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# Muscle Spindle Feedback Directs Locomotor Recovery and Circuit Reorganization after Spinal Cord Injury

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## SUMMARY

Spinal cord injuries alter motor function by disconnecting neural circuits above and below the lesion, rendering sensory inputs a primary source of direct external drive to neuronal networks caudal to the injury. Here, we studied mice lacking functional muscle spindle feedback to determine the role of this sensory channel in gait control and locomotor recovery after spinal cord injury. High-resolution kinematic analysis of intact mutant mice revealed proficient execution in basic locomotor tasks but poor performance in a precision task. After injury, wild-type mice spontaneously recovered basic locomotor function, whereas mice with deficient muscle spindle feedback failed to regain control over the hindlimb on the lesioned side. Virus-mediated tracing demonstrated that mutant mice exhibit defective rearrangements of descending circuits projecting to deprived spinal segments during recovery. Our findings reveal an essential role for muscle spindle feedback in directing basic locomotor recovery and facilitating circuit reorganization after spinal cord injury.

## INTRODUCTION

Spinal cord injury has an immediate and devastating impact on the control of movement. The origin of motor impairments lies in the physical disconnection of descending pathways from spinal circuits below the lesion, depriving them of synaptic input essential for the generation and regulation of motor output. Despite the failure of severed axons to regenerate at long distance (Ramon y Cajal, 1928; Tello, 1907), partial lesions of the human spinal cord are frequently associated with spontaneous functional improvement (Curt et al., 2008). One of many challenges in restoring motor control after spinal cord injury is to re-establish a sufficient level of task-specific excitability within disconnected local spinal circuits to drive motor neurons caudal to the injury.

Recent studies on incomplete spinal cord injury animal models uncovered some of the mechanisms that may contribute to spontaneous motor recovery (Ballermann and Fouad, 2006; Bareyre et al., 2004; Courtine et al., 2008; Jankowska and Edgley, 2006; Rosenzweig et al., 2010; Zörner et al., 2014). These investigations showed that recovery correlates with the establishment of intraspinal detour circuits. Such alternative pathways through the spared tissue form novel functional bridges across the lesioned spinal segments. At present, circuit-level mechanisms promoting the formation of detour circuits to restore control of movement remain elusive, even though such insight might play a pivotal role in developing interventions that enhance locomotor recovery after spinal cord injury.

Various studies suggest that sensory information plays a critical role in gait control and in locomotor recovery after spinal cord injury (Edgerton et al., 2008; Pearson, 2008; Rossignol et al., 2006; Rossignol and Frigon, 2011; Windhorst, 2007). The most common medical practice used to facilitate motor recovery of paraplegic patients is weight-supported locomotor rehabilitation (Dietz and Fouad, 2014; Knikou and Mummidisetty, 2014; Roy et al., 2012). Repetitive movement during rehabilitative training likely enhances glutamatergic dorsal root ganglia (DRG) sensory feedback, which constitutes the primary extrinsic source of excitation entering the spinal cord below injury to engage local spinal circuits. This interpretation is supported by evidence from animal models in which spinal cord injury coupled to partial or complete elimination of sensory input impairs gait control and locomotor recovery (Bouyer and Rossignol, 2003; Lavrov et al., 2008). However, the DRG neuron subtype promoting locomotor recovery and the mechanisms by which this process takes place are unclear.

Proprioceptive sensory neurons innervate sense organs in the muscle and transmit information about muscle contraction to the spinal cord (Brown, 1981; Rossignol et al., 2006; Windhorst, 2007). Their influence on the activity of central circuits is essential for modulation and adjustment of motor output (Pearson, 2008; Rossignol et al., 2006). Muscle spindle afferents constitute a subset of proprioceptors contacting muscle spindle sense organs. They exhibit the most widespread central projection pattern of all DRG sensory neurons and establish synaptic contacts with motor neurons and various classes of interneurons implicated in motor control (Brown, 1981; Eccles et al., 1957; Rossignol et al., 2006; Windhorst, 2007). Muscle spindle afferents are thus in a prime position to convey direct excitation to spinal circuits relevant to the regulation of motor behavior, especially under conditions of disconnected descending input.

The zinc-finger transcription factor Egr3 is expressed selectively by muscle spindle-intrinsic intrafusal muscle fibers, and its mutation results in early postnatal degeneration of muscle spindles (Tourtellotte and Milbrandt, 1998). This defect abolishes normal function of muscle spindle afferents as assessed electrophysiologically (Chen et al., 2002) and leads to gait ataxia (Tourtellotte and Milbrandt, 1998). *Egr3* mutant mice thus represent a genetic model with DRG sensory neuron dysfunction selectively restricted to muscle spindle afferents. They provide an opportunity to investigate how this feedback channel contributes to gait control in intact mice and influences locomotor recovery and circuit reorganization after spinal cord injury.

To address this question, we conducted kinematic analyses in wild-type and *Egr3* mutant mice. Deficiency of muscle spindle feedback did not affect basic motor abilities in intact *Egr3* mutant mice beyond specific gait features. However, lack of muscle spindle feedback severely restricted spontaneous recovery after incomplete spinal cord injury. *Egr3* mutant mice also exhibit a markedly reduced ability to establish descending detour circuits restoring access to spinal circuits below spinal cord injury. We conclude that muscle spindle feedback is a key neuronal substrate to direct circuit rearrangement necessary for locomotor recovery after incomplete spinal cord injury.

## RESULTS

### Proficient Basic Locomotion in Absence of Muscle Spindle Feedback

We performed high-resolution video recordings to reconstruct hindlimb kinematics in wild-type and *Egr3* mutant mice (Figures 1A and 1B). We focused on task-dependent contributions of muscle spindle input to hindlimb motor control with the aim to establish a baseline to which we could compare the locomotor recovery process after spinal cord injury.

We first assessed hindlimb motor control during basic overground locomotion. Wild-type and *Egr3* mutant mice performed this task with reciprocal activation of flexor and extensor muscles and alternation between left and right hindlimbs (Figure 1B; Movie S1 available online). However, *Egr3* mutant mice exhibited gait ataxia as reported previously (Tourtellotte and Milbrandt, 1998). To characterize gait patterns, we computed >100 kinematic parameters that provide a comprehensive quantification of locomotor features (Figure S1) (Courtine et al., 2008). We subjected all measured parameters to a principal component (PC) analysis (van den Brand et al., 2012) (averaged values of 10–25 step cycles/hindlimb/mouse; n = 22 wild-type and n = 19 *Egr3* mutants; Figure S2). We then visualized gait patterns in the new space created by PC1–3, where PC1 explained the highest variance (18%) and distinguished the two genotypes (Figure 1C). The locomotor phenotype observed in *Egr3* mutant mice was limited to distinct gait features represented in PC1 and approximately 65% of all parameters did not correlate with this genotype-specific PC1 (Figure S3A).

To evaluate the ability of *Egr3* mutant mice to adjust gait patterns to changing locomotor velocities, we tested mice during stepping on a treadmill. Both wild-type and *Egr3* mutant mice were capable of stepping across the entire range of tested treadmill speeds (7–23 cm/s; Figures 1D and S3B). PC1 captured adjustment of gait patterns with increasing speed in mice of both genotypes (16% of explained variance; Figures 1D and S3C), whereas PC2 segregated genotypic differences independent of velocity (10% explained variance; Figures 1D and S3C). Electromyogram (EMG) recordings of ankle extensor and flexor muscles revealed that both genotypes showed appropriate speed-dependent adjustments in burst duration (Figure 1D). These findings resonate with work demonstrating that the flexion phase of the step cycle remains constant, whereas the extension phase progressively shortens with increased locomotor speed (Arshavskiĭ et al., 1965; Halbertsma, 1983), a property we now demonstrate to be independent of muscle spindle sensory feedback.

In summary, both wild-type and *Egr3* mutant mice are able to perform basic locomotor tasks proficiently, but mutant mice display specific gait alterations concordant with the previously proposed role of muscle spindle feedback in control and adjustment of locomotion (Pearson, 2008; Rossignol et al., 2006; Windhorst, 2007).

### Muscle Spindle Feedback Is Essential for Locomotor Precision Task and Swimming

Next, we tested mice of both genotypes during walking on a horizontal ladder, requiring precision in foot placement. Whereas wild-type mice progressed across the ladder with ease, *Egr3* mutants frequently slipped off or missed rungs, which was reflected in aberrant bouts of EMG activity (Figure 2A; Movie S2). Quantification of foot positioning relative to successive rungs revealed that wild-type mice targeted rungs precisely, whereas *Egr3* mutant mice displayed near-random foot placement (Figure 2B). These findings demonstrate an essential role for muscle spindle feedback circuits in the regulation of accurate foot placement in a locomotor precision task.

*Egr3* mutant mice exhibit selective defects of muscle spindle feedback, but other sensory feedback is preserved (Tourtellotte and Milbrandt, 1998). During swimming, afferents from Golgi tendon organs are attenuated due to reduced weight load (Gruner and Altman, 1980). Proprioceptive signaling therefore relies almost exclusively on muscle spindle feedback. We found that during swimming, wild-type mice displayed well-coordinated alternation of left and right hindlimbs with reciprocal activity of ankle flexor and extensor muscles (Figures 2C and 2D). In contrast, *Egr3* mutant mice were unable to keep afloat and showed uncoordinated hindlimb movements with extensive coactivation of antagonistic muscles (Figures 2C and 2D). Together, these findings stress the pivotal function of muscle spindle feedback in the control of swimming, a condition when Golgi tendon organ and cutaneous feedback circuits only play a limited task-related function.