Behavioral/Systems/Cognitive

The Differential Role of Motor Cortex in Stretch Reflex Modulation Induced by Changes in Environmental Mechanics and Verbal Instruction

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The motor cortex assumes an increasingly important role in higher mammals relative to that in lower mammals. This is true to such an extent that the human motor cortex is deeply involved in reflex regulation and it is common to speak of "transcortical reflex loops." Such loops appear to add flexibility to the human stretch reflex, once considered to be immutable, allowing it to adapt across a range of functional tasks. However, the purpose of this adaptation remains unclear. A common proposal is that stretch reflexes contribute to the regulation of limb stability; increased reflex sensitivity during tasks performed in unstable environments supports this hypothesis. Alternatively, before movement onset, stretch reflexes can assist an imposed stretch, opposite to what would be expected from a stabilizing response. Here we show that stretch reflex modulation in tasks that require changes in limb stability is mediated by motor cortical pathways, and that these differ from pathways contributing to reflex modulation that depend on how the subject is instructed to react to an imposed perturbation. By timing muscle stretches such that the modulated portion of the reflex occurred within a cortical silent period induced by transcranial magnetic stimulation, we abolished the increase in reflex sensitivity observed when individuals stabilized arm posture within a compliant environment. Conversely, reflex modulation caused by altered task instruction was unaffected by cortical silence. These results demonstrate that task-dependent changes in reflex function can be mediated through multiple neural pathways and that these pathways have task-specific roles.

Introduction

Control of the human arm is achieved through a combination of rapid involuntary responses and slower, more complex voluntary commands. When a joint is rapidly displaced, the earliest muscle responses (~20 ms for biceps brachii) result from the involuntary activation of monosynaptic reflexes that excite the lengthened muscles (Liddell and Sherrington, 1924). The earliest voluntary muscle activation in response to mechanical taps occurs after 90–100 ms in the biceps (Hammond, 1956; Marsden et al., 1978). Between these extremes of involuntary and voluntary control, "long-latency" muscle responses are commonly observed (Marsden et al., 1972, 1976a,b). These occur sufficiently rapidly to be considered involuntary while possessing modulatory capacity reminiscent of voluntary control.

Evidence of long-latency reflex modulation during postural tasks performed in different mechanical contexts has prompted suggestions that modulation serves to regulate limb stiffness (Doemges and Rack, 1992; Dietz et al., 1994; Perreault et al., 2008). An alternative hypothesis is that modulation of long-latency responses represents the superposition of reflex activity and voluntary activity triggered early by joint perturbations (Crago et al., 1976; Koshland and Hasan, 2000). In this manner desired motions may be hastened by appropriate stimuli, allowing rapid initiation of task-appropriate movements.

Given the two potential functions of the long-latency reflex, posture maintenance and action initiation, it is conceivable that each is subserved by a different neural substrate. Although cortical and subcortical elements may contribute to long latency reflexes in humans (Matthews, 1991; Lewis et al., 2004), the role of the cortex seems to change with task. Recordings from corticomotoneuronal cells suggest that they contribute to the long-latency stretch reflex (Cheney and Fetz, 1984), but their role in reflex regulation appears to differ between postural or precision tasks and those involving ballistic movements. Based on recordings from non-human primate pyramidal tract neurons, Evarts and Fromm (1978) suggested that transcortical reflex loops may be most relevant during precision tasks. A lack of cortical involvement in reflex modulation during ballistic tasks also has been noted in human studies (MacKinnon et al., 2000; Lewis et al., 2006).

To date, there have been no human studies assessing the role of the cortex in reflex modulation observed during both action initiation and postural precision tasks. Transcranial magnetic stimulation (TMS) provides a means to accomplish this goal. TMS can be used to induce a period of cortical inhibition (Inghilleri et al.,

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1993). The role of cortical cells in the reflex modulation reported in ballistic and precision tasks can therefore be probed by eliciting long-latency reflexes during the period of cortical inhibition following TMS (Kimura et al., 2006).

This study examined the contribution of primary motor cortex to modulation of long-latency stretch reflexes induced by changes in task instruction, corresponding to how the subject should react to a perturbation, or by the stability of the task environment. Our hypothesis was that inhibition of the motor cortex would eliminate modulation of the long-latency stretch reflex when the stability of the task environment was altered but not when the instructed response to perturbations was altered.

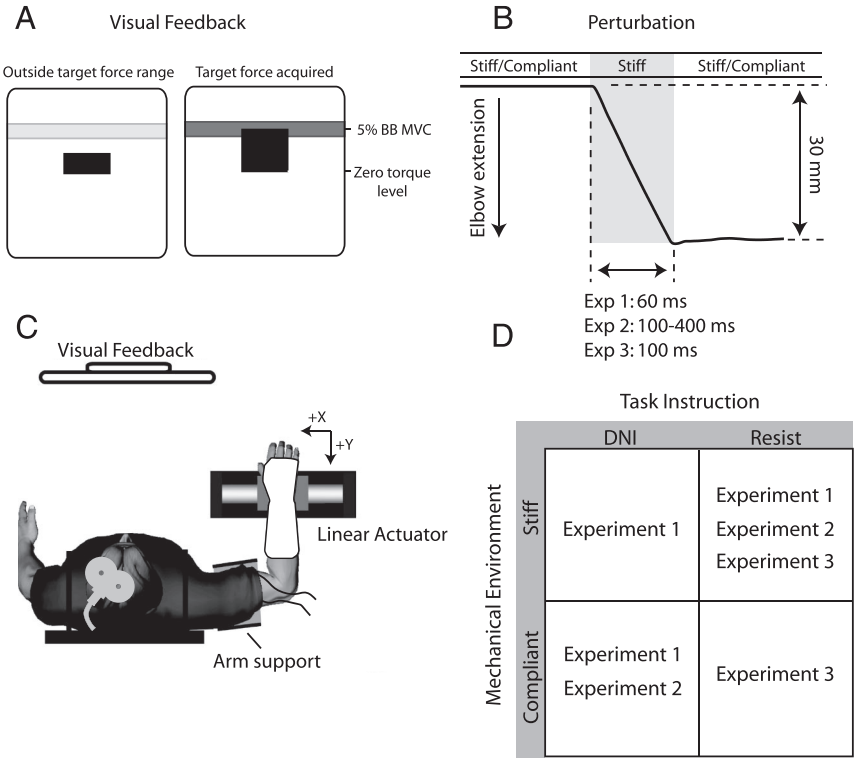

Figure 1. ***A***, Participants were required to reach and hold a target level of endpoint force before a perturbation was triggered. Endpoint force was measured along the *x*-axis of the linear actuator. Visual feedback was provided as shown with a green column representing the instantaneous force level and a horizontal bar indicating the target force range (5 ± 1% elbow flexion MVC). ***B***, Ramp-and-hold perturbations delivered by the linear actuator moved the wrist 30 mm along the −*x*-axis, thus extending the elbow joint and stretching the biceps brachii muscle. The actuator controller remained stiff throughout Stiff:DNI and Stiff:Resist trials and switched rapidly from compliant to stiff during Compliant:DNI trials to ensure consistent joint displacements. The duration of the perturbation was different in each experiment to alter the amplitude of the stretch response and avoid contamination of the reflex response by the end stop of the perturbation. ***C***, Participants were seated comfortably facing a visual display at a distance of ~1 m on which the force feedback was provided. Their arm was supported along the humerus by a cradle and at the wrist by a linear actuator. ***D***, The three experiments in the current study examined the effect of changes in task instruction ("Do not intervene" and "Resist" the perturbation) and mechanical environment (Stiff and Compliant) on the amplitude of the long latency stretch response. Experiment 1 compares experimental conditions in which one of each of the task variables is altered from the baseline (Stiff:DNI) condition. Experiments 2 and 3 represent control experiments that serve to eliminate potential confounding variables.

Materials and Methods

Participants

Eight participants (31 ± 11 years of age) with no history of neurological disorder or upper limb impairment were recruited for each experiment. All protocols were approved by the Northwestern University Institutional Review Board (IRB protocol 1322-001), and required informed consent of the subjects.

Equipment

Elbow joint actuator. A linear actuator (Copley ThrustTube TB3806; Copley Controls) was used in this study to induce rapid stretches of the biceps brachii (BB) muscle by extending the elbow joint. Participants were seated comfortably with their trunk secured to an adjustable chair (Biodex) using padded straps. The participant's right arm was positioned in a plane parallel to the floor with the shoulder at 90° abduction and flexion and the elbow at 90° of flexion (Fig. 1*C*). The forearm was fully pronated with the wrist fixed in a neutral position with a thermoplastic cast. The cast was attached at the wrist to the linear actuator. A steel plate located on the underside of the cast, centered at the wrist joint, was secured to the top surface of the actuator via a precision bearing that allowed rotation in the horizontal plane. The actuator was mounted at shoulder height such that perturbations were applied in the horizontal plane in a direction orthogonal to forearm orientation. The upper arm was placed in an adjustable trough support to ensure a constant shoulder joint position. Displacement of the linear actuator resulted in rotation at the elbow joint in the flexion/extension axis while the upper arm remained stationary. The actuator was instrumented with a linear encoder (RGH24; Renishaw) to provide position information (resolution 1 μm) and was configured during the appropriate portions of the study as either a stiff position servo or a compliant load easily moved by the subject. These different mechanical environments were implemented using an admittance control algorithm implemented in Matlab xPC (MathWorks). This algorithm was used to simulate a second-order, critically damped mechanical system with a mass of 1 kg. The stiffness in the position servo mode was 250 kN/m; in the compliant mode, it was 10 N/m. Perturbations were identically matched in each mechanical environment by transiently switching the actuator to the position servo mode at the start of each perturbation, as we have used in portions of a previous study (Perreault et al., 2008). This timing is illustrated in Figure 1*B*. Transitions between the compliant and stiff modes occurred within 0.2 ms, immediately before perturbation onset.

Electromyography. Disposable bipolar electrodes (Noraxon) were attached to the arm to record electromyographic (EMG) activity from the biceps and triceps brachii muscles in the right arm before and after stretch of the same muscle. EMG was recorded from the triceps brachii muscle to monitor antagonist activation before each perturbation. The skin overlying each muscle belly was cleaned with ethanol and abrasive gel before the application of the electrodes. Surface EMGs were amplified and conditioned using a Bortec AMT-8 (Bortec Biomedical Ltd) with high- and low-pass cutoff frequencies of 10 and 1000 Hz, respectively. The resulting signals were anti-aliased filtered using fifth order Bessel filters with a cutoff frequency of 500 Hz and then sampled at 2.5 kHz for subsequent analysis.

TMS

TMS was applied to the primary motor cortex to induce a period of cortical inhibition during the period within which afferent information elicited by the muscle stretch would be traversing the cortex. TMS was administered with a MagStim 200 (Magstim Co.) via a figure-of-eight coil (coil diameter 70 mm). The coil was positioned over the subject's head with the handle pointing posteriorly and oriented 45° from the mid-sagittal line. The optimal site for stimulation was located by moving the coil in discrete steps across the scalp until the site eliciting the largest

responses in the biceps muscle was located. The optimal stimulation site was marked on the scalp, and coil position was visually monitored by the operator during each experiment. For all three experiments the stimulation intensity used before muscle stretch perturbations was determined as the intensity at which a 150 ms period EMG silence (as measured from the stimulus trigger) in the tonically active (5% of a maximal voluntary contraction [MVC]) BB was observed following the motor evoked potential in 10 consecutive stimuli. The long-latency stretch response was timed to occur within the latter portion of the induced silent period (>100 ms after TMS trigger) to evaluate cortical effects on the stretch response. This technique has previously been shown to reduce task-specific stretch reflex modulation without eliminating the reflex response, suggesting that it affects cortical neurons involved in regulating reflex sensitivity without disrupting the primary reflex pathway (Kimura et al., 2006). The separability of spinal and cortical inhibitory effects within the TMS-induced silent period is based on evidence that H-reflexes elicited within the silent period recover to baseline levels before the end of the silent period (Fuhr et al., 1991) and that stimulation of descending motor pathways at the level of the cervicomedullary junction induces a silent period of ~50 ms, significantly shorter than that induced by TMS (Inghilleri et al., 1993).

The application of TMS results in an auditory "click" that may have influenced the subjects' reaction time when instructed to resist an imposed perturbation. We controlled for this possibility in our third experiment by masking the sound of the TMS click. This was accomplished by repetitively firing both a sham TMS coil and the active TMS coil in all conditions. The sham coil (70 mm Placebo Coil, Magstim Co.) produced only the clicking noise, without a corresponding magnetic field. It was discharged repetitively at 5 Hz starting from 1 to 3 s before perturbation onset, with a sound intensity matched to the active coil. During non-TMS trials, the sham coil was positioned on the scalp and the active coil on top of the sham coil, resulting in no TMS-induced silent period. The position of the coils was reversed in the TMS trials. Trial order was randomized and subjects were not aware of whether or not TMS would be applied in any given trial. Subjects also wore ear plugs during these experiments to reduce auditory acuity.

Protocols

At the start of each experimental session, participants were asked to perform a biceps MVC while positioned in the actuator. In all subsequent trials, the target endpoint force level was set to 5 ± 1% MVC. Subjects were provided with a visual display of the elbow flexion moment along with the target moment range (4–6% MVC). Perturbations and magnetic stimuli were delivered when endpoint force had been maintained within the target range for 1 s. Trials were separated by random intervals ranging from 3 to 7 s. Responses to joint perturbations and to TMS were collected in a series of three experiments, as outlined below. Not all subjects participated in all experiments, and experiments were conducted on separate days.

Experiment 1: Influences of cortical silence on task-dependent modulation of the long-latency stretch reflex response. In this experiment we evaluated the effects of changes in the mechanical environment and task instruction on the amplitude of the long-latency reflex in the biceps brachii muscle. We also assessed the effects of cortical silence on the reflex modulation observed across task conditions. Reflexes were elicited in each participant with perturbations that were 30 mm in amplitude and occurred with a velocity of 500 mm/s. The duration of each perturbation was therefore 60 ms, sufficient to elicit consistent long-latency responses (Lewis et al., 2005). Twenty perturbations were applied in each of three task conditions: a baseline condition in which subjects interacted with a stiff mechanical environment and received an instruction of "Do not intervene" with the perturbation (Stiff:DNI), a stiff environment with instructions altered to "Resist the perturbation" (Stiff:Resist), and a mechanical environment with reduced stiffness (Compliant) with the "Do not intervene" instruction (Compliant:DNI) (Fig. 1*D*). The task conditions were chosen to enable comparisons between conditions in which either the mechanical environment (Stiff or Compliant) or task instructions ("Do not intervene" or "Resist the perturbation") were altered independently, allowing for comparison with previous studies into long-latency reflex modulation. In addition, a novel condition in which both mechanical environment and task instruction (Compliant:Resist) differed from the baseline (Stiff:DNI) condition was investigated in experiment 3. Blocks of 20 trials in each task condition were performed with and without the application of TMS applied 50 ms before the perturbation. The order of task conditions was randomized for each participant.

During each block of 20 perturbations participants were provided with visual feedback of the endpoint force, along with the desired target force (equivalent to 5 ± 1% MVC). The instantaneous endpoint force being generated was displayed as a vertical bar on the screen while the target force level was represented as a horizontal bar that changed color when the target was achieved (Fig. 1*A*).

Experiment 2: Controlling for the amplitude of long-latency stretch reflex across tasks. For the matched perturbations used in experiment 1, the long-latency reflexes elicited in the Stiff:Resist task typically were larger than those in the Compliant:DNI task. In experiment 2, we examined the possibility that observations made in experiment 1 were attributable to those differences. To rule out this possibility we deliberately matched the amplitude of the long-latency response in each of these conditions without TMS before repeating them with TMS applied, as in experiment 1 (Fig. 1*D*). As part of this investigation we also wanted to rule out the possibility that the amplitude of the long-latency response was influenced by the end of the perturbation at 60 ms. To ensure that this was not the case, we increased the perturbation duration to a minimum of 100 ms. Perturbations with a velocity of 300 mm/s and duration of 100 ms were used in the Compliant:DNI condition. The velocity of the perturbations in the Stiff:Resist was decreased from this starting value until the amplitude of long-latency stretch reflex was matched to that in the Compliant:DNI condition. An amplitude of 30 mm was maintained for all conditions. Blocks of 20 trials in each condition were randomly ordered for each participant.

Experiment 3: Controlling for auditory cues associated with TMS. In experiment 3 we investigated the possibility that the effect of cortical silence on the amplitude of the long-latency stretch response may have been masked in the Stiff:Resist condition by the early release of a motor response, triggered by the auditory click associated with TMS discharge. Since the TMS unit discharged 50 ms before the stretch perturbation it is feasible that the early warning of an upcoming perturbation triggered the instructed "resist" response despite additional instructions being given to participants to use the perturbation as the signal to respond. In this experiment we therefore masked the sound of the TMS discharge with repetitive (5 Hz) sham TMS, as described above. Equivalent muscle responses elicited with and without TMS would indicate no effect of the cortical disruption on muscle response. Conversely, a significant difference in the amplitude of the muscle response between the two conditions would indicate that cortical inhibition has an effect on stretch responses beyond that caused by the auditory click associated with coil discharge.

In addition, we used this experiment to examine a Compliant:Resist condition to determine whether additional reflex modulation above the Stiff:Resist condition would be observed and whether it could be altered by cortical silence. Comparisons between the Stiff:Resist and Compliant:Resist conditions provide information about the interaction between task instruction and mechanical environment on the long latency response and the effect of cortical silence.

Twenty perturbations were applied in each of the TMS and no TMS conditions while participants performed the Stiff:Resist or Compliant:Resist task. TMS and no TMS trials were presented in random order. Although each block of 40 trials (20 with TMS, 20 without TMS) was performed within a single mechanical environment (Stiff or Compliant), the order in which Compliant and Stiff mechanical environments were presented was randomized. Participants reported no detectable difference between the sounds associated with the TMS and no TMS conditions. The perturbations used in experiment 3 were constant across participants, being 30 mm in amplitude and 100 ms in duration.

Data processing and analysis

EMG recordings were rectified and averaged across all 20 trials in each experimental condition before further processing. All EMGs are expressed in mV of electrical activity recorded at the skin. Background

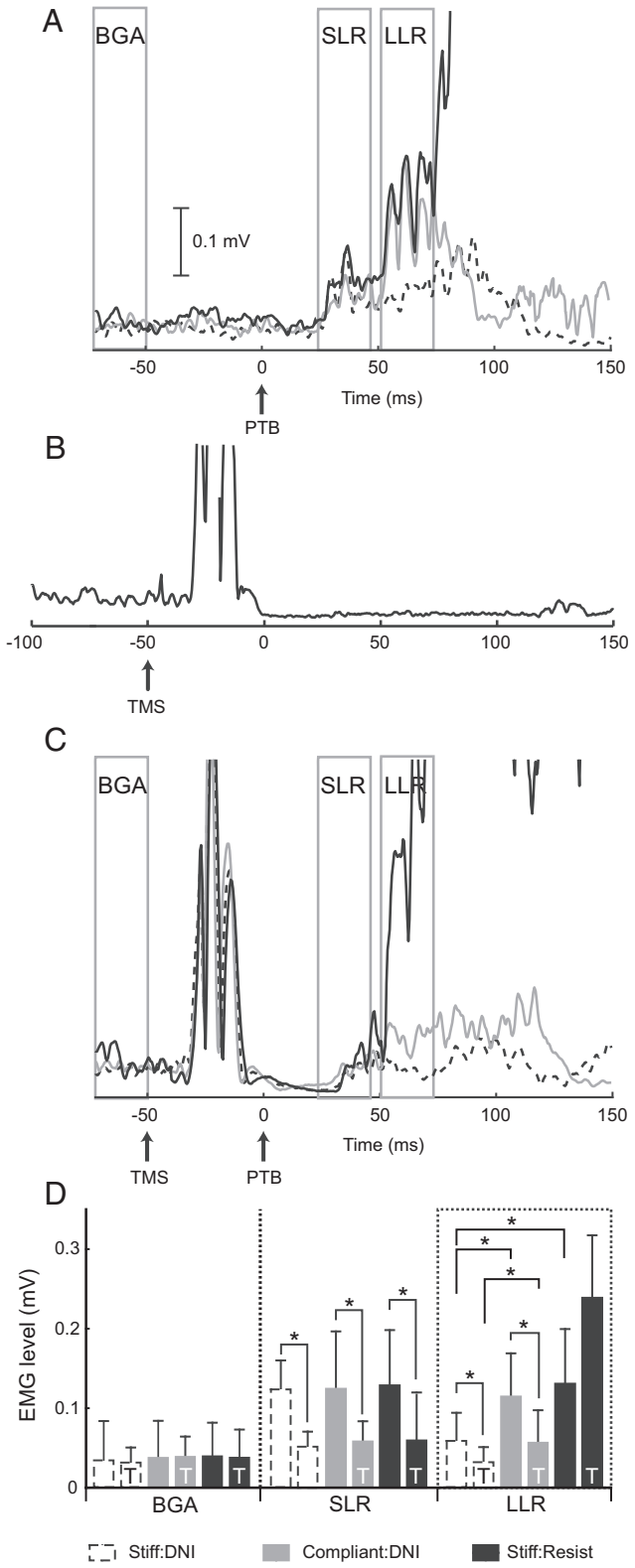

Figure 2. Cortical silence reduces the modulation of long-latency stretch responses due to changes in mechanical environment but not task instruction. ***A***, Representative data from a single participant showing the response of the BB to stretches imposed at time 0 during low-level (5% MVC) activation. Mean data obtained from 20 consecutive trials are shown in this figure. Short-latency reflex (SLR) and long-latency reflex (LLR) components are evident at latencies of 23 ms and 62 ms respectively. ***B***, A single trial is shown in which TMS was applied during a contraction of the biceps brachii at 5% MVC. The silent period following the excitatory motor evoked potential lasts longer than 150 ms following the TMS trigger. ***C***, Data from the same participant as in ***A*** show reductions in the LLRs obtained within a period of cortical silence in the Stiff:DNI and Compliant:DNI conditions. No reduction in LLR amplitude is evident in the Stiff:Resist condition. ***D***, Group means (±95% confidence intervals) are shown for background muscle activity (BGA), SLRs, and LLRs in each experimental condition. The 20 ms windows used to determine the amplitudes at each time point are shown in ***A*** and ***C***. *Statistically significant difference ($p < 0.05$).

EMG was quantified as the root-mean-square (RMS) of the averaged EMG within the period of 50–70 ms before perturbation onset. This is the period immediately before the application of TMS in the TMS trials, and was selected so that it could be used in all experimental conditions (see Fig. 2*A* for example). The onset of the short-latency stretch response 0–50 ms after perturbation onset was determined by visual inspection for each participant based on data from each experimental condition without TMS. The onset of the long-latency response also was determined visually 50–100 ms after perturbation onset as EMG activity often did not return to baseline after the short-latency response. The onsets of long latency responses were determined from data obtained in each no TMS condition and the same onset applied to trials with TMS. For each perturbation, short and long-latency response amplitudes were quantified as the RMS of the rectified EMG signal over a 20 ms time window after response onset. For trials without TMS, reflex amplitudes were quantified relative to the background EMG before perturbation onset. For the Perturbation + TMS trials, reflexes were quantified relative to the RMS EMG measured during the silent period of the TMS-only trials, corresponding to the time period used for reflex calculations. Levels of background activity were matched in each experiment and trials were eliminated off-line if the background muscle activity exceeded the mean of 20 trials ± 1.5 SD (~5% of trials).

One-way ANOVAs were used to compare the background activity across the tested conditions within each experiment to ensure that there were no significant differences. Paired *t* tests were used to compare reflexes across the tested experimental conditions. One-sided tests were used to assess the influence of TMS within a specific task of experiments 1 and 2 (e.g., Compliant:DNI) because we hypothesized that TMS would reduce long-latency reflexes mediated by cortical circuits contributing to the TMS-induced silent period. Two-sided tests were used to assess the influence of TMS in experiment 3, since our specific control made increases and decreases in the long latency response equally probable. In all cases, our conclusions were robust whether one- or two-sided tests were performed. Two-sided *t* tests with Bonferroni correction were used to compare reflex amplitudes across tasks (e.g., Compliant:DNI compared with Stiff:Resist), with or without the presence of TMS since these predictions were not always directed; the adjusted α values are reported when Bonferroni correction was used. Differences at an overall α level <0.05 were considered to be significant. Results are reported as mean ± 95% confidence interval.

Results

Stretch reflex characteristics

Stretch reflexes were elicited in both stiff and compliant environments by applying a positional perturbation to produce extension at the elbow joint. Typical responses recorded from the BB during the perturbation are shown in Figure 2*A*. These responses were recorded as the participant produced an elbow flexion moment at 5% MVC, resulting in large reflex responses in the BB. Two components of the response were identifiable in all participants. The onset of the first component (referred to as the short-latency stretch response) occurred at 22 ± 2 ms (mean ± SD), consistent with the monosynaptic stretch reflex (Lewis et al., 2006). The second consistently identifiable response (referred to as the long-latency stretch response) had a latency of 62 ± 8 ms. This long-latency stretch response has been observed previously and attributed to a reflex pathway that is polysynaptic and potentially transcortical (Palmer and Ashby, 1992). In this study we conducted three experiments to test the idea that the neural sub-

strate underlying modulation of the long-latency stretch response is critically dependent on the task being performed.

Experiment 1: Influences of cortical silence on task-dependent modulation of the long-latency stretch reflex response

Consistent with previous reports of stretch reflex modulation, increases in the long-latency stretch reflex were observed during the Compliant:DNI and Stiff:Resist conditions relative to the Stiff:DNI condition (Fig. 2*A*). Comparisons between conditions revealed an increase in the amplitude of the long-latency stretch response during the Compliant:DNI condition (0.12 ± 0.04 mV) relative to that elicited during the Stiff:DNI condition (0.06 ± 0.03 mV; $t_{(7)} = 3.11$, $p = 0.017$, $\alpha_{corrected} = 0.025$). An increase in the long-latency response was also evident in the Stiff:Resist condition (0.13 ± 0.06 mV) relative to the Stiff:DNI condition ($t_{(7)} = 3.23$, $p = 0.014$, $\alpha_{corrected} = 0.025$). In each case the increase in amplitude of the long-latency stretch response occurred without corresponding changes in the level of background muscle activity (which was deliberately matched across experimental conditions) or in the amplitude of the short-latency stretch response (Fig. 2*D*).

To address our hypothesis regarding the cortical origin of long-latency reflex modulation it was critical that transcranial magnetic stimulation (TMS) induced a period of electromyographic silence in the preactivated BB of at least 100 ms after the excitatory motor evoked potential. This was achieved with a mean stimulation intensity of 163 ± 14% of each individual's active motor threshold. A representative example of the response to TMS is shown in Figure 2*B*.

The influence of TMS on the long-latency stretch reflex varied with experimental condition (Fig. 2*C*). When the long-latency stretch response occurred in the portion of the TMS-induced silent period thought to be mediated by cortical inhibition, a decrease in response amplitude was observed in both the Stiff:DNI (No TMS: 0.06 ± 0.03, TMS: 0.037 ± 0.02; $t_{(7)} = 2.56$, $p = 0.019$) and Compliant:DNI (No TMS: 0.12 ± 0.04, TMS: 0.06 ± 0.03; $t_{(7)} = 2.39$, $p = 0.024$) conditions. Although the difference in long-latency response amplitude remained significantly different between the Stiff:DNI and Compliant:DNI conditions with TMS ($t_{(7)} = 3.33$, $p = 0.013$, $\alpha_{corrected} = 0.025$), the size of this difference (Compliant:DNI − Stiff:DNI = 0.023 mV) was reduced considerably compared with the modulation observed without TMS in the same conditions (Compliant:DNI − Stiff:DNI = 0.06 mV). No decrease in amplitude was seen in long-latency responses elicited in the Stiff:Resist condition (Fig. 2*D*). Consistent with the idea that the initial portion of the TMS-induced silent period (up to 50 ms) is attributable to refractoriness and inhibition of spinal level motoneurons and interneurons, a decrease in the amplitude of the short-latency stretch response (No TMS: 0.13 ± 0.05, TMS: 0.06 ± 0.02; $t_{(23)} = 4.33$, $p = 0.0001$) was always observed following TMS (Fig. 2*D*). The maintenance of the long-latency response in the Stiff:Resist condition suggests that the spinal inhibition contributing to attenuation of the short-latency stretch response is subsequently released before the long-latency response. The observed changes in reflex amplitude were not due to changes in background muscle activity before perturbation onset, since these were matched across all TMS and non-TMS conditions (Fig. 2*D*).

While demonstrating an interesting difference between the interaction of cortical silence and long-latency response amplitude across the experimental conditions, the protocol used for experiment 1 had two potential limitations. First, these initial experiments matched the imposed perturbations across each of the tested conditions, but this resulted in unmatched long-latency responses for each participant. Given this, it remains possible that the differential effects of TMS on the long-latency responses were driven in part by the difference in long-latency response amplitude in the absence of TMS. For example, it is conceivable that small responses are more susceptible to the inhibition caused by cortical stimulation than larger responses. Second, since our muscle stretch perturbation lasted 60 ms and the shortest monosynaptic response time is ~20 ms, part of the long-latency response we observed in the data may be attributable to a short-latency response to the rapid limb deceleration at the end of the perturbation. To ameliorate concern regarding these two issues, we performed a control experiment in which the amplitudes of the long-latency responses were specifically matched between the Compliant:DNI and Stiff:Resist conditions and a stretch perturbation with a duration of at least 100 ms was used.

Experiment 2: Controlling for the amplitude of long-latency stretch reflex across tasks

With the target elbow moment or bias force held constant, the amplitude of both short and long-latency responses in the Stiff:Resist condition was attenuated by reducing the velocity of the stretch perturbation. This process allowed us to match the amplitude of the long-latency stretch response in the Compliant:DNI (0.082 ± 0.04) and Stiff:Resist (0.074 ± 0.03; $t_{(7)} = -1.01$, $p = 0.35$) conditions while maintaining matched levels of background BB activity before the perturbation (Fig. 3). Because of the reduction in perturbation velocity, the amplitudes of the short-latency response were not matched, although the difference in short-latency response amplitude between conditions was not generally large.

As in experiment 1, the application of TMS 50 ms before the onset of the stretch perturbation resulted in the attenuation of the long-latency response in the Compliant:DNI condition (TMS: 0.05 ± 0.03; $t_{(7)} = -2.21$, $p = 0.03$) but no attenuation of the same response in the Stiff:Resist condition (TMS: 0.26 ± 0.06; $t_{(7)} = -8.88$, $p = 1.0$). An increase in amplitude of the long-latency stretch response following TMS in the Stiff:Resist condition was observed that was proportionally larger than that observed in experiment 1, possibly due to the higher perturbation velocities used to elicit responses in the first experiment. Although these results confirmed those obtained in experiment 1, the increase of the long-latency stretch response in the Stiff:Resist condition (Fig. 3*C*) gave rise to concerns that the auditory click associated with the discharge of the TMS unit 50 ms before the onset of the stretch perturbation may induce participants to produce a voluntary response in the Stiff:Resist condition earlier than would normally be possible without prior warning. Such behavior could mask any suppression of cortical contributions to the long latency reflex observed in the Stiff:Resist paradigm. Therefore, a second control experiment was performed using sham repetitive TMS to mask the sound of the real TMS coil. We also used this final experiment to examine reflex modulation in a Compliant:Resist condition, to determine whether this would result in reflex modulation above that observed in the Compliant:DNI or the Stiff:Resist conditions alone.

Experiment 3: Controlling for auditory cues associated with TMS

In experiment 3, we used an auditory masking procedure to minimize the release of anticipatory voluntary activity based on the sound of the TMS pulse. Using this procedure it was possible to

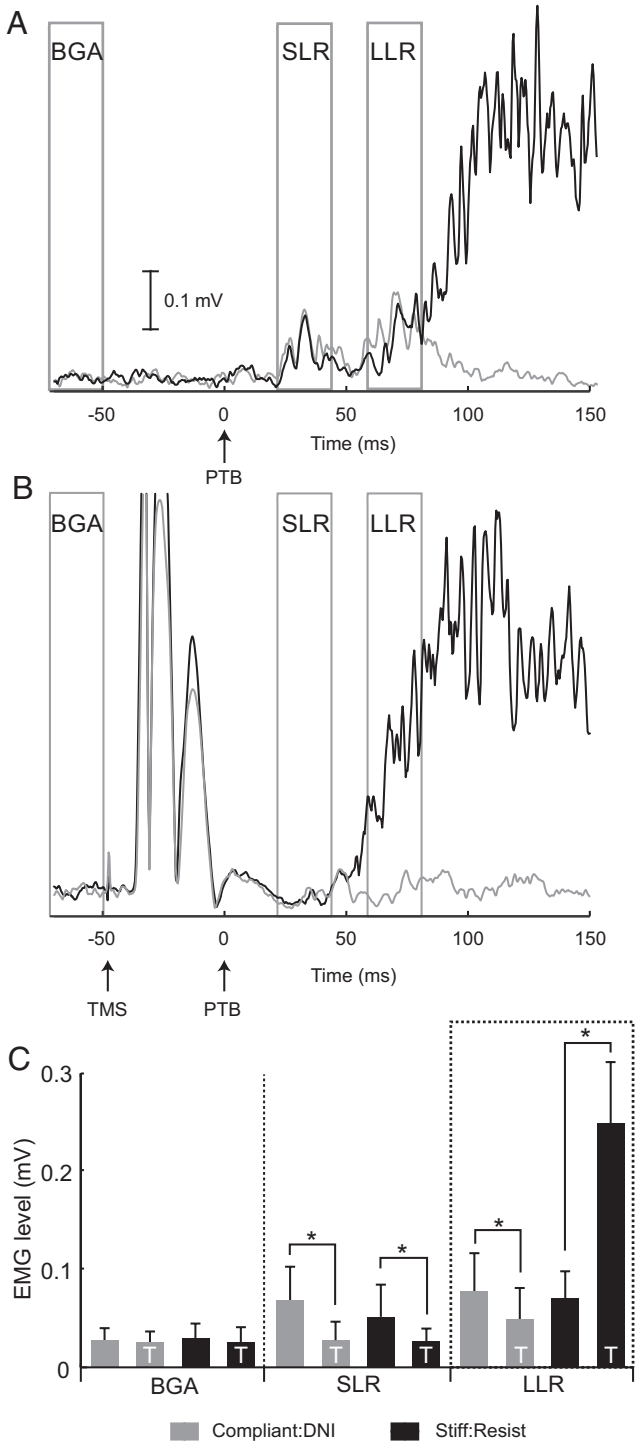

Figure 3. Cortical silence reduces long-latency stretch responses in the Compliant:DNI but not Stiff:Resist condition. ***A***, Representative data from a single participant demonstrates the equivalence of background levels of BB activity and the amplitude of the LLR to a series of imposed 100 ms muscle stretches. Mean responses from 20 trials in each condition are shown in this figure. ***B***, During the period of TMS-induced cortical silence the LLR response is reduced during the Compliant:DNI condition but increased during the Stiff:Resist condition. ***C***, Group means (±95% confidence intervals) demonstrate reductions in SLR amplitude following TMS. Statistically significant (*$p < 0.05$) differences between LLR amplitudes with and without TMS were also observed in both conditions.

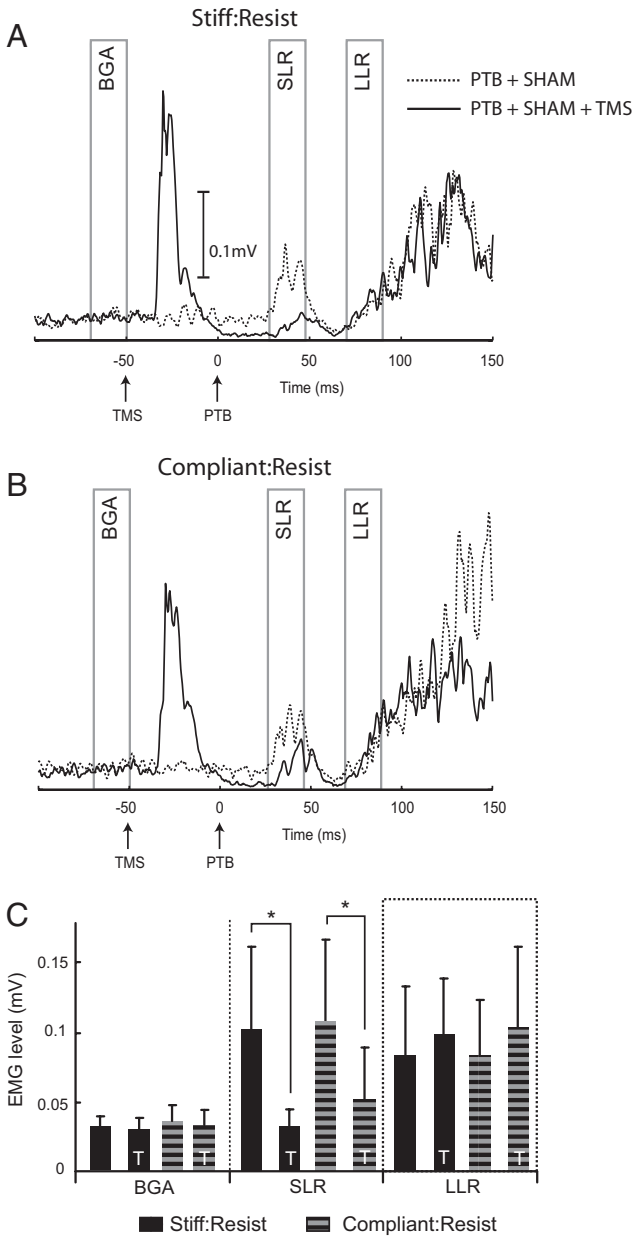

Figure 4. The amplitude of long-latency responses in the Stiff:Resist condition is independent of cortical silence. ***A***, Representative data from a single participant demonstrates similar long-latency responses in the Stiff:Resist condition whether or not the elbow perturbation is preceded by TMS. ***B***, Representative data from the same participant as in ***A*** demonstrate that long-latency responses in the Compliant:Resist condition also remain similar with and without TMS. ***C***, Group mean data show that the amplitude of the long-latency response is not influenced by cortical inhibition. A reduction in the amplitude of the SLR is observable, however, indicating a pattern of TMS-induced inhibition similar to that observed in experiments 1 and 2.

match the onset of the long latency stretch response in conditions without TMS (Fig. 4*A*). In contrast to the results of experiment 2 the amplitude of long latency responses did not differ between TMS and non-TMS trials in the Stiff:Resist condition (No TMS: 0.085 ± 0.048, TMS: 0.096 ± 0.043; $t_{(7)} = -0.66$, $p = 0.53$). The long-latency response was similarly matched in the Compliant:Resist trials (Fig. 4*B*), which were performed only in this experiment (No TMS: 0.085 ± 0.04, TMS: 0.11 ± 0.056; $t_{(7)} = -1.68$, $p = 0.137$). Two of the 8 tested subjects contributed to the small, nonsignificant increase in the long-latency response with TMS (Fig. 4*C*), leading to a non-normal distribution; nonparametric Wilcoxon rank sum tests provided significance values of $p = 0.80$ for the Stiff:Resist trials and 0.88 for the Compliant:Resist trials. The increased long-latency response in these two subjects was due to a change in amplitude, not onset latency. The average

change in long-latency onset between the no TMS and TMS conditions in experiment 3 was 0.3 ± 2.6 ms with a maximum of 5.6 ms. In contrast, TMS resulted in an average shortening of the long-latency onset of 16 ± 30 ms in experiment 1, with a maximum of 39.6 ms. The increased variability in long-latency onset for experiments 1 and 2 is consistent with an auditory startle (Nieuwenhuijzen et al., 2000). The reduced variability in experiment 3 further suggests that the auditory click was not triggering the subjects' reactions in this final experiment.

We also found no difference between the amplitude of the long-latency reflexes elicited in the Stiff:Resist condition (0.085 ± 0.048) compared with those in the Compliant:Resist condition (0.085 ± 0.04; $t_{(7)} = -0.16, p = 0.88$). Hence, the equivalency of the long-latency response with and without TMS in Compliant:Resist condition is congruent with the similar effect of TMS during the Stiff:Resist condition.

Discussion

As shown previously, our results demonstrate that different experimental conditions can lead to modulation of the long-latency stretch reflex. Long-latency reflexes were larger when participants interacted with a compliant mechanical environment compared with a stiff environment, a task requiring the precise maintenance of posture. The amplitude of long-latency reflexes was also larger when subjects were instructed to oppose a postural perturbation as rapidly as possible, a task in which a ballistic motor program is triggered by a somatosensory stimulus, compared with when they were instructed not to intervene. By using TMS to inhibit cells within the cortex, we have provided evidence that reflex modulation observed in the postural maintenance task (Compliant: DNI) involves the cells in the motor cortex inhibited by TMS, whereas the modulation observed in the action initiation task (Stiff:Resist) does not involve these inhibited cells. These results demonstrate that the pathways contributing to the motor responses often grouped together as long-latency stretch reflexes can vary with task, and may contribute to our understanding of the functions attributable to this fundamental motor response.

Distinct neural pathways for modulation of the long-latency stretch response

Suprathreshold TMS applied during tonic muscle contractions induces an initial excitatory response followed by a period of silence in the target muscle (Inghilleri et al., 1993). Several investigators have demonstrated that the latter portion (from 50 ms after the onset of EMG silence) of the TMS-induced silent period is due to cortical rather than subcortical or spinal-level inhibition (Fuhr et al., 1991; Cantello et al., 1992; Inghilleri et al., 1993). The period of cortical inhibition caused by TMS has been attributed to either recurrent collateral connections from pyramidal tract neurons in layer V of motor cortex or inhibitory intracortical circuits (Werhahn et al., 1999; Terao and Ugawa, 2002). In the current experiment, long-latency responses were induced within the latter portion of the TMS-induced silent period, and influences of TMS on their behavior were attributed to these cortical mechanisms. Short-latency reflexes were induced within the early portion of the silent period (<50 ms after the onset of EMG silence) and influences of TMS on their behavior likely resulted from spinal-level inhibition (Fuhr et al., 1991; Inghilleri et al., 1993).

Our data show a decrease in the amplitude of long-latency stretch responses during TMS-induced cortical inhibition for both experimental conditions in which individuals were instructed not to respond to applied elbow perturbations (i.e., Stiff:DNI and Compliant:DNI). These results from the biceps brachii are consistent with the idea that neurons within the motor cortex contribute to long-latency responses observed in more distal upper limb muscles during postural control tasks (Matthews, 1991; Palmer and Ashby, 1992; Capaday et al., 1994). Reductions in the amplitude of the long-latency response following TMS were greatest when "do not intervene" tasks were performed within a compliant mechanical environment. This is consistent with previous findings that TMS-induced cortical inhibition reduces the anticipatory modulation of involuntary stretch responses during multijoint reaching (Kimura et al., 2006). Together, the results of the current study and those of Kimura et al. (2006) provide evidence that the motor cortex is involved in regulating, in an anticipatory manner, the gain of the long-latency stretch response to compensate for changes in the mechanical environment. This regulation may occur in the cortex, or indirectly via a cortically mediated change in reflex excitability as could occur by altering gamma bias. The present experiments cannot distinguish between these possibilities, although the equivalence of the short-latency reflex in the Stiff:DNI and the Compliant:DNI suggest that changes in the drive to gamma motoneurons is not the only mechanism contributing to the observed long-latency reflex modulation between these tasks.

Contrary to effects observed during postural maintenance tasks, TMS-induced cortical inhibition either increased (experiments 1 and 2) or had no effect (experiment 3) on the amplitude of the long-latency response when participants were instructed to rapidly resist the perturbation. This was true even when actions were prepared during interactions with a compliant environment (experiment 3), suggesting that stabilizing actions may be subservient to preplanned, rapid motor responses. It has been demonstrated that "involuntary" responses to rapid perturbations retain many features of voluntary actions when the perturbations are applied before action initiation (Koshland and Hasan, 2000; Kurtzer et al., 2008; Pruszynski et al., 2008). The similarities between long-latency responses and voluntary activity have been suggested to result from superposition of reflex activity and the triggered release of early voluntary activation (Crago et al., 1976; Rothwell et al., 1980). The inability of TMS-induced cortical inhibition to reduce the amplitude of the long-latency stretch response in this task supports the idea that different neural mechanisms contribute to long-latency stretch reflexes during the precise maintenance of posture and action initiation. It also suggests that the "triggering" of planned motor actions following rapid joint perturbations occurs outside the motor cortical areas affected by TMS. This is consistent with evidence that the activity of human motor cortical circuits immediately preceding the long-latency stretch response is not altered with changes in the instructed response to applied joint perturbations (MacKinnon et al., 2000; Lewis et al., 2006).

The differential effect of TMS-induced cortical inhibition on long-latency reflexes in the Compliant:DNI and Stiff:Resist conditions is also consistent with cortical recordings in non-human primates. Evarts and Fromm (1978) demonstrated that pyramidal tract neurons, involved in voluntary control of both small corrective movements and larger ballistic movements, respond much stronger to perturbations applied during the small movements required for postural control than immediately before ballistic movements, which are similar to the rapid motor responses required during our Resist tasks. In contrast, the differential effects of cortical inhibition in the current study are less consistent with earlier work by Evarts and Tanji (1974, 1976) demonstrating that the intended movement direction in a Resist task influences

the modulation of activity in pyramidal tract neurons following postural perturbations. These differences may arise from the specific movement instructions used in each experiment, especially the speed at which the intended movements were instructed to occur, or from changes in the excitability of the motoneurons pool before perturbation. Evarts and Tanji (1974, 1976) noted an instruction-dependent modulation of short-latency reflexes, which may have contributed to the corresponding cortical observations. In contrast, we controlled the amplitude of the short-latency reflex across conditions by matching the background muscle activity before perturbation onset (see experiment 1), as done in previous human studies (MacKinnon et al., 2000; Lewis et al., 2006). Although the later work by Evarts and Fromm (1978) agrees with our findings, it is important to note that TMS is thought to influence nonpyramidal tract neurons (Werhahn et al., 1999; Terao and Ugawa, 2002). Hence, comparisons between our results and those from direct pyramidal tract neuron recordings must consider those differences.

Similarities exist between the timing of motor actions triggered by proprioceptive inputs and responses to auditory startle stimuli, which preferentially excite brainstem neurons (Colebatch and Porter, 1987; Lingenhöhl and Friauf, 1992; Yeomans et al., 2002). When applied to individuals who are preparing a motor action, startle stimuli release the intended action earlier than the voluntary reaction time, while the intended pattern of agonist-antagonist muscle activity is preserved (Rothwell et al., 2002). It is possible therefore, that rapid, preplanned motor actions triggered by rapid limb perturbations are dependent on similar subcortical structures as actions triggered by an auditory startle. This idea is supported by evidence that removal of the support surface during feline walking produces startle-like muscle responses which are preceded by activity within neurons of the pontomedullary reticular formation (Stapley and Drew, 2009). Similar startle-like muscle responses are observed during human walking when an unanticipated change in surface height is encountered (van der Linden et al., 2007). The integration of proprioceptive signals in the human brainstem, similar to that observed in the cat, could provide a mechanism for the early release of prepared resistive responses following joint perturbations in the current study.

Task-dependent functions of the long-latency stretch response

The results of the current study suggest a parsimonious explanation for the observed differences in function of the long-latency stretch response. When the motor system encounters changes in the stability of the environment with which it is interacting, the long-latency response is modulated in a manner consistent with the regulation of limb stiffness, as is needed to maintain precise control of posture. This response appears to involve the motor cortex and may be mediated by a closed neural circuit of the type commonly associated with a "reflex." When joint perturbations are applied during the preparation of an action, the perturbation appears to act like an auditory startle in that it triggers the early release of the stored motor action. In this situation, the motor cortical areas contributing to precise control of posture are less involved in the long-latency reflex and it is possible that subcortical structures contribute to the increased response. The planned pattern of muscle activity triggered by the perturbation would be superimposed on activity derived from the transcortical 'reflex' circuit to produce the observed long-latency stretch response.

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