigm. Cats were implanted with EMG electrodes in the main flexor and extensor muscles of the forelimbs and hindlimbs bilaterally as presented in the preceding section. After a control period of 3 weeks to collect baseline values, a left hemisection was performed at T10. For the next 6 weeks, 6 cats were trained on the LTM and the 3 others on the FTM 5 times per week for 30 minutes. Once a week, for 6 weeks, the stepping patterns (EMG, Kinematics) were recorded together with synchronized video of the left side of the animal at 0, 5 m/s on the FTM and on the LTM. To compare both training methods (LTM versus FTM) locomotor sequences were recorded and analysed only during FTM stepping in both groups of cats. Thus, any differences

between the two training groups observed in kinematics record on the FTM will probably represent changes in the way the animal walk due to a specific preceding training. At the end of the experimental series, cats were prepared to record fictive locomotion (decerebration, curarization, electroneurographic (ENG) recordings). The results of fictive locomotion are however not presented here.

3.1.2 Spinal lesions

The general procedure for spinal lesions was similar to that described in previous papers (Barrière et al. 2008a, 2010; Martinez et al. 2011). T10-T11 vertebrae were exposed and a small laminectomy was performed to approach the spinal cord dorsally. A small incision of the dura was first made and a few drops of a local anesthetic (2% xylocaine) were put on the surface of the exposed spinal cord. Some anesthetic was then injected directly into the targeted segment to reduce brisk movements during the actual section and thus better control the extent of the lesion. Hemisections on the left side were achieved with micro-scissors under a microscope. The wound was then closed in anatomic layers.

3.1.3 Histology

Six weeks after spinalization, the animals were prepared for acute experiments (not presented in this report). These experiments, dealing mainly with peripheral nerve recordings, did not interfere with the histological procedures. Cats were given a lethal dose of intravenous pentobarbital sodium solution. A piece of spinal cord segment between T8 and L1 (to include the hemisection and non-lesioned tissue) was carefully dissected out and fixed in 10% paraformaldehyde for several weeks. The blocks were cryoprotected by successive transfers into increasing concentrations (10, 20, and 30%) of sucrose solution in 0.1 M phosphate buffer for 72 h at 4°C.

For histological examination, the spinal cord was frozen and 40 µm thick coronal sections of a spinal cord segment of 4 mm centered on the lesion were taken using a cryostat. Every section was mounted on a slide and stained with cresyl violet. Under microscope visualization, a qualitative and quantitative evaluation of the damaged area was performed by using all the coronal sections in which the lesion was visible (Fig. H2).

3.1.4 Kinematics analysis

After the spinal lesion, foot drag analysis was added to the previous kinematic analysis (Step cycle characteristics, angular excursions, foot trajectories) described in the preceding chapter.

Foot drag was quantified as the period of time where the dorsal part of the distal phalanx of a given hindpaw was in contact with the treadmill belt during the early swing phase. In order to compare both training methods, cats were regrouped by the similarity of their spinal lesions. Histological analyses were made to determine the extension of the lesions and foot drag deficits were compared between FTM and LTM trained groups with similar lesions.

3.1.5 Statistical analysis

Statistical analysis to quantify foot drag from each animal was averaged from a minimum of 14 consecutive locomotor cycles. Analyses were performed using SigmaPlot software (Systat software Inc., San Jose, USA). A Student' t-test was used to determine whether foot drag data comparing FTM and LTM conditions were statically different. When data showed non-normal distribution, the non-parametric U-test of Mann–Whitney was used, as equivalent of the parametric t-test. Statistical significance between conditions is indicated by an asterisk. To compare the performances after the FTM and LTM training

a p value of < 0.05 was considered statistically significant. Results are presented as means ± standard error.

3.2 Results

Limb kinematics and angular excursions 42 days after left hemisection adopted by the left hindlimb on a flat treadmill after 6 weeks of LTM training (orange lines) compared to kinematics before spinal hemisection (blue lines) are shown in Fig. H3. Angular excursions of the hip, knee, ankle and MTP were superimposed and synchronized on the left hindpaw contact to visually extract differences or similitudes between these two delays. During training sessions on the LTM after spinal lesion the hindlimb on the side of the hemisection walked between the rungs while all the 3 other limbs walked on the rungs. All cats showed the same behaviour even though some of them were able to perform very few irregular steps (approximately 2 or 3 out of 20 steps) with the paw placed on the rungs. These small sequences were not taken for analysis because they interrupted the regularity of the gait. As shown in Fig. H3.A, limb kinematics after LTM training (orange lines) of the left hindlimb (side of the lesion) results in a hyperflexion during the swing phase which was used during training to overcome the next rung. In consequence increased flexion in all angular excursion were seen, especially in the knee, ankle and MTP during the swing phase and at the moment of the paw contact (fig.H3.B). These findings were consistent among LTM trained cats. In some cats, the ankle joint showed a greater yield during the whole stance phase (for instance see cat 3 show in Fig. H3). We hypothesized that these findings were due to placement of the paw between rungs during training.

Paw trajectory. Fig. H4 shows the paw trajectories comparing one LTM trained cat and one FTM trained cat with an intact spinal cord and 42 days after left hemisection. This last delay was taken to assess whether prolonged training on the LTM induces changes of the paw trajectory on the FTM stepping that are different than walking only on the FTM. It should be recalled that these trajectories are analyzed from walking sequences on the FTM

as indicated by the figurine at the bottom. The two other figurines illustrate how the cats were trained (on LTM or FTM).

We found that the FTM trained cat (Fig.H4 right panel) preserved the same shape paw trajectory but not the same amplitude than the one it had before the spinal hemisection. Paw trajectory shape in both delays (before and after lesion) were characterized by an evident flexion at the beginning of the swing phase that slowly decreased until it reaches the paw contact with the treadmill. Interestly, the LTM trained cat (Fig.H4 left panel) while stepping on the FTM showed a completely different trajectory 42 days after the training compared to that than before spinal lesion. The lift-paw was followed not by a flexion but by a flat ascending plateau that finished in a large flexion and then sudden vertical drop to contact the treadmill. Changes in the shapes of the trajectories appeared 7 days after the lesion and were maintained throughout the whole experimental period.

Even though paw trajectories were different for both cats, the "hook" shape representing a refinement on the foot placement (see preceding chapter) observed before the lesion (Fig.4 black arrows) disappeared in both cats after spinal hemisection. In both cases, this "hook" shape was replaced by a sudden vertical drop of the paw at the moment of the contact with the treadmill (compare foot trajectory just before landing to the left of the graph in both cases).

Changes of EMG activity in relation to adaptation to the LTM. In order to study changes induced by the spinal hemisection on the "voluntary" adaptations seen during LTM stepping in cats with an intact spinal cord (See preceding chapter), average EMG activity of the implanted muscles during LTM stepping before the lesion was superimposed to the LTM stepping 42 days after spinal hemisection. One of the main changes in EMG activity was seen in the flexor muscle St discharge around the foot contact. The first St burst was always present even form the earliest recording delay of the experiment i.e. 7 days after the lesion. ***However, the second burst necessary to brake the swing phase never reappeared during the whole experiment after the hemisection***. On the contrary, in the non lesioned side (right side walking on the rung) the second burst was always present during LTM locomotion. Figure H5 shows these findings compared to LTM walking before the lesion. EDB muscle also showed a decreased (or disappearance in some cats) of the first burst on

the side of the lesion (see for instance orange line in Fig.H5 panel B, black arrow) while it was always present on the contralateral non lesioned (right) side. No important shape changes were seen in the extensor group (VL, GM, Srt) or in the rest of the flexors (TA).

Foot drag. Fig.H6 shows 2 cats with similar spinal hemisections and exposed to FTM or LTM training methods. We observed that 7 days after the lesion more than 70% of the whole step cycle was dominated by foot drag deficits (Fig.H6.A). Several weeks after training we observed a reduction of foot drag in both conditions. However, the step cycle of the LTM trained cat presented 30% less foot drag compared to the FTM trained cat. 42 days after the training foot drag disappeared on the LTM trained cat while the FTM trained cat still showed foot drag deficits.

When comparing the duration of foot drag during the swing phase (fig.H6.B) we noticed a significant difference (U test; $p < 0.05$) 7 days after the lesion. For instance, 48% of the swing phase on the LTM trained cat was dominated by foot drag, while only 40% of the swing phase presented foot drag on the FTM trained cat. 28 days after the lesion these differences were reverted (U test; $p < 0.05$). The LTM trained cat showed an important decrease in the duration of the foot drag. Thus, only 22% of the swing phase was dominated by foot drag compared to the FTM trained cats in which foot drag was present on 54% of the swing phase. After 6 weeks of training i. e. 42 days after spinal hemisection, foot drag completely disappeared on the LTM trained cat while in the FTM trained cat it was still dominating 41% of the swing phase.

Overall, the main changes observed after spinal lateral hemisection correspond to an interruption of voluntary commands for precise foot placement. We found changes in angular excursion which tended to be more flexed in all joints after LTM training and also changes in paw trajectory which lost the refinement at the moment of the paw contact with the treadmill. This last finding was specific to the lesion and not to the training method since both groups of cats (LTM and FTM trained cats) presented this deficit. We also found EMG changes such as the disappearance of the second St burst, an interesting finding only highlighted by the LTM task since in normal condition (and since this burst is not always present in FTM stepping) it would not have been possible to observe these changes. LTM training induced kinematic changes in paw trajectory presenting an initial plateau at the

beginning of the swing to finish in an important flexion before contacting the treadmill. Training on the LTM led to an improvement in the distal deficit of paw placement (foot drag) probably by an improvement in the descending control of distal musculature.

Figures

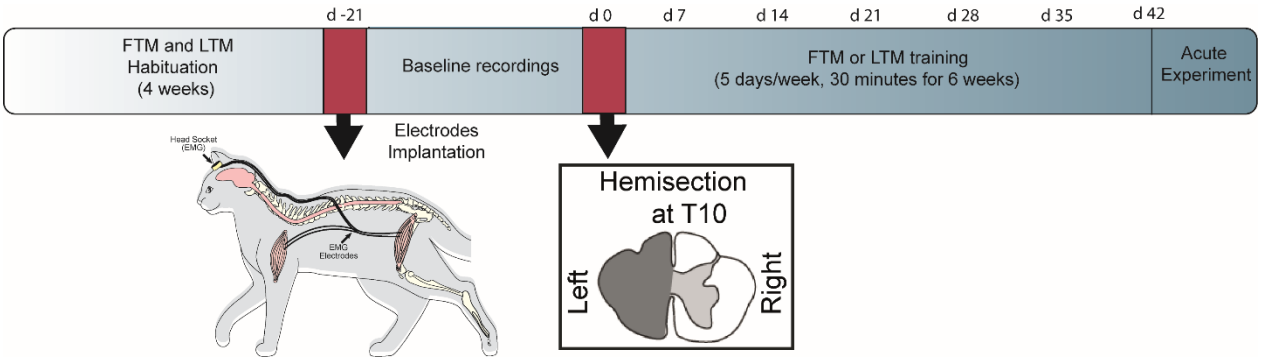

Fig. H1. Schematic overview of the experimental protocol. After a period of habituation of 4 weeks cats were selected based on their ability to walk regularly for several minutes on the flat treadmill (FTM) and on the ladder treadmill (LTM). EMG electrodes were implanted in flexor and extensor muscles in the forelimbs and hindlimbs. A left hemisection of the spinal cord was performed at T10. 4 days after spinal lesion cats were trained during 30 minutes, 5 days a week to walk on the LTM or on the FTM and were recorded once every week during 42 days. At the end of the experimental series, cats were prepared to record fictive locomotion (decerebration, curarization, electroneurographic (ENG) recordings).

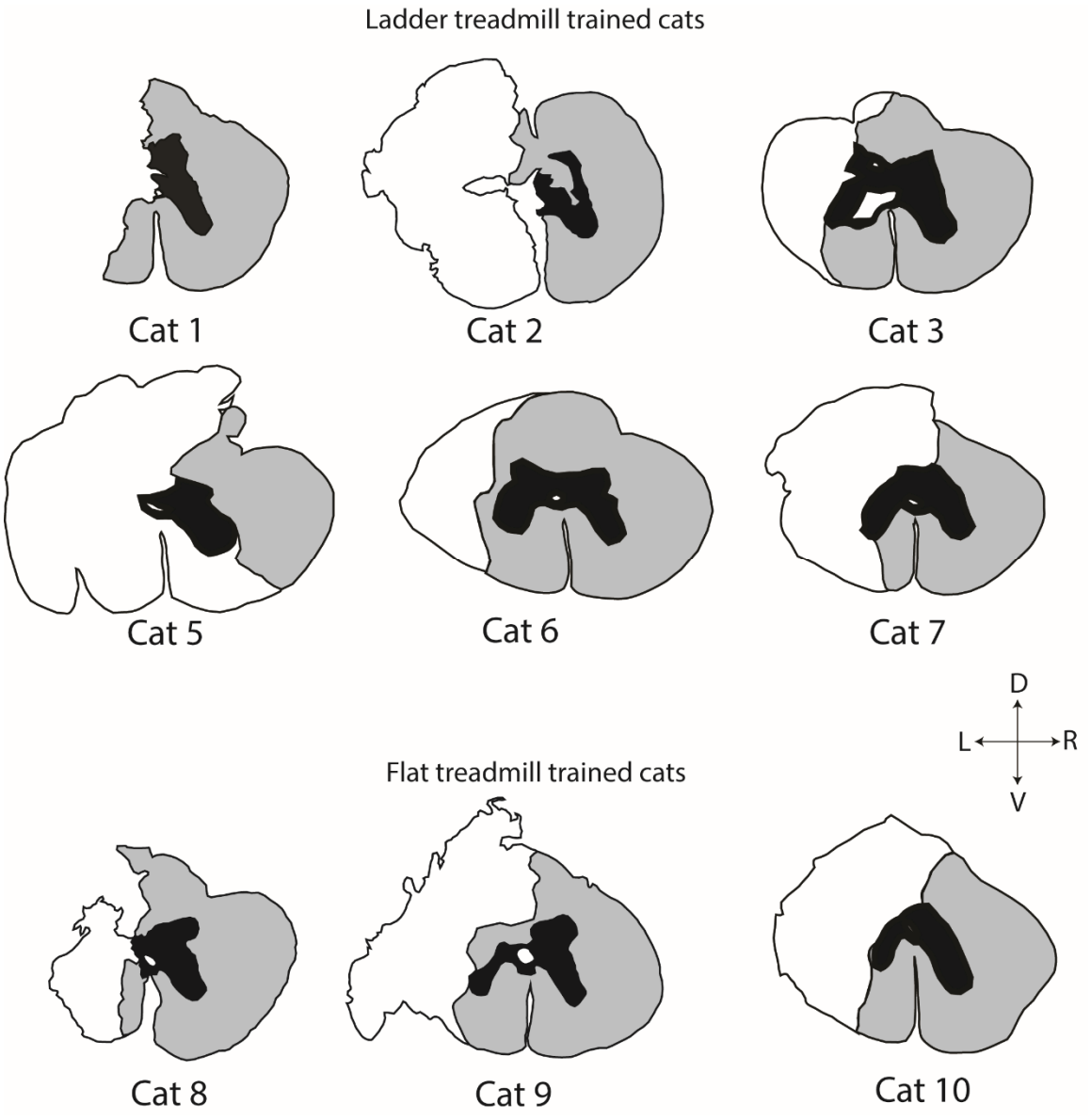

Fig. H2. Schematic drawings of the hemisections at T10 in individual cats. The damaged tissue is represented in white. D, dorsal; V, ventral; L, left; R, right.

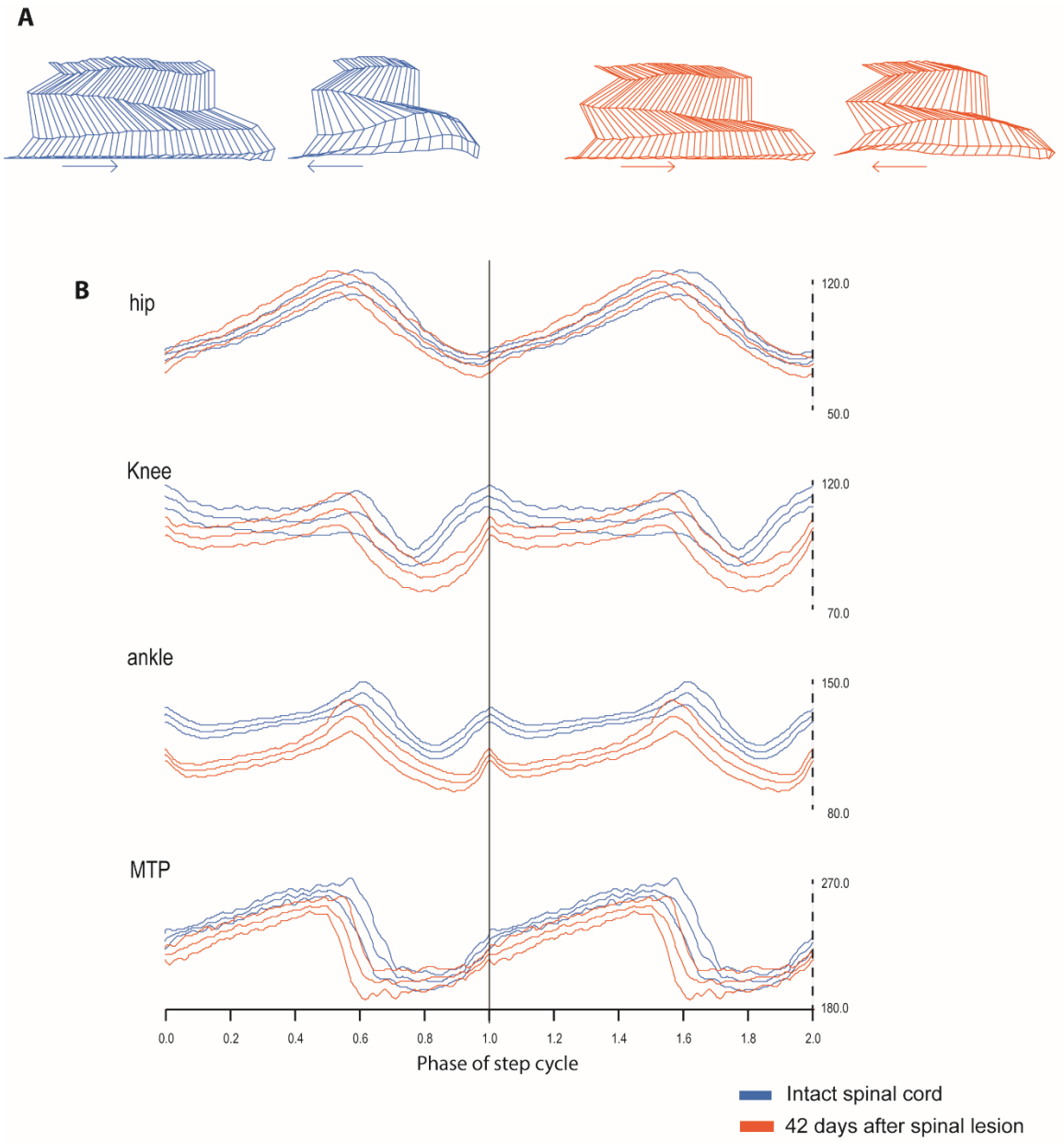

Fig. H3. A. Stick figures of swing and stance phase from one step cycle of the left hindlimb of cat 3, during FTM stepping 42 days after spinal hemisection and trained on the LTM (orange lines) compared to the same kinematic parameters of the same cat before spinal lesion (blue lines). **B.** Averaged angular excursion of the hip, knee, ankle and MTP joints of the left hindlimb synchronized on the left paw contact for the same cat on the FTM before and 6 weeks after training on the LTM.

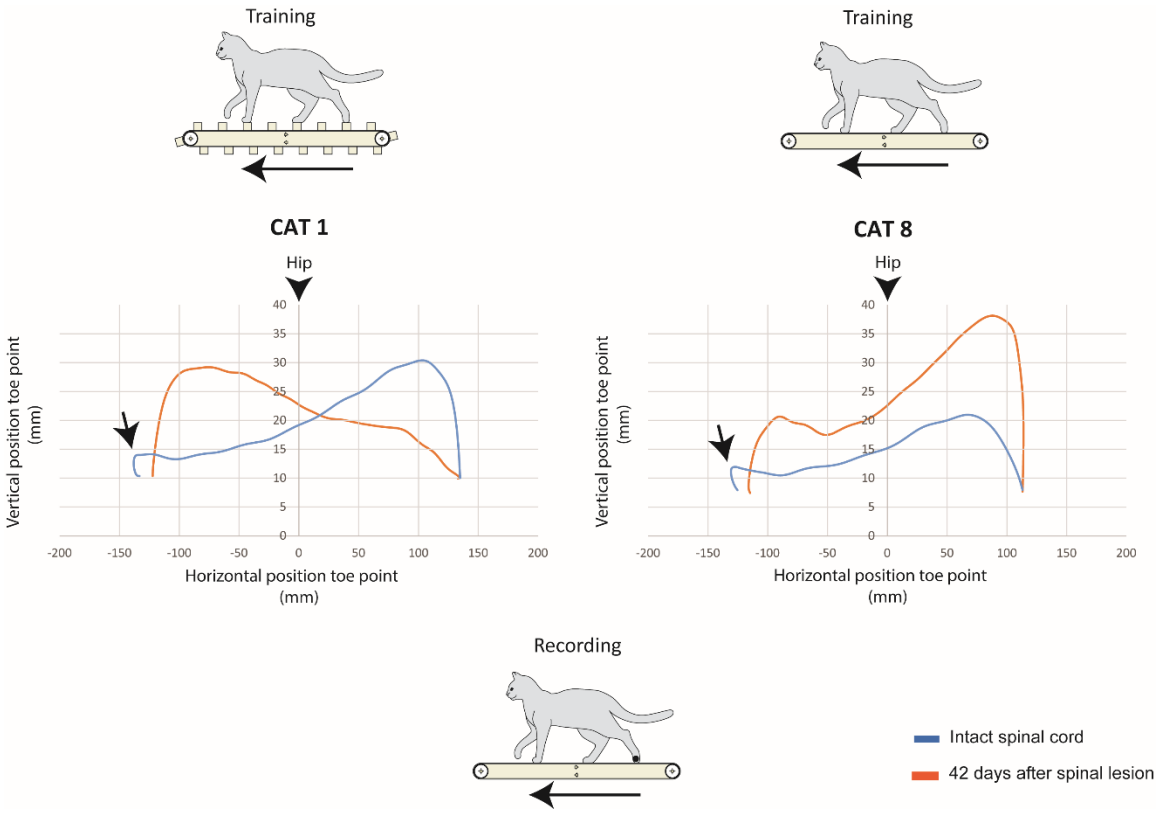

Fig. H4. Graphical 2d representations of left toe trajectories during FTM stepping before (blue lines) and after (orange lines) spinal hemisection. Cat 1 trained on the LTM (left panel) and cat 8 trained on the FTM (right panel). A "hook" shape (black arrow) was seen before spinal hemisection as the foot prepared to land whereas this hook disappears after hemisection. Cat figurines represent training conditions for each panel. Arrows below figurines represent the direction of stepping.

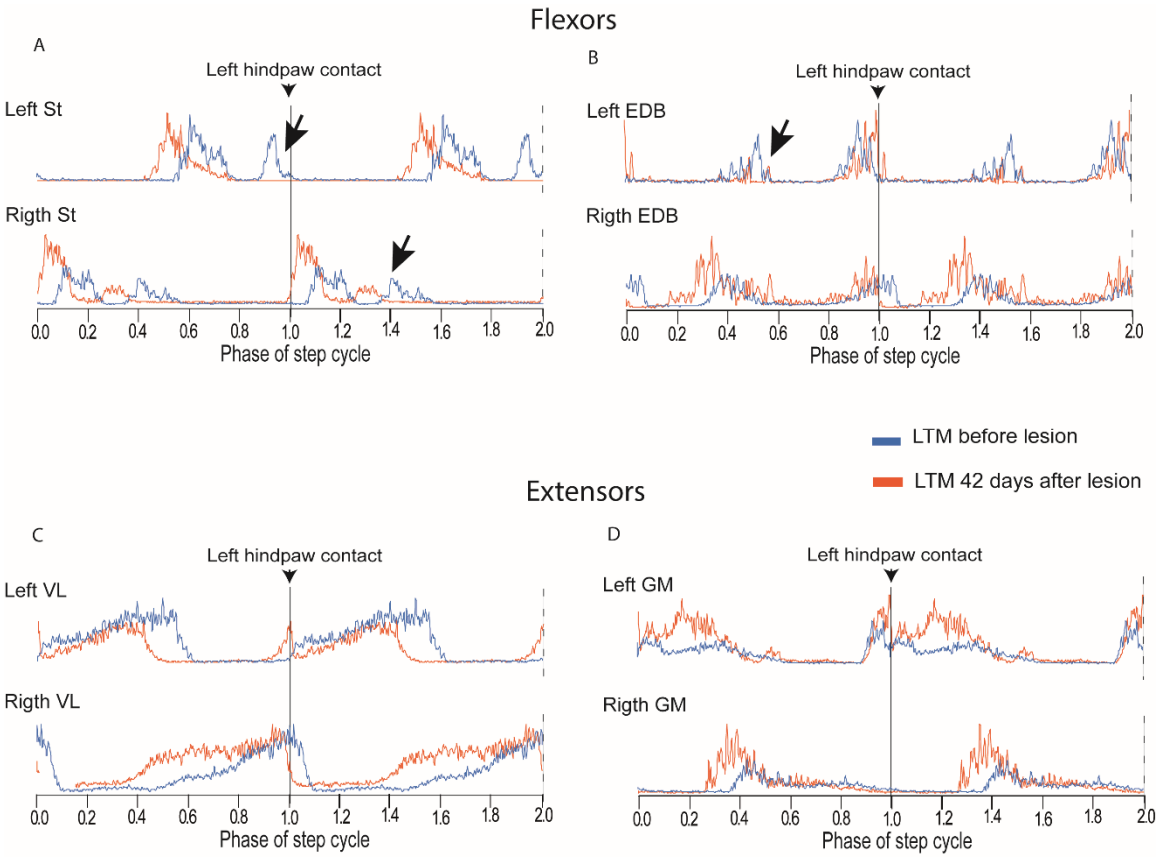

Fig. H5. Averaged rectified EMG locomotor bursts synchronized on the left paw contact in cat 1 (panels A, C and D) and cat 3 (panel B) comparing stepping on the LTM before (blue lines) and 42 days after a left lateral hemisection (orange lines). Black arrows point to 3 major changes: 1) the second burst of activity in the St muscle on the left disappears after the hemisection. 2) the second burst of activity on the right is still present after the hemisection and 3) an important decreased (or disappearance in some cats) of the EDB`s first burst on the side of the lesion. St= semitendinosus, VL= vastus lateralis, EDB= extensor digitorum brevis, GM= gastrocnemius medialis.

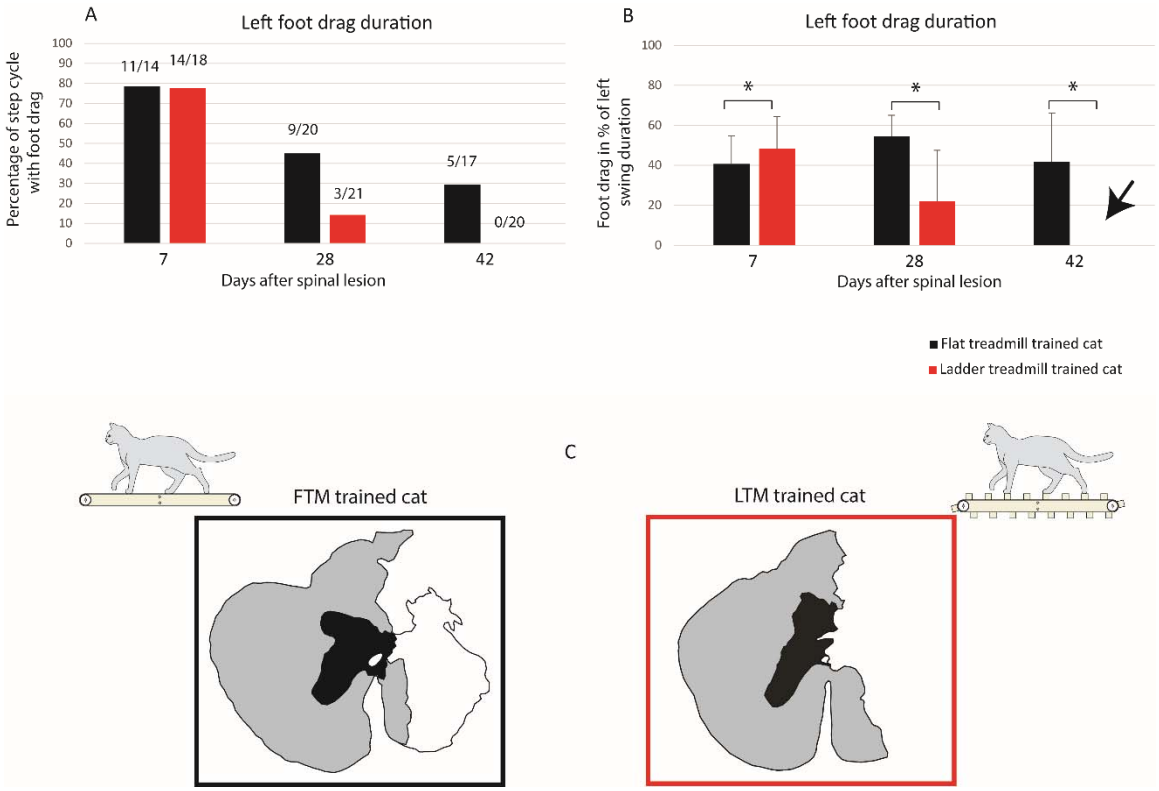

Fig. H6. Comparisons of the frequency (**A**) and duration (**B**) of foot drag between a hemisected cat trained on the LTM (red) and a cat trained on the FTM (black) at 3 different delays after spinal lesion. Measurements of drag are made during locomotion on FTM walking at 0.5 m/s. Notice the absence of foot drag 42 days after the lesion on the LTM trained cat (black arrow). Values on the top of bars are number of left foot drag vs the total number of steps. Values are means; error bars represents SD. *P < 0.05. **C.** Histology of the partial lesion on the left T-10 segment on the LTM trained cat (Cat 1) and the FTM trained cat (Cat 8).

Chapter 4: Discussion

Although the locomotor movements required to move through a changing environment are the product of a variety of commands and interactions at various levels within the nervous system, skilled locomotion involving precise foot placement in animals has shown the importance and dependence of supraspinal structures (Armstrong, 1988;Drew, 1991b) to modulate the basic locomotor patterns at the spinal cord level.

Studies in cats have shown that precision walking on a horizontal ladder or obstacle clearance depends not only on visual control of the movements (calculation of the distance, site of the foot placement) which is mainly controlled by the parietal cortex (Andersen, 1987;Drew, Andujar et al., 2008), but also on the motor cortex (MC) which influences the locomotor pattern through direct pathways to the spinal cord (Asanuma, 1981).

The main idea of the project was to develop a treadmill training condition using skilled locomotion task and therefore increase the inputs from supraspinal structures to the locomotor CPG, and study its effects on the locomotor recovery after an incomplete spinal lesion. The advantage of this LTM task compared with the evaluation of skilled locomotion on a horizontal fixed ladder is that it allowed maintaining the same environmental sensory inputs expected on a FTM with an added constraint to walk on rungs. Parameters such as speed are maintained so that most of the sensory inputs remain constant for the two tasks (LTM and FTM). Also, confinement to a treadmill box while walking on a moving ladder allowed studying locomotor parameters (EMG and kinematics) in an exact same ways as with a conventional treadmill. Changes on kinematics or EMGs may then be attributed to modifications within the locomotor control systems.

4.1 Kinematics

We have shown that with LTM-stepping in cats with intact spinal cord, kinematic parameters such as step length, step duration and limb trajectories are largely maintained for a given speed in order to adapt efficiently to a more demanding environment. The amplitudes of the angular excursions were generally increased (more flexion) for all cats. Modifications in walking gait (interlimb combination) were found in order to maintain balance to successfully adapt to the task.

Similarity in step length and step duration during LTM and FTM might be explained by the fact that distance between rungs was determined in accordance to the comfortable stride length for each cat as described in previous work (Beloozerova, Farrell et al., 2010;Beloozerova and Sirota, 1993). Comfortable stride length was chosen so that cats could be easily trained for long sessions. However this may have limited the voluntary efforts required, and therefore less supraspinal contributions, to adjust limb trajectories.

After lateral hemisection of the spinal cord, we found that the limb on the lesioned side did not recover stepping on the rungs, but instead the paw was placed between the rungs. This produced a major change in limb trajectory with a hyperflexion of the knee and ankle on the left side.

Changes in the amplitude of angular excursion comparing FTM and LTM task before spinal lesion might represent a compensatory mechanism which allows cats to adapt to a challenging environment to preserve the overall locomotion function i.e. the dynamics of the whole limb kinematics close to normal walking. For example, compensatory changes after peripheral nerve injury includes changes in angular excursions but not on the general limb kinematics (trajectories and step length) leading to the hypothesis that stabilization of limb kinematics might come from supraspinal commands (Chang, Auyang et al., 2009). These descending signals reach the spinal circuitry which in return selects and regulates individual joint kinematics to keep the kinematic limb plan.

The “hook” shape seen at the very end of the swing phase in the toe trajectories may represent a refinement in locomotor control in order to achieve proper foot placement

required for this task (Fig. 4). Such changes in paw trajectory before contact were reported during downslope walking even though such trajectory changes were not seen on level walking (Smith, Carlson-Kuhta et al., 1998). We observed these changes in all cats and we do not think that this hook shape is attributable to a specific training or task because it was present in both the LTM trained group and the FTM trained group. We believe that this "hook" originated from supraspinal structures because it disappeared after spinal hemisection on that side. The disappearance of the hook in toe trajectory corresponded to a free-fall of the paw making a clear sound at the impact on the treadmill. Among the supraspinal structures possibly involved in generating the hook toe trajectory, we believe that descending systems that control of distal musculature, like cortico- and/or rubrospinal pathways are prime candidates.

One of the main findings comparing FTM vs LTM tasks was the change in walking gait. According to Hildebrand (1980), animals select combinations of paws (two or three paws) so that balance is always maintained. To our knowledge walking gaits were little studied on ladder walking. One could imagine that the preservation of kinematic parameters signifies similar walking gaits. Interestingly, the analysis of double support periods (two paws on the ground at the same time), showed that the diagonal couplets prevailed during the LTM stepping. This type of diagonal coordination obviously gives more support when balance might be compromised while stepping on narrow rungs.

Maintenance of kinematic parameters and changes observed in walking gaits during LTM walking are the result of complex changes in the muscular activity dynamics. Such EMG changes allow us to hypothesize that these modifications were driven by supraspinal structures in order to maintain a kinematic plan close to the FTM locomotion.

Indeed, research from episodic goal directed arm movement in humans (Georgopoulos, 1991;Kalaska and Drew, 1993) where the cortical level of activity was correlated with a specific kinematic plan, supports the idea that locomotor patterns are planned at a kinematic level in order to adapt efficiently to a changing environment. Also, a study of artificial neural networks in humans, based on a model to correlate a desired kinematic movement plan and the required muscle activation, showed that different gait conditions required changes in both the timing and magnitude of muscle activity of different muscles

(Prentice, Patla et al., 2001). These studies allow us to speculate that, based on either intrinsic or extrinsic reference frames, the nervous system could transform visual and sensory information into the appropriate motor commands for individual muscles.

4.2 EMG adaptations

Most of the important changes in EMG activity were seen in events occurring around foot contact with the treadmill or ladder. One of the most important changes was the increased and almost doubled amplitude of the second St burst on the LTM, whose function is to decelerate both the hip and knee at the end of swing and thus prepare the foot for contact (Smith, Chung et al., 1993;Wisleder, Zernicke et al., 1990). Positioning of the limb during placement on ground is the most important event in the step cycle (Halbertsma, 1983). Therefore, it is not surprising that there might be a more precise muscle control and therefore changes on the EMG activity during locomotor tasks requiring more precise foot placement. Studies using recordings of motor cortex have shown that the mean activity of cortical neurons was elevated in late stance and early swing during accurate stepping, suggesting that cortical regulation of paw placement was achieved by modifying the activity of specific groups of muscles during different parts of the swing phase (Beloozerova, Farrell et al., 2010;Drew, Jiang et al., 2002b).

Interestingly after hemisection, the second St burst disappeared on the lesioned (left) side while it was maintained on the non-lesioned (right) side. The most likely explanation is that the St discharge is in part controlled by supraspinal pathways that transmit the voluntary command to the specific muscles to adapt and brake the swing for precise paw placement on a particular place on the rungs. An interesting anecdotal observation is that some hemisected cats succeeded to position the left hindfoot on the rung (for example, 3 out of 20 steps). To clarify the question whether the second St burst might be the result of sensory feedback specific to the LTM task, these particular sequences were analyzed in details (not shown). We observed that the step on the rung occurred without a reappearance of the second burst in St muscle showing that this second burst in St was truly of supraspinal origin and not the result of sensory information to the spinal cord.

Changes in distal muscles such as EDB and the maintenance of its two bursts of activity only on the LTM might imply that a more precise control is needed on this task for this muscle to propel the paw forward during the onset of the swing phase and to secure the landing on the rungs at paw contact. Interestingly after spinal hemisection, the first burst disappeared from the lesioned (left) side even on the LTM task while the second burst was always present (Fig. H5). Again, control of distal musculature, such as EDB, may be necessary for adequate paw off or at the foot contact. Because the paw was placed between rungs, no precise control was needed for paw off. This, in turn, could partially explain the disappearance or the important decrease of the first EDB burst.

Changes in EMG extensor activity such as VL could also be due to supraspinal commands. During walking over obstacles or on a fixed horizontal ladder the mean discharge rate and the depth of frequency modulation of the activity in the majority of MC neurones is greater than on the flat surface (Beloozerova and Sirota, 1986;Drew, 1988). Also, stimulation of the motor cortex during stance phase can increase the activity of extensor muscles (Armstrong and Drew, 1985;Leblond, Menard et al., 2001).

Not all flexor or extensor muscles showed important changes (for example TA, GM and Srt). But this does not mean that changes in other muscles (not implanted in this study) were not changed to successfully adapt to the demanding environment while maintaining predetermined kinematics. This can be considered for future studies, in which muscular electrodes implantation of more distal muscles acting in the moment of the foot contact are necessary to complement the present study.

Evidence that training on the LTM induces different plastic changes on the spinal cord and therefore in the patterns of locomotion are showed by our results on foot trajectory after the lesion. The idea that the type of training could modify differently the recovery of locomotion is supported by experiments where spinalized cats received a specific training, one treadmill step-trained group and other stand trained group, i.e. only by standing on a platform. This last group did not recover locomotor walking on a treadmill (Edgerton, de Guzman et al., 1991). In our work, differences on the foot trajectory when comparing both trained groups (LTM and FTM groups) on the FTM walking are an indication that adaptations to the training task changed the way the cat walked. This is an important

finding, because even though at first glance the cats looked like walking similarly as before the lesion, the adapted spinal circuits to the new task (LTM) somehow reconfigured differently the trajectory of the paw. We do not know if this situation can be reversible, i.e. if changing the training to a regular FTM training will result in a trajectory similar to the FTM group.

Changes in the spinal circuitry were studied through fictive locomotion experiments. We first looked at the effect of hemisection on rhythmogenesis and found that ENG recordings muscle nerves from the hemisected side (left) were active and showed an alternate, rhythmic activity while those on the right side showed minimal or no locomotor activity. After an acute complete section at T13, the left-right asymmetry was still present. Because these cats were not trained, it is clear that these changes were due to the spinal hemisection where the lesioned side becomes somehow more autonomous because of the loss of communication with supraspinal structures (Gossard, Delivet-Mongrain et al., 2015). It would be interesting to see if the different training methods, LTM or FTM, could modify the left-right asymmetry in rhythmogenesis.

4.3 Foot drag

Deficits in the control of distal musculature due to the hemisection may have functional consequences for stepping on a flat surface as well. As stated before, following lesions of the dorsolateral system of the spinal cord in cats, the most easily noticeable long-term deficit is the inability to adequately lift the paw from the treadmill surface during the swing phase, resulting in a clear foot drag. This deficit could be explained as the interruption of descending signals to the motoneurons that controls distal musculature. It has been suggested that changes in timing relationship between the hip flexor Sartorius and the St muscle might contribute to the foot drag (Bélanger, Drew et al., 1996;Drew, Jiang et al., 2002b). Others suggested that muscles participating in the propulsion during the onset of the stance phase, like the soleus muscle, might rapidly decrease their activity which, in turn, would lead to a premature onset of the swing phase and contribute to the foot drag (Lovely, Gregor et al., 1990).

We observed foot drag in 5 out of 9 cats. Such differences could be explained by the different sizes of the lesions: some being too small to interrupt the dorsolateral pathways (3 cats) or too big, reaching to the contralateral grey matter (on 2 cats), see Fig. H2. This forces the cat to find other strategies (hip hyper flexion for example) to be able to walk on the FTM and, in consequence, diminish foot drag. In order to avoid bias we compared cats with LTM or FTM training with similar damage from the spinal hemisection. The most similar damage to the dorsolateral funiculi and similar deficits a few days after the hemisection were found between cat 1 and cat 8 which were used to compare foot drag frequency and recovery between the LTM and FTM training conditions (Fig. H6). Cat 7 and cat 10 also presented similar lesions (Fig. H2). However, cat 7 did not present foot drag at any recorded delay.

Our results showed that the foot drag only disappeared in the LTM trained cat. This suggests that LTM training may have involved an enhanced supraspinal descending activity on locomotor networks which, in turn, improved the control of distal musculature. It is important to mention that our criteria for foot drag included objective and precise deficits, i.e. the distal phalanx had to be in contact with the treadmill to be considered as foot drag.

4.4 Training and human SCI

Animal studies have shown that after a complete spinal lesion the recovery is mediated by modification of the spinal circuitry (Edgerton, Leon et al., 2001;Ribotta, Provencher et al., 2000;Rossignol, Bélanger et al., 2000;Rossignol, Chau et al., 2003;Rossignol, Dubuc et al., 2006). By contrast, in humans recovery of independent walking through training only occurs when there is a preservation of voluntary leg movement (Dietz, Colombo et al., 1994;Wernig and Muller, 1992). Increased activity of the spared descending pathways may also be a very important component to recover functional stepping (Thomas and Gorassini, 2005).

The loss of motor function after an incomplete spinal lesion in humans is primarily due to the interruption of the lateral system, more specifically of the corticospinal tract (Nathan, Smith et al., 1990). The importance of the corticospinal tract in humans makes it necessary to find, first in animal models, methods to increase the strength of remaining descending connections to improve stepping. Rehabilitation strategies use exercises like treadmill training to induce plasticity and remodel supraspinal inputs to the spinal circuits. Residual or even new pathways may contribute to the plasticity if neural repair strategies are coupled with rehabilitation therapies (Dobkin and Havton, 2004).

Studies in humans have shown that allowing subjects to activate their muscles independently while walking on a treadmill at lower speeds improved their overground walking function (Thomas and Gorassini, 2005). Increasing the function of the spared corticospinal tract could induce improvements in walking. It is therefore important to assess the efficiency of methods in which voluntary commands are needed while maintaining the treadmill training conditions as shown here.

4.5 Limitations

One of the main limitation of this study was the inconsistency between strategies adopted by cats after the spinal lesion to adapt to the LTM situation during locomotor training. For instance we still do not know if the left paw placement between bars represented a strategy for specific cats because 3 out of six cats had actually started to improve such placement on the rungs by the end of the experiments. Ideally we would have need more time to see if such variabilities could reach a plateau. Also we would have need more cats (an equal number of cats on each group) to compare better differences between FTM and LTM trained groups. Another limitation was the variability on the spinal lesions (see Fig. H2) among cats. Although it is difficult to produce the exact same lesion for all cats (because of factors we cannot control such as edema, bleedings during or after the surgery and other biological variables) such differences did not allows us to see the same deficits among cats. For instance only 4 out of 9 cats presented foot drag and 3 of them

were included for training on the LTM which did not allow us to compare on more cats differences on the recovery of this distal deficit.

4.6 Concluding remarks and future work

Future experimental work should concentrate on identification of supraspinal structures implicated during LTM stepping. Knowledge about the adaptation of the supraspinal system after a partial SCI may also improve our understanding of voluntary motor commands. Also, it is of great importance in the future to investigate more distal muscles acting on the lift and the landing of the paw. Muscles such as extensor digitorum longus (which show synchronous activity with TA muscle), flexor digitorum longus (which discharges as an extensor in synchronous activity with VL) and plantaris (an ankle extensor and digit flexor) should be recorded in order to see how voluntary commands change muscular activity.

The main perspective of this work lies in the possibility to reorganize spinal circuits through different training methods in order to get a more functional walking after SCI such as minimizing foot drop in humans with SCI. Many questions about training on the LTM remain. For instance, why is it that the cat was never able to place the paw correctly on the rungs? Is it a strategy to regain stability or it is because cats do not really have any voluntary control over the paw? Could this be also due to a high level sensory deficit in which the cats cannot evaluate the exact position of the limb and paw in space? After all, the lateral hemisection cuts through important ascending sensory systems for tactile and proprioceptive information. Will the cats regain foot placement on the rungs if they were trained for many more weeks? Will an interruption of the LTM training and substitution for a FTM training result in changes in the paw trajectory? In other words, will these plastic changes induced by the LTM training be retained after the training is over?

Locomotor interventions after a partial spinal injury should aim at restituting innate capacities which allow the same neural pathways to be used after injury (Dobkin and Havton, 2004).

Nowadays, we know that reflexes are modified after SCI, and these changes could also be targeted as part of combinatorial therapeutic approaches (Thuret, Moon et al., 2006). Studies have shown that treadmill training could normalize load and cutaneous pathways in the cat (Côté and Gossard, 2004;Côté, Menard et al., 2003). This, in itself, could provide easily accessible clues to test new rehabilitation approaches in patients. Indeed, in human SCI patients, the basis of locomotor training is to provide sensory cues consistent with normal walking (Harkema, 2001), and these approaches are primarily derived from work on adult cat locomotion.

Approaches taking advantage of the interactions of the tripartite system (dynamic interactions between spinal CPG, sensory inputs and descending commands) controlling locomotion are beginning to emerge. For instance, studies in humans after iSCI have shown that adaptation to multisensory feedback i.e. visual cues adaptations during locomotion combined with proprioceptive feedback (applying a resistance to the leg during the swing phase) improve locomotor recovery compared to individually applying these stimuli (Yen, Landry et al., 2014). Other approaches include feedback-error learning, taking advantage of different motor and sensory adaptations trough locomotor resistance training to improve stepping (Chisholm, Peters et al., 2015).

Even when promising research on drug treatments, stem cell inoculations, electrical stimulation and many other important approaches, could give us hope of finding the best treatment to reestablish walking, locomotor training will probably always be a fundamental component of rehabilitation aiming at re-establishing a functional locomotor recovery.

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