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Cortical neuroprosthesis-mediated functional ipsilateral control of locomotion in rats with spinal cord hemisection

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eLife Assessment

The contributions of ipsilateral cortical pathways to motor control are yet not fully understood. Here, the authors present **important** insights into their role in locomotion following unilateral spinal cord injury. Their data provide **convincing** evidence in rats that stimulation of ipsilateral motor cortex improves the injured side's ability to support weight and leads to improved locomotion, a result that may inspire new treatments for spinal or cerebral injuries.

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Abstract

Control of voluntary limb movement is predominantly attributed to the contralateral motor cortex. However, increasing evidence suggests the involvement of ipsilateral cortical networks in this process, especially in motor tasks requiring bilateral coordination, such as locomotion. In this study, we combined a unilateral thoracic spinal cord injury (SCI) with a cortical neuroprosthetic approach to investigate the functional role of the ipsilateral motor cortex in rat movement through spared contralesional pathways. Our findings reveal that in all SCI rats, stimulation of the ipsilesional motor cortex promoted a bilateral synergy. This synergy involved the elevation of the contralateral foot along with ipsilateral hindlimb extension. Additionally, in two out of seven animals, stimulation of a sub-region of the hindlimb motor cortex modulated ipsilateral hindlimb flexion. Importantly, ipsilateral cortical stimulation delivered after SCI immediately alleviated multiple locomotor and postural deficits, and this effect persisted after ablation of the homologous motor cortex. These results provide strong evidence of a causal link between cortical activation and precise ipsilateral control of hindlimb movement. This study has significant implications for the development of future neuroprosthetic technology and our understanding of motor control in the context of spinal cord injury.

Introduction

Cortical commands primarily regulate contralateral limb movements. This lateralization bias is reflected (1) anatomically, by a majority of crossed cortico-spinal tract (CST) projections (Hicks & D'Amato, 1975), (2) electrophysiologically, by a predominance of contralateral muscle recruitments by cortical stimulation (Kwan et al., 1978), (3) functionally, by contralateral deficits induced by cortical lesions (Passingham et al., 1983). However, lateralization of cortical control is incomplete, yet there is limited evidence on the functional significance of cortical ipsilateral regulation of movement (Montgomery et al., 2013). Ipsilateral impairments have been reported after unilateral cortical injury or transient interference (e.g., via transcranial magnetic stimulation) accompanied with increased cortical activity from the opposite hemisphere (Blasi et al., 2002; Chen, Gerloff, et al., 1997; Johansen-Berg et al., 2002; Jones et al., 1989; Kim et al., 2003; Marque et al., 1997; Yarosh et al., 2004). Nevertheless, the function of the ipsilateral motor cortex is unclear and its role in the recovery of motor control after injury remains controversial (Caramia et al., 2000; Chen, Cohen, et al., 1997; Dancause et al., 2006; Hallett, 2001; Hummel & Cohen, 2006; Jankowska & Edgley, 2006; Serrien et al., 2004; Stoeckel & Binkofski, 2010; Turton et al., 1996). While the most prominent feature of motor cortex pathways is their contralateral organization, unilateral or bilateral movements are well represented in the ipsilateral hemisphere (Aizawa et al., 1990; Ames & Churchland, 2019; Bundy et al., 2018; Cisek et al., 2003; Diedrichsen et al., 2013; Donchin et al., 1998; Ganguly et al., 2009; Ghacibeh et al., 2007; Heming et al., 2019; Kawashima et al., 1994; Merrick et al., 2022; Tinazzi & Zanette, 1998; Wisneski et al., 2008). Imaging studies have shown that lower extremities movements and walking, which require efficient bilateral coordination, are associated with bilateral activity in primary sensorimotor cortices and supplementary motor areas (Miyai et al., 2001). Yet cortical dynamics underlying locomotion have been primarily studied in relation to contralateral kinematics (Barroso et al., 2019; Bonizzato et al., 2018; Brown & Martinez, 2021; DiGiovanna et al., 2016; Song et al., 2009; Yin et al., 2014). The relationship between cortical commands and locomotion has received attention in the last decades (Amboni et al., 2013). In recent studies, we have shown that, after a unilateral spinal cord injury in rats (Bonizzato & Martinez, 2021) and large spinal contusion injuries in cats (Duguay et al., 2023), microstimulation delivered to the contralesional motor cortex in phase coherence with locomotion immediately alleviated contralateral hindlimb deficits. Other studies have shown that not only the cortex proactively controls high-level and goal-oriented motor planning but it is also involved during stereotyped locomotion (Artoni et al., 2017; Bretzner & Drew, 2005; Song & Giszter, 2011; Song et al., 2009). Nevertheless, there is still limited evidence showing that cortical networks can be interfaced to probe and augment control of hindlimb movements, especially with respect to ipsilateral cortical contribution.

To address this knowledge gap, we designed a behavioral neuromodulation framework to assess the gait-phase-specific effects of intracortical neurostimulation on ipsilateral hindlimb kinematics during locomotion. We evaluated the immediate modulation of hindlimb trajectory and posture both in intact rats and after a unilateral hemisection SCI. This side-specific lesion preserves most crossed projections from the ipsilateral motor cortex while maximizing the loss of direct efferences from the contralateral motor cortex. In most cases, this lesion also disrupts all spinal tracts descending on the same side as the cortex under investigation at the thoracic level, meaning that the transmission of cortical commands to the ipsilesional hindlimb must depend on crossed descending tracts (**Fig. S1**). As early as one week after injury, different modalities of ipsilateral cortical neuroprosthetic stimulation immediately alleviated SCI-induced deficits, including lack of hindlimb support, weak hindlimb extension and flexion, and dragging (see **Fig. S1** for a graphical representation).

Our functional causal approach to ipsilateral movement directly challenges the classical view whereby ipsilateral motor cortex control of movement is epiphenomenal and functionally limited. We demonstrate that the ipsilateral motor cortex has functional control of hindlimb motor synergies and that its action can reverse SCI locomotor deficits, independently from the homologous motor cortex. We then sought to provide a parallel description of the time course of ipsilateral cortico-spinal transmission and spontaneous recovery of locomotor function after SCI. We longitudinally acquired and scored hindlimb movements evoked by intracortical stimulation, obtaining chronic ipsilateral "motor maps" in awake rats. Finally, by unilaterally delivering longer cortical stimulation trains we show activation of bilateral flexion-extension rhythms, and that this control property is transiently lost and then recovered in our SCI model.

Results

In this study, we developed cortical neurostimulation protocols to investigate the role of the motor cortex in controlling ipsilateral hindlimb movements. Our primary objectives were to determine whether this stimulation could modulate ongoing locomotor patterns in intact conditions and immediately alleviate motor deficits following hemiparesis induced by a lateralized spinal cord injury (SCI). To achieve this, we applied intracortical stimulation in synchrony with specific gait phases, precisely timed to coincide with either the contralateral or ipsilateral foot lift. We used real-time processing of muscle activity to predict the timing of these gait events, as in our previous work (Bonizzato & Martinez, 2021). Our experimental model involved inducing selective unilateral hindlimb deficits through a thoracic lateral hemisection of the spinal cord (Brown & Martinez, 2019). This injury results in transient paralysis of the hindlimb on the side of the spinal lesion due to the loss of major supraspinal inputs. Importantly, the ipsilesional motor cortex, which corresponds to the left motor cortex and left leg in our study, retains most of its crossed connections to the sublesional spinal circuits.

For clarity, throughout the manuscript, we will use the terms "ipsilateral" and "ipsilesional" to refer to the left implanted motor cortex and the left leg, both located on the same side of the spinal hemisection. Conversely "contralateral" and "contralesional" will exclusively pertain to the right motor cortex and right leg. In brief, left = ipsi-; right = contra-.

Phase-coherent stimulation in intact rats

Online detection of muscle activation was used to predict gait events and consequently trigger short-train intracortical microstimulation (ICMS) (40 ms, 330Hz) through a 32-channel intracortical array implanted into the left motor cortex. The specific channel within the array was chosen based on its ability to generate the strongest contralateral ankle flexion. Stimulation through these channels produced a strong whole-leg flexion movement, with an evident distal component. From visual inspection, all responding electrodes in the array produced contralateral leg flexion, although with different strength of contraction for a fixed stimulation intensity (100μA). Moreover, some sites did not present a distal movement component, failing in eliciting ankle flexion and resulting in a generally weaker proximal flexion.

To assess the effects of intracortical microstimulation on leg trajectory and locomotor behavior, we conducted experiments with six intact rats (**Fig. 1A**). Our findings demonstrated that the modulation of gait was dependent on the timing of the stimulation. The most significant effects were observed when stimuli were delivered during the preparation and early execution of the right swing phase of gait. These effects included an increase in right hindlimb flexion (**Fig. 1B**).

Furthermore, when we synchronized the stimulation with the timing of the right foot lift (within ±75ms, referred to as 'phase-coherence,' as previously described in Bonizzato & Martinez, 2021), we observed specific alterations in gait patterns. These alterations included an increase in right

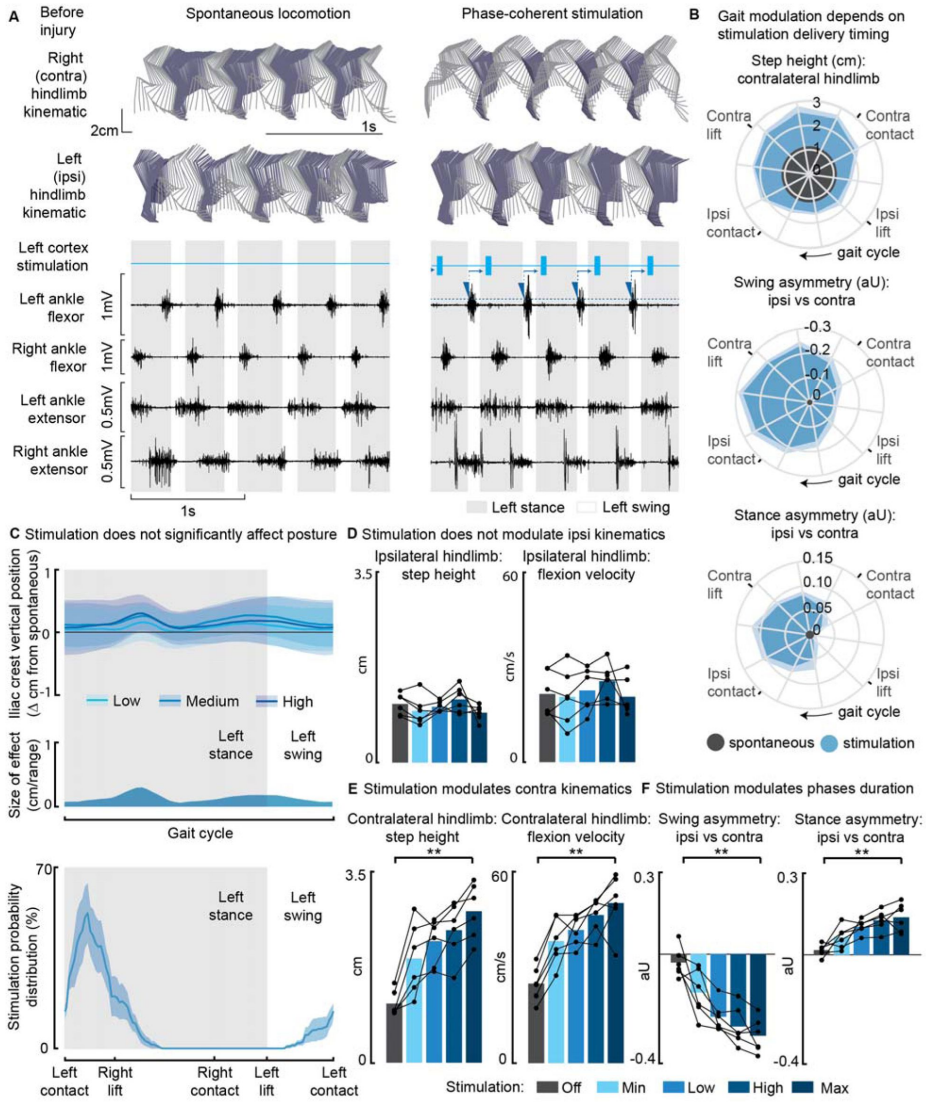

Fig. 1.

Phase-coherent intracortical stimulation modulated contralateral kinematics in intact rats (n=6 rats).

(A) Stick diagrams and electromyographic (EMG) activity during spontaneous locomotion and phase-coherent stimulation. The stimulation was triggered by ipsilateral ankle flexor activation and was delivered during the late contralateral stance (early ipsilateral stance). On the left ankle trace, the dotted line represents a threshold value for the ankle EMG to cause a trigger, represented by a blue triangle. The stimulation delivery follows a fixed delay indicated with an arrow. **(B)** Polar plots showing contralateral step height in cm and gait phase asymmetries in arbitrary units (aU, calculated as Δ% length) for stimulation delivered with different timings along the whole gait cycle. Positive asymmetry index values refer to ipsilateral-side dominance. For ease of reading, the radial axis of the swing symmetry plot has been inverted (outer values are negative). For the three polar plots, the most effective kinematic neuromodulation corresponds to the largest radius. The gait cycle progresses clockwise. **(C)** Analysis of the effects of cortical stimulation on the posture of rats (top) and experimental stimulation distribution (bottom). Posture is shown as the height of the ipsilateral iliac crest during the gait cycle, which was not modulated by increasing cortical stimulation amplitude, indicated as Low, Medium and High to represent 33, 66 and 100% of the functional stimulation range, defined from motor threshold to maximum comfortable stimulus. **(D)** Characterization of ipsilateral kinematics. Ipsilateral step height and flexion speed were not affected by increasing cortical stimulation amplitudes. **(E)** Modulation of contralateral kinematics. Contralateral step height and flexion speed were linearly increased with greater stimulation amplitudes. **(F)** Modulation of bilateral gait phase duration. The absolute values of swing and stance asymmetry indexes were linearly increased with greater stimulation amplitudes. Positive asymmetry index values refer to ipsilateral-side dominance. The data are represented as the mean ± SEM. ** $p < 0.01$.

step height (+119±37% of the spontaneous level, p=4E-4, t-test, phase-coherent vs. off-timing). Additionally, the gait pattern modifications resulted in a contralateral swing dominance (i.e., significantly longer than that of the opposite limb by +22±6%, p=0.03, t-test) and an ipsilateral stance dominance (+10±3%, p=0.01, t-test) (**Fig. 1B** and **S2B**).

In our investigation of the effects of modulating phase-coherent stimulation amplitudes, we observed that there were no discernible effects on posture (**Fig. 1C**) or ipsilateral kinematics (**Fig. 1D**) across all intact rats. However, the stimulation did have a notable impact on various aspects of contralateral limb movement during walking. Specifically, as we increased the stimulation amplitudes, we observed linear increases in several parameters related to contralateral hindlimb movement. These parameters included right step height (+157±13%, p=4E-5, t-test), flexion velocity (+107±21%, p=2E-4, t-test), and swing (30±3%, p=5E-4, t-test) and stance (14±2%, p=0.0026, t-test) asymmetry indexes. The relationships between stimulation amplitudes and these parameters were characterized by high variance accounted for (VAF) values, ranging from [79±5, 78±6, 80±4, 73±9]% (**Fig. 1E-F**).

Phase-coherent stimulation in SCI rats

We then tested the immediate impact of cortical stimulation in modulating locomotor output in seven rats, each exhibiting various hemisection profiles (**Fig 2A**, blue star) and ladder scores at week 1 (**Fig. 2B**).

Following a left spinal hemisection at the thoracic level (**Fig. 3A**), rats displayed ipsilateral hindlimb motor deficits corresponding to the same side as the lesion. About one week after the injury (5 to 10 days depending on the injury severity), once the animal had regained alternated hindlimb stepping (**Fig. 3B-C**), we assessed treadmill locomotion. The observed deficits included a lack of ipsilateral hindlimb support, as well as weaker ipsilateral flexion and extension, leading to asymmetries in the gait pattern (ipsilateral swing dominance 29±4% and right stance dominance 16±3%, **Fig. 3E**).

Phase-coherent stimulation of the ipsilateral motor cortex (see scheme in **Fig. 3A**) enhanced contralateral step height (**Fig. 3E**). This effect was behaviorally expressed as a bilateral synergy, characterized by a contralateral hindlimb flexion and an ipsilateral extension. Consequently, ipsilateral weight-bearing was intensified and prolonged, leading to a reversal of the motor deficits and the restoration of a balanced gait phase distribution between the ipsilateral and contralateral hindlimb (**Fig. 3B-C**, Video 1). The most significant effects were obtained when stimuli were delivered during the preparation and early execution of the right swing, similar to what was observed in the intact condition (**Fig. S2A**). These strong effects included an increase in contralateral step height (+94±43%, p=0.001, t-test, phase-coherent vs. off-timing stimuli) as well as a counterbalance between swing (p=2E-5, t-test) and stance durations (p=0.0017, t-test) effectively reversing the asymmetry deficit up to 116±11% and 115±9% respectively, compared to intact walking (**Fig. 3D** and **S2B**). When delivered during the late right or early ipsilateral stance, stimulation amplitude linearly (VAF=85±4%) modulated right step height (+172±30%, p=1E-4, t-test) and flexion velocity (+115±22%, p=7E-4, t-test, linear fit VAF=86±4%). In addition, the swing (deficit reversed up to 123±10%, p=0.0018, t-test, linear fit VAF=80±7%) and stance (deficit reversed up to 125±10%, p=9E-4, t-test, linear fit VAF=86±5%) asymmetry indexes proportionally decreased (**Fig. 3E**).

We confirmed that the modulation of ipsilateral hindlimb kinematics described in this experiment persisted even one month after SCI, with all effects in hindlimb extension and swing/stance asymmetry remaining consistent (**Fig. S3A-C**). The stimulation currents needed to achieve this modulation decreased over time (**Fig. S3D**), while electrode impedances generally remained stable (**Fig. S3E**).

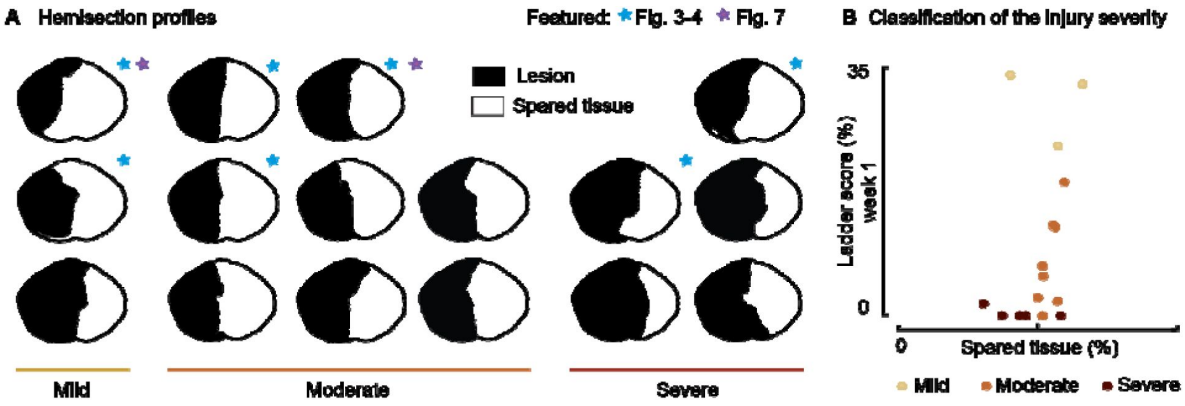

Fig. 2.

Spinal lesion severity (n=16 rats). Related to Fig. 3-4 and Fig. 7.

(A) Hemisection profiles at the epicenter level. **(B)** Classification of the injury severity. Injury severity groups were defined according to skilled locomotion performance during ladder crossing 7 days after injury. The injuries were classified as mild: left hindlimb > 20% correct paw placement, moderate: left hindlimb < 20% correct paw placement and right hindlimb > 75% correct paw placement and severe: right hindlimb < 75% correct paw placement (bilateral deficits).

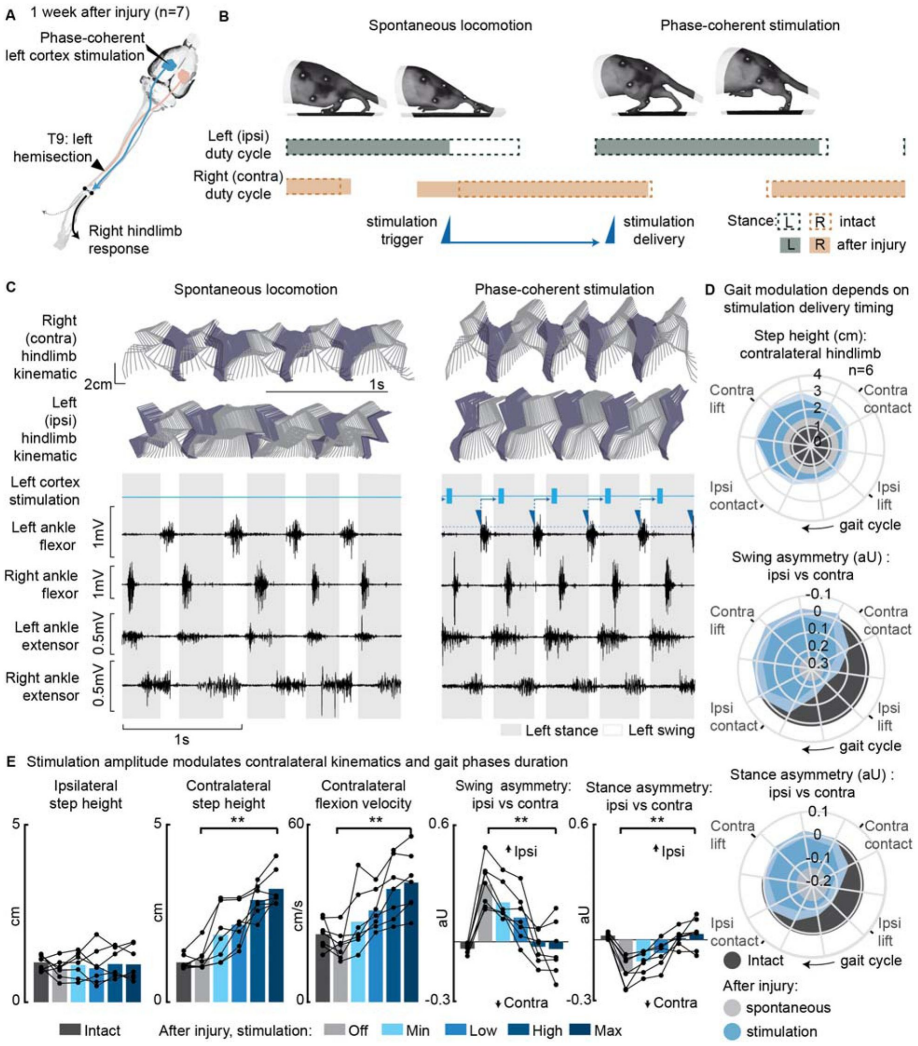

Fig. 3.

Phase-coherent intracortical stimulation alleviated locomotor deficits 1 week after injury (n=7 rats).

(A) A schematic representation of the injury and neurostimulation model showing the thoracic left hemisection (T9) and left (ipsilesional) motor cortex stimulation. **(B)** A schematic representation of spontaneous locomotion and phase-coherent stimulation effects on postural changes, gait phase duration and alternation as well as stimulation trigger and delivery timings. The stimulation, triggered in correspondence with the ipsilateral lift and delivered just before the contralateral lift, resulted in a stronger contralateral swing and a synchronous stronger ipsilateral stance. **(C)** Stick diagrams and EMG activity during spontaneous locomotion and phase-coherent stimulation. The stimulation was triggered by ipsilateral ankle flexor activation and was delivered during the late contralateral stance (early ipsilateral stance). **(D)** Polar plots showing contralateral step height (cm) and gait phase asymmetry variations (aU, calculated as Δ% length) for stimulation delivered at different timings along the whole gait cycle. Positive asymmetry index values refer to ipsilateral-side dominance. For ease of reading, the radial axis of the swing symmetry plot has been inverted (outer values are negative). For the three polar plots, the condition of strongest neuromodulation corresponded to the largest radius. Gait phase symmetry, highly affected during spontaneous locomotion, was recovered for stimulation delivered after the ipsilateral contact and before the contralateral contact. The gait cycle progresses clockwise. **(E)** The contralateral kinematics and gait phase durations were linearly modulated with increasing stimulation amplitudes. Positive asymmetry index values refer to ipsilateral-side dominance. Phase-coherent stimulation generated an increase in the step height and flexion speed of the contralateral hindlimb and mediated the recovery of the physiological symmetry between the ipsilateral and contralateral swing and stance phases. The data are represented as the mean ± SEM. ** $p < 0.01$. The hemisection profiles of the seven rats are identified by a blue star in **Fig. 2A**.

Next, we examined the effects of phase-coherent stimulation on muscle activity (**Fig. 4A**). Following the injury, the ipsilesional ankle extensor muscle exhibited significant alterations during spontaneous locomotion (-72±4% burst duration, p=0.007, t-test, -92±2% total activation, p=0.002, t-test, compared to intact conditions, **Fig. 4B**). However, phase-coherent stimulation reinstated the function of this muscle, leading to increased burst duration (recovered 90±18% of the lost burst duration, p=0.004, t-test, **Fig. 4B**) and total activation (recovered 56±13% of the total activation, p=0.014, t-test, **Fig. 3B**), with the degree of improvement linearly correlated with the applied stimulus amplitude (VAF=[84±7, 84±10]%).

After the injury, rats displayed a low posture due to the loss of weight acceptance on their ipsilateral hindlimb, and the severity of these postural deficits depended on the SCI severity (**Fig. 5A**). However, during the recovery process, postural compensation occurred, leading to a notably elevated posture one month after SCI (**Fig. 5B**). Phase-coherent stimulation, administered in the early ipsilateral stance phase, immediately alleviated postural deficits one week after injury, and the iliac crest height increased proportionally with higher stimulation amplitudes (p=0.03, t-test, VAF=76±9%, **Fig. 5C**).

Awake motor maps

In twelve awake rats allowed to spontaneously recover in their cage, we collected cortical maps, measuring from the ipsilesional motor cortex, which served as for measuring a proxy of cortico-spinal transmission to both hindlimbs for 8 weeks following SCI (**Fig. 6A**).

By visually inspecting the responses elicited by stimulation delivered through each of the array electrodes, we categorized movements as proximal or distal. This classification was based on whether the ankle participated in the evoked response or if the movement was restricted to the proximal hindlimb. Each leg was scored independently.

Initially, the injury led to a substantial decrease in cortico-spinal transmission on both sides: 5 days after the injury, the ipsilateral (left motor cortex to left hindlimb) and contralateral (left motor cortex to right hindlimb) transmission decreased by approximately -90±7% and -53±13% (p=[2 E-4, 5 E-4], Wilcoxon signed rank test, **Fig. 6B**), respectively. The size of the contralateral map (i.e., the number of responding sites on the implanted array) substantially increased by 2 weeks (+264±20%) and remained consolidated 8 weeks after injury (+250±21%, p=2E-4, Wilcoxon signed rank test, **Fig. 6B**). Over time, the representation of ipsilateral hindlimb movements significantly increased compared to the intact condition (+115±26%, p=0.002, Wilcoxon signed rank test, **Fig. 6B**).The upregulation of cortico-spinal transmission and postural changes during spontaneous locomotion predominantly occurred within the same timeframe, specifically 1 to 2 weeks after SCI (**Fig. 6A-D**). Between weeks 1 and 2, 91±22% of the overall postural correction (**Fig. 5B**, 6C) and 71±2% of the overall locomotor score recovery occurred (**Fig. 6D**). Furthermore, 83±7% of the increment in size of the ipsilateral map took place within the same time interval (**Fig. 6B**).

For each individual rat, the trend of locomotor score improvement measured during the first 3 weeks after SCI correlated with both ipsilateral (VAF: 84±3%) and contralateral (VAF: 82±4%) hindlimb representations in the left cortex. This result is consistent with our previous finding on the contralateral representation in the right cortex, the one opposite to the lesion side (VAF: 70±24%, Bonizzato & Martinez, 2021).

However, it is important to note that for any given day post-injury, the size of the left motor map across rats did not predict locomotor performance measured in an open field. The VAF was 3±2% for ipsilateral transmission and 8±4% for contralateral transmission (**Fig. 6E-G**), compared to 56±5% VAF when considering the opposite cortex (Bonizzato & Martinez, 2021). Additionally, the size of the left motor map did not correlate with lesion size (**Fig. 6F**), unlike the opposite cortex,

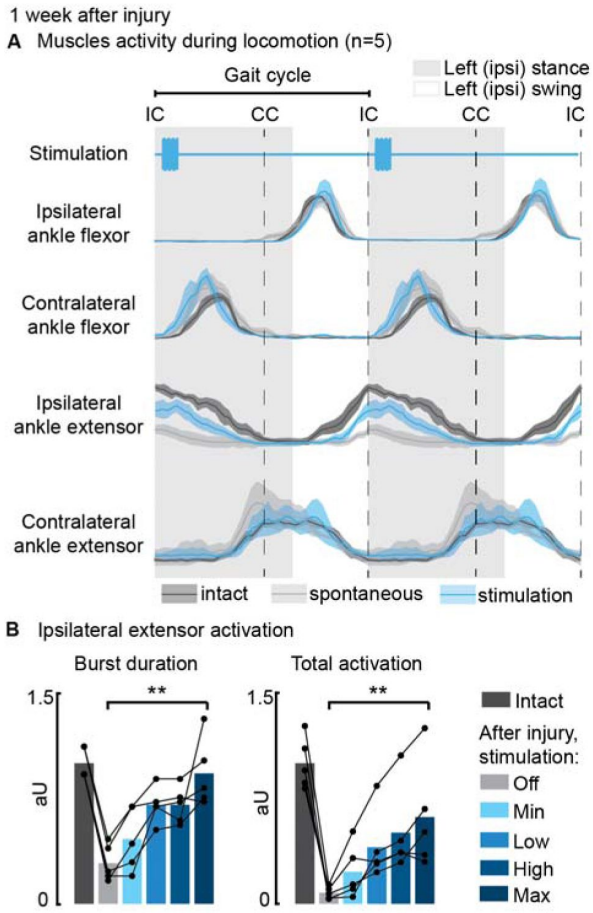

Fig. 4.

Phase-coherent intracortical stimulation reinstated ipsilateral extension muscle activity 1 week after injury (n=5 recordings for each muscle, from a total of 7 rats, with some muscles unavailable due to implant failure).

(A) EMG envelopes during spontaneous locomotion before and after injury as well as phase-coherent stimulation after injury. Activities of the ipsilateral and contralateral ankle flexor (tibialis anterior) and ipsilateral and contralateral ankle extensor (medial gastrocnemius). The gait event references are reported as LC: left contact, RC: right contact. (B) Left medial gastrocnemius activity was modulated by the stimulation. The burst duration and the level of muscle activation were linearly increased with greater stimulation amplitudes. The data are represented as the mean ± SEM. ** $p < 0.01$. The hemisection profiles of the seven rats are identified by a blue star in **Fig. 2A**.

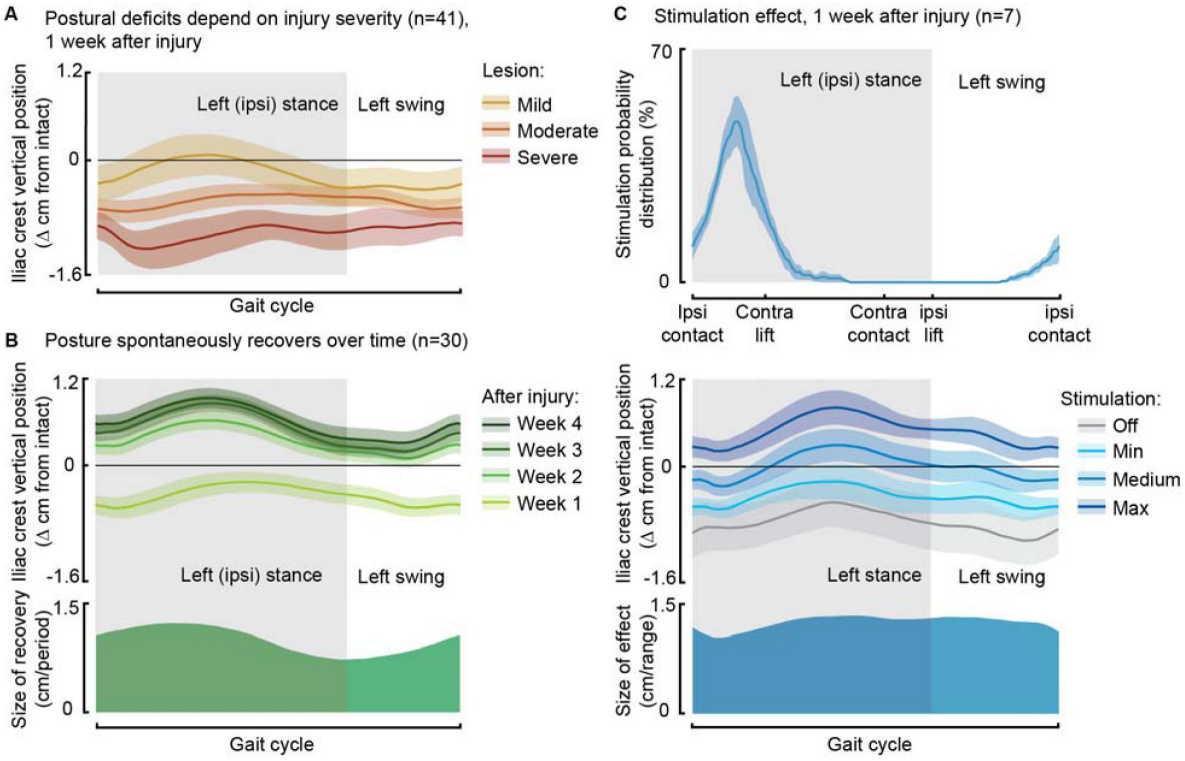

Fig. 5.

Phase-coherent stimulation improved posture 1 week after injury.

Posture is shown as the height of the ipsilateral iliac crest during locomotion with respect to the spontaneous condition before injury. The data are represented as the mean ± SEM. **(A)** Postural deficits depend on injury severity. Rats with severe SCI exhibit a weaker posture 1 week after injury. **(B)** Variation over 1 month of spontaneous recovery. Posture is raised and overcompensated. **(C)** Effect of phase-coherent stimulation 1 week after injury. Posture is increasingly raised with greater stimulation amplitudes. n=41, 30 or 7 rats, indicated in each panel.

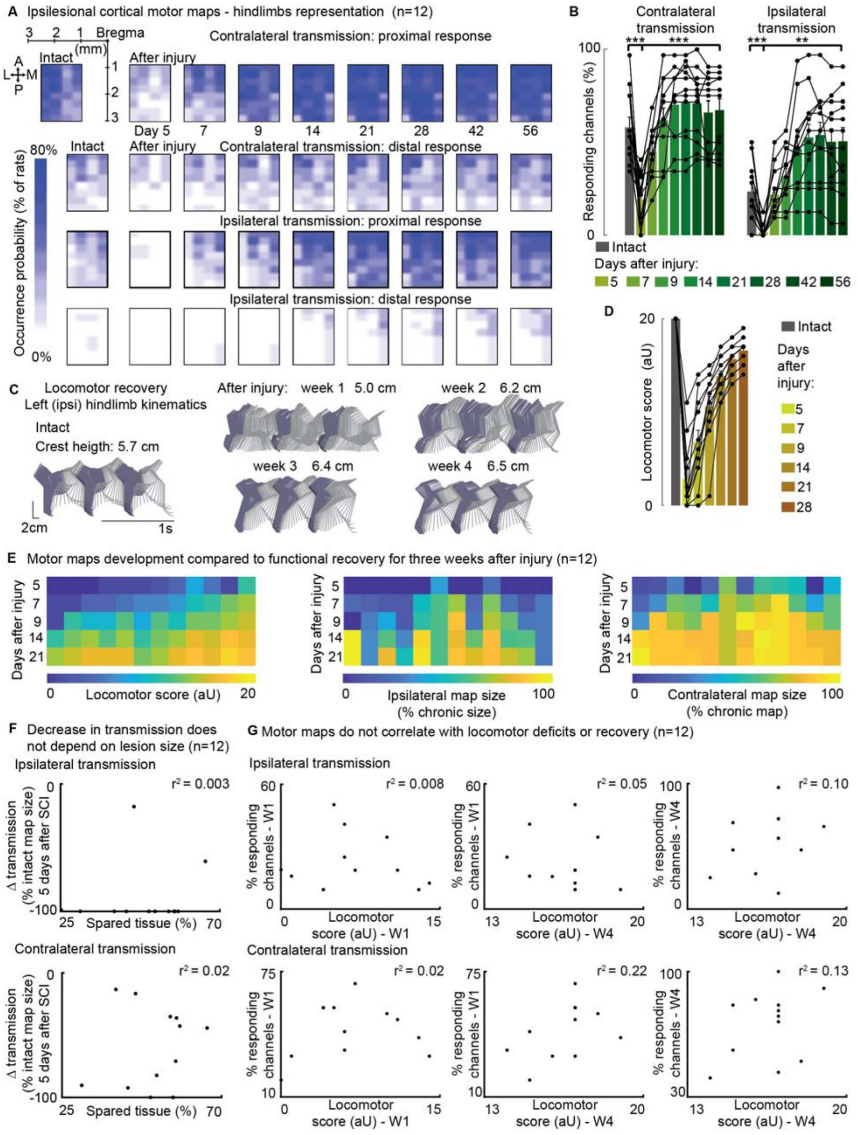

Fig. 6.

Ipsilateral motor representation of the affected hindlimb was increased in the ipsilesional motor cortex after injury but does not reflect functional recovery (n=12 rats).

The term 'transmission' in figure indicates a quantification of the number of responding sites within the array, which is the surface whereby a stimulus transmission to the muscles resulted in a visible hindlimb contraction. **(A)** Awake cortical motor map representation before injury and up to 2 months after injury. The color intensity is proportional to the probability of evoking proximal/distal ipsi-/contra-lateral responses when stimulating a given site, across twelve animals. Bilateral representation of hindlimb movements increased over time compared to the intact condition. The top left sub-panel carries a representation of the electrode array positioning within the left motor cortex. **(B)** Quantification of responding channels from the intact condition and up to 2 months after injury. **(C)** Stick diagrams from treadmill locomotion and iliac crest height before injury and during the first 4 weeks after injury. **(D)** Quantification of locomotor score from the intact condition and up to 1 month after injury. **(E)** Cortico-spinal transmission and locomotor performance. Single rats (columns) are sorted by day-7 locomotor score. The same sorting is maintained for the three sub-panels. Absolute map size (i.e., responding sites on the implanted array, indicated as % of the final, chronic, size) did not correlate with higher or lower locomotor scores across rats, whereas the individual rats' trend of map size increase for 3 weeks paralleled locomotor recovery. **(F)** Lack of correlation between map size and lesion size 5 days after injury. An ipsilateral and contralateral decrease in transmission did not parallel the spared tissue at the lesion epicenter. **(G)** Lack of correlation between map size and locomotor score. Time points are reported as W1: week 1 and W4: week 4. Ipsilateral and contralateral transmission did not correlate with global locomotor recovery measured in an open field. Bars: mean of individual data points.

where a correlation was observed (Bonizzato & Martinez, 2021). Overall, with a left hemisection, both the left and right cortical motor maps expand in size with a similar temporal pattern to locomotor recovery. However, the absolute size of the left motor map does not predict lesion size or locomotor deficits, whereas the right motor map does.

When assessing the correlation between ipsilateral motor maps and skilled locomotor performance on the ladder task, we found that the return of distal representations within the ipsilateral motor maps correlated with the recovery of fine motor control 3 weeks after SCI (**Fig. S4A**). This correlation disappeared four weeks after SCI (**Fig. S4B**) and was not observed when including proximal movements (**Fig. S4A-B**). Additionally, the representation of the ipsilateral limb was notably variable between subjects.

Ipsilateral neuromodulation of hindlimb flexion

Cortical control of hindlimb movements in behaving rats has been primarily associated with contralateral limb flexion and elevation (Bonizzato & Martinez, 2021; Bonizzato et al., 2018; Brown & Martinez, 2021; DiGiovanna et al., 2016; Rigosa et al., 2015). However, in our study, we observed a unique motor response in two out of the seven rats tested for phase-coherent stimulation (**Fig. 7A**, **Table S1**). Specifically, in these two rats, tested two weeks after SCI, stimulation of specific array sites within a medial area of the hindlimb motor cortex (1.1 mm mediolateral from Bregma, **Fig. 7D**) preferentially evoked ipsilateral flexor responses (**Fig. 7B-D**, rat#1: 3 channels with 271±36% ipsilateral dominance, rest of responding channels 43±4%, p=0.003, Wilcoxon rank sum test, rat#2: 6 channels 452±87%, all others 18±4%, Wilcoxon rank sum test, p=2E-4).The site with the highest ipsilateral dominance (rat#1: 327±109%, rat#2: 692±84%) was chosen to test the modulation of ipsilateral swing trajectories (**Fig. 7B**). Stimuli delivered during the late ipsilateral stance resulted in kinematic modulation: step height (+133±18,+99±23%, p=[1E-4,0.001], Wilcoxon rank sum test) and flexion velocity (+46±19,+101±19%, p=[0.01,1E-4], Wilcoxon rank sum test) increased linearly (rat#1 VAF=[90, 91]%, rat#2 VAF=[95, 86]%) with greater stimulation amplitudes (**Fig. 7E**, Video 2). As a result, dragging was immediately alleviated (-46±6, -100%, p=[1E-6,7E-4], Wilcoxon rank sum test). This result was unique for ipsi-dominant cortical sites; no other tested electrode produced ipsilateral flexion facilitation (see **Fig. 3E**). The sites produced a similar functional effect as contralesional cortical stimulation (Bonizzato & Martinez, 2021).

The ipsilesional motor cortex does not modulate ipsilesional movements through the homologous motor cortex

In three rats we combined thoracic unilateral SCI with a surgical ablation of the contralateral motor cortex (**Fig. 8A**) to determine whether contralateral cortical networks are necessary to ipsilateral hindlimb modulation. In all three rats, modulation of leg extension was readily obtained through phase-coherent ipsilateral cortical neurostimulation one week after SCI. All three rats displayed postural deficits that were immediately alleviated by phase-coherent stimulation of the ipsilesional motor cortex (**Fig. 8B-C**).

Cortical neuromodulation of hindlimb alternated rhythms

We next investigated whether long-train intracortical stimulation in awake, resting rats could evoke complex multi-modal motor responses (Graziano et al., 2002) and whether the effects on hindlimb movement are bilateral. The stimulation lasted 250ms, approximately matching the time scale of locomotor movement preparation and initiation (Bonizzato & Martinez, 2021). In six intact rats, we found that long-train stimulation of one motor cortex evoked locomotor-like rhythms (**Fig. 9A-B**, Video 3), characterized by bilateral alternated whole-leg movements.

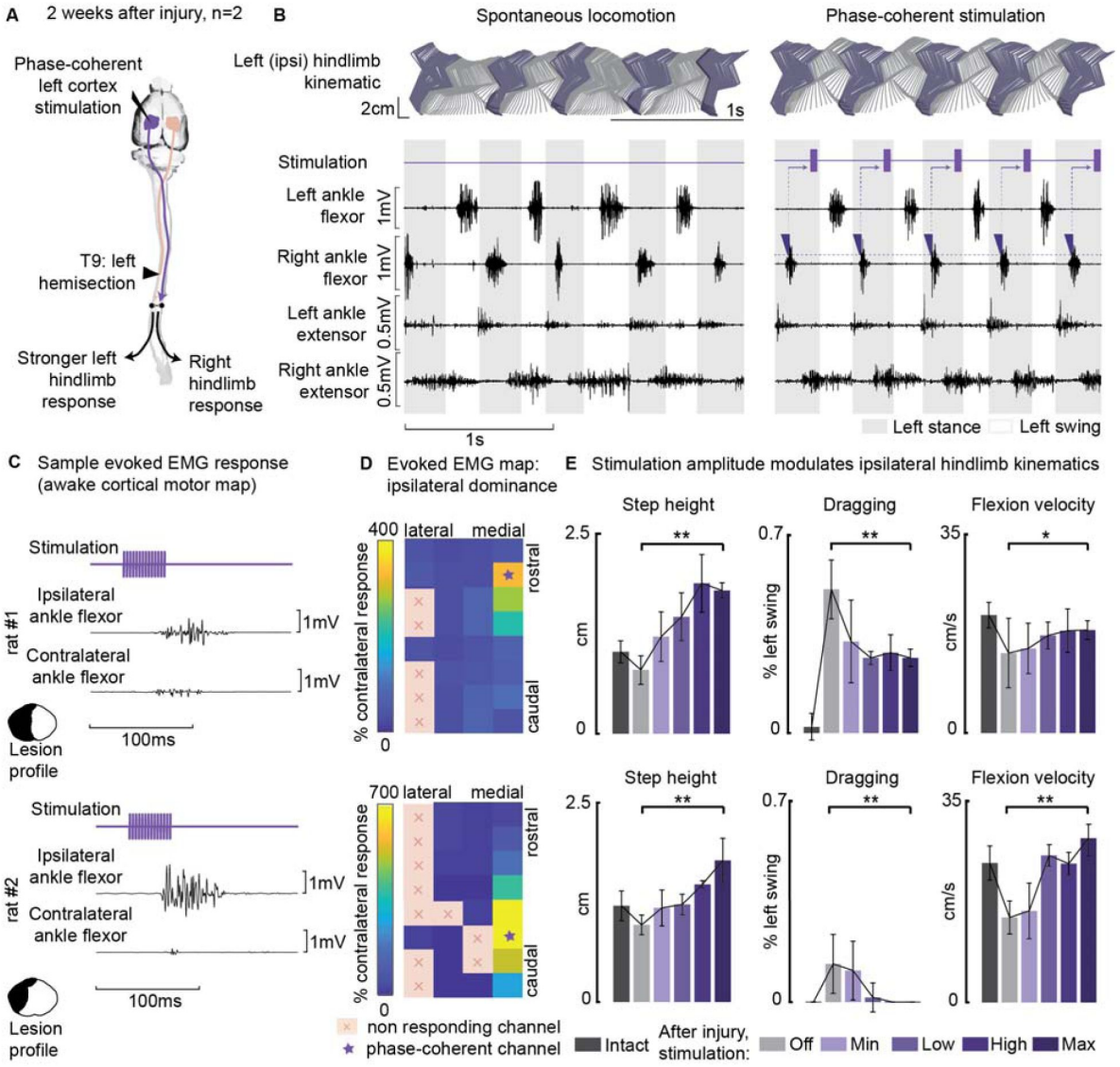

Fig. 7.

Ipsilesional motor cortex stimulation modulated ipsilateral hindlimb movements 2 weeks after injury (n=10 steps, experiment repeated in 2 rats).

(A) A schematic representation of the injury and neurostimulation model. After lateral hemisection, ipsilesional motor cortex stimulation evoked ipsilateral responses. **(B)** Stick diagrams and EMG activity during spontaneous locomotion and phase-coherent stimulation. The stimulation was triggered by contralateral ankle flexor activation and was delivered during the late ipsilateral stance. **(C)** Samples of single train stimulation of specific channels that preferentially evoked ipsilateral muscle activation in two different animals. **(D)** Ipsilateral dominance of EMG responses. Awake cortical motor maps were obtained as a ratio between ipsilateral and contralateral tibialis anterior activation. Channels that presented an ipsilateral preference were located in the most medial region of the map and identified by a star. **(E)** Phase-coherent stimulation modulated ipsilateral kinematics and reduced the foot drop deficit. Ipsilateral step height, flexion speed, and dragging alleviation linearly increased with greater stimulation amplitudes. Two subjects are presented independently, ten steps per condition. The hemisection profiles of the two rats are identified by a purple star in **Fig. 2A**. The data are represented as the mean ± SEM. *, ** $p < 0.05$ and < 0.01, respectively.

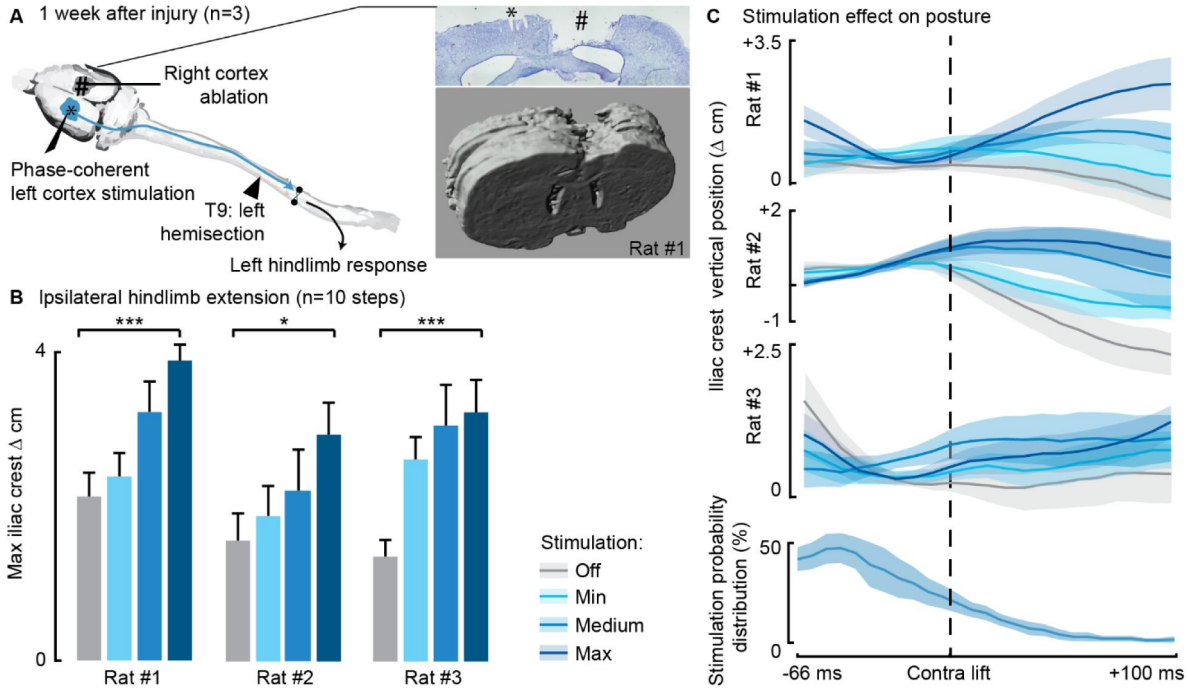

Fig. 8.

Phase-coherent stimulation of the ipsilesional motor cortex alleviated locomotor deficits even after ablation of the contralateral motor cortex (n=3 rats).

(A) Schematic representation of the injury and neurostimulation model. After lateral hemisection, ipsilesional motor cortex stimulation evoked ipsilateral responses, even when the contralateral motor cortex was ablated. Right inset: top, Cresyl violet staining of a coronal brain slice of rat #1. *, electrode traces in the left cortex. #, right cortex ablation. **(B-C)** Phase-coherent stimulation modulated ipsilateral kinematics. Posture linearly increased with greater stimulation amplitudes. Three rats are presented independently, ten steps per condition. The data are represented as the mean ± SEM. *, *** $p < 0.05$ and < 0.001, respectively.

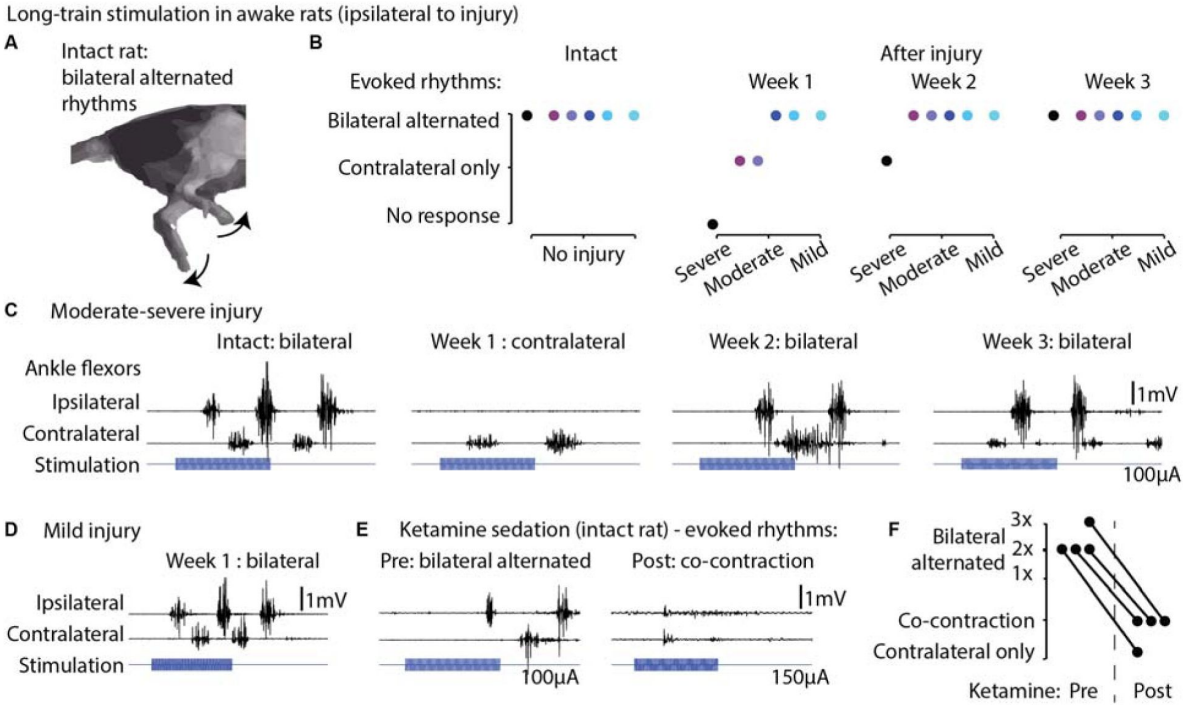

Fig. 9.

Long-train intracortical stimulation in awake rats elicited alternated bilateral rhythms.

(A) Schematic representation of the locomotor-like rhythmic movements evoked by long-train (250ms) cortical stimulation (amplitude 100 µA). Evoked rhythms are characterized by alternated hindlimb movements. **(B)** In six intact rats, stimulation of the left motor cortex generated bilateral alternated hindlimb rhythms. After SCI, rats are sorted by injury severity, using their ladder score at week 1 for ranking. One week after injury, long-train cortical stimulation failed to evoke bilateral alternated rhythms in half of the cohort. In two of these rats, contralateral rhythms were still present and bilateral alternated rhythms were recovered by week 2. In the most severe rat, contralateral-only rhythms were evoked on week 2 and bilateral alternated rhythms on week 3. For the remaining half of the cohort, long-train cortical stimulation recruited bilateral alternated rhythms at all tested time points. **(C)** Stimulus-synchronized ankle flexor EMG traces from one rat with a moderate-severe injury, showing loss (week 1) and following recovery (week 2-3) of ipsilateral evoked hindlimb rhythms. **(D)** Stimulus-synchronized EMG trace from one rat with mild injury, showing that bilateral alternated evoked rhythms are preserved at week 1. **(E)** Stimulus-synchronized EMG trace from four intact rats before and after ketamine sedation, showing transient loss of bilateral alternated rhythms. **(F)** Loss of bilateral alternated rhythms in four rats after ketamine sedation. 1X, 2X, 3X: number of complete repetitions of alternating movements produced by long-train cortical stimulation (amplitude 150 µA)

Subsequently, we assessed whether one week after unilateral hemisection SCI, long-train stimulation of the ipsilesional motor cortex could induce bilateral rhythms. We observed that in half of the tested rats with more severe injuries and lower ladder crossing performance, bilateral alternated locomotor-like rhythms did not emerge immediately after injury. However, by week 2 or 3 post-injury, these bilateral rhythms returned (**Fig. 9C**). In contrast, the remaining three rats with less severe injuries exhibited bilateral alternated hindlimb rhythms when receiving ipsilesional cortical stimulation as early as one week after injury (**Fig. 9D**). Classically, studies of cortical control and recovery of movement are often conducted under ketamine sedation (Brown & Martinez, 2018; Nudo, Wise, et al., 1996). To illustrate the well-known absence of rhythmic hindlimb activity after ketamine sedation, we tested and recorded four intact rats before and after ketamine injection, confirming the suppression of rhythmic hindlimb responses (**Fig. 9E-F**).

Discussion

A cortical neuroprosthesis facilitates the control of ipsilateral hindlimb extension

In this study we demonstrated that, after a lateralized SCI, the ipsilesional motor cortex (with most of its crossed efferences preserved) played a prominent role in controlling bilateral hindlimb movements. Our ipsilateral cortical neuroprosthesis effectively alleviated SCI-induced locomotor and postural deficits across different levels of injury severity (**Fig. 2**, **Table S1**), even after removal of the homologous motor cortex (**Fig. 8**). The lateralized lesion model and phase-coherent cortical stimulation revealed functional ipsilateral motor control. The evoked movement was characterized by contralateral hindlimb flexion accompanied by simultaneous ipsilateral hindlimb extension. Thus, the ipsilesional motor cortex can activate and influence bilateral lumbar synergistic networks through descending connections spared by the injury. We propose that the acute expression of this bilateral synergy (1 week after injury) is compatible with an adaptive or compensatory upregulation of pre-existing functional networks after SCI. Rapid onset of postural compensation is also displayed behaviorally by rats during the same timeframe (**Fig. 5B**). Although this outcome may reflect the participation of several supralesional networks, lateralized injuries highlight the role of the ipsilesional motor cortex in voluntary postural and weight-bearing adjustments. We hypothesize that this phenomenon is indicative of the necessity to preserve the functional role of the motor cortex in modulating contralateral step height during locomotion. In the absence of appropriate support from the opposite hindlimb due to the injury, the ability to elevate the foot would be compromised. Therefore, cortex-driven descending pathways may increase the excitatory transmission to ipsilesional extensor networks, thus facilitating the restoration of appropriated hindlimb support and precise functional control of contralateral step height. We postulate that this may represent either a demonstration of redundancy emerging with the lesion, and/or a specific compensatory strategy.

Ipsilesional motor map progression after SCI did not correlate with spontaneous recovery

After a unilateral cortical injury, plastic changes are observed in the opposite hemisphere (Axelson et al., 2013; Dancause et al., 2005; Mansoori et al., 2014; Rehme et al., 2012; Shimizu et al., 2002; Strens et al., 2003; Witte et al., 2000). Laterally unbalanced SCIs induce dynamic changes in the contralesional and ipsilesional motor cortex, which may participate in functional recovery or compensation mechanisms (Bonizzato & Martinez, 2021; Brown & Martinez, 2018; Brown & Martinez, 2021; Ghosh et al., 2010; Ghosh et al., 2009; Nishimura et al., 2007; Schmidlin et al., 2005). The relationship between map plasticity and motor recover is, however, complex: motor maps are static representations of a dynamic and modifiable system that is under the influence of experience (Milliken et al., 2013; Nudo, Milliken, et al., 1996; Oza & Giszter, 2015; Singleton et al., 2021) and interconnected circuits state (Ethier et al., 2007). Some

studies have shown that the reorganization of motor maps does not correlate with the time-course of behavioural recovery (Eisner-Janowicz et al., 2008; Nishibe et al., 2015; Plautz et al., 2023; Wang et al., 2010). In this study, we derived cortical maps in awake animals to investigate the time-course of ipsilateral transmission between the motor cortex and spinal circuits. The main advantages of awake mapping are twofold: first, this technique allows to longitudinally track motor cortex plasticity in the same animal; second, awake mapping unveils non-pyramidal transmission, which is suppressed by ketamine anesthesia (Bonizzato & Martinez, 2021). We tracked the progression of motor representation of both hindlimbs in the ipsilesional motor cortex and found that in all rats, cortico-spinal transmission significantly decreased after injury (**Fig. 6B**), independently from the subject-specific size of the injury (**Fig. 6F**). This finding is consistent with a major loss of connectivity, including damage to the uncrossed ventral CST (Weidner et al., 2001) and ipsilateral cortico-reticulo-spinal transmission (Bonizzato & Martinez, 2021). The loss of excitability quickly recovered within 2 weeks (**Fig. 6B**), with a return of cortico-spinal transmission consistent with the upregulation of the descending pathways spared by the injury. However, the subject-specific size of cortical motor maps did not correlate with the behaviorally expressed global motor performance measured in an open field (**Fig. 6G**). Conversely, we previously showed that contralesional cortical map size across subject correlated with locomotor recovery measured in an open field (Bonizzato & Martinez, 2021). Comparison of these two results suggested that recovery of hindlimb movement after SCI may be more tightly connected to changes in the contralateral cortical motor representation rather than the ipsilateral cortical motor representation, even in fully lateralized thoracic injuries, which disproportionally affect the crossed projections from the contralateral motor cortex. Nevertheless, we found that the return of ipsilateral distal transmission paralleled the recovery of fine motor control assessed in the ladder task, but this effect was transitory and restricted to the third week after SCI (**Fig. S4**). This is in line with our previous work showing that acute cortical inactivation immediately reinstated bilateral hindlimb deficits on the ladder task but only 3 weeks after SCI (Brown & Martinez, 2018). These combined results suggested that, although not a precise predictor of motor performance, the return of bilateral cortico-spinal transmission from the ipsilesional motor cortex after SCI is an important excitatory drive that supports bilateral skilled hindlimb movement. Given that bi-cortical interactions in shaping descending commands are established (Brus-Ramer et al., 2009), and considering the changes we observed in ipsilesional motor cortex excitability, the potential role of the ipsilateral cortex in mediating or supporting functional descending commands from the contralateral cortex -particularly in the immediate increase in flexion of the affected hindlimb and the long-term recovery of functional control (Bonizzato & Martinez, 2021)- warrants further investigation.

A cortical neuroprosthesis facilitates the control of ipsilateral hindlimb flexion

We observed a unique case of ipsilateral hindlimb flexion modulation in two rats that deserves specific consideration. These rats had arrays positioned at precisely the same brain coordinates ([1.1 mm posterior, 1.1.mm lateral] from bregma) and depth (1,5 mm). However, their lesion profiles were substantially different (see **Fig. 2A**, purple stars and **Table S1**). In one rat, all descending tracts were interrupted on one side while in the other rat, the lesion spared CST pathways and non-pyramidal ventral tracts. Interestingly, the remaining five rats (see **Fig. 2A**, blue stars), used to test immediate modulation of movement under cortical stimulation did not exhibit ipsilateral hindlimb flexion, despite having variable lesion profiles. Thus, we did not find a relationship between spared pathways on the lesioned side and the capacity to neuroprosthetically achieve ipsilateral flexion modulation. Additionally, all rats had their right hemicord preserved, suggesting that pathways traveling on the intact side are also unlikely to be involved in the observed results in two out of seven rats.

The localization of the specific channels closest to the interhemispheric fissure (**Fig. 7D**) may suggest the involvement of transcallosal interactions in mediating the transmission of the cortical command generated in the ipsilateral motor cortex (Brus-Ramer et al., 2009). While ablation experiments (**Fig. 8**) refute this hypothesis for ipsilateral extension control, they do not conclusively determine whether a different efferent pathway is involved in ipsilateral flexion control in this specific case.

As an alternative hypothesis to explain the low incidence of rats presenting electrodes evoking ipsilateral flexion, one might consider inter-individual variability in cortical motor maps. We know from our previous work that the localization of hindlimb motor maps varies between rats (around 0,5 mm medial from Bregma between rats) (Bonizzato & Martinez, 2021; Brown & Martinez, 2018; Brown & Martinez, 2021). Since the channels generating ipsilateral flexion were found to be the most medial in the map, it is possible that more of these responses could be obtained if the array was positioned more medially. However, performing the craniotomy and inserting the array at such coordinates would be challenging due to the risk to damage the superior sagittal sinus and inducing hemorrhage.

Further experiments are necessary to understand the mechanism(s) underlying this unconventional instance of cortical control of movement and whether they are mediated by cortical efferences, transcallosal communication, brainstem relays or spinal networks. A compelling research question arising from these results is whether similar findings can be found in the primate motor cortex.

A cortical neuroprosthesis unveiled ipsilateral functional control of movement

Numerous hypotheses have been proposed to explain ipsilateral motor cortical activity during movement, and our study contributes to this ongoing debate by establishing specific causal links in brain-behavior interactions (Silvanto & Pascual-Leone, 2012). These hypotheses include:

1. An abstract, limb-independent representation of movement (Porro et al., 2000).
2. The presence of an efference copy of signals generated by the contralateral motor cortex (Ganguly et al., 2009).
3. The existence of uncrossed descending connectivity (Brinkman & Kuypers, 1973; Nathan et al., 1990; Rosenzweig et al., 2009; Stecina & Jankowska, 2007; Weidner et al., 2001).
4. Bilateral termination of crossed descending connectivity (Becker et al., 2010; Lacroix et al., 2004; Rosenzweig et al., 2009).
5. Distributed (Ames & Churchland, 2019; Cisek et al., 2003; Li et al., 2016) or overlapping (Gazzaniga, 2000; Merrick et al., 2022; Parsons et al., 1998; Schaefer et al., 2007; Volpe et al., 1982) motor cortical computations across the two hemispheres.

Our study provides evidence of cortical-mediated control of functional, complex, and diverse ipsilateral movements in rats, even after the ablation of the homologous motor cortex. These findings challenge the view that ipsilateral motor cortex activity is solely epiphenomenal, a purely abstract representation, or a mere efference copy. Importantly, our results indicate that the complex bilaterality of cortical descending projections, as suggested in hypothesis (4), persists and does not rely solely on uncrossed pathways, as lateralized injury completely abolished all uncrossed descending connectivity in some animals while the observed effects persisted. This complexity highlights the intricate nature of neural control of movement and raises questions about the interplay of various neural pathways in motor control.

This hypothesis gains further supported from the observation that in rats, ipsilesional cortical inactivation immediately reinstates leg control deficits three weeks after hemisection (Brown & Martinez, 2018). While the mechanisms through which the ipsilateral motor cortex influences spinal circuits are likely multifaceted, several studies have proposed that the upregulation of indirect cortico-reticulospinal pathways, which are partially spared in our rats, may serve as a neural substrate for transmitting cortical drive (Asboth et al., 2018; Bonizzato & Martinez, 2021). Following hemisection SCI in rats, it has been proposed that the ipsilesional reticular formation could influence locomotor functions either through reciprocal connections with its contralesional counterpart (Zorner et al., 2014) or detour pathways involving relay interneurons within the spinal cord (Cowley et al., 2008). Given that the brainstem's reticular formation is known to control bilateral flexor and extensor synergies during locomotion, intracortical stimulation may gate the modulation of flexion and extension-related outputs of pattern generation in a phase-dependent manner through preserved cortico-spinal or cortico-reticulospinal pathways (Bretzner & Drew, 2005; Drew, 1991; Drew & Rossignol, 1984; Dyson et al., 2014; Fortier-Lebel et al., 2021; Lemieux & Bretzner, 2019). The recruitment of ipsilateral synergies through intracortical stimulation after spinal hemisection likely also involves spinal plasticity and changes in excitability (Martinez et al., 2011, 2012; Martinez et al., 2013). Spinal circuits contain sets of couple oscillators, one for flexion and one for extension, which are reciprocally connected but independently regulated (McCrea & Rybak, 2008). After a spinal hemisection, activity within extensors decreases on the lesion side while mirror effects occur in the intact limb, altering the balance between flexion and extension rhythms generators (Brown & Martinez, 2021; Martinez & Rossignol, 2013). These functional changes after hemisection are highly sensitive to activity within remaining pathways on the intact side of the spinal cord (Martinez et al., 2012), including residual corticospinal projections (Brown & Martinez, 2021; Brustein & Rossignol, 1998; Gorska et al., 1993). Therefore, increasing ipsilateral cortical drive with intracortical stimulation during locomotion may have recruit new synergies or uncover novel modes of locomotor control. Given that corticospinal-interneuronal connections primarily mediate movements and reflexes in adult rodents and primates (Lemon, 2008), corticospinal inputs mainly influence bilateral motor synergies through these interneurons. These interneurons are significantly affected by sensory inputs that are crucial in reflex pathways, essential for coordinating limb movements and maintaining posture. Mutual inhibitory interactions between interneuronal networks that control opposite actions, such as Ia inhibition of either flexors or extensors (Hultborn et al., 1976a, 1976b), as well as commissural interneurons connecting the left and right sides (Harrison et al., 1986), modulate motoneuronal activity and work together to coordinate movements between the left and right limbs and to manage crossed reflex loops. These circuits are sensitive to supraspinal inputs, and depending on the activation of these pathways, they may support various motor outputs, including motor learning, unskilled, and skilled movements.

However, it is essential to note a distinction between the specificity of the ipsilateral cortical role in functional motor control and motor recovery. Control and recovery are distinct physiological processes. While our results demonstrate the ability of the motor cortex to specifically control ipsilateral extension (**Fig. 1**, 3-5) and flexion (**Fig. 7**) movements linearly with increasing stimulation amplitudes, we did not observe a clear predictive relationship between changes in motor transmission from the ipsilesional cortex and functional measures of global locomotor recovery assessed in an open field (**Fig. 6**). In contrast, this type of analysis yielded positive results when applied to the contralateral motor cortex (Bonizzato & Martinez). Further investigation could clarify how corticospinal neurons contribute to the recruitment of ipsilateral spinal networks and the broader implications for motor behavior, particularly in the context of neuroprosthetic interventions and SCI recovery.

Long-train cortical stimulation recruits spinal locomotor circuits

The brief duration of the stimulus train typically used in phase-coherent stimulation experiments may limit the display of complete and coordinated movements that can be evoked and modulated by cortical networks when activated for longer periods (Baldwin et al., 2017; Baldwin et al., 2018; Bonazzi et al., 2013; Brown et al., 2022; Brown & Teskey, 2014; Graziano et al., 2002; Halley et al., 2020; Mayer et al., 2019). This limitation contrasts with the endogenous movement initiation process, which operates over hundreds of milliseconds (Bonizzato & Martinez, 2021). To address this, we employed long-train cortical stimulation in resting animals to elicit complex locomotor-like rhythms. These evoked movements exhibited high coordination bilaterally across the entire hindlimb system. While afferent inputs are known to play a crucial role in spinal locomotion (Alluin et al., 2015; Barthélemy et al., 2007; Slawinska et al., 2012), our study demonstrates that unilateral cortical drive can activate spinal locomotor circuits, leading to the generation of alternated "air-stepping" in awake rats even in the absence of cutaneous interaction with a ground surface. Furthermore, we observed that thoracic hemisection initially restricts the effects of cortical excitation to the unilateral generation of spinal rhythms. This suggests that cortical projections recruit independent rhythm-generating spinal units, which can be side-specific (Grillner & Wallen, 1985). However, the recovery of bilateral alternated rhythms within 2-3 weeks after hemisection implies changes within the spinal circuitry below the lesion, possibly mediated by the persistent interaction between commissural interneurons and efferences responsible for cortico-spinal transmission (Gossard et al., 2015; Martinez et al., 2011). The role of supraspinal drive on spinal locomotor circuits has been previously discussed with respect to "fictive" locomotion (decerebrate) preparations. In the cat, pyramidal stimulation was found to reset the locomotor rhythm by initiating bursts of activity in either extensor (Leblond et al., 2001) or flexor muscles (Orlovsky, 1972), but repetitive burst stimulation was required to evoke repeated hindlimb responses and structured them into a rhythm. This falls short to the rhythms-evoking capacity we demonstrated through long-train cortical stimulation in awake rats.

Ipsilateral cortical control of movement

Our findings reveal that brief, phasic cortical stimulation generates specific cortical commands, either for flexion or extension, and these commands are transmitted to both hindlimbs when applied during the appropriate phase of the locomotor cycle. In contrast, prolonged cortical stimulation activates spinal locomotor circuits, effectively converting unilateral cortical neuromodulation into a bilateral alternating output. This transformation demonstrates the complex executive relationship between the rodent motor cortex and spinal networks responsible for cortical initiation and modulation of ongoing movement. This interaction allows for a bilateral efferent transmission, effectively integrating and regulating spinal states. Movement generation involves the coordinated activity of distributed cortical, subcortical, brainstem, and spinal networks, each strongly interconnected with its contralateral counterpart. Multiple cortical networks contributing to movement generation have been shown to activate in a limb-independent manner. In the dorsal stream of visuomotor processing, for instance, the posterior parietal cortex contributes to grasping (Kermadi, 2000) or locomotor movements such as obstacle avoidance (Andujar et al., 2009), with neurons responding to both left and right limb movements predominating. Similarly, premotor cortical areas contain neurons that become activated during ipsilateral movement (Cisek et al., 2003; Kermadi, 2000; Michaels & Scherberger, 2018). Our results suggest that this bilaterality is not extinguished in the cortical line of sensorimotor integration. Instead, it is selectively preserved in the functional network properties of the primary motor cortex, the ultimate cortical actuator of movement.

Cortical neuroprostheses

These findings, in addition to shedding light on the intricate ipsilateral control of movement in rats, carry promising translational implications for the future development of neuroprosthetic solutions. Our previous work has demonstrated that phase-dependent cortical stimulation applied to the contralesional motor cortex immediately ameliorates dragging deficits following SCI by specifically enhancing contralateral hindlimb flexion (Bonizzato & Martinez, 2021; Duguay et al., 2023). Given that ipsilesional cortical stimulation induces a bilateral synergy, leading to the improvement of the affected limb's extension, this approach has the potential to effectively complement contralesional cortical stimulation. The ultimate aim would be to promptly reverse both postural and locomotor deficits associated with lateralized lesions. As per our previous study (Bonizzato & Martinez, 2021), future work could embed ipsilateral stimulation into rehabilitative training and evaluate its long-term impact over locomotor recovery. Since ipsilesional cortical stimulation immediately alleviated motor deficits in rats, and effects were maintained after the ablation of the contralateral cortex, it may also promote more efficient movement execution in individuals with lateralized SCI or hemiparesis due to cortical or subcortical stroke. Improved motor performance may lead to a broad range of potential benefits, including better and more sustained access to activity-based training. A limitation of this potential strategy is the invasive nature of the intracortical interface utilized in the rats. Less invasive solutions exist including transcranial magnetic stimulation, which requires further targeted research since (1) it has not yet been tested as a 'priming' agent for movement in the subacute phases of neurotrauma (Smith & Stinear, 2016) and (2) it is usually intended as an inhibitory agent for the non-lesioned cortex (Nowak et al., 2009), in line with the interhemispheric inhibition stroke model. A clear trade-off between invasiveness and efficacy of neurostimulation techniques needs to be established to determine which set of neurostimulation methods holds the potential to improve the generation of cortical motor commands in individuals with neurotrauma.

Materials and Methods

Experimental model and subject details

Animals

All procedures adhered to the guidelines established by the Canadian Council of Animal Care and received approval from the Comité de déontologie de l'expérimentation sur les animaux (CDEA), the animal ethics committee at Université de Montréal. A total of sixteen female Long-Evans rats (Charles River Laboratories, line 006, weighing between 270-350 g, as detailed in **Table S1**), were utilized for this study. Additional rats (n=25) were included in the analysis of spontaneous postural changes following injury (**Fig. 5A-B**).

Following an acclimatization period and habituation to handling, the rats were trained to ambulate on a motorized treadmill using positive reinforcement in the form of food rewards. Prior to surgery, the rats were housed by groups of three, but after implantation, they were housed individually. The blinding approach was not applicable in this case, as kinematic analysis was automatically conducted by DeepLabCut. The output data underwent curation to rectify any detection errors, and such corrections accounted for less than 0.5% of the conditioned points.

Study design

The number of animals used in this study was determined through a power analysis. The primary objective of this study was to assess the immediate effects of phase-coherent intracortical stimulation on modulating ipsilateral movements both before and after a unilateral SCI. The

specific aims were to use ipsilesional motor cortex stimulation to enhance the extension/stance phase and to improve weight support of the affected hindlimb after unilateral SCI. At the outset of the study, a pilot experiment involving two animals revealed that ipsilesional phase-coherent intracortical stimulation resulted in an increase of over 80% in the duration of the contralateral stance phase when compared to intra-subject variability. Based on this finding, a power analysis was conducted, which estimated a 97% probability of achieving statistically significant results (α=0.05) with a sample size of n=5 subjects and a 99% probability with n=6 subjects (one-sided, paired t-test). Initially, we characterized a total of six intact animals. For subjects with SCI, we expanded the sample size to n=7 to ensure an adequate power for electromyographic (EMG) investigations. Subsequently, after excluding data from recordings that exhibited poor signal quality, we conducted an EMG analysis with five animals for each muscle.

Method details

Surgical procedures

All surgical procedures were performed under isoflurane general anesthesia. Lidocaine (2%) was administered at the incision sites for local anesthesia. Analgesic (buprenorphine) and antibiotic (Baytril) medications were administrated for 3-4 days following surgery to ensure the animals' comfort and prevent infection.

During the initial surgery, we implanted the EMG electrodes and the intracortical array. Differential EMG wires were inserted into the left and right tibialis anterior and medial gastrocnemius muscles, while common ground wires were subcutaneously placed around the torso. A craniotomy was performed, and the dura mater was removed from the left motor cortex hindlimb area. Subsequently, a Tucker-Davis Technologies 32-channel array (consisting of 8 rows and 4 columns, measuring 1.125 x 1.75 mm) was inserted into cortical layer V at a depth of 1.5 mm, with the top-right site of the array positioned at coordinates [1.1 mm posterior, 1.1 mm lateral] from bregma. The EMG connector and intracortical array were then embedded in dental acrylic and secured on the head using four screws.

In the second surgery, spinal cord injury (SCI) was induced in the rats. This involved performing a partial T9 laminectomy and using 2% lidocaine to reduce spinal reflexes. Subsequently, a left spinal cord hemisection procedure was performed as described by (Brown & Martinez, 2019). In cases where rats experienced difficulty with micturition, their bladders were manually expressed for several days following the injury until they regained spontaneous control of micturition.

Behavioral assessments

The motor performance of the rats was assessed using three tasks: i) ladder crossing, ii) open-field, and iii) treadmill.

1. recorded at a frame rate of 100 frames per second while crossing a horizontal ladder measuring 130 cm in length, with rungs (3 mm diameter) regularly spaced at 2 cm intervals. In each session, trials involving consecutive steps were analyzed, and the results of five trials per rat were averaged. Each trial consisted of approximately 10 steps. The scoring system was based on the foot fault score (Metz & Whishaw, 2002). Seven days after the induction of the lesion, the performance was used as a reference to classify the severity of the animal's injury. Injuries were categorized based on the number of partial or correct paw placements on the rungs relative to the total number of steps (referred to as paw placements). Consequently, injuries were classified as follows: (1) mild (left hindlimb > 20% paw placement), (2) moderate (left hindlimb < 20% paw placement and right hindlimb > 75% paw placement), and (3) severe (bilateral deficit, right hindlimb < 75% paw placement).

2. To assess the spontaneous recovery of global locomotor and postural abilities, rats underwent evaluation in an open field using an adapted version of a neurological scoring scale originally developed for assessing locomotor function after cervical SCI (Brown & Martinez, 2019; Martinez et al., 2009). During this test, rats were recorded at a frame rate of 30 frames per second while engaging in 4 minutes of spontaneous locomotion within a circular Plexiglas arena with a diameter of 96 cm and wall height of 40 cm, featuring an anti-skid floor. The locomotor score was assigned based on the Martinez scale, which took into account specific parameters: 1) Articular movement amplitude of hip, knee, and ankle (0 = absent, 1 = slight, 2 = normal); 2) Stationary and active weight support of the limb (0 = absent, 1 = present); 3) Digit position of hindlimb (0 = flexed, 1 = atonic, 2 = extended); 4) Paw placement at initial contact (0 = dorsal, 1 = internal/external rotation, 2 = parallel); 5) Paw orientation during lift (1 = internal/external rotation, 2 = parallel); 6) Movement during swing (1 = irregular, 2 = regular); 7) Coordination between the fore- and hindlimb (0 = absent, 1 = occasional, 2 = frequent, 3 = consistent); and 8) tail position (0 = down, 1 = up) for a maximum of 20 points.
3. The treadmill task was employed to assess the effects of stimulation on hindlimb kinematics, posture, and muscular activity. Each trial involved the analysis of 10 consecutive steps, with the treadmill set at a speed of 23 cm/s. Kinematics were recorded at a rate of 119.2 Hz using six reflective markers placed on key anatomical points, including the iliac crest, trochanter, knee, fifth metatarsal, and fourth toe tip. The kinematic data were processed using DeepLabCut (Mathis et al., 2018) and underwent manual curation to correct any misidentifications. Gait analysis was subsequently performed to identify important locomotor performance parameters. *Stance* was defined as the phase of the gait between foot contact and the subsequent lift, while *swing* was defined as the phase between lift and the following foot contact. *Swing asymmetry* (left vs right) was defined as 1-(SwLeft/SwRight). *Stance asymmetry* (left vs right) was defined as 1-(StLeft/StRight). SwLeft or SwRight, StLeft or StRight indicate the duration of swing and stance phases, respectively. Negative values indicated that the left leg had a shorter duration than the right, while positive values indicated the opposite. *Flexion velocity* referred to the maximum vertical speed of the foot during hindlimb flexion occurring in the swing phase. *Step height* was calculated by subtracting the average vertical position of the foot during stance from its maximum vertical displacement during swing. Additionally, the posture of the rats was evaluated by measuring the height of the iliac crest during the gait cycle and comparing it to intact rats. It's important to note that the ladder and open-field scoring, as well as kinematic analysis, were conducted offline.

Awake motor maps

Motor maps were performed in awake animals, as in (Bonizzato & Martinez, 2021). In contrast to traditional cortical mapping, which is performed under ketamine anesthesia and during terminal experiments, we implanted 32-channel electrode arrays chronically within the motor cortex of rats. We monitored changes in cortico-spinal transmission by recording hindlimb movements evoked by intracortical stimulation. Awake mapping offers two primary advantages: firstly, it enables the longitudinal tracking of motor cortex plasticity in the same animal, and secondly, it reveals non-pyramidal transmission, which is suppressed by ketamine anesthesia (Bonizzato & Martinez, 2021).

The 32 channels cortical implant was connected to a 32-channel stimulator (Tucker–Davis Technologies). A 40-ms pulse train (330 Hz, biphasic, 200 µs/phase) was delivered to each site, and hindlimb responses were visually assessed while the animal was at rest and supported by trunk support. We initiated testing with stimulation amplitudes of 100 µA, evaluating the response type (proximal or distal), and identifying the minimum amplitude that evoked a visible twitch as the threshold value. Testing was interrupted when no response was detected. A joint motor map was constructed using data from twelve subjects, selecting the most frequent response for each site

across the population (**Fig. 6**). In the case of two rats, wherein specific channels predominantly evoked ipsilateral motor responses, we recorded EMG signals during an additional 10 rounds of testing for all channels. Following the normalization of each muscle activity to spontaneous locomotion, we quantified the ipsilateral dominance of muscle activation as the ratio of the left and right tibialis anterior evoked responses (**Fig. 7D**).

Phase-coherent cortical stimulation

The phase-coherent neurostimulation strategy has been previously described in detail (Bonizzato & Martinez, 2021). During treadmill locomotion, EMG activity was processed online, and a trigger event was detected when the signal crossed a manually selected activation threshold. Subsequently, a biphasic 40 ms train at 330 Hz was delivered with a specific delay. Among the 32 sites of the cortical array, the stimulation channel that evoked the strongest right hindlimb flexion (or left hindlimb flexion in the case of ipsilateral modulation) during motor maps assessment was chosen.

For amplitude characterization, the left flexor served as the synchronization signal and the delay was fixed, corresponding to 140-190 ms depending on the rat's gait pattern. In this protocol, the stimulation was delivered in the late right stance or the corresponding early left stance. The amplitude values were linearly spaced within a functional range, defined from a minimum visible effect (40-100 μA before injury, 25-70 μA after injury) to a maximum comfortable value for the animal (125-300 μA before injury, 70-200 μA after injury). For each episode of locomotion, a single, fixed stimulation amplitude was selected from within the defined range, and all amplitude values within the range were systematically tested in subsequent episodes.

Regarding timing characterization, synchronization was alternatively based on the right flexor and the left flexor or right extensor activity. The amplitude was fixed and equal to a medium value of the functional range. The delay varied among trials to ensure that stimulation complementarily covered the entire gait cycle (0-200 ms for the flexors, 80-280 ms for the extensor, in steps of 40 ms).

In specific cases involving ipsilateral kinematics modulation, the trigger was detected from the right flexor signal, the delay was fixed (160 ms, corresponding to late left stance), and the amplitudes varied within the functional range (lower bound 50 and 100 μA, upper bound 200 and 175 μA). Random permutation of trials was employed whenever possible in each characterization.

Long-train cortical stimulation

For each tested rat, we initially selected the cortical channel that elicited the strongest hindlimb responses through visual observation. A total of six awake resting rats were involved in the experiments. During these experiments, we provided manual support to the rats at the torso and forelimbs, allowing the hindlimbs to remain relaxed without any support. We recorded the hindlimb responses to long-train stimuli, which consisted of 250ms duration, 330 Hz frequency, biphasic pulses with cathodic first phases, and a pulse width of 200 μs/phase. These responses were captured using a camera recording at 120 Hz. In three of the rats, we also collected electromyography (EMG) data from both ankle flexor muscles (Tibialis Anterior) concurrently, with a sampling rate of 6 kHz. The stimulus amplitude for all long-train experiments was fixed at 100 μA, following established protocols (Brown & Teskey, 2014; Singleton et al., 2021). These experiments were conducted in both the intact state and weekly for three weeks after spinal cord injury (SCI). In addition, we administered a single dose of ketamine (120mg/kg, IP) to four intact rats to confirm the absence of alternated evoked rhythms under ketamine-induced sedation. These rats were tested 10 minutes after the ketamine injection, during a moderately sedated state where corneal and paw withdrawal reflexes were preserved, but no overt spontaneous movement occurred. In this ketamine-administered condition, a stimulus amplitude of 150 μA was used.

Current spread

To investigate the potential propagation of current to the homologous motor cortex, we conducted experiments involving the ablation of the contralateral motor cortex following SCI. We assessed the immediate effects of ipsilesional motor cortex stimulation on movement modulation and posture in three rats (**Fig. 8**).

Histology

At the conclusion of the experiments, euthanasia was performed on the rats using pentobarbital administration (Euthanyl, 100 mg/kg, intraperitoneal). Transcardiac perfusion was carried out using a 0.2% phosphate-buffered saline (PBS) solution, followed by a 4% paraformaldehyde (PFA) solution (pH 7.4). The spinal cords were then extracted and initially placed in a 4% PFA solution, followed by immersion in a 20% sucrose solution. To assess the extent of lesions, spinal sections around the T9 segment were sliced into 40 μm sections, and tissue damage was examined under a microscope. Lesion profiles at the epicenter level were reconstructed, and the extent of healthy and damaged tissue was quantified.

Quantification and statistical analyses

All results are presented as the mean value ± the standard error of the mean (SEM). The statistical analyses were conducted as follows: First, we assessed whether each population could be considered normally distributed or not. In cases where a population was trivially non-normally distributed (e.g., low amounts of dragging saturate to zero), non-parametric tests were applied. For all other cases, we did not make an automatic presumption of normality. Additionally, a one-sample Kolmogorov-Smirnov test was performed to test for normality.

After determining the distribution, statistical tests were carried out between populations. For all analyses where replicates were individual rats and both populations' normality could not be excluded, we used the paired Student's t-test. In cases involving other populations or where normality could be excluded, the Wilcoxon signed-rank test was employed for paired population samples.

In analyses where replicates were individual gait cycles and both populations' normality could not be excluded, we used the unpaired Student's t-test. For other populations or cases where normality could be excluded, the Wilcoxon rank-sum test was utilized for non-paired tests.

The specific test used is always indicated alongside the p-value. All tests were one-sided, as our hypotheses were strictly defined to predict motor improvement. Specifically, we hypothesized that delivering an extension-inducing stimulus would enhance leg extension, and delivering a flexion-inducing stimulus would enhance leg flexion. Consequently, any potentially statistically significant result in the opposite direction (e.g., inhibition) would not be considered. However, no such occurrences were observed.

The study was powered for comparison between no stimulation and maximum stimulation only, which was the only statistical comparison performed in each figure. When replicates were rats, power analysis assumed effect sizes of 2.5 times the sample variability, requiring n=5 for a $\beta=0.8$ probability of obtaining significant effects ($\alpha<0.05$). When replicates were single gait cycles, power analysis assumed effect sizes of 1.50 times the sample variability, necessitating n=7 for a $\beta=0.8$ probability of obtaining significant effects. However, ten gait cycles were used to provide extra room for unexpected variability. Intermediate stimulation values are reported to demonstrate proportionality and were assessed with linear fits, with adequacy measured using Variance Accounted For (VAF). Samples with $p < \alpha$ were considered statistically significant.

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Additional information

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Author contributions

M.B. and M.M. conceived the research; E.M., M.B. and M.M. designed the experiments; M.B. developed the overall system integration; E.M. and M.B. performed the surgeries and collected the experimental data; E.M., M.B., I.D.J., R.D., M.M. analyzed the data; E.M. and M.B. drafted the manuscript; E.M., M.B., M.M., edited, revised the manuscript and approved its final version.

Competing interests

M.B. and M.M. submitted an international patent application (U.S. No. 62/880,364) covering a device allowing performing coherent cortical stimulation during locomotion. They are also shareholders of a start-up company focused on developing neurostimulation technologies, 12576830 Canada Inc.

Supplemental figures

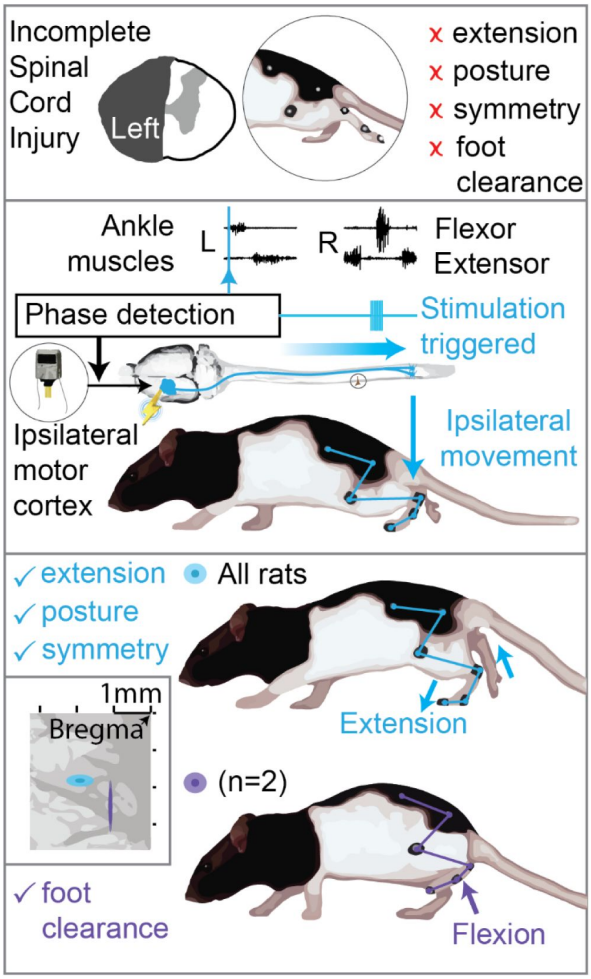

Fig. S1.

Graphical scheme summarizing the results. Related to Fig. 3 and Fig. 7.

Top: hemisection profiles at the epicenter level. Unilateral SCI causes leg flexion and extension deficits, affecting posture, symmetry and causing leg dragging. Middle: phase-coherent cortical stimulation is obtained by monitoring leg muscle activity and triggering cortical stimulation by reference muscle pattern recognition. In this work we utilize phase-coherent stimulation to unveil ipsilateral cortical control of movement. Bottom: ipsilateral phase-coherent cortical stimulation improves leg extension in all rats and flexion, alleviating foot drop, in two rats displaying a cluster of active sites with unique flexor properties.

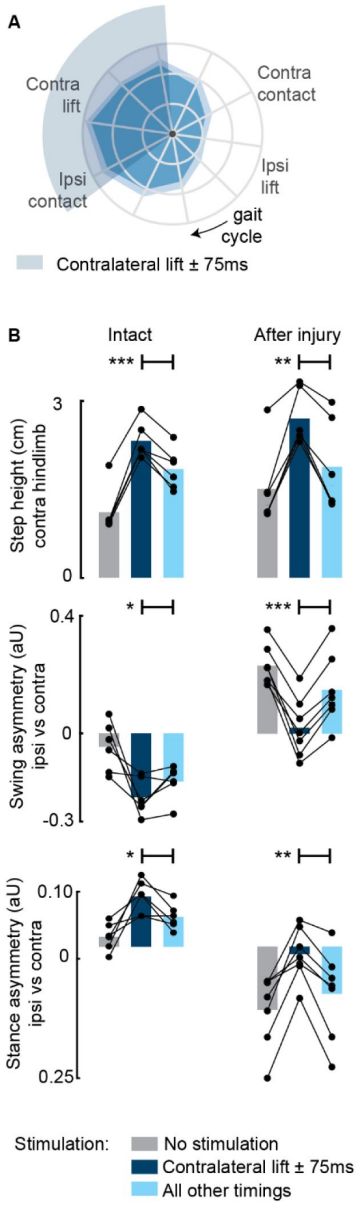

Fig. S2.

Aggregate timing characterization of phase-coherent stimulation (n=7 rats). Related to Fig. 3.

(A) Polar plots showing contralateral step height (cm) for stimulation delivered at different timings along the whole gait cycle. The contralateral lift start time ± 75ms is highlighted with a shaded area. The gait cycle progresses clockwise. **(B)** Contralateral step height was maximized by stimuli delivered around the time of the contralateral lift. Gait phase symmetry, highly affected during spontaneous locomotion, was recovered for stimulation delivered around the time of the contralateral lift. Positive asymmetry index values refer to left-side dominance. The data are represented as mean values and individual replicates. *, **, *** $p < 0.05$, $p<0.01$ and $p< 0.001$, respectively.

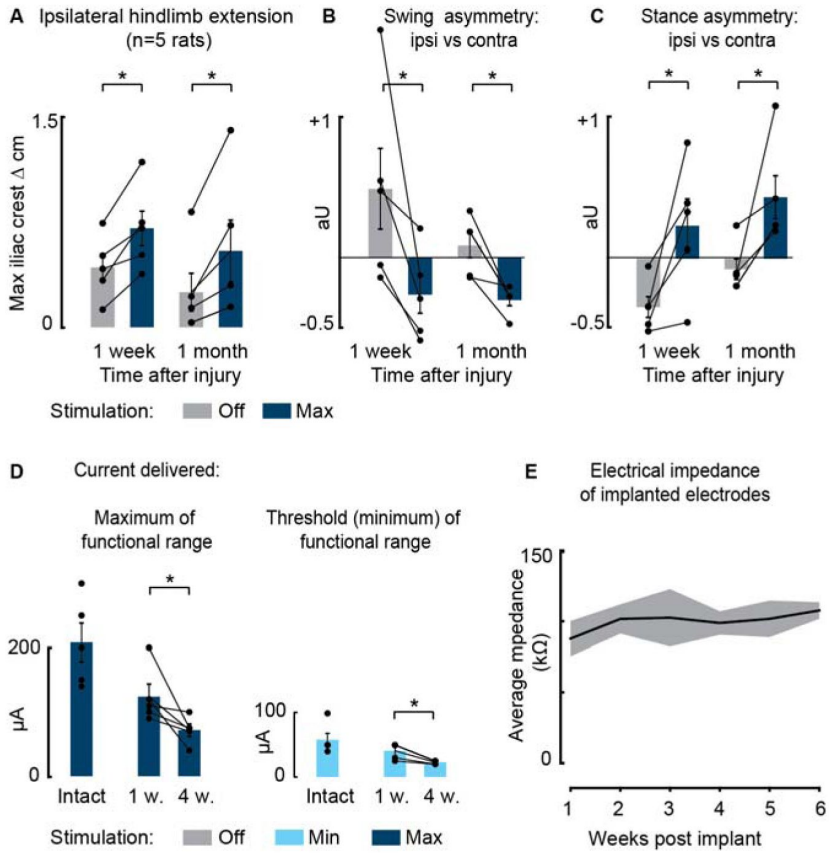

Fig. S3.

Persistent effects of phase-coherent cortical stimulation one month after SCI (n=5 rats). Related to Fig. 4.

(A-C) Comparison of maximal stimulation effects on posture **(A)**, swing asymmetry **(B)** and stance asymmetry **(C)**, one week vs. one month after SCI. **(D)** Current delivered to obtain ipsilateral modulation of movement (functional range from the minimal threshold value to visualize motor effect to the maximum value that the rat was able to integrate without discomfort). **(E)** Average impedance recorded at each electrode array over 6 weeks post implantation. Data are shown as mean ± SEM. *, $p < 0.05$.

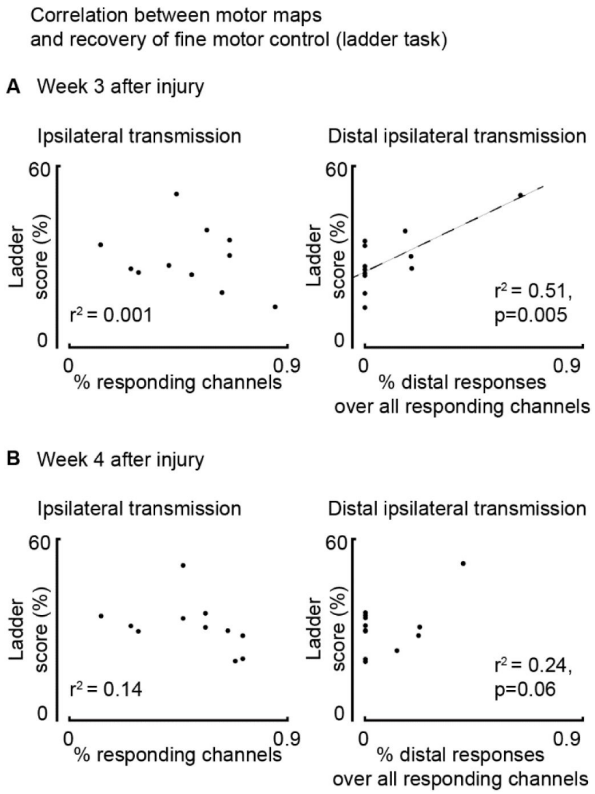

Fig. S4.

Assessment of correlation between motor maps and recovery of fine motor control evaluated as ladder score (n=12 rats).

Related to Fig. 6. (A) Correlation between map size and ladder score three weeks after injury. Return of ipsilateral distal transmission (but not general transmission: proximal and distal) paralleled recovery of fine motor control, as assessed by the ladder crossing task. **(B)** The same assessment performed four weeks after injury.

Rat #	Kinematics: intact rats (Fig. 1)	Kinematics: SCI rats (Fig. 3)	EMG analysis (Fig. 4)	Long-term efficacy (Fig. S3)	Posture: SCI severity (Fig. 5)	Posture: recovery (Fig. 5)	Motor maps (Fig. 6)	Ipsilateral modulation (Fig. 7)	Contra ablation (Fig. 8)	Long-train stimulation (Fig. 9)	Injury severity group
1	✓	✓	✓	✓	✓	✓	✓				moderate
2	✓	✓	✓	✓	✓	✓	✓				severe
3	✓				✓	✓	✓			✓	moderate
4	✓	✓	✓		✓		array failure				moderate
5	✓	✓	✓		✓		✓	✓		✓	mild
6	✓	✓	✓		✓		✓			✓	severe
7		✓	✓		✓		✓				mild
8		✓	✓		✓		✓	✓		✓	moderate
9					✓	✓	array failure			✓	moderate
10					✓	✓	✓			✓	moderate
11					✓	✓	✓				severe
12					✓	✓	✓				moderate
13					✓	✓	✓				mild
14					✓	✓	array failure				severe
15					✓	✓	array failure				moderate
16					✓	✓	✓				severe
17										ketamine	
18				✓						ketamine	
19				✓						ketamine	
20				✓						ketamine	
21									✓		
22									✓		
23									✓		
24*					✓						severe
25*					✓						moderate
26*					✓						moderate
27*											mild
28*					✓						moderate
29*					✓						moderate
30*					✓	✓					moderate
31*					✓	✓					mild
32*					✓	✓					moderate
33*					✓	✓					moderate
34*					✓	✓					moderate
35*					✓	✓					moderate
36*					✓	✓					mild
37*					✓	✓					moderate
38*					✓	✓					severe
39*					✓	✓					mild
40*					✓	✓					moderate
41*					✓	✓					moderate
42*					✓	✓					severe
43*					✓	✓					moderate
44*					✓	✓					moderate
45*					✓	✓					moderate
46*					✓	✓					moderate
47*					✓	✓					mild
48*					✓	✓					moderate

Table S1.

List of animals engaged in each experiment.

Rats marked with * did not receive left motor cortex implantation. They were included in the study for establishing spontaneous changes in posture over time (**Fig. 5A-B**).

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Reviewer #2 (Public review):

Summary:

The authors long term goals are to understand the utility of precisely phased cortex stimulation regimes on recovery of function after spinal cord injury (SCI). In prior work the authors explored effects of contralesion cortex stimulation. Here, they explore ipsilesion cortex stimulation in which the ipsilesion corticospinal fibers that cross at the pyramidal decussation are spared. The authors explore the effects of such stimulation in intact rats and rats with a hemisection lesion at thoracic level ipsilateral to the stimulated cortex. The appropriately phased microstimulation enhances contralateral flexion and ipsilateral extension, presumably through lumbar spinal cord crossed extension interneuron systems. This microstimulation improves weight bearing in the ipsilesion hindlimb soon after injury, before any normal recovery of function would be seen. The contralateral homologous cortex can be lesioned in intact rats without impacting the microstimulation effect on flexion and extension during gait. In two rats ipsilateral flexion responses are noted, but these are not clearly demonstrated to be independent of the contralateral homologous cortex remaining intact.

Strengths:

This paper adds to prior data on cortical microstimulation by the authors' laboratory in interesting ways. First, the strong effects of the spared crossed fibers from ipsi-lesional cortex in parts of the ipsi-lesion leg's step cycle and weight support function are solidly demonstrated. This raises the interesting possibility that stimulating contra-lesion cortex as reported previously may execute some of its effects through callosal coordination with the ipsi-lesion cortex tested here. This is also now discussed by the authors and may represent a significant aspect of these data. The authors demonstrate solidly that ablation of the contra-lesional cortex does not impede the effects reported here. I believe this has not been shown for the contra-lesional cortex microstimulation effects reported earlier, but I may be wrong. Effects and neuroprosthetic control of these effects are explored well in the ipsi-lesion cortex tests here.

Weaknesses:

Some data is based on only a few rats. For example (N=2) for ipsilateral flexion effects of microstimulation. N=3 for homologous cortex ablation, and only ipsi extension is tested it seems. However, these data clearly point the way and replication is likely.

Likely Impacts:

This data adds in significant ways to prior work by the authors, and an understanding of how phased stimulation in cortical neuroprosthetics may aid in recovery of function after SCI, especially if a few ambiguities in writing and interpretation are fully resolved.

https://doi.org/10.7554/eLife.92940.2.sa2

Reviewer #3 (Public review):

Summary:

This article aims to investigate the impact of neuroprosthesis (intracortical microstimulation) implanted unilaterally on the lesion side in the context of locomotor recovery following thoracic spinal hemisection.

Strength:

The study reveals that stimulating the left motor cortex, on the same side as the lesion, not only activates the expected right (contralateral) muscle activity but also influences unexpected muscle activity on the left (ipsilateral) side. These muscle activities resulted a substantial enhancement in lift during the swing phase of the contralateral limb and improved trunk-limb support for the ipsilateral limb. They used different experimental and stimulation condition to show the ipsilateral limb control evoked by the stimulation. This outcome holds significance, shedding light on the engagement of the contralateral-projecting corticospinal tract (CST) in activating a not only contralateral but also ipsilateral spinal network.

The experimental design and findings align with the investigation of the stimulation effect of contralateral projecting CSTs. They carefully examined the recovery of ipsilateral limb control with motor maps. And they also tested the effective sites of cortical stimulation. The study successfully demonstrates the impact of electrical stimulation on the contralateral projecting neurons on ipsilateral limb control during locomotion, as well as identifying importance stimulation spots for such effect. These results contribute to our understanding of how these neurons influence bilateral spinal circuitry. The study's findings contribute valuable insights to the broader neuroscience and rehabilitation communities.

Weakness:

The term "ipsilateral" lacks a clear definition in some cases, potentially causing confusion for the reader. Readers can potentially link ipsilateral cortical network to ipsilateral-projecting CSTs, which is less likely to play a role to ipsilateral limb control in this study since this tract is disrupted by the thoracic hemisection.

Specific comments:

Abstract: Line 1-4: Consider refining the initial sentences of the abstract to reduce ambiguity around the term 'ipsilateral lesion' and its potential conflation with ipsilateral projecting cortical neurons.

The abstract begins with 'Control of voluntary limb movement is predominantly attributed to the contralateral motor cortex.' This is followed by, 'However, increasing evidence suggests the involvement of ipsilateral cortical networks in this process, especially in motor tasks requiring bilateral coordination, such as locomotion.'

The phrase 'ipsilateral cortical networks' remains somewhat unclear. Readers may mistakenly interpret it as referring to the ipsilateral projecting corticospinal tract (CST), which is not the focus of this study.

Shifting the focus away from 'ipsilateral cortical control' and instead highlighting ipsilateral limb control following a spinal hemisection would improve clarity. This adjustment would also align the title and abstract more closely with the study's primary focus.

Introduction:
It is suggested to revise the introduction to more closely align with the study's experimental design and outcomes, placing emphasis on the stimulation effects observed in contralateral projecting tracts rather than implying a primary focus on ipsilateral projecting CST neurons.

Line 30-32: "Nevertheless, the function of the ipsilateral motor cortex is unclear and its role in the recovery of motor control after injury remains controversial. " This still gives the impression that ipsilateral projecting CST is the topic of the research here. Also, some of the cited references contains discuss ipsilateral projecting CSTs.

Line 34-36: "While the most prominent feature of motor cortex pathways is their contralateral organization, unilateral or bilateral movements are well represented in the ipsilateral hemisphere." This sentence is unclear to me. It would be helpful to specify what 'ipsilateral hemisphere' refers to-ipsilateral to what? Clarifying whether it's ipsilateral to the lesion or another reference point would make the statement more precise."

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Author response:

The following is the authors' response to the original reviews.

> ***eLife Assessment***
>
> *This manuscript reveals important insights into the role of ipsilateral descending pathways in locomotion, especially following unilateral spinal cord injury. The study provides solid evidence that this method improves the injured side's ability to support weight, and as such the findings may lead to new treatments for stroke, spinal cord injuries, or unilateral cerebral injuries. However, the methods and results need to be better detailed, and some of the statistical analysis enhanced.*

Thank you for your assessment. We incorporated various text improvements in the final version of the manuscript to address the weaknesses you have pointed out. The specific improvements are outlined below.

> ***Public Reviews:***
>
> ***Reviewer #1 (Public Review):***
>
> *Summary:*
>
> *This manuscript provides potentially important new information about ipsilateral cortical impact on locomotion. A number of issues need to be addressed.*

> *Strengths:*
>
> *The primary appeal and contribution of this manuscript are that it provides a range of different measures of ipsilateral cortical impact on locomotion in the setting of impaired contralateral control. While the pathways and mechanisms underlying these various measures are not fully defined and their functional impacts remain uncertain, they comprise a rich body of results that can inform and guide future efforts to understand cortical control of locomotion and to develop more effective rehabilitation protocols.*
>
> *Weaknesses:*
>
> *(1) The authors state that they used a cortical stimulation location that produced the largest ankle flexion response (lines 102-104). Did other stimulation locations always produce similar, but smaller responses (aside from the two rats that showed ipsilateral neuromodulation)? Was there any site-specific difference in response to stimulation location?*

We derived motor maps in each rat, akin to the representation depicted in Fig 6. In each rat, alternative cortical sites did, indeed, produce distal or proximal contralateral leg flexion responses. Distal responses were more likely to be evoked in the rostral portion of the array, similarly to proximal responses early after injury. This distribution in responses across different cortical sites is reported in this study (Fig. 6) and is consistent with our prior work. The Results section has been revised to provide additional clarification of the passage you indicated and context for the data presented in Figure 6:

On page 4, we have clarified: “Stimulation through these channels produced a strong whole-leg flexion movement, with an evident distal component. From visual inspection, all responding electrodes in the array produced contralateral leg flexion, although with different strength of contraction for a fixed stimulation intensity (100μA). Moreover, some sites did not present a distal movement component, failing in eliciting ankle flexion and resulting in a generally weaker proximal flexion.”

On page 12, we have further noted: “By visually inspecting the responses elicited by stimulation delivered through each of the array electrodes, we categorized movements as proximal or distal. This classification was based on whether the ankle participated in the evoked response or if the movement was restricted to the proximal hindlimb. Each leg was scored independently.”

> *(2) Figure 2: There does not appear to be a strong relationship between the percentage of spared tissue and the ladder score. For example, the animal with the mild injury (based on its ladder score) in the lower left corner of Figure 2A has less than 50% spared tissue, which is less spared tissue than in any animal other than the two severe injuries with the most tissue loss. Is it possible that the ladder test does not capture the deficits produced by this spinal cord injury? Have the authors looked for a region of the spinal cord that correlates better with the deficits that the ladder test produces? The extent of damage to the region at the base of the dorsal column containing the corticospinal tract would be an appropriate target area to quantify and compare with functional measures.*

In Fig. S6 of our 2021 publication "Bonizzato and Martinez, Science Translational Medicine", we investigated the predictive value of tissue sparing in specific sub-regions of the spinal cord for ladder performance. Among others, we examined the correlation between the accuracy of left leg ladder performance in the acute state and the preservation of the corticospinal tract (CST). Our results indicated that dorsal CST sparing serves as a mild predictor for ladder deficits, confirming the results obtained in this study.

> *(3) Lines 219-221: The authors state that "phase-coherent stimulation reinstated the function of this muscle, leading to increased burst duration (90{plus minus}18% of the deficit, p=0.004, t-test, Fig. 4B) and total activation (56{plus minus}13% of the deficit, p=0.014, t-test, Fig. 3B). This way of expressing the data is unclear. For example, the previous sentence states that after SCI, burst duration decreased by 72%. Does this mean that the burst duration after stimulation was 90% higher than the -72% level seen with SCI alone, i.e., 90% + -72% = +18%? Or does it mean that the stimulation recovered 90% of the portion of the burst duration that had been lost after SCI, i.e., -72% * (100%-90%)= -7%? The data in Figure 4 suggests the latter. It would be clearer to express both these SCI alone and SCI plus stimulation results in the text as a percent of the pre-SCI results, as done in Figure 4.*

Your assessment is correct; we intended to report that the stimulation recovered 90% of the portion of the burst duration that had been lost after SCI. This point has been clarified (see page 9):

"…leading to increased burst duration (recovered 90±18% of the lost burst duration, p=0.004, t-test, Fig. 4B) and total activation (recovered 56±13% of the total activation, p=0.014, t-test, Fig. 3B)"

> *(4) Lines 227-229: The authors claim that the phase-dependent stimulation effects in SCI rats are immediate, but they don't say how long it takes for these effects to be expressed. Are these effects evident in the response to the first stimulus train, or does it take seconds or minutes for the effects to be expressed? After the initial expression of these effects, are there any gradual changes in the responses over time, e.g., habituation or potentiation?*

The effects are immediately expressed at the very first occurrence of stimulation. We never tested a rat completely naïve to stimuli, as each treadmill session involves prior cortical mapping to identify a suitable active site for involvement in locomotor experiments. Yet, as demonstrated in Supplementary Video 1 accompanying our 2021 publication on contralateral effects of cortical stimulation, "Bonizzato and Martinez, Science Translational Medicine," the impact of phase-dependent cortical stimulation on movement modulation is instantaneous and ceases promptly upon discontinuation of the stimulation. We did not quantify potential gradual changes in responsiveness over time, but we cannot exclude that for long stimulation sessions (e.g., 30 min or more), stimulus amplitude may need to be slightly increased over time to compensate habituation.

> *(5) Awake motor maps (lines 250-277): The analysis of the motor maps appears to be based on measurements of the percentage of channels in which a response can be detected. This analytic approach seems incomplete in that it only assesses the spatial aspect of the cortical drive to the musculature. One channel could have a just-above-threshold response, while another could have a large response; in either case, the two channels would be treated as the same positive result. An additional analysis that takes response intensity into account would add further insight into the data, and might even correlate with the measures of functional recovery. Also, a single stimulation intensity was used; the results may have been different at different stimulus intensities.*

We confirm that maps of cortical stimulation responsiveness may vary at different stimulus amplitudes. To establish an objective metric of excitability, we identified 100μA as a reliable stimulation amplitude across rats and used this value to build the ipsilateral motor representation results in Figure 6. This choice allows direct comparison with Figure 6 of our 2021 article, related to contralateral motor representation. The comparison reveals a lack of

correlation with functional recovery metrics in the ipsilateral case, in contrast to the successful correlation achieved in the contralateral case.

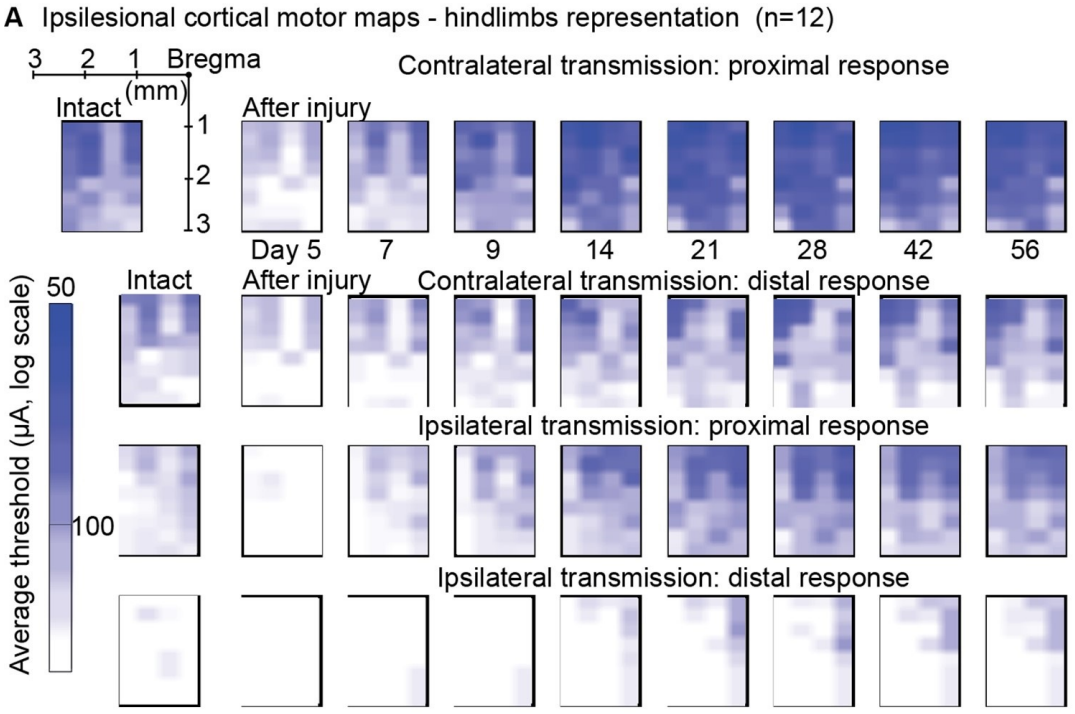
Regarding the incorporation of stimulation amplitudes into the analysis, as detailed in the Method section (lines 770-771), we systematically tested various stimulation amplitudes to determine the minimal threshold required for eliciting a muscle twitch, identified as the threshold value. This process was conducted for each electrode site.

Upon reviewing these data, we considered the possibility of presenting an additional assessment of ipsilateral cortical motor representation based on stimulation thresholds. However, the representation depicted in the figure did not differ significantly from the data presented in Figure 6A. Furthermore, this representation introduced an additional weakness, as it was unclear how to represent the absence of a response in the threshold scale. We chose to arbitrarily designate it as zero on the inverse logarithmic scale, where, for reference, 100 µA is positioned at 0.2 and 50 µA at 0.5.

In conclusion, we believe that the conclusions drawn from this analysis align substantially with those in the text. The addition of the threshold analysis, in our assessment, would not contribute significantly to improving the manuscript.

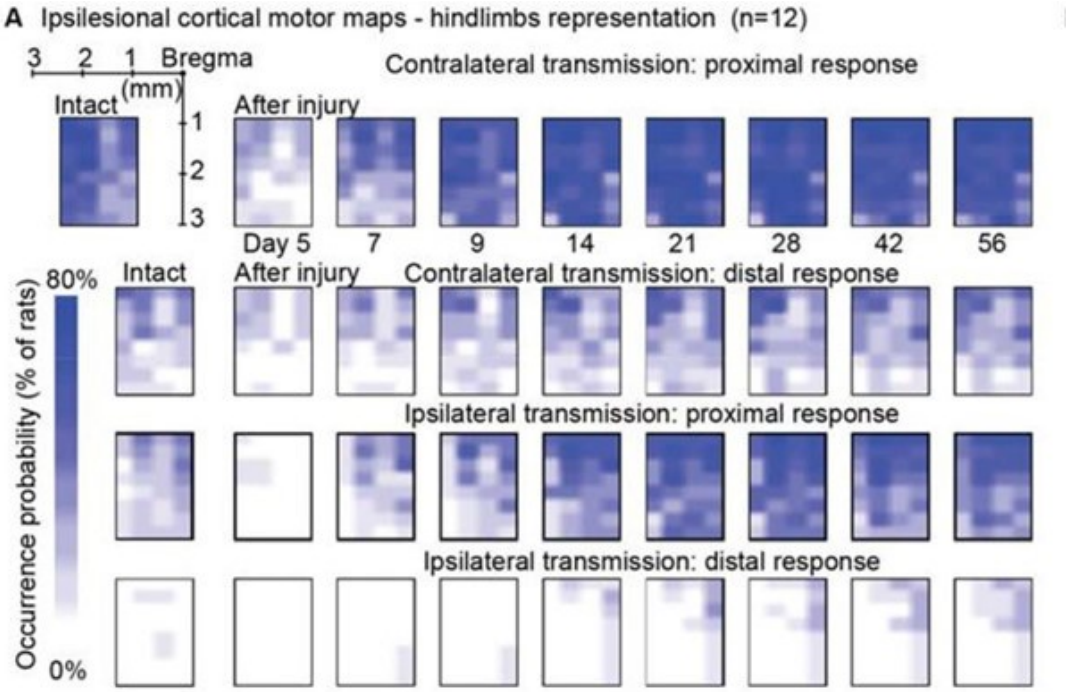
Author response image 1.

Threshold analysis



Author response image 2.

Occurrence probability analysis, for comparison.



> *(6) Lines 858-860: The authors state that "All tests were one-sided because all hypotheses were strictly defined in the direction of motor improvement." By using the one-sided test, the authors are using a lower standard for assessing statistical significance that the overwhelming majority of studies in this field use. More importantly, ipsilateral stimulation of particular kinds or particular sites might conceivably impair function, and that is ignored if the analysis is confined to detecting improvement. Thus, a two-sided analysis or comparable method should be used. This appropriate change would not greatly modify the authors' current conclusions about improvements.*

Our original hypothesis, drawn from previous studies involving cortical stimulation in rats and cats, as well as other neurostimulation research for movement restoration, posited a favorable impact of neurostimulation on movement. Consistent with this hypothesis, we designed our experiments with a focus on enhancing movement, emphasizing a strict direction of improvement.

It's important to note that a one-sided test is the appropriate match for a one-sided hypothesis, and it is not a lower standard in statistics. Each experiment we conducted was constructed around a strictly one-sided hypothesis: the inclusion of an extensor-inducing stimulus would enhance extension, and the inclusion of a flexion-inducing stimulus would enhance flexion. This rationale guided our choice of the appropriate statistical test.

We acknowledge your concern regarding the potential for ipsilateral stimulation to have negative effects on locomotion, which might not be captured when designing experiments based on one-sided hypotheses. That is, when hypothesizing that an extensor stimulus would enhance extension (a one-sided hypothesis) in a functional task, and finding an opposite result (inhibition), statistical rigor would impose that we cannot present that result as significant. This concern is valid, and we explicitly mentioned our design choice it in the method section, Quantification and statistical analyses:

“All tests were one-sided, as our hypotheses were strictly defined to predict motor improvement. Specifically, we hypothesized that delivering an extension-inducing stimulus

would enhance leg extension, and delivering a flexion-inducing stimulus would enhance leg flexion. Consequently, any potentially statistically significant result in the opposite direction (e.g., inhibition) would not be considered. However, no such occurrences were observed."

As a final note, even if such opposite observations were made, they could serve as the basis for triggering an ad-hoc follow-up study.

Reviewer #1 also provided several detailed suggestions in the section "Recommendations for the authors". We estimated that each of them was beneficial for the correctness or for the readability of the text, and thus all were incorporated into the final version.

> ***Reviewer #2 (Public Review):***
>
> *Summary:*
>
> *The authors' long-term goals are to understand the utility of precisely phased cortex stimulation regimes on recovery of function after spinal cord injury (SCI). In prior work, the authors explored the effects of contralesion cortex stimulation. Here, they explore ipsilesion cortex stimulation in which the corticospinal fibers that cross at the pyramidal decussation are spared. The authors explore the effects of such stimulation in intact rats and rats with a hemisection lesion at the thoracic level ipsilateral to the stimulated cortex. The appropriately phased microstimulation enhances contralateral flexion and ipsilateral extension, presumably through lumbar spinal cord crossed-extension interneuron systems. This microstimulation improves weight bearing in the ipsilesion hindlimb soon after injury, before any normal recovery of function would be seen. The contralateral homologous cortex can be lesioned in intact rats without impacting the microstimulation effect on flexion and extension during gait. In two rats ipsilateral flexion responses are noted, but these are not clearly demonstrated to be independent of the contralateral homologous cortex remaining intact.*
>
> *Strengths:*
>
> *This paper adds to prior data on cortical microstimulation by the laboratory in interesting ways. First, the strong effects of the spared crossed fibers from the ipsi-lesional cortex in parts of the ipsi-lesion leg's step cycle and weight support function are solidly demonstrated. This raises the interesting possibility that stimulating the contra-lesion cortex as reported previously may execute some of its effects through callosal coordination with the ipsi-lesion cortex tested here. This is not fully discussed by the authors but may represent a significant aspect of these data. The authors demonstrate solidly that ablation of the contra-lesional cortex does not impede the effects reported here. I believe this has not been shown for the contra-lesional cortex microstimulation effects reported earlier, but I may be wrong. Effects and neuroprosthetic control of these effects are explored well in the ipsi-lesion cortex tests here.*

In the revised version of the manuscript, we incorporated various text improvements to address the points you have highlighted in your review. Additionally, we have integrated the suggested discussion topic on callosal coordination related to contralateral cortical stimulation. The discussion section now incorporates:

"Since bi-cortical interactions in sculpting descending commands are known (Brus-Ramer et al., 2009), and in light of the changes we report in ipsilesional motor cortex excitability, the role of the ipsilateral cortex in mediating or supporting functional descending commands from the contralateral cortex, particularly the immediate increase in flexion of the affected hindlimb and long-term recovery of functional control (Bonizzato & Martinez, 2021), could be further explored."

The localization of the specific channels closest to the interhemispheric fissure (Fig. 7D) may suggest the involvement of transcallosal interactions in mediating the transmission of the cortical command generated in the ipsilateral motor cortex (Brus-Ramer, Carmel, & Martin, 2009). “While ablation experiments (Fig. 8) refute this hypothesis for ipsilateral extension control, they do not conclusively determine whether a different efferent pathway is involved in ipsilateral flexion control in this specific case."

> *Weaknesses:*
>
> *Some data is based on very few rats. For example (N=2) for ipsilateral flexion effects of microstimulation. N=3 for homologous cortex ablation, and only ipsi extension is tested it seems. There is no explicit demonstration that the ipsilateral flexion effects in only 2 rats reported can survive the contra-lateral cortex ablation.*

We agree with this assessment. The ipsilateral flexion representation is here reported as a rare but consistent phenomenon, which we believe to have robustly described with Figure 7 experiments. We underlined in the text that the ablation experiment did not conclude on the unilateral-cortical nature of ipsilateral flexion effects, by replacing the sentence with the following:

“While ablation experiments (Fig. 8) refute this hypothesis for ipsilateral extension control, they do not conclusively determine whether a different efferent pathway is involved in ipsilateral flexion control in this specific case."

> *Some improvements in clarity and precision of descriptions are needed, as well as fuller definitions of terms and algorithms.*
>
> *Likely Impacts: This data adds in significant ways to prior work by the authors, and an understanding of how phased stimulation in cortical neuroprosthetics may aid in recovery of function after SCI, especially if a few ambiguities in writing and interpretation are fully resolved.*

The manuscript text has been revised in its final version, and we sought to eliminate all ambiguity in writing and data interpretation.

In the section “Recommendations for the authors” Reviewer #2 also suggested to better define multiple terms throughout the manuscript. A clarification was added for each.

The Reviewer pointed out that we might have overlooked a correlation between locomotor recovery and motor maps increase in Figure 6. We re-approached this evaluation and found that the reviewer is correct. We were led to think that there was no correlation by “horizontally” looking at whether motor map size across rats would predict locomotor scores (as it did in the case of contralateral cortex mapping, Bonizzato and Martinez, 2021). However we now found a strong correlation between changes that happen over time for each rat and locomotor recovery, a result that was only hinted with no appropriate quantification in the previous version of the manuscript. We have now reformulated the results of Figure 6 on page 12, to include this result, and we would like to thank the reviewer for having noticed this opportunity.

Finally, we have expanded the discussion to include the following points:

The possibility that hemi-cortex coordination of contralesional microstimulation inputs may explain the Sci Transl Med results for contralesional cortex ICMS, which warrants further investigation.

The recognition that the ablation experiments do not provide conclusive evidence regarding ipsilateral flexion control and whether an alternative efferent pathway might be involved in this specific case.

> ***Reviewer #3 (Public Review):***
>
> *Summary:*
>
> *This article aims to investigate the impact of neuroprosthesis (intracortical microstimulation) implanted unilaterally on the lesion side in the context of locomotor recovery following unilateral thoracic spinal cord injury.*
>
> *Strength:*
>
> *The study reveals that stimulating the left motor cortex, on the same side as the lesion, not only activates the expected right (contralateral) muscle activity but also influences unexpected muscle activity on the left (ipsilateral) side. These muscle activities resulted in a substantial enhancement in lift during the swing phase of the contralateral limb and improved trunk-limb support for the ipsilateral limb. They used different experimental and stimulation conditions to show the ipsilateral limb control evoked by the stimulation. This outcome holds significance, shedding light on the engagement of the "contralateral projecting" corticospinal tract in activating not only the contralateral but also the ipsilateral spinal network.*
>
> *The experimental design and findings align with the investigation of the stimulation effect of contralateral projecting corticospinal tracts. They carefully examined the recovery of ipsilateral limb control with motor maps. They also tested the effective sites of cortical stimulation. The study successfully demonstrates the impact of electrical stimulation on the contralateral projecting neurons on ipsilateral limb control during locomotion, as well as identifying important stimulation spots for such an effect. These results contribute to our understanding of how these neurons influence bilateral spinal circuitry. The study's findings contribute valuable insights to the broader neuroscience and rehabilitation communities.*

Thank you for your assessment of this manuscript. The final version of the manuscript incoporates your suggestions for improving term clarity and we enhanced the discussion on the mechanisms of spinal network engagement, as outlined below.

> *Weakness:*
>
> *The term "ipsilateral" lacks a clear definition in the title, abstract, introduction, and discussion, potentially causing confusion for the reader.*
>
> *[and later] However, in my opinion, readers can easily link the ipsilateral cortical network to the ipsilateral-projecting corticospinal tract, which is less likely to play a role in ipsilateral limb control in this study since this tract is disrupted by the thoracic spinal injury.*

In order to mitigate the risk of having readers linking the effects of ipsilateral cortical stimulation with ipsilateral-projecting corticospinal tract, we specified:

In the abstract, we precise that our goal was: “to investigate the functional role of the ipsilateral motor cortex in rat movement through spared contralesional pathways.”

In the introduction: “In most cases, this lesion also disrupts all spinal tracts descending on the same side as the cortex under investigation at the thoracic level, meaning that the

transmission of cortical commands to the ipsilesional hindlimb must depend on crossed descending tracts (Fig. S1)."

> *The unexpected ipsilateral (left) muscle activity is most likely due to the left corticospinal neurons recruiting not only the right spinal network but also the left spinal network. This is probably due to the joint efforts of the neuroprosthesis and activation of spinal motor networks which work bilaterally at the spinal level.*

We agree with your assessment and the discussion section now emphasizes the effects of supraspinal drive onto spinal circuits.

In the section "Recommendations for the authors" Reviewer #3 suggested to provide an early reminder to the reader that the focus is on exploring the control of the ipsilateral limb through the corticospinal tract of the same side, projecting contralaterally. We did so in the abstract and introduction, as presented above.

The reviewer also suggested that the discussion could be shorter. While we recognize it covers diverse subjects that may appeal to different readers, we believe omitting some sections could limit its overall scope. The manuscript underwent three revisions and a thorough dialogue with reviewers from diverse backgrounds, and we are hesitant to undo some of these improvements.

> *Moreover, the section falls short of fully exploring the involvement of contralateral projecting corticospinal neurons in spinal networks for diverse motor behaviors. It could potentially delve into aspects like the potential impact of corticospinal inputs on gating the cross-extensor reflex loop and elucidating the mechanisms underlying the recruitment of the ipsilateral spinal network for generating ipsilateral limb movements. Is it a direct control on motor neurons or via existing spinal circuits?*

The discussion section now includes the potential spinal circuits through which corticospinal neurons may affect motor control and reflexes.

Reviewer #3 also provided several detailed suggestions in the sub-section "Minor points". We estimated that all of them were beneficial for the correctness or for the readability of the text, and thus were incorporated into the final version. Some of the questions raised were answered directly in the text (defining "% of chronic map" and rephrasing the original Line 479). We would like to answer here below two remaining questions:

> *Fig. 3C I wonder what is the average latency between stimulation onset and onset of right ankle flexor activity. Is the latency fixed, or variable (which probably indicates that the Cortical activation signal is integrated with spinal CPG activity.)*

ICMS trains, unfortunately, do not allow for precise dissection of transmission timing. Single pulses at 100 μA are insufficient to generate motoneuron responses and require multiple pulses to build up cortical transmission. Alstermark et al. (Journal of Neurophysiology, 2004) used two to four stimuli with higher amplitudes to investigate forelimb transmission timing. In our 2021 Science Translational Medicine paper, we employed single pulses at 1 mA to establish transmission delays from the contralateral cortex to the ankle flexor. However, the circuits recruited at 1 mA are not directly comparable to those activated by shorter trains.

In this study, we used cortical trains of approximately 14 pulses, typical of ICMS protocols. Each pulse could potentially be the first to generate a response volley in the ankle flexor, with delays measured at 30 to 60 ms from ICMS train onset. While we believe that cortical commands are necessarily integrated with spinal CPG activity—as indicated in Figures 1B and 3D, where timing is crucial and descending commands can be gated out if delivered off-

phase—the variability in latency that we recorded could be attributed to any of the following factors: cortical activation build-up, integration within reticular relay networks, or CPG integration.

> *Fig. 4A. Why is the activity of under contralateral ankle flexor intact condition is later than the stimulation condition?*

We timed the stimulation to coincide with the contralateral leg lift and did not adjust its onset relative to spontaneous walking in SCI rats. Although stimulation could induce leg lift, as shown in Fig. 4A, SCI rats exhibited a slightly earlier and stronger activation of the right (contralateral) ankle flexor muscle even during spontaneous walking. This phenomenon is attributed to the deficits observed on the left side. The stronger right leg bears the body weight, as illustrated in Fig. 3, and thus, during body advancement, the right leg is engaged sooner and more rapidly (with a shorter swing phase) to provide support (right foot forward).

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