Supplementary Information for
Forelimb force direction and magnitude independently controlled by spinal modules in the macaque

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**Supplementary Information Text**

**Other factors that may influence the direction and magnitude of force fields and their summation**

**Electrode depth and stimulus current**

We stimulated intraspinal sites at different depths using electrode arrays with three different lengths (2, 3, or 4 mm). Figure S2 compares the magnitude (S2A) and direction (S2B) of individual force vectors evoked by single-site stimulation, as well as the SIs representing the supra-linear summation of force field magnitude (S2C) and the directional similarity to the linear sum (S2D), all recorded when stimulating at different depths. While the parameters related to force field direction (the direction and the similarity to directional linear sum) were not affected, those related to force field magnitude (x-y force and SI) depended on the electrode depth. Specifically, the size of the SI (S2C) exhibited a consistent relationship with the depth of electrodes, with superficial electrodes producing larger, supra-linear facilitation of force field magnitude. The result is the following linear model: $SI = 5 - 0.96x$, where $x$ represents the electrode depth in mm ($F$-statistic vs. constant model: $F_{(204)} = 31.7$, $P < 0.01$).

One possible reason for this is that supra-linear summation of force field magnitude might represent a particular motor output mechanism found in the dorsal part of the cervical spinal cord (e.g., output through premotor interneurons) (1,2,3,4). However, this bias toward a higher SI during co-stimulation using more superficial electrodes might also be influenced by other experimental constraints, such as the current required to evoke a minimum motor output measurable at the wrist. For example, we found that the actual amplitude of stimulation current applied differed depending on electrode depth: 437.5 µA ± 61.64 for 2 mm, 253.75 ± 56.80 for 3 mm, and 133.12 ± 103 for 4 mm (mean ± SD). This could have been because we adjusted the current around the motor threshold. For example, stimulating closer to the motoneuron pool (the deepest electrode) might require a lower stimulus current, while generating a measurable output field using premotor interneurons might require a bigger set of interneurons, which would require a larger current. Therefore, the depth bias shown in Figure S2C could represent the fact that intraspinal stimulation with a large current will increase the excitability of a greater number of subthreshold premotor neurons, irrespective of the electrode depth. Co-stimulation in this scenario, particularly in the case of spatial facilitation, would eventually recruit a large number of premotor neurons and motoneurons, which would then generate supra-linear summation.
Alternatively, the higher stimulus current required for the dorsal electrode might have been an artifact of the length of time the electrode array had been implanted; electrode efficacy is known to become degraded owing to scarring and possible neuronal migration during chronic electrode use (5,6,7). Our recordings were conducted 7 to 72 days after the spinal implant surgery, and the recording using the shortest electrodes (2 mm) was conducted on the last (72nd) day. Therefore, although a higher absolute current was used for these electrodes, the actual number of recruited neurons might have been smaller than expected from the high current, and the efficacy in this situation might be comparable to that for one of our stimulations using a deeper electrode and a lower current.

When we checked for effects of electrode depth, current, and days-post-implant on the SI during co-stimulation using a linear mixed-effects model (Matlab routine “fitlme”), we found that depth and current both affected the SI (depth: $F_{(1,1115)} = 7.23, p < 0.01$, current: $F_{(1,1115)} = 7.68, p < 0.01$), with days-post-implant to a less-likely degree ($F_{(1,1115)} = 3.92, p < 0.05$), and no significant cross-factor effect. Therefore, we cannot separate the effect of stimulating at a particular depth within the spinal cord from the current used at those depths to evoke a motor response. Thus, nonlinear magnitude summation should not be ascribed exclusively to a characteristic of a focal, dorsal region in the primate cervical spinal cord.

**Position in the workspace**

Afferent feedback might particularly bias spinal output to muscles in an outstretched limb configuration (8) and influence multiple nodes in the hierarchy of the spinal motor module (9). Therefore, as shown in Figure S3, we tested the influence of afferent feedback bias on the magnitude (S3A) and direction (S3B) of each force vector, as well as the scaling index (S3C) and the directional similarity to the linear sum (S3D) in the force field evoked by co-stimulation. When we compared these variables among the seven different wrist positions irrespective of the stimulation site, we found no systematic difference among the fields (Magnitude, A: $F_{(6, 121)} = 0.21, p = 0.91$; Direction, B: $F_{(6, 73)} = 0.6, p = 0.73$; Scaling index, C: $F_{(6, 203)} = 0.65, p = 0.69$; Cosine similarity, D: $F_{(6, 203)} = 1.35, p = 0.24$). To examine the interaction between hand location and the effect of co-stimulation on the magnitude, we used a linear mixed-effects model (Matlab routine fitlme) with stimulation type and hand position at seven set locations as fixed factors. Although we found a highly significant effect of stimulation type: ($F_{(1, 406)} = 50.8, p < 0.01$), location had no effect on magnitude ($F_{(6, 406)} = 0.02, p > 0.05$) and the two factors did not interact ($F_{(6, 406)} = 1.73, p > 0.05$). Therefore, the difference in afferent input that depends on hand position did not have a position-consistent systematic influence on force field direction or magnitude, or on the interaction between magnitude and SI.
**Reafference signal from the hand and finger**

We allowed limited hand and finger movement because completely immobilizing these joints would transmit their muscle activity to the force transducer. As a consequence, we needed to determine whether or not the afferent feedback generated by these subtle movements of the hand and fingers had a significant effect on the linear summation of vector direction (summarized in Fig. 4F) or the supra-linear summation of vector magnitude (in Fig. 5H). To make this assessment, we repeated the analysis described in Figures 4F and 5H using only the EMG response generated by the first stimulus pulse in each train (S4a, 20 ms), the rationale being that the chance for the reafference signal to significantly influence the response was virtually zero because it was evoked before the onset of hand or finger movement.

As shown in Figure S4, we found no major differences when we compared the response to the first pulse with the total response induced by all 25 pulses in the train (Figs. 4F and 5H). We again found that the highest directional similarity (S4B) was in the top-two muscle group (similarity: 0.92 ± 0.02; mean chance level: 0.29), followed by a significantly lower similarity in the shoulder-muscle group (similarity: 0.77 ± 0.03, chance level: 0.26; top-two vs. shoulder, corrected for different chance levels: p < 0.05). The similarities were again even lower in the wrist/finger- and elbow-muscle groups (wrist/finger similarity: 0.65 ± 0.05, chance level 0.25; elbow similarity: 0.57 ± 0.06, chance level: 0.25), with elbow similarity being significantly lower than shoulder-muscle similarity (p < 0.01). The lowest similarity (but still significantly higher than chance level) was for a vector comprising all muscles (similarity: 0.5 ± 0.06; chance level: 0.24). These characteristics resemble those shown in Figure 4E. As for the supra-linear summation in the amplitude (S4C), the SIs for the wrist and finger (1.8 ± 0.13, p < 0.001) as well as for the elbow muscles (1.8 ± 0.13, p < 0.05) were significantly larger than those for the top-two muscles (0.34 ± 0.13). These results also confirm the original results shown in Figure 5H. Overall, the similarity between the results compiled using responses evoked by the first pulse and those from the full 25-stimulus pulse train suggest that the reafference signal from hand and finger movements evoked by ISMS did not significantly affect the results.

**Stimulation history**

We compared the force field direction and magnitude evoked by the first stimulation to each intraspinal site (and pair) in the first cycle after changing the wrist position with that evoked by the last stimulation in the last (i.e., sixth) cycle of repetition before changing the wrist position. The rationale for this test is that the influence from the previous stimulation sequence should be lowest in the first cycle because of the time required to change the wrist position (i.e., greatest time since the last stimulation) and highest in the last
cycle (largest number of stimulations applied without a break). The results are shown in Figure S5. We did not find any significant difference between the force fields evoked in the first and last cycles in terms of the direction ($p = 0.09$) or magnitude ($p = 0.21$) for single-site stimulation, the directional similarity ($p = 0.51$), or the SI for magnitude ($p = 0.26$). Consequently, we conclude that the stimulation history had little influence.

**Anesthesia**

Although anesthesia is known to change the excitability of spinal neurons (10), we used an animal preparation with an intact CNS, and thus an anesthetic drug was required. As described in the Methods, the level of anesthesia during each recording session was carefully and continuously monitored by veterinary staff and was kept at a relatively constant depth to ensure minimal discomfort related to animal positioning or the stimulation itself. However, subtle fluctuations in depth are unavoidable for any anesthesia and might have had some influence on the results. To test this possibility, we compared the aggregated, background EMG signal from all muscles under the assumption that it might exhibit a subtle change related to the muscle relaxants that we used (ketamine and medetomidine). In Figure S6, we plot the aggregated background EMG activity in varying intervals from the time of anesthesia injection (S6A). We compared EMG activity just before and after injections ($n = 2$ to $3$ per recording session) during each experimental session (S6B) and found that the variance in EMG activity was quite small (no statistically significant changes), and that there were no systematic trends in EMG level. These results indeed confirm that the level of anesthesia was stable throughout the experiment. We can then assume a stable level of spinal neuron excitability throughout each recording session. We also checked to see if the anesthetic affected the synergistic recruitment of interneurons during co-stimulation, but found no correlation between time after the last injection and the SI ($r = 0.1, p = 0.14$). This suggests that the observed supra-linear summation effects were not substantially influenced by the depth of anesthesia.

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**FIGURE S1**

**Correlation of EMG SI with force SI.** (A) An example of force SI measured in monkey TE in response to stimulation at intraspinal sites #1 and #3 (depth: 4 mm) at seven hand positions, ordered by the force SI. (B) An example of EMG SI from the lateral head of the triceps brachii (TLA) recorded at the same time as the force in (A). (C) Force SI (A) and EMG SI (B) were significantly and positively correlated ($r = 0.7, p < 0.05$). (D) The mean ($\pm$ SE) of the absolute correlation coefficients between the EMG SI in response to stimulation at a single intraspinal site and the corresponding force SI, computed for each muscle. (E) Same data as (D), divided into groups of related muscles. *, $p < 0.05$; **, $p < 0.01$.

| Muscle Group | Force SI | EMG SI | Absolute Correlation |
|--------------|----------|--------|----------------------|
| Wrist/ Finger | 0.1      | 0.2    | 0.1                  |
| Shoulder     | 0.3      | 0.4    | 0.3                  |
| Elbow        | 0.5      | 0.6    | 0.5                  |
| Top two      | 0.7      | 0.8    | 0.7                  |
Influences of intraspinal depth for each stimulus.

(A) Box plot showing the distribution of force magnitude for all intraspinal sites at different depths: 2 mm (n = 33), 3 mm (n = 99), and 4 mm (n = 83). No significant differences were found between the groups (ANOVA, $F_{(2,212)} = 1.13, p > 0.05$). (B) Box plot showing the distribution of force directions (in relation to the east), for all intraspinal sites at different depths: 2 mm (n = 9), 3 mm (n = 41), and 4 mm (n = 30). No significant differences were found between the groups in A and B (ANOVA, $F_{(2,77)} = 0.23, p > 0.05$). (C) Box plot showing the distribution of SIs (the natural logarithm of the scaling coefficient between the observed and expected responses to stimulation), for all intraspinal sites (n = 169) at different depths: 2 mm (n = 41), 3 mm (n = 83), and 4 mm (n = 82). The horizontal gray line shows the mean SI for all depths. The black dotted line represents the regression fit line for the three electrode depths. Differences between the groups were significant (ANOVA, $F_{(2,203)} = 26.75, p < 0.01$). (D) Box plot showing the distribution across all intraspinal sites (n = 30) of the cosine similarities for direction between a force vector generated by co-stimulation and that estimated by the linear-sum of EMG vectors generated by each single-site stimulation. Depths: 2 mm (n = 6), 3 mm (n = 12), and 4 mm (n = 12). No significant differences were found between the groups (ANOVA, $F_{(2,27)} = 1.35, p > 0.05$). (A-D) The gray boxes are centered on the median of the distribution (red line) and its top and bottom edges indicate the 25th and 75th percentiles of the distribution, respectively. The dashed red line represents the mean. Outliers are marked by + symbols, and the whiskers show the extent of all data that are not considered outliers. The notches represent 5% confidence intervals around the medians.
FIGURE S3

Influences from different wrist positions.

(A) A 3-D bar plot showing the distribution of force magnitudes across the seven standard wrist positions for all intraspinal stimulation sites (n = 122). Red lines represent ±SE. An ANOVA (F(6,121) = 0.34, p > 0.05) for the effect of position showed no significant differences between the groups. (B) A 3-D bar plot showing the distribution of force directions (in relation to the east), for all intraspinal sites (n = 80). Red lines represent ±SE. An ANOVA (F(6,73) = 0.6, p > 0.05) for the effect of position showed no significant differences between the groups. (C) A 3-D bar plot showing the SI distribution for all intraspinal site combinations (n = 210). Red lines represent ±SE. An ANOVA (F(6,203) = 0.65, p > 0.05) for the effect of position showed no significant differences between the groups. (D) A 3-D bar plot showing the distribution of cosine similarity for all intraspinal site combinations (n = 210). Red lines represent ±SE. ANOVA (F(6,203) = 1.35, p > 0.05) for the effect of position showed no significant differences between the groups.
FIGURE S4

Analysis using EMG responses evoked by the first stimulus

(A) Illustration showing the entire series of EMG responses evoked by a stimulation train (in this case the ECR muscle from monkey NE) and the part selected for additional analysis (following just the first pulse).

(B) Mean (± SE) of the cosine similarity between the EMG vectors generated by the first co-stimulation pulse and those expected by linear summation of the vectors generated by the first single-stimulation pulse, computed for each group of muscles. Solid gray lines indicate chance level similarity and dashed lines indicate the 5th and 95th percentiles of the distribution. (C) Box plot showing the distribution of EMG SIs evoked by the first stimulus pulse for the different muscle groups. Outliers are marked by + symbols, and the whiskers show the extent of all data that are not considered outliers. The notches represent 5% confidence intervals around the medians. * p < 0.05, ** p < 0.005, *** p < 0.0005.
Influences from stimulation history.

(A) Box plot showing the distribution of force magnitudes for all intraspinal sites at the first (n = 147) and last (n = 148) repetitions. No differences were found between the two (t-test, \( p > 0.01 \)). (B) Box plot showing the distribution of force direction (in relation to the east) for all intraspinal sites at the first (n = 109) and last (n = 109) repetitions. No differences were found between the two (t-test, \( p > 0.01 \)). (C) Box plot showing the distribution of SIs for all intraspinal site combinations at the first (n = 141) and last (n = 142) repetitions. No differences were found between the two (t-test, \( p > 0.01 \)). (D) Box plot showing the distribution of cosine similarity of force vectors for all intraspinal site combinations at the first (n = 30) and last (n = 30) repetitions. No differences were found between the two (t-test, \( p > 0.01 \)).

(A–D) The gray boxes are centered on the median of the distribution (red line) and its top and bottom edges indicate the 25th and 75th percentiles of the distribution, respectively. The dashed red line represents the mean. Outliers are marked by + symbols, and the whiskers show the extent of all the data that are not considered outliers. The notches represent 5% confidence intervals around the medians.
FIGURE S6

Influences from fluctuations in anesthesia depth

(A) Example (monkey NE, depth: 2 mm) of background EMG activity as an indicator of anesthesia depth at different intervals after the anesthesia injection. Each bar represents the average aggregated EMG of all muscles over 2 seconds when there was no stimulation. The black line represents the time when a second injection was applied. There was no significant correlation between injection time and rectified EMG area. Error bars are ±SE.

(B) Mean rectified EMG activity (2-second periods during a time when there was no stimulation) recorded from all muscles and all days immediately before and after a second injection. No differences were found between the two (t-test, \( p > 0.01 \), \( n = 120 \) for each).