Intracortical inhibition during volitional inhibition of prepared action

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Abstract

Volitional inhibition is the voluntary prevention of a prepared movement. Here we ask whether primary motor cortex (M1) is a site of convergence of cortical activity associated with movement preparation and volitional inhibition. Volitional inhibition was investigated by presenting a stop signal before execution of an anticipated response that requires a key lift to intercept a revolving dial. Motor evoked potentials (MEPs) were elicited in intrinsic hand muscles by transcranial magnetic stimulation (TMS), to assess corticomotor excitability and short interval intracortical inhibition (sICI) during task performance. The closer the stop cue was presented to the anticipated response, the harder it was for subjects to inhibit their response. Corticomotor pathway excitability was temporally modulated during volitional inhibition. Using subthreshold TMS, corticomotor excitability was reduced for ‘Stop’ trials relative to ‘Go’ trials from 140 ms after the cue. sICI was significantly greater for ‘Stop’ trials compared with ‘Go’ trials at a time that preceded the onset of muscle activity associated with the anticipated response. These results provide evidence that volitional inhibition is exerted at a cortical level and that inhibitory networks within M1 contribute to volitional inhibition of prepared action.

Keywords

Motor Cortex; Go/Nogo; Response inhibition; Stop task; Transcranial magnetic stimulation


Introduction

Primate research provides convincing evidence that primary motor cortex (M1) pyramidal cells begin to discharge prior to movement onset (Cheney and Fetz 1980; Evarts 1966, 1968; Porter and Lewis 1975; Thach 1975). For simple movements of the wrist, corticomotoneuronal firing begins up to 120 ms before the electromyography (EMG) burst onset in the target muscle and is maximal about 50 ms prior to EMG onset (Cheney and Fetz 1980). Similarly, studies using transcranial magnetic stimulation (TMS) to non-invasively investigate the corticomotoneuronal pathway in human subjects, have shown increased excitability of the agonist M1 representation in the 100 ms preceding EMG onset (Chen and Hallett 1999; Chen et al. 1998; Hashimoto et al. 2004; Leocani et al. 2000; MacKinnon and Rothwell 2000; McMillan et al. 2004). Furthermore, Reynolds and Ashby (1999) provided evidence that the increase in excitability prior to a voluntary contraction was preceded by a reduction in intracortical inhibition directed to the agonist muscle representation.

During movement preparation, a selective release of inhibition within M1 may mediate which muscle representations receive subsequent excitatory drive.

There are some situations where it is necessary to rapidly prevent a prepared movement from occurring. For example, in cricket or baseball it is often necessary to prevent striking at the approaching ball. The term volitional inhibition is used here to refer to the ability to inhibit prepared action. Converging evidence from primate (Sasaki et al. 1989), lesion (Aron et al. 2003b), and neuroimaging (Kawashima et al. 1996; Konishi et al. 1998; Rubia et al. 2003) studies show the right inferior frontal cortex (IFC) region of the prefrontal cortex (PFC) is fundamentally involved in volitional inhibition (for review see Aron and Poldrack 2005; Aron et al. 2004). The
PFC is postulated to countermand prepared movement via the basal ganglia (Aron and Poldrack (in press); Aron et al. 2004; Band and van Boxtel 1999). Given its prominent role in movement initiation, M1 is a likely site of convergence of cortical activity associated with movement preparation and volitional inhibition.

Stinear and Byblow (Stinear and Byblow 2004b) have recently reported a behavioural deficit in subjects with focal hand dystonia (FHD) when performing a volitional inhibition task. FHD subjects were less able to inhibit a prepared action than age-matched controls. When patients were able to successfully inhibit the anticipated response, they were more likely to show a small amplitude/duration agonist EMG burst in the absence of overt movement. The threshold for intracortical inhibition within M1 is elevated in FHD at rest (Stinear and Byblow 2004a) and the level of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) is reduced in M1 contralateral to the affected hand (Levy and Hallett 2002). Deficient cortical inhibition as a result of basal ganglia dysfunction has been put forward as a mechanism underlying dystonia (Hallett 1998) and it has been shown that in FHD there is impaired modulation of intracortical inhibition when engaged in functional tasks (Butefisch et al. 2005; Gilio et al. 2003; Stinear and Byblow 2004c). The observed volitional inhibition deficit in FHD may therefore be related to altered intracortical inhibitory function within M1.

Similarly, in patients with attention deficit hyperactivity disorder (ADHD), the ability to volitionally inhibit a motor response is impaired relative to controls (Aron et al. 2003a; Oosterlaan et al. 1998). ADHD is hypothesized to be related to dysfunction of right-sided prefrontal-striatal structures (Aron and Poldrack 2005; Casey et al. 1997; Castellanos et al. 1996; Rubia et al. 1999). Using magnetic resonance imaging (MRI), the volumes of the prefrontal cortex and basal ganglia structures of the right
hemisphere have been shown to be significantly smaller in ADHD boys than matched controls (Castellanos et al. 1996) and anatomical measures of the right prefrontal cortex and caudate nuclei are significantly correlated with tasks requiring volitional inhibition in children with ADHD but not controls (Casey et al. 1997). In addition, using functional MRI (FMRI), Rubia et al. (1999) observed less activation in the right inferior frontal cortex and caudate nucleus in ADHD adolescents compared with age matched controls during performance of a stop signal task. Within M1, intracortical inhibition is impaired in drug-naïve ADHD children relative to age matched controls (Moll et al. 2000). These studies suggest that in both FHD and ADHD, impaired volitional inhibition is associated with abnormal inhibitory function within M1.

Few studies have used TMS to investigate the excitability of the corticomotoneuronal pathway during volitional inhibition of planned action (Hoshiyama et al. 1997; Hoshiyama et al. 1996; Leocani et al. 2000; Sohn et al. 2002; Waldvogel et al. 2000; Yamanaka et al. 2002). These studies employed Go/Nogo paradigms that require a motor response to a ‘Go’ stimulus but for no response to be made to the ‘Nogo’ stimulus. Suppressed motor evoked potential (MEP) amplitude to single pulse TMS applied after the ‘Nogo’ stimulus has been interpreted as reflecting a strong active inhibition of the pyramidal tract (Hoshiyama et al. 1997; Hoshiyama et al. 1996; Leocani et al. 2000). However, single pulse TMS provides no direct evidence for the involvement of intracortical inhibitory circuits in volitional inhibition. Suppressed MEP amplitude could be the result of either increased inhibition or reduced excitation, or both. Paired-pulse stimulation techniques, used to investigate inhibitory function within M1, are required to implicate an increase in inhibition in the process by which a prepared movement is countermanded. Paired pulse techniques involve preceding a test stimulus (TS) suprathreshold for evoking
descending corticospinal volleys with a subthreshold conditioning stimulus (CS) (Kujirai et al. 1993). At short interstimulus intervals (1 – 5 ms), the addition of the CS reduces the MEP amplitude compared with that evoked by the TS alone. There is convincing evidence that this is a cortical phenomenon, provided that the CS used is subthreshold for evoking a MEP in active muscle (Di Lazzaro et al. 2003; Di Lazzaro et al. 1998; Hanajima et al. 1998; Kujirai et al. 1993; Ridding et al. 1995b). The current understanding is that when the interstimulus interval (ISI) is set to between 2 and 3 ms, the CS imposes an inhibitory effect via excitation of low threshold GABAergic inhibitory interneurons within M1 (Fisher et al. 2002; Ilic et al. 2002).

Only two studies have attempted to investigate intracortical inhibition during volitional inhibition (Sohn et al. 2002; Waldvogel et al. 2000). Both employed the Go/Nogo paradigm. Waldvogel et al. (2000) reported increased intracortical inhibition following ‘Nogo’ stimulus presentation in two subjects. In another investigation, Sohn et al. (2002) concluded that intracortical inhibition is a candidate mechanism underlying reduced corticomotor excitability during volitional inhibition. For there to be strong evidence of an involvement of GABAergic inhibitory networks during volitional inhibition, the increase in inhibition would need to be present before the onset of ‘Go’ related EMG activity (and account for nerve conduction time) in order to prevent its occurrence. In the study by Sohn and colleagues (2002), stimulation was delivered after the onset of the ‘Go’ response in 80% of trials. Thus, it remains to be elucidated whether intracortical inhibitory circuits within M1 are engaged during volitional inhibition and whether this occurs prior to the time when movement onset would have occurred.

An alternative paradigm to investigate volitional inhibition involves presenting the stop signal before the subject executes an anticipated response (Slater-Hammel
In this paradigm, subjects view a sweep dial that completes a single revolution in 1 second. The target response is to stop the sweep dial at a specified point in its revolution by releasing a key. On a proportion of trials, the sweep dial is unexpectedly stopped before the target, in which case the subject is required to prevent the anticipated key release from occurring. Young, healthy participants are able to volitionally inhibit a prepared response (to a level better than chance) when the sweep dial is stopped at least 166 ms prior to the target (Slater-Hammel 1960). Importantly, this paradigm enables the examination of volitional inhibition during preparation of an internally generated movement rather than inhibition in reaction to a ‘Nogo’ cue.

The aim of this study was to investigate the role of intracortical inhibitory circuits within M1 when a prepared action is terminated abruptly. This was achieved by recording MEPs in response to single- and paired-pulse TMS, while subjects were engaged in an anticipated response task that required volitional inhibition on a proportion of trials. It was hypothesized that the increase in corticomotor excitability that precedes movement would be absent on trials where volitional inhibition was required, depending on the time of stimulation. It was also hypothesized that excitability of intracortical inhibitory networks would be selectively increased when a prepared action was volitionally inhibited.

Method

Participants

Nineteen subjects with no neurological impairments participated in three experiments. Fourteen subjects participated in experiments 1 and 3 (5 male; aged 21 - 40 years) and 8 subjects participated in experiment 2 (4 male; aged 21 – 36). Fifteen subjects were right handed, and four were left handed (mean laterality quotients
91.8% and -86.2%) as determined from the Oldfield Handedness Inventory (Oldfield 1971). The right hand was tested for all subjects. Informed consent was obtained prior to their participation. The University of Auckland Human Participants Ethics Committee approved the experiment in accordance with the Declaration of Helsinki. Subjects who participated in experiments 1 and 3 were tested on separate days. Experiment 1 was behavioural whereas experiments 2 and 3 involved single- and paired-pulse TMS delivered during task execution.

**Preparation**

For all experiments, subjects were comfortably seated in front of a personal computer with the distal aspect of their right index finger positioned over the ‘0’ key on the number pad of the keyboard. The forearm was resting on a table, positioned midway between pronation and supination, and cushioned with a foam support. The keyboard was raised to eliminate radial or ulnar deviation of the wrist. The medial aspect (with respect to the anatomical position) of the index finger was used to depress the key (index finger adduction) (Fig 1a). Surface EMG recordings using pairs of 10 mm diameter Ag-AgCl Hydrosport electrodes (Physiomertix, N. Billerica, MA) were made from the first dorsal interosseus (FDI), abductor pollicis brevis (APB), and first volar (FV) muscles. The FDI was the primary muscle of interest as an index finger abduction agonist, APB is a surrounding thumb muscle that does not functionally contribute to task performance. The signals from FDI and APB were recorded using bipolar electrode configurations over each muscle belly while the signal from FV was recorded using a belly-to-tendon montage. The FV electrode configuration attempted to pick up the activity of deep index finger adductors using surface EMG, this set up was not optimal and results should be interpreted with
caution. An earth electrode was placed over the ulna styloid. The EMG signals were amplified using Grass P511AC EMG amplifiers (Grass Instrument Division, RI), bandpass filtered between 30 Hz and 1 kHz, and sampled at 1 kHz for experiment 1 and 2 kHz for experiments 2 and 3 using a 16 bit National Instruments A/D acquisition system. The EMG signal was displayed using custom LabVIEW software and stored to disk for offline analysis. For experiment 2, high gain auditory feedback of the FDI muscle was provided throughout the experiment.

**Transcranial Magnetic Stimulation**

Single-pulse TMS was applied using a figure-of-eight coil (7 cm diameter) connected to a Magstim200 unit (Magstim, Whitland, UK). Paired-pulse TMS was applied through the same coil via a Bistim unit, connected to two Magstim200 units (Magstim, Whitland, UK). Stimulation was applied to the motor cortex contralateral to the right hand and positioned to optimally stimulate the muscle representation of FDI. Using a slightly suprathreshold stimulus intensity the optimal position was ascertained by moving the coil in 1 cm increments as determined by a grid marked on a tight fitting cotton cap worn by the subject. The position eliciting MEPs of the largest amplitude was then marked to ensure consistent coil placement. The coil was held perpendicular to the central sulcus (~45° with respect to the vertex) and tangentially to the surface of the scalp thus inducing a current flow from posterior to anterior. This orientation is optimal for activating the corticospinal pathways transynaptically (Kaneko et al. 1996).

For experiment 2, the subject’s motor threshold (MT) was determined for both FDI and APB. MT was determined with the subject in the experimental position and depressing the ‘0’ key. MT was defined as the minimum stimulus intensity required to
evoke MEPs of ≥50 µV amplitude in at least 5 out of 10 stimuli (Rossini et al. 1994). The TS intensity was set at 90% of the subject’s FDI MT and increased by 1-2% of maximal stimulator output (MSO) if necessary to ensure a measurable response was evoked immediately preceding EMG onset. The subthreshold TS was then held constant for data collection.

For experiment 3, the subject’s active motor threshold (AMT) was determined. AMT was defined as the minimum stimulus intensity required to evoke MEPs of ≥100 µV amplitude in at least 5 out of 10 stimuli (Rossini et al. 1994) and was determined by having the subject maintain an isometric FDI contraction (~5% of maximal voluntary contraction). The TS intensity was determined while the subject carried out the primary ‘Go’ task. Stimulation occurred 600 ms before the target, prior to any assumed M1 movement preparation associated with the key release. During setting of the stimulation parameters, custom LabVIEW software was used to only accept trials where pre-trigger root mean square EMG (rmsEMG) in the 50 ms prior to the stimulus was less than twice the resting level of rmsEMG (i.e. less than 10 µV). An online average of accepted peak-to-peak MEP amplitudes was displayed. The stimulus intensity was initially set at a level evoking a MEP of about 1.5 mV in the FDI at rest. As expected, MEP amplitude was greater when the subject was engaged in the task and stimulation disrupted subjects’ ability to perform the task. The intensity was thus decreased to a level where performance was minimally affected and a consistent trial to trial MEP amplitude was observed, so as to be confident that the TS was on the linear portion of the stimulus-response curve. Short interval intracortical inhibition (sICI) was examined using an ISI of 2.5 ms, which has been shown to be optimal for examining excitability of GABAergic intracortical inhibitory circuits (Fisher et al. 2002; Roshan et al. 2003). The CS intensity was set to produce
50% inhibition of the non-conditioned MEP (CS50) in FDI to enable observation of both increases and decreases in sICI, thus eliminating the potential of a floor effect (Fisher et al. 2002; Stinear and Byblow 2003). To find CS50, the conditioning stimulus intensity was initially set to 90% AMT, for maximal inhibition (Orth et al. 2003; Ziemann et al. 1996), and was subsequently decreased in 1% increments of MSO under the same task and stimulation conditions mentioned above for setting the TS. Once the TS and CS50 were determined, the stimulator intensities were held constant for the remainder of the experiment.

Experiment 1

Procedure

The ability of the subject to inhibit a prepared action was tested using a custom-built LabVIEW program based on a paradigm first used by Slater-Hammel (1960). The subjects viewed a display similar to an analogue clock face with the exception that there were only ten digits, evenly spaced around the circumference (Fig 1a). Situated above the sweep-dial display (above the ‘10’) were two lights. Illumination of the first light indicated that the subject could depress the ‘0’ key, thus initiating the trial. The subject was instructed to use only as much force needed to maintain the key in its depressed state. Once the key was depressed, the second light became illuminated, and 1 s later the sweep indicator began its clockwise revolution. The sweep indicator completed a full revolution in 1 s. Subjects were instructed that lifting their finger off the key would stop the sweep indicator and that their primary task was to lift their finger from the key so that the indicator would stop at the ‘8’ (i.e. 800 ms after the beginning of the sweep). These trials are referred to as ‘Go’ trials. A
pop-up display provided the subject with instant feedback of their performance, indicating the time, in milliseconds, that they released the key relative to the ‘8’. It was emphasised to the subject that they were to perform this task as accurately as possible.

The subject observed a demonstration and then completed a series of practice trials to become familiar with the protocol. After several practice trials, the subject was informed that on a proportion of trials, the sweep indicator may stop on its own prior to reaching the ‘8’, and that for these trials they were not to lift their finger from the key. These trials are referred to as ‘Stop’ trials. If the sweep indicator is stopped late in the movement preparation period, the subject will sometimes lift their finger anyway. Subjects were instructed that this may occur and were informed to do their best to keep the key depressed but not to be too concerned if they did lift their finger. This was reinforced by the feedback given in the pop-up display upon completion of the trial. If the subject did not lift their finger from the key, the feedback stated ‘Response inhibited’. If the subject did lift their finger from the key, the feedback stated ‘The clock hand stopped too late for the response to be inhibited’. The series of practice trials was continued (with the sweep indicator stopping prior to the ‘8’ on a proportion of the trials) to enable the subject to become familiar with the additional instruction.

A preliminary block consisting of 30 ‘Go’ trials was performed (‘Go-only’). Subjects were informed that the sweep dial would not stop unexpectedly. This block served as additional practice at stopping the indicator at the ‘8’. At the end of the block, a pop-up display presented the mean lift time relative to the ‘8’, the standard deviation, and a histogram of lift times. This served as an indication of primary task performance and the subject was instructed that performance on subsequent blocks
was to be sustained at this level. The experiment consisted of 11 blocks, each consisting of 30 trials. Within each block, both ‘Go’ and ‘Stop’ trials were presented in a randomised order. Feedback, as above, was presented at the end of the block for performance on ‘Go’ trials only. There were 330 trials in total, 225 of which were ‘Go’ trials (~70% of trials) and 105 were ‘Stop’ trials (~30% of trials). The high proportion of ‘Go’ trials served to develop a predisposition to respond. For the ‘Stop’ trials, the sweep indicator stopped randomly 245, 230, 215, 200, 185, 170, and 155 ms before the target, with a total of 15 trials for each stop time presented throughout the experiment (Fig 2a). This enabled investigation of the ability to inhibit a pre-planned response in the temporal domain. Trials were self-paced, with a minimum inter-trial interval of 1.5 s. The EMG collection system was triggered by the start of each sweep of the dial and recorded from FDI, APB and FV for 1.2 s.

**Data Analysis**

Data were analyzed using custom software. Integrated EMG was calculated between 300 ms and 500 ms after the onset of each trial, reflecting the baseline level of activity. The EMG burst onsets in the FDI muscle were determined using an algorithm that detected where the rmsEMG first deviated by more than 5 standard deviations above the baseline (300 – 500 ms) window rmsEMG. Onsets determined by the algorithm were verified using a manual cursor for every trial. The experimenter was blinded to the type of trial (whether the trial required the subject to ‘Go’ or ‘Stop’ and at what time the sweep dial stopped). In addition to recording the latency of burst onset, the integrated EMG of all channels was calculated for 200 ms from burst onset. For trials where no EMG burst was detected, integrated EMG from all channels was recorded for 200 ms beginning 700 ms into the sweep. This was considered to
coincide with the time in the sweep where a burst would be expected had the sweep dial not stopped. The variables extracted from each trial were then aligned with the behavioural data (lift time and trial type) for further analysis. Trials were separated into ‘Go’ and ‘Stop’ trials, and ‘Stop’ trials were sorted by stop time.

For ‘Go’ trials, the mean burst onset and lift time were calculated for each subject along with the electromechanical delay (EMD), mean error, absolute error, and variable error. EMD refers to the time between EMG onset and the subsequent key lift. The mean error refers to the mean of the deviations from the specified lift time, the absolute error refers to the mean of the absolute deviations from the specified lift time, and the variable error refers to one standard deviation of the distribution of lift times.

For the ‘Stop’ trials, the probability of inadvertently responding, the probability of a partial response and the partial response size as a percentage of the mean ‘Go’ burst integrated EMG were determined as a function of stop time. A partial response is defined as a burst of agonist muscle EMG that is of short duration (50 – 100 ms) and that does not lead to an overt behavioural response i.e. the key lift is successfully inhibited. The probability of a partial response and partial response size were analysed for stop times where the probability of making a response was less than 0.5 (when subjects inhibited their responses more often than not) to ensure these variables were calculated from a sufficient number of responses. In addition, an estimate of stop signal reaction time (SSRT) was determined. For each subject, the stop time where the probability of responding was 50 % was determined by linear interpolation. This time was then subtracted from their mean ‘Go’ trial lift time to determine SSRT.
Experiment 2

Procedure

A practice block of 30 trials was performed. Within this block, ‘Go’ and ‘Stop’ trials were presented in a pseudorandom order. A pop-up display presented the mean lift time relative to the ‘8’, the standard deviation, and a histogram of lift times. This served as an indication of primary task performance and at this point the experimenter decided if further practice was required before beginning the experiment. Stimulation was thereafter delivered during performance of the task.

The experiment consisted of 12 blocks, each consisting of 30 trials. Within each block, both ‘Go’ and ‘Stop’ trials were presented in a randomised order. Feedback, as above, was presented at the end of the block for performance on ‘Go’ trials only. There were 360 trials in total, 240 of which were ‘Go’ trials (67% of trials) and 120 were ‘Stop’ trials (33% of trials). The high proportion of ‘Go’ trials served to develop a predisposition to respond. For the ‘Go’ trials, stimulation was delivered in the time leading up to the anticipated response (Fig 2b). There were 12 stimuli delivered 230, 210, 190, 170, 70, and 50 ms before the target and 24 stimuli delivered 150, 140, 130, 120, 110, 100 and 90 ms before the target. The increased number of stimuli for the times near the anticipated EMG onset served to offset the increased probability of discarding the trial due to muscle activity during or immediately preceding the MEP. For the ‘Stop’ trials, 20% served as catch trials where the sweep dial stopped either 300 or 150 ms before the target. For the remaining 80% of ‘Stop’ trials, the sweep indicator stopped 250 ms prior to the target. The catch trials ensured that subjects could not revert to preparing a response in reaction to the dial passing the 250 ms stop time. For ‘Stop’ trials, 12 stimuli were delivered 190, 170, 150, 130, 110,
90, 70, and 50 ms prior to the target. For all trials, the stimulation time was randomised. Trials were self-paced, with a minimum inter-trial interval of 1.5 s. The EMG collection system was triggered by the stimulator and EMG was recorded for 800 ms, 400 ms of which was pre-trigger.

Data Analysis

Trials were visually inspected for pre-MEP EMG and discarded if there was activity between the stimulus and the onset of the MEP. The experimenter was blinded to the time of stimulation. Peak-to-peak MEP amplitude within a 15 ms window beginning at the subject’s individual MEP latency and pre-trigger rmsEMG over the 50 ms immediately prior to the stimulus were determined using custom software. If the level of pre-trigger rmsEMG exceeded 10 µV for the FDI muscle (two times the resting rmsEMG) that trial was rejected from the analysis. Trials were sorted by condition (‘Go’ or ‘Stop’) and stimulation time. Mean MEP amplitudes were determined for each subject, condition, and stimulation time. To reduce between subject variability, the MEP amplitudes from each condition were normalized to the largest MEP amplitude of that muscle from ‘Go’ trials (a value of 1 was assigned and all other values expressed relative to this value). This permitted analysis of variance without a disproportionate influence from subjects who produced larger responses.

Experiment 3

Procedure

Twenty non-conditioned (NC) and 20 conditioned (C) trials were collected while subjects’ performed only the ‘Go’ task and stimulation was presented 600 ms
prior to the target (‘Go-only’ condition). Subsequently, TMS was delivered during task performance on half of the trials. For each trial, EMG was collected for 500 ms of which 200 ms were prior to the stimulus. The experiment consisted of 8 NC blocks and 8 C blocks presented in alternating order, each consisting 15 trials. Stimulated and non-stimulated trials were delivered in a randomised order. The type of trial (‘Go’ or ‘Stop’) was also randomised. There were 240 trials in total, with TMS delivered on 120 trials.

Stimuli were delivered at two times for ‘Go’ stimulation trials; 600 ms before the target (‘Early’ condition) and immediately prior to the mean EMG burst onset for each subject (‘Go’ condition). Even though stimulation for the ‘Early’ condition was given on what would eventually become a ‘Go’ trial, at the time of stimulation the subject was unaware of the trial type. The mean EMG burst onset for each subject was determined from analysing the experiment 1 ‘Go’ trials and this onset was categorised as early (> 90 ms), intermediate (90 – 80 ms), or late (< 80 ms). The stimulation time for each category was 120, 110, or 100 ms before the target for early, intermediate and late burst onsets respectively (Fig 2c). Thus, stimuli were delivered, on average, immediately prior to ‘Go’ descending volleys from M1 for each subject. There were 20 NC and 20 C trials collected for each stimulation time.

For the non-stimulated ‘Stop’ trials, the dial stopped 200 or 100 ms prior to the target, 20 times each, randomly over the course of the experiment. These trials served as catch trials. Based on research by Hoshiyama and colleagues (1997; 1996), changes in sICI were expected 150 ms after the stop cue. Thus, for the stimulated ‘Stop’ trials, the dial stopped 270, 260, or 250 ms before the target and stimuli were delivered 150 ms later, 120, 110, or 100 ms prior to the target (Fig 2c). Twenty NC and 20 C stimuli were delivered at these times (‘Stop’ condition).
There were 160 ‘Go’ trials in total (66%) and 80 ‘Stop’ trials (33%). Stimulation was delivered on 80 ‘Go’ trials and on 40 ‘Stop’ trials. ‘Stop’ and ‘Go’ trials were presented in a random order. Subjects did not know whether or not (or when) a stimulus would be delivered on any given trial. Feedback was presented at the end of each block. The mean ‘Go’ lift time for non-stimulated trials was used as an indicator of primary task performance.

**Data Analysis**

Trials were visually inspected for pre-MEP EMG and discarded if there was activity between the stimulus and the onset of the MEP. Pre-trigger rmsEMG over the 50 ms immediately prior to the stimulus and peak-to-peak MEP amplitudes were determined using custom software. Trials where pre-trigger rmsEMG exceeded 10 µV for the FDI muscle (two times the resting rmsEMG) were rejected from the analysis. Trials were sorted by trial type, stimulation time, and condition (Early, Go, and Stop). A trimmed mean procedure, established *a priori*, was used to enhance the validity of the data set and increase statistical power. This procedure is considered optimal for small data sets where outliers may skew the mean for some conditions but not others (Wilcox 2001). sICI was determined for each subject, muscle, and condition using the following formula:

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\text{sICI} (%) = [1 – (C/NC)] \times 100
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where C is the average conditioned MEP amplitude and NC is the average non-conditioned MEP amplitude. Thus, 100% inhibition reflects complete abolition of the non-conditioned response while 0% inhibition reflects no effect of the CS.

Statistical analysis
For experiment 1, the mean error, absolute error and variable error were compared between ‘Go-only’ and ‘Go’ using two-tailed paired t-tests.

For experiment 2, a linear regression and correlation coefficient were determined for mean lift time as a function of stimulation time. rmsEMG and MEP amplitude data were analysed using repeated measures analyses of variance (ANOVA). Stimulation times (70 and 50 ms prior to the target) confounded by voluntary muscle activity were not included in the analysis. The pre-trigger rmsEMG was analysed with a 2 x 2 x 6 ANOVA with within-group factors of Condition (Go, Stop), Muscle (FDI, APB) and Stimulation time (190, 170, 150, 130, 110, and 90 ms prior to the target). ‘Go’ trial mean MEP amplitude was analysed with a 2 x 11 ANOVA, with factors of Muscle (FDI, APB) and Stimulation time. Post-hoc analysis of log-transformed FDI MEP amplitude was carried out by comparing the first stimulation time to each subsequent stimulation time. To investigate the effect of condition within muscle, a 2 x 6 ANOVA was performed on the normalised MEP amplitudes for FDI and APB. The within-group factors were Condition (Go, Stop) and Stimulation time (190, 170, 150, 130, 110, and 90 ms prior to the target). Planned contrasts were performed to investigate whether normalised MEP amplitude was significantly less during ‘Stop’ trials than ‘Go’ for stimulation times at least 100 ms after the sweep dial stopped (150, 130, 110, and 90 ms prior to the target).

For experiment 3, FDI MEP amplitude and sICI were compared between ‘Go-only’ and ‘Early’ conditions using paired t-tests. rmsEMG, NC MEP amplitude and percent sICI were analysed using repeated measures ANOVA. rmsEMG was analysed using a 3 x 3 x 2 ANOVA with within-group factors of Condition (Early, Go, Stop), Muscle (FDI, APB, FV), and Stimulation type (NC,C). For NC MEP amplitude and % sICI, data were analysed with 3 x 3 ANOVA with factors of Condition (Early, Go,
Stop) and Muscle (FDI, APB, FV). An α level of 0.05 was used as a criterion for statistical significance for all analyses. Bonferroni corrected p-values are reported where post-hoc comparisons of significant effects or interactions have been made. All results are shown as group means ± standard errors (S.E.).

Results

Experiment 1

Task performance

The typical behavioural and EMG response to the anticipated response task is shown in Fig 3 (‘Go’, far right). An EMG burst in the agonist FDI and a small amount of motor overflow to APB are present when the depressed key is released 800 ms into the sweep dial revolution. As can be seen in Fig 1b, holding the key depressed required very low levels of muscle activation in all three muscles and there was an increase in activity after burst onset that was most pronounced in the agonist FDI muscle. Analysis of primary ‘Go’ task performance revealed that subjects tended to release the key slightly later when the possibility of the sweep dial stopping was introduced. This is evidenced by the significant difference in mean error between ‘Go-only’ and ‘Go’ task performance (Table 1). There was no difference in the variability of primary task performance or in the EMD between the two conditions.

Fig 3 illustrates trials where volitional inhibition of the anticipated response was required. When the dial stopped 245 ms prior to the target, the response was inhibited and no muscle activity was present in FDI. As the sweep dial was stopped closer to the target and thus later in movement preparation, partial responses appeared
in FDI on successfully inhibited trials. For ‘Stop’ trials, as expected, the probability of responding increased as the sweep dial was stopped closer to the target (Fig 4a). When the sweep dial stopped at least 200 ms prior to the target, subjects almost always inhibited the anticipated response. The probability of a partial response being present increased (Fig 4b) and the size of the partial responses increased (Fig 4c) as the sweep dial was stopped closer to the target. By determining each subject’s 50% probability of inhibition stop time and subtracting this value from their mean ‘Go’ trial lift time, SSRT was calculated to be 192 ± 3.8 ms (183 ms prior to the target).

Experiment 2

Stimulation parameters

MT was 40.8 ± 2.4% MSO for FDI and 42.1 ± 2.4% MSO for APB. The TS intensity used was, on average, 38 ± 2.4% MSO. Therefore the TS was 93.1 ± 1.0% of MT for FDI and 90.2 ± 2.2% of MT for APB. For the practice block, subjects released the key 3 ± 2.8 ms after the target with a variable error of 29.7 ± 2.8 ms.

Task performance

The probability of responding on catch trials was 0.034 ± 0.034 and 0.86 ± 0.05 when the dial stopped 300 and 150 ms prior to the target, respectively. This indicates that TMS did not affect subjects’ performance of the task. As expected, the number of trials discarded due to pre-MEP and pre-trigger rmsEMG increased as stimulation was delivered closer to the mean EMG burst onset time determined in experiment 1 (see Table 1). The percentage of discards on ‘Go’ trials was 3.1 ± 1.5% when stimulation was 570 ms into the sweep, and 57.6 ± 4.1% when stimulation was
delivered 710 ms into the sweep. The increase in discarded trials at later stimulation times was offset by presenting more stimuli at times closer to the target.

**Pre-trigger EMG**

The ANOVA of pre-trigger rmsEMG revealed no significant main effects or interactions. The mean pre-trigger rmsEMG collapsed over time for ‘Go’ trials was 6.0 ± 0.4 µV in FDI and 5.8 ± 0.7 µV in APB, and for ‘Stop’ trials was 6.1 ± 0.4 µV in FDI and 6.0 ± 0.8 µV in APB. Therefore, differences in corticomotor excitability cannot be explained by differences in pre-trigger EMG.

**MEP amplitude**

Representative EMG traces with MEPs from one subject are shown for the FDI muscle in Fig 5a. The ‘Go’ trial MEP amplitudes for FDI and APB are shown in Fig 6a. As expected, MEP amplitude in the time leading up to EMG burst onset increased substantially for the agonist FDI muscle but not in the surround muscle APB. The ANOVA comparing the ‘Go’ trial MEP amplitude across muscles revealed significant main effects of muscle \( F = 7.1, p = 0.03 \), time \( F = 7.0, p = 0.02 \) and a significant muscle by time interaction \( F = 6.3, p = 0.03 \). Post-hoc comparisons investigating the temporal profile of FDI MEP amplitude revealed significantly enhanced MEP amplitude for all stimulation times less than 170 ms from the target (Fig 6a).

The subthreshold stimulation affected the mean lift time for ‘Go’ trials. As shown in Fig 6b, the closer in time stimuli were delivered relative to the target, the quicker the mean lift time \( r = 0.93 \). In experiment 1, mean lift time was 809 ± 1.7 ms.
after the start of the sweep dial revolution. Thus, subthreshold TMS tended to speed up the response relative to trials where no stimulation was delivered (Fig 6b).

Of particular interest in experiment 2 were differences between the ‘Go’ and ‘Stop’ conditions in the temporal modulation of corticomotor excitability. Fig 7 illustrates normalised MEP amplitudes for ‘Go’ and ‘Stop’ trials as a function of stimulation time for FDI (Fig 7a) and APB (Fig 7b). The ANOVA of the normalised FDI MEP amplitude revealed significant main effects of condition \( (F = 48.2, p < 0.001) \), time \( (F = 19.8, p < 0.001) \) and a significant condition by time interaction \( (F = 22.4, p < 0.001) \). Planned contrasts revealed that corticomotor excitability was significantly less for ‘Stop’ trials relative to ‘Go’ trials when stimuli were delivered at least 140 ms after the sweep dial had stopped its revolution \( (p < 0.001) \).

For APB, the ANOVA of normalised MEP amplitude revealed a non significant main effect of condition, however, the main effect of time was significant \( (F = 8.4, p = 0.002) \) as was the condition by time interaction \( (F = 5.6, p = 0.01) \). Planned contrasts revealed that corticomotor excitability was significantly less for ‘Stop’ trials relative to ‘Go’ trials when stimuli were delivered 160 ms after the sweep dial had stopped its revolution \( (p < 0.05) \).

Experiment 3

**Stimulation parameters**

Mean AMT was 34.6 ± 1.3% MSO. The mean TS intensity used was 55.9 ± 2.1% MSO, which equates to 162.8 ± 5.3% AMT. The mean CS intensity used was 27.3 ± 1.1% MSO, which equates to 79.6 ± 3.1% AMT. Overall, 12.9 ± 2.3% of stimulation trials were discarded on the basis of excessive pretrigger rmsEMG or
preMEP EMG. The discard rate differed according to condition with $3.0 \pm 0.7\%$ of Early trials, $23.6 \pm 2.8\%$ of Go trials, and $12.0 \pm 2.3\%$ of Stop trials being discarded.

Task performance

For ‘Go’ trials where no stimulation was delivered behavioural variables did not differ significantly from experiment 1 (Table 1) in terms of average lift time ($805 \pm 0.2$ ms), mean error ($5.2 \pm 2.3$ ms), absolute error ($23.4 \pm 1.5$ ms), and variable error ($28.5 \pm 1.6$ ms). For ‘Stop’ trials where no stimulation was delivered, the probability of responding was $0.18 \pm 0.04$ and $0.99 \pm 0.005$ when the dial stopped 200 and 100 ms prior to the target, respectively. This indicates that the possibility of magnetic stimulation during the sweep did not affect the subjects’ primary or stop task performance.

Response uncertainty

Stimulation parameters were set in the ‘Go-only’ condition, 600 ms prior to the target, where the subject was certain that they would make an anticipated response. In the ‘Early’ condition, stimulation was also delivered 600 ms prior to the target, however, there was now a degree of uncertainty regarding whether an anticipated response would be required. To investigate the effect of uncertainty, FDI MEP amplitude and percent sICI were compared between the ‘Go-only’ and ‘Early’ conditions. Average NC MEP amplitude was $1.46 \pm 0.17$ mV and $1.53 \pm 0.19$ mV in the ‘Go-only’ and ‘Early’ conditions respectively, and a paired two-tailed t-test indicated no difference in MEP amplitude. There was however, a significant difference in percent inhibition between the two conditions ($p = 0.008$) with more sICI present in ‘Go-only’ ($59.8 \pm 3.7\%$) than ‘Early’ ($40.2 \pm 7.3\%$).
Pre-trigger EMG

The ANOVA of rmsEMG revealed significant main effects of condition ($F = 6.9, p = 0.01$) and muscle ($F = 18.5, p < 0.001$). There was no significant main effect of stimulation type (NC or C) and there were no significant interactions. Mean rmsEMG for ‘Early’, ‘Go’, and ‘Stop’ conditions respectively was $6.3 \pm 0.4$, $6.4 \pm 0.4$, $6.5 \pm 0.4$ µV in FDI, $5.1 \pm 0.5$, $5.4 \pm 0.6$, $5.4 \pm 0.6$ µV in APB, and $11.7 \pm 1.3$, $12.8 \pm 1.6$, $13.2 \pm 1.6$ µV in FV. Post-hoc comparisons revealed that the main effect of muscle was the consequence of significantly greater rmsEMG in FV than FDI ($p = 0.003$) and APB ($p = 0.001$). The rmsEMG was not significantly different between FDI and APB. The main effect of condition arose due to significantly greater rmsEMG for ‘Stop’ compared to ‘Early’ ($p = 0.02$). rmsEMG was not significantly different between ‘Go’ and ‘Stop’ ($p > 0.7$).

MEP amplitude

Representative APB EMG traces with MEPs from one subject are shown in Fig 5b. For NC MEP amplitude (Fig 8a), the ANOVA revealed significant main effects of condition ($F = 9.6, p = 0.001$) and muscle ($F = 8.2, p = 0.002$). The interaction between condition and muscle was also significant ($F = 10.0, p < 0.001$). The interaction arose from lower MEP amplitude in ‘Stop’ compared with ‘Go’ for FDI ($p = 0.0004$) but not for APB or FV.

Intracortical inhibition

For percent inhibition (Fig 8b), the ANOVA revealed a significant main effect of condition ($F = 8.5, p = 0.003$). The main effect of muscle was not significant nor
was the interaction between condition and muscle. Post-hoc comparisons revealed that sICI was increased for ‘Stop’ compared to ‘Go’ for both FDI ($p = 0.013$) and APB ($p = 0.022$) and there was a non-significant trend for sICI to be greater in APB for ‘Early’ than ‘Go’ ($p = 0.078$).

**Discussion**

Consistent with the original findings of Slater-Hammel (1960), the closer the sweep dial was stopped to the target, the harder it was for subjects to inhibit their response (Fig 4a). In addition, partial responses became more likely (Fig 4b) and increased in size (Fig 4c) with later sweep dial stop times. During task performance, volitional inhibition modulated corticomotor excitability. In experiment 2, subthreshold TMS demonstrated that corticomotor excitability was reduced for ‘Stop’ trials relative to ‘Go’ trials. This difference was significant when stimuli were delivered at least 140 ms after the sweep dial had stopped (Fig 7a). In experiment 3, using suprathreshold TMS, excitability was significantly suppressed in the agonist FDI for ‘Stop’ trials compared with ‘Go’ trials. In contrast, excitability did not modulate in APB, a nearby muscle not involved in performance of the task. Intracortical inhibition (sICI) was significantly greater for ‘Stop’ trials compared with ‘Go’ trials in both FDI and APB (Fig 8b). This indicates that volitional inhibition may be achieved by a focal reduction in excitability and a less specific increase in inhibition. The increase in GABAergic inhibition for ‘Stop’ trials was present at a time that preceded the mean EMG onset associated with the anticipated response. This result provides evidence that volitional inhibition is exerted via GABAergic inhibitory networks within M1.
Experiment 1 – A novel hypothesis of partial responses

Performance of the primary ‘Go’ task was influenced by the introduction of ‘Stop’ trials. With the introduction of trials requiring volitional inhibition, subjects responded significantly later. However, there was no effect on the variability of task performance (Table 1). This finding is consistent with the original report of Slater-Hammel (1960). Slowing of primary task performance has also been shown to occur in the Stop signal paradigm, increasing with increased probability of presenting a stop signal (Logan 1981), and is insensitive to monetary reward (McGarry and Franks 1997).

Several psychologists (De Jong et al. 1990; Logan et al. 1984; McGarry and Franks 1997; Osman et al. 1986) have conceptualized volitional inhibition as a ‘Horse Race’ between the independent processes of excitation and inhibition, which race to a hypothetical finish line or ‘point of no return’. De Jong et al. (1990) proposed that volitional inhibition occurs downstream from M1 and McGarry and Franks (1997) propose a phantom point of no return at the spinal motor neuron pool. An alternative argument regarding the location of the point of no return is that it occurs in the temporal domain within M1.

On successfully inhibited trials, EMG bursts were sometimes observed in the agonist FDI muscle in the absence of an overt behavioural response. These partial responses became more prevalent the closer the sweep dial was stopped relative to the target (Fig 4b). While partial responses have been reported previously (McGarry and Franks 1997; Stinear and Byblow 2004b), they remain undetected in purely behavioural analyses. Interestingly, partial responses have been shown to be more prevalent in patients with FHD than age matched controls (Stinear and Byblow
a neurological disorder characterized by impaired inhibition within M1 (Butefisch et al. 2005; Gilio et al. 2003; Ridding et al. 1995a; Stinear and Byblow 2004c) resulting from basal ganglia dysfunction (Hallett 1998; Mink 2003).

There is mounting evidence that volitional inhibition originates in the PFC (Aron et al. 2003b; Aron et al. 2004; Rubia et al. 2003; Sasaki et al. 1989) and subsequently involves the basal ganglia (Aron and Poldrack (in press); Aron et al. 2004; Band and van Boxtel 1999). High resolution FMRI has recently detected activation in the vicinity of the STN during performance of the stop signal paradigm (Aron and Poldrack (in press)). Furthermore, convincing evidence for basal ganglia involvement was recently provided by Kuhn et al. (2004) who demonstrated event related synchronisation within the subthalamic nucleus during ‘Nogo’ trials that preceded ‘Go’ trial movement onset. Focal disinhibition of the agonist M1 muscle representation via the direct pathway through the basal ganglia (Mink 1996; Nambu 2004; Nambu et al. 2002) could potentially explain the partial response phenomenon. Alternatively, partial responses may arise from an inhibitory signal generated in the inferior frontal cortex (Aron et al. 2004; Rubia et al. 2003) arriving at M1 via the basal ganglia late in the movement preparation period. Thus, a reduction in excitatory thalamocortical drive may exert an effect after descending volleys from M1 sufficient to depolarise the motoneuron pool have occurred, but in time to countermand M1 output before innervation of the agonist muscle is sufficient to overcome the inertial properties of the limb. In either case, deficient intracortical inhibition within M1 would explain the finding that partial responses are more prevalent in FHD (Stinear and Byblow 2004b).
Experiment 2 – Non-specific suppression of MI excitability observed with subthreshold stimulation

In the present study, subjects lifted their index finger off a key to intercept a moving target. In experiment 1 it was shown that EMG onset occurred, on average, 84 ms prior to the target (Table 1). Consistent with Chen et al. (1998) and Rossini et al. (1988) corticomotor excitability increased prior to EMG onset when subthreshold TMS was applied (Fig 6a) and was shown to be specific to the prime mover. This experiment demonstrated that corticomotor excitability differed between volitional inhibition and movement preparation. When stimuli were delivered up until 100 ms after the sweep dial stopped, excitability increased as for ‘Go’ trials (Fig 7a). This indicates that during sensory processing of the volitional inhibition cue, movement preparation activity within M1 continues uninterrupted. After this time, the temporal profile of excitability differs for ‘Stop’ trials relative to ‘Go’ trials. Previous studies using Go/Nogo paradigms have reported significant decreases in MEP amplitude 100 – 200 ms after the presentation of the Nogo stimulus (Hoshiyama et al. 1997; Hoshiyama et al. 1996; Yamanaka et al. 2002). In the present study, ‘Stop’ trial FDI MEP amplitude first became significantly less than ‘Go’ trial amplitude 140 ms after the cue to inhibit movement (Fig 7a). Thus, cortical processing of the visual cue to inhibit movement converges on the corticospinal pathway within 140 ms. An additional observation worth mentioning concerns the apparent dip in the excitability profile of FDI muscle during ‘Go’ trials 120 ms after the ‘Stop’ signal presentation time (Fig 6a and 7a). This may reflect uncertainty about the possible presentation of a ‘Stop’ signal.

The APB was not directly involved in performance of the task. This is shown by the relatively small amount of muscle activation associated with task performance.
(Figs 1b and 3). In experiment 2, excitability of this nearby muscle with a cortical representation in the ‘surround’ of FDI did not increase to the same extent during movement preparation (Fig 6a). However, when responses were normalised to the maximal response during ‘Go’ trials, a significant suppression of excitability was evident for ‘Stop’ trials 160 ms after the cue to inhibit (Fig 7b). This finding provides further evidence for non-selective suppression of M1 excitability, at least for tasks where the ‘Stop’ condition requires the subject not to move at all, and is consistent with previous research (Hoshiyama et al. 1997; Hoshiyama et al. 1996; Leocani et al. 2000).

While TMS was primarily used here to probe the excitability of the corticomotoneuronal pathway at the time of stimulation, it can also be applied to disrupt ongoing neural processing in various areas of human cortex, including M1. Suprathreshold stimulation close to presumed movement onset (EMG onset for non-stimulated trials) prolongs reaction time (Burle et al. 2002; Hashimoto et al. 2004; Leocani et al. 2000; McMillan et al. 2004) and this effect is proportional to the intensity of the stimulus (Ziemann et al. 1997). In contrast, subthreshold TMS has been shown to speed responses in simple reaction time and Go/Nogo paradigms (Hashimoto et al. 2004; Sawaki et al. 1999). In the present study, subthreshold TMS was shown to decrease the mean lift time as stimuli were delivered at times closer to the target, and speed up responses relative to the mean lift time of trials where no stimulation was delivered (Fig 6b). The speeding of responses by subthreshold TMS may be ascribed to intersensory facilitation (Terao et al. 1997), however, given the close temporal proximity of TMS to movement onset in the present study, this explanation seems unlikely.
Experiment 3 – Response uncertainty modulates intracortical inhibition

An interesting and unexpected finding in experiment 3 was the modulation of sICI by response uncertainty when stimulation was presented 600 ms prior to the target. Intracortical inhibition was greater when subjects were certain that a response was required (i.e. in the ‘Go-only’ condition) compared with stimulation at the same time but with uncertainty as to whether or not a response would be required (i.e. the ‘Early’ condition). This may be related to a greater level of arousal for trials with movement uncertainty, however, this interpretation is speculative.

Experiment 3 – Selective suppression of excitability and non-selective inhibition within M1

Experiment 2 revealed dynamic changes in M1 excitability and these changes were temporally divergent depending on whether a response was executed or inhibited. In experiment 3 we sought to provide evidence that intracortical inhibition within M1 contributes to the suppression of MEP amplitude observed on ‘Stop’ trials. Using paired-pulse TMS, we probed the excitability of intracortical inhibitory networks at the time immediately preceding response initiation. On ‘Stop’ trials, the cue to inhibit movement was presented at least 250 ms before the target. Thus, the probability of inhibiting the response was high and the probability of a partial response was low (Fig 4). TMS was delivered 150 ms after the ‘Stop’ cue allowing sufficient time for processing related to volitional inhibition to exert an effect on M1.

As expected, excitability in the agonist FDI muscle was enhanced in the ‘Go’ condition relative to ‘Early’. This result did not reach statistical significance and is likely due to a general increase in excitability for the ‘Early’ condition due to the subjects being actively engaged in the task. Excitability was significantly suppressed
for ‘Stop’ trials relative to ‘Go’ trials (Fig 8a). This finding is consistent with the results of experiment 2 and of previous studies (Hoshiyama et al. 1997; Hoshiyama et al. 1996; Yamanaka et al. 2002). In contrast, the suppression of MEP amplitude in experiment 3 was confined to the agonist muscle. There was no suppression observed in the antagonist, FV, or in a surrounding muscle, APB. This result contradicts the findings of experiment 2 and previous studies (Hoshiyama et al. 1997; Hoshiyama et al. 1996; Leocani et al. 2000) providing evidence for a selective reduction in excitability. The difference between experiments 2 and 3 can be explained by the difference in stimulation intensity and the normalisation procedure used in experiment 2. Increasing stimulation intensity likely alters the relative balance of induced inhibitory and excitatory inputs to corticospinal neurons. Inhibitory interneurons are thought to have a lower threshold than excitatory interneurons (Ilic et al. 2002). Thus with subthreshold TMS, MEP amplitude would be more sensitive to the effect of inhibitory inputs. In contrast, the amplitude of MEPs produced by suprathreshold stimulation would be more sensitive to the effect of excitatory inputs. Compared with the studies of Hoshiyama and colleagues (1997; 1996) the selective reduction in excitability may be ascribed to differences in the task performed and the muscles tested. In the present experiment, the ‘Stop’ task required the subject to keep the key depressed whereas the Go/Nogo task requires the subject to remain completely at rest. Additionally, Hoshiyama et al. (1997; 1996) investigated wrist flexors and extensors using a non-focal circular coil.

The principle finding of experiment 3 was that inhibition was enhanced in both FDI and APB for ‘Stop’ trials relative to ‘Go’ at a time that preceded the onset of movement for ‘Go’ trials (Fig 8b). A previous study by Sohn et al. (2002) reported increased sICI in both the agonist extensor indices proprius (EIP) and in a
surrounding muscle abductor digiti minimi (ADM) following a ‘Nogo’ cue relative to baseline. However, these authors investigated sICI after the onset of the ‘Go’ response in 80% of trials and interpreted their findings as evidence sICI is recruited in M1 nonselectively during volitional inhibition. Unfortunately, they increased the CS intensity for ‘Nogo’ trials compared with that used to examine baseline (Sohn et al. 2002). Since sICI increases with increasing CS intensity up to AMT (Orth et al. 2003; Ziemann et al. 1996), the finding of increased sICI during ‘Nogo’ (Sohn et al. 2002) may have been a manifestation of the stronger CS intensity used. The present study provides the first evidence that excitability of M1 inhibitory networks mediating sICI increases for ‘Stop’ trials before onset of the ‘Go’ response. In support of this interpretation is the finding that increased excitability of intracortical inhibitory circuits precedes voluntary muscle relaxation (Buccolieri et al. 2004). Intracortical inhibition appears to play an important role in the termination of ongoing movements and in preventing prepared movements from occurring.

In experiment 3, there was no attempt to precisely match non-conditioned MEP amplitude across conditions (Fig 8a). Given the dynamic nature of this task, precise matching would have been extremely difficult to achieve. Recent experiments by Rosenkranz and colleagues (2003; 2005) have reported that adjusting the test stimulus to account for MEP augmentation resulting from muscle vibration has no effect on the amount of sICI. Studies that have investigated the independent effect of test stimulus intensity on sICI have shown no significant effect on the amount of inhibition for test MEP amplitudes between 1 – 4 mV (Daskalakis et al. 2002; Ridding et al. 1995b; Roshan et al. 2003; Sailer et al. 2002; Sanger et al. 2001). Thus, there is evidence to support the interpretation that enhanced sICI in FDI for ‘Stop’ trials relative to ‘Go’ trials cannot be explained by the difference in NC MEP
amplitude. In addition, the increase in sICI for APB occurred in the presence of well matched NC MEP amplitudes. The differences in sICI between ‘Stop’ and ‘Go’ trials cannot be explained by pre-trigger EMG as there was no difference in rmsEMG between the two conditions. The results indicate that sICI is enhanced in FDI and APB but not in the antagonist FV. This indicates that inhibition appears to exert a non-selective effect, but not a ‘global’ effect across hand muscle representations, when a selective response is volitionally inhibited.

**Conclusion**

Volitional inhibition leads to a suppression of corticomotor excitability for ‘Stop’ trials relative to ‘Go’ trials in response to subthreshold TMS, and an increase in intracortical inhibition as measured by paired-pulse TMS. Both findings can be explained by an increase in the excitability of inhibitory interneurons within M1 acting to reduce the output of the corticospinal pathway. These findings lend support to the hypothesis that inhibition within M1 contributes to the volitional inhibition of prepared action.
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References


### Tables

#### Table 1.

‘Go’ Task performance

<table>
<thead>
<tr>
<th></th>
<th>Go-only (ms)</th>
<th>Go (ms)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Lift time</td>
<td>795.6 ± 2.2</td>
<td>809.3 ± 1.7</td>
<td>-</td>
</tr>
<tr>
<td>FDI Burst onset</td>
<td>699.4 ± 7.9</td>
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<td>-</td>
</tr>
<tr>
<td>EMD</td>
<td>96.2 ± 6.8</td>
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<td>Mean error</td>
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<tr>
<td>Absolute error</td>
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<td>26.0 ± 1.4</td>
<td>ns</td>
</tr>
<tr>
<td>Variable error</td>
<td>34.9 ± 2.9</td>
<td>32.0 ± 1.5</td>
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</tbody>
</table>

Task performance for experiment 1. *Go-only* – initial block consisting entirely of Go trials, *Go* – subsequent blocks when Go and Stop trials were presented in a random order. See experiment 1 method for EMD and error term definitions. Values mean ± SE. *ns* Non-significant.
Figure Legends

Fig 1
A) Schematic of the sweep dial display. Subjects sat in front of a computer screen viewing the display. Illumination of the Green indicator above the ‘10’ signaled that the trial was ready to commence. By depressing a key, the Red indicator was illuminated for 1 s, followed by the start of the 1 s sweep dial revolution. The anticipated response task required the subject to release the key, stopping the sweep dial as close to the arrow (positioned 800 ms into the sweep) as possible. If the sweep dial stopped unexpectedly during its revolution, subjects were instructed to keep depressing the key. B) Integrated EMG for the ‘Go’ condition in experiment 1. The unfilled bars show the baseline level of activity (300 – 500 ms). The filled bars show the activity for 200 ms from burst onset. FDI first dorsal interosseus; APB abductor pollicis brevis; FV first volar. Error bars SE.

Fig 2
Schematic of experiment 1, 2, and 3 design. Time is represented by horizontal lines (not drawn to scale). The vertical dashed line at 800 ms represents the target. To the far right is the trial type with total number of trials in parentheses. For ‘Stop’ trials, the sweep dial stop times are shown by vertical bars. In experiments 2 and 3, vertical arrows represent the times where stimuli were delivered. In experiment 2, short arrows indicate 12 subthreshold stimuli were delivered and long arrows indicate 24 subthreshold stimuli were delivered. In experiment 3, the solid arrow heads indicate that 20 NC and 20 C responses were collected. For ‘Go’ and ‘Stop’ trials, stimuli were delivered at one of three times, determined by the subjects’ behavioural
performance in experiment 1. Horizontal dashed lines separate the catch trial stop times. Numbers above arrows or stop bars indicate time in milliseconds prior to the target. In some instances only the first and last times are labeled.

Fig 3
Example EMG traces from one subject during experiment 1. EMG is shown from the beginning of the sweep dial revolution. Schematic at the top shows where the sweep dial stopped in its revolution. On ‘Go’ trials (see far right) the key release stopped the sweep dial. Key position of the key, with upward deflection representing release of the key; FDI first dorsal interosseus, agonist muscle for the key release; APB abductor pollicis brevis, in the surround of FDI. Note the appearance and increase in size of partial responses (agonist EMG burst in the absence of an overt behavioural response) as a function of where the sweep dial is stopped.

Fig 4
‘Stop’ condition performance for experiment 1. A) The probability of releasing the key when this response should have been inhibited; B) The probability of a partial response in FDI; C) Area of partial EMG burst in FDI expressed as a percentage of the ‘Go’ condition EMG activity. Partial response data is only shown for stop times where the probability of responding is less than 0.5 to ensure a sufficient number of data points. The abscissa shows the sweep dial stop time (ms) relative to the anticipated response target. Error bars SE.
Fig 5
A) Representative FDI EMG traces from one subject in experiment 2. The time of stimulation relative to the target (in milliseconds) is shown to the left. From left-right, arrows identify stimulus artifact, MEP, and voluntary EMG. For Go trials MEP amplitude increases in response to the subthreshold stimulation indicating an increase in corticomotor excitability. For Stop trials, no response is made and corticomotor excitability modulates in a time dependent manner. The stimulation times shown correspond to 60, 100, 120, and 160 ms after the sweep dial stopped revolving. B) Representative APB EMG traces from one subject in experiment 3. Go stimulation presented immediately prior to the subjects’ mean EMG onset for ‘Go’ trials in the behavioural session; Stop stimulation presented at the same time as for ‘Go’ but preceded by the sweep dial stopping 150 ms prior to stimulation indicating that the prepared action should be inhibited. Note the matched NC MEP amplitude and the increase in sICI for the ‘Stop’ condition.

Fig 6
Experiment 2 group results. A) ‘Go’ trial MEP amplitudes as a function of stimulation time. Stimulation times less than 90 ms from the target were discarded as the MEP occurred within the EMG burst. MEP amplitude increases in the agonist FDI muscle over the 100 ms leading up to EMG onset. MEP amplitude does not increase to the same extent in the APB muscle. Times where FDI MEP amplitude is significantly greater than the first stimulation time is shown by * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Error bars SE. B) Mean lift time as a function of stimulation time. The horizontal line represents the mean lift time when no stimulation was presented. A linear regression line is fitted to the data. $Y = 0.091x + 792.6, R^2 = 0.86, r = 0.93$. 
Fig 7
Experiment 2 group results showing normalized MEP amplitude as a function of stimulation time for the FDI (A) and APB (B) muscles. For each muscle, MEP amplitude was normalized to the maximum mean MEP amplitude for ‘Go’ trials. Times where MEP amplitude is significantly greater for ‘Go’ trials compared to ‘Stop’ trials is shown by * $p < 0.05$; *** $p < 0.001$. Error bars SE.

Fig 8
Experiment 3 group results. A) NC MEP amplitude; B) sICI, 100% reflecting complete inhibition of the NC response. Black bars FDI, grey bars APB, white bars FV. The schematic at the top represents the position of the sweep dial when TMS was delivered for each condition. The time of TMS is represented by a lightning bolt. The arrow represents the anticipated response target. Early stimulation delivered 600 ms into the target, prior to knowledge of the trial type; Go stimulation delivered immediately prior to the subjects’ mean burst onset for ‘Go’ trials in experiment 1; Stop stimuli delivered at the same time as for ‘Go’ but preceded by the sweep dial stopping 150 ms prior to stimulation. Error bars SE. * $p < 0.05$; *** $p < 0.001$. 


Figures

Fig 1.
Fig 2.

**Experiment 1**

- ‘TARGET’
- ‘Go’ (225)
- ‘Stop’ (105)

**Experiment 2**

- ‘Go’ (240)
- ‘Stop’ (24)
- ‘Stop’ (96)

**Experiment 3**

- ‘Go’ (80)
- ‘Early’ (40)
- ‘Stop’ (40)
Fig 3.

Key

FDI

APB

0.5 mV

200 ms
Fig 4.

A

P (respond)

B

P (partial)

C

Partial size (%)
Fig 5.

A

Go

190

Stop

50 ms

1 mV

B

Go

NC

Stop

0.5 mV

50 ms
Fig 6.

A

Stimulation time relative to target (ms) vs. MEP amplitude (mV).

FDI — APB

B

Stimulation time relative to target (ms) vs. Lift time (ms).
Fig 7.

A

B