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Recovery of locomotion after partial spinal cord lesions in cats: assessment using behavioral, electrophysiological and imaging techniques

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This short review summarizes experimental findings made after spinal cord injury, mainly in cats. After a complete spinal injury, cats re-express hindlimb locomotion after 2–3 weeks because of a spinal locomotor circuitry named the central pattern generator or CPG. To investigate whether such circuits are also implicated in the recovery of locomotion after partial spinal lesions, we have used a dual spinal lesion paradigm. Essentially, after an initial unilateral hemisection, cats spontaneously recover quadrupedal locomotion. When a complete section is then performed 3 weeks after this hemisection, cats can walk with the hindlimbs within 24 hours compared to 2–3 weeks in cats with single complete spinal lesions demonstrating the importance of spinal mechanisms after partial lesions. Using kinematic and electromyographic methods to evaluate the changes throughout the dual lesion paradigm, we could show that the spinal cord reorganizes spontaneously without locomotor training or with training provided between the partial and complete spinal lesion. To assess spinal lesions we have used histology and magnetic resonance imaging (MRI). We will describe some advanced MRI techniques such as diffusion and magnetization transfer, which provide higher specificity to axon degeneration and demyelination. Examples of advanced MRI techniques in cats and humans are described, including the current limitations and perspectives.

Key words: spinal lesions, hemisection, electromyography, locomotor training, kinematics, MRI, DTI

INTRODUCTION

This paper aims at summarizing the recovery of locomotor function after a complete or a partial spinal lesion at the low thoracic level in cats. We have used electrophysiological, behavioral and imaging methods to document various aspects of this research. It is believed that a short descriptive summary of locomotor recovery after various types of lesions is needed to understand the evolution of our understanding of this recovery after complete and partial spinal cord lesions. This is by no means an exhaustive review of the subject but a canvass of the experiments as they evolved in our group (mainly) on this topic. We have kept the physiological evaluation as a whole and the MRI as a separate section to preserve the internal coherence of each approach but we refer reciprocally to each part as deemed important.

COMPLETE SPINAL CORD SECTION

After a complete spinal cord injury (SCI) at T13, we and others have previously demonstrated that adult cats can recover hindlimb locomotion on a treadmill while the forelimbs are maintained stationary on a fixed platform above the belt. Locomotor training for 2–3 weeks is important for this recovery (Barbeau and Rossignol 1987, Lovely et al. 1990). Hindlimb locomotion in this spinal state can be enhanced by noradrenergic agonists such as clonidine or can be blocked by noradrenergic antagonists such as yohimbine (Barbeau et al. 1987, Barbeau and

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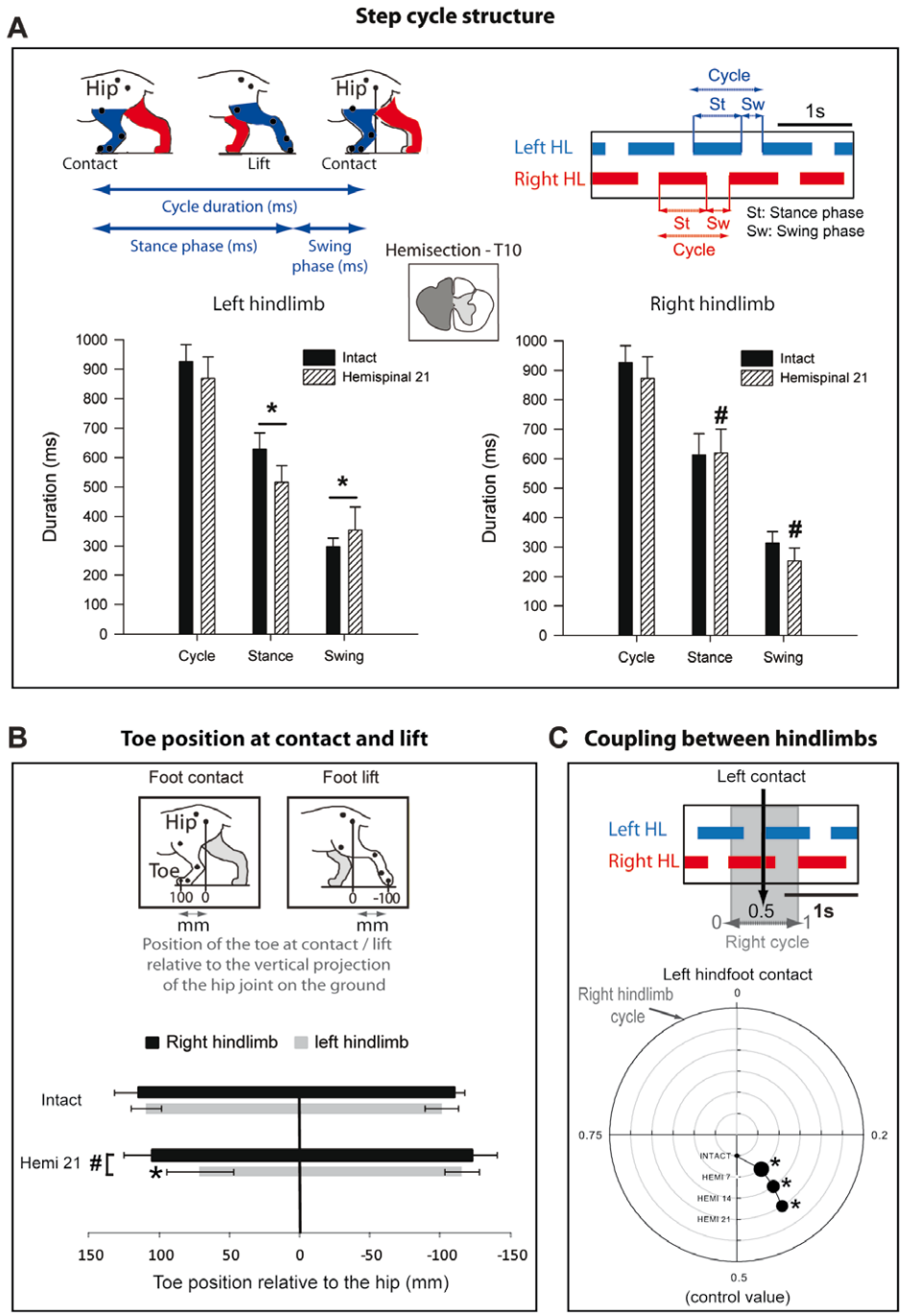

Fig. 1. Kinematic changes after a spinal hemisection on the left side at T10 during treadmill walking at 0.4 m/s. Evolution of the step cycle structure (A), toe position at contact and lift (B) and hindlimb coupling values (C) after hemisection. (A) To reduce the time spent by the hindlimb on the affected side (left) after hemisection, the stance phase was shortened in that limb and this was achieved by a phase advance of the foot contact and a necessarily longer swing phase. (B) While the magnitude of forward and backward movements remained unchanged on the right side after hemisection, the capacity to perform correct forward placements was diminished on the left side. (C) The coupling between hindlimbs was also affected after hemisection such that the affected hindlimb phase-advanced its weight support. Error bars represent SD. Statistical differences between the intact and hemispinal state are shown by the symbols * and differences between the left and right hindlimbs are shown by the symbol #. (Adapted from Martinez et al. 2011, 2012b with permission form American Psychological Society).

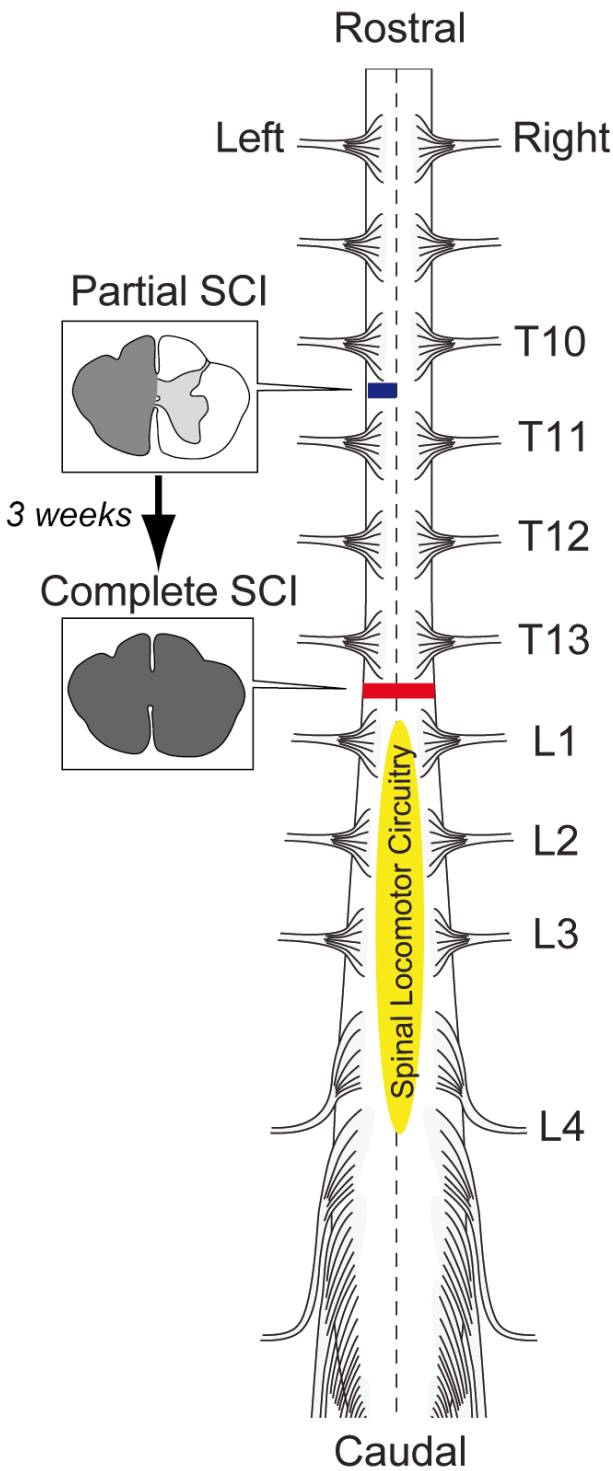

Fig. 2. The dual lesion paradigm. A partial spinal cord injury (SCI) consisting in a unilateral hemisection of the cord is first performed at T10–11 and is followed, 3 weeks later, by a second complete SCI at T13 to isolate the spinal locomotor circuitry from residual supraspinal inputs and reveal the spinal changes induced by the previous hemisection. (T) Thoracic; (L) Lumbar.

Rossignol 1990, 1994, Chau et al. 1998b, Giroux et al. 2003). Combination of electrical spinal stimulation and pharmacological stimulation can enhance the expression of spinal locomotion (Chau et al. 1998a, Barthélemy et al. 2006, 2007). These observations confirm the concept of spinal generation of hindlimb locomotion by neural circuits at the lumbo-sacral level. Indeed, the isolated spinal cord of adult decerebrated and curarized spinal cats can generate an elaborate bilateral and alternating rhythmic activity in flexor and extensor muscle nerves when stimulated by l-DOPA (Grillner and Zangger 1979, Grillner 1981) or even without pharmacological stimulation but with training (Pearson and Rossignol 1991, Frigon and Gossard 2009).

What mechanisms underlie the re-expression of hindlimb locomotion after complete spinalization? Even though 'passive' electrical properties such as membrane resistance do not change much in motoneurons (Baker and Chandler 1987), their responsiveness is greatly modified, namely because of the initial lack of calcium-mediated persistent inward currents (PICs) leading to plateau potentials that will however gradually return with time or can re-appear with injection of monoaminergic agonists (Hounsgaard et al. 1988, Li and Bennett 2003, Hultborn et al. 2003, Rossignol and Frigon 2011). Furthermore, we and others have documented changes in the receptors of various neurotransmitter systems using immunohistochemistry (Giroux et al. 1999, Tillakaratne et al. 2002). Pharmacological stimulation is necessary early after spinalization but "fictive locomotion" can be recorded even without pharmacological stimulation in trained adult spinal cats (Pearson and Rossignol 1991). From these observations we can state that the lumbo-sacral spinal cord of adult cats has an intrinsic neuronal circuitry responsible for generating the essential locomotor pattern and that this circuitry can reorganize after SCI and exposure to locomotor training (Lovely et al. 1986, de Leon et al. 1998). Although intrinsic changes must undoubtedly occur within the spinal cord circuitry suddenly deprived of all descending pathways as mentioned above, there is little doubt that repetitive locomotor training adds a further beneficial effect on locomotor performance. This effect is probably related to the movement-related activation of sensory afferents that can participate in the regulation of muscle discharge amplitude and the control of step cycles characteristics (onset/offset of swing/stance) and adaptation to external demands. The importance played by sensory inputs in reshaping/reactivating the

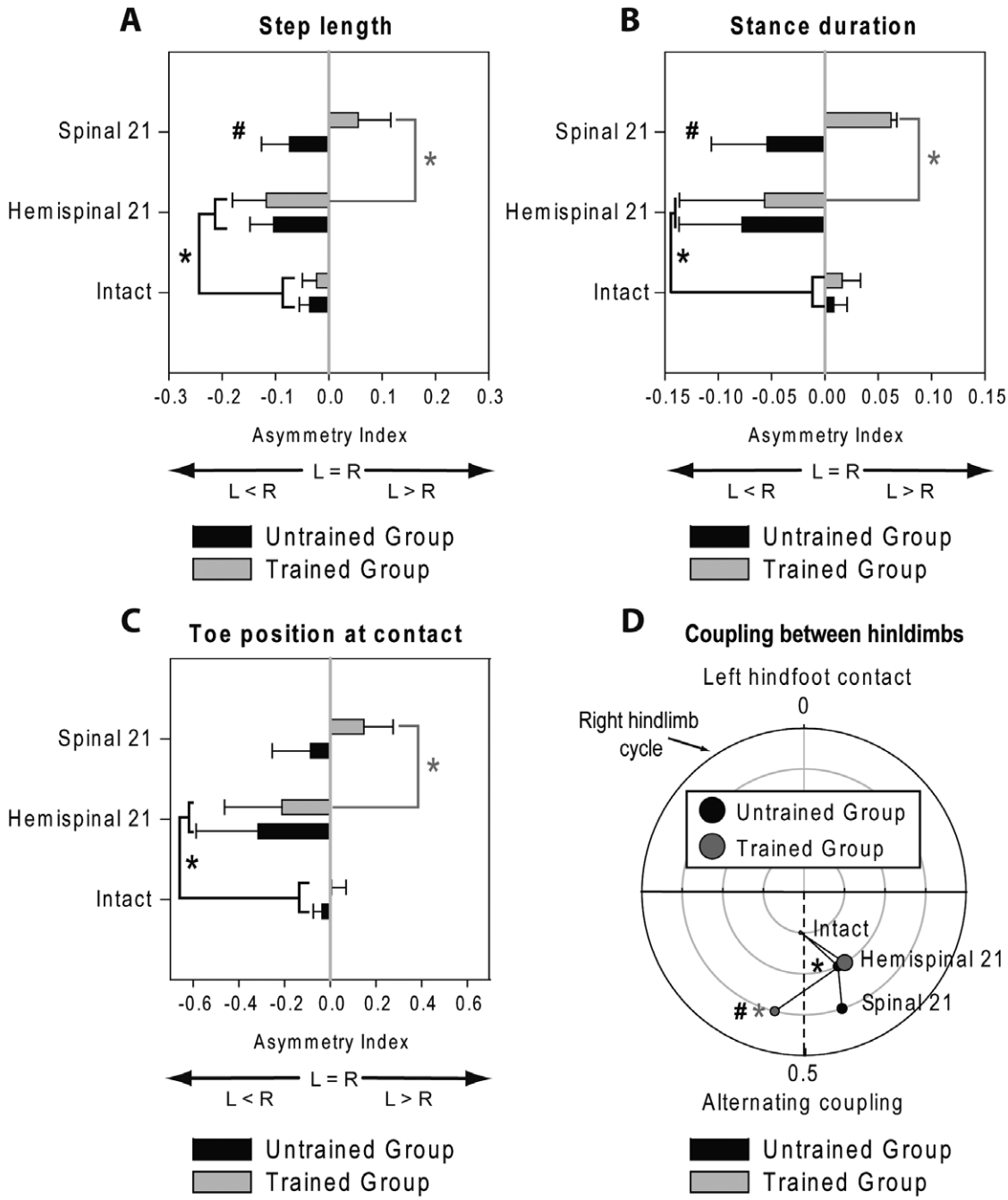

Fig. 3. Effect of locomotor training on hindlimb kinematics in completely spinalized cats previously submitted to a spinal hemisection on the left side. Evolution of the asymmetry indices calculated for step length (A), stance duration (B) and toe position at contact (C) and hindlimb coupling values (D) in cats trained to locomote after spinalization and untrained cats over the entire dual lesion paradigm. In the intact state, the two groups of cats exhibited a symmetrical walking pattern (AIs=0±0.05) and an alternating coupling between hindlimbs (~0.5). 21 days after hemisection, both groups displayed an asymmetrical walking pattern and abnormal hindlimb coupling values due to the deficits of the left hindlimb (side of the lesion). Note that both groups were comparable before the spinalization and the beginning of training. 21 days after spinalization, the asymmetrical walking pattern displayed by all cats after hemisection was retained in untrained cats while it reversed in trained cats. Statistical differences between delays are indicated by the symbol *. Statistical differences between groups are indicated by the symbol #. (L) left; (R) right.

spinal circuitry has been stressed by us (Rossignol et al. 2006, 2011, Rossignol and Frigon 2011) and others (Edgerton et al. 2004). Of clear relevance however are the results on changes in muscle and cutaneous reflexes excitability following treadmill training in spinal cats (Côté et al. 2003, Côté and Gossard 2004). This work has shown that the recovery of spinal locomotion coincides with a normalization of excitability in specific reflex pathways which tend otherwise to be overexcited or poorly modulated after spinalization.

PARTIAL SPINAL CORD LESIONS

Although the role of spinal mechanisms is inescapable after complete spinal lesions, one can ask the question of whether such mechanisms are involved in locomotor recovery after partial spinal lesions in which some supraspinal pathways remain intact and can still access to the spinal circuitry below the lesion. Indeed, following partial SCI, various compensatory mechanisms occur within the whole

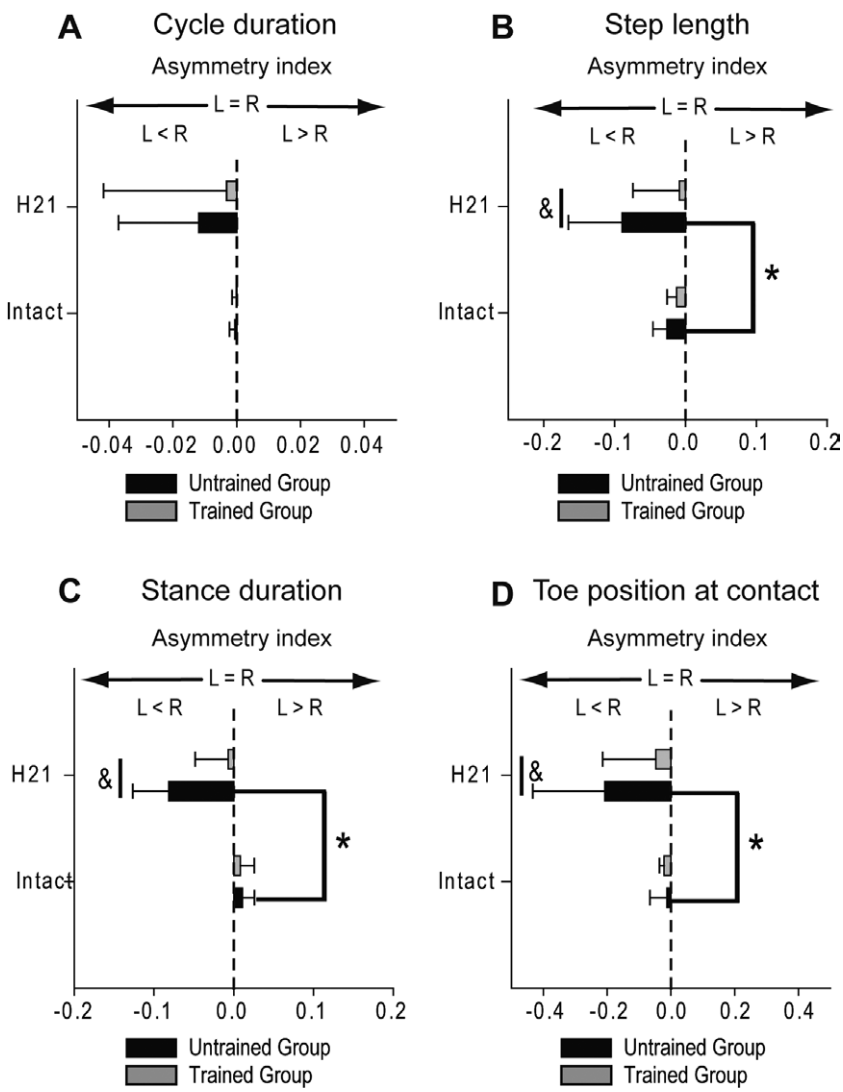

Fig. 4. Effect of locomotor training on the step cycle structure after hemisection. Evolution of the asymmetry indices calculated for cycle duration (A), step length (B), stance duration (C) and toe position at contact (D) in cats trained to locomote after hemisection and untrained cats. In the intact state, the two groups of cats exhibited a symmetrical walking pattern (AIs=0±0.05). 21 days after hemisection, the Untrained Group displayed an asymmetrical walking pattern. By contrast, submitting cats to training after hemisection restored a symmetrical walking pattern. Statistical differences between the intact and hemispinal values are indicated by the symbol *. Statistical differences between groups are indicated by the symbol &.

neuraxis to optimize locomotor recovery (Nudo 2006, Rossignol 2006, Martinez and Rossignol 2011). These mechanisms were better studied in rats and mice. It is actually well known that after a partial SCI in various animal species, intact and/or damaged pathways reorganize above and below the lesion. Rostrally, plastic changes can take place in the brainstem and cerebral cortex (Jain et al. 1997, Fouad et al. 2001, Ghosh et al. 2009, Martinez et al. 2009, 2010) as well as in propriospinal neurons (Zaporozhets et al. 2006, Courtine et al. 2008, Cowley et al. 2008). These changes can result from various mechanisms involving sprouting of undamaged fibers (Fouad et al. 2001, Weidner et al. 2001, Raineteau et al. 2002, Bareyre et al. 2004, Ballermann and Fouad 2006, Ghosh et al. 2010), regeneration of damaged axons (Bregman, 1998) and/or changes in synaptic efficiency (Tillakaratne et al. 2000, Thomas and Gorassini 2005). However, caudal to the spinal lesion, intrinsic changes can also occur within the spinal locomotor circuitry as it will be shown later using the dual spinal lesion paradigm (Frigon and Rossignol 2006, Rossignol et al. 2006, 2009, Barrière et al. 2008, 2010, Martinez et al. 2011, 2012a,b).

The changes observed above and below the partial SCI also depend on the specific types of partial lesions as briefly summarized here.

Dorsolateral lesions

After dorsal/dorsolateral SCIs impacting bilaterally the cortico- and rubrospinal fibers, cats can recover voluntary quadrupedal locomotion while maintaining their weight and equilibrium after a 3–10 day period. Cats have some long-lasting deficits such as foot drag (resulting from impaired coupling between hip and knee flexors) and they lose the capacity for anticipatory modifications when negotiating obstacles placed on the treadmill (Jiang and Drew 1996). Despite these defects, the cats do walk remarkably well with all four limbs on the treadmill.

Ventrolateral lesions

With small ventral/ventrolateral lesions, cats can walk voluntarily at speeds of up to 0.7 m/s with all four limbs 1–3 days after the lesion (Brustein and Rossignol 1998, 1999, Rossignol et al. 1999). With much larger

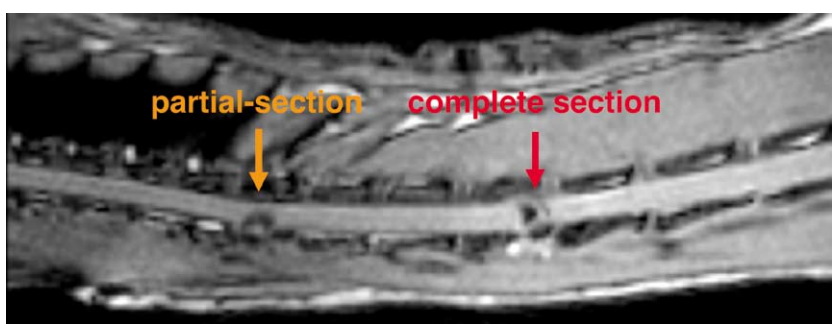

Fig. 5. Sagittal view of a T1-weighted anatomical MRI of a cat with hemi-section at T10 and complete section at T13. Lesions are well identified, however this type of MRI contrast is not sensitive to microstructural damage occurring above and below the lesion.

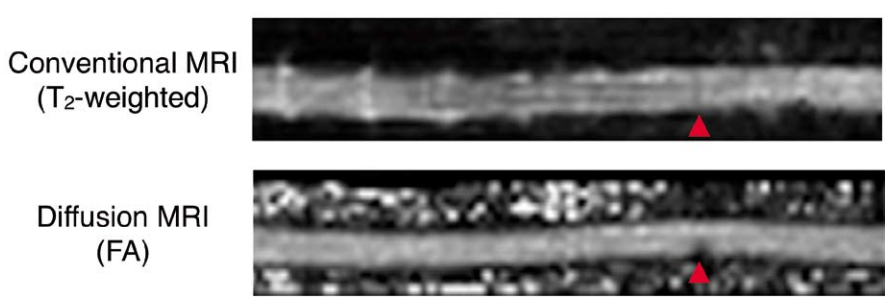

Fig. 6. Coronal views of T2-weighted (top) and maps of fractional anisotropy (FA) from DTI in a cat with a dorsal left section of the spinal cord. Decrease of FA is visible in the dorsal-left aspect at T11 level (red arrow) whereas no abnormality is visible on the conventional MRI.

lesions, sparing only part of one dorsal column, cats behave initially as complete spinal cats. However, with regular treadmill training, the animals could walk but not faster than 0.4 m/s

Hemisections

Following a unilateral thoracic hemisection, the ascending and descending communication between the brain and spinal cord is interrupted on one side only (Murray and Goldberger 1974, Kato 1992, Helgren and Goldberger 1993, Barrière et al. 2008, 2010, Rossignol et al. 2009). During the first 2–3 days, the hindlimb ipsilateral to the lesion shows flaccid paresis and animals adopt a tripod gait. Stepping activity greatly improves during the first 3 weeks post-hemisection. However, some deficits persist at this postoperative time. To minimize the time spent on the affected hindlimb, the support time is reduced on the side of the lesion while the swing phase is increased (Fig. 1A). Concomitantly, the burst duration of extensors decrease while those of the flexors increase on the side of the lesion (Martinez et al. 2011). The neural control of locomotion is also altered such that the intrinsic structure of the cycle is disrupted on both sides (Martinez et al. 2012b). Moreover, the capacity to perform correct forward placements is diminished on the side of the lesion (Fig. 1B). Left/right hindlimb coupling is altered (Fig. 1C) and cats exhibit an asymmetrical gait (Martinez et al. 2011). However, despite these residual deficits, animals can in most cases regain a functional locomotor pattern.

From studies on partial spinal lesions we can conclude that, whatever the size and type of lesions, the basic hindlimb locomotor pattern is always re-expressed despite deficits relative to the disruption of specific pathways. What are the mechanisms involved in hindlimb locomotor re-expression after partial SCI Possibilities include: (1) several supraspinal structures that can compensate for the loss of other descending pathways provided they can somehow reach the spinal locomotor circuitry (Fouad et al. 2001, Weidner et al. 2001, Ballermann and Fouad 2006, Ghosh et al. 2010, Martinez et al. 2010, Martinez and Rossignol 2011), (2) the spinal locomotor circuitry below the partial lesion that can assume a greater role in generating the hindlimb locomotor pattern. To identify where in the central nervous system some of the basic changes occur, we have devised a unique dual lesion paradigm (for a review, see Martinez and Rossignol 2013).

ROLE OF SPINAL MECHANISMS: THE DUAL LESION PARADIGM

In the dual spinal lesion paradigm (Barrière et al. 2008, 2010, Martinez et al. 2011), a unilateral hemisection of the cord is first performed at T10–11 and is followed by a second complete spinal lesion at T13 i.e. two levels below the first one and at the level where we made our complete spinal lesions in previous studies (Barbeau and Rossignol 1987, Bélanger et al. 1996, Chau et al. 1998a) (Fig. 2). The main idea of this paradigm is that if intrinsic changes occurred within the

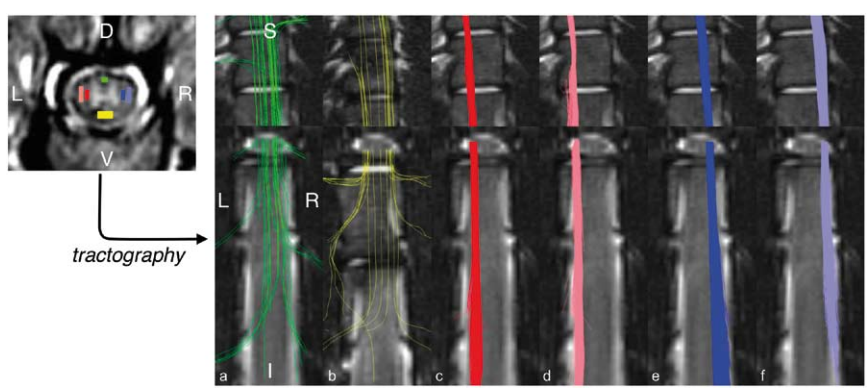

Fig. 7 Selective tractography showing dorsal column (a), ventral column (b) and lateral tracts (c–f). The regions of interest (ROI) used for the tractography (seed points) are shown on the top right panel. Top ROI includes dorsal columns, bottom ROI includes ventral columns, both lateral-median ROIs overlap the corticospinal tracts and the rubrospinal tract, both most lateral ROIs overlap the dorso-ventral spinocerebellar tract, and part of the spinothalamic tract. (L) left, (R) right, (S) superior, (I) inferior, (D) dorsal, (V) ventral. (Modified from Cohen-Adad J, Benali H, Hoge RD, Rossignol S (2008) In vivo DTI of the healthy and injured cat spinal cord at high spatial and angular resolution. NeuroImage 40 (2), p. 685–697, (c) 2008 with permission from Elsevier).

spinal cord itself during locomotor recovery after the initial hemisection, these changes could probably be retained and expressed very early after this second and complete spinalization a few segments below. The locomotor behavior of hemisected cats after this second spinalization could thus be different from that of cats with a single complete spinalization. The first major finding was that within 24 hours (i.e. the first testing session) following complete spinalization, 6/11 cats (55%) expressed a bilateral hindlimb locomotion albeit with some left-right asymmetry, 3/11 expressed a unilateral pattern on the side of the previous hemisection and 2/11 were not able to walk (Martinez et al. 2011). It should be recall that under normal circumstances, the re-expression of locomotion requires 2–3 weeks of intense treadmill training in cats with a single complete spinalization (Barbeau and Rossignol 1987). As these cats were not trained to walk during the interim between the two lesions, these results demonstrate that, after hemisection, the spinal locomotor circuitry reorganizes spontaneously with a limited level of afferent inputs and within a short period of time (3 weeks).

To determine how the spinal circuits reorganize after hemisection, we investigated, over the entire dual lesion paradigm, the evolution of specific locomotor parameters mainly controlled by the spinal locomotor circuitry (Martinez et al. 2012b). By plotting the relationships between the cycle and its sub-phases at various treadmill speeds, we showed that the intrinsic structure of the step cycle was altered in both hindlimbs during the hemisected period such that the cycle period changed with speed by adapting the durations of both phases instead of only the stance phase (Halbertsma 1983, Frigon and Gossard 2009). Second, some asymmetries seen after hemisection were retained for several weeks after spinalization. Similarly, some changes that occurred in cutaneous reflexes after the partial lesion were also retained following spinalization (Frigon et al. 2009). This suggests a durable asymmetric reorganization at the spinal level resulting from the previous partial spinal lesion. The carry-over of changes that result from the previous partial SCI indicated that the spinal cord has an intrinsic capacity to be imprinted by past experiences.

However, can a spinal cord previously modified by past experience (such as a hemisection) again adapt to new demands or are these changes immutable? To address this question, we evaluated the effect of increasing the level of sensory inputs by providing cats with

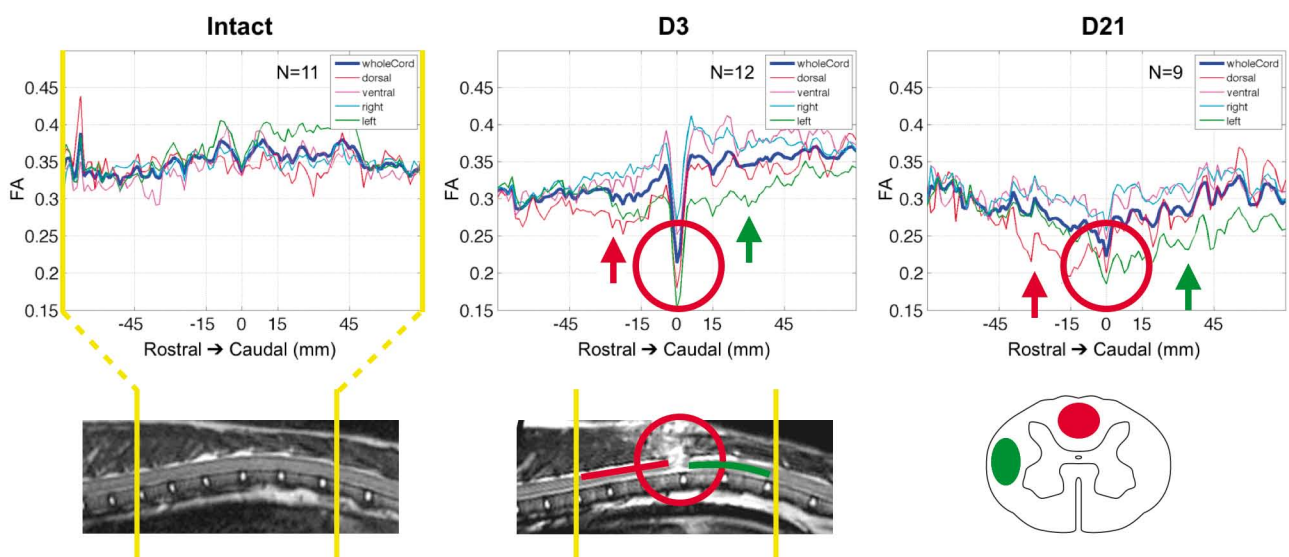

Fig. 8. Group results of quantitative tractography showing mean FA along the dorsal, ventral, right and left aspect of the spinal cord for the three sessions: Intact (left), D3 (middle) and D21 (right). The mean FA across quadrants is shown in thick blue line. Standard deviation is not shown for clarity purpose. Interestingly, lower FA is noticeable on the dorsal aspect rostrally to the lesion (red arrow), and on the left aspect caudally to the lesion (green arrow). These trends are both observed at D3 and D21 and may be associated with degeneration of ascending fibers rostral to the lesion, and of descending fibers caudally to the lesion.

treadmill training after the complete SCI (Martinez et al. 2012a). While in untrained cats the asymmetrical locomotion observed after hemisection was retained for 3 weeks after the complete SCI, training cats after spinalization reversed the direction of asymmetries (Fig. 3) suggesting that new plastic changes occurred within the spinal cord in response to training. Moreover, training cats after spinalization was shown to improve the locomotor performance of the hindlimb previously affected by the hemisection. These results demonstrate that a spinal cord previously modified by past experience (such as after an hemisection) can remarkably adapt to new demands imposed by increased sensory inputs and suggest that locomotor training can be beneficial regardless of the previous experience of the spinal cord.

Considering the role of locomotor training in shaping intraspinal plasticity in spinal cats previously submitted to hemisection, we evaluated whether the locomotor deficits and asymmetrical intraspinal changes observed after hemisection could be compensated by locomotor training. By using the dual lesion paradigm described above, we compared the locomotor capacities of 8 cats trained to walk for 3 weeks after hemisection and 8 untrained cats which served as control (Martinez et al. 2013). We showed that a 3-week period of locomotor training after hemisection promotes the recovery of voluntary quadrupedal locomotion and restores a symmetrical locomotor pattern (Fig. 4). Furthermore, locomotor training enhanced plasticity in the spinal cord below the lesion because 100% of the trained cats re-expressed a high level of bilateral hindlimb locomotion immediately after spinalization compared to 60% of untrained cats. This study highlights the beneficial role of locomotor training on facilitating adaptive plastic changes within the spinal circuitry and in promoting locomotor recovery after hemisection.

From these studies using kinematic and electromyographic approach in cat models of spinal cord injury, we demonstrated that (1) the spinal locomotor circuitry has a remarkable potential of plasticity; (2) this plasticity is involved in the recovery of locomotion after partial SCI; (3) locomotor training improves recovery by re-establishing an adequate left/right balance within the spinal circuits; (4) the spinal cord has not only the ability to be modified, but also to retain information even when disconnected from the brain.

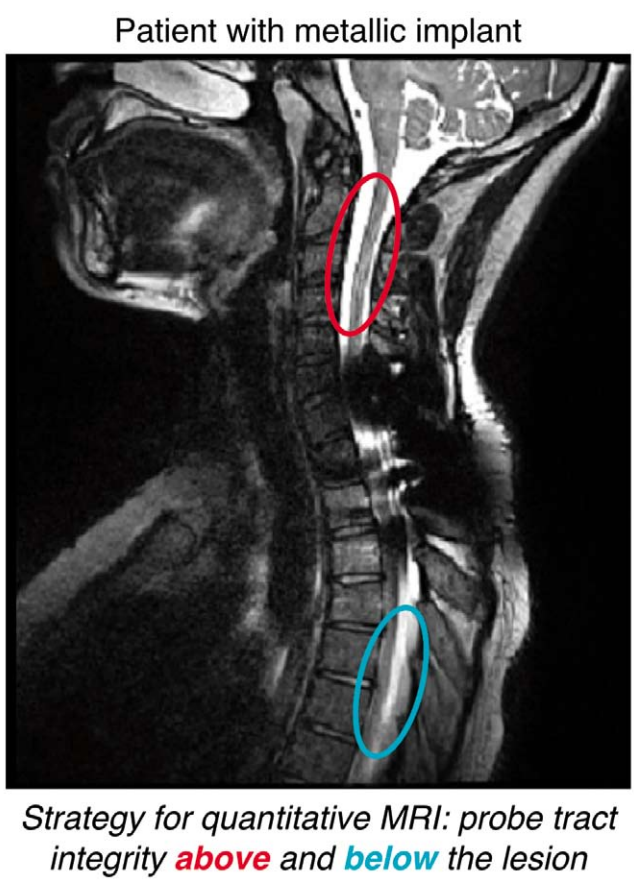

Fig. 9. Multi-parametric MRI of the spinal cord. Illustration of atrophy measurement, high-resolution images for precise location of pathologies, diffusion-weighted MRI and magnetization transfer imaging. Combining all these modalities takes less than 20 minutes of acquisitions on a 3T system and provides more insight into spinal cord damage.

IMAGING SPINAL CORD INJURY WITH MRI

While kinematic and electromyographic methods are particularly powerful to assess the time-course of recovery-related mechanisms after spinal cord injuries, the use of advanced magnetic resonance imaging techniques should provide a complementary approach in determining more precisely, in a non-invasive way, the extent of anatomical damage to the spinal cord. For instance, after a partial spinal lesion, the variability of recovery may depend not on the intended size of the surgical lesion but on the effective resulting size of the lesion. This may depend on several factors, namely secondary spinal injuries which cannot be controlled even with precise surgical lesions since these secondary changes depend on vascular changes and neuro-

toxic mechanisms. It was thus deemed important to develop non-invasive imaging techniques to evaluate the size of the lesions in cats that may be kept for several months after injury. It is important to assess this damage while the animals are still alive and while experimental course might be changed knowing that the lesions are either much bigger or much smaller than intended

CONVENTIONAL MRI AND ITS LIMITATIONS

Magnetic resonance imaging (MRI) can be used for assessing the extent and the location of SCI (Cadotte and Fehlings 2013). Moreover, since it is non-invasive, MRI enables longitudinal follow-up. Conventional imaging includes T_1, T_2 and proton density (Laule et al. 2007, Neema et al. 2007). These contrasts are based on intrinsic magnetic relaxation properties of the tissue and provide useful information to the clinician to assess tissue damage. For example, Figure 5 shows a T_1-weighted MRI image of a cat that experienced a dual-lesion paradigm as described in the previous paragraphs. Here, the locations of the primary sites of the hemi- and complete sections are clearly visible. What is less visible however is the degeneration resulting from these spinal sections. Because locomotor deficits are related to the type of pathways that have been damaged, it is important to be able to quantify their degeneration with high precision and sensitivity. Conventional MRI does not provide quantitative measures of microstructural damage and is poorly sensitive to this type of degeneration. For example, an increase of T_2 signal can be caused by edema, demyelination or hemorrhage. These limitations motivated the development of new MRI biomarkers such as diffusion tensor imaging.

DIFFUSION TENSOR IMAGING

Diffusion MRI measures the random microscopic motion (diffusion) of water protons (Stejskal and Tanner 1965). During the application of opposite magnetic gradients, static protons will produce a high spin-echo signal, while moving protons will have small residual transverse magnetization and hence will produce low signal. In white matter, the coherent orientation of myelinated axons causes water molecules to diffuse along axon bundles (Beaulieu and Allen 1994). This anisotropic diffusion is often modeled as a tensor (diffusion tensor imaging – DTI) (Basser and Pierpaoli 1996) and was shown to correlate with demyelination or axonal loss (Song et al. 2005, Klawiter et al. 2011). The example in Figure 6 shows a comparison between a conventional T_2-weighted MRI and a map of fractional anisotropy (FA) that was calculated from DTI. Although widely applied to the brain, DTI is challenging at the spinal level because of: (1) the small cross-sectional size of the spinal cord requiring higher spatial resolution and thus decreasing the signal-to-noise ratio (SNR), (2) physiological motions (respiration, cardiac), which can reduce image quality and (3) geo-

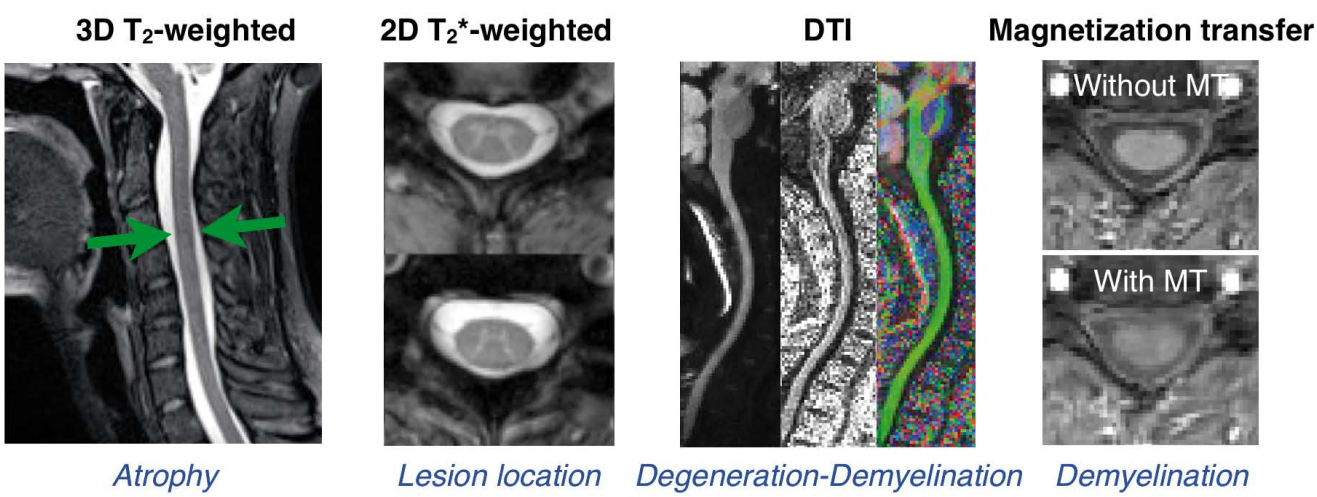

Fig. 10. Example of a patient with metallic implants. Since this is not feasible with the current hardware and sequence to obtain sufficient image quality at the site of the lesion, our strategy is to probe tract integrity above and below the lesion, assuming the presence of diffuse degeneration in case of spinal cord section or compression.

metric distortions arising from magnetic field inhomogeneities in nearby inter-vertebral disks and lungs (Kharbanda et al. 2006). Techniques such as reduced field of view (Wilm et al. 2007, Dowell et al. 2009) or parallel imaging (Griswold et al. 2002) can overcome this later issue.

DTI OF SPINAL CORD INJURY IN CATS

One goal of *in vivo* neuroimaging is the detection of neurodegenerative processes and anatomical reorganizations after SCI. Non-invasive examination of white matter fibers in the living spinal cord can be conducted using DTI. In one study (Cohen-Adad et al. 2008), DTI was performed *in vivo* in the healthy and injured spinal cord of five cats. High spatial resolution (1.1×1.1×1.1 mm^3) was used to isolate sub-segments of the spinal cord and to minimize partial volume effect between the spinal cord and the surrounding CSF. As a result, fiber tractography enabled the identification of various axonal trajectories including dorsal and ventral columns as well as lateral tracts (Fig. 7). Also, fiber bundles showed robust disruption at the site of spinal cord injuries (partial and complete) *via* tractography, accompanied with significantly lower fractional anisotropy values at the site of lesions. An important step forward was the *in vivo* assessment of axonal integrity following experimental SCI.

In another study (Cohen-Adad et al. 2011b), we acquired *in vivo* high angular resolution diffusion imaging data in cats submitted to partial SCI. Cats were imaged before, 3 and 21 days after injury. The goal here was to evaluate the sensitivity of DTI to detect primary and secondary lesions. Spatial resolution was 1.5x1.5x1.0 mm^3. Regions of interest were generated using tractography in the dorsal, ventral, right and left quadrants, to extract DTI metrics within each of these regions. Results showed significant changes of MRI metrics at the lesion epicenter (P<0.005). More interestingly, significant changes were also found several centimeters from the lesion epicenter at both 3 and 21 days (see Fig. 8). These changes were specific to the type of fibers, i.e.

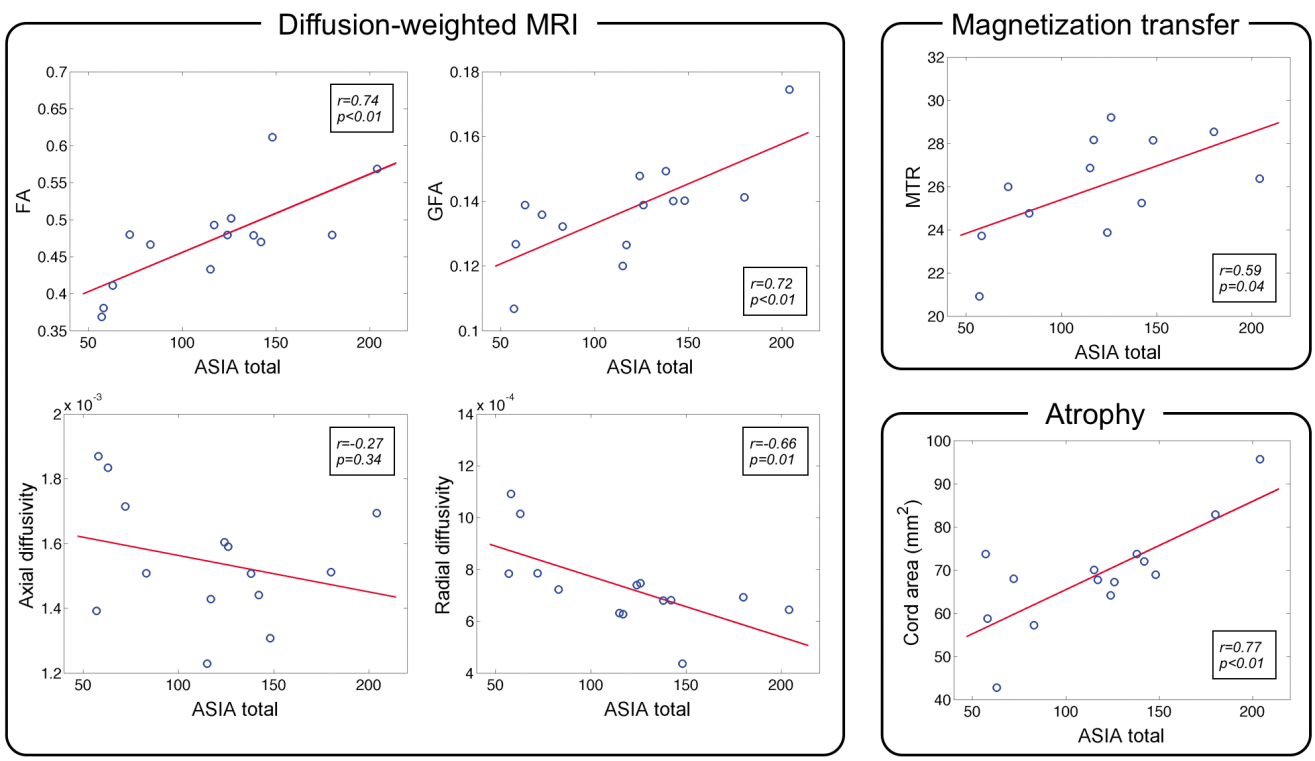

Fig. 11. Pearson's correlations between total clinical score (motor and sensorial) and MRI metrics. MRI metrics were averaged in the normal appearing spinal cord white matter of patients. (FA) Fractional anisotropy, (GFA) Generalized Fractional Anisotropy (calculated from q-ball imaging methods), axial and radial diffusivities are calculated from DTI. (Modified from Cohen-Adad J, El Mendili MM, Lehericy S, Pradat PF, Blancho S, Rossignol S, Benali H (2011) Demyelination and degeneration in the injured human spinal cord detected with diffusion and magnetization transfer MRI. NeuroImage 55 (3), p. 1024–1033, (c)2011, with permission form Elsevier.

rostrally to the lesion on the dorsal aspect of the cord and caudally to the lesion on the ipsilateral aspect of the spinal cord. These changes are in accordance with an anterograde Wallerian degeneration, as also been shown by other groups using diffusion MRI (Zhang et al. 2009, Farrell et al. 2010) .

MULTI-PARAMETRIC MRI OF SPINAL CORD INJURY IN HUMANS

As noted in the previous paragraphs, one limitation of DTI in the characterization of white matter integrity however, is the lack of specificity for determining demyelination and axonal loss. Several physical parameters can influence diffusion metrics including myelination, axonal density, axonal diameter, or orientation of fiber bundles (Beaulieu 2002, Sen and Basser 2005,). Therefore combining DTI with an independent measure that is sensitive to demyelination would increase the certainty of diagnosis. Multi-parametric MRI consists in combining several quantitative methods based on MRI, in order to gain confidence in assessing structural impairment after spinal cord injury. These methods include: atrophy measurement, high resolution images for precise location of pathologies, diffusion-weighted MRI and magnetization transfer imaging. Figure 9 illustrates these different types of measures and their specific role for assessing spinal cord damage.

Magnetization transfer describes the interaction between protons from free water and those bound to macromolecules (such as the lipids contained in myelin), thereby providing an indirect biomarker for myelin content (Kucharczyk et al. 1994, Pike et al. 2000). The magnetization transfer (MT) can be elicited by applying a pulse at a particular frequency, which aims at selectively saturating protons from macromolecules, but not protons from water. Doing so, the longitudinal magnetization of water trapped in myelin sheath is reduced, yielding lower signal during imaging. By taking the ratio of images with and without an off-resonance pulse, the MT ratio (MTR) can be measured, providing a useful semi-quantitative measure of myelination (Pike et al. 2000, Schmierer et al. 2004). Over the years, quantitative MT methods have been designed to provide more specific marker for myelin (Sled and Pike 2001, Levesque et al. 2010).

Translating these developments from cats to human, the combination of diffusion MRI and MT was performed in patients with SCI, in order to gain specificity in assessing demyelination and degeneration (Cohen-Adad et al. 2011a). Since most chronic SCI patients have metallic implants which cause large image artifact, the strategy used in this study was to probe the integrity of white matter tracts above and below the lesion, as illustrated in Figure 10.

Significant differences were detected between patients and controls in the normal-appearing white matter for fractional anisotropy (FA, $P<0.0001$), axial diffusivity ($P<0.05$), radial diffusivity ($P<0.05$), magnetization transfer ratio (MTR, $P<0.0001$) and cord area ($P<0.05$). No significant difference was detected in mean diffusivity ($P=0.41$), T1-weighted ($P=0.76$) and T2-weighted ($P=0.09$) signals. These metrics were remarkably well correlated with clinical disability (Pearson's correlations, FA: $P<0.01$, radial diffusivity: $P=0.01$, MTR: $P=0.04$ and atrophy: $P<0.01$), as illustrated in Figure 11. Stepwise linear regressions showed that measures of MTR in the dorsal spinal cord predicted the sensory disability (ASIA score) whereas measures of MTR in the ventro-lateral spinal cord predicted the motor disability. However, diffusion metrics were not specific to the sensorimotor scores. Due to the specificity of axial and radial diffusivity and MT measurements, results suggested the detection of demyelination and degeneration in SCI patients. The same methodology was applied to quantify the degree of degeneration in patients with amyotrophic lateral sclerosis (Cohen-Adad et al. 2013). Combining diffusion-weighted MRI, MT imaging and atrophy measures is a clinically feasible method and increases the specificity for the characterization spinal cord pathways in spinal cord pathologies.

CONCLUSION

This brief summary outlines the usefulness of using an integrative approach combining kinematics, electromyography and magnetic resonance imaging to accurately assess the reorganization and contribution of spinal circuits after spinal cord injuries. Our work highlights the importance of spinal mechanisms in locomotor recovery after complete and partial spinal lesions and the powerful effect of rehabilitative approaches in influencing these mechanisms. The innovative *in vivo* imaging techniques described herein are particularly useful to correlate spinal damages with the prognosis of recovery. Importantly, these techniques, which can be applied to humans, represent a major diagnostic tool for SCI patients.

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