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

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

Running title: Inhibition contributes to motor preparation

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ABSTRACT

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

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

SIGNIFICANCE STATEMENT

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

INTRODUCTION

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

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

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

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

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

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

MATERIALS AND METHODS

Subjects

A total of 59 right-handed healthy human volunteers (30 males; age 24 ± 1 years, range 19-42 years), who reported no contraindications to TMS (Rossi et al., 2011), provided written informed consent prior to participating in the study which was approved by University 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 200^2,
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-

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

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

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

Peripheral nerve stimulation

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

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

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

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

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

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

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

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

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

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

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

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

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

cue and TMS timing, and the inter-trial interval was set to 5 ± 0.5 s. Five minutes rest separated each block.

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

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

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

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

Experiment 4: Go/No Go task with directional TMS

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

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

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

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

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

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

Data analysis

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

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

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

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

Statistical analyses

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

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

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

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

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

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

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

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

Experiment 4: Go/No Go task with directional TMS

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

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

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

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

RESULTS

Thresholds and baseline response amplitudes

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

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

H-reflex amplitude

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

There was no difference in the amplitude of the unconditioned H-reflex at the time of the WS compared to that at the IS (1.10 ± 0.41 versus 1.03 ± 0. 36 mV; $t_{[10]} = 1.382$, $P = 0.197$). The statistics showed a significant time × afferent-corticospinal volley delay interaction ($F_{[7,70]} = 5.881$, $P < 0.001$). Subsequent paired *t*-tests revealed a smaller conditioned H-reflex amplitude for IS versus WS time point at a 4 ms delay, though comparisons at 2 and 3 ms

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

Reaction time

Reactions times for the unconditioned H-reflex condition were 181 ± 6ms and 179 ± 6ms when stimuli were delivered at the WS and IS, respectively. rmANOVA showed no main effect of time ($F_{[1,10]}$ = 1.121, P = 0.315) or afferent-corticospinal volley delay ($F_{[7,70]}$ = 1.441, P = 0.203), and no time × afferent-corticospinal volley delay interaction ($F_{[3.228,32.284]}$ = 1.037, P = 0.393).

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

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

MEP amplitude

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

(normalised to WS) was greater than that of PA-evoked MEPs at the time of the IS and at $70\%_{RT}$.

MEP latency

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

Voluntary RMS EMG amplitude

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

Reaction time

As expected from previous work (Pascual-Leone et al., 1992), reactions times were shortened for right hand responding and non-responding trials (i.e. left hand responses), irrespective of current direction, when TMS was delivered around the time of the IS consistent with an effect of intersensory facilitation (Nickerson, 1973). Additionally, reaction times were increased for right hand responding trials when delivered at $70\%_{RT}$ (Fig. 6). This was supported by a significant interaction of hand × time in the rmANOVA (Table 2.). This may relate to the silent period that follows the MEP in contracting muscle (see also (Day et al., 1989b). There 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%$_{RT}$ 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%$_{RT}$ 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 × current direction × 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%$_{RT}$
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%$_{RT}$ 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%$_{RT}$.
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%$_{RT}$ and 70%$_{RT}$ 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 × 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 $35\%_{RT}$
681 compared to at the WS for right hand responding and non-responding trials (all $P \leq 0.01$), and
682 at $70\%_{RT}$ 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 × 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 $35\%_{RT}$ and $70\%_{RT}$ was therefore normalised to those at the IS. Results showed that AP and
701 PA MEPs were suppressed to a similar extent at $70\%_{RT}$ in successful No Go trials and, as
702 expected, were facilitated to a similar extent in the Go trials at $70\%_{RT}$ (Fig. 5B). Three-way
703 rmANOVA revealed main effects of trial type and time, and a significant trial type × 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

indicated a significant suppression of MEPs at 70%$_{RT}$ compared to the IS for No Go trials (P = 0.031), and a significant facilitation in Go trials (P = 0.011).

MEP latency

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

Voluntary RMS EMG amplitude

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

Reaction time

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

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

MEP amplitude

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

Correlation between reaction times and MEP suppression

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

Reaction time

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

DISCUSSION

Selective inhibition of synaptic inputs to corticospinal neurones during motor preparation

These experiments made use of the fact that TMS can activate different sets of excitatory I-wave inputs to the corticospinal neurones. The novel finding is that, if the muscle is potentially involved in a forthcoming movement, late I-waves are selectively suppressed between the warning and imperative signal while early I-waves are unaffected. Experiment 1 provided evidence for this using the H-reflex conditioning technique (van der Linden and Bruggeman, 1993; Niemann et al., 2016). At the time of the "go" cue, H-reflex facilitation was reduced at long afferent-corticospinal volley delays, which we interpret as reflecting a reduced contribution of late I-waves to the overall facilitation of spinal motoneurones. We then corroborated this by comparing the responses to PA and AP TMS using our new method (D'Ostilio et al., 2016; Hannah and Rothwell, 2017), and showed that AP MEPs were selectively inhibited whilst PA MEPs were largely unchanged. These effects were observed in a right/left choice reaction time task (experiment 2), a simple reaction task in which the right hand always responded (experiment 3) and Go/No Go task (experiment 4). The results suggest that when the timing of the imperative stimulus is highly predictable, selected inputs to the corticospinal neurones are suppressed rather than suppressing the whole of the output pathway. We conclude that the data rule out the simplest version of the subthreshold hypothesis that postulates that inhibition prevent premature release of excitatory inputs corticospinal neurones. They are more compatible with more nuanced hypotheses of the role of inhibition in which there is a change in the balance of excitatory input to corticospinal neurones, rather than a simple inhibitory gating of corticospinal output. When the imperative 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%$_{RT}$).
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-120μs and AP-30μs 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

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

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

Functional significance of motor cortex inhibition

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

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

Conclusions

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

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995 **TABLES**

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_{1mV} (%MSO)	31 ± 1	89 ± 2	32 ± 1	91 ± 1	32 ± 2	85 ± 2	92 ± 2
A_{1mV}/AMT (%)	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_{1mV}, active 1mV; AP, anterior-posterior; NR, non-responding; %MSO, % of maximum stimulator output; R, responding.

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

Current direction × Time	$1.757_{[4,56]}$	0.150	$0.778_{[4,48]}$	0.545
Hand × current direction × Time	$1.424_{[4,56]}$	0.238	$0.864_{[2.32,27.838]}$	0.447
Reaction time				
Hand	$4.727_{[1,14]}$	0.047	$4.593_{[1,12]}$	0.053
Current direction	$0.002_{[1,14]}$	0.963	$3.807_{[1,12]}$	0.075
Time	$22.292_{[1.981,27.737]}$	<0.001	$74.832_{[4,48]}$	< 0.001
Hand × Current direction	$0.047_{[1,14]}$	0.831	$0.389_{[1,12]}$	0.545
Hand × Time	$6.284_{[4,56]}$	< 0.001	$9.461_{[4,48]}$	< 0.001
Current direction × Time	$0.726_{[4,56]}$	0.578	$1.802_{[4,48]}$	0.144
Hand × current direction × Time	$0.660_{[4,56]}$	0.622	$1.216_{[4,48]}$	0.317

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Table 3. Results of rmANOVAs conducted for experiment 4.

	Experiment 4: Go/No Go (n=12)			
	WS versus IS (preparatory)		35%$_{RT}$ and 70%$_{RT}$ (after the IS)	
	$F_{[DF,error]}$	P	$F_{[DF,error]}$	P
MEP amplitude				
Trial type			$29.750_{[1,11]}$	< 0.001
Current direction	$0.039_{[1,11]}$	0.847	$1.147_{[1,11]}$	0.307
Time	$2.805_{[1,11]}$	0.122	$16.925_{[1,11]}$	0.002
Current direction × Time	$8.05_{[1,11]}$	0.016	$0.157_{[1,11]}$	0.700
Trial type × Time			$27.276_{[1,11]}$	< 0.001
Trial type × Current direction			$0.102_{[1,11]}$	0.755
Trial type × current direction × Time			$0.810_{[1,11]}$	0.387
Voluntary RMS EMG				

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

FIGURE LEGENDS

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

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

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

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

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

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

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

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

Figure 7. Correlation between mean MEP amplitude change and simple reaction time arranged in consecutive 10 percentile bins ($0\text{-}10^{th}$, $10\text{-}20^{th}$ etc.).