Supplemental Material

MVA

Participants were instructed to perform isometric plantarflexion contractions as hard and as fast as they could. As plantarflexion contractions can be quite powerful, a customized binding was used to maintain isometric positioning. Contractions were held until the degree of muscle activation no longer increased (about 3 - 10 seconds). Verbal encouragement and visual feedback was provided. The maximum activity of rectified EMG, averaged over 1s, was calculated for each trial. Multiple trials (typically 3-4 trials) with at least 30 seconds of rest between trials were performed until the two highest trials were within 10% of each other. MVA was defined as the average of these two trials. The electrical noise recorded at rest (1-2 μV) was a large proportion of the MVA in some participants with CP (7-10 μV). Thus, electrical noise was subtracted from all measures of voluntary muscle activity (i.e., MVA and pre-stimulus background EMG) in each participant. The maximal voluntary activity (MVA) was 24 (7 – 85) μV in the group with CP and 185 (73 – 297) μV in the NI group ($p<0.001$). N.B. Measures of strength using maximal torque, or less directly with maximal EMG activation, frequently use time frames in the range of seconds like the 1 second time frame used to determine MVA here. However, when looking at the degree of voluntary activation just prior to a stimulus, shorter time frames (50 - 100 ms) are used. This is thought to better characterize the level of activity in the nervous system influencing the response to stimulation and thus, we measured the voluntary EMG activation over the 100ms prior to TMS.

M_{max}

The maximal soleus M-wave (M_{max}) was evoked using monopolar tibial nerve stimulation (1-ms pulse width, DS7A constant-current stimulator NL703, Digitimer, Welwyn Garden City, UK)

applied with a cathode probe in the popliteal fossa over the tibial nerve and a large anode over the patellar tendon. M_{max} values were used to normalize MEP values in all but 3 participants (1CP, 2NI) due to contamination of the M-wave with the stimulus artifact. The maximal possible evoked response (M_{max}) was 5.1 (1.3 – 9.8) mV in the group with CP and 9.8 (4.5 – 12.2) mV in the NI group (p=0.003).

Screening Procedures for TMS and MRI

Participants were screened for relative contraindications to TMS using a standard screening questionnaire[1]. As per investigator and physician judgment, no participants had absolute contraindications to TMS as recommended by Rossi et al. 2011[2]. Examples of potential contraindications included unstable epilepsy, intracranial or cardiac metal implants.

Participants were screened for contraindications to MRI using the standard screening procedures at the Peter S. Allen MR Research Centre (Edmonton, Alberta). Potential contraindications included the presence of MR incompatible metal in or on the body, pregnancy or claustrophobia. One participant CP-4 had a contraindication to MRI. All participants could lie still in the MRI without sedation except for CP-11 who had involuntary lip and jaw movements during some sequences.

Transcranial Magnetic Stimulators

Two stimulators were used in this study due to a technical failure with one of the stimulators. As a result, 8/16 participants in the CP group and 13/15 participants in the NI group were tested using a MagStim 200 stimulator and the remainder were tested using its BiStim module. The same batwing coil was used with both stimulators. Due to potential differences in the absolute levels of stimulator output (%MSO) between the two stimulators used in this study, no comparison of active motor thresholds was repeated here though thresholds are known to be higher in people

with CP[3-7]. Further, all measures of intensity are expressed relative to the individual's active motor threshold (aMT) and are thus not impacted by the stimulator differences.

Hotspot Localization

Participants wore a cap containing a 1-cm grid aligned to their vertex. Four submaximal stimuli were applied in 1-cm increments anterior and posterior to the start position of 2 cm lateral to vertex until surrounding sites of activation were less than the location producing the largest MEP responses. TMS was kept at least 2 cm lateral to vertex to reduce current spread to the opposite cortex as per[8]. Pilot testing in neurologically intact participants revealed that if the hotspot was first determined to be 1 cm or at midline, moving the coil to 2 cm lateral did not alter the maximum MEP that could be evoked.

MEP_{max}

Both high levels of background muscle activity and high levels of stimulation intensity produce large MEPs up to a point beyond which responses plateau or decrease. Ideally, to determine an individual's maximal MEP, one would explore the complete array of responses created by varying both the simulator intensity and the background activity. However, that experiment would be prohibitively long and would be confounded by fatigue. Therefore, we restricted our protocol to determine MEP_{max} to: 1) systematically varying the stimulus intensity at one level of voluntary activation (20% MVA) (i.e. generated a stimulus-recruitment curve) and by varying the voluntary activation at two levels of stimulus intensity at 2) 1.2 x aMT and 3) the intensity that produced the largest MEP amplitude in the stimulus-recruitment curve. To determine the largest MEPs evoked while exploring the impact of voluntary modulation, the MEPs evoked with a given stimulus intensity were plotted against the corresponding level of background activity and a moving-window, running average of 5 MEPs was calculated. These plots were generated at both stimulation

intensities, 1.2 x aMT and the intensity that produced the largest MEP amplitude in the stimulus-recruitment curve. MEP_{max} was defined as the largest average of 5 MEPs from either of these plots or from the stimulus-recruitment curve at 20% MVA.

Diffusion Tractography

DTI sequences were pre-processed, and motion distortion was corrected in ExploreDTI v4.8.3 [9]. Deterministic whole brain tractography was performed using a fractional anisotropy threshold of 0.2 and an angle threshold of 50°. The corticospinal pathway contralateral to the target leg was analyzed. A 'seed' region of interest (ROI) was placed around the posterior limb of the internal capsule and a 'target' ROI around the corticospinal pathway below the basis pontis [10]. Due to technical factors, in CP-14 the contralateral, right corticospinal pathway could not be reliably followed to the basis pontis, requiring placement of the 'target' ROI at the level of the cerebral peduncle. 'Not' ROI(s) were used to exclude spurious fibres. Partial tract analysis was performed on the tract segment between the 'seed' and 'target' ROIs only, rather than following the tracts all the way up to the cortex. This was done to control for the high variability in tract length in the CP group, as reflected in the larger standard deviation of tract length in the CP group (±27%) compared to the NI group (±10%). The greater variability in streamlines near the cortex may be due to a larger number of crossing fibres near the cortical lesions and/or from the lesions themselves. A recent study showed stronger association between tractography parameters and motor function using partial tract analysis rather than full tracts[11]. Note that the two DTI parameters FA and perpendicular diffusivity have greater correlation to white matter abnormalities compared to MD and parallel diffusivity.[12]

Heterogeneity in the Participants with CP

In 4 participants with CP, it was not possible to consistently evoke MEPs from any location over the contralateral motor cortex at the maximum stimulator output (MEPs = 0.0 mV, Fig. 2C), although responses from stimulation to the ipsilateral cortex were present (see MEP_{max} values). Note that all differences in MEP measures between the NI and CP groups remained statistically significant when data from these 4 participants (CP-2, CP-6, CP-8 and CP-15) were excluded.

There is little evidence regarding the effect of chronic medication use on TMS measures. Six participants with CP were chronically taking neuromodulatory medications that, in a single dose, have been shown to, or theoretically may, alter responses evoked by TMS.[13] In specific, the participants taking neuromodulator medication were: CP-2 baclofen & citalopram, CP-4 venlafaxine & cyclobenzaprine, CP-6 citalopram & tolterodine, CP-11 clonazepam & sertraline, CP-12 clonazepam, escitalopram & quetiapine, CP-15 amitriptyline, citalopram, phenytoin & flunarizine. To ensure *chronic* use of these medications did not influence the results, all statistical tests involving TMS were repeated without these 6 participants. The statistical results were unchanged even as the statistical power was reduced.

Peripheral factors, such as variability in reduced muscle size and increased subcutaneous tissue, could have contributed to the reduced and variable MEP amplitudes in the CP group. People with CP are known to have smaller muscles with less contractile tissue.[14,15] This likely contributes, in part, to the known differences in maximal voluntary activity (MVA) and maximal evoked responses (M_{max}) that were also observed here. However, previous studies have demonstrated that muscle structure cannot completely explain these differences.[16,17] Further, when accounting for differences in the amount of muscle that can be activated by an imposed stimulus by normalizing the data to M_{max}, the differences in MEP amplitudes persisted. Therefore, the reduced MEP

amplitudes observed in the participants with CP in this study were likely driven by decreased activation of high and low threshold corticospinal pathways which contribute to decreased muscle activation and weakness.

The presented findings from this study are all as inclusive as possible. People with cerebral palsy, even the subset of people with bilateral spastic cerebral palsy represented by this study are a large and heterogeneous group. The steps described above were undertaken to ensure that potentially confounding factors did not drive the findings of reduced MEPs and impaired voluntary modulation of MEPs measured in this study. Thus, the presented results include the entire populations studied here to remain representative of people with bilateral spastic CP.

Figure S1

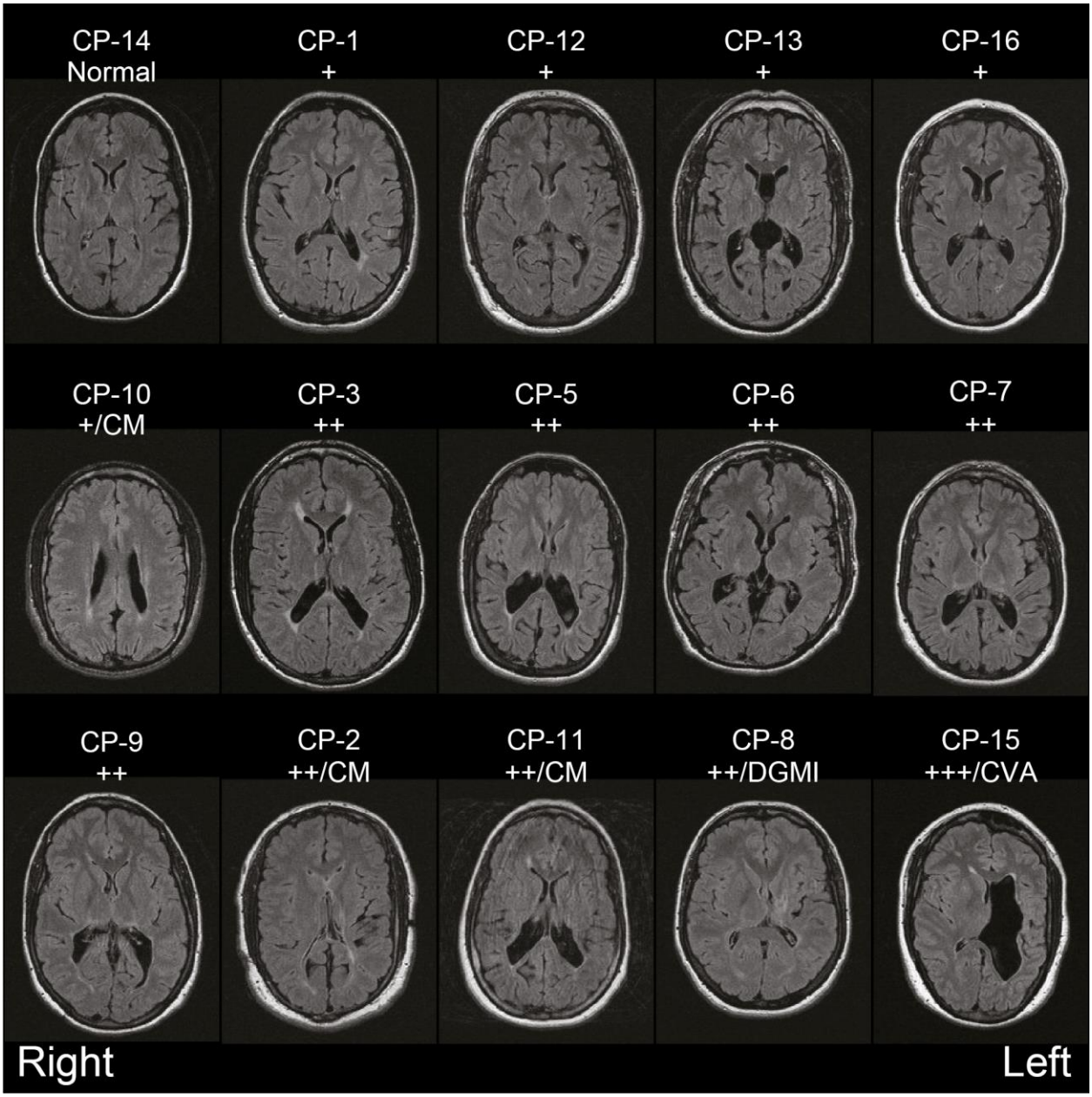

Figure S1. MRI of Participants with CP. Axial FLAIR slices illustrating the anatomical findings for each participant with CP imaged (15/16). One participant had normal imaging. This is known to occur in CP with 1/15 representing a lower rate of normal imaging than is found in population based registries.[18] All remaining participants had evidence of mild (+), moderate (++), or severe (+++) periventricular white matter injury (PVWMI) on both sides of the brain. Additional abnormalities were noted in 5 participants. Three participants had cerebral malformations (CM) in the form of polymicrogyria (CP-10 & CP-11) and partial corpus callosum agenesis (CP-2). CP-8 had deep gray matter injury (DGMI, particularly notable on the left. The image of CP-15 is consistent with a periventricular cerebrovascular accident (CVA) in the left hemisphere with evidence of periventricular white matter injury in the right hemisphere and she had bilateral motor impairments. No contralateral MEP responses were recorded in CP-15 in addition to CP-2, CP-6 and CP-8 (Table 1). Figure adapted from previous publication.[19]

Figure S2

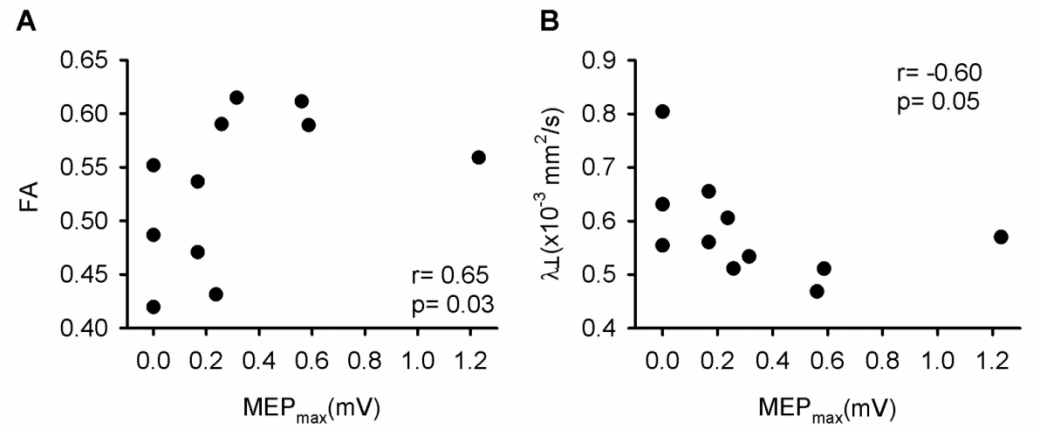

Figure S2. Association between DTI parameters and MEP$_{max}$. FA **(A)** and perpendicular diffusivity **(B)** plotted against corresponding MEPmax values (in raw voltage) for participants with CP.

References

1. Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol.* Dec 2009;120(12):2008-2039.
2. Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Screening questionnaire before TMS: an update. *Clin Neurophysiol.* Aug 2011;122(8):1686.
3. Berweck S, Walther M, Brodbeck V, et al. Abnormal motor cortex excitability in congenital stroke. *Pediatr Res.* Jan 2008;63(1):84-88.
4. Eyre JA, Smith M, Dabydeen L, et al. Is hemiplegic cerebral palsy equivalent to amblyopia of the corticospinal system? *Annals of neurology.* Nov 2007;62(5):493-503.
5. Heinen F, Kirschner J, Fietzek U, Glocker FX, Mall V, Korinthenberg R. Absence of transcallosal inhibition in adolescents with diplegic cerebral palsy. *Muscle Nerve.* Feb 1999;22(2):255-257.
6. Koerte I, Pelavin P, Kirmess B, et al. Anisotropy of transcallosal motor fibres indicates functional impairment in children with periventricular leukomalacia. *Dev Med Child Neurol.* Feb 2011;53(2):179-186.
7. Vry J, Linder-Lucht M, Berweck S, et al. Altered cortical inhibitory function in children with spastic diplegia: a TMS study. *Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale.* Apr 2008;186(4):611-618.
8. Maegaki Y, Maeoka Y, Ishii S, et al. Central motor reorganization in cerebral palsy patients with bilateral cerebral lesions. *Pediatr Res.* Apr 1999;45(4):559-567.
9. Leemans A, Jeurissen G, Sijbers J, Jones DK. ExploreDTI: a graphical toobox for processing, analyzing, and visualizing diffusion MR data. *17th Annual Meeting of Intl Soc Mag Reson Med*. Hawaii, USA2009:3537.
10. Rha DW, Chang WH, Kim J, Sim EG, Park ES. Comparing quantitative tractography metrics of motor and sensory pathways in children with periventricular leukomalacia and different levels of gross motor function. *Neuroradiology.* Jun 2012;54(6):615-621.
11. Hodge J, Goodyear B, Carlson H, Wei XC, Kirton A. Segmental Diffusion Properties of the Corticospinal Tract and Motor Outcome in Hemiparetic Children With Perinatal Stroke. *J Child Neurol.* May 2017;32(6):550-559.
12. Concha L, Livy DJ, Beaulieu C, Wheatley BM, Gross DW. In vivo diffusion tensor imaging and histopathology of the fimbria-fornix in temporal lobe epilepsy. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* Jan 20 2010;30(3):996-1002.
13. Ziemann U. Pharmaco-transcranial magnetic stimulation studies of motor excitability. *Handbook of clinical neurology.* 2013;116:387-397.
14. Noble JJ, Fry NR, Lewis AP, Keevil SF, Gough M, Shortland AP. Lower limb muscle volumes in bilateral spastic cerebral palsy. *Brain & development.* Apr 2014;36(4):294-300.
15. Mathewson MA, Lieber RL. Pathophysiology of muscle contractures in cerebral palsy. *Phys Med Rehabil Clin N Am.* Feb 2015;26(1):57-67.
16. Rose J, McGill KC. Neuromuscular activation and motor-unit firing characteristics in cerebral palsy. *Dev Med Child Neurol.* May 2005;47(5):329-336.
17. Hussain AW, Onambele GL, Williams AG, Morse CI. Muscle size, activation and coactivation in adults with cerebral palsy. *Muscle Nerve.* Apr 5 2014;49(1):76-83.
18. Reid SM, Dagia CD, Ditchfield MR, Carlin JB, Reddihough DS. Population-based studies of brain imaging patterns in cerebral palsy. *Developmental medicine and child neurology.* Mar 2014;56(3):222-232.
19. Condliffe EG, Jeffery DT, Emery DJ, Gorassini MA. Spinal inhibition and motor function in adults with spastic cerebral palsy. *J Physiol.* Feb 4 2016.