

1 **EPIDURAL ELECTRICAL STIMULATION OF THE CERVICAL DORSAL ROOTS RESTORES**
2 **VOLUNTARY UPPER LIMB CONTROL IN PARALYZED MONKEYS**

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23 DOI : 10.1038/s41593-022-01106-5

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25

26 **ABSTRACT**

27 Regaining arm control is a top priority for people with paralysis. Unfortunately, the complexity of
28 the neural mechanisms underlying arm control has limited the effectiveness of neurotechnology
29 approaches. Here, we exploited the neural function of surviving spinal circuits to restore voluntary
30 arm and hand control in three monkeys with spinal cord injury, using spinal cord stimulation. Our
31 neural interface leverages the functional organization of the dorsal roots to convey artificial
32 excitation via electrical stimulation to relevant spinal segments at appropriate movement phases.
33 Stimulation bursts targeting specific spinal segments produced sustained arm movements,
34 enabling monkeys with arm paralysis to perform an unconstrained reach-and-grasp task.
35 Stimulation specifically improved strength, task performances and movement quality.
36 Electrophysiology suggested that residual descending inputs were necessary to produce
37 coordinated movements. The efficacy and reliability of our approach hold realistic promises of
38 clinical translation.

39

40 **MAIN TEXT**

41 **INTRODUCTION**

42 More than 5 million people in the US currently live with some form of motor paralysis¹. Stroke and
43 spinal cord injury (SCI) are the main causes with hundreds of thousands of new cases per year².
44 Impairments of the hand and arm are particularly problematic, representing a major unmet need
45 for both SCI and stroke patient populations^{3,4}. Indeed, even mild deficits in hand function lead to
46 significant degradation of quality of life. Unfortunately, recovery of hand and arm motor function
47 is still an unsolved clinical challenge.

48 Generated in the cerebral cortex, upper limb motor commands are relayed to subcortical and
49 spinal circuits that activate motoneurons and regulate sensory inputs to produce skilled motor
50 actions^{5–7}. Spinal cord injury (SCI), or stroke, damage these communication pathways generating
51 impairments in sensory regulation and motor functions that lead to motor paralysis.

52 Historically, neurotechnologies were conceived around the idea of restoring movements in
53 paralyzed subjects via a technological bypass. Such solution would use signals from cortical
54 areas as inputs and artificially compensate for lack of motoneuron activation by producing desired
55 muscle activity below the lesion⁸. For example, both intraspinal micro stimulation^{9–11} (ISMS) and
56 functional electrical stimulation¹² (FES) were used to activate arm muscles in response to
57 intracortical neural activity from the motor cortex. While ISMS was never attempted in human
58 subjects, brain-controlled FES allowed paralyzed humans to perform voluntary grasping tasks^{13,14}.
59 However, translation of these concepts into daily clinical practice is hindered by two distinct
60 limitations. First, the artificial motoneuron recruitment order generated by FES induces muscle
61 fatigue¹⁵ which is particularly problematic for arm movements. Indeed, fatigue prevents the
62 generation of sustained forces and consequently FES fails to enable sustained three-dimensional
63 arm movements that are required for daily activities. Second, since FES bypasses surviving
64 circuits in the spinal cord, complex stimulation protocols and sophisticated decoding algorithms¹
65 are required to orchestrate the activation of multiple muscles and produce functional movements.
66 As a result, these systems require an articulated combination of hardware and software.
67 Unfortunately, this complexity does not cope well with dynamic clinical environments that need
68 robust and practical solutions for a rapid set up and large-scale use.

69
70 In contrast, epidural electrical stimulation (EES) of the lumbar spinal cord exploits surviving spinal
71 circuits and supra-spinal connections after injury to produce movements¹⁶. Similar to intraspinal
72 stimulation^{11,17}, EES engages motoneurons via direct recruitment of large sensory afferents¹⁸
73 leading to widespread excitatory post-synaptic potentials in the spinal cord. More importantly,
74 since motoneurons are recruited via natural synaptic inputs, EES generates a natural recruitment
75 order¹⁹ that is resistant to artificial fatigue. This enables the production of forces that can sustain
76 the whole-body weight²⁰. Moreover, engagement of motoneurons from pre-synaptic pathways
77 allows residual descending inputs and spinal circuits to control motoneurons excitability and
78 produce voluntary movement after complete motor paralysis²¹.

79
80 Building on animal models^{22–24}, recent clinical studies have shown that continuous stimulation
81 delivered through epidural implants on the dorsal aspect of the lumbosacral spinal cord increased
82 muscle strength, voluntary muscle activation and single joint movements in people with complete
83 leg paralysis^{21,25}. More strikingly, when coupled with targeted physical rehabilitation protocols,
84 continuous EES restored weight bearing locomotion in subjects with severe SCI^{26,27}. These
85 outstanding clinical results prompted experimental studies aiming at verifying whether EES could
86 be used to promote also upper limb movements after SCI²⁸. Unfortunately, while clinical studies

88 showed some success in improving hand grip force with both epidural and non-invasive
89 approaches^{29,30}, continuous EES did not produce results of similar outstanding efficacy as those
90 observed for the lower limbs^{26,27}. In fact, clinical outcomes were similar to those obtained with
91 surface FES³¹.

92 Reasons for this discrepancy may stem from the complexity of upper limb motor control and
93 biomechanics compared to locomotion. Indeed, in contrast to pattern-driven³² and repetitive
94 locomotor movements, upper limb movements are composed by a non-repetitive and task-
95 dependent combination of movement modules which are highly dependent from sophisticated
96 cortico-spinal control^{6,33-35} and accurate sensory feedback^{36,37}. Because of this intrinsic
97 complexity, non-specific neuromodulation could limit the efficacy of EES by exciting all spinal
98 segments simultaneously, irrespectively of movement phase. More importantly, unspecific and
99 continuous stimulation of the sensory afferents through EES disrupts natural sensory inputs¹⁹
100 thus hindering spinal regulation of movements which is critical in dexterous upper limb control^{36,37}.
101 We and others have shown that it is possible to direct electrical stimulation of the spinal cord to
102 target restricted segments during appropriate times^{17,38}. These spatio-temporal stimulation
103 protocols enabled voluntary locomotion in monkeys with SCI as early as day 6 post injury without
104 any physical training³⁹ and within 2 weeks post implantation in humans with complete leg
105 paralysis²⁰. This approach exploits the somato-topography of the spinal sensory system to
106 selectively engage restricted spinal regions^{18,38}. Unfortunately, non-invasive technologies and
107 clinically approved electrodes are unfit for this scope⁴⁰ because of their limits in selectivity.
108 Therefore, we hypothesized that a neural interface, specifically designed to target the cervical
109 dorsal roots, could enable the administration of spatio-temporal stimulation patterns to the cervical
110 spinal cord. We tested this hypothesis in three monkeys with a unilateral cervical SCI. We
111 designed a personalized epidural interface to target primary afferents within the cervical dorsal
112 roots. We hypothesized that the electrical stimulation of the roots with bursts linked to movement
113 attempts would enable voluntary motor control and improve functional deficits of the arm and hand
114 that emerge after SCI. Specifically we tested for improvements in muscle strength, dexterity and
115 ability to execute three-dimensional functional tasks in full independence. Finally, we verified that
116 the mechanisms enabling the voluntary recruitment of motoneurons in the cervical spinal cord
117 were similar to those occurring during EES of the lumbosacral circuits.

118 119 **Results**

120

121 **Natural arm movements**

122 Clinically effective systems should enable truly functional arm movements rather than simplified
123 tasks such as single-joint movements. A functional arm movement entails a coordinated activation
124 of arm muscles to achieve a desired movement while supporting the arm weight at all times. Most
125 of daily activities require arm extension (reach) and flexion (pull), combined with a hand-grasp
126 without a constrained timing or structure. Consequently, we developed a robotic platform allowing
127 the quantification of reach, grasp and pull movements⁴¹ that would feel natural and unconstrained
128 to monkeys both in trajectory and timings (**Figure 1A**). We trained three adult *Macaca fascicularis*
129 monkeys to reach for, grasp, and pull an instrumented object placed on the end effector of our
130 robotic arm (**Figure 1B**). Movement trajectories were not constrained neither kinematically nor in
131 time. Monkeys waited for the go signal, reached for the object and pulled to receive a food or juice
132 reward when the object crossed a pre-defined displacement threshold⁴¹. Monkeys intuitively and
133 rapidly learned this task by developing their own individual kinematic strategies (**Extended Data**
134 **Figure 1**) and personal movement speeds. We then designed a battery of electrophysiology and
135 kinematic measurements to evaluate functional outcomes on task performances, muscle
136 activation, muscle strength and movement dexterity. Specifically, we quantified full-limb 3D

137 kinematics (Vicon Motion Systems, Oxford, UK), pulling forces, and electromyographic (EMG)
138 signals from intramuscular leads in eight arm muscles (**Figure 1A, Extended Data Figure 1**).
139 Before SCI, we observed clear bursts of EMG activity from all hand and arm muscles during the
140 three movement phases: reach, grasp, and pull in all monkeys. Finally, to document the
141 involvement of cortical neurons during movement enabled by EES and to extract signals that
142 could also be used to link stimulation bursts to movement phase onset, we implanted multi-
143 microelectrode arrays (Blackrock Microsystems, Salt Lake City, USA) in the arm/hand region of
144 the right sensorimotor (M1, S1) and ventral premotor (PMv) cortex. We validated these recordings
145 by verifying that neural activity was consistently modulated with kinematics pre-injury and with the
146 three movement phases as largely expected⁴¹ (**Figure 1, Extended Data Figure 1**). In summary,
147 we analyzed natural arm movements in monkeys and concluded that in order for stimulation
148 protocols to be effective, it was important to support reach, grasp and pull independently with
149 specific parameters for each animal.

150

151

152 **Personalized spinal interface**

153 To design an optimal interface, we studied the anatomy of the monkey cervical spinal cord. We
154 extrapolated available anatomical information from literature and found that, similar to humans,
155 motoneurons innervating arm muscles in the monkeys are segmentally organized⁴⁰ (**Figure 1C**).
156 We previously showed that stimulation of a single cervical dorsal root will recruit motoneurons
157 that receive direct afferent inputs from that root⁴⁰. Exploiting this property allows to obtain a
158 segmental recruitment order of motoneurons that can be targeted to promote specific movement
159 phases^{20,38,42}. Therefore, we designed a spinal interface that could target each root independently.
160 We achieved this by placing contacts on the lateral aspect of the cord to target the entry zone of
161 each individual root⁴⁰. Since each monkey displayed a unique anatomy, we tailored the design of
162 our interface to each specific subject. For this, we measured white matter diameter and vertebral
163 canal features from computed tomography (CT) and magnetic resonance imaging (MRI). We then
164 spaced the electrodes rostro-caudally and medio-laterally to match the transversal and
165 longitudinal dimensions of the cord of each animal (**Extended Data Figure 2A, 2B**). This allowed
166 us to simplify the neural interface architecture by minimizing the number of contacts while
167 maintaining high muscle recruitment specificity⁴³. We then designed a surgical strategy to position
168 the epidural interface between the C6 and T1 dorsal roots (**Figure 1D**). We performed
169 laminectomies between the T1 and T2 vertebrae and the C5 and C6 vertebrae, then pulled the
170 neural interface through the intermediate epidural space with the help of a custom soft inserter⁴³.
171 We verified that the position of the array remained stable for the entire duration of the study (up
172 to 3 weeks) through repeated X-ray imaging (**Figure 1D, Extended Data Figure 2C**). During the
173 same surgery, we performed a unilateral spinal cord injury at the C5/C6 segments (**Figure 1E**)
174 aiming at transecting the cortico-spinal tract that is located on the lateral aspect of the white matter
175 in monkeys. This type of lesion is amply described in literature and induces unilateral arm and
176 hand paralysis¹ while preserving important bodily functions such as bladder control. Postmortem
177 immunohistochemistry analysis of the spinal cords showed that the spinal interface did not
178 damage the cervical cord in any of the three monkeys but did reveal that Mk-Br received an
179 unplanned compression injury at the insertion site (T3 spinal segment). Given the caudal position
180 of this contusion it is likely for it to have occurred during implantation (**Extended Data Figure 2D**).
181 Since the T3 segment is below the innervation of the arm motoneurons, this lesion did not affect
182 the phenotype of arm and hand motor deficits which did not differ from the other monkeys (see
183 Methods).

184 In summary, we designed a spinal interface to selectively recruit the cervical dorsal roots. We
185 tailored the interface to the specific anatomy of each monkey and designed a surgical strategy to
186 perform a consistent and stable implantation.

187

188 **EES can produce functional movements and grasp**

189 We next assessed the selectivity of the epidural interface. In propofol anaesthetized monkeys, we
190 delivered asymmetric, charge-balanced biphasic pulses of EES at low repetition rate (1Hz) at
191 various current amplitudes from each contact. Minimum and maximum amplitude values were
192 selected as the first subthreshold and first saturation current value respectively. As predicted⁴⁰,
193 different stimulation contacts generated muscle recruitment patterns that mirrored the segmental
194 organization of cervical motoneurons (**Figure 2A, Extended Data Figure 3**). Specifically,
195 contacts located at C8/T1 level (caudal) elicited spinal reflexes mostly in the hand and forearm
196 muscles, contacts located at C7 level elicited triceps and contacts located at C5/C6 recruited
197 biceps and deltoids (rostral). Those results were consistent in all animals (**Figure 2B, Extended**
198 **Data Figure 3**). To ensure that this segmental selectivity translated into separate functional arm
199 and hand movements, we delivered supra-threshold stimulation at various frequencies (20-120
200 Hz) from each contact in two animals (Mk-Br and Mk-Yg). Indeed, since recruitment of
201 motoneuron is pre-synaptic, EES may not be able to produce sustained muscle activation
202 because of frequency dependent suppression⁴⁶. This effect is an observed substantial
203 suppression of muscle evoked potentials during repetitive stimulation of the afferents. Instead, we
204 observed large and sustained arm movements during EES bursts. Muscle selectivity was
205 preserved during long stimulation trains (**Figure 3C, F**) and different contacts elicited distinct
206 functional joint movements (**Figure 3A, B, D, E, Video 1**) such as shoulder abduction, elbow
207 extension and whole hand grasp. When looking at the energy of the EMGs, we found a monotonic
208 relationship between muscle activation and stimulation frequency in most of the upper arm
209 muscles (**Figure 3C, F**). However, not all muscles showed such clear frequency dependent
210 responses (**Extended Data Figure 4A**). Moreover, peak-to-peak responses (**Extended Data**
211 **Figure 4B**) were generally decreased during a burst at high frequency but were not abolished
212 and tended to vary during the burst and while the movement was produced. We used these
213 observations to optimize stimulation parameters to be used in a behavioral reach and grasp task
214 (see Methods and **Extended Data Figure 5**). In summary, we found that single contacts of our
215 spinal interface elicited segmental recruitment of arm flexors, extensors and hand flexors. Bursts
216 of stimulation from these contacts produced sustained joint movements that were graded by
217 stimulation frequency (**Extended Data Figure 6**).

218

219 **EES improves arm and hand function after spinal cord injury**

220

221 We next tested whether our stimulation protocol could improve functional outcomes of upper limb
222 movements after SCI. Specifically, we tested the efficacy of EES to improve muscle activation,
223 pulling forces, functional task performance, and kinematic quality of three-dimensional
224 movements after SCI when stimulation was on against stimulation off as a control. In all monkeys,
225 the lesion led to substantial motor deficits of the left arm and hand.

226 While each monkey retained the ability to activate proximal shoulder and biceps muscles, elbow
227 extension and hand functions were severely compromised. Severity of the impairment and extent
228 of spontaneous recovery (**Extended Data Figure 7**) varied across monkeys because of the
229 variability in lesion size (**Figure 1E**). Generally, animals showed severe paralysis immediately
230 after lesion, and then gradually regained some movement capabilities (**Extended Data Figure 7**).
231 Due to the initial impairment, immediately after the lesion, monkeys were not able to perform the

232

behavioral task. Consequently, during the first week, we simplified the task by presenting an object close to the monkeys and triggering stimulation bursts manually to encourage the animal to perform the task. After the first week, all monkeys spontaneously attempted to perform the task, making it possible to link the delivery of movement-specific stimulation bursts to real-time detection of movement onset using intra-cortical signals. Whenever the monkeys strived for a reach, grasp or pull movement, we delivered bursts of stimulation promoting reach or grasp/pull respectively (movement specific EES). Outcomes were computed for each animal independently and compared between EES on and EES off. In terms of functional task performances, without stimulation Mk-Br and Mk-Yg were more capable of completing reach movements than grasp and pull, which is consistent with their lesion severity. Stimulation improved overall movement performances but differentially according to specific animal's deficits. We found that the majority of times that a monkey had completed a portion of the task it had done so during EES (**Figure 4B**). More specifically, Mk-Sa suffered the most severe deficits and could only reach during EES and never without (**Figure 4B**). Similarly, Mk-Yg was never able to grasp or pull without EES, whereas Mk-Br could perform only a handful of grasps and pulls without stimulation. Interestingly, performance changed overtime. For example, in both Mk-Br and Mk-Yg improvements in grasps and pull emerged only later when the animals spontaneously recovered some movement capacity (**Figure 4B, Video 2,3,4**). More specifically Mk-Br improved grasp and pull only after 2 weeks with stimulation while Mk-Yg could grasp with stimulation by the end of week 1 (**Figure 4B**) which was unfortunately the last day we could test this task because the grasp contact E6 failed after (see Methods). Instead, when we used our interface to deliver continuous EES that was not related to movement onsets, only non-significant and modest improvements were observed in Mk-Br while Mk-Yg did not show ability to grasp and pull during continuous EES (**Extended Data Figure 8A**). Moreover, we analyzed trials in which stimulation bursts were not triggered at movement onset, for example when pull stimulation was erroneously triggered during reach. In these trials the reach movement was abruptly interrupted, and the animal did not complete the task (**Extended Data Figure 8B, Video 5**).

During phase dependent stimulation, EES enhanced muscles activity and forces (**Figure 5A,B**) compared to no stimulation. In terms of movement quality, EES bursts triggered at movement onset significantly improved the overall quality of arm movements (**Figure 5B**). Indeed, principal component analysis (PCA) of three-dimensional kinematic parameters (i.e., timing, force, arm trajectories, joint angles) revealed that during EES, movement kinematics were significantly closer to pre-lesion kinematics than the few successful movements performed without stimulation (distance from pre-lesion performances in the multi-parametric kinematic space, **Figure 5B**). Notably, animals sustained the weight of the arm and lifted their elbow more, performed wider movements, and generated stronger forces (**Figure 5B**), getting closer to normal kinematic trajectory patterns without any long-term training.

In summary, we showed that EES bursts triggered at movement phase onsets, improved muscle strength, task performance and quality of arm movements. This allowed monkeys to perform reach, grasp and pull movements that were otherwise not able to perform without EES.

Sensory inputs can decrease EES-induced motor output

We then investigated the role of spinal circuits and sensory inputs in the production of the movements that we observed. Indeed, since activation of motoneurons was pre-synaptic, spinal reflexes and sensory inputs can influence EES evoked spinal reflexes in the legs³⁸. In order to exclude influences of residual supraspinal voluntary inputs, we conducted experiments under propofol anesthesia (**Figure 6A**) with Mk-Br. We then delivered bursts of EES from the contact eliciting elbow flexion at varying stimulation frequencies in two distinct conditions (**Figure 6B**): in

isometric and unconstrained conditions. In the isometric condition, we constrained the wrist, elbow and shoulder of the animal and measured force production at the wrist joint. Under unconstrained conditions we left the arm free to move under the effect of stimulation. This setup only differs from the sensory feedback generated at the load when pull forces are produced by EES. We found that EES induced EMG activity during unconstrained movement that was significantly different from the EMG activity induced during isometric movements (**Figure 6B**). In particular, overall EMGs and peak-to-peak amplitudes of elicited spinal reflexes were significantly lower when the arm was attached to a load (isometric) compared to when it was free to move. Albeit present at all frequencies, this difference was particularly important within the 40 to 60Hz range, thus overlapping with the functional frequency ranged that we selected for our study. These results show that force loads at the hand decreased EMG activity induced by EES as compared to no load applied at the hand. Under anesthesia, only changes on spinal circuit excitability induced by sensory inputs can explain the observed changes on EES evoked muscle activity.

Residual cortical input is necessary
The influence of spinal sensory inputs showed that EES output may be decreased because of spinal sensory inputs when loads are applied at the hand. This would decrease the efficacy of EES which is supposed to enhance force production. Therefore, to explain the results we obtained in behaving monkeys (**Figure 6**) we investigated the contribution of residual cortical inputs in the production of forces and movements during EES. Specifically, since cortical inputs actively modulate spinal circuits, they should be able to both enhance and suppress EES output by modulating spinal circuit excitability²¹. Since we showed that monkeys could use EES to amplify their movement and forces (**Figure 6D**) we focused on demonstrating that cortical inputs could also suppress unwanted EES-generated movements. We hypothesized that if monkeys did not want to move, EES would not produce the large joint movements that we observed when the monkeys were anesthetized. Therefore, we identified trials in which our decoder detected a false-positive reach movement (**Figure 6C**). In this situation our system would deliver a burst of stimulation even if the animal was not attempting to execute the task. We then compared intracortical activity from the primary motor cortex (M1) of Mk-Br and Mk-Yg during these false-positive trials to the signals recorded during correctly detected trials. We identified trials where EES was present and the monkey moved, and trials when EES was present but the monkey did not move (**Figure 6D**). We verified that the same neural units were present in both conditions and found that the overall firing rates of all units in motor cortex was significantly higher when EES produced movement (**Figure 6E**) than when it did not. This suggested that movement happened only if the motor cortex was active, despite EES was delivered at amplitudes that generated large joint movements when the same monkey was anesthetized. To further validate this hypothesis we applied dimensionality reduction using Principal Component Analysis to the firing rates in each electrode and reduced the M1 population activity to low-dimensional states⁴⁷. In this low-dimensional space each point represents the global neural state of the motor cortex at a given time point (**Figure 6F**). We compared the neural states present when EES was associated movements and those when EES was not associated movement with the neural states associated to rest, e.g. when the monkeys were resting before the go signals between trial repetitions. When looking at the spatial distribution of neural states, trials in which EES was not associated to movement seemed to overlap with states of rest. We then computed the distance between each neural state to the subspace representing neural states at rest and found that the neural states associated to movements during EES were significantly further away from neural states at rest than neural states associated to EES and no movement. In summary, we found that the motor cortex activity was similar to the activity at rest whenever we delivered EES but the monkey did

331 not move (**Figure 6F**). Instead, the monkey moved when the motor cortex was significantly active.
332 This implies that the residual cortical inputs via direct and indirect pathway can either suppress
333 or enable movement during EES.

334
335

336 **Discussion**

337 We showed that EES of cervical spinal cord immediately enhanced muscle activation and strength,
338 task performances and movement quality during a natural-like reach and grasp task in monkeys
339 with unilateral cervical SCI compared to no stimulation controls in three monkeys. Importantly,
340 our technique allowed monkeys to support the weight of their arm during reach, grasp and pull
341 movements. These results are important in light of clinical translation of our technology. Stronger
342 forces and better arm weight bearing can empower patients with the capacity to perform a larger
343 spectrum of movements than they would normally be capable of doing without the need of support.
344 This may provide for more independence in daily living as well as better outcomes of physical
345 therapy.

346

347 **Exploiting subject-specific anatomy to simplify technology**

348 We obtained our results with relatively simple stimulation protocols that engaged up to three
349 monopolar contacts (one for reach, one for grasp and one for pull). The combination of simple
350 bursts through these contacts enabled whole arm multi-joint movements. We believe that the
351 design of our interface was key to achieve this result. The dorsal roots are a robust anatomical
352 target that we could easily identify through standard imaging to personalize surgical planning and
353 interface design. A similar surgical planning approach can be imagined in humans where MRIs
354 and CT can guide surgical planning²⁰.

355 Our results were enabled by the relative mapping between each dorsal root and the rostro-caudal
356 distribution of motoneurons in the cervical spinal cord, which is similar in monkeys and humans⁴⁰.
357 The anatomical separation of roots in the cervical enlargement allowed us to recruit each root
358 independently which generated distinct joint movements to a degree that was not observed in
359 applications of EES for the lower limbs³⁸. Stimulation of the C6 root elicited distinct arm flexion,
360 C7 stimulation produced arm extension and C8/T1 stimulation produced hand grasp. However,
361 similarly to other spinal cord stimulation studies we could not identify contacts that selectively
362 produced finger extension^{11,48}. This is likely caused by the overlap of extensor motor-pools in the
363 forearm⁴⁰ but possibly also because flexors may be biomechanically stronger and dominate hand
364 kinematics in the case of co-contraction at rest. Despite these limitations in specificity, we were
365 able to restore a whole three-dimensional arm movement by solely detecting movement onset
366 signals to trigger pre-determined stimulation bursts through two or three contacts. Unlike FES,
367 this is possible because EES activates cervical motoneurons via pre-synaptic inputs thus allowing
368 modulation of elicited muscle responses that can compensate for reduced specificity³⁸.

369

370 **Supporting arm movement phases independently**

371 Contrary to previous pilot applications of epidural and transcutaneous spinal cord stimulation of
372 the cervical spinal cord^{29,30}, we utilized a soft epidural interface that allowed selective and
373 independent support of each movement phase rather than providing continuous stimulation to the
374 whole spinal cord. This approach is not possible with transcutaneous technologies⁴⁹ or current
375 design of human leads⁴⁰ and would require new interfaces designed for the cervical cord.
376 Selective spatiotemporal stimulation was shown to be more effective in animal models and
377 humans than continuous stimulation in the sense that it was able to immediately produce
378 coordinated locomotion compared to continuous stimulation that instead required long training
379 periods^{23,38,42}. In the case of the upper limb we believe that this approach was critical. Indeed,

380 while continuous stimulation did provide some level of facilitation, it failed to entirely promote
381 grasp and pull in one of the monkeys. Perhaps the intrinsically unstructured nature of arm and
382 hand control makes a continuous stimulation approach less effective than it is in locomotion that
383 instead has an intrinsic repetitive structure³². For example, stimulation parameters that promote
384 grasp, may impair reach if they are delivered continuously throughout movement. Indeed, when
385 a pull stimulation was triggered at mid-reach it generated the interruption of the reach movement.
386 Perhaps a different interface design or lower stimulation amplitudes could be used to optimize
387 continuous stimulation protocols, but it would be at the expense of power of elicited movements
388 potentially preventing the weight bearing component necessary for three-dimensional movements.
389 In summary, the complex articulation of arm and hand movements may exacerbate the difference
390 in efficacy between continuous and phase-specific stimulation protocols that was already
391 observed for EES in locomotion, possibly explaining the difference in effect size that was obtained
392 so far for application in the upper limb.

393

394 The role of sensory feedback and residual cortical inputs

395 We showed that sensory feedback when the hand was constrained to a force load reduced the
396 EMG power produced by EES compared to free movements. This is likely caused by afferent
397 inhibitory feedback coming from Ib afferents⁵⁰. Unfortunately, lower muscle power while resisting
398 a force load would decrease the clinical usability of this technology. We believe that this
399 phenomenon is particularly relevant for the upper limb. Indeed, also during EES of the
400 lumbosacral cord, the EES motor output is influenced by sensory inputs³⁸, however sensory inputs
401 are instrumental for locomotion and heavily contribute to the generation of the repetitive
402 movement patterns that are required to walk^{16,19,32,51}. Therefore, in the case of locomotion these
403 inputs amplified and sustained EES-induced activity^{16,19,23}. Instead arm and hand movements are
404 produced by an unstructured sequence of primitive movements³³ and reflexes³⁶ in parallel with a
405 sophisticated gating of sensory inputs through mechanisms such as pre-synaptic inhibition⁷.
406 Therefore, residual cortical inputs become instrumental to obtain arm and hand movement with
407 EES as shown by our analysis of intra-cortical signals during the production of movement
408 combined with the observation that functional grasp was achieved only when the animals had
409 recovered some level of function. Indeed, our lesions were non-complete and while most of the
410 cortico-spinal tract was transected, multiple residual descending pathways were spared. These
411 indirect inputs could have been used by the animals to mediate the inputs required to integrate
412 EES and sensory inputs to produce voluntary movements. In summary, we believe that even
413 during phase-specific EES residual cortical inputs play a critical role in enabling arm movement
414 for cervical EES.

415

416 Clinical significance and challenges

417 The most important challenge for clinical translation of EES to humans concerns the role of
418 residual inputs. Our data show that some level of residual inputs and of function is required to
419 enable movement, first because in awake animals EES did not initiate movements, and second
420 because it lacks the selectivity to achieve selective finger activity. However, previous studies
421 showed that even completely paralyzed subjects retain residual but functionally silent descending
422 inputs-. Therefore, while overall efficacy may depend on injury severity, even severely injured
423 patients may obtain benefits from cervical EES. After a period of physical training combined with
424 EES⁵² these subjects may be able to use EES to achieve simple but functional grasp. Alternatively,
425 more selective technologies targeting hand muscles such as FES could be combined with EES
426 to obtain powerful yet selective movements.

427 The adaptation of EMG output to stimulation frequency that we observed in consequence of pre-
428 synaptic activation of motoneurons may lead to a reduction in efficacy during long-term clinical

429 use. Additionally, stimulation of afferent fibers may cause uncontrolled reflexes which may affect
430 function. While we did not observe these phenomena in our data, this may be due to the relatively
431 small size of the lesion compared to severe contusion in humans. However, data in humans with
432 SCI suggest that stimulation protocols can be adapted to be functional even in subjects with
433 chronic severe thoracic lesions^{20,26}, therefore we expect that this will be the case also for cervical
434 lesions. At any rate both risks can be reduced by accurate stimulation tuning and real-time
435 adaptation of stimulation patterns⁵³.

436 Concerning complexity of our system, in our study we detected movement onsets from
437 intracortical activity which may be seen as a limitation for a realistic implementation of our protocol
438 in clinical settings. However, given the simplicity of our protocol which is essentially constituted
439 by alternation of pre-defined bursts, brain recordings may not be required in clinics. Indeed, most
440 patients suffer from a severe but incomplete paralysis²⁰, which spares some residual muscle
441 activity in few muscles. While this residual activity is not sufficient to produce functional
442 movements, it can be reliably detected and used to trigger stimulation bursts with standard clinical
443 technologies^{20,38}. In summary, we believe that by exploiting the functionality of residual spinal
444 circuits and supra-spinal inputs, cervical EES constitutes a simple yet robust approach to the
445 restoration of arm motor control with significant translational potential.

446

Acknowledgements

447 The authors would like to thank Jacques Maillard and Laurent Bossy for the care provided to the
448 animals, Dr Eric Schmidlin and Dr Simon Borgognon for their help with anaesthesia and surgery
449 preparations, Dr Marion Badi for her help and advice during experiment preparations and
450 experimental procedures, Dr. Andrina Zbinden for her contribution to the health survey of the
451 monkeys, André Gaillard and Andrea Francovich for their help with the implementation of the
452 hardware and the students of the University of Fribourg Amélie Jeanneret, Alen Jelusic, Laora
453 Marie Jacquemet and Samia Borra for their help in processing data.

455

Funding

456 The authors would like to acknowledge the financial support from the Wyss Center grant (WCP
457 008) to MC, GC and TM, an industrial grant from GTX medicals to GC and MC; the Bertarelli
458 Foundation (Catalyst Fund Grant to MC and TM and funds to SL) a Swiss National Science
459 Foundation Ambizione Fellowship (No. 167912 to MC), a Swiss National Science Foundation
460 Doc-Mobility Grant (No. 188027 to BB), The European Union's Horizon 2020 research and
461 innovation program under the Marie Skłodowska-Curie grant agreement no. 665667 (GS) the
462 Swiss National foundation grant BSCGI0_157800 (SL), a Whitaker International Scholars
463 Program fellowship to MGP, and an internal pilot grant of the University of Fribourg to MC.

464

Author Contributions

465 MC, BB and SC conceived the study; BB, MGP, and TM designed and implemented the hardware
466 and software tools; SC designed the behavioral task and training strategy; GS and SL designed
467 and manufactured the implantable interface; BB, SC, MGP and MC conducted the experiments;
468 BB, SC, MGP and KZ performed the data analysis; SC, MD and MK trained the animals; SC, KG,
469 NJ and QB processed the histological data; JB, GC and MC designed surgical implantation
470 strategies and stimulation strategies. GC and JB, performed surgical implantations and lesions.
471 EMR and MC implemented and supervised procedures on monkeys; MC, BB, SC and MGP wrote
472 the manuscript; all authors edited the manuscript; SL, TM, JB, GC and MC secured funding for
473 the study; MC supervised the study.

474

476

477 **Competing Interests**

478 G.C., J.B., S.L., M.C., B.B. and K.Z. hold various patents in relation to the present work. G.C.,
479 S.L. and J.B. are founders and shareholders of Onwarrd medical, a company developing an EES-
480 based therapy to restore movement after spinal cord injury. MC is founder and shareholder of
481 Reach Neuro, Inc. a company developing spinal cord stimulation technologies for stroke. All the
482 other authors declare no financial interest.

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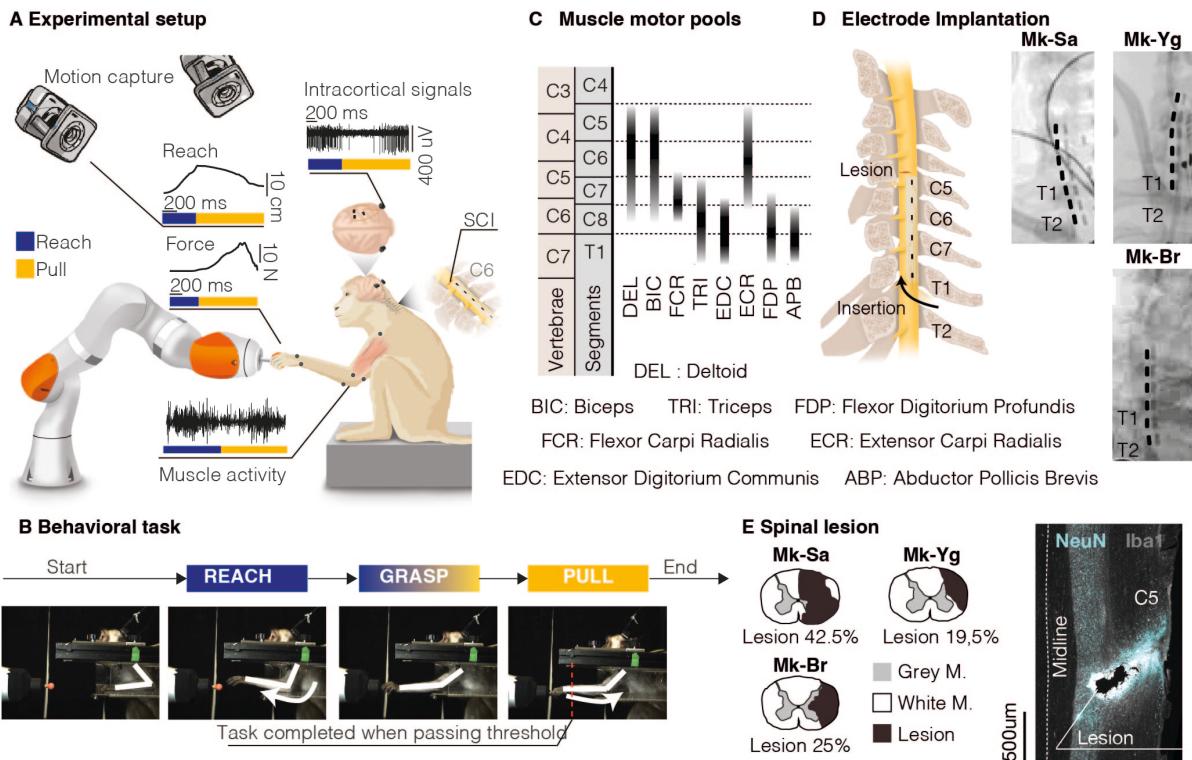
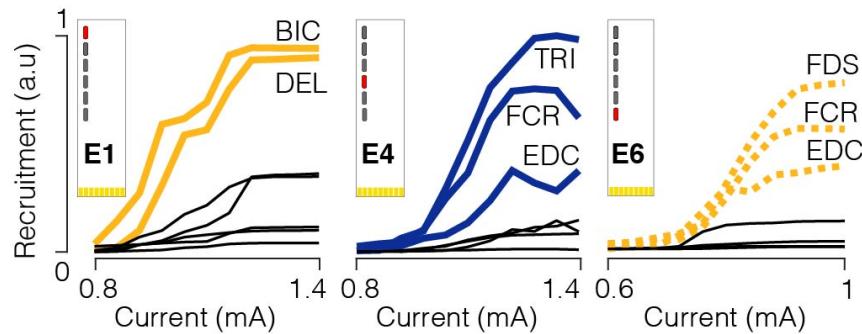


Figure 1. Experimental framework. **(A)** Schematic of the behavioral experimental platform. While the animals were performing a robotic reach, grasp and pull task, we measured 3D forces applied to the robot joints, full-limb kinematics, electromyographic (EMG) activity from eight muscles of the arm and hand, and intra-cortical signals from sensorimotor areas. **(B)** Schematic illustration of the task. Monkeys were trained to reach for, grasp, and pull a target object placed at the end effector of a robotic arm. We considered a movement complete when a target spatial threshold was crossed during pull. **(C)** Motoneurons pool distribution of arm and hand muscles in the cervical spinal cord in relation to vertebrae and spinal segments (adapted from Jenny and Inukai, 1983). Deltoid (DEL), Biceps Brachii (BIC), Flexor Carpi Radialis (FCR), Triceps Brachii (TRI), Extensor Digitorum Communis (EDC), Extensor Carpi Radialis (ECR), Flexor Digitorum Profundis (FDP), Abductor Pollicis Brevis (ABP). **(D)** Schematic representation of spinal implant positioning and X-ray scans of the epidural implant in the three monkeys (Mk-Sa, Mk-Br and Mk-Yg). **(E)** Anatomical reconstruction of the cervical spinal cord lesion (black area) for the 3 monkeys, shown on a transversal section (the percentage indicates the portion of the total spinal cord area that was injured on this transversal plane). On the right, representative image of longitudinal section of the spinal cord of Mk-Br around the lesion site stained with NeuN (neuronal cell bodies) and Iba1 (microglia). Copyright Jemère Ruby.

A Muscle recruitment during single pulse

Mk-Yg — C5 Stim — C6/C7 Stim. - - - T1 Stim.

**B Similar muscle recruitment in three monkeys**

■ C6/C7 Stim. ■ T1 Stim.

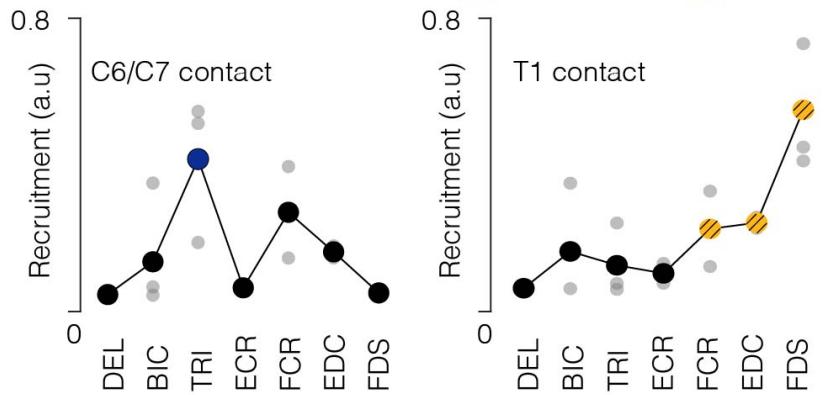


Figure 2. Muscle recruitment of spinal stimulation. (A) Examples of muscle recruitment obtained by stimulating (1 Hz) at C5, C6/C7, and T1 spinal segments (Mk-Yg). (B) Average muscle activations elicited from C6/C7 and T1 contacts in n=3 monkeys (grey bullets: for each animal, average recruitment across all stimulation currents. Big bullets: mean of average recruitments across animals).

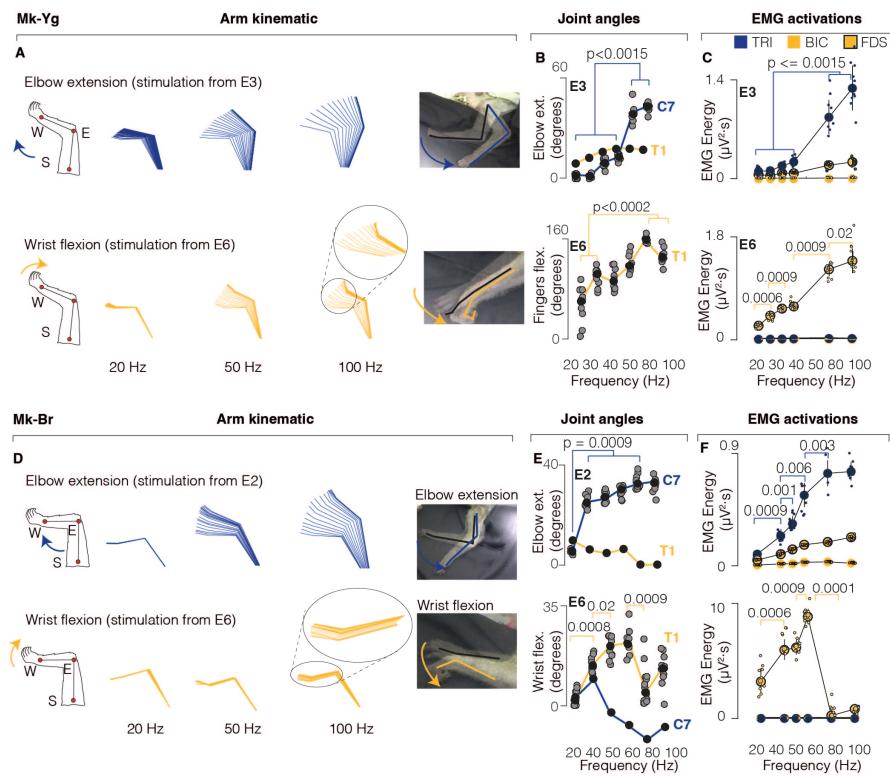


Figure 3. EES produces functional joint movements in anesthetized animals. (A) Stick diagram schematic of elbow extension and wrist flexion movements elicited by pulse-trains of stimulation in anesthetized conditions in Mk-Yg from the electrode contacts 3 and 6 (E3 and E6). (B) Modulation of maximal joint angles achieved by pulse-trains of stimulation at different frequencies, in anesthetized conditions in Mk-Yg. Stimulation was delivered at C7 (blue, n = 12, 10, 11, 10, 10, 10 independent samples) and T1 (yellow, n = 11, 10, 10, 10, 10, 13 independent samples). Black bullets represent median values, gray bullets are individual datapoints. Statistics performed with two-sided Wilcoxon Ranksum test and Bonferroni correction. (C) Triceps (blue), biceps (yellow), and flexor digitorium superficialis (yellow with black border) activity elicited by pulse-trains of stimulation at different frequencies, in anesthetized conditions in Mk-Yg. Top, activation during stimulation from electrode contact E3 (from left to right, n = 12, 10, 11, 10, 10, 10 independent samples). Bottom, activation during stimulation from electrode contact E6 (from left to right, n = 11, 10, 10, 10, 10, 13 independent samples). Bullets represent median values and bars are standard deviation. Statistics performed with two-sided Wilcoxon Ranksum test and Bonferroni correction. (D) Stick diagram schematic of elbow extension and wrist flexion movements elicited by pulse-trains of stimulation in anesthetized conditions in Mk-Br from the electrode contacts 2 and 6 (E2 and E6). (E) Modulation of maximal joint angles achieved by pulse-trains of stimulation at different frequencies, in anesthetized conditions in Mk-Br. Stimulation was delivered at C7 (blue, n = 10, 10, 10, 10, 10 independent samples) and T1 (yellow, n = 10, 11, 10, 10, 15, 11 independent samples). Statistics performed with two-sided Wilcoxon Ranksum test and Bonferroni correction. Black bullets represent median values, gray bullets are individual datapoints. (F) Triceps (blue), biceps (yellow), and flexor digitorium superficialis (yellow with black border) activity elicited by pulse-trains of stimulation at different frequencies, in anesthetized conditions in Mk-Br. Top, activation during stimulation from electrode contact E2 (from left to right, n = 10, 10, 10, 10, 10 independent samples). Bottom, activation during stimulation from electrode contact E6 (from left to right, n = 10, 11, 10, 10, 15, 11 independent samples). Bullets represent median values and bars are standard deviation. Statistics performed with two-sided Wilcoxon Ranksum test and Bonferroni correction.

A Example of task performance, Mk-Yg

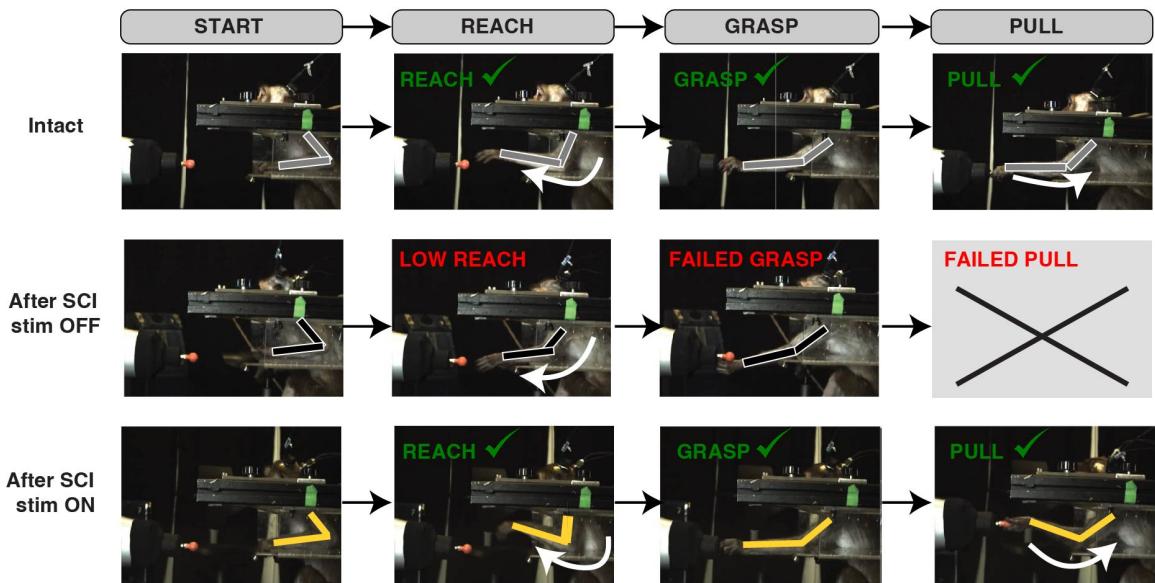
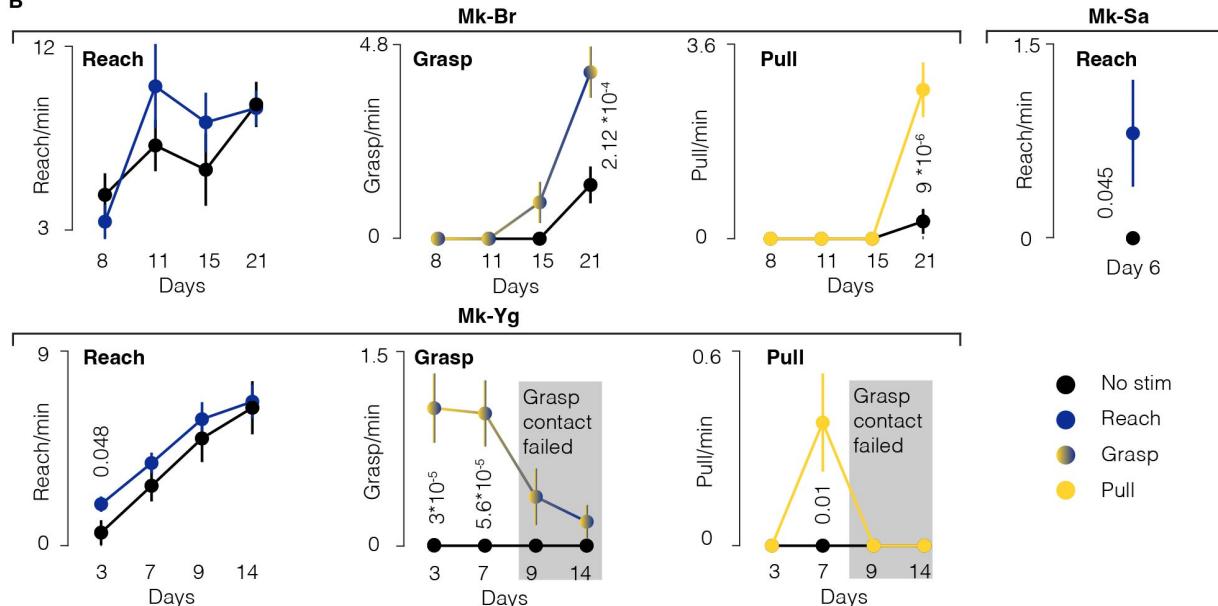
**B**

Figure 4. EES improves task performance. (A) Snapshots of Mk-Yg performing the task before SCI, after SCI without EES, and after SCI with EES. A full successful trial is composed of a reach, a grasp, and a pull. After SCI, Mk-Yg could only perform reaching movements without EES, while when EES was delivered the full task could be performed. (B) Task rate performance rate over different sessions, computed as the number of successful movements per minute. Performance rate are shown for reach (blue), grasp (yellow to blue gradient) and pull (yellow movements). Data are shown as mean (bullets) and standard deviation (bars). Statistics and significance evaluated by estimating two side residuals via Bootstrap. Asterisks: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

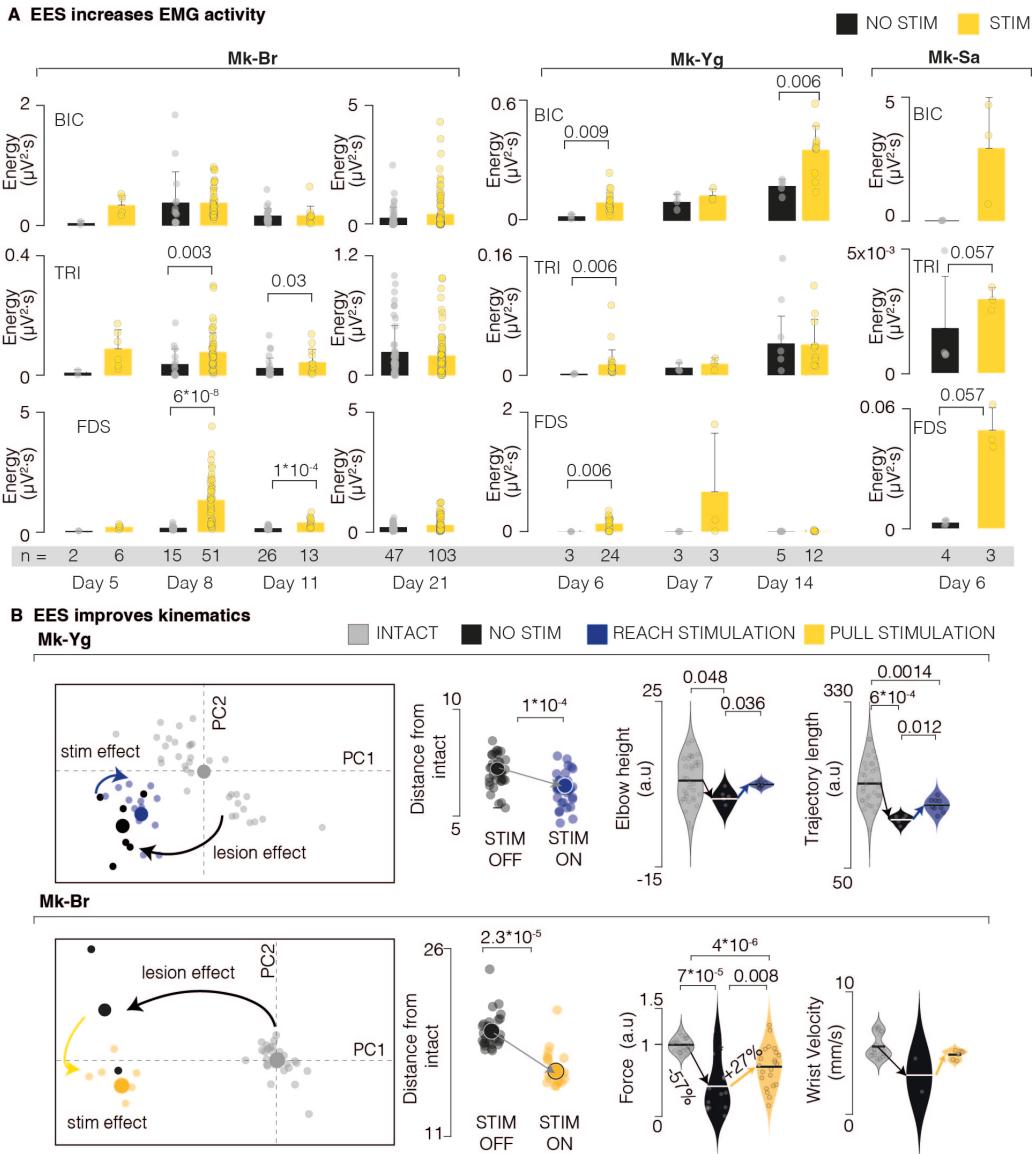


Figure 5. EES improves muscle strength and movement quality. (A) Bar plots of signal energy of biceps, triceps and FDS EMG profiles during movement with no stimulation (black) and stimulation (yellow). Data are shown for different sessions (one for each week) in Mk-Br and Mk-Yg. Mk-Sa performed only one session. Data are shown as mean \pm STD. All individual data points are represented by bullets. Statistical analysis with two-sided Wilcoxon Ranksum test. (B) Kinematic features for Mk-Yg (top) and Mk-Br (bottom) are displayed in a new space created by principal component 1 (PC1) and principal component 2 (PC2). From left to right: (1) first and second PC space. Each bullet represents one trial. Trials performed after injury (black) are consistently separated from the trials performed in intact conditions, highlighting a change in the quality of resulting kinematics. Trials performed with the support of stimulation (blue for reach and yellow for pull) are located closer to the intact trials in the PC space, denoting an improvement in kinematic features. (2) Euclidean distance in the feature space of trials without stimulation (black) and with stimulation (blue for Mk-Yg, yellow for Mk-Br) from the centroid of the trials in intact condition. Statistical analysis with two-sided Wilcoxon Ranksum test. (3) example violin plots of movement quality features in the three conditions: intact, after SCI with stimulation. Statistical analysis with two-sided Wilcoxon Ranksum test.

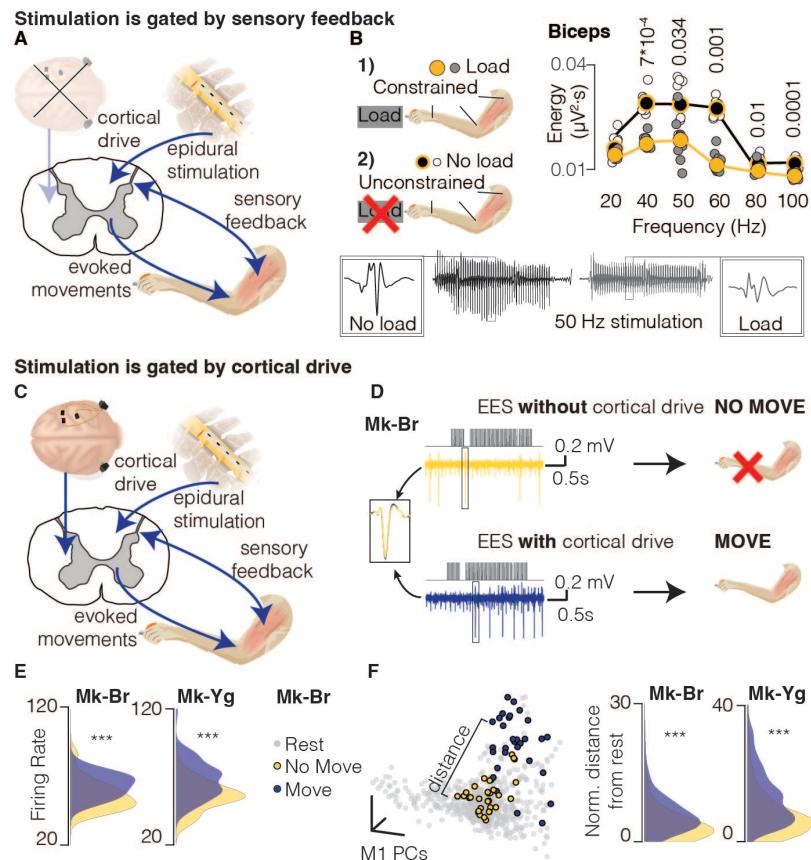
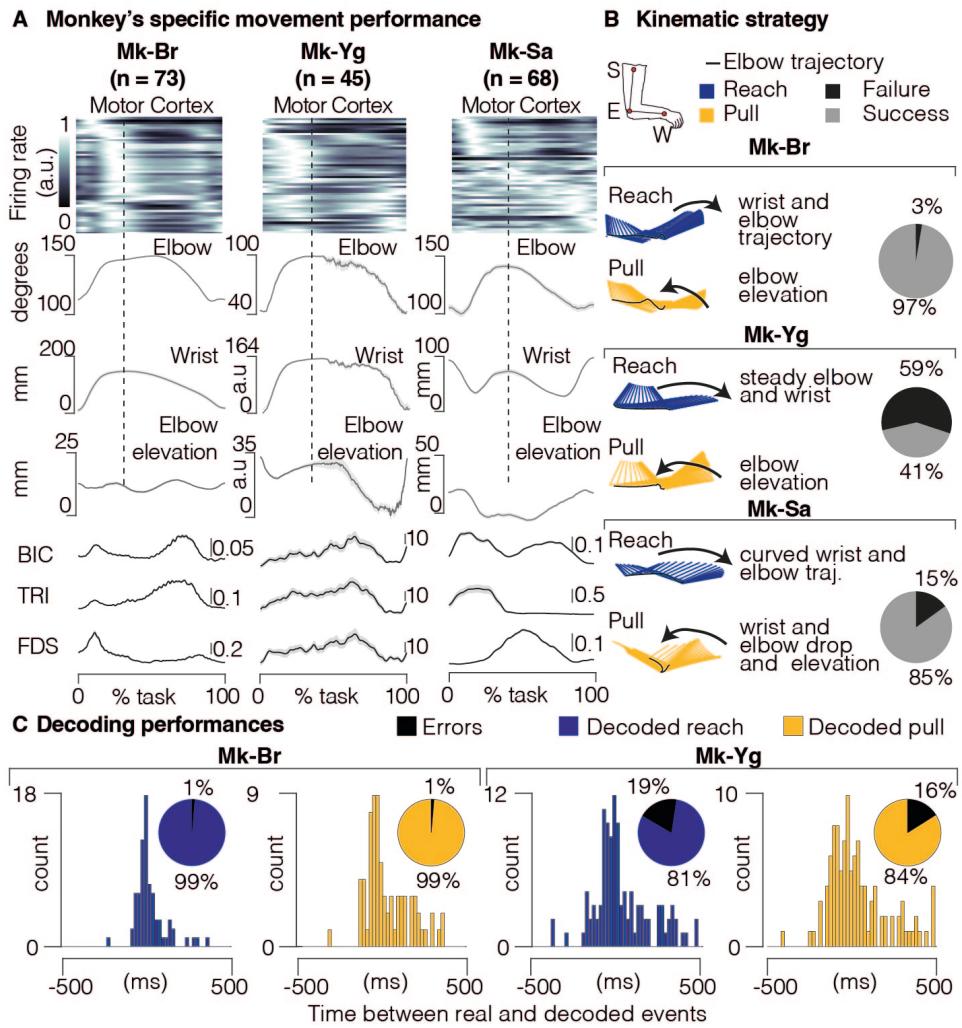
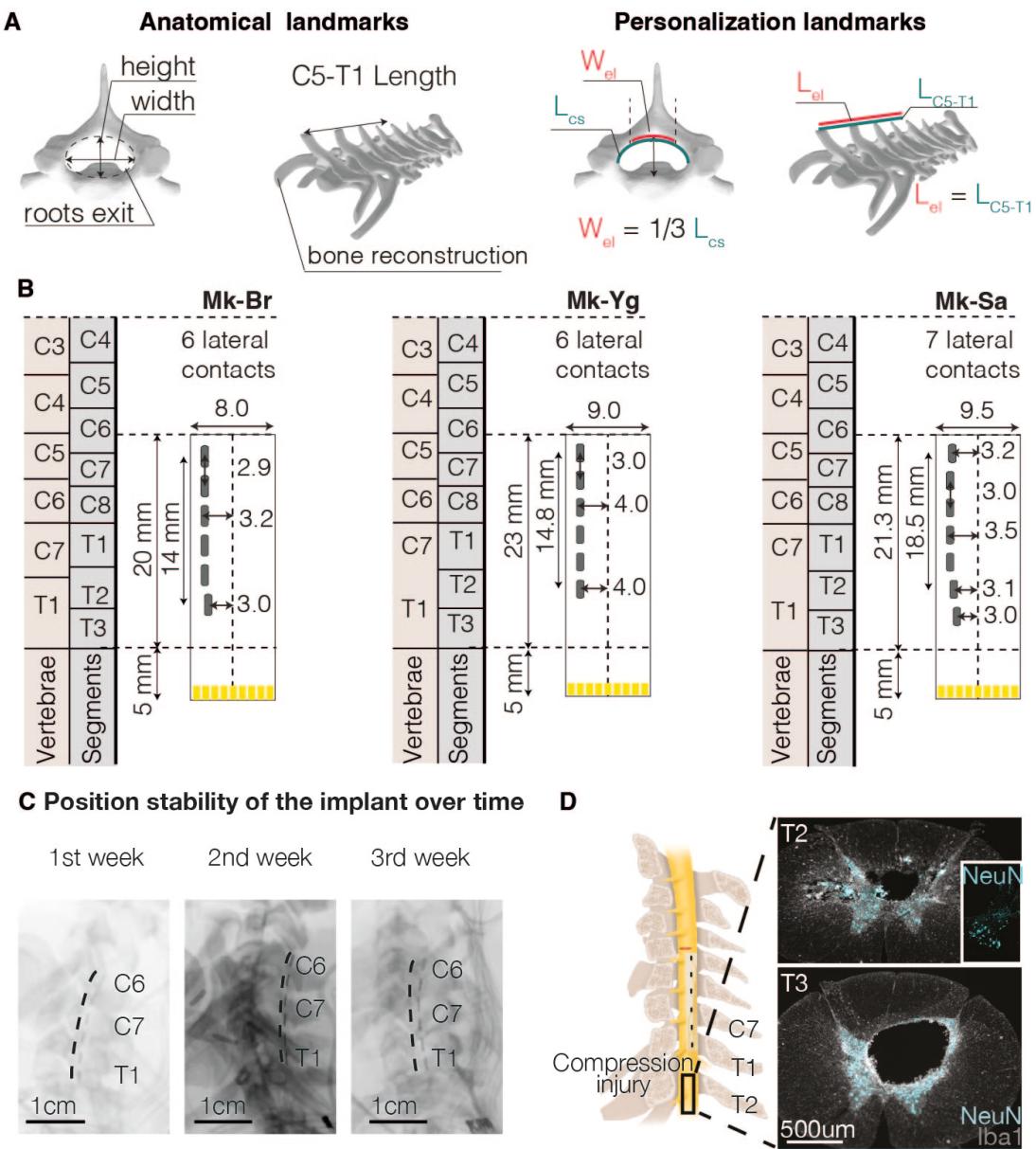


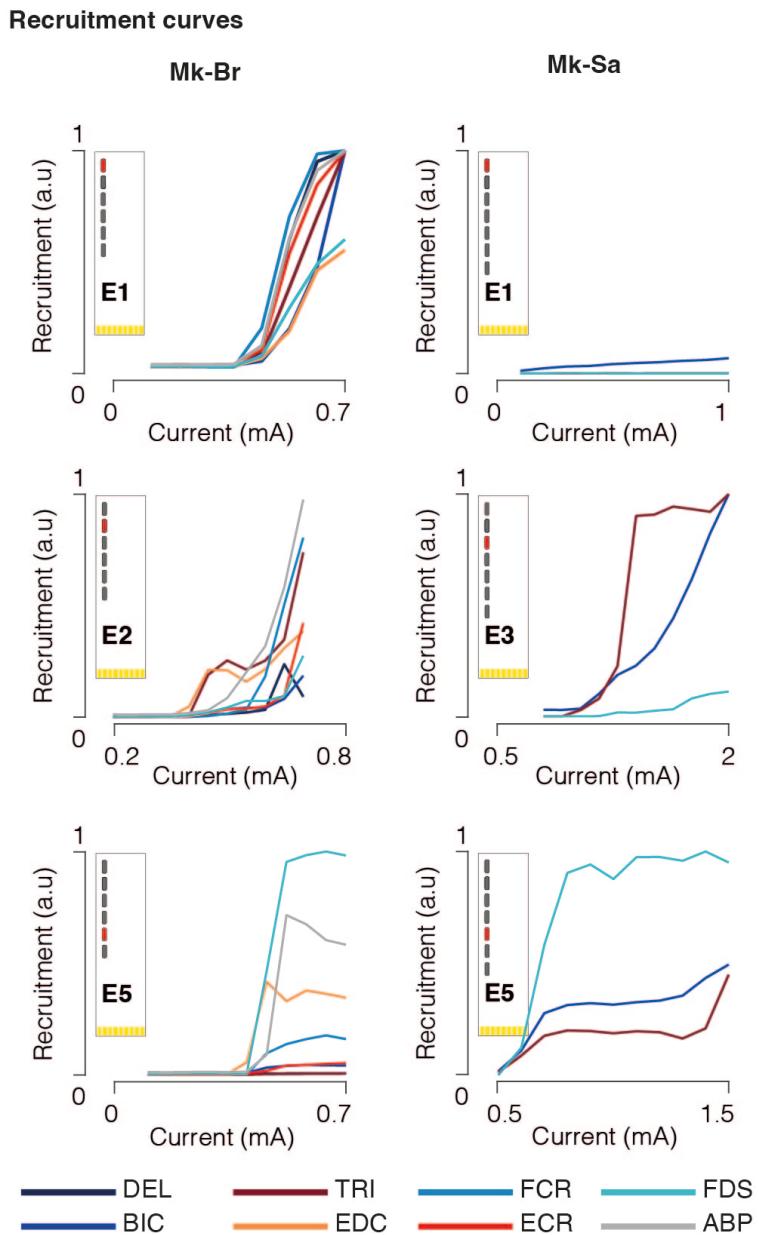
Figure 6. EES must be synchronized with motor intention. (A) interactions between EES and residual neural structures during anesthesia. During anesthesia, cortical inputs are absent, therefore EES interacts solely with spinal sensory feedback. (B) Quantification of EMG activity: unconstrained arm (no load, black); arm constrained by load applied at the hand (load, gray). White and grey bullets: individual data points for no load and load conditions. Black and yellow bullets: median values for no load and load conditions. Black and yellow lines: interpolation of median values for no load and load conditions. On the bottom, example of EMG traces obtained during stimulation in the no-load (black) and load (gray) conditions. Stimulation artifacts have been removed. Data from Mk-Br (C) Interactions between EES and residual neural structures in awake monkeys. EES interacts with residual descending cortical drive after SCI and with spinal sensory inputs. (D) Schematic illustrating the kinematic outcome of the interaction between EES and residual cortical inputs. The same EES pulse train (top) applied to Mk-Br can result in different motor outputs: no movement output when the cortex is silent (yellow, top), movement is produced when the cortex is active (blue, bottom). (E) Distribution of average firing rates across all M1 channels during stimulation trains that evoked no movement (yellow) and movement (blue). Statistical analysis with two-sided Wilcoxon Ranksum test (Mk-Br, $p = 1.19e^{-18}$; Mk-Yg, $p = 2.82e^{-118}$) (F) Left: State space view of M1 activity for all time points during rest (gray), successful stimulation (blue) and unsuccessful stimulation (yellow). The brain states during unsuccessful stimulation (yellow) overlapped with the rest states, while the successful stimulation (blue) did not. Right: we computed a relative Mahalanobis distance between the two stimulation conditions and the cluster of neural states at rest. For both monkeys, neural states during stimulation periods with no movement were close to rest. Statistical analysis with two-sided Wilcoxon Ranksum test (Mk-Br, $p = 5.69e^{-96}$; Mk-Yg, $p = 5.36e^{-11}$).



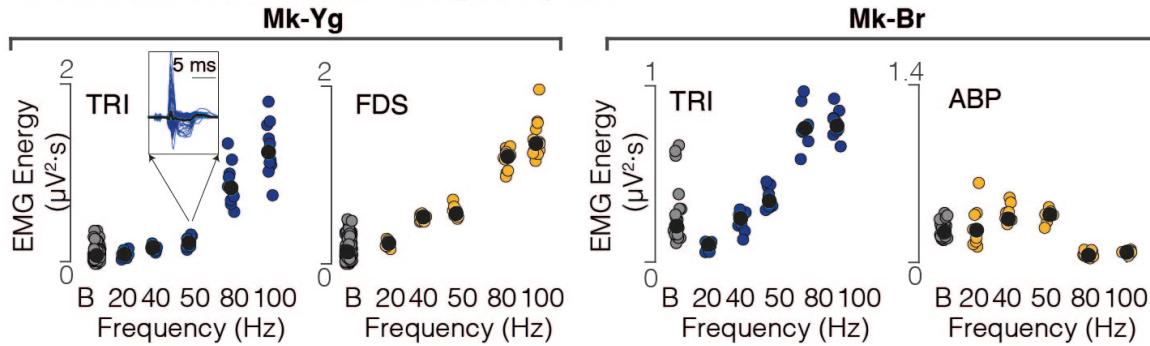
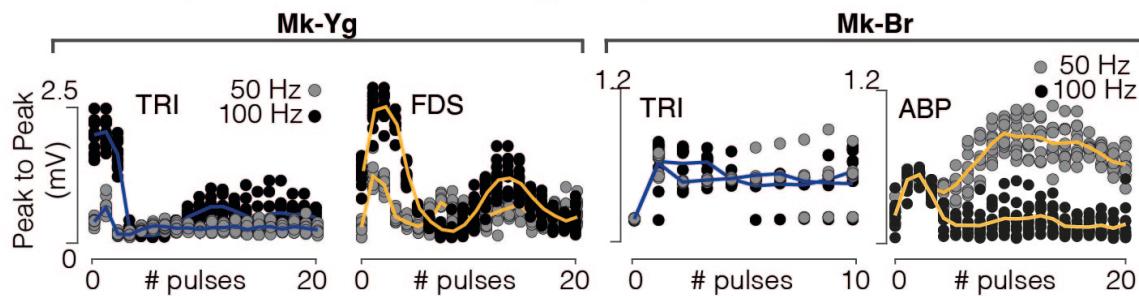
Extended Data Figure 1. **(A)** Portfolio of signals recorded during intact movement for each animal. These signals have been recorded during the experimental session prior to the lesion. Motor cortex recordings show firing rate profiles for the 64 microelectrodes. Each row shows the firing rate of a specific electrode. Electrodes are displayed from top to bottom by order of first activation in a reference trial. Arbitrary units in motor cortex recording indicate normalized firing rate for each electrode (see methods). In kinematic and EMG plots, black lines correspond to the mean profile across all trials, shaded area shows the SEM across all trials. Kinematic scales are expressed in mm. For Mk-Yg, arbitrary units on kinematic plots represent displacement units derived by the count of video pixels. EMG scales are expressed in mV. **(B)** Kinematic strategies implemented by each monkey. Stick diagrams representations of the arm kinematic during reach (blue) and pull (yellow). The black line highlights the elbow trajectory. Pie charts represent the percentage of success and failure in task performance before lesion. **(C)** Offline decoding performance for Mk-Br and Mk-Yg before lesion. Histograms show timing accuracy of reach (blue) and pull (yellow) event decoding. The height of bars (y coordinate) illustrates the amount of events decoded with a specific timing accuracy (x coordinate). Pie charts (inset) show the percentage of correctly identified (true positive) reaches (blue) and pulls (yellow), across all decoded events. The black portion of the pie chart highlights the percentage of false positive decoded events.



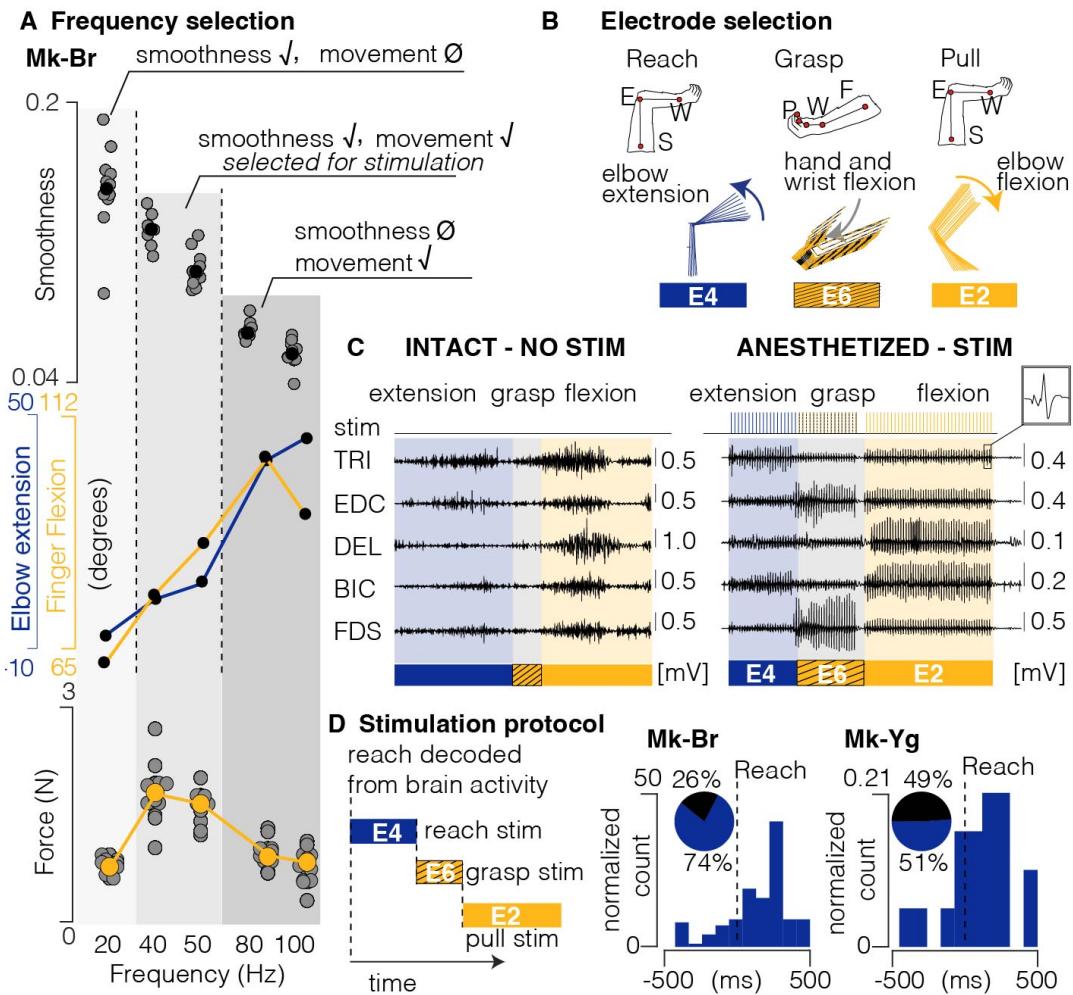
Extended Data Figure 2. **(A)** Anatomical landmarks used to tailor the epidural interface to each monkey's anatomy (Length of dorsal aspect of spinal canal L_{cs} , length of C5-T1 spinal segment L_{C5-T1} , electrode width W_{el} , electrode length L_{el}). Three-dimensional reconstructions of vertebrae are obtained by CT-reconstruction (Osirix, Pixmeo, Switzerland). **(B)** Personalized design of the epidural implant for each animal. All measures are in millimeters. Yellow traces at the bottom of the electrode identify connectors. **(C)** Position stability of the epidural array over time, illustrated through X-rays imaging taken during 3 consecutive weeks after the implantation, images from Mk-Yg **(D)** Compression injury at the insertion level of the array (T2-T3 segment) in Mk-Br, discovered post-mortem, stained with NeuN (neuronal cell bodies) and Iba1 (microglia).



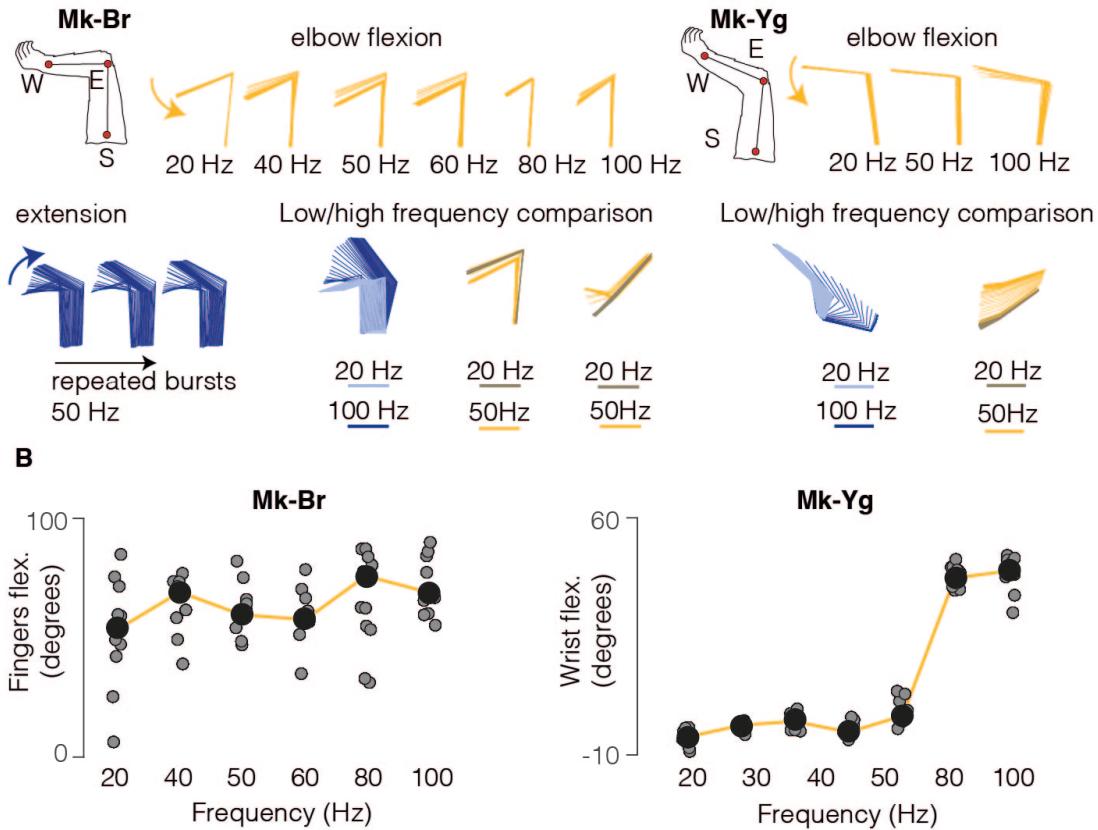
Extended Data Figure 3. Muscle recruitment obtained by stimulating, through different electrode contacts (E1, E2, E3, E5), at 1 Hz at C5, C6/C7, and T1 spinal segments for Mk-Br and Mk-Sa. Mk-Sa only had three muscles implanted: biceps, triceps, and flexor digitorium superficialis.

A Graded muscle activation during train pulses**B Muscle responses are modulated at higher frequencies**

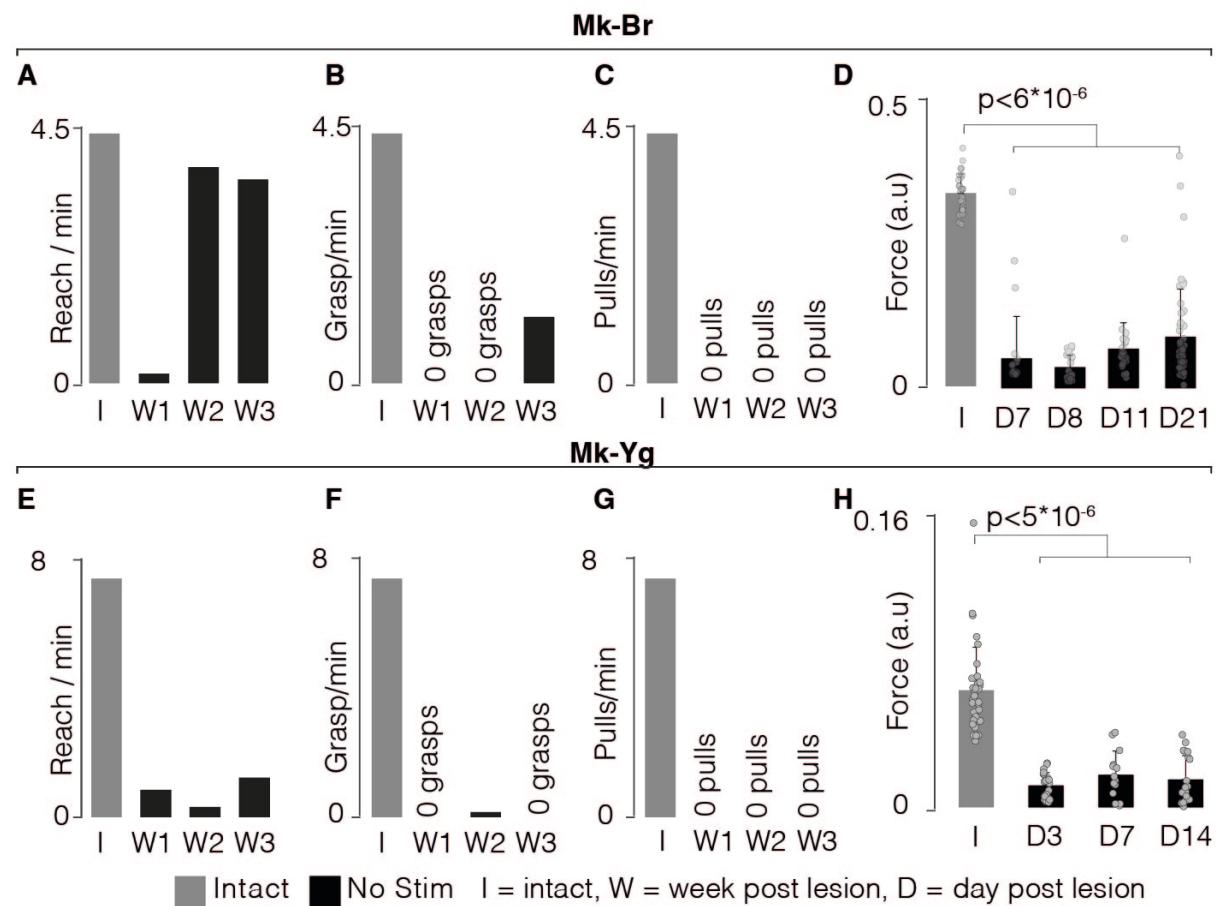
Extended Data Figure 4. (A) Energy of EMG signals of triceps (Mk-Br and Mk-Yg), Flexor Digitorium Superficialis (Mk-Yg) and abductor pollicis (Mk-Br) muscles, following pulse-train stimulation at different frequencies (on the x-axis). Black bullets represent mean values. **(B)** Evolution over time of the peak-to-peak value of stimulation evoked responses during a stimulation burst. Each plot shows the evolution for a specific muscle following pulse-train stimulation at 50 and 100Hz. Triceps is shown for Mk-Br and Mk-Yg, Flexor Digitorium Superficialis for Mk-Yg and abductor pollicis for Mk-Br. Each data point is represented as a bullet and lines represent mean values over time.



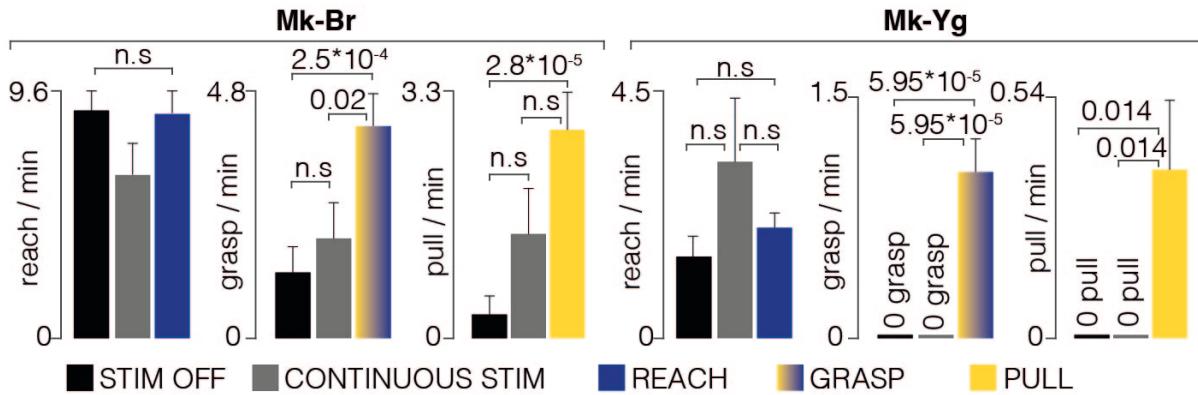
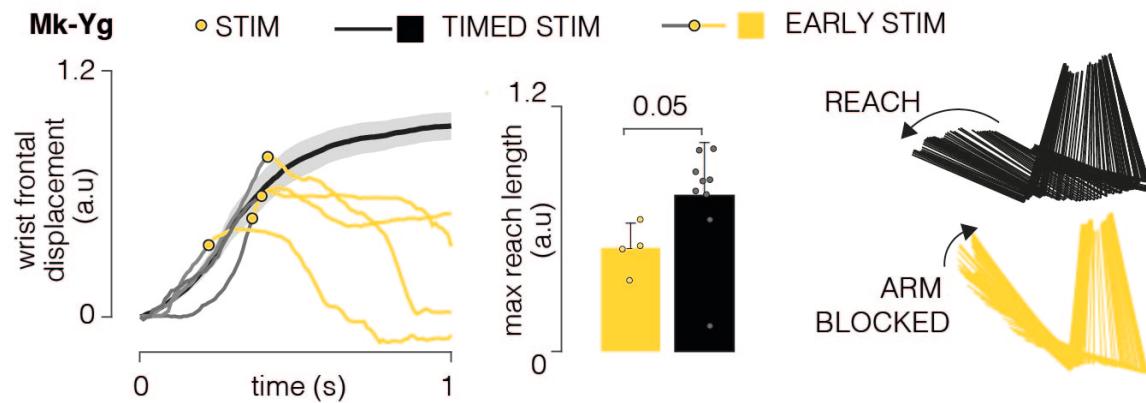
Extended Data Figure 5. **(A)** Combined representation of movement smoothness, elbow and finger flexion, and pulling force during anesthetized stimulation. Shades of gray highlight three frequency ranges that produce: (1) smooth trajectory, but little movement and low force (20Hz), (2) smooth trajectory, extended movement and medium force (40 and 50Hz), (3) abrupt and very extended movement and low force (80 and 100Hz). Kinematics and force reported here were measured in different experiments, kinematics was unconstrained, force data were acquired in isometric conditions (see Methods). The range 40-50 Hz was selected as the best optimization of sufficient movement, smoothness and force production. **(B)** Schematic representation of arm and hand kinematics during stimulation delivered from the selection of three contacts to produce elbow extension (blue), hand and wrist flexion (yellow and black), and elbow flexion (yellow). **(C)** Example of comparison between EMG activity during intact movement (left) and movement elicited by chaining stimulation from the three selected contacts (right). **(D)** Scheme illustrating how stimulation is triggered from movement-related intra-cortical signals. On the right, online performances of movement attempt decoder in two animals with SCI. Pie charts represent percentage of predicted (blue) and unpredicted (black) reach events by our decoder.

A Arm movement is modulated by stimulation frequency


Extended Data Figure 6. (A) Stick diagram schematic of movements elicited by pulse-trains of stimulation in anesthetized conditions. Mk-Br: on the left, arm kinematic obtained by delivering stimulation at different frequencies from contact number 5, on the bottom-left, arm kinematics obtained by repetitive delivery of a burst at 50 Hz; on the bottom right, superimposition of stick diagrams obtained with stimulation at 20 Hz and at higher frequencies (50 or 100 Hz) from different contacts. For Mk-Yg: arm kinematic obtained by delivering stimulation at different frequencies from contact number 2 and superimposition of stick diagrams obtained with stimulation at 20 Hz and at higher frequencies (50 or 100 Hz) from different contacts. **(B)** On the left, finger flexion produced by stimulation at different frequencies from the grasp contact in Mk-Br. Black bullets represent the mean value across different pulse-trains. On the right, wrist flexion obtained by stimulation at different frequencies from the grasp contact in Mk-Yg.



Extended Data Figure 7. **(A)** Evolution (in weeks) of rates at which Mk-Br performed reach movements after SCI (black), compared to the performances before injury (gray). **(B)** Evolution (in weeks) of rates at which Mk-Br performed grasp movements after SCI (black), compared to the performances before injury (gray). **(C)** Evolution (in weeks) of rates at which Mk-Br performed pull movements after SCI (black), compared to the performances before injury (gray). **(D)** Evolution (in days) of pull force after SCI without stimulation for Mk-Br. Values are plotted as the mean \pm STD (from left to right, n = 28, 29, 22, 26, 51 independent samples). Statistical analysis was carried out with two-sided Wilcoxon Ranksum test and Tuckey-Cramer correction. **(E)** Evolution (in weeks) of rates at which Mk-Yg performed reach movements after SCI (black), compared to the performances before injury (gray). **(F)** Evolution (in weeks) of rates at which Mk-Yg performed grasp movements after SCI (black), compared to the performances before injury (gray). **(G)** Evolution (in weeks) of rates at which Mk-Yg performed pull movements after SCI (black), compared to the performances before injury (gray). **(H)** Evolution (in days) of pull force after SCI without stimulation for Mk-Yg. Values are plotted as the mean \pm STD. (from left to right, n = 35, 23, 14, 20, independent samples). Statistical analysis was carried out with two-sided Wilcoxon Ranksum test and Tuckey-Cramer correction.

A Continuous EES**B Effect of stimulation timing**

Extended Data Figure 8. (A) Bar plots report the rate of successful movements after SCI, without stimulation (black), with continuous stimulation (gray) and with phase-dependent stimulation (blue or yellow) for Mk-Br and Mk-Yg. Data are presented as mean \pm STD and normalized on the mean value in stimulation condition. Significance evaluated by estimating two side residuals via Bootstrap. **(B)** Left: wrist frontal displacement in trials in which pull stimulation was erroneously triggered during reach (gray and yellow), compared to trials in which pull stimulation was not delivered (black, solid line represents the mean and shaded area represents the SEM). Yellow bullets highlight the instant at which stimulation was delivered: yellow lines highlight the trajectories during and after stimulation. Middle: barplot of the length of the reach movement when pull stimulation was erroneously delivered ($n = 4$) and when pull stimulation was not delivered ($n = 9$). Data are presented as mean \pm STD. Statistics performed with two-sided Wilcoxon Ranksum test. Right: stick diagram of arm kinematics during reach without (black) and with (yellow) erroneous pull stimulation.

646 **METHODS**

647

648 Animals involved in the study

649

650 All procedures were carried out in accordance to the Guide for Care and Use of Laboratory
651 Animals and the principle of the 3Rs. Protocols were approved by local veterinary authorities of
652 the Canton of Fribourg (veterinary authorization No 2017_04_FR and 2017_04E_FR), including
653 the ethical assessment by the local (cantonal) Survey Committee on Animal Experimentation and
654 final acceptance by the Federal Veterinary Office (BVET, Bern, Switzerland). Three adult female
655 *Macaca Fascicularis* monkeys were involved in the study (Mk-Sa 9 years old, 4.0 kg, Mk-Br 3
656 years old, 3.4 kg, Mk-Yg 3 years old, 4.0 kg). Animals were not food deprived, could freely access
657 water at any time and were housed in collective rooms designed in accordance to the Swiss
658 guidelines (detention in groups of 2-5 animals in a room of at least 45 m³). Rooms were enriched
659 with toys, food puzzles, tree branches and devices to climb and hide, as well as access to an
660 outdoor space of 10-12 m³ (see www.unifr.ch/spccr/about/housing). Detailed information on which
661 animals were involved in specific experimental procedures are reported in **Supplementary Table
662 1.**

663 Surgical procedures

664 For each animal, we performed three surgical procedures, (1) intracortical electrodes implantation,
665 (2) intramuscular electrodes implantation, and (3) epidural implant insertion and spinal cord injury.
666 Mk-Sa deviated from this protocol. Mk-Sa was first implanted with the epidural interface before
667 injury, however an infection occurred and resulted in the explantation of the lead to treat the
668 infection. After recovery, the animal was re-implanted, and lesion performed following the same
669 protocol of Mk-Br and Mk-Yg. All the surgical procedures were performed under full anesthesia
670 induced with midazolam (0.1 mg/kg, i.m.), methadone (0.2 mg/kg, i.m.), and ketamine (10 mg/kg,
671 i.m.) and maintained under continuous intravenous infusion of propofol (5 ml/kg/h) and fentanyl
672 (0.2-1.7 ml/kg/h) using standard aseptic techniques. A certified neurosurgeon (Dr. Jocelyne Bloch,
673 CHUV, Lausanne, Switzerland) performed all the surgical procedures. A detailed description of
674 each surgical procedure is reported in the Supplementary information.

675 Data acquisition

676 For Mk-Sa and Mk-Br, we acquired three-dimensional spatial coordinates of arm and hand joints
677 using a 14-camera motion tracking system (Figure 1, Vicon Motion Systems, Oxford, UK) that
678 tracked the Cartesian position of 6 infrared reflective markers (6 to 9 mm in diameter each, Vicon
679 Motion Systems, Oxford, UK) at a 100 Hz framerate. All markers were placed on the left arm, one
680 below the shoulder, three on the elbow (proximal, medial and distal position), and two on the left
681 and right side of the wrist. For each subject, a model of the marker placement was calibrated in
682 Vicon's Nexus software at the beginning of each experimental session. For Mk-Yg spatial
683 coordinates of arm and hand joints were recorded using two cameras placed parallel to the sagittal
684 and transversal plane of the animal (Vicon Motion Systems, Oxford, UK). The 3D coordinates of
685 the arm and hand joints were extracted using DeepLabCut⁵⁵. Due to the reduced informative
686 content extracted from the camera parallel to the transverse plane, we then only used 2D
687 coordinates on the animals' sagittal plane. The training set needed for automatic data labeling
688 was created by manually labeling a subset of recorded videos. An investigator was blinded to the
689 experimental condition and was instructed to mark four anatomical landmarks that mirrored the
690 position of markers in Mk-Sa and Mk-Br (shoulder, medial elbow, left and right wrist). Neural

691 signals were acquired with a Neural Signal Processor (Blackrock Microsystems, USA) using the
692 Cereplex-E headstage with a sampling frequency of 30 kHz. Electromyographic signals were
693 acquired with a Behavioral Neurophysiology chronic recording system (RZ2 BioAmp Processor,
694 Tucker-Davis Technologies, USA) at a sampling frequency of 12207 Hz.

695

696 Electrophysiology in sedated monkeys

697 Monkeys were sedated with a continuous intravenous infusion of propofol (5 ml/kg/h) that
698 minimizes effects on spinal cord stimulation⁵⁶. We delivered single pulses of cathodic, charge
699 balanced, asymmetric square pulses (0.3 ms, 1 Hz) from each electrode contact while recording
700 compound potentials from all implanted arm and hand muscles. Electromyographic signals were
701 acquired with a Behavioral Neurophysiology chronic recording system (RZ2 BioAmp Processor,
702 Tucker-Davis Technologies, USA) at a sampling frequency of 12207 Hz. We then delivered 10
703 repetitions of pulse trains from each contact, at several frequencies ranging from 20 to 120 Hz.
704 We recorded compound potentials from all implanted arm and hand muscles and arm kinematics
705 through two high resolution cameras (Sony FDR-X3000 Action Cam 4K). Through this procedure
706 we identified three contacts that primarily elicited (1) arm flexors, (2) arm extensors and (3) hand
707 flexors. In a reduced set of trials, we also recorded the force produced by arm flexion through a
708 10 N range force sensor (Dual-Range Force Sensor, DFS-BTA, Vernier, Beaverton, Oregon,
709 USA). To record the pulling force produced during isometric arm flexion, the hand was fixated to
710 the sensor hook through a string, and the sensor and the elbow were kept in place by two
711 experimenters, to optimally capture the strength produced by muscle contraction.

712 Behavioral experimental recordings

713 All animals were trained to perform a three-dimensional robotic reach, grasp and pull task,
714 previously described in detail in (Barra 2019⁴¹) and briefly recalled in the supplementary
715 information.

716

717 For Mk-Sa, data presented in this paper were collected several weeks pre lesion and 1 week post
718 lesion, unfortunately a severe infection of the spinal array and EMGs that recurred after day 7
719 lead to the premature euthanasia of the monkey before the study could be completed, in
720 agreement with the endpoints in our veterinary authorization. For Mk-Br and Mk-Yg data
721 presented in this paper were collected several weeks pre lesion and until 3 weeks post lesion. At
722 the end of week 3 post lesion, Mk-Br had 2 episodes of self-mutilation on the foot ipsi-lateral to
723 the lesion. In consequence we euthanized the animal before the end of the protocol according to
724 the endpoints in our veterinary authorization. As described in the results section, we found post-
725 mortem that Mk-Br had a medial spinal cord contusion at the T3 level. While this lesion did not
726 affect motor control of the legs or the arms, it may have generated neuropathic pain. Mk-Yg could
727 perform the entire protocol without any adverse event, however after day 7, the caudal contact of
728 the spinal interface (E8) identified to promote grasp failed, thus preventing us to perform
729 experiments with optimal stimulation configuration and impacting the efficacy of grasp movements.

730

731

732 *Optimization of EES parameters*

733 To optimize stimulation parameters we exploited the frequency/kinematic relationship that we
734 observed during single contact stimulation (**Figure 3B,E**). We then analyzed single joint
735 movements at different frequencies and contacts and weighted joint excursion angles against
736 movement smoothness⁵⁷, we found that stimulation frequencies of 50-60 Hz (**Extended Data**
737 **Figure 5**) produced smooth⁵⁷ and full-range movements and maximal forces. Instead, movements
738 elicited at frequencies lower than 40 Hz were too weak to complete a full joint movement while
739 frequencies higher than 60 Hz produced either abrupt movements or incomplete movements
740 (**Extended Data Figure 5A**). Next, we identified among all the tested contacts, those that could
741 consistently elicit arm extension (reach), hand flexion (grasp) and arm flexion (pull) (**Extended**
742 **Data Figure 5B**). We chose these contacts and 50-60Hz to sustain full arm and hand movement
743 and tested their effect in anesthetized animals by sequentially executing bursts on each of these
744 three contacts. We verified that the sequence triggered whole arm and hand movements that
745 mimicked smooth⁵⁷ and natural multi-joints movements (**Extended Data Figure 5C, Video 1**).
746 Specifically, extension, grasping and pulling movements produced clear EMG bursts as well as
747 robust and smooth kinematics. These stimulation protocols could be triggered by an operator at
748 the beginning of each reach movement or automatically from intra-cortical signals in real-time.
749 Therefore, we verified that movement onset could be detected from intra-cortical signals even
750 after SCI (**Extended Data Figure 5**).
751

752 *Stimulation during three-dimensional reach and pull task in injured monkeys*

753 All monkeys were recorded after injury as soon as they could independently move in their housing,
754 feed themselves autonomously and did not show signs of discomfort. This corresponded to 3, 5
755 and 6 days after injury respectively for Mk-Yg, Mk-Br and Mk-Sa. After injury, the animals were
756 reluctant to perform the task which required intense manual activity by the trainers to encourage
757 them with the use of special positive rewards. Moreover, in consequence of the arm and hand
758 impairments animals were quickly exhausted. As a result, the output of consistent behavior/day
759 was low, and we were able to collect robust data in about 1day/week per animal after SCI. Each
760 session was organized as follows. First, we executed two blocks without stimulation, each of the
761 duration of approximately 2 minutes. During those blocks we visually evaluated the impairment
762 level of the animal and the performance of the brain decoder. Second, we used the brain decoder
763 to trigger specific stimulation patterns. Contacts used to elicit those functions were defined
764 through the experiments described in the previous paragraph and combined together to create
765 stimulation protocols that allowed the animal to perform a full reach, grasp and pull movement.

766 *Identification and classification of arm movements for kinematic analysis*

767 We defined the movement performed by the animals as composed of three different phases:
768 reach, grasp and pull. The identification of the reach phase was done by marking the moment in
769 which the left hand left the metallic bar to when the hand closed around the object secured to the
770 robot hand effector (the grasp event). The grasp phase was considered to be a window of 100
771 ms around the moment in which hand closed around the object. The pull phase started from the
772 grasp event and finished when the animal accomplished the task by pulling the object towards its
773 body and placed the hand back on the resting bar. Events related to the 3 phases of the movement
774 (movement onset: reaching, grasp onset: grasping and release of the object, and pulling) were
775 identified manually by inspecting video recordings from Vicon Motion Systems (Oxford, UK).

776 Moreover, a blind experimenter manually inspected the same video recordings from Vicon Motion
777 System to mark successful and complete performance of reach, grasp and pull movements as
778 events. A successful reach was defined as a complete extension of the arm that brought the hand
779 at the position of the target (even when grasp could not be performed). A successful grasp was
780 defined as a successful closure of the hand around the target. A successful pull was defined as
781 the accomplishment of a flexion movement that brought the target towards the animal. Events
782 were then extracted from Vicon and used to perform analysis on the kinematic of the movements
783 and to train the brain decoder by automatic routines (Matlab 2019b). All the analysis was
784 conducted as blinded experiments.

785 *Decoding motor states from intracortical signals*

786 We designed a neural decoder that detected reaching and grasping events using intracortical
787 spiking activity. To detect spikes, we set a threshold on each channel of -4 times the root-mean-
788 square voltage recorded during a brief period while the monkey was at rest. We estimated firing
789 rates in each of the motor cortical array channels by summing the multiunit spikes with a 150 ms
790 history every 0.5 ms. We used these multiunit firing rate estimates to compute a twenty-
791 dimensional neural manifold capturing the majority of population variance⁴⁷. We projected the
792 spiking activity onto this manifold to calibrate a multiclass regularized linear discriminant analysis
793 decoder³⁹ that predicted the labeled timing of reach and grasp events. The decoder used 500 ms
794 of past neural activity and output the probability of observing the reach and grasp events. During
795 calibration, we defined a probability threshold for each event ranging from 0.8 to 0.99 to optimize
796 predictions of the timing of each event using cross-validation. Since the monkeys could not
797 complete the task after SCI, we were unable to consistently acquire labeled training data. We
798 therefore calibrated a decoding algorithm using reaches from a recording session of a healthy
799 monkey. We then manually labeled attempted reaches after SCI by manual inspection of video
800 recordings. Using canonical correlation analysis, we aligned the neural dynamics⁵⁸ preceding
801 reaches on the healthy sessions to the observed neural dynamics preceding attempted reaches
802 after SCI. These aligned dynamics were used to control the decoder trained on the healthy
803 reaches.

804 We implemented a custom C++ software application running a control suite that used the
805 decoding algorithm to trigger EES stimulation in real-time. The application received neural data
806 over UDP and made predictions using the decoding algorithm at 15 ms intervals. When the output
807 probabilities crossed the defined threshold, the application triggered preprogrammed patterns of
808 EES.

809 *Analysis of muscle recruitment curves*

810 Electromyographic activity was bandpass filtered between 30 and 800 Hz with an offline 3rd order
811 Butterworth filter and stimulus artifact were removed. For each animal, stimulation contact, muscle
812 and stimulation amplitude, we extracted compound potentials from 50ms-long segments of
813 electromyographic activity following a stimulation pulse. We then computed the peak-to-peak
814 amplitude of compound potentials. Since we gave four pulses of stimulation for each selected
815 current amplitude, we averaged across values corresponding to the same stimulation amplitude
816 and represented as the mean recruitment value of each muscle as a function of the injected
817 current. For each muscle, recruitment values have been subsequently normalized by the
818 maximum value obtained for that specific muscle, provided that we obtained response saturation
819 (and therefore maximal contraction) in at least one occasion during the session. In addition, we

820 computed a selectivity index for each muscle⁵⁹.

821 In order to obtain a comprehensive measure of muscle recruitment for each contact that would
822 allow to compare across animals, we computed, for each animal, each muscle and each contact,
823 an Average Recruitment Index (ARI) as the average of the recruitment values across all
824 stimulation amplitudes used from a specific stimulation site.

825 To compute muscle recruitment during the delivery of pulse train stimulation, we computed the
826 energy of the EMG signal during the duration of stimulation. We then applied the same
827 normalization procedure described above for single pulse recruitment.

828 *Analysis of muscle activity during EES*

829 Electromyographic activity was bandpass filtered between 30 and 800 Hz with an offline 3rd order
830 Butterworth filter and stimulus artifact were removed. In all animals we computed the energy EMG
831 signals, for each implanted muscle. Energy of EMG signals during stimulation was computed on
832 each segment in which stimulation was delivered after the animal started a movement attempt,
833 with the formula here below:

834
$$EN_{EMG} = \frac{1}{N} \sum_i^N \|EMG_i\|^2 dt$$

835 Where EMG_i is the value of EMG activity at sample i , N is the number of samples in the signal
836 and dt is the sampling resolution.

837 Energy of EMG signals without stimulation was computed on each segment in which stimulation
838 was not delivered and the animal started a movement attempt. A movement attempt was defined
839 as an increased EMG activity of the Biceps and Deltoid muscles.

840

841 *Analysis of task and kinematics performance*

842 We computed task performance as the rate of each movement expressed in events per minute.
843 Successful movements were identified by a blind experimenter as movements performed skillfully
844 and that had functional relevance (see above, *Identification and classification of arm movements*
845 *for kinematic analysis*). First, we have identified all the sections of the recording during which the
846 animal was actively attempting the task. All dead times, i.e. moments in which animals directed
847 their attentions elsewhere than the task itself, were discarded from the analysis. Second, we
848 computed the task performance frequency as the rate of successful movements per unit of time.
849 In order to do this, we subdivided sessions in time bins of 1 second and we marked the presence
850 or absence of successful trials, both with and without stimulation. We then used bootstrap to
851 analyze significance of those results. Sessions when the animal did not perform the task were
852 discarded from this analysis. Next, we performed Principal Component Analysis (PCA) on a large
853 set of kinematic features. Details of this analysis are explained in the Supplementary information.

854

855 *Processing of cortical signals*

856 We identified spiking events on each channel when the band-pass filtered signal (250 Hz–5kHz)
857 exceeded 3.0–3.5 times its root-mean-square value calculated over a period of 5s. We removed
858 artifacts by deleting all the spikes that synchronously in at least 30 channels. We computed the
859 firing rate of each channel as the number of spikes detected over non-overlapping bins of 10ms.
860 Whenever we showed average firing rate activity, we sorted channels in order of activation in one
861 reference trial, and subsequently applied the same ordering method to all other trials. Finally, we
862 normalized the activity of each channel by its maximum firing rate.

863 *Comparison of motor cortical activity during EES evoking movement and no movement*

864 To study how motor cortical activity interacted with EES, we analyzed the neural recordings from
865 Mk-Br and Mk-Yg. We identified periods where EES pulse trains produced no discernible
866 movements by setting a threshold on hand velocity. We compared multi-unit neural firing rates on
867 each channel in this period to neural firing rates in the previously identified trials where EES
868 enabled reaching and grasping. First, we counted the number of spikes within the window of
869 stimulation and divided by the duration of stimulation. We then averaged across stimulus
870 repetitions of the movement and no movement conditions and pooled across recording sites in
871 motor cortex.

872 We next computed instantaneous estimates of multi-unit firing rates on each channel by counting
873 the number of spikes in non-overlapping 20 ms bins and convolving with a gaussian kernel of 50
874 ms width. We applied Principal Component Analysis (PCA) to compute 10-dimensional neural
875 manifolds spanning this multi-unit population activity⁴⁷. We projected the neural activity onto these
876 manifold axes during the periods where EES evoked either movement or no movement. We then
877 identified periods where the monkey was at rest with no EES, as well as periods where the
878 monkey attempted movements of the arm with no EES. To compare the similarity of neural activity
879 between these conditions, we computed the Mahalanobis distance between activity at rest and
880 the three other periods: EES with movement, EES with no movement, and attempted movements
881 with no EES.

882 *Histology*

883 Monkeys were deeply anesthetized (lethal dose of pentobarbital, 60mg/kg, injected i.v.) and
884 transcardially perfused with saline (about 200 ml), followed by 3 liters of 4% paraformaldehyde
885 (PFA). Dissected spinal cord were post-fixed in 4% PFA overnight, and then immersed in 30%
886 sucrose solution for 2 weeks. 50µm transverse or horizontal sections were cut using a cryostat
887 and kept in 0.1M PBS azide (0.03%) at 4°C. Primary antibodies were: rabbit anti-Iba1 (1:1000,
888 Wako) and guinea pig anti-NeuN (1:300, Millipore). Fluorescence secondary antibodies were
889 conjugated to: Alexa fluor 647 (1:200, Life technologies) and Alexa fluor 555 (1:300, Life
890 technologies). Sections were coverslipped using Mowiol. Immunofluorescence was imaged
891 digitally using a slide scanner (Olympus VS-120). Lesions were reconstructed using image
892 analysis software (Neurolucida) to trace the lesion over serial sections (200 µm apart).

893 *Statistical procedures*

894 All data are reported as mean values ± standard error of the mean (s.e.m.) or mean values ±
895 standard deviation (std). The choice is highlighted directly in the figures or in the relative
896 caption. Significance was analyzed using the non-parametric Wilcoxon rank-sum test. In the
897 comparisons shown in Figure 3 we subsequently applied the Bonferroni correction. In only one

898 case (Figure 4A, 4B), significance was analyzed using bootstrap. The level of significance was
899 set at * $p<0.05$, ** $p<0.01$, *** $p<0.001$.
900 No statistical methods were used to pre-determine sample sizes instead number of animals used
901 in this study are consistent to other works that involved similar procedures in monkeys^{9,10,42}.
902

903 **Data availability**

904 Due to the sensitive nature of the dataset, which contains graphic information on monkeys, raw
905 data, including videos, will be available upon reasonable request to the corresponding author and
906 after authorization from the Swiss cantonal authorities. A set of pre-processed data will be
907 deposited on the open-data commons for spinal cord injury (<https://odc-sci.org>).
908

909 **Code availability**

910 Software routines utilized for data analysis will be deposited on GitHub under search keyword
911 NN-A75365C.
912

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