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ORIGINAL ARTICLE

Spinal cord associative plasticity improves forelimb sensorimotor function after cervical injury

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Associative plasticity occurs when two stimuli converge on a common neural target. Previous efforts to promote associative plasticity have targeted cortex, with variable and moderate effects. In addition, the targeted circuits are inferred, rather than tested directly. In contrast, we sought to target the strong convergence between motor and sensory systems in the spinal cord.

We developed spinal cord associative plasticity, precisely timed pairing of motor cortex and dorsal spinal cord stimulations, to target this interaction. We tested the hypothesis that properly timed paired stimulation would strengthen the sensorimotor connections in the spinal cord and improve recovery after spinal cord injury. We tested physiological effects of paired stimulation, the pathways that mediate it, and its function in a preclinical trial.

Subthreshold spinal cord stimulation strongly augmented motor cortex evoked muscle potentials at the time they were paired, but only when they arrived synchronously in the spinal cord. This paired stimulation effect depended on both cortical descending motor and spinal cord proprioceptive afferents; selective inactivation of either of these pathways fully abrogated the paired stimulation effect. Spinal cord associative plasticity, repetitive pairing of these pathways for 5 or 30 min in awake rats, increased spinal excitability for hours after pairing ended. To apply spinal cord associative plasticity as therapy, we optimized the parameters to promote strong and long-lasting effects. This effect was just as strong in rats with cervical spinal cord injury as in uninjured rats, demonstrating that spared connections after moderate spinal cord injury were sufficient to support plasticity. In a blinded trial, rats received a moderate C4 contusive spinal cord injury. Ten days after injury, they were randomized to 30 min of spinal cord associative plasticity each day for 10 days or sham stimulation. Rats with spinal cord associative plasticity had significantly improved function on the primary outcome measure, a test of dexterity during manipulation of food, at 50 days after spinal cord injury. In addition, rats with spinal cord associative plasticity had persistently stronger responses to cortical and spinal stimulation than sham stimulation rats, indicating a spinal locus of plasticity. After spinal cord associative plasticity, rats had near normalization of H-reflex modulation. The groups had no difference in the rat grimace scale, a measure of pain. We conclude that spinal cord associative plasticity strengthens sensorimotor connections within the spinal cord, resulting in partial recovery of reflex modulation and forelimb function after moderate spinal cord injury. Since both motor cortex and spinal cord stimulation are performed routinely in humans, this approach can be trialled in people with spinal cord injury or other disorders that damage sensorimotor connections and impair dexterity.

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Abbreviations: AAV = adeno-associated virus; CNO = clozapine-*N*-oxide; CST = corticospinal tract; DREADD = Designer Receptor Exclusively Activated by Designer Drug; DRG = dorsal root ganglion; IBB = Irvine, Beatties and Bresnahan scale; MEP = motor evoked potential; SCAP = spinal cord associative plasticity; SCI = spinal cord injury

Introduction

Integration of sensory feedback with motor commands is required for skilled movement. Neuromodulation strategies have been developed to target sensorimotor integration.[1] The most common application, paired associative stimulation, repetitively pairs median nerve stimulation with motor cortex stimulation timed to increase cortical excitability in healthy people[2] and improve arm and hand function after injury.[3,4] Using a similar logic but targeting the spinal cord, epidural stimulation has been used to drive activation of spinal afferents that can be timed to converge with descending motor activation. This strategy helped to restore walking after spinal cord injury (SCI), but only when spinal stimulation was timed to coincide with motor cortex signals arriving in the spinal cord.[5–7] Our goal was to use associative learning through pairs of electrical stimuli to strengthen residual connections after cervical SCI, the most common type.[8] For people with cervical SCI, recovery of arm and hand function is the highest priority.[9]

Previous trials of paired stimulation targeting the spinal cord have targeted the motor system alone. Electrical stimulation of motor cortex has been paired with peripheral nerve stimulation to backfire spinal motor neurons. This motor system stimulation promotes lasting changes in motor evoked potentials (MEPs) and improved grip strength and finger control in people.[10–12] Intraspinal stimulation in monkeys has been timed with cortical activity so endogenous brain activity and exogenous spinal cord stimulation converge.[13,14] Repetitive pairing induced a small but significant increase in evoked potentials.

In contrast to paired stimulation targeting cortex or the motor system in spinal cord, we targeted sensorimotor interactions in spinal cord with paired stimulation of motor cortex and cervical spinal cord.[15] The convergence of subthreshold spinal cord electrical stimulation with a descending cortical volley robustly augmented the cortical MEPs. When this pairing was performed repeatedly, there was an increase in excitability that lasted for at least an hour. This plasticity required paired stimulation delivered at the proper time, but the exact neural circuits that mediate the sensorimotor interactions are not known. Knowing the targets is critical to ensure strong and selective engagement and to understand the anatomical basis for spinal cord associative plasticity (SCAP). It is also not known whether the sparse connections that are spared after SCI are sufficient to support SCAP and recovery of function. Together, the experiments in this study were designed to improve our understanding of SCAP mechanisms and to test its efficacy for the recovery of dexterity after SCI.

Materials and methods

In awake and freely moving rats, we tested SCAP and its efficacy on physiology and function in rats with SCI. We used custom spinal stimulating electrodes that can be inserted into the thin epidural space over the cervical spinal cord, are supple enough to conform to the spinal cord and move with the neck.[16,17] First, we tested the motor cortex and spinal cord stimulation timing with the hypothesis that convergence in the spinal cord would produce the largest changes in physiology. Second, we tested the necessity of corticospinal and segmental afferent connections for paired stimulation by selectively inactivating each using chemogenetic tools. We then paired motor cortex and spinal cord stimulation repeatedly to induce plasticity (SCAP). We optimized the protocol by varying the frequency, number and pattern of stimuli in uninjured rats. We tested SCAP in rats with SCI. Finally, we performed a randomized, blinded preclinical trial of 10 days of SCAP versus sham stimulation to determine the effects on physiology and behaviour after a moderate C4 contusion SCI. The primary outcome measure was a behavioural test of food manipulation; several physiological, behavioural and anatomical measures were secondary.

Timing

In adult Sprague-Dawley female rats, we implanted three sets of electrodes: epidural screw electrodes over each motor cortex, EMG electrodes in each biceps muscle and spinal epidural electrodes over the midline of dorsal C5-C6 spinal cord. To determine whether subthreshold spinal cord stimulation augments cortical MEPs, we compared cortex stimulation only to motor cortex stimulation paired with spinal cord stimulation. We determined the threshold for provoking an MEP from motor cortex and from the spinal cord. We measured the time from motor cortex stimulation to the spinal cord dorsum potential [Fig. 1A(i)]. We then quantified the biceps MEP as the area under the curve in response to stimulation at 110% of threshold intensity [Fig. 1A(ii)]. In uninjured rats, this was compared against motor cortex stimulation paired with spinal cord stimulation at 90% of threshold intensity [Fig. 1A(iii) and B]. This experiment quantified the size of the MEP with paired stimulation relative to cortex stimulation alone using unpaired *t*-tests with Bonferroni correction. We also tested whether stimulating motor cortex at 90% of threshold with spinal cord stimulation also at 90% would provoke an MEP together when neither produced a response on their own. The magnitude of the MEP was compared to that of motor cortex stimulation at 110% of threshold (Fig. 1C). Finally, we tested the effects of paired stimulations in rats, weeks after a moderate C4 contusion injury (Fig. 1D, using the same methods as Fig. 1B but in rats with SCI).

Inactivation

To target the corticospinal tract (CST) selectively, a Cre-dependent adeno-associated virus (AAV1) encoding the Designer Receptor Exclusively Activated by Designer Drug (DREADD) was injected into the motor cortex and another encoding Cre recombinase was injected into the cervical spinal cord [Fig. 2A(i)]. Thus, only doubly infected neurons express the DREADD [Fig. 2A(ii)], which can be inactivated by its ligand, clozapine-*N*-oxide (CNO).[18,19] One week after AAV injections, animals were trained on the vermicelli handling task.[20,21] After reaching a stable baseline performance, the number of forepaw manipulations was measured before, during and after inactivation [Fig. 2A(iii)]. One week after the behavioural

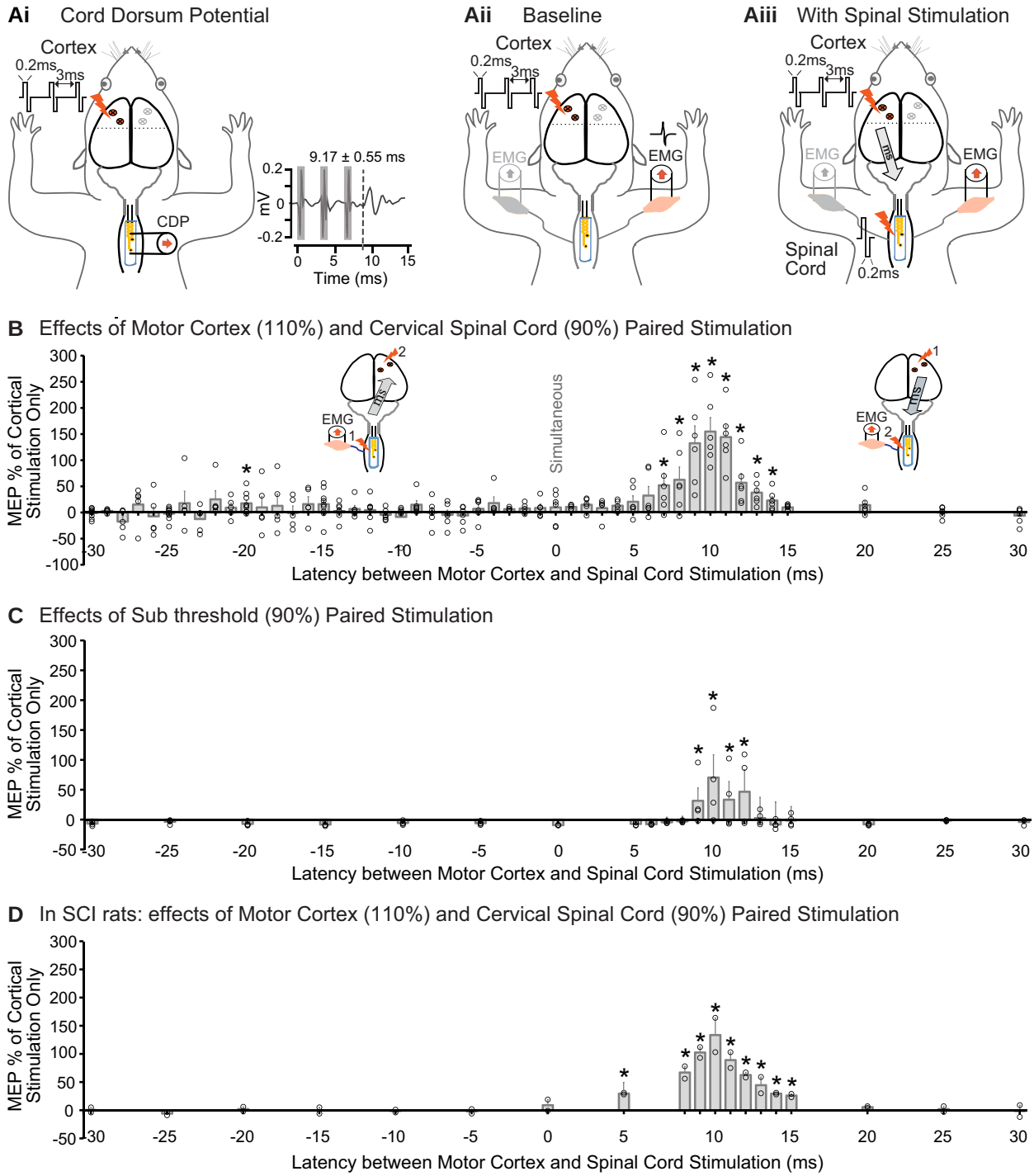

Figure 1 The immediate effects of pairing depend on timing. [**A(i)**] Schematic of cervical spinal cord dorsum potential recording. After the onset of three pulses of motor cortex stimulation at 110% of motor threshold, the latency of the response was 9.17 ± 0.55 ms ($n = 3$). (**ii**) Schematic of baseline testing after M1 stimulation at 110% of cortical threshold and EMG recorded from contralateral biceps. (**iii**) Immediate effect testing. Suprathreshold (110% of cortical threshold) stimulation of the motor cortex produces an MEP that is modulated by subthreshold (90% of spinal threshold) spinal cord stimulation. (**B**) Immediate effects of paired stimulation depend on latency. We tested different latencies between motor cortex and spinal cord stimulation from −30 to 30 ms. As indicated by the *insets*, negative times indicate that spinal cord was stimulated before motor cortex stimulation and positive times indicate that spinal cord was stimulated after motor cortex stimulation. Maximum augmentation was found when spinal cord stimulation was delivered 10 ms after motor cortex stimulation. MEPs were significantly elevated at the time points indicated, as measured by *t*-test with Bonferroni correction compared to no spinal stimulation baseline, asterisks represent $P < 0.05$ ($n = 9$). (**C**) Immediate effects when both motor cortex and spinal cord stimulations were paired with their 90% of threshold intensities. The paired stimulations with latencies between ±30 ms were applied, and MEP responses were recorded. This figure shows the percentage increase in the MEPs as a function of latencies. MEP responses started to appear with latencies ∼10 ms, and cortical convergence does not seem to cause any MEP response. MEP response becomes visible once the baseline EMG activity is doubled ($n = 4$). (**D**) We further tested this hypothesis in animals with C4 spinal contusion. A moderate C4 contusion injury was performed in two rats, and spinal electrode arrays were inserted over C5–C6. Two weeks later, paired stimulation was performed. In SCI rats when 110% cortical stimulation were paired with 90% spinal stimulation, we observed a similar augmentation in MEP as observed in uninjured rats with peak at 10 ms latency (134 ± 20%, $P = 0.0001$). Circles represent individual animals and error bars show SEM.

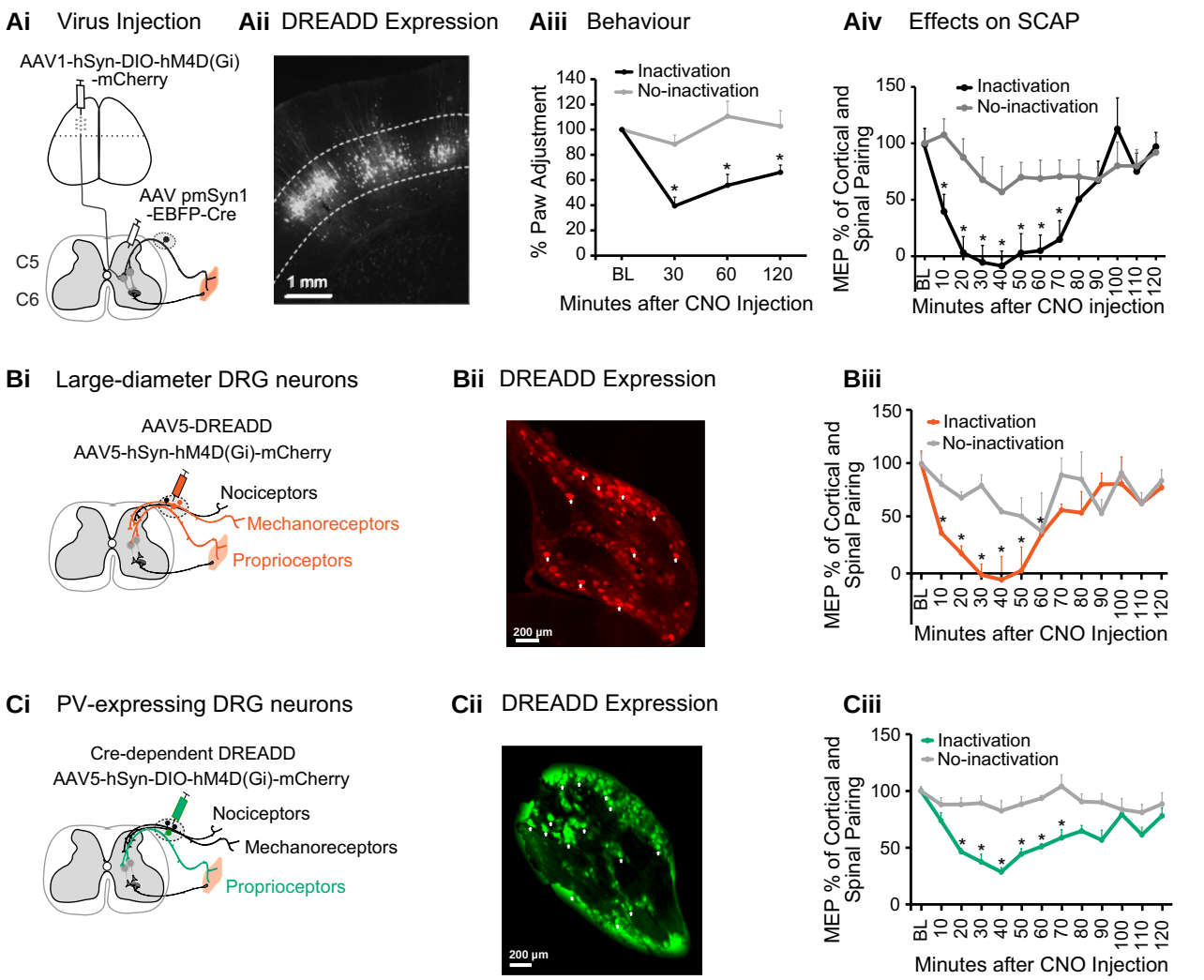

Figure 2 The CST and spinal afferents are necessary for paired stimulation. (**A**) CST inactivation. (**i**) AAV1-hSyn-DIO-hM4D(Gi)-mCherry was injected into the forelimb area of the left motor cortex and AAV2/retro-pmSyn1-EBFP-Cre was injected into the right side of the C5 and C6 spinal segments. (**ii**) Dual virus transduction drives robust mCherry expression in corticospinal neurons located in motor cortex layer 5 (dotted lines). (**iii**) CST inactivation impairs skilled forepaw use. The number of adjustments of both forepaws was counted while the animal was eating uncooked pasta before and at 30, 60 and 120 min after CNO injection. Inactivation significantly reduces the paw manipulations in the paw controlled by the inactivated CST ($n = 3$). (**iv**) CST inactivation abrogates the effect of paired stimulation. One week after the behavioural test, the CST was inactivated by CNO injection. The augmentation of paired stimulation was fully abrogated at 20–60 min in the inactivation side and gradually recovered ($n = 3$). (**B**) Large-diameter afferent inactivation. (**i**) An AAV5 encoding the inactivating DREADD was injected into DRG on same site as the targeted side. (**ii**) DREADD expression in neurons (arrowhead), infected with the virus measured 67.9 ± 18.5 µm in diameter, similar to neurons that mediate touch, proprioception and muscle length/tension. (**iii**) Inactivation abrogates paired stimulation in rats infected with AAV5-DREADD, inactivation side (vermilion line) compared to non-inactivated side (grey line, $n = 2$), 100% suppression at 40 min after CNO injection. (**C**) Proprioceptor inactivation. (**i**) A Cre-dependent DREADD was injected into the DRG on the inactivation side of rats expressing Cre under control of parvalbumin, which is expressed selectively by proprioceptors. (**ii**) DREADD in proprioceptive neurons (arrowhead), infected with the virus. (**iii**) Inactivation abrogates paired stimulation in PV-Cre rats, inactivation side (blue-green line) compared to baseline ($n = 3$); >70% suppression of paired stimulation effect was observed at 40 min after CNO injection. More selective inactivation (blue-green line) resulted in 25.8 ± 3.8% suppression of sensory afferents while non-selective (vermilion line; −1.5% ± 10.5%) inactivation caused more robust suppression of paired stimulation responses on the targeted side of forelimb after CNO injection. Vehicle (normal saline) injection did not change MEPs compared to baseline on the inactivated side (data shown in Supplementary Fig. 1). Error bars show SEM.

test, rats were anaesthetized and effects of inactivation on the immediate effects of paired motor cortex and spinal cord stimulation were tested [Fig. 2A(iv)]. The details are in the Supplementary material.

The necessity of afferents for the immediate effects of paired stimulation was tested using two inactivation strategies. For the first strategy, large-diameter dorsal root ganglion (DRG) neurons were inactivated [Fig. 2B(i)]. Large DRG neurons mediate touch, proprioception and muscle length/tension.[22] The specificity of large-diameter DRG neurons was provided by the tropism of the AAV5.[23] An AAV5-hSyn-hM4D(Gi) was injected into each DRG at C5, C6 and C7 on one side of the spinal cord after partial laminectomy [Fig. 2B(ii)]. At the time of virus injection, cortical, EMG and spinal electrodes were implanted as described earlier. CNO was administered as described in the Supplementary material, and responses to paired stimulation was measured [Fig. 2B(iii)].

For the second strategy, we targeted the proprioceptive afferents, since these mediate the effects of spinal cord stimulation

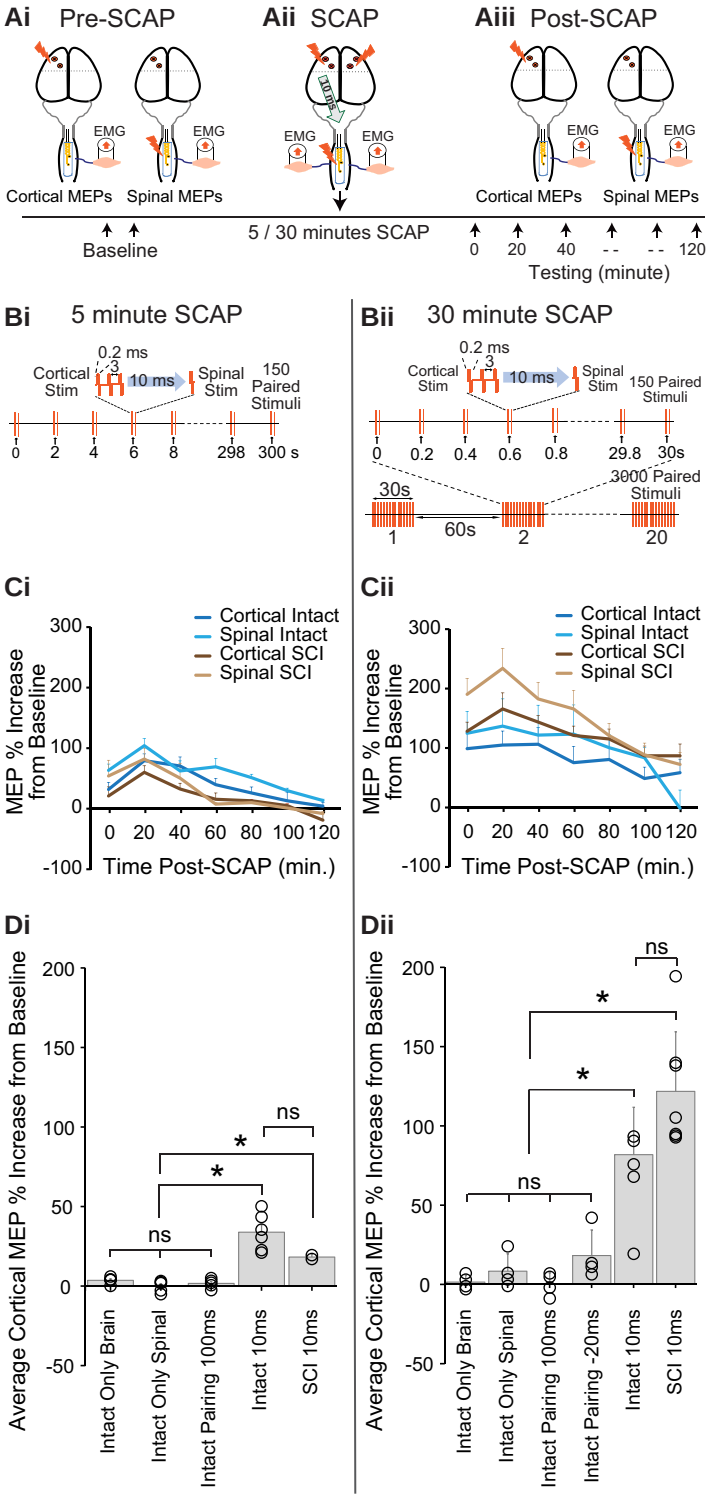

Figure 3 SCAP protocol. [**A**(**i**)] Baseline measurements of cortical MEP, spinal MEPs and thresholds before repetitive pairing. Cortical and spinal stimulation at 110% of the cortical and spinal thresholds. MEPs recorded from the biceps. Threshold was determined by adjusting stimulation intensity to provoking a short-latency EMG in >50% of trials. (**ii**) Repetitive paired stimulation, repetitive pairing motor cortex and spinal cord stimulation at 10 ms latency for either 5 or 30 min. (**iii**) Testing the effects of both 5 and 30 min SCAP, cortical, spinal MEPs and thresholds were measured as was done before SCAP. [**B**(**i**)] The 5-min SCAP protocol (0.5 Hz, 150 pairs). (**ii**) A 30-min SCAP paradigm (5 Hz, 150 pairs of cortical and spinal stimuli 20 times with 1-min gap between bursts, delivering a total of 3000 pairs). [**C**(**i** and **ii**] SCAP causes lasting changes in cortical and spinal cord MEPs in uninjured rats and rats with SCI. (**i**) Cortical MEP augmentation after 5-min SCAP in uninjured awake rats ($n = 6$) as well as in rats with C4 SCI ($n = 2$). In both injured and uninjured groups of rats after 5 min of repetitive pairing, cortical evoked responses were augmented for >1 h and this augmentation persisted even after SCI, then returned to the baseline. A similar effect was also observed with the spinal MEP and excitability increased after pairing (data shown in Supplementary Fig. 4A). (**ii**) Cortical MEP augmentation after 30-min SCAP in uninjured awake rats ($n = 4$) and in SCI rats ($n = 7$).

(Continued)

[Fig. 2C(i)].[24–26] We used a genetic strategy to target DRG neurons that express parvalbumin, which are proprioceptors.[27,28] In transgenic rats that express Cre recombinase under the control of parvalbumin promoter PV-Cre rats [LE-Tg(Pvalb-iCre)2Ottc], we injected a Cre-dependent DREADD [AAV5-hSyn-DIO-hM4D(Gi)-mCherry][29] into the C5, C6 and C7 DRGs as described previously [Fig. 2C(ii)]. Inactivation with CNO and physiology measurement were also identical [Fig. 2C(iii)].

Repetitive pairing: SCAP

We tested brain and spinal cord responses at baseline and then after the period of repetitive pairing [Fig. 3A(i–iii)], as we did in Mishra *et al.*,[15] but in awake rats with implanted electrodes. This differs from the immediate effects of stimulation [Fig. 1A(iii)], as we examined the lasting effects of paired stimulation. Before modulation, we measured the stimulation threshold for both motor cortex and spinal cord, defined as the stimulation intensity needed to produce an MEP in 50% of trials. We then stimulated with 110% of threshold to determine cortical and spinal MEPs, which were quantified as the area under the curve. At baseline, cortical and spinal MEPs were measured singly and independently [Fig. 3A(i)]. For repetitive pairing [Fig. 3A(ii)], motor cortex stimulation was performed at 110% of the threshold for evoking a cortical MEP followed 10 ms later by spinal cord stimulation performed at 90% of the threshold for generating a spinal MEP. After repetitive pairing [Fig. 3A(iii)], the same measures at baseline were taken immediately after pairing and every 20 min thereafter up to 120 min. We quantified excitability as 1/threshold and expressed it as percentage change from baseline.

Optimization

To maximize the efficacy of the SCAP protocol [5-min SCAP, Fig 3B(i)], we varied four stimulation parameters: frequency, number of stimuli, number of trains and time between trains. We kept the total stimulation period to 30 min, a period that could be used for stimulation in people. In two rats implanted with cortical and spinal electrodes, we first varied frequency by delivering 10 stimuli at 5, 2, 1, 0.5 and 0.2 Hz and measured the size of the MEPs (Supplementary Fig. 3A). For the other parameters, we measured the change in cortical MEPs and cortical and spinal excitability. Spinal excitability was used since this was less variable than spinal MEPs, and out of concern that eliciting spinal MEPs might induce plasticity. For these trials, we used 5 Hz pairing and varied the number of stimuli by comparing 150, 75 and 38 pulses [Supplementary Fig. 3B, C and D(i–iii)]. For subsequent trials, we used 150 stimuli, which we term a train, and varied the number of trains to either 10 or 20 [Supplementary Fig. 3B, C and F(i–iii)]. For subsequent trials, we used 20 trains and varied the time between trains to either 1 or 5 min [Supplementary Fig. 3B, C and E(i–iii)].

Cervical spinal cord contusion: SCI

The C4 spinal cord contusion surgery was performed as previously described.[30] Briefly, the C3 to C5 vertebrae were exposed by skin incision and muscle separation, and laminectomy of C4 was performed. An Infinite Horizon impactor (IH-0400, Precision Systems & Instrumentation, LLC), was used to deliver 200 kilodynes of force onto the spinal cord with a 3.5-mm diameter tip. The force curve of the impactor was recorded (Fig. 7A). After the injury, the spinal electrode array was implanted as described previously and rats received intense care, as described in the Supplementary material.

Preclinical trial

We designed a preclinical efficacy trial; the timeline is shown in Fig. 4A. Ten days after SCI, rats were randomized to receive real or sham SCAP for 10 days, a period that we have previously shown to be therapeutic.[30,31] The daily stimulation duration (30 min) and the number of sessions (10) were designed to be clinically viable. The primary outcome measure was the dexterity [Irvine, Beatties and Bresnahan (IBB) scale[32]] score, and secondary outcomes included performance of skilled walking, changes in MEPs (immediate effects), thresholds, lasting effects of pairing in stimulated rats, H-reflex modulation (a measure of hyperreflexia) and a pain measure (Rat Grimace Scale). The study was powered based on the effect size we observe of a different neuromodulation strategy.[30] *A priori* power analysis suggested we need eight rats in each group to achieve a power of 0.8. Rats were pseudorandomized into real (Stim) and sham (Control) groups with each cohort of up to eight rats having rats assigned to each group. Rats were randomized immediately after SCI surgery, and experiments were carried out with blinded measures.

Behaviour

Food manipulation task

To assess the rat's ability to manipulate, we used the IBB scale,[32,33] as we did previously.[30]

Horizontal ladder walking task

The horizontal ladder walking task measured paw placement accuracy on irregularly spaced rungs, a task that requires the CST.[31,34–36]

Rat Grimace Scale

The Rat Grimace Scale is used to quantify pain based on rat facial characteristics in a way that is commonly used to assess pain in people.[37]

Figure 3 Continued
In both SCI and intact (uninjured) groups of rats after 30 min of repetitive pairing, cortical evoked responses were augmented for >2 h and this augmentation persisted even after SCI, similarly the spinal MEP and excitability increased for 2 h after pairing (data shown in Supplementary Fig. 4B). **[D(i)]** Average MEP augmentation over 120 min with 5 min SCAP conditions. Only motor cortex (4 ± 7%) or only spinal cord stimulation (8 ± 6%) or pairing at an inappropriate latency (100 ms; 2 ± 5%), do not result in elevated MEP responses. **(ii)** Average MEP augmentation over 120 min with different 30-min SCAP conditions. Only motor cortex (1.3 ± 6%) or only spinal cord stimulation (8 ± 4%) or pairing at an inappropriate latency (100 ms; 0.2 ± 3%), do not result in elevated MEP responses. Pairing at −20 ms aiming at cortical convergence, does not cause a strong MEP response (18 ± 5%). Circle represents individual animals and error bars shows SEM. A one-way ANOVA was done for overall comparison and was highly significant followed by Bonferroni correction for multiple comparisons. *$P < 0.001$, NS = non-significant.

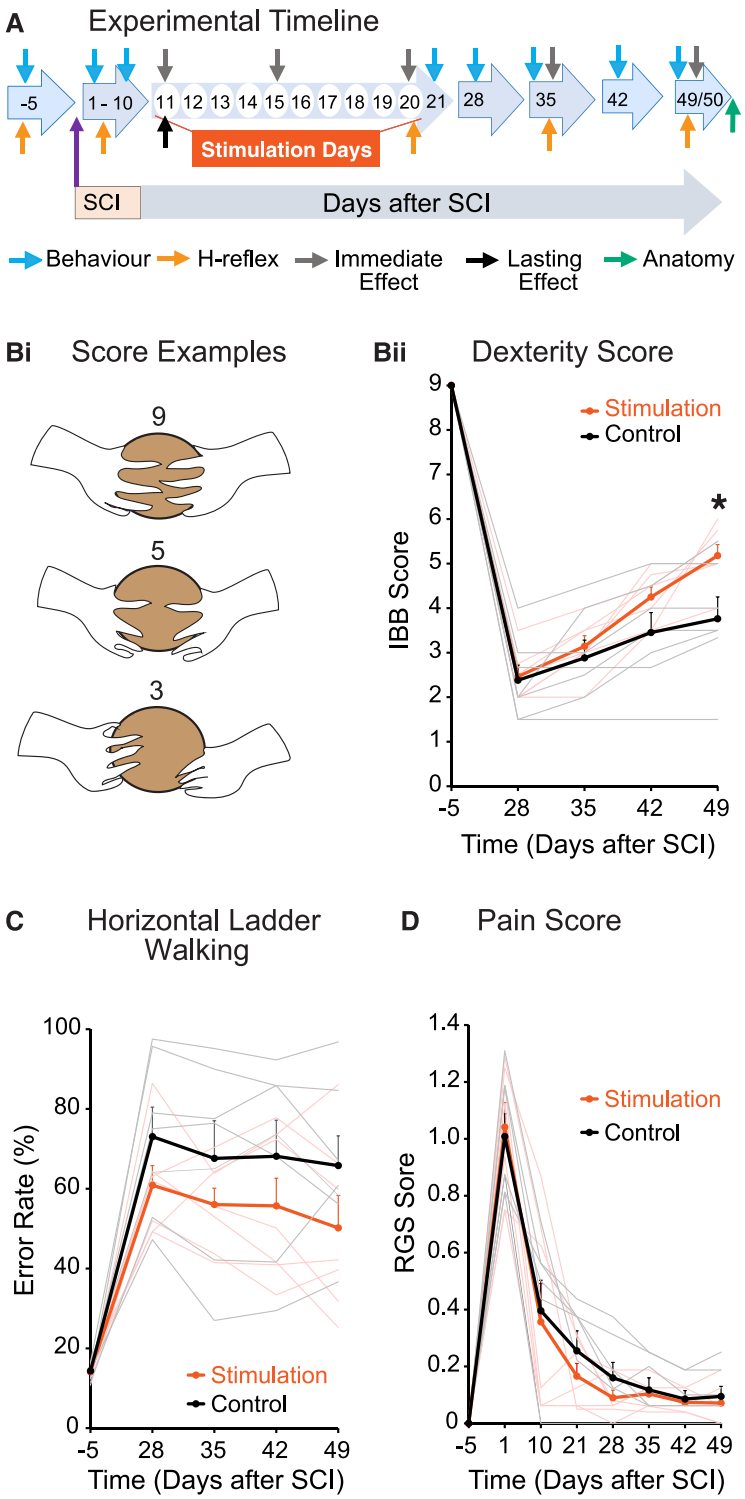

Figure 4 SCAP promotes motor recovery after SCI and does not affect pain. (**A**) Experimental timeline: Rats were trained on the food manipulation task and then subjected to C4 contusion injury. Rats were randomized to SCAP daily for 10 days (stimulation group) versus sham (no) stimulation (control group). Physiology and behaviour testing was done before stimulation and then weekly after stimulation. After 50 days, animals were sacrificed and anatomical analysis was done. [**B(i)**] Schematics showing characteristic features of rat forelimbs during cereal manipulation task, the primary outcome measure. Images of a representative stimulation rat were generated from frames of live videos with the image tracing tool of Adobe Illustrator software. Score 9 at baseline before SCI: Volar (palm) support, paw adapts to the shape of the cereal. Score 5 after stimulation: Volar support, digit 2 contributes to cereal manipulation, other digits extended. Score 3 after injury, before stimulation: Lack of volar support, extended digits and paw does not adapt to the shape of the cereal. (**ii**) Average and individual rat's IBB scores. At each time point, the IBB score was averaged over four pieces of cereals for cereal manipulation trials. Thick red and black line are the average IBB scores for all stimulation group rats and controls respectively. Thin light red and black lines indicate individual stimulation and control group rats, respectively. Error bar represents SEM. *Unpaired *t*-test, $P = 0.024$, at 49 days after SCI

(Continued)

Physiology

The immediate effects of pairing [Fig. 1A(iii)] as well as thresholds were tested before the 30-min SCAP on each day of stimulation and every 2 weeks thereafter. The lasting effect of SCAP [Fig. 3A(i)] was measured on Day 11 after SCI.

H-reflex testing

To track H-reflex modulation, we measured the H-reflex at baseline, 1 week after SCI, just after 10 days of stimulation, and every 2 weeks after stimulation (Fig. 4A). First, we tested responses to increasing stimulus intensities up to twice threshold, which activates low threshold afferent fibres. Next, we tested the frequency-dependent depression of the H-reflex.[38]

Anatomy

To determine the severity of SCI, we measured impact force during spinal cord contusion. We quantified the volume of spared grey and white matter at the injury site. We also quantified spared axons below the lesion in both groups. For CST axon quantification, BDA was injected into cortex and histology was performed on sections above (C3) and below (C7) the lesion site as in our previous studies.[30] For sensory axon quantifications, AAV1 was injected in C5-C7 DRG, and sensory afferent axons were quantified below the lesion segments.

Statistical analyses

All the comparisons between stimulation conditions were done with paired/*t*-tests within group and unpaired *t*-tests between groups with Bonferroni correction for single time points or one-way ANOVA with Bonferroni corrections for multiple time points. Cohen's *d* was used for the effect size. All the statistical analysis was performed with SPSS (SPSS Statistics for Windows, v.27; IBM Corp., Armonk, NY, USA). We tested the normality of the data using the Shapiro-Wilk test. All data are expressed as mean ± SEM.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Results

We hypothesized that repetitive pairing of motor cortex and spinal cord would augment spinal excitability and improve function in rats with SCI through spinal sensory and descending motor convergence in the spinal cord. This hypothesis was tested in steps. First, we tested whether convergence of paired stimulation was more effective in cortex or the spinal cord by altering the timing for paired stimulation. Second, we tested whether the connections preserved by cervical SCI were sufficient to enable paired stimulation. Third, to determine whether the CST and proprioceptors were necessary, we selectively inactivated each. Fourth, we optimized the repetitive application of paired stimulation (SCAP) protocol. Fifth, we tested this optimized protocol in rats with SCI. Finally, we tested whether 10 days of repetitive SCAP improved physiology and behaviour after SCI.

Spinal stimulation augments cortical responses when properly timed

We hypothesized that stimulating the spinal cord at the time that descending motor potentials arrive would most strongly augment cortical MEPs. The latencies shown in Fig. 1A(i) are the averages of 20 responses each from three animals, while the waveforms are for a representative trial in one animal. Recorded C5–C6 spinal cord responses to cortical stimulation in awake rats are shown in Fig. 1A(i). After the initiation of a train of three stimuli in the motor cortex, the cord dorsum potential arrived at 9.17 ± 0.55 ms (mean ± SEM, $n = 3$). Spinal cord stimulation augmented cortical MEPs at multiple time points (Fig. 1B) with a strong peak when the spinal cord was stimulated 10 ms after the motor cortex (155 ± 27%, d.f. = 13, Cohen's $d = 40.86$, $P < 0.0001$). There were also smaller increases in MEPs when the spinal cord was stimulated 20 ms before cortex (17 ± 6%, d.f. = 16, Cohen's $d = 13.50$, $P = 0.019$).

If the effects of motor cortex and spinal cord stimulation are synergistic, then subthreshold stimulation at each site may result in a suprathreshold response if they are properly timed. To test this hypothesis, stimulation intensities for both cortical and spinal stimulations were set to 90% of the threshold. Stimulation of the spinal cord only and motor cortex only produced no responses. Paired subthreshold stimulation generated an MEP with a peak effect at 10 ms. The response was larger than cortical stimulation only at 110% of threshold (66 ± 38% larger) of 110% cortical threshold ($P = 0.001$; paired *t*-test). Thus, the synergistic effects of subthreshold stimulation were observed only with convergence in the spinal cord.

Neural circuits spared by SCI are sufficient to mediate the effects of paired stimulation

To determine whether the neural circuits spared by SCI were sufficient to enable paired stimulation, we tested paired stimulation in rats with moderate C4 spinal contusion. Just as in Fig. 1A, 110% cortical stimulation was paired with 90% spinal stimulation. Augmentation of MEPs was observed with a peak at 10 ms latency (Fig. 1D; 134 ± 20%, $P = 0.0001$, paired *t*-test). No augmentation was observed at any latency in which spinal cord was stimulated before cortex. This supports the hypothesis that the spinal cord pathways spared by contusion can mediate the effects of sensory-motor convergence in the spinal cord.

Figure 4 Continued
($n = 7$ in both stimulation and control groups). (**C**) Locomotor behaviour on horizontal ladder shows percentage error that rats made while walking across the horizontal ladder over 20–24 trials at each time point, averaged over left and right forelimbs. Thick red and black lines are the average error rate for all stimulation group rats and controls, respectively. Thin light red and black lines indicate individual stimulation and control group rats, respectively. There was a decreased trend in percentage error rate in the rats in stimulation groups as compared to controls but was not significantly deferent between the groups at the final end point of testing (Day 49, unpaired *t*-test, $P = 0.182$, $n = 7$ in each group). (**D**) Pain scores quantified pain using the rats' facial characteristics. Thick red and black lines are the average pain scores for all stimulation group rats and controls, respectively. Thin light red and black lines indicate individual stimulation and control group rats, respectively. We observed no increase in pain score with 10 days of repetitive stimulation, rats in both the groups did not experience pain during the stimulation phase (unpaired *t*-test, $P = 0.646$, $n = 7$ in each group). Error bars show SEM.

Effects of paired stimulations are mediated by corticospinal and proprioceptive afferents

To test the hypothesis that descending corticospinal circuits and large-diameter afferents are necessary for effects of pairing, we selectively inactivated each with a chemogenetic tool. In rats with injections into motor cortex and spinal cord [Fig. 2A(i)], layer 5 neurons in the motor cortex were selectively and robustly labelled [Fig. 2A(ii)]. These neurons were inactivated by administration of CNO, the ligand for the inhibitory DREADD. At the time of maximum inactivation [30 min; Fig. 2A(iii)] the number of manipulations the rat made while eating uncooked pasta with the paw targeted for inactivation diminished to 39 ± 7%. The number of manipulations partially recovered by 120 min, as the CNO washed out. Manipulations of the non-targeted paw were not highly affected. We then tested whether this inactivation abrogated the effects of paired stimulation [Fig. 2A(iv)]. At baseline, subthreshold spinal cord stimulation delivered 10 ms after suprathreshold motor cortex stimulation strongly augmented biceps MEPs (>100%). Administration of CNO caused the MEP augmentation to be diminished compared to baseline, and at 30 min (–6.3% ±23%, d.f. = 4, Cohen's $d = 5.35$, $P = 0.003$), spinal cord stimulation had no augmenting effect on motor cortex MEPs on the inactivated side. The effects of pairing recovered by the end of the 120-min testing period. On the non-inactivated side, there was a less pronounced and more delayed effect of inactivation. Thus, corticospinal neurons are necessary for the effects of paired stimulation.

To determine whether paired stimulation also depends on spinal afferents, we inactivated DRG neurons using two methods. First, we targeted large-diameter neurons; an AAV5 encoding an inactivating DREADD was injected into DRGs of C5 and C6 on one side [Fig. 2B(i)]. Cells infected with the virus [Fig. 2B(ii)] measured 68 ± 18 µm in diameter, such as neurons that mediate touch, proprioception and muscle length or tension.[22] Before inactivation, the pairing of the motor cortex and spinal cord stimulation caused a large augmentation of the biceps MEP [>100%; Fig. 2B(iii), red] compared to cortical stimulation only. Thirty minutes after CNO injection, the paired stimulation effect was abolished (–1.5% ± 10.5%, d.f. = 4, Cohen's $d = 2.34$, $P = 0.013$) and largely returned by 120 min. There was no significant effect on the side without inactivation.

For the second approach, we selectively targeted the proprioceptors. A Cre-dependent DREADD was injected into the DRGs [Fig. 2C(i)] of transgenic rats expressing Cre under control of parvalbumin, which is expressed selectively by proprioceptors [Fig. 2C(ii)]. Inactivation caused a large-scale, but incomplete, abrogation of paired stimulation [28.6% ± 3.8%, d.f. = 4, Cohen's $d = 11.81$, $P = 0.017$; Fig. 2C(iii), green]. Inactivation also decreases the motor cortex excitability (Supplementary Fig. 1A), spinal MEPs (Supplementary Fig. 1B) and spinal excitability (Supplementary Fig. 1C) in PV-Cre rats. The loss of paired stimulation effect with inactivation of proprioceptors was less than inactivation of many large-diameter DRG neurons (including mechanoreceptors and others). Thus, large-diameter sensory afferents, and proprioceptors in particular, are necessary for the effects of SCAP.

To ensure that the effects of inactivation were due to loss of function of specific circuits, we conducted two control experiments. We tested whether CNO injection itself changed physiological responses. In a blinded test of rats without viral injection, CNO had no effect on physiological responses (Supplementary Fig. 2A). In addition, administration of saline in rats with DREADD injection showed no change in paired stimulation effects on cortical MEPs, cortical excitability, spinal MEPs or spinal excitability [Supplementary Fig. 2B(i–iv)]. Thus, neither CNO without DREADD administration nor DREADD injection without CNO affected physiological responses.

SCAP caused lasting changes in cortical and spinal cord evoked responses in awake rats with and without SCI

In addition to the immediate effects of paired stimulation, repeatedly pairing motor cortex and cervical spinal cord stimulation at 10 ms latency promotes SCAP. In these experiments, cortical and spinal responses were tested before and after a period of SCAP (Fig. 3A). SCAP delivered over 5 min [Fig. 3B(i)] nearly doubled the size of both the cortical and spinal MEPs [Fig. 3C(i), dark and light brown lines], an effect that ebbed over 120 min. These changes were of a similar magnitude but much longer duration than we previously reported; anaesthetized rats had no increase in MEPs after 60 min.[15]

We also tested the effects of SCAP in rats with SCI. Following the 5-min SCAP protocol, rats with SCI also showed lasting increases in the size of cortical and spinal MEPs [Fig. 3C(i), dark and light blue lines]. Similar effects were also observed with cortical and spinal thresholds in intact and SCI rats (Supplementary Fig. 4A). We compared the average effects of paired stimulation delivered at 10 ms latency versus several control conditions: motor cortex stimulation alone, spinal cord stimulation alone, pairing at 100 ms latency and 10 ms pairing in intact and SCI rats [Fig. 3D(i)]. The control conditions did not induce plasticity (ANOVA (analysis of variance) with Bonferroni correction; $P = 0.65$). However, both intact and SCI rats had significant increased cortical MEPs compared with these control conditions (ANOVA with Bonferroni correction $P < 0.001$); there was no significant difference between intact rats and rats with SCI (Intact = 38% ± 11%; SCI = 18% ± 10%; ANOVA with Bonferroni correction; $P = 0.11$). The change in cortical MEP was averaged across the 120 min of testing. Thus, the circuits spared by cervical SCI were sufficient to enable SCAP.

In anticipation of using SCAP in a preclinical therapeutic trial, we optimized the protocol. We kept the duration to a maximum of 30 min, a period that is tolerable for clinical neuromodulation.[39] We tested four parameters: frequency of pairing, pulse number (length of train), time between trains and number of bursts/trains (Supplementary Fig. 3). We tested five frequencies to determine whether each pair of stimuli would cause inhibition or augmentation of subsequent stimuli. The 5 and 0.2 Hz frequencies produced little change between stimuli, and we selected 5 Hz to minimize the total duration of stimulation. We also observed that 1 Hz was strongly inhibitory. Using the 5 Hz frequency, we varied the number of stimuli; 150 stimuli were better than the others tested. We tested the time interval between bursts of 150 stimuli at 5 Hz; we selected 1 min to balance effect size and the total duration of stimulation. Finally, we tested the total number of bursts. We observed that 20 trains of stimulation had a more durable effect than 10 trains with a similar overall magnitude. This resulted in the paradigm pictured in Fig. 3B(ii), which delivers 3000 pairs of stimuli over 30 min.

We tested the effects of 30-min SCAP in intact rats and rats with SCI. In intact rats, cortical and spinal MEPs were doubled immediately after 30-min SCAP [Fig. 3C(ii), dark and light blue lines], and they were still ~50% elevated at 120 min. In SCI rats, following the 30-min SCAP protocol [Fig. 3B(ii)] the size of both the cortical and spinal MEPs was increased 100–200% [Fig. 3C(ii), dark and light brown lines], and the MEPs were still almost double at 120 min.

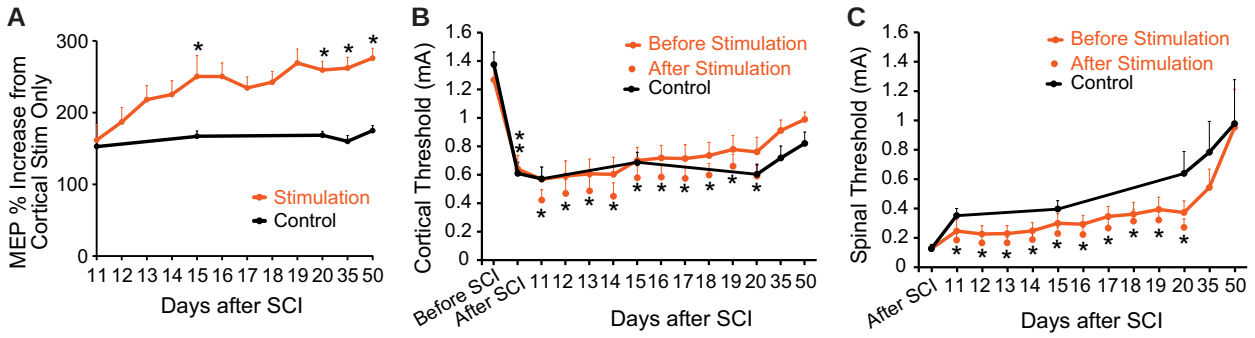

Figure 5 Ten days of SCAP produces augmented MEPs for at least 50 days after SCI. (**A**) The immediate effects were not different before stimulation (Day 11) in between the stimulation and control groups, in the stimulation group ($n = 7$), cortical MEP augmentation increased over the 10-day periods of stimulation to 259 ± 12%, while the control group ($n = 7$) did not change (168 ± 5.4%). The difference between groups was maintained at 50 days after SCI (Stim = 258% ± 21% versus Control = 169% ± 10%, d.f. = 12, Cohen's $d = 3.10$, $P = 0.0001$). This indicates that repetitive pairing over a 10-day period creates a durable change in physiology. (**B**) Cortical thresholds before SCI were above 1 mA in both stimulation (vermilion line) as well as control (black line) rats; after SCI there was a 50% drop in the cortical thresholds in both groups of rats, which may be due to hyperreflexia. There was gradual increase in cortical threshold after SCI but still below the before SCI value. The vermilion line indicated the change in cortical thresholds in each session of repetitive paired stimulation (30-min SCAP). After each session of 30 min of SCAP the cortical threshold decreased (vermilion dots) compared to that of the prestimulation value, indicating increased cortical excitability after each session. (**C**) Spinal thresholds after SCI were low in both stimulation (vermilion line) as well as control (black line) rats, the spinal threshold was measured only after SCI since spinal electrodes were implanted just after spinal contusion. There was a gradual increase in spinal threshold after SCI that we observed with our spinal electrodes due to an increase in impedance post implantation. The red line indicates the change in the spinal thresholds in each session of repetitive paired stimulation (30-min SCAP). After each session of 30 min of SCAP, the spinal threshold decreases (vermilion dots) as compared to that of prestimulation value, indicating increase spinal excitability after each session. Error bars show SEM.

Similar effects were also observed with cortical and spinal excitability in both intact rats and those with SCI (Supplementary Fig. 4B). The physiological effects of the 30-min SCAP paradigm are more robust than the 5-min SCAP paradigm in both intact and injured animals. Further, we compared the efficacy of 30-min SCAP to control conditions (motor cortex alone, spinal cord alone or paired with spinal cord stimulation after a 100 ms delay) and convergence in motor cortex. Neither the control conditions nor convergence at cortex increased cortical or spinal MEPs [ANOVA with Bonferroni correction; $P = 0.20$; Fig. 3D(ii)] or changed cortical or spinal thresholds (Supplementary Fig. 5). However, both intact and SCI rats had a significant increased cortical MEPs after 30-min SCAP compared with these control conditions [ANOVA with Bonferroni correction $P < 0.0001$; Fig 3D(ii)]. There was no significant difference between intact rats and rats with SCI (Intact = 82% ± 9%; SCI = 121% ± 11%; ANOVA with Bonferroni correction; $P = 0.15$). Finally, the similar magnitude and duration of effects in intact and injured rats suggest that circuits spared by SCI are sufficient to mediate pairing effects.

Repetitive SCAP after SCI improved forelimb dexterity without changing affective pain

To determine whether SCAP might be used to restore sensorimotor function after SCI, we performed a preclinical efficacy trial (Fig. 4A). The prespecified primary outcome measure was the IBB test, a scale that measures paw function while eating pieces of cereal [Fig. 4B(i)]. Before SCI, all rats performed at the top of the 9-point scale [Fig. 4B(ii)]. Twenty-eight days after SCI, the rats in each group showed severe impairment (stimulation = 2.46 ± 21; control = 2.38 ± 34), with difficulty holding and manipulating the cereal. By 50 days after injury, the last time tested, control rats scored 3.76 ± 0.49 and rats with stimulation scored 5.18 ± 0.25 (50 days; d.f. = 12, Cohen's $d = 2.62$, $P = 0.024$) demonstrating better grasp and support of the cereal [Fig. 4B(ii)].

Our secondary behavioural outcomes were walking performance on the horizontal ladder (Fig. 4C) and observed pain (Fig. 4D). Rats performed equally before the injury. For walking, by 28 days, the first time that rats reliably perform the task, control rats made 73 ± 7% errors, while rats with stimulation made 61 ± 5% errors. At the end of the testing period, control rats made 66 ± 8% errors and rats with stimulation made 50 ± 8% errors; this difference was not significant (50 days: d.f. = 12, $P = 0.18$). To test the effects of stimulation on pain, we used the Rat Grimace Scale, which uses facial features to rate severity on a 3-point scale. All rats exhibited pain after SCI (Fig. 4D), despite being given analgesics, which diminished by 10 days and stayed low. There was no difference in pain scores between the groups at the end of the trial (50 days: d.f. = 9, $P = 0.65$). Thus, 10 days of SCAP improved dexterity and did not worsen pain after SCI.

SCAP caused lasting increases in evoked responses and largely restored H-reflex modulation over weeks

We hypothesized that SCAP delivered daily over 10 days in rats with SCI would strengthen sensorimotor connections, as measured by evoked responses. We measured the immediate effect of paired stimulation; how strongly spinal stimulation augmented the cortical MEP as the time they are paired. Since paired MEPs are always compared to cortical stimulation alone, this measure is stable and reflects the strength of convergent stimuli. In rats with SCI, the immediate effect of pairing was large at 11 days after SCI (Stim = 162% ± 22%, Control = 153±11%) in both groups before the start of stimulation (Fig. 5A). In the stimulation group, cortical MEPs increased over the 10-day period to 259 ± 12%, while the control group did not change (168 ± 5.4%). The difference between groups was maintained at 50 days after SCI (Stim = 258 ± 21% versus Control = 169 ± 10%, d.f. = 12, Cohen's $d = 3.10$, $P = 0.0001$). This indicates that repetitive pairing over a 10-day period creates a durable change in MEPs from paired stimulation.

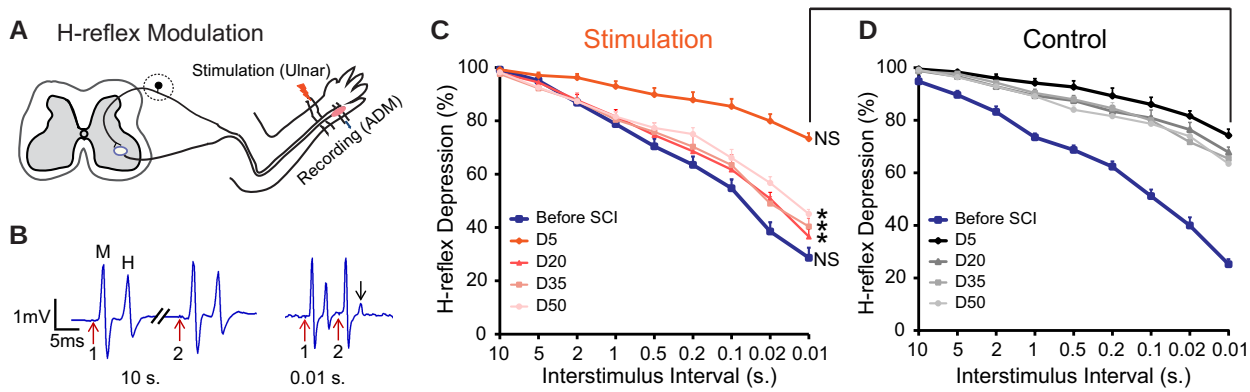

Figure 6 H-reflex modulation largely restored with SCAP. (**A**) Method of H-reflex testing. (**B**) The second H-reflex is diminished (arrow) at short interstimulus intervals. (**C**) Rats with stimulation ($n = 7$). Less H-reflex depression in rats 5 days after SCI (dark red) compared to before (blue). After paired stimulation H-reflex depression was largely restored and maintained in the stimulation group. (**D**) Control rats ($n = 7$) do not show restoration of H-reflex modulation. M = M-wave; H = H-reflex. Error bars show SEM.

We also measured cortical and spinal thresholds over the study. Cortical thresholds before SCI were above 1.2 mA in both stimulation (vermilion line) and control (black line) rats (Fig. 5B). After SCI there was ~50% drop in the cortical thresholds in both groups of rats (ANOVA, $P < 0.001$). After each 30-min SCAP session, the cortical threshold (vermilion dot) was lower than when it began (ANOVA, $P < 0.001$; red line). There was a gradual increase in cortical threshold until the end of the study period. Similarly, the spinal thresholds (Fig. 5C) immediately after SCI were low in both stimulation (vermilion line) as well as control (black line) rats (electrodes were implanted just after SCI). There was a lower threshold in the rats with stimulation compared to control rats (ANOVA, $P < 0.001$, vermilion line versus black line). There was a gradual increase in spinal threshold over the study period, as we have observed in implanted arrays.[16]

We hypothesized that repetitive stimulation would help restore spinal reflex modulation. We tested H-reflex modulation before and after SCI (Fig. 6A). H-reflexes were elicited at different interstimulus intervals to modulate responses (Fig. 6B). Before injury in both rats with SCAP (Fig. 6C) and controls (Fig. 6D), shorter intervals between H-reflexes caused the second response to be diminished (blue lines). After SCI, the rate-dependent decrease in the H-reflex was diminished (top lines). Ten days of SCAP largely restored rate-dependent depression of the H-reflex (Fig. 6C; bright vermilion lines). In contrast, control rats had little to no recovery of H-reflex modulation (Fig. 6D; grey lines). This effect was still persistent at 50 days after SCI (control 63 ± 1.5% versus Stim 45 ± 1.7%; d.f. = 12, Cohen's $d = 4.33$, $P = 0.0001$). Thus, SCAP largely restored H-reflex modulation after SCI.

No difference in SCI and tissue sparing between stimulation and control groups

To determine severity of SCI, we quantified the peak force rats received during C4 spinal contusion and the tissue preserved at the injury site at the end of the trial. The peak force that rats received is shown in Fig. 7A. There was no significant difference between the stimulation group (203.57 ± 5.81 kilodynes) and the control group (209.1 ± 7.13 kilodynes; *t*-test, $P = 0.28$). We also compared the amount of tissue spared at the site of injury. Representative spinal cord sections are shown in Fig. 7B and representative lesion reconstruction (Fig. 7C, vermilion). There was no significant difference in tissue sparing at the lesion epicentre between the control (3.13 ± 0.46 mm^2) and the stimulation group (2.76 ± 0.53 mm^2, independent *t*-test, $P = 0.61$; Fig. 7D). We concluded that the injuries rats received are similar between stimulation and control groups. We quantified the length of the spared descending motor and sensory afferent axons below the lesion in both groups. We did not observe a significant difference in total axon length of both descending motor or segmental sensory afferent axon lengths in between stimulation and control groups (Supplementary Fig. 6). We conclude no difference in tissue-sparing or anatomical connections between the groups after SCI.

Discussion

Our main hypothesis proved true: repetitive pairing of motor cortex and spinal cord stimulation augmented spinal excitability and improved function in rats with SCI through spinal sensory and descending motor convergence. Paired stimulation produces effects on three timescales: immediate, hours and weeks. The immediate effects (Fig. 1) do not represent plasticity; they operate only at the time that subthreshold spinal stimulation augments cortical MEPs. For paired stimulation to promote plasticity, it must be repeated (SCAP). Our optimized repetitive stimulation protocol produces robust augmentation that was still 50% greater than baseline at 2 h after 30 min of pairing. Thus, some effects are short-lived, on the order of hours. However, when SCAP was delivered for 10 days, both function and physiology were improved as long as they were measured, to 50 days. Dexterity improved with no increase in pain (Fig. 4). Physiology changes included stronger MEPs with paired stimulation (Fig. 5) and largely restored H-reflex modulation (Fig. 6). These represent two critical functions of the sensorimotor system, to drive voluntary movement and to modulate segmental reflexes.[39,40]

SCAP depends on sensorimotor convergence in the spinal cord

Our model of SCAP as the convergence of descending sensory and segmental proprioceptors at the level of the spinal cord is supported by three lines of evidence. First, there is striking concordance between the time that motor potentials arrive in the spinal cord [9.17 ± 0.55 ms; Fig. 1A(i)] and the optimal latency for pairing for the immediate effects (10 ms). Second, this immediate effect

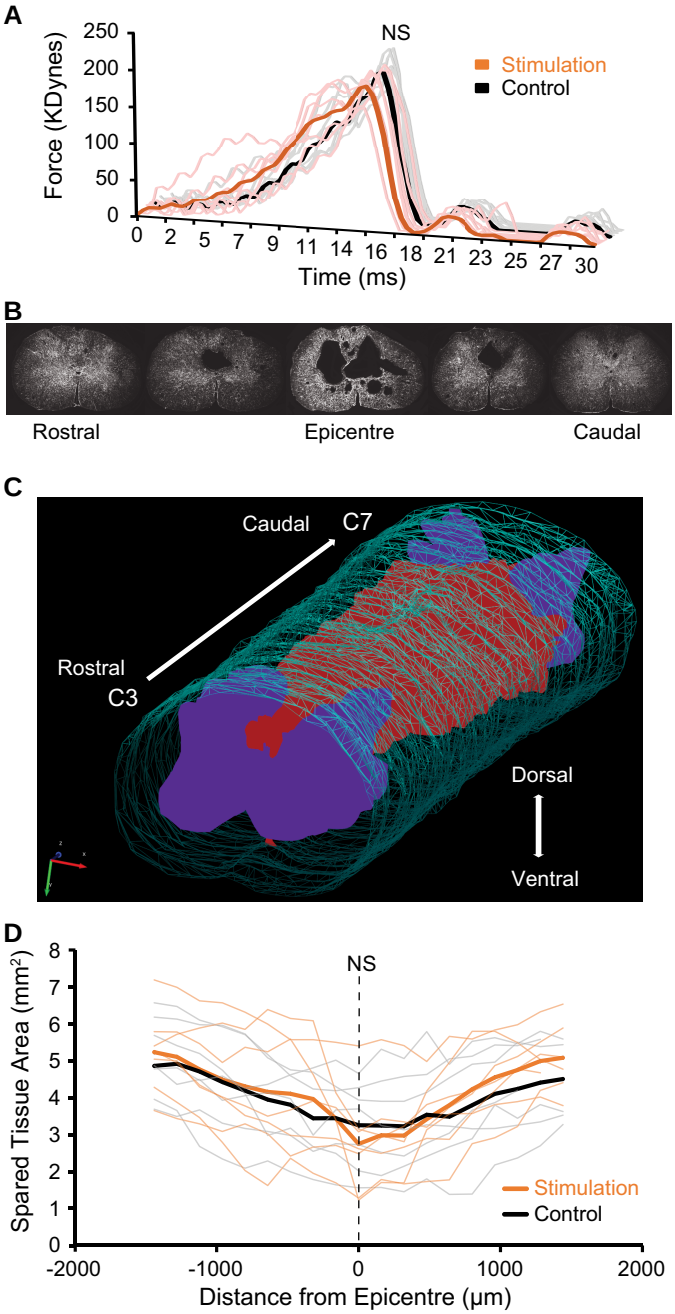

Figure 7 The injury force and the spared tissue did not differ between stimulation and control animals. (**A**) Force applied during cervical contusion ($n = 7$ for stimulation, $n = 7$ for control rats). (**B**) Representative spinal cord sections showing the extent of the lesion. Scale bar = 1 mm. (**C**) A rostro-caudal view of the lesion reconstruction. This 3D representation was created from cross sections through the site of spinal contusion. Lesion is shown in red, grey matter in purple and the tissue border in blue-green. Shows full degeneration of the main CST caudal to the site of injury, with partial sparing of the ventral pathways. (**D**) Spared tissue area through the lesioned spinal cord ($n = 7$ for stimulation, $n = 7$ for control rats).

depends on the CST and proprioceptors, as demonstrated by abrogation of the effect with selective inactivation of each of these pathways (Fig. 2). Finally, pairing of motor cortex and spinal cord at a latency that has no immediate effect (100 ms) also produces no plasticity when applied repetitively [Fig. 3D(ii) and Supplementary Fig. 5].

We compared the effects of sensorimotor convergence at the level of the spinal cord with convergence in cortex, and the results challenge the most common clinical approach of targeting cortex. Paired afferent stimulation, which targets cortex, has been used to improve function in people, but the protocol does not work in all individuals and the physiological effects are moderate in size.[3,41] In contrast, convergence in the spinal cord had a much larger immediate and lasting effect in the rats. Several groups have applied paired stimulation of the descending (upper) motor system and segmental (lower) motor system.[12–14,42–45] SCAP differs from

these approaches by targeting descending motor and segmental afferents—sensorimotor integration rather than the motor only connections in the spinal cord.

SCAP effective in rats with moderate cervical SCI

SCAP was at least as effective in rats with C4 SCI as uninjured rats, indicating that sparse descending motor circuits mediate the plasticity effect, along with the intact sensory system below injury. The C4 contusion injury obliterates the grey matter and leaves just a thin rim of white matter, like human injury.[46,47] Interestingly, we observed sparing of <1% of the CST in rats with this injury.[30] This suggests that other descending motor pathways may be involved, as they are in recovery after human SCI.[48] Targeting the reticulospinal tract, which is slightly better preserved by experimental SCI in rats, results in improvement of motor function, both in rodents[23,49] and in humans.[11,50]

There is a striking concordance of physiological changes induced by SCAP at the cortical and spinal cord levels. This is likely explained by changes in responses in the spinal cord, possibly in the common target of descending motor and segmental afferent connections. These results contribute to our working model that convergent inputs to spinal neurons cause the synapses or the cellular targets themselves to undergo lasting change. Synchrony of input is critical since paired stimulation with an inappropriate time difference between motor cortex and spinal cord stimulation was ineffective in producing plasticity.

Conclusion

This study provides important considerations for the application of paired stimulation to enhance sensorimotor function in people with CNS injury. The interactions between descending motor pathways and large-diameter afferents are largely conserved between rats and humans,[51,52] but the direct connections between motor cortex and spinal motoneurons in humans may alter these interactions. In addition, the scale of the two species is very different, and it will be important to determine the proper timing of paired stimulation in people. Finally, it will be important to consider the differences in the location of tracts within the spinal cord and the pattern of injury to the spinal cord between human injury and experimental SCI in rats.

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Competing interests

J.C. is a co-inventor of a patent for the use of softening spinal electrodes. He also has equity in Backstop Neural, which seeks to commercialize the devices for humans. The authors declare no other competing financial interests.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Asan AS, McIntosh JR, Carmel JB. Targeting sensory and motor integration for recovery of movement after CNS injury. *Front Neurosci*. 2022;15:791824.
2. Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain*. 2000;123 Pt 3:572–584.
3. Suppa A, Quartarone A, Siebner H, *et al*. The associative brain at work: Evidence from paired associative stimulation studies in humans. *Clin Neurophysiol*. 2017;128(11):2140–2164.
4. Ling YT, Alam M, Zheng Y-P. Spinal cord injury: Lessons about neuroplasticity from paired associative stimulation. *Neuroscientist*. 2020;26(3):266–277.
5. Wagner FB, Mignardot J-B, Le Goff-Mignardot CG, *et al*. Targeted neurotechnology restores walking in humans with spinal cord injury. *Nature*. 2018;563(7729):65–71.
6. Angeli CA, Boakye M, Morton RA, *et al*. Recovery of over-ground walking after chronic motor complete spinal cord injury. *N Engl J Med*. 2018;379(13):1244–1250.
7. Gill ML, Grahn PJ, Calvert JS, *et al*. Neuromodulation of lumbosacral spinal networks enables independent stepping after complete paraplegia. *Nat Med*. 2018;24(11):1677–1682.
8. NSCISC. National Spinal Cord Injury Statistical Center (Facts and Figures at a Glance). 2016.
9. Anderson KD. Targeting recovery: Priorities of the spinal cord-injured population. *J Neurotrauma*. 2004;21(10):1371–1383.
10. Bunday KL, Perez MA. Motor recovery after spinal cord injury enhanced by strengthening corticospinal synaptic transmission. *Curr Biol*. 2012;22(24):2355–2361.
11. Bunday KL, Urbin MA, Perez MA. Potentiating paired corticospinal-motoneuronal plasticity after spinal cord injury. *Brain Stimulat*. 2018;11(5):1083–1092.
12. Jo HJ, Perez MA. Corticospinal-motor neuronal plasticity promotes exercise-mediated recovery in humans with spinal cord injury. *Brain*. 2020;143:1368–1382.
13. Nishimura Y, Perlmutter SI, Eaton RW, Fetz EE. Spike-timing-dependent plasticity in primate corticospinal connections induced during free behavior. *Neuron*. 2013;80(5):1301–1309.
14. Nishimura Y, Perlmutter SI, Fetz EE. Restoration of upper limb movement via artificial corticospinal and musculospinal connections in a monkey with spinal cord injury. *Front Neural Circuits*. 2013;7:57.
15. Mishra AM, Pal A, Gupta D, Carmel JB. Paired motor cortex and cervical epidural electrical stimulation timed to converge in the spinal cord promotes lasting increases in motor responses. *J Physiol (Lond)*. 2017;595(22):6953–6968.
16. Garcia-Sandoval A, Pal A, Mishra AM, *et al*. Chronic softening spinal cord stimulation arrays. *J Neural Eng*. 2018;15(4):045002.
17. Garcia-Sandoval A, Guerrero E, Hosseini SM, *et al*. Stable softening bioelectronics: A paradigm for chronically viable ester-free neural interfaces such as spinal cord stimulation implants. *Biomaterials*. 2021;277:121073.
18. Park HG, Carmel JB. Selective manipulation of neural circuits. *Neurotherapeutics*. 2016;13(2):311–324.

19. Wahl AS, Omlor W, Rubio JC, *et al*. Neuronal repair. Asynchronous therapy restores motor control by rewiring of the rat corticospinal tract after stroke. *Science*. 2014;344:1250–1255.
20. Allred RP, Adkins DL, Woodlee MT, *et al*. The vermicelli handling test: A simple quantitative measure of dexterous forepaw function in rats. *J Neurosci Methods*. 2008;170:229–244.
21. Wen T-C, Lall S, Pagnotta C, *et al*. Plasticity in one hemisphere, control from two: Adaptation in descending motor pathways after unilateral corticospinal injury in neonatal rats. *Front Neural Circuits*. 2018;12:28.
22. Giuffrida R, Rustioni A. Dorsal root ganglion neurons projecting to the dorsal column nuclei of rats. *J Comp Neurol*. 1992;316:206–220.
23. Vulchanova L, Schuster DJ, Belur LR, *et al*. Differential adeno-associated virus mediated gene transfer to sensory neurons following intrathecal delivery by direct lumbar puncture. *Mol Pain*. 2010;6:31.
24. Takeoka A, Vollenweider I, Courtine G, Arber S. Muscle spindle feedback directs locomotor recovery and circuit reorganization after spinal cord injury. *Cell*. 2014;159:1626–1639.
25. Takeoka A. Proprioception: Bottom-up directive for motor recovery after spinal cord injury. *Neurosci Res*. 2020;154:1–8.
26. Asboth L, Friedli L, Beauparlant J, *et al*. Cortico-reticulo-spinal circuit reorganization enables functional recovery after severe spinal cord contusion. *Nat Neurosci*. 2018;21:576–588.
27. Ichikawa H, Deguchi T, Nakago T, Jacobowitz DM, Sugimoto T. Parvalbumin, calretinin and carbonic anhydrase in the trigeminal and spinal primary neurons of the rat. *Brain Res*. 1994;655:241–245.
28. Walters MC, Sonner MJ, Myers JH, Ladle DR. Calcium imaging of parvalbumin neurons in the dorsal root ganglia. *eNeuro*. 2019;6:ENEURO.0349-18.2019.
29. Krashes MJ, Koda S, Ye C, *et al*. Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *J Clin Invest*. 2011;121:1424–1428.
30. Yang Q, Ramamurthy A, Lall S, *et al*. Independent replication of motor cortex and cervical spinal cord electrical stimulation to promote forelimb motor function after spinal cord injury in rats. *Exp Neurol*. 2019;320:112962.
31. Carmel JB, Berrol LJ, Brus-Ramer M, Martin JH. Chronic electrical stimulation of the intact corticospinal system after unilateral injury restores skilled locomotor control and promotes spinal axon outgrowth. *J Neurosci*. 2010;30:10918–10926.
32. Irvine K-A, Ferguson AR, Mitchell KD, *et al*. The Irvine, Beatties, and Bresnahan (IBB) forelimb recovery scale: an assessment of reliability and validity. *Front Neurol*. 2014;5:116.
33. Irvine K-A, Ferguson AR, Mitchell KD, Beattie SB, Beattie MS, Bresnahan JC. A novel method for assessing proximal and distal forelimb function in the rat: The Irvine, Beatties and Bresnahan (IBB) forelimb scale. *J Vis Exp*. 2010;46:2246.
34. Carmel JB, Kimura H, Martin JH. Electrical stimulation of motor cortex in the uninjured hemisphere after chronic unilateral injury promotes recovery of skilled locomotion through ipsilateral control. *J Neurosci*. 2014;34:462–466.
35. Carmel JB, Martin JH. Motor cortex electrical stimulation augments sprouting of the corticospinal tract and promotes recovery of motor function. *Front Integr Neurosci*. 2014;8:51.
36. Metz GA, Whishaw IQ. The ladder rung walking task: A scoring system and its practical application. *J Vis Exp*. 2009;28:1204.
37. Sotocinal SG, Sorge RE, Zaloum A, *et al*. The Rat Grimace Scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*. 2011;7:55.
38. Tan AM, Chakrabarty S, Kimura H, Martin JH. Selective corticospinal tract injury in the rat induces primary afferent fiber sprouting in the spinal cord and hyperreflexia. *J Neurosci*. 2012;32:12896–12908.
39. Fitzgerald PB. An update on the clinical use of repetitive transcranial magnetic stimulation in the treatment of depression. *J Affect Disord*. 2020;276:90–103.
40. Porter R, Lemon R. *Corticospinal function and voluntary movement*. Oxford University Press; 1993.
41. Wolpaw JR. The negotiated equilibrium model of spinal cord function. *J Physiol (Lond)*. 2018;596:3469–3491.
42. Carson RG, Kennedy NC. Modulation of human corticospinal excitability by paired associative stimulation. *Front Hum Neurosci*. 2013;7:823.
43. Taylor JL, Martin PG. Voluntary motor output is altered by spike-timing-dependent changes in the human corticospinal pathway. *J Neurosci*. 2009;29:11708–11716.
44. Harel NY, Carmel JB. Paired stimulation to promote lasting augmentation of corticospinal circuits. *Neural Plast*. 2016;2016:7043767.
45. Chandrasekaran S, Nanivadekar AC, McKernan G, *et al*. Sensory restoration by epidural stimulation of the lateral spinal cord in upper-limb amputees. *eLife*. 2020;9:e54349.
46. Bunge RP, Puckett WR, Becerra JL, Marcillo A, Quencer RM. Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. *Adv Neurol*. 1993;59:75–89.
47. Kakulas BA, Kaelan C. The neuropathological foundations for the restorative neurology of spinal cord injury. *Clin Neurol Neurosurg*. 2015;129:S1–S7.
48. Baker SN, Perez MA. Reticulospinal contributions to gross hand function after human spinal cord injury. *J Neurosci*. 2017;37(40):9778–9784.
49. Moritz CT. Now is the critical time for engineered neuroplasticity. *Neurotherapeutics*. 2018;15:628–634.
50. Sangari S, Lundell H, Kirshblum S, Perez MA. Residual descending motor pathways influence spasticity after spinal cord injury. *Ann Neurol*. 2019;86:28–41.
51. Curfs MH, Gribnau AA, Dederen PJ, Bergervoet-Vernooij IW. Induction of c-Fos expression in cervical spinal interneurons after kainate stimulation of the motor cortex in the rat. *Brain Res*. 1996;725:88–94.
52. Pierrot-Deseilligny E, Burke D. *The circuitry of the human spinal cord: Its role in motor control and movement disorders*. Cambridge: Cambridge University Press; 2005.