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**Research Articles: Systems/Circuits**

**Selective suppression of local interneuron circuits in human motor cortex contributes to movement preparation**

**Ricci Hannah, Sean E Cavanagh, Sara Tremblay, Sara Simeoni and John C Rothwell**

*Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, London, UK*

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Corresponding author: Ricci Hannah, UCL Institute of Neurology, London, WC1N 3BG, UK, email: [r.hannah@ucl.ac.uk](mailto:r.hannah@ucl.ac.uk)

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2 contributes to movement preparation

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4 **Running title:** Inhibition contributes to motor preparation

5

6

7 **Authors:** Ricci Hannah, Sean E Cavanagh, Sara Tremblay, Sara Simeoni & John C Rothwell

8

9 **Affiliations:** Sobell Department of Motor Neuroscience and Movement Disorders, UCL  
10 Institute of Neurology, London, UK

11

12 **Corresponding author:** Ricci Hannah, UCL Institute of Neurology, London, WC1N 3BG,  
13 UK, email: [r.hannah@ucl.ac.uk](mailto:r.hannah@ucl.ac.uk)

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31 **ABSTRACT**

32 Changes in neural activity occur in the motor cortex prior to movement, but the nature and  
33 purpose of this preparatory activity is unclear. To investigate this in the human (male and  
34 female) brain non-invasively, we used transcranial magnetic stimulation (TMS) to probe the  
35 excitability of distinct sets of excitatory inputs to corticospinal neurones during the warning  
36 period of various reaction time tasks. Using two separate methods (H-reflex conditioning and  
37 directional effects of TMS), we show that a specific set of excitatory inputs to corticospinal  
38 neurones are suppressed during motor preparation, whilst another set of inputs remain  
39 unaffected. To probe the behavioural relevance of this suppression, we examined whether the  
40 strength of the selective preparatory inhibition in each trial was related to reaction time.  
41 Surprisingly, the greater the amount of selective preparatory inhibition, the faster the reaction  
42 time was. This suggests that the inhibition of inputs to corticospinal neurones is not involved  
43 in preventing release of movement but may in fact facilitate rapid reactions. Thus, selective  
44 suppression of a specific set of motor cortical neurones may be a key aspect of successful  
45 movement preparation.

46

47 **Key words:** motor cortex; motor preparation; transcranial magnetic stimulation;  
48 corticospinal; inhibition

49

50

51 **SIGNIFICANCE STATEMENT**

52 Movement preparation evokes substantial activity in the motor cortex despite no apparent  
53 movement. One explanation for the lack of movement is that motor cortical output in this  
54 period is gated by an inhibitory mechanism. This notion was supported by previous non-  
55 invasive TMS studies of human motor cortex indicating a reduction of corticospinal  
56 excitability. On the contrary, our data supports the idea that there is a coordinated balance of  
57 activity upstream of the corticospinal output neurones. This includes a suppression of specific  
58 local circuits that supports, rather than inhibits, the rapid generation of prepared movements.  
59 Thus, the selective suppression of local circuits appears to be an essential part of successful  
60 movement preparation, instead of an external control mechanism.

61

62

63

64 **INTRODUCTION**

65 Neural activity in motor cortex occurs not only during execution of movement but also in the  
66 preparatory period prior to movement (Tanji and Evarts, 1976; Riehle and Requin, 1989;  
67 Kaufman et al., 2014). However, the nature of this preparatory activity is still unclear. A  
68 common assumption, dating back to classic studies (e.g. Tanji and Evarts, 1976), is that it  
69 represents a *subthreshold* version of the activity that accompanies movement. The  
70 preparatory activity is prevented from generating movement by a presumed “gating”  
71 mechanism.

72

73 Initial experiments with transcranial magnetic stimulation (TMS) appeared to be consistent  
74 with this idea. Rather than finding a subtle increase in excitability during the preparatory  
75 period as expected by the subthreshold hypothesis, many studies reported a paradoxical  
76 reduction (Hasbroucq et al., 1997; Touge et al., 1998; Duque and Ivry, 2009) which was  
77 originally interpreted as an inhibitory signal that prevents premature expression of pre-  
78 movement activity (Touge et al., 1998; Duque and Ivry, 2009). Effectively, corticospinal  
79 neurones were envisaged as being inhibited so that they could not respond to a gradually  
80 increasing amount of preparatory excitation. However, other explanations were also put  
81 forwards. Hasbroucq et al. (1997) thought inhibition might increase the signal-to-noise ratio  
82 in motor cortex by suppressing unwanted inputs that were irrelevant to the task. Others  
83 suggested that inhibition may be important in action selection for example, by preventing  
84 certain inputs from driving a muscle in an inappropriate way (Bestmann and Duque, 2016;  
85 Duque et al., 2017). However, neither of these explanations addresses the question of why  
86 preparatory activity in motor areas is not accompanied by a detectable change in motor  
87 output.

88

89 The *dynamical systems approach* provides an alternative way of viewing preparatory activity.  
90 It analyses the activity of populations of neurones without any assumptions about the  
91 particular role of individual cells. Individual neural firing rates are subsumed into a  
92 dynamically evolving population output. The approach highlights the fact that the activity of  
93 many single neurones is tuned differently in the preparatory and movement epochs meaning  
94 that the preparatory activity cannot be a subthreshold version of the movement command  
95 (Churchland et al., 2010; Kaufman et al., 2010; Elsayed et al., 2016). Instead, it is suggested  
96 that preparatory activity represents a separate, initial neural state that will evolve into the  
97 movement (Churchland et al., 2010; Kaufman et al., 2014; Elsayed et al., 2016). In this

98 scenario there is a balance of excitatory (and inhibitory) input to corticospinal neurones  
99 during the pre-movement period that facilitates preparation, but ultimately cancels out so that  
100 no movement occurs (Kaufman et al., 2014). The activity then evolves to produce a  
101 movement upon receipt of an imperative command (Kaufman et al., 2016). It is important to  
102 note that this population-based description of neural activity can in principle accommodate  
103 the idea that sub-populations behave according to a “signal-to-noise” or “action selection”  
104 hypothesis.

105

106 The purpose of the present experiments was to test the inhibitory gating version of the  
107 “subthreshold hypothesis”. At its simplest this predicts that an external inhibitory input  
108 prevents release of an evolving excitatory corticospinal command. If this is true then we  
109 predict that the corticospinal response to any facilitatory input ought to be suppressed. In  
110 contrast, if there is a patterned suppression of inputs, as predicted by the *dynamical systems*  
111 *hypothesis*, or the more nuanced versions of a subthreshold hypothesis, we may be able to  
112 demonstrate that only a proportion of these inputs are suppressed. A second prediction is that  
113 if inhibition prevents premature release of movement, then less preparatory inhibition might  
114 be expected to speed movement onset. Alternatively, if inhibition is an essential part of  
115 preparatory activity, then we might expect movements to take longer to evolve when  
116 preparatory inhibition fails to occur.

117

118 We used novel TMS methods to activate two different separate subsets of excitatory inputs  
119 that drive corticospinal neurones (D’Ostilio et al., 2016; Hannah and Rothwell, 2017). We  
120 could then examine whether each of these was suppressed to the same extent during  
121 movement preparation. In addition we could ask whether the degree of suppression  
122 correlated, in each individual, with the reaction time on that trial. Finally we tested whether  
123 movements requiring more explicit inhibition such as a Go/No Go task have similar effects  
124 on corticospinal inputs.

125

## 126 **MATERIALS AND METHODS**

### 127 ***Subjects***

128 A total of 59 right-handed healthy human volunteers (30 males; age  $24 \pm 1$  years, range 19-42  
129 years), who reported no contraindications to TMS (Rossi et al., 2011), provided written  
130 informed consent prior to participating in the study which was approved by University  
131 College London Ethics Committee.

132

133 ***Reaction time tasks***

134 Participants were seated 60 cm in front of coloured (red or green) light emitting diodes  
135 (LEDs) presented against a black background. They performed one of three different types of  
136 warned reaction time task: simple reaction time task (SRTT; Fig. 1A), choice reaction time  
137 task (CRTT; Fig. 1B) and Go/No Go task (Fig. 1C). In each of the tasks, a visual or auditory  
138 warning signal (WS) preceded a visual imperative signal (IS) by a fixed interval, and the  
139 latter signal cued a response. In experiment 1, participants were positioned with their right  
140 hand and wrist supported in an isometric dynamometer, with the shoulder in slight abduction,  
141 the elbow semi-flexed and the forearm semi-pronated. They responded by attempting to flex  
142 the wrist “as quickly as possible”. In experiments 2-5 participants were positioned with their  
143 hands resting palm down on a table surface and the fingertips of the index fingers resting on a  
144 load cell. They responded by attempting to flex the index finger against a load cell “as  
145 quickly as possible”. Prior to the main experimental blocks in each task, all participants  
146 completed two blocks without TMS: a practice block followed by another block which was  
147 used to estimate their mean baseline reaction time. Stimulus timings were controlled via  
148 Signal v5.10 software (RRID: SCR\_009601) connected to a data acquisition system  
149 (Power1401; CED, Cambridge, UK).

150

151 ***Surface electromyogram (EMG)***

152 In experiment 1, surface EMG electrodes (WhiteSensor 40713, Ambu®, Denmark) were  
153 placed 2 cm apart over the right flexor carpi radialis (FCR) muscle, with the ground  
154 positioned over the medial epicondyle of the humerus. In experiments 2-5 electrodes were  
155 placed in a belly-tendon arrangement over the first dorsal interosseous (FDI) muscle of the  
156 left and right hand. The ground electrode was over the styloid process of the radius. Signals  
157 were amplified with a gain of 1000 (Digitimer, UK), band-pass filtered (5 - 3000 Hz),  
158 digitised at 5 kHz (Power1401; CED, Cambridge, UK), and analysed with Signal v5.10  
159 software. EMG recordings enabled measurement of reaction times and H-reflexes or motor  
160 evoked potentials (MEPs).

161

162 ***Transcranial magnetic stimulation (TMS)***

163 In experiment 1, a standard TMS device connected to a figure-of-eight coil (Magstim 200<sup>2</sup>,  
164 The Magstim Co. Ltd., UK) was used to stimulate the FCR representation of the left primary  
165 motor cortex (M1). The coil was held tangentially on the scalp at an angle of 45° to the mid-

166 sagittal plane to induce a posterior-anterior (PA) current across the central sulcus (Fig. 1A).  
167 The motor hot spot was found by searching for the position where slightly suprathreshold PA  
168 currents produced the largest and most consistent MEPs in FCR at rest. The position was  
169 marked on a cap worn by the participants. Resting motor threshold with a PA current was  
170 defined as the lowest intensity to evoke an MEP of at least 0.05 mV in five of 10 consecutive  
171 trials while subjects were at rest. Thereafter, TMS was used to condition H-reflexes (van der  
172 Linden and Bruggeman, 1993; Niemann et al., 2016), rather than to elicit MEPs (see below).  
173 Stimulus intensity during the experiment was therefore below RMT (90% of RMT), i.e. at a  
174 level sufficient for evoking activity in the corticospinal tract, but producing only sub-  
175 threshold depolarisation of spinal motoneurons which can be detected by changes in H-  
176 reflex amplitude.

177

178 For experiments 2-5, MEPs in the dominant right FDI were evoked using a prototype  
179 controllable pulse parameter TMS device (cTMS3; Rogue Resolutions Ltd., UK) [see also  
180 (Peterchev *et al.*, 2014)], connected to a standard figure-of-eight coil (wing diameter 70 mm;  
181 The Magstim Co. Ltd., UK). The coil was held to induce either a PA current across the  
182 central sulcus (Fig. 1A), or an oppositely directed anterior-posterior (AP) current, whereby  
183 the position of the coil handle was reversed around the intersection of coil windings (Sakai  
184 *et al.*, 1997). PA and AP currents tend to activate the corticospinal tract via different sets of  
185 excitatory synaptic inputs (Di Lazzaro *et al.*, 2001) (see below). Here, we used different pulse  
186 durations for PA and AP current directions: long duration (120  $\mu$ s) pulses in the PA direction  
187 and short duration (30  $\mu$ s) pulses in the AP direction. It was recently shown that these  
188 combinations of current direction and pulse duration achieve the greatest distinction in the  
189 recruitment of these distinct synaptic inputs (D'Ostilio *et al.*, 2016; Hannah and Rothwell,  
190 2017).

191

192 The motor hot spot for the FDI was defined in a similar manner as for the FCR. The active  
193 motor threshold (AMT) with PA and AP currents was defined as the lowest intensity to evoke  
194 a discernible MEP in five of 10 consecutive trials while subjects maintained slight voluntary  
195 contraction (5-10% of maximum voluntary EMG amplitude during isometric finger flexion).  
196 Stimulation intensity during experiments 2-5 was set to that which produced a mean MEP  
197 amplitude of  $\sim$ 1mV ( $A_{1mV}$ ) during slight voluntary contraction (5-10% maximum voluntary  
198 EMG amplitude) for each of the PA and AP currents.

199



200 ***Peripheral nerve stimulation***

201 In experiment 1, square wave (1 ms pulses) were delivered to the median nerve just proximal  
202 to the elbow via cup electrodes (cathode proximal), which were connected to a constant-  
203 current stimulator (DS7A, Digitimer, UK). Initially, stimulus intensity was gradually  
204 increased in order to obtain maximal H-reflex and M-wave responses in the FCR. Then the  
205 stimulus intensity was set to evoke H-reflexes with an amplitude of >5% of maximal M-wave  
206 amplitude (Pierrot-Deseilligny and Burke, 2012). Unconditioned H-reflex amplitudes at the  
207 warning and imperative signals were  $17 \pm 3 \%$  and  $16 \pm 3 \%$  maximal M-wave amplitude,  
208 respectively.

209

210

211 ***Experimental design: Assessing excitatory synaptic inputs to corticospinal neurones with***  
212 ***H-reflex conditioning***

213 A single TMS pulse can activate separate excitatory synaptic inputs to the corticospinal  
214 neurones which arrive at different latencies and produce temporally distinct discharges in the  
215 pyramidal tract (I-waves) (Kaneko et al., 1996; Di Lazzaro et al., 1998). We employed a  
216 method of conditioning the H-reflex with TMS to test for selective suppression of the inputs  
217 responsible for early and late I-wave discharges (Niemann et al., 2016; van der Linden and  
218 Bruggeman, 1993) during the preparatory period of a simple reaction time task. The rationale  
219 for the paradigm is that TMS-evoked I-waves descending the corticospinal tract will produce  
220 excitatory post-synaptic potentials (EPSPs) at the spinal motoneurones. The TMS intensity is  
221 set below RMT so that the I-waves produce only subliminal depolarisation of the spinal  
222 motoneurones, which increases the probability of them firing in response to another  
223 excitatory input. Thus if a Ia afferent volley arrives at the same time or shortly after the TMS-  
224 evoked corticospinal volleys the resulting H-reflex will be facilitated compared to control H-  
225 reflexes where no TMS is delivered (van der Linden and Bruggeman, 1993; Niemann et al.,  
226 2016). Similarly, if the interval between the conditioning TMS stimulus and test H-reflex  
227 stimulus is altered so that the afferent volley reaches the spinal motoneurones before the TMS  
228 volleys arrive, the H-reflex will be unaffected since the efferent response will already have  
229 been generated. The interval between the conditioning TMS stimulus and the test H-reflex  
230 stimulus that produced coincident arrival of the corticospinal and afferent volleys at the  
231 spinal motoneurones, and thus facilitated the H-reflex, can be considered to be 0 ms (i.e.  
232 there is zero delay between their arrivals). Positive values for the afferent-corticospinal volley  
233 delay (e.g. +1 ms) then reflect delayed arrival of the afferent compared to corticospinal

234 volleys, whilst negative values (e.g. -1 ms) reflect the earlier arrival of the afferent volleys  
235 compared the corticospinal volleys.

236

237

238 It is important to note that the time of arrival of the early and late I-waves at the spinal  
239 motoneurons differs by several milliseconds (Day et al., 1989a; Sakai et al., 1997; Di  
240 Lazzaro et al., 1998), thus their contribution to the period of H-reflex facilitation can be  
241 partly dissociated by using different conditioning-test intervals. Facilitation at intervals  
242 resulting from near coincident arrival of the first corticospinal volleys (early I-waves) and  
243 afferent volleys (e.g. 0 and +1 ms) should correspond to EPSPs generated by those same  
244 early I-waves, whilst facilitation at longer intervals (e.g. +3, +4 and +5 ms) should receive an  
245 important contribution from EPSPs generated by later arriving I-waves. Consequently,  
246 changes in the level of H-reflex facilitation at different conditioning-test intervals throughout  
247 the pre-movement period (i.e. from the warning to the imperative signal) would, all other  
248 things being equal, be expected to reflect changes in I-wave composition. For example,  
249 greater facilitation at 0 ms and reduced facilitation at +4 ms would reflect an increased  
250 presence of early I-waves and a reduced presence of late I-waves, respectively. The  
251 dynamical systems approach posits that during movement preparation there is an overall  
252 balance of suppression and facilitation of inputs to corticospinal neurones. However, it seems  
253 unlikely that inhibition and facilitation would be equally distributed to early and late I-wave  
254 inputs. We therefore proposed that the early (early I-waves) and later period of H-reflex  
255 facilitation (late I-waves) would be differentially, and potentially oppositely, affected at the  
256 time of the imperative by comparison with the warning signal.

257

258 *Experiment 1: Simple reaction time task (SRTT) with H-reflex conditioning*

259 We studied reflexes in the FCR because it can be difficult to reliably evoke H-reflexes in  
260 hand muscles (Mazzocchio et al., 1995). Single median nerve stimulation pulses were used to  
261 evoke test H-reflexes in the right FCR muscle in separate trials at either the time of the  
262 warning or the imperative signal. In some trials, a conditioning stimulus consisting of  
263 subthreshold TMS of the left M1 was delivered at different times relative to the median nerve  
264 stimulus, from 3 ms prior to 5 ms after in 1 ms increments. Note that the earliest facilitation  
265 of the H-reflex, resulting from coincidental arrival of corticospinal and afferent volleys (0 ms  
266 as mentioned above), typically occurs when the TMS follows the peripheral nerve stimulus  
267 by 3 ms because of the faster conduction to the spinal motoneurons in the corticospinal

268 pathway compared to the peripheral afferent pathway. The experiment was performed at rest,  
269 i.e. no background muscle contraction, and with the application of near threshold PA  
270 currents, which we presumed would recruit a mixture of early and late I-waves (Day et al.,  
271 1989a; Di Lazzaro et al., 1998).

272

273 Eleven individuals participated in the experiment. The main experiment consisted of 2 blocks  
274 of 122 trials (244 trials in total) of right wrist flexor responses. Unconditioned control H-  
275 reflexes were evoked in the right FCR at the warning and at the imperative signal (20 and 20  
276 trials in total, respectively). 10 trials were included for each conditioning-test interval of the  
277 conditioned H-reflexes (180 trials in total), and 24 catch trials with no stimulation or  
278 imperative signal were also included. Trial order was randomised and the inter-trial interval  
279 was set to 8 s. Five minutes rest separated each block.

280

281 ***Experimental design: Assessing excitatory synaptic inputs to corticospinal neurones with***  
282 ***directional TMS***

283 Many factors can contribute to the time course of H-reflex facilitation produced by a  
284 subthreshold TMS pulse. The initial millisecond or so is probably dominated by the  
285 interaction between monosynaptic inputs from the fastest corticospinal and Ia afferent  
286 pathways. Thereafter, in addition to arrival of late corticospinal I-waves, there can be  
287 contributions from slower conducting fibres, Ib afferents activated by the H-reflex stimulus,  
288 presynaptic effects and indirect inputs from cortex coming via propriospinal, reticulospinal or  
289 even segmental interneuronal pathways. Changes in the contribution from any of these  
290 pathways in the preparation for movement could contribute to the results in experiment 1,  
291 although they would not easily account for the specificity of the timing. Thus, in order to  
292 provide more support for our hypothesis that these effects were likely to be related to  
293 suppression of late I-wave inputs we added a second series of experiments using directional  
294 effects of TMS.

295

296 These experiments investigated differential changes in the amplitudes of PA- and AP-evoked  
297 MEPs during movement preparation. PA and AP currents recruit different proportions of  
298 early and late I-waves, and thus comparing the relative changes in MEP amplitudes can help  
299 reveal differential changes in the activity of different I-waves (Hanajima et al., 1998; Hannah  
300 and Rothwell, 2017). Practically, this method also allowed us to more fully investigate the  
301 time-course of changes in cortical excitability during movement preparation by including a

302 greater number of stimulus time points. In each experiment, single pulse TMS was delivered  
303 over the FDI representation of the left motor cortex in separate trials, and at various times, to  
304 evoke MEPs in the right FDI muscle.

305

306 Experiments 2-5 were performed with slight background muscle contraction, ensuring that  
307 MEPs could be evoked by low intensity stimulation. This was necessary because differences  
308 in MEP latencies between PA and AP currents are obscured at higher intensities since pulses  
309 then recruit a mixture of I-waves (Day et al., 1989a; Sakai et al., 1997; Di Lazzaro et al.,  
310 2001). Participants received intermittent verbal feedback regarding voluntary RMS EMG  
311 amplitude (target 5-10% maximum) to ensure they maintained a consistent level of voluntary  
312 muscle activity throughout the tasks by lightly flexing the index fingers against the load cell.  
313 Feedback was given in between trials in relation to the action that was required (increase or  
314 decrease activity) and the hand it related to (left, right, both), and only when activity was  
315 consistently outside the bounds for three or more consecutive trials.

316

317 *Experiment 2: Choice reaction time task (CRTT) with directional TMS*

318 Previous studies adopting a CRTT in which an uninformative WS precedes an informative IS  
319 reported a suppression of MEPs in all response-relevant muscles towards the time of the IS  
320 (Touge *et al.*, 1998; Duque and Ivry, 2009), for example, in both left and right hand muscles.  
321 The present experiment served two purposes. The first was to confirm the data from the  
322 previous experiment by showing that late I-waves (AP MEPs) in the eventual responding  
323 hand are suppressed more than early I-waves (PA MEPs) in the preparatory period. The  
324 second was to extend these results and ask whether the same is true in the other potential  
325 respondent muscle, i.e. the non-responding hand (Fig 1B).

326

327 Fifteen individuals participated in the experiment. The main experiment consisted of eight  
328 blocks, with TMS delivered in the four blocks with a PA current and four blocks with an AP  
329 current. The order of blocks alternated between PA and AP, and the first block was randomly  
330 assigned either PA or AP. Each block consisted of fifty trials: twenty-five each of left and  
331 right index cues. Each combination of response hand and TMS timing was repeated five  
332 times per block, and therefore twenty times over the course of four blocks each for PA and  
333 AP currents, resulting in 20 MEPs per time point for each current direction and response cue.  
334 The order of trials was pseudo-randomised across the ten different combinations of response

335 cue and TMS timing, and the inter-trial interval was set to  $5 \pm 0.5$  s. Five minutes rest  
336 separated each block.

337

338 *Experiment 3: Simple reaction time task (SRTT) with directional TMS*

339 Preparatory inhibition of MEPs has been reported in the responding effector during warned  
340 SRTTs towards the time of the imperative signal (Hasbroucq et al., 1997; Touge et al., 1998;  
341 Greenhouse et al., 2015). Surprisingly, preparatory inhibition of MEPs has also been reported  
342 in “response-irrelevant” muscles, for example, a homologous or non-homologous muscle on  
343 the contralateral side of the body that is not a response option (Greenhouse et al., 2015).  
344 Preparatory inhibition here, where it may be desirable to fully suppress the output neurones  
345 of the response-irrelevant muscle representation, might be enacted through a less selective  
346 mechanism, e.g. somatic inhibition of corticospinal output neurones that could resemble the  
347 sort of gating mechanism implied by the subthreshold hypothesis. This would be expected to  
348 suppress the response to all excitatory I-wave inputs, and might therefore affect PA and AP  
349 MEPs similarly. We compared preparatory motor inhibition in the absence of choice between  
350 response options, i.e. where there is only one response option, and when the muscle  
351 representation was or was not a potential response option.

352

353 Thirteen individuals participated in the experiment. The main experiment consisted of four  
354 blocks (Fig. 1C), two blocks with each hand and with TMS delivered in one block with a PA  
355 current and the other with an AP current. The order of blocks alternated between PA and AP.  
356 Each block consisted of only right or left index responses and participants were told prior to  
357 each block which hand they were required to respond with. Blocks consisted of one hundred  
358 and twenty trials. In two blocks MEPs were evoked in the right hand when it was the  
359 responding (response-relevant) hand, and in the other two blocks MEPs were evoked in the  
360 right hand when it was the non-responding (response-irrelevant) hand, i.e. when left hand  
361 response was required. In order to prevent anticipation of the IS and premature responses,  
362 catch trials (20 in total for PA and AP conditions) were included where a warning appeared  
363 but no imperative signal was presented and no TMS was delivered, and participants were  
364 instructed not to respond on these trials. This design resulted in 20 MEPs per time point for  
365 each current direction and response hand. The order of trials within each block was pseudo-  
366 randomised across the five different TMS timings, and the inter-trial interval was set to  $5 \pm$   
367  $0.5$  s. A two minute break was given after the first fifty trials of each block and five minutes

368 rest separated each block in order to prevent fatigue due to the sustained voluntary muscle  
369 contraction.

370

371 *Experiment 4: Go/No Go task with directional TMS*

372 Several studies have reported that during successful outright suppression of a response in  
373 reaction to a sudden Stop or No Go signal involves a broad “global” inhibition of response-  
374 relevant and –irrelevant muscle representations after the IS, at around the time when a  
375 volitional muscle activity would be otherwise have been expected (Hoshiyama et al., 1997;  
376 Badry et al., 2009; Greenhouse et al., 2015). We hypothesised that successful stopping in a  
377 Go/No Go task would involve direct (e.g. somatic inhibition) of corticospinal output neurones  
378 and be reflected by a similar suppression of both PA- and AP-evoked MEPs.

379

380 Twelve individuals participated in the experiment. The main experiment consisted of eight  
381 blocks (Fig. 1D), with TMS delivered in the four blocks with a PA current and four blocks  
382 with an AP current, the order of blocks alternating between PA and AP. Since any  
383 preparatory inhibition prior to the imperative might confound attempts to explore subsequent  
384 inhibition after this time, we attempted to minimise any preparatory inhibition by increasing  
385 the interval between the warning and imperative to 2 s (Touge et al., 1998). We also used an  
386 auditory warning in the present experiment in order to ensure that it was unambiguous and  
387 distinct from the two possible visual imperative signals.

388

389 In total there were 70 trials per block. Trials included: TMS alone trials delivered at the time  
390 of the WS, though without the presentation of the WS or IS (10 per block); Go trials with no  
391 TMS (10); Go with TMS at the IS (12), 35%<sub>RT</sub> (12) and 70%<sub>RT</sub> (12); as well as No Go trials  
392 with TMS at 35%<sub>RT</sub> (7) and 70%<sub>RT</sub> (7). Thus blocks consisted of 10 trials with TMS at the  
393 WS, serving as the baseline measure of corticospinal excitability, along with 46 Go trials and  
394 14 No Go trials which resulted in Go/No Go ratio of 3.3/1. Four blocks were performed for  
395 each TMS current direction to ensure an adequate number of MEPs at each time point for the  
396 No Go trials (24 each). The order of trials within each block was pseudo-randomised across  
397 the seven different types of trial, and the inter-trial interval was set to  $5 \pm 0.5$  s.

398

399 *Experiment 5: Relationship of reaction times and trial-by-trial variability in MEPs assessed*  
400 *with AP TMS*

401 Following on from the previous experiments, we wanted to test the validity of the assumption  
402 that the preparatory inhibition reflected a mechanism for preventing movement during  
403 preparation. We hypothesised that if individuals do employ such a mechanism then it should  
404 be observable on a trial-by-trial basis: trials with greater suppression of MEPs would be  
405 associated with extended reaction times. Supra-threshold TMS around the time of the  
406 imperative signal can potentially delay contralateral responses (Day et al., 1989b) and impair  
407 detection of EMG-derived reaction time because of the silent period following the MEP in a  
408 pre-activated muscles. We therefore employed a bilateral response version of the SRTT (Fig  
409 1A) so that reaction times on the side ipsilateral to the TMS (left hand) could be used as a  
410 surrogate of the actual reaction time on the contralateral (right hand) side (Schneider et al.,  
411 2004).

412 Eleven individuals participated in the experiment. They performed an initial familiarisation  
413 consisting of 20 trials without TMS, followed by a further 60 practice trials (55 response  
414 trials and 5 catch trials in total) in order to obtain stable reaction times. The main experiment  
415 consisted of three blocks of the SRTT with AP TMS delivered in each. Blocks consisted of  
416 one hundred and twelve trials (336 trials in total) of simultaneous right and left index  
417 responses. MEPs were evoked in the right hand at the time of the warning signal (120 trials in  
418 total) and at the imperative signal (120 trials in total), since the latter was most often  
419 associated with the greatest preparatory MEP suppression (experiments 2-3). Catch trials (36  
420 trials in total) and trials without TMS (60 trials in total) were included as before. Trial order  
421 was pseudo-randomised across the four different trial types, and the inter-trial interval was set  
422 to  $5 \pm 0.5$  s. A two minute break was given after the first sixty-six trials of each block and  
423 five minutes rest separated each block.

424

#### 425 *Data analysis*

426 EMG data were analysed offline using Signal v5.10. For experiment 1, two dependent  
427 variables were measured on a trial-by-trial basis and used to create a mean value for each  
428 time point (WS and IS) and conditioning-test interval: (i) H-reflex peak-to-peak amplitude;  
429 and (ii) reaction time measured from the onset of the IS to the onset of volitional muscle  
430 activity.

431

432 For experiments 2-5, four dependent variables were measured on a trial-by-trial basis and  
433 used to create a mean value for each response hand (responding versus non-responding,  
434 experiments 2 and 3), current direction, time point of TMS and trial type (Go and No Go,

435 experiment 3): (i) MEP peak-to-peak amplitude; (ii) MEP onset latency measured from the  
436 time of TMS pulse delivery to the onset of the MEP; (iii) voluntary RMS EMG amplitude  
437 over the 100 ms prior to the TMS pulse; and (iv) reaction time measured as above. The onset  
438 of volitional muscle activity was defined as an increase in the RMS EMG (5 ms time  
439 constant) amplitude that exceeded the pre-TMS RMS EMG (100 ms) by  $\geq 2$  SD for at least 10  
440 ms. The onset of MEPs was determined visually from the raw EMG traces (Day et al., 1989a;  
441 Hamada et al., 2013)(Day *et al.*, 1989a; Hamada *et al.*, 2013). MEP latencies were measured  
442 for both current directions and at all TMS time points for experiment 2 to verify that any  
443 differences between current directions persisted throughout the task. In experiments 3 and 4,  
444 MEP latencies were measured for each current direction only at the earliest TMS time point  
445 (WS). Measurement of the voluntary RMS EMG amplitude 100 ms prior to each TMS pulse  
446 enabled comparison of the level of volitional muscle activity across different current  
447 directions and TMS pulse timings, to ensure that any differences in the amplitudes of MEPs  
448 were not confounded by differences in volitional muscle activity.

449

450 In experiment 1, trials were included for analysis if they met the following criteria: (i) RT  
451 was  $>80$  ms and within 3 SD of the mean; and (ii) RMS EMG in the 100ms prior to the IS  
452 was within  $\pm 2$ SD of the mean for that block. For experiments 2-5, trials were included for  
453 further analysis if they met the following criteria: (i) RT was  $>80$  ms and within 3 SD of the  
454 mean; (ii) response was correct (e.g. left index response only for trials with left cues, or no  
455 response in No Go trials); (iii) voluntary RMS EMG prior to the TMS pulse was within  $\pm$   
456 2SD of the mean for that block. The average number of trials removed per individual in each  
457 experiment: 6%, experiment 1; 7%, experiment 2; 9%, experiment 3; 6%, experiment 4 (4%  
458 in Go trials versus 15% in No Go trials); and 22% of IS trials, experiment 4 leaving  $94 \pm 5$   
459 trials for analysis.

460

#### 461 ***Statistical analyses***

462 Data are reported as group mean  $\pm$  standard error of the mean (SEM). Repeated measures  
463 ANOVA (rmANOVA) was used to evaluate the majority of the data, with Bonferroni-  
464 corrected, repeated measures *t*-tests used to follow up significant main effects or interactions.  
465 *P* values  $< 0.05$  were considered significant. Where necessary, the Greenhouse-Geisser  
466 procedure was applied to correct for violations of sphericity in ANOVA.

467

468 *Experiment 1: Simple reaction time task (SRTT) with H-reflex conditioning*



469 Data were assessed to identify the first conditioning-test interval at the WS time point where  
470 the mean conditioned H-reflex amplitude exceeded the mean unconditioned H-reflex  
471 amplitude by at least 2SEM of all 20 unconditioned trials. Conditioning-test intervals were  
472 then re-aligned on an individual basis such that this interval (afferent-corticospinal volley  
473 delay) corresponded to 0 ms, reflecting presumed coincident arrival of the afferent and  
474 corticospinal volleys at the spinal motoneurons (i.e. zero delay between their arrivals) as  
475 described earlier. Because of the different onsets of facilitation across individuals, analyses  
476 were limited to the unconditioned response and conditioned responses at re-aligned intervals  
477 between -1 to +5 ms.

478

479 Two-way rmANOVA was used to determine the effects of time point (WS, IS) and afferent-  
480 corticospinal volley delay (unconditioned, -1, 0, 1, 2, 3, 4, 5) on absolute H-reflex amplitudes  
481 and RTs. For *post hoc* analyses assessing the effect of afferent-corticospinal volley delay on  
482 the H-reflex, *t*-tests were performed on absolute conditioned H-reflexes by comparing them  
483 to the unconditioned H-reflex at the same time point, which served as the baseline measure of  
484 spinal motoneurone excitability. When comparing H-reflexes across different stimulation  
485 time points for a given afferent-corticospinal volley delay, data at each delay were normalised  
486 at each time point by expressing the mean conditioned H-reflex amplitude relative to the  
487 mean unconditioned H-reflex amplitude. This controlled for potential differences in baseline  
488 H-reflex amplitude at the WS and IS. Paired *t*-tests were performed on the normalised data.

489

490 *Experiment 2: Choice reaction time task (CRTT) with directional TMS*

491 Three-way rmANOVA was used to determine the effects of hand (right hand responding,  
492 right hand non-responding), current direction (PA, AP) and time of TMS (WS, WP, IS,  
493 35%<sub>RT</sub>, 70%<sub>RT</sub>) on absolute MEP amplitudes, MEP latencies, voluntary RMS EMG  
494 amplitude and RTs. For *post hoc* analyses assessing effects of time point on MEPs within a  
495 particular response hand and current direction, *t*-tests were performed on absolute MEPs by  
496 comparing them to those at the WS, which served as the baseline measure of corticospinal  
497 excitability. When comparing current directions at each time point for a given hand, data at  
498 each time point were normalised by expressing the mean MEP size as a ratio relative to the  
499 mean MEP size at the WS, to control for potential differences in baseline MEP amplitude,  
500 and paired *t*-tests were performed on the normalised data.

501

502 *Experiment 3: Simple reaction time task (SRTT) with directional TMS*

503 Data were analysed in a similar manner as experiment 2, whereby three-way rmANOVA was  
504 used to determine the effects of hand (right hand responding, right hand non-responding),  
505 current direction (PA, AP) and time of TMS (WS, WP, IS, 35%<sub>RT</sub>, 70%<sub>RT</sub>) on absolute MEP  
506 amplitudes, voluntary RMS EMG amplitude and RTs. However, since MEP latencies were  
507 only measured at the WS time point, a two-way rmANOVA was used to determine the effects  
508 of hand (right hand responding, right hand non-responding) and current direction (PA, AP).

509

510 *Experiment 4: Go/No Go task with directional TMS*

511 We analysed the data in two stages. First we wanted to test for the presence of preparatory  
512 suppression of MEPs at the IS, and examine whether this was different for PA and AP current  
513 directions. Two-way rmANOVA was used to assess the effects of current direction (PA, AP)  
514 and time (WS, IS) on absolute MEP amplitudes. For the second analysis, we were  
515 particularly interested in whether the suppression of MEPs after the IS in the No Go  
516 condition was different between AP and PA currents. To minimise any bias introduced by  
517 potential preparatory suppression of MEPs at the IS, we chose to normalise the amplitude of  
518 MEPs at 35%<sub>RT</sub> and 70%<sub>RT</sub> to those at the IS, and did this for both Go and No Go trials.  
519 Three-way rmANOVA was used to examine the effects of trial type (Go, No Go), current  
520 direction (PA, AP) and time (35%<sub>RT</sub>, 70%<sub>RT</sub>) on normalised MEP amplitudes. For *post hoc*  
521 analyses assessing effects of time on MEPs within a trial type and current direction, *t*-tests  
522 were performed on absolute MEPs by comparing them to those at the IS. When comparing  
523 current directions at each time for a trial type, paired *t*-tests were performed on the  
524 normalised MEP amplitudes data. Voluntary RMS EMG data were analysed in the same  
525 manner as MEPs. MEPs latencies were only measured at the time of the WS, and thus a  
526 paired *t*-tests was performed to compare them for PA and AP currents. A two-way  
527 rmANOVA was used to evaluate the effects of current direction (PA, AP) and time (Go  
528 alone, IS, 35%<sub>RT</sub>, 70%<sub>RT</sub>) on RTs in Go trials.

529

530 *Experiment 5: Relationship of reaction times and trial-by-trial variability in MEPs assessed*  
531 *with AP TMS*

532 For each individual, right (responding) hand MEP amplitudes during IS trials and WS trials  
533 were first normalised to the EMG amplitude preceding the TMS pulse in each trial, to  
534 account for variations in background muscle activity. Normalised MEP amplitudes from IS  
535 trials were then each expressed as a percentage change relative to the average amplitude of  
536 normalised MEPs from the WS trials. Left hand reaction times from IS trials were ranked

537 within each individual, expressed at a percentage of the total number of trials and then binned  
538 according to each consecutive 10 percentile window (i.e. 0-10<sup>th</sup>, 10<sup>th</sup>-20<sup>th</sup>... 90<sup>th</sup>-100<sup>th</sup>, in  
539 which the 0-10<sup>th</sup> percentile would contain the fastest 10% of reaction times etc.). The  
540 corresponding average MEP amplitude changes from the right hand were plotted as a  
541 function of reaction time percentile bins, and Pearson bivariate correlations were used to  
542 assess the relationship between them at both the individual and group average level.

543

544

545

## 546 **RESULTS**

### 547 *Thresholds and baseline response amplitudes*

548 Resting motor threshold in experiment 1 was  $55 \pm 5$  % maximum stimulator output, such that  
549 the 90% RMT conditioning stimulus was  $50 \pm 5$  % maximum stimulator output. Motor  
550 thresholds measured at the start of experiments 2-5 and absolute MEP amplitudes measured  
551 at the control TMS time point (WS) in each experiment are shown in table 1. AP pulses  
552 required much greater stimulus intensities than PA currents (all  $P < 0.001$ ). This was to be  
553 expected given: (i) thresholds are greater for AP pulses even when similar pulse durations are  
554 applied (D'Ostilio et al., 2016; Hannah and Rothwell, 2017); and (ii) the strength-duration  
555 behaviours of PA- and AP-sensitive inputs are different (D'Ostilio et al., 2016). The level of  
556 background muscle activity, quantified as the root mean square amplitude, was typically  
557  $\sim 0.05$  mV during experiments 2-5.

558

### 559 *Experiment 1: Simple reaction time task (SRTT) with H-reflex conditioning*

#### 560 *H-reflex amplitude*

561 At afferent-corticospinal volley delays corresponding to the earliest facilitation of the H-  
562 reflex by TMS, there was no change in the level of facilitation during the warning period.  
563 However, at delays corresponding to the later periods of H-reflex facilitation, there was a  
564 decrease in the level of facilitation during the warning period (Fig. 2).

565

566 There was no difference in the amplitude of the unconditioned H-reflex at the time of the WS  
567 compared to that at the IS ( $1.10 \pm 0.41$  versus  $1.03 \pm 0.36$  mV;  $t_{[10]} = 1.382$ ,  $P = 0.197$ ). The  
568 statistics showed a significant time  $\times$  afferent-corticospinal volley delay interaction ( $F_{[7,70]} =$   
569  $5.881$ ,  $P < 0.001$ ). Subsequent paired  $t$ -tests revealed a smaller conditioned H-reflex  
570 amplitude for IS versus WS time point at a 4 ms delay, though comparisons at 2 and 3 ms

571 delays did not survive the Bonferroni correction. Comparison of conditioned H-reflex  
572 amplitudes with respect to unconditioned H-reflex amplitudes at each time point indicated  
573 that responses were significantly facilitated at 0 ms at both the WS and IS, and at 3 ms for the  
574 WS and 2 ms for the IS time points. The remaining intervals did not survive the Bonferroni  
575 correction.

576

#### 577 *Reaction time*

578 Reactions times for the unconditioned H-reflex condition were  $181 \pm 6$ ms and  $179 \pm 6$ ms  
579 when stimuli were delivered at the WS and IS, respectively. rmANOVA showed no main  
580 effect of time ( $F_{[1,10]} = 1.121$ ,  $P = 0.315$ ) or afferent-corticospinal volley delay ( $F_{[7,70]} =$   
581  $1.441$ ,  $P = 0.203$ ), and no time  $\times$  afferent-corticospinal volley delay interaction ( $F_{[3,228,32.284]} =$   
582  $1.037$ ,  $P = 0.393$ ).

583

584 Many descending and afferent pathways could potentially contribute to the time course of H-  
585 reflex facilitation produced by a subthreshold TMS pulse, and changes in any of their  
586 contributions could thus influence the results in experiment 1. We therefore attempted to  
587 verify that these results were specifically related to suppression of late I-wave inputs by  
588 adding a second series of experiments using the directional effects of TMS.

589

#### 590 ***Experiment 2: Choice reaction time task (CRTT) with directional TMS***

##### 591 *MEP amplitude*

592 MEPs evoked by AP pulses were suppressed to a greater extent than PA-evoked MEPs  
593 during the warning period of a choice reaction time task, both when the right hand was the  
594 eventual responding hand and non-responding hand (Fig. 3A and B). The facilitation of  
595 MEPs in the right hand immediately prior to movement was similar for PA and AP MEPs.  
596 This was supported by a significant hand  $\times$  current direction  $\times$  time interaction in the  
597 rmANOVA (Table 2). Subsequent paired *t*-tests for right hand responses revealed that AP-  
598 evoked MEPs, but not PA MEPs, were suppressed at the time of the IS and 35%<sub>RT</sub> compared  
599 to those at the WS, but both PA and AP MEPs were facilitated just prior to volitional EMG  
600 onset at 70%<sub>RT</sub> (Fig. 3A). Additionally, comparison of normalised MEP amplitudes indicated  
601 a greater suppression of AP MEPs compared to PA MEPs at the time of the IS (Fig. 3A).  
602 When the right hand was the non-responding hand, paired *t*-tests revealed that AP-evoked  
603 MEPs were suppressed at all time points compared to the WS, whereas PA MEPs were only  
604 suppressed at 70%<sub>RT</sub> (Fig. 3B). Furthermore, the suppression of AP-evoked MEPs

605 (normalised to WS) was greater than that of PA-evoked MEPs at the time of the IS and at  
606 70%<sub>RT</sub>.

607

#### 608 *MEP latency*

609 The latency of AP-evoked MEPs was greater than that of PA-evoked MEPs for right hand  
610 responding and non-responding trials at nearly all time points (Fig 3C and D). In the  
611 statistics, rmANOVA revealed an interaction of hand × current direction × time (Table 2).  
612 Subsequent paired *t*-tests suggested this was driven by the generally greater latency of AP  
613 versus PA MEPs except when evoked during right hand responses at 70%<sub>RT</sub> (Fig. 3C), where  
614 both AP and PA MEPs were strongly facilitated (Fig. 3A). This confirms we achieved  
615 selective recruitment of AP and PA inputs through the majority of the task, especially at the  
616 time when preparatory inhibition was observed.

617

#### 618 *Voluntary RMS EMG amplitude*

619 The voluntary RMS EMG amplitude in the right hand was generally consistent across current  
620 directions, right hand responding and non-responding trials, and time points (Fig 3A and B),  
621 as indicated by a general lack of main effects and interactions in the rmANOVA (Table 2).  
622 Although an interaction of hand × time was suggestive of a small decrease in Voluntary RMS  
623 EMG amplitude at 70%<sub>RT</sub> for right hand responding trials versus non-responding trials,  
624 irrespective of current direction, a paired *t*-test on the pooled EMG amplitudes of AP and PA  
625 conditions revealed no significant difference between responding and non-responding trials  
626 ( $P = 0.139$ ). Thus the differences observed between AP and PA pulses in MEP amplitudes  
627 and latencies are unlikely to have been confounded by potential differences in the level of  
628 voluntary muscle activity.

629

#### 630 *Reaction time*

631 As expected from previous work (Pascual-Leone et al., 1992), reactions times were shortened  
632 for right hand responding and non-responding trials (i.e. left hand responses), irrespective of  
633 current direction, when TMS was delivered around the time of the IS consistent with an effect  
634 of intersensory facilitation (Nickerson, 1973). Additionally, reaction times were increased for  
635 right hand responding trials when delivered at 70%<sub>RT</sub> (Fig. 6). This was supported by a  
636 significant interaction of hand × time in the rmANOVA (Table 2.). This may relate to the  
637 silent period that follows the MEP in contracting muscle (see also (Day et al., 1989b). There  
638 was no effect of current direction or any interactions with current direction. Follow-up paired

639 *t*-tests showed that, when collapsed across current directions, reaction times were shortened  
640 when TMS was delivered at the IS and 35%<sub>RT</sub> compared to at the WS for right hand  
641 responding trials (both  $P \leq 0.002$ ) and at the IS for non-responding trials ( $P < 0.001$ ), and  
642 lengthened when delivered at 70%<sub>RT</sub> during right hand responses ( $P = 0.001$ ; Fig 6).

643

### 644 ***Experiment 3: Simple reaction time task (SRTT) with directional TMS***

#### 645 *MEP amplitude*

646 The suppression of MEPs during the preparatory period of the simple reaction time task  
647 depended on which hand was responding: AP-evoked MEPs were preferentially suppressed  
648 when preparing a response with the right hand (Fig. 4A), whereas both PA and AP MEPs  
649 were similarly suppressed during the preparation of left hand responses (i.e. right hand was  
650 non-responding) (Fig. 4B). This was supported by the rmANOVA showing a significant hand  
651  $\times$  current direction  $\times$  time interaction (Table 2). Follow-up paired *t*-tests for right hand  
652 responses revealed that AP-evoked MEPs were suppressed at the time of the IS and 35%<sub>RT</sub>  
653 compared to those at the WS, and though there appeared to be a small suppression of PA  
654 MEPs the comparison did not survive the Bonferroni correction (Fig. 4A). At 70%<sub>RT</sub> both PA  
655 and AP MEPs were facilitated (Fig. 4A). Additionally, comparison of normalised MEP  
656 amplitudes indicated a greater suppression of AP MEPs at the time of the IS and at 35%<sub>RT</sub>.  
657 This pattern of results is similar to those obtained for right hand responses in the choice  
658 reaction time task (experiment 2; Fig. 3A). When the right hand was non-responding hand,  
659 paired *t*-tests revealed that AP- and PA-evoked MEPs were suppressed at WP (PA MEPs  
660 only), IS, 35%<sub>RT</sub> and 70%<sub>RT</sub> by comparison with those evoked the time of the WS (Fig. 4B).  
661 There were no differences between PA and AP MEPs amplitudes at any time point.

662

#### 663 *MEP latency*

664 The latency of MEPs assessed at the time of the WS was greater for AP-evoked MEPs than  
665 PA-evoked MEPs for both right hand responding and non-responding trials, (Fig. 4C). This  
666 was supported by a main effect of current direction in the rmANOVA (Table 2), and again  
667 highlighted the selective recruitment of PA and AP inputs. There was also a main effect of  
668 hand (Table 2), indicating that MEP latencies were slightly longer (0.2 ms on average) in  
669 right hand responding versus non-responding trials.

670

#### 671 *Voluntary RMS EMG amplitude*

672 The Voluntary RMS EMG amplitude in the right hand was generally consistent across  
673 current directions, right hand responding and non-responding trials, and time points (Fig. 4A  
674 and B), as indicated by a lack of main effects or interactions in the rmANOVA (Table 2).

675

#### 676 *Reaction time*

677 Reaction times during the simple reaction time task were influenced both by the responding  
678 hand and the time of the TMS pulse (Fig. 6B), as indicated by a significant hand  $\times$  time  
679 interaction (Table 2). Follow-up paired *t*-tests showed that, when collapsed across current  
680 directions, reaction times were shortened when TMS was delivered at the IS and 35%<sub>RT</sub>  
681 compared to at the WS for right hand responding and non-responding trials (all  $P \leq 0.01$ ), and  
682 at 70%<sub>RT</sub> for non-responding trials ( $P < 0.01$ ; Fig 6B).

683

#### 684 ***Experiment 4: Go/No Go task with directional TMS***

##### 685 *MEP amplitude*

686 We first assessed whether a selective anticipatory suppression of AP MEPs was observed at  
687 the IS. There were no main effects of current direction or time; however, there was a  
688 significant current direction  $\times$  time interaction (Table 3). *Post hoc* paired *t*-tests revealed no  
689 difference in the absolute amplitude of PA and AP MEPs at WS (Table 1,  $P = 0.47$ ).  
690 However, MEPs were suppressed at the IS compared to WS for AP currents, but not PA  
691 currents (Fig. 5A). Furthermore, a paired *t*-test on the normalised (to WS) amplitude of MEPs  
692 at the IS further illustrated greater suppression of AP- compared with PA-evoked MEPs (Fig.  
693 5A). The suppression of AP MEPs here is less than half of that observed in the choice  
694 (experiment 2) and simple reaction time (experiment 3) tasks, and could be a consequence of  
695 the longer warning period used here to minimise preparatory inhibition and emphasise  
696 reactive inhibition or could reflect the different task requirements.

697

698 For the second analysis, we were interested in whether the suppression after the IS in the No  
699 Go condition was different between AP- and PA-evoked MEPs. The amplitude of MEPs at  
700 35%<sub>RT</sub> and 70%<sub>RT</sub> was therefore normalised to those at the IS. Results showed that AP and  
701 PA MEPs were suppressed to a similar extent at 70%<sub>RT</sub> in successful No Go trials and, as  
702 expected, were facilitated to a similar extent in the Go trials at 70%<sub>RT</sub> (Fig. 5B). Three-way  
703 rmANOVA revealed main effects of trial type and time, and a significant trial type  $\times$  time  
704 interaction (Table 3). There was no main effect of current direction or any interactions  
705 involving current direction (Table 3). *Post hoc* paired *t*-tests on the pooled AP and PA MEPs

706 indicated a significant suppression of MEPs at 70%<sub>RT</sub> compared to the IS for No Go trials ( $P$   
707 = 0.031), and a significant facilitation in Go trials ( $P = 0.011$ ).

708

#### 709 *MEP latency*

710 A paired  $t$ -test on MEP latencies at the WS showed them to be significantly greater for AP  
711 ( $23.3 \pm 0.5$  ms) versus PA MEPs ( $22.1 \pm 0.5$  ms) ( $P < 0.001$ ).

712

#### 713 *Voluntary RMS EMG amplitude*

714 The level of volitional muscle activity was analysed in the same manner as for MEP  
715 amplitudes, and it was found to be consistent across different current directions, trial types  
716 and time points (Fig 5A and B). First, two-way rmANOVA revealed no main effects of  
717 current direction or time, or an interaction of current direction  $\times$  time (Table 3). Subsequent  
718 three-way rmANOVA revealed no main effects of current direction, trial type or time, nor  
719 any interactions (Table 3).

720

#### 721 *Reaction time*

722 Reactions times were affected by the time at which TMS pulses were delivered (Fig 6C).  
723 Two-way rmANOVA showed a main effect of time, but no effect of current direction or  
724 interaction of current direction  $\times$  time (Table 3). Compared to the Go alone trials with no  
725 TMS, paired  $t$ -tests showed RTs were significantly shortened when TMS was delivered at the  
726 IS ( $P < 0.001$ ) and increased when delivered at 70%<sub>RT</sub> ( $P = 0.014$ ).

727

### 728 ***Experiment 5: Relationship of reaction times and trial-by-trial variability in MEPs*** 729 ***assessed with AP TMS***

#### 730 *MEP amplitude*

731 On average, MEPs in the right hand decreased by  $28 \pm 2\%$  at the IS compared to the WS ( $P <$   
732  $0.01$ ).

733

#### 734 *Correlation between reaction times and MEP suppression*

735 Greater preparatory suppression of AP-evoked MEPs at the IS was associated with slightly  
736 faster reaction times (Fig 7). This was supported by a significant correlation at the group level  
737 between reaction time percentile bin and average MEP amplitude change (Fig 7). Significant  
738 positive correlations were observed at the individual level in 6/11 participants, with no  
739 significant correlation being observed in the remaining 5 participants.



740

741 *Reaction time*

742 Reactions times were affected by the time at which TMS pulses were delivered (Fig 6D).  
743 Two-way rmANOVA showed a main effect of time ( $F_{[2,20]} = 35.34, P < 0.001$ ), but no effect  
744 of response hand ( $F_{[1,10]} = 0.00, P = 0.99$ ), indicating the reaction times were faster with TMS  
745 (WS and IS) compared to without (Go alone). There was a significant interaction of response  
746 hand  $\times$  time ( $F_{[2,20]} = 4.64, P = 0.022$ ) but *post hoc* tests revealed no differences between  
747 hands at any time (all  $P \geq 0.14$ ) and the mean difference at each time point was extremely  
748 small ( $\pm 3$ ms), so the meaningfulness of this is questionable.

749

750

751 **DISCUSSION**752 *Selective inhibition of synaptic inputs to corticospinal neurones during motor preparation*

753 These experiments made use of the fact that TMS can activate different sets of excitatory I-  
754 wave inputs to the corticospinal neurones. The novel finding is that, if the muscle is  
755 potentially involved in a forthcoming movement, late I-waves are selectively suppressed  
756 between the warning and imperative signal while early I-waves are unaffected. Experiment 1  
757 provided evidence for this using the H-reflex conditioning technique (van der Linden and  
758 Bruggeman, 1993; Niemann et al., 2016). At the time of the “go” cue, H-reflex facilitation  
759 was reduced at long afferent-corticospinal volley delays, which we interpret as reflecting a  
760 reduced contribution of late I-waves to the overall facilitation of spinal motoneurones. We  
761 then corroborated this by comparing the responses to PA and AP TMS using our new method  
762 (D’Ostilio et al., 2016; Hannah and Rothwell, 2017), and showed that AP MEPs were  
763 selectively inhibited whilst PA MEPs were largely unchanged. These effects were observed  
764 in a right/left choice reaction time task (experiment 2), a simple reaction task in which the  
765 right hand always responded (experiment 3) and Go/No Go task (experiment 4). The results  
766 suggest that when the timing of the imperative stimulus is highly predictable, selected inputs  
767 to the corticospinal neurones are suppressed rather than suppressing the whole of the output  
768 pathway. We conclude that the data rule out the simplest version of the subthreshold  
769 hypothesis that postulates that inhibition prevent premature release of excitatory inputs  
770 corticospinal neurones. They are more compatible with more nuanced hypotheses of the role  
771 of inhibition in which there is a change in the balance of excitatory input to corticospinal  
772 neurones, rather than a simple inhibitory gating of corticospinal output. When the imperative  
773 signal occurs the population activity evolves into a state where there is net facilitation of all

774 inputs to corticospinal neurones, which results in a similar facilitation of PA and AP MEPs  
775 near to the onset of movement (70%<sub>RT</sub>).

776

777 At first sight the results of our PA and AP TMS experiments might seem to contradict  
778 previous studies which reported that PA-evoked MEPs were suppressed during the warning  
779 period of reaction time tasks (Hasbroucq et al., 1997; Touge et al., 1998; Duque and Ivry,  
780 2009; Greenhouse et al., 2015). Our explanation for previous results is that PA currents are  
781 not very selective in their recruitment of particular I-wave inputs and thus PA MEPs,  
782 particularly when evoked using the high stimulus intensities needed at rest, must be generated  
783 by a mixture of both early and late I-wave activity. The effects seen in previous experiments  
784 were therefore likely due to a reduced contribution of late I-waves to the generation of PA  
785 MEPs. The results of our H-reflex conditioning experiment, performed at rest with  
786 subthreshold PA currents, are fully compatible with this explanation. In fact, there was a  
787 suggestion of weak suppression of PA MEPs when preparing for a right hand response in  
788 experiment 3 which also supports this idea. The trick in our experiments is that brief AP  
789 currents are quite specific in their recruitment of late I-waves (Hannah and Rothwell, 2017),  
790 and so the comparison with PA-evoked MEPs allows us to dissociate changes in the relative  
791 excitability of early and late input pathways. Our interpretation relies on the assumption that  
792 the neural subpopulations recruited by PA and AP currents are equally sensitive to the tonic  
793 muscle contraction employed to lower motor thresholds in the latter experiments. Whilst we  
794 did not measure resting and active motor thresholds here, our unpublished observations based  
795 on a previous data set (D'Ostilio et al., 2016) suggest that the PA-120 $\mu$ s and AP-30 $\mu$ s pulses  
796 show similar relative reductions in threshold from rest to muscle contraction (17% and 14%;  
797  $P = 0.14$ ). Thus it seems unlikely that the present results could be explained by differential  
798 effects of muscle activity on PA- and AP-sensitive neuronal subpopulations.

799

800 A potential concern when evaluating changes in MEP size is that the site of any changes  
801 could be located at a cortical or spinal level. There is evidence of concurrent changes in the  
802 spinal H-reflex as well as MEPs during the warning period of reaction time tasks (Duque *et*  
803 *al.*, 2010), implying that changes in spinal excitability could contribute to the smaller MEP.  
804 However, three features suggest that the selective inhibition of AP MEPs described here is of  
805 cortical origin. First, the main difference between current orientations is thought to be in how  
806 they activate corticospinal neurones in M1 (Day et al., 1989a; Hanajima et al., 1998; Di  
807 Lazzaro and Rothwell, 2014). Second, the latency differences between PA and AP currents

808 can be observed in the same motor unit (Day et al., 1989b; Sakai et al., 1997; Hanajima et al.,  
809 1998; Hannah and Rothwell, 2017), so that any inhibition at the spinal level would be  
810 expected to affect AP and PA MEPs in the same way. Finally, and in line with recent data  
811 (Lebon et al., 2016), we found no evidence that the unconditioned H-reflex was suppressed in  
812 the warning period during a SRTT, which argues against a major role of spinal mechanisms  
813 in the suppression of the MEP under the present conditions.

814

815 ***Broad inhibition of synaptic inputs to corticospinal neurones during outright response***  
816 ***suppression***

817 In contrast to the selective inhibition of AP MEPs, we also found evidence for suppression of  
818 both PA and AP MEPs in the right FDI when a response of the right index had to be  
819 completely suppressed or aborted. These effects were observed soon after the warning  
820 stimulus in blocks of the SRTT where only a left index response was being prepared and the  
821 right index was response-irrelevant (experiment 3, non-responding). Note that this contrasts  
822 with the selective suppression of AP MEPs in the non-responding hand during the CRTT.  
823 The similar suppression of PA and AP MEPs was also observed after the imperative signal  
824 ( $70\%_{RT}$ ) in trials where the right index is response-relevant but the No Go signal indicated  
825 that initiation of a prepared response of the right index had to be stopped (experiment 4). This  
826 suggests that when the situation demands that a response must be suppressed, whether it is  
827 known in advance or not of the imperative, there is a broad suppression of corticospinal  
828 output that affects response-relevant and –irrelevant muscle representations, as well as early  
829 and late I-wave inputs in both output zones.

830

831 It perhaps seems surprising that there was preparatory inhibition of the right FDI in a task that  
832 only involved a response of left index (experiment 2, non-responding). The most likely  
833 explanation is that in the present experiments participants had to maintain a slight  
834 background contraction of both left and right FDI muscles (in order to lower the threshold for  
835 stimulation) and so the right FDI was still relevant for the task. Inhibition in this case might  
836 prevent potential mirror movements in the right index when preparing a response with the left  
837 index (Duque et al., 2005). Alternatively, Greenhouse et al. recently suggested that broad  
838 suppression of the motor system was general feature of the response preparation process that  
839 helped resolve “competition resolution” by reducing noise to enhance signal processing and  
840 in turn enhance the gain of a selected response (Greenhouse et al., 2015). This argument  
841 cannot fully explain our results, however, since we saw a differential regulation of PA and

842 AP MEPs depending on whether the right index was response-relevant or –irrelevant  
843 (experiment 3, responding versus non-responding).

844

845 The contrast between targeted inhibition of specific inputs to corticospinal neurones and  
846 broader inhibition of both input pathways was illustrated particularly well in the Go/No Go  
847 task (experiment 4). Selective inhibition of AP MEPs at the time of the imperative signal was  
848 replaced by inhibition of both PA and AP MEPs after the IS during successful response  
849 cancellation in No Go trials. The less selective inhibition when completely suppressing a  
850 response might be suggestive of somatic inhibition of the corticospinal neurones.

851

### 852 ***Functional significance of motor cortex inhibition***

853 The results of experiment 5 demonstrated a relationship between the extent of preparatory  
854 inhibition of MEPs and response times. We found that greater preparatory suppression of the  
855 corticospinal pathway was associated with slightly *faster* reaction times. Importantly,  
856 experiment 5 was similar to experiment 3 in that it involved response preparation with the  
857 index fingers of both the left and right hands. In both cases, inhibition seems to target a  
858 specific set of inputs to the corticospinal neurones (late I-waves), rather than the corticospinal  
859 neurone cell body. These data seem to argue against the hypothesis that preparatory inhibition  
860 of M1 output neurones serves to brake the initiation of the movement being prepared (Touge  
861 et al., 1998; Duque and Ivry, 2009), since one might have expected preparatory inhibition to  
862 slow response times. However they would be highly compatible with the dynamical systems  
863 concept that coexistence of balanced excitation and inhibition is an essential part of  
864 successful movement preparation. They also fit well with recent data showing that in addition  
865 to neurones showing excitation, there is a specific population of layer II-III neurones in  
866 mouse motor cortex that are suppressed during the waiting period prior to movement  
867 (Hasegawa et al., 2017). In fact, the amount of suppression correlated well with reaction time.

868

869 Cancelling a movement altogether, as in the non-responding/No-Go trials of experiments 3  
870 and 4, seems to involve a different process to the coordinated change in activity patterns  
871 described above, and instead might rely on the direct suppression of M1 corticospinal output  
872 neurones. This would be akin to an inhibitory gate that prevents any build-up of excitatory  
873 activity from driving corticospinal neurones and thus causing unwanted movement.

874

875

876 ***Conclusions***

877 The experiments suggest that pre-movement suppression of MEPs is not caused by  
878 suppression of corticospinal output that prevents premature release of an excitatory motor  
879 command. Instead it seems to affect only specific inputs to the corticospinal system and is  
880 compatible with the idea that suppression of specific sets of cortical neurones is an essential  
881 part of successful movement preparation.

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887 **REFERENCES**

- 888 Badry R, Mima T, Aso T, Nakatsuka M, Abe M, Fathi D, Foly N, Nagiub H, Nagamine T,  
889 Fukuyama H (2009) Suppression of human cortico-motoneuronal excitability during the  
890 Stop-signal task. *Clin Neurophysiol* 120:1717–1723.
- 891 Bestmann S, Duque J (2016) Transcranial magnetic stimulation: Decomposing the process  
892 underlying action preparation. *Neurosci* 22:392–405.
- 893 Churchland MM, Cunningham JP, Kaufman MT, Ryu SI, Shenoy K V (2010) Cortical  
894 preparatory activity: representation of movement or first cog in a dynamical machine?  
895 *Neuron* 68:387–400.
- 896 D’Ostilio K, Goetz SM, Hannah R, Ciocca M, Chieffo R, Chen J-CA, Peterchev AV,  
897 Rothwell JC (2016) Effect of coil orientation on strength-duration time constant and I-  
898 wave activation with controllable pulse parameter transcranial magnetic stimulation.  
899 *Clin Neurophysiol* 127:675–683.
- 900 Day B, Dressler D, Maertens de Noordhout A, Marsden C, Nakashima K, Rothwell J,  
901 Thompson P (1989a) Electric and magnetic stimulation of human motor cortex: surface  
902 EMG and single motor unit responses. *J Physiol* 412:449–473.
- 903 Day B, Rothwell J, Thompson P, De Noordhout AM, Nakashima K, Shannon K, Marsden D  
904 (1989b) Delay in the execution of voluntary movement by electrical or magnetic brain  
905 stimulation in intact man: evidence for the storage of motor programs in the brain. *Brain*  
906 112:649–663.
- 907 Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali PA,  
908 Rothwell JC (1998) Comparison of descending volleys evoked by Transcranial magnetic  
909 and electric stimulation in conscious humans. *Electroencephalogr Clin Neurophysiol*  
910 109:397–401.
- 911 Di Lazzaro V, Oliviero A, Saturno E, Pilato F, Insola A, Mazzone P, Profice P, Tonali P,  
912 Rothwell JC (2001) The effect on corticospinal volleys of reversing the direction of  
913 current induced in the motor cortex by transcranial magnetic stimulation. *Exp Brain Res*  
914 138:268–273.
- 915 Di Lazzaro V, Rothwell JC (2014) Corticospinal activity evoked and modulated by non-  
916 invasive stimulation of the intact human motor cortex. *J Physiol* 592:4115–4128.
- 917 Duque J, Greenhouse I, Labruna L, Ivry RB (2017) Physiological Markers of Motor  
918 Inhibition during Human Behavior. *Trends Neurosci*.
- 919 Duque J, Ivry RB (2009) Role of corticospinal suppression during motor preparation. *Cereb*  
920 *Cortex* 19:2013–2024.

- 921 Duque J, Mazzocchio R, Dambrosia J, Murase N, Olivier E, Cohen LG (2005) Kinematically  
922 specific interhemispheric inhibition operating in the process of generation of a voluntary  
923 movement. *Cereb Cortex* 15:588–593.
- 924 Elsayed GF, Lara AH, Kaufman MT, Churchland MM, Cunningham JP (2016)  
925 Reorganization between preparatory and movement population responses in motor  
926 cortex. *Nat Commun* 7:13239.
- 927 Greenhouse I, Sias A, Labruna L, Ivry RB (2015) Nonspecific Inhibition of the Motor  
928 System during Response Preparation. *J Neurosci* 35:10675–10684.
- 929 Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC (2013) The role of interneuron  
930 networks in driving human motor cortical plasticity. *Cereb Cortex* 23:1593–1605.
- 931 Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, Kanazawa I (1998)  
932 Paired-pulse magnetic stimulation of the human motor cortex: differences among I  
933 waves. *J Physiol* 509 :607–618.
- 934 Hannah R, Rothwell JC (2017) Pulse Duration as Well as Current Direction Determines the  
935 Specificity of Transcranial Magnetic Stimulation of Motor Cortex during Contraction.  
936 *Brain Stimul* 10:106–115.
- 937 Hasbroucq T, Kaneko H, Akamatsu M, Possamai C-A (1997) Preparatory inhibition of  
938 cortico-spinal excitability: a transcranial magnetic stimulation study in man. *Cogn Brain*  
939 *Res* 5:185–192.
- 940 Hasegawa M, Majima K, Itokazu T, Maki T, Albrecht U-R, Castner N, Izumo M, Sohya K,  
941 Sato TK, Kamitani Y, Sato TR (2017) Selective Suppression of Local Circuits during  
942 Movement Preparation in the Mouse Motor Cortex. *Cell Rep* 18:2676–2686.
- 943 Hoshiyama M, Kakigi R, Koyama S, Takeshima Y, Watanabe S, Shimojo M (1997)  
944 Temporal changes of pyramidal tract activities after decision of movement: a study  
945 using transcranial magnetic stimulation of the motor cortex in humans.  
946 *Electroencephalogr Clin Neurophysiol* 104:255–261.
- 947 Kaneko K, Kawai S, Fuchigami Y, Morita H, Ofuji A (1996) The effect of current direction  
948 induced by transcranial magnetic stimulation on the corticospinal excitability in human  
949 brain. *Electroencephalogr Clin Neurophysiol* 101:478–482.
- 950 Kaufman MT, Churchland MM, Ryu SI, Shenoy K V (2014) Cortical activity in the null  
951 space: permitting preparation without movement. *Nat Neurosci* 17:440–448.
- 952 Kaufman MT, Churchland MM, Santhanam G, Yu BM, Afshar A, Ryu SI, Shenoy K V  
953 (2010) Roles of monkey premotor neuron classes in movement preparation and  
954 execution. *J Neurophysiol* 104:799–810.

- 955 Kaufman MT, Seely JS, Sussillo D, Ryu SI, Shenoy K V., Churchland MM (2016) The  
956 Largest Response Component in the Motor Cortex Reflects Movement Timing but Not  
957 Movement Type. *eNeuro* 3.
- 958 Lebon F, Greenhouse I, Labruna L, Vanderschelden B, Papaxanthis C, Ivry RB (2016)  
959 Influence of Delay Period Duration on Inhibitory Processes for Response Preparation.  
960 *Cereb Cortex* 26:2461–2470.
- 961 Mazzocchio R, Rothwell JC, Rossi a (1995) Distribution of Ia effects onto human hand  
962 muscle motoneurons as revealed using an H reflex technique. *J Physiol* 489 ( Pt 1:263–  
963 273.
- 964 Nickerson RS (1973) Intersensory facilitation of reaction time: Energy summation or  
965 preparation enhancement? *Psychol Rev* 80:489–509.
- 966 Niemann N, Wiegel P, Rothwell J, Leukel C (2016) The effect of subthreshold transcranial  
967 magnetic stimulation on the excitation of corticospinal volleys with different conduction  
968 times. *bioRxiv*.
- 969 Pascual-Leone A, Brasil-Neto JP, Valls-Sol J, Cohen LG, Hallett M (1992) Simple reaction  
970 time to focal transcranial magnetic stimulation comparison with reaction time to  
971 acoustic, visual and somatosensory stimuli. *Brain* 115:109–122.
- 972 Pierrot-Deseilligny E, Burke D (2012) General methodology. In: *The Circuitry of the Human*  
973 *Spinal Cord: Spinal and Corticospinal Mechanisms of Movement*. Cambridge:  
974 Cambridge University Press.
- 975 Riehle A, Requin J (1989) Monkey primary motor and premotor cortex: single-cell activity  
976 related to prior information about direction and extent of an intended movement. *J*  
977 *Neurophysiol* 61:534–549.
- 978 Rossi S, Hallett M, Rossini PM, Pascual-Leone A (2011) Screening questionnaire before  
979 TMS: An update. *Clin Neurophysiol* 122:1686.
- 980 Sakai K, Ugawa Y, Terao Y, Hanajima R, Furubayashi T, Kanazawa I (1997) Preferential  
981 activation of different I waves by transcranial magnetic stimulation with a figure-of-  
982 eight-shaped coil. *Exp Brain Res* 113:24–32.
- 983 Schneider C et al. (2004) Timing of cortical excitability changes during the reaction time of  
984 movements superimposed on tonic motor activity. *J Appl Physiol* 97:2220–2227.
- 985 Tanji J, Evarts E V (1976) Anticipatory activity of motor cortex neurons in relation to  
986 direction of an intended movement. *J Neurophysiol* 39:1062–1068.
- 987 Touge T, Taylor JL, Rothwell JC (1998) Reduced excitability of the cortico-spinal system  
988 during the warning period of a reaction time task. *Electroencephalogr Clin Neurophysiol*



989 109:489–495.

990 van der Linden C, Bruggeman R (1993) Multiple descending corticospinal volleys  
991 demonstrated by changes of the wrist flexor H-reflex to magnetic motor cortex  
992 stimulation in intact human subjects. *Muscle Nerve* 16:374–378.

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994

**Table 1.** Motor thresholds and baseline response amplitudes for experiments 2-5

	Exp. 2: CRTT (n=15)		Exp. 3: SRTT (n=13)		Exp. 4: Go / No Go (n=12)		Exp. 5: Bilateral SRTT (n = 11)
	PA	AP	PA	AP	PA	AP	AP
AMT (%MSO)	26 ± 1	78 ± 2	27 ± 1	76 ± 1	27 ± 2	74 ± 2	74 ± 2
A <sub>1mV</sub> (%MSO)	31 ± 1	89 ± 2	32 ± 1	91 ± 1	32 ± 2	85 ± 2	92 ± 2
A <sub>1mV</sub> /A MT (%)	117 ± 1	115 ± 1	123 ± 2	121 ± 2	119 ± 2	116 ± 1	125 ± 3
MEP amplitude at WS (mV)	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1 (R) 1.1 ± 0.1 (NR)	1.2 ± 0.1 (R) 1.2 ± 0.1 (NR)	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
AMT, active motor threshold; A <sub>1mV</sub> , active 1mV; AP, anterior-posterior; NR, non-responding; %MSO, % of maximum stimulator output; R, responding.							

996

997

**Table 2.** Results of rmANOVAs conducted for experiments 2 and 3.

	Experiment 2: CRTT ( $n=15$ )		Experiment 3: SRTT ( $n=13$ )	
	$F_{[DF,error]}$	P	$F_{[DF,error]}$	P
<i>MEP amplitude</i>				
Hand	1.114 <sub>[1,14]</sub>	0.178	16.551 <sub>[1,12]</sub>	0.002
Current direction	30.133 <sub>[1,14]</sub>	< 0.001	0.222 <sub>[1,12]</sub>	0.646
Time	21.389 <sub>[1.89,26.532]</sub>	< 0.001	17.337 <sub>[1.86, 22.274]</sub>	< 0.001
Hand × Current direction	2.417 <sub>[1,14]</sub>	0.142	0.256 <sub>[1,12]</sub>	0.616
Hand × Time	34.991 <sub>[4,56]</sub>	< 0.001	27.486 <sub>[2.041,24.487]</sub>	< 0.001
Current direction × Time	3.170 <sub>[4,56]</sub>	0.020	0.656 <sub>[2.11,25.275]</sub>	0.535
Hand × current direction × Time	4.609 <sub>[1.69,56]</sub>	0.025	2.930 <sub>[4,48]</sub>	0.015
<i>MEP latency</i>				
Hand	7.959 <sub>[1,14]</sub>	0.014	5.212 <sub>[1,12]</sub>	0.041
Current direction	51.152 <sub>[1,14]</sub>	< 0.001	41.485 <sub>[1,12]</sub>	<0.001
Time	9.723 <sub>[2.52,35.295]</sub>	< 0.001		
Hand × Current direction	1.513 <sub>[1,14]</sub>	0.239	1.706 <sub>[1,12]</sub>	0.216
Hand × Time	18.292 <sub>[4,56]</sub>	< 0.001		
Current direction × Time	1.131 <sub>[4,56]</sub>	0.351		
Hand × current direction × Time	3.126 <sub>[2.39,56]</sub>	0.049		
<i>Voluntary RMS EMG amplitude</i>				
Hand	4.325 <sub>[1,14]</sub>	0.056	3.483 <sub>[1,12]</sub>	0.087
Current direction	0.018 <sub>[1,14]</sub>	0.895	0.131 <sub>[1,12]</sub>	0.723
Time	0.325 <sub>[1.834,25.682]</sub>	0.059	2.596 <sub>[2.248,26.981]</sub>	0.087
Hand × Current direction	2.418 <sub>[1,14]</sub>	0.142	0.016 <sub>[1,12]</sub>	0.900
Hand × Time	4.512 <sub>[4,56]</sub>	0.026	0.782 <sub>[2.298,27.575]</sub>	0.483

Current direction × Time	1.757 <sub>[4,56]</sub>	0.150	0.778 <sub>[4,48]</sub>	0.545
Hand × current direction × Time	1.424 <sub>[4,56]</sub>	0.238	0.864 <sub>[2,32,27.838]</sub>	0.447
<i>Reaction time</i>				
Hand	4.727 <sub>[1,14]</sub>	0.047	4.593 <sub>[1,12]</sub>	0.053
Current direction	0.002 <sub>[1,14]</sub>	0.963	3.807 <sub>[1,12]</sub>	0.075
Time	22.292 <sub>[1.981,27.737]</sub>	<0.001	74.832 <sub>[4,48]</sub>	< 0.001
Hand × Current direction	0.047 <sub>[1,14]</sub>	0.831	0.389 <sub>[1,12]</sub>	0.545
Hand × Time	6.284 <sub>[4,56]</sub>	< 0.001	9.461 <sub>[4,48]</sub>	< 0.001
Current direction × Time	0.726 <sub>[4,56]</sub>	0.578	1.802 <sub>[4,48]</sub>	0.144
Hand × current direction × Time	0.660 <sub>[4,56]</sub>	0.622	1.216 <sub>[4,48]</sub>	0.317

998

<b>Table 3.</b> Results of rmANOVAs conducted for experiment 4.				
Experiment 4: Go/No Go ( <i>n</i> =12)				
WS versus IS (preparatory)			35% <sub>RT</sub> and 70% <sub>RT</sub> (after the IS)	
	<i>F</i> <sub>[DF,error]</sub>	P	<i>F</i> <sub>[DF,error]</sub>	P
<i>MEP amplitude</i>				
Trial type			29.750 <sub>[1,11]</sub>	< 0.001
Current direction	0.039 <sub>[1,11]</sub>	0.847	1.147 <sub>[1,11]</sub>	0.307
Time	2.805 <sub>[1,11]</sub>	0.122	16.925 <sub>[1,11]</sub>	0.002
Current direction × Time	8.05 <sub>[1,11]</sub>	0.016	0.157 <sub>[1,11]</sub>	0.700
Trial type × Time			27.276 <sub>[1,11]</sub>	< 0.001
Trial type × Current direction			0.102 <sub>[1,11]</sub>	0.755
Trial type × current direction × Time			0.810 <sub>[1,11]</sub>	0.387
<i>Voluntary RMS EMG</i>				

<i>amplitude</i>				
Trial type			1.071 <sub>[1,11]</sub>	0.323
Current direction	0.021 <sub>[1,11]</sub>	0.888	0.483 <sub>[1,11]</sub>	0.501
Time	0.057 <sub>[1,11]</sub>	0.816	0.291 <sub>[1,11]</sub>	0.600
Current direction × Time	0.045 <sub>[1,11]</sub>	0.836	0.049 <sub>[1,11]</sub>	0.829
Trial type × Time			0.035 <sub>[1,11]</sub>	0.856
Trial type × Current direction			0.088 <sub>[1,11]</sub>	0.772
Trial type × current direction × Time			1.577 <sub>[1,11]</sub>	0.235
<i>Reaction time</i>				
Current direction			0.214 <sub>[1,11]</sub>	0.653
Time			50.402 <sub>[3,33]</sub>	< 0.001
Current direction × Time			2.344 <sub>[3,33]</sub>	0.091

999

1000

1001 **FIGURE LEGENDS**

1002

1003 **Figure 1.** Reaction time tasks and stimulus timings. **(A)** For the SRTT in experiment 1,  
1004 participants performed the task with their right wrist, and median nerve stimulus (MNS) and  
1005 TMS stimulus timings were limited to warning signal (WS) and imperative signal (IS) time  
1006 points. **(B)** For the CRTT in experiment 2, a non-informative visual WS (left and right LEDs  
1007 lit for 150 ms) preceded a left or right IS (75 ms duration), which cued a response with either  
1008 left and right index, respectively. **(C)** In experiment 3, participants performed separate blocks  
1009 of the SRTT with their left and right index fingers. They received a visual WS (150 ms  
1010 duration) prior to a visual IS (75 ms duration). **(D)** For the Go/No Go task in experiment 4, an  
1011 auditory WS (500 Hz tone, 150 ms duration) preceded either a green (Go) or red (No Go)  
1012 visual stimulus (75 ms duration), which cued the execution of a right index response and  
1013 withholding of a response, respectively. Within each experiment stimuli were delivered at  
1014 one of several time points in a trial: at the WS, in the warning period (WP) 0.25 s after the  
1015 WS and before the IS (A and B), at the IS, and after the IS at 35% and 70% of the mean  
1016 baseline reaction time (35%<sub>RT</sub>, 70%<sub>RT</sub>). TMS was delivered with the coil positioned to induce  
1017 PA currents (see A) only in experiment 1, and both PA and AP (position coil handle rotated  
1018 180° around the intersection of coil windings) currents in experiments 2-4. Note that for trials  
1019 cueing a right hand response, MEPs were recorded from the (right) responding hand; and for  
1020 trials cueing a left hand response, MEPs were recorded from the (right) non-responding hand.  
1021 An example raw EMG trace is shown at the bottom to illustrate the MEP against the  
1022 background voluntary muscle activity during experiments 2-5.

1023

1024 **Figure 2.** H-reflexes conditioned with TMS during the simple reaction time task. The interval  
1025 between the conditioning TMS stimulus and the test H-reflex stimulus that produced  
1026 coincident arrival of the corticospinal and afferent volleys at the spinal motoneurons, and  
1027 thus facilitated the H-reflex, was considered to be 0 ms (i.e. the afferent-corticospinal volley  
1028 delay is zero). Positive values for the delay (e.g. +1 ms) then reflected delayed arrival of the  
1029 afferent compared to corticospinal volleys, whilst negative values (e.g. -1 ms) reflected the  
1030 earlier arrival of the afferent volleys compared the corticospinal volleys. During the simple  
1031 reaction time task, H-reflexes in the FCR muscle were facilitated to a lesser extent at the IS  
1032 than the WS specifically when the arrival of the afferent volleys at the spinal motoneurons  
1033 was delayed relative to the corticospinal volleys (4 ms). By contrast, H-reflexes were  
1034 facilitated to a similar extent at the IS and WS when the afferent and corticospinal volleys

1035 arrived coincidentally at the spinal motoneurons (0 ms).  $*P < 0.05$ , compared to  
1036 unconditioned (Unc.) H-reflex within each time point (WS and IS);  $++P < 0.01$ , IS versus  
1037 WS.

1038

1039 **Figure 3.** During the choice reaction time task, MEP amplitudes in the right FDI shown  
1040 normalised to the WS time point (coloured lines, left y-axis), were suppressed more for AP  
1041 currents than PA currents at the IS during right hand responding trials (**A**) and at the IS and  
1042 70%<sub>RT</sub> in right hand non-responding trials (**B**). The facilitation of MEPs in right hand  
1043 responding trials at 70%<sub>RT</sub> was similar for both current directions (**A**). Voluntary RMS EMG  
1044 (coloured bars, right y-axis) measured prior to the TMS pulses is shown normalised to values  
1045 at the WS, and was similar for PA and AP currents across different time points for right hand  
1046 responding (**A**) and non-responding trials (**B**). MEP latencies were longer for AP currents  
1047 compared with PA currents in both right hand responding (**C**) and non-responding (**D**) trials  
1048 at all time points except 70%<sub>RT</sub> in responding trials.  $**P < 0.01$ ,  $***P < 0.001$ , compared to  
1049 WS time point within each current direction;  $++P < 0.01$ ,  $+++P < 0.001$ , AP versus PA.

1050

1051 **Figure 4.** During the simple reaction time task, MEP amplitudes in the right FDI shown  
1052 normalised to the WS time point (coloured lines, left y-axis), were suppressed more for AP  
1053 currents than PA currents at the IS and 35%<sub>RT</sub> during right hand responding blocks (**A**). The  
1054 facilitation of MEPs in the same block at 70%<sub>RT</sub> was similar for both current directions.  
1055 However, for right hand non-responding blocks, normalised MEP amplitudes were  
1056 suppressed to a similar extent for AP and PA currents at all times following the WS (**B**).  
1057 Voluntary RMS EMG (coloured bars, right y-axis) measured prior to the TMS pulse is shown  
1058 normalised to values at the WS, and was similar for PA and AP currents across different time  
1059 points for right hand responding (**A**) and non-responding blocks (**B**). MEP latencies measured  
1060 at the WS were longer for AP currents compared with PA currents in both right hand  
1061 responding and non-responding blocks (**C**).  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ , compared  
1062 to WS time point within each current direction;  $+P < 0.05$ ,  $++P < 0.01$ ,  $+++P < 0.001$ , AP  
1063 versus PA.

1064

1065 **Figure 5.** During the Go/No Go task, MEP amplitudes in the right FDI, shown normalised to  
1066 the WS time point (coloured lines, left y-axis), were suppressed more for AP currents than PA  
1067 currents at the IS compared to the WS (**A**), indicating a selective anticipatory suppression in  
1068 response to the WS. However, during successful No Go trials of the Go/No Go task, MEP

1069 amplitudes normalised to the IS were suppressed to a similar extent for AP currents than PA  
1070 currents at 70%<sub>RT</sub> when compared to those at the IS (B), indicating a similar reactive  
1071 suppression in response to the No Go signal. The facilitation of MEPs in Go trials at 70%<sub>RT</sub>  
1072 was similar for both current directions. Voluntary RMS EMG measured prior to the TMS  
1073 pulse (coloured bars, right y-axis) is shown normalised to values at the WS (A) and IS (B),  
1074 and was similar for PA and AP currents across different time points for Go and No Go trials.  
1075 \* $P < 0.05$ , \*\* $P < 0.01$ , compared to IS time point within each current direction; + $P < 0.05$ ,  
1076 AP versus PA.

1077

1078 **Figure 6.** Mean EMG-determined reaction times shown for correct response trials and both  
1079 PA and AP current directions in CRTT (A), SRTT (B), Go/No Go (C) and bilateral SRTT  
1080 tasks (D). For the legends in (A, B), subscript R denotes right hand responding trials and  
1081 subscript NR denotes right hand non-responding trials (i.e. reaction times determined from  
1082 the left hand). For legend in (D), subscript R and L denotes right and left hand responses in  
1083 the same trial. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , compared to WS time point in (A, B)  
1084 and to Go alone (C, D).

1085

1086

1087 **Figure 7.** Correlation between mean MEP amplitude change and simple reaction time  
1088 arranged in consecutive 10 percentile bins (0-10<sup>th</sup>, 10-20<sup>th</sup> etc.).

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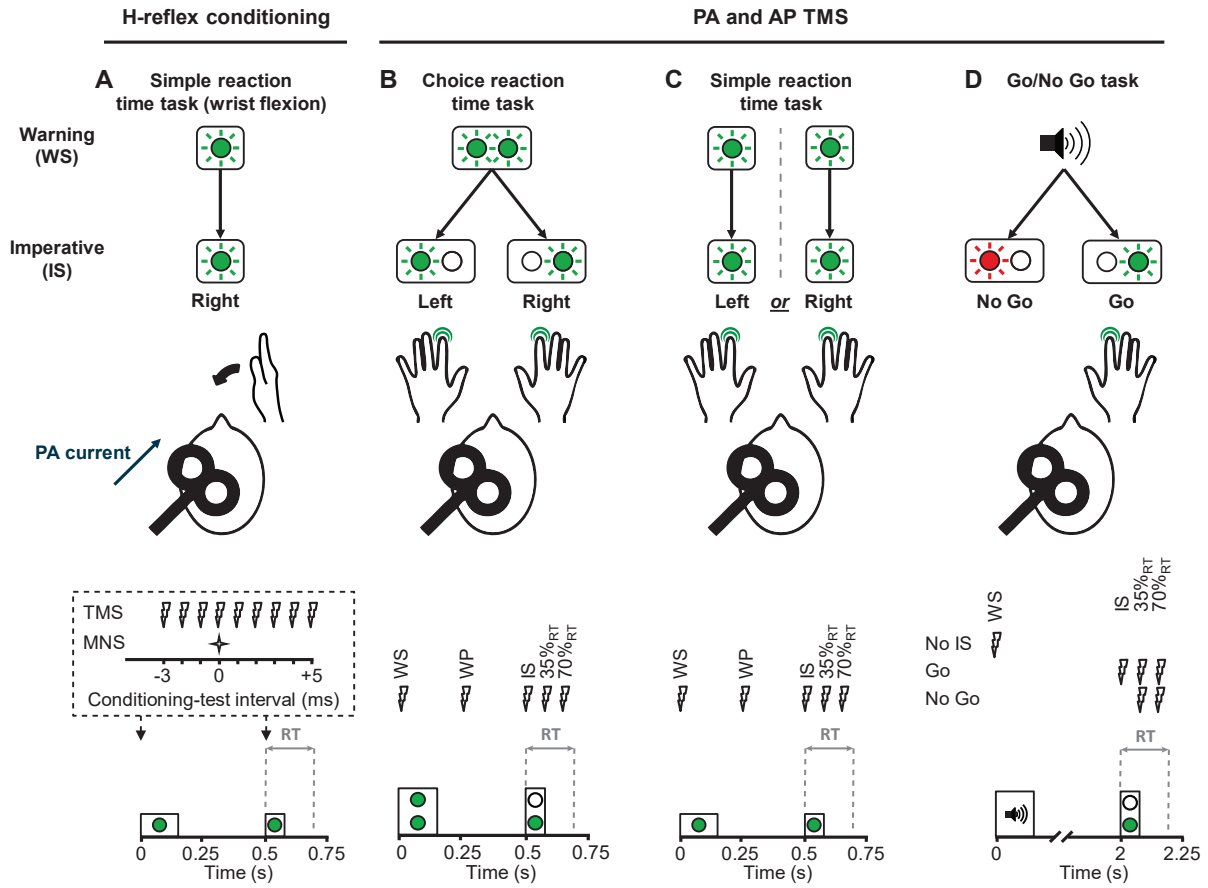
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Raw EMG trace illustrating an MEP against background activity



