Development/Plasticity/Repair

Spike Timing-Dependent Plasticity in the Long-Latency Stretch Reflex Following Paired Stimulation from a Wearable Electronic Device

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The long-latency stretch reflex (LLSR) in human elbow muscles probably depends on multiple pathways; one possible contributor is the reticulospinal tract. Here we attempted to induce plastic changes in the LLSR by pairing noninvasive stimuli that are known to activate reticulospinal pathways, at timings predicted to cause spike timing-dependent plasticity in the brainstem. In healthy human subjects, reflex responses in flexor muscles were recorded following extension perturbations at the elbow. Subjects were then fitted with a portable device that delivered auditory click stimuli through an earpiece, and electrical stimuli around motor threshold to the biceps muscle via surface electrodes. We tested the following four paradigms: biceps stimulus 10 ms before click (Bi-10ms-C); click 25 ms before biceps (C-25ms-Bi); click alone (C only); and biceps alone (Bi only). The average stimulus rate was 0.67 Hz. Subjects left the laboratory wearing the device and performed normal daily activities. Approximately 7 h later, they returned, and stretch reflexes were remeasured. The LLSR was significantly enhanced in the biceps muscle (on average by 49%) after the Bi-10ms-C paradigm, but was suppressed for C-25ms-Bi (by 31%); it was unchanged for Bi only and C only. No paradigm induced LLSR changes in the unstimulated brachioradialis muscle. Although we cannot exclude contributions from spinal or cortical pathways, our results are consistent with spike timing-dependent plasticity in reticulospinal circuits, specific to the stimulated muscle. This is the first demonstration that the LLSR can be modified via paired-pulse methods, and may open up new possibilities in motor systems neuroscience and rehabilitation.

Key words: plasticity; rehabilitation; reticulospinal; STDP; stretch reflex; wearable electronic device

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Significance Statement

This report is the first demonstration that the long-latency stretch reflex can be modified by repeated, precisely timed pairing of stimuli known to activate brainstem pathways. Furthermore, pairing was achieved with a portable electronic device capable of delivering many more stimulus repetitions than conventional laboratory studies. Our findings open up new possibilities for basic research into these underinvestigated pathways, which are important for motor control in healthy individuals. They may also lead to paradigms capable of enhancing rehabilitation in patients recovering from damage, such as after stroke or spinal cord injury.

Introduction

The reticulospinal tract is a major descending motor pathway, which is typically considered to convey commands for gross motor functions, such as maintaining posture, locomotion, and

reaching movements (Matsuyama and Drew, 2000; Prentice and Drew, 2001; Buford and Davidson, 2004; Davidson and Buford, 2006; Dyson et al., 2014). Recent work in primates has shown that the reticulospinal tract may also contribute to hand function, in parallel with the more prominent corticospinal tract (Riddle et al., 2009; Riddle and Baker, 2010; Baker, 2011; Soteropoulos et al., 2012). Following corticospinal damage, connections from the reticulospinal tract to motoneurons controlling the upper limb strengthen selectively to flexors (Zaaimi et al., 2012). This probably underlies the selective recovery of function seen after stroke or spinal cord injury, when extensors remain weak but flexors regain strength, sometimes even to the extent of an unhelpful spasticity.

These observations motivate the search for principled interventions to modify reticulospinal connections, which could enhance functional recovery after lesion. Previous work has induced plastic

changes in the cortex by consistently pairing stimuli that act on a common circuit. By the principles of spike timing-dependent plasticity, if a postsynaptic neuron is activated consistently after a presynaptic input, that input is strengthened; reversing this timing weakens the input (Markram et al., 1997). Plasticity protocols can use pairs of stimuli delivered using microelectrodes (Bi and Poo, 2001), or can time one stimulus at a fixed delay after spontaneous neural spikes (Jackson et al., 2006). It is also possible to induce plasticity using noninvasive stimuli. Example paradigms targeting the cortex pair transcranial magnetic brain stimulation with peripheral nerve stimulation (Stefan et al., 2000) or stimulation of the motor points of two hand muscles (Ridding and Uy, 2003). To date, no reports have attempted to induce plasticity in reticulospinal pathways.

An essential prerequisite to induce spike timing-dependent plasticity is to find two stimuli that converge on a common target circuit. It is well known that the brainstem nuclei that give rise to the reticulospinal tract receive extensive afferent input (Leiras et al., 2010); electrical stimulation in the periphery will thus generate robust synaptic input. We recently demonstrated that primate reticular neurons fire bursts of action potentials after loud auditory clicks (Fisher et al., 2012). We therefore hypothesized that precisely timed pairing of peripheral shocks with clicks may lead to plasticity in reticulospinal circuits.

Experimental study of plasticity finally requires a way to measure any changes. For the corticospinal system, motor-evoked potentials following transcranial magnetic brain stimulation are typically used to assay connectivity. Similarly unambiguous noninvasive measures of reticulospinal function are not available. One option may be to assess the long-latency stretch reflex (LLSR). For distal muscles acting on the digits or wrist, the LLSR appears to have a substantial component passing via the primary motor cortex and the corticospinal tract (Cheney and Fetz, 1984; Matthews et al., 1990), although even the LLSR in finger muscles has a reticulospinal contribution (Soteropoulos et al., 2012). For muscles acting on the elbow and shoulder, although there is undoubtedly a corticospinal contribution (Evarts and Tanji, 1976; Pruszynski et al., 2011b), there is evidence that this is reduced compared with more distal muscles (Fellows et al., 1996) and that there may also be a subcortical component (Kimura et al., 2006). We therefore hypothesized that LLSR in a more proximal muscle might partially measure reticulospinal output (Kurtzer, 2015), and that paired stimuli targeted to induce plasticity in reticulospinal pathways might modify the LLSR.

To date, most experiments on synaptic plasticity have paired stimuli for only short periods, working within the confines of a laboratory setting. While changes may be induced, they typically fade after approximately an hour. We wished instead to develop protocols that could be applied for many hours while the subject went about their normal daily activities. To this end, we developed a wearable electronic device that is capable of delivering the required stimuli in a portable system. In this report, we describe the successful induction of plasticity using this device measured as a change in LLSR, which may partially reflect spike timing-dependent processes within the brainstem.

Materials and Methods

Results were obtained from 74 healthy volunteers (22 male; age range, 19–84 years) from >89 experiments. All procedures were approved by the local ethical committee of Newcastle University Medical School, and full written consent was obtained from each participant.

Measurement of stretch reflex. Subjects were seated in a rigid chair, fitted with a five-point harness to prevent trunk and shoulder move-

ments. An electromyogram (EMG) was recorded from the biceps and brachioradialis muscles of the right arm, using adhesive surface electrodes (brachioradialis muscle: model H59P, Kendall; biceps muscle: model H91SSG, Kendall) placed over the muscle belly with an interelectrode spacing of 2 cm for the brachioradialis and 3–5 cm for the biceps. Electrodes were connected to a Digitimer D360 amplifier (gain, 1000; bandpass filter, 30 Hz to 2 kHz). The arm was fitted into a robotic device, which measured elbow flexion angle and could generate torques around the elbow using a powerful motor (part #353301, Maxon; with a 25:1 planetary gearhead and a further 1.6:1 reduction ratio generated by the gear wheels and belt drive linking the motor to the drive shaft). The forearm was partially pronated, and the shoulder was flexed at 45° and abducted at 90° (Fig. 1A). Subjects were instructed to maintain a 90° flexion against a background torque by moving a cursor related to elbow angle into a target displayed on a computer screen. With a delay of 1.5–2 s after the target was acquired (chosen at random from a uniform distribution), the motor torque increased to a high level (~86 Nm, measured as a static torque when activating the motor for a prolonged period) for 150–200 ms, generating an elbow extension movement with a near-constant velocity of 150–300°/s. This was a little lower than the expected velocity obtained by taking the specified free-running speed of the motor and correcting for the gearing (404°/s). It is likely therefore that the large torque generated by the motor rapidly accelerated the arm to a terminal velocity, in which friction in the gears and bearings was equal and opposite to the motor torque. These perturbations evoked consistent short- and long-latency reflexes (Thilmann et al., 1991; Trumbower et al., 2013) in the recorded muscles. Subjects were told to return the arm to the central target after each perturbation, but were not required to do this within any time constraints. A total of 60 trials was recorded for each session, comprising 20 trials at each of three levels of background torque. Levels of background torque were determined individually for the subject to allow comfortable task performance, and ranged from 0 to 4.5 Nm; the same levels were used for that subject in both recording sessions.

EMG, elbow displacement, and motor torque signals together with markers indicating task events were captured with a personal computer (5 kHz sampling rate) using a 1401 interface (Cambridge Electronic Design). Subsequent analysis involved constructing averages of rectified EMG, using custom scripts written in the MATLAB environment.

Experimental protocol. All experiments followed the same general pattern (Fig. 1B). Subjects came to the laboratory before 9:30 A.M., and a set of stretch reflex recordings were made. They were then fitted with a wearable electronic device, designed to deliver electrical and auditory stimuli. The wearable device generated constant-current electrical stimuli to the biceps muscle (surface electrodes and placement as above; 220 V compliance; 150 μs pulse width; more proximal electrode negative). The intensity was adjusted to be just below the motor threshold (defined as a visible muscle twitch); subjects reported a weak paresthesia produced by the stimulus. Auditory stimuli were generated by delivering a 0.1-ms-wide, 12-V-square excitation pulse into a miniature earpiece; this produced a brief click with an intensity of 110 dB SPL. Based on calculations provided by Rosengren et al. (2010), this intensity delivered at 0.66 Hz for 8 h corresponds to an A-weighted intensity of 68 dB L_{Aeq} (equivalent continuous level), which is well below the recommended safe limit for hearing of 85 dB L_{Aeq} given by the UK Control of Noise at Work Regulations (The Stationery Office, 2005). The earpiece was placed in either the left or the right ear. We found no consistent differences that were dependent on which ear was stimulated; recordings from monkey reticular formation also showed that cells could be activated by clicks delivered to a wide area of the scalp by a bone vibrator (K. M. Fisher, B. Zaaimi, and S. N. Baker, personal communication). Stimuli were delivered with an interstimulus interval of 1.25–1.75 s (chosen at random from a uniform distribution). Subjects then left the laboratory and continued their usual daily activities. As most subjects were staff or students in the university, this typically involved office or laboratory tasks, such as typing and soldering. After 5:00 P.M., they returned, the wearable device was removed, and a further set of stretch reflex recordings was made. Because the electrodes over the biceps muscle were used for both recording reflex responses and for stimulation, they were kept in place for the whole day, ensuring consistency between the morning and evening re-
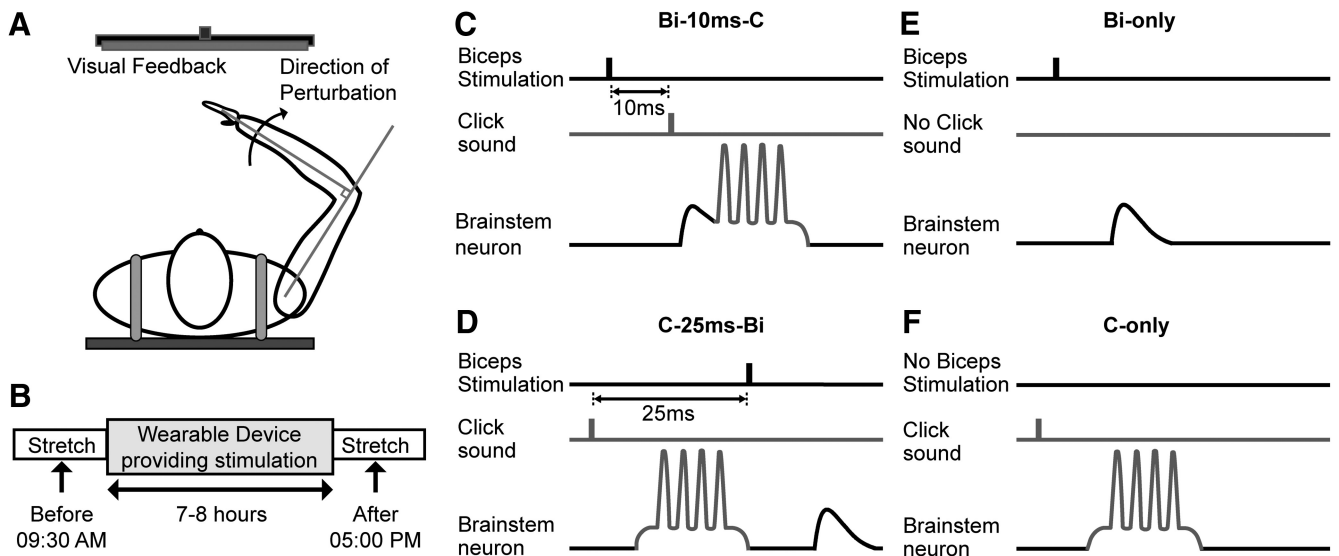
Figure 1. Schematic diagram showing the experimental setup and wearable device stimulus conditions. ***A***, Subjects were strapped into a chair, with their right arm attached to a robotic device capable of delivering extension perturbations at the elbow joint. A computer screen provided visual feedback of the elbow angle. ***B***, The general experiment protocol. ***C–F***, The four different stimulus conditions implemented by the wearable device, and their hypothesized effects on a reticulospinal neuron. ***C***, Biceps stimulation 10 ms before the click (Bi-10ms-C); the EPSP elicited by the afferent input arrives just before the click-induced discharge, which should potentiate synapses conveying the EPSP. ***D***, Click 25 ms before biceps stimulation (C-25ms-Bi); the afferent EPSP arrives after the click-induced discharge, which should lead to depression of the EPSP. ***E***, Biceps stimulation alone (Bi-only). ***F***, Click stimulation alone (C-only). With no stimulus pairing, we expect no change in EPSP amplitude.

cordings. For the brachioradialis muscle, either the electrodes were also left in place all day or their location was marked after the morning session with a UV fluorescent marker pen; this ensured that electrodes could be replaced in exactly the same location for the evening reflex recording.

Four different stimulus combinations were tested in different experiments; these are illustrated in Figure 1C–F, together with a schematic that indicates the effects in the brainstem, which we hypothesized each would generate, given previous work. The first paradigm placed the electrical stimulus 10 ms before the click. We expect the biceps stimulus to generate an EPSP within the reticular formation in a human subject with a latency of ~10 ms (see Discussion). The click should generate an action potential burst after ~7 ms (Fisher et al., 2012). We predicted therefore that this timing would lead to the EPSP consistently arriving just before the spike burst, and should lead to long-term potentiation. By contrast, when the click preceded the electrical stimulus by 25 ms (Fig. 1D), this should place the EPSP after the spike burst, and generate long-term depression. Two control conditions (Fig. 1 E,F) tested the effect of giving electrical or auditory stimuli alone. Some subjects participated in more than one of these paradigms; at least 1 week separated different experiments in the same subject.

Analysis. Figure 2A illustrates 10 sweeps of raw EMG from a single subject following the perturbation. An average of the elbow displacement trace revealed a nearly linear ramp perturbation (Fig. 2B). One problem that we encountered was considerable variation in the level of background EMG before the perturbation onset. Since it is known that the stretch reflex scales with background activation (Matthews, 1986), an inconsistent background would render comparisons of the morning and evening reflex recordings invalid.

Figure 2C illustrates the approach taken to this problem. We first measured the background EMG in each of the 60 available sweeps over the 50 ms before perturbation onset, for both morning and evening recordings. The interval from the largest to the smallest background measured was divided into 20 equally spaced bins, and each sweep was allocated to one of these bins. This led to a distribution histogram, as shown in Figure 2C. For each bin, we took the minimum count between the morning and evening recordings. For the session that had this minimum, all sweeps falling in that bin were used. For the other session, a number of sweeps equal to the minimum count were chosen at random. Following this procedure for all bins led to selection of a subset of sweeps, with equal numbers in both morning and evening sessions,

and with a very similar mean background EMG level. These sweeps were averaged together, generating traces, as shown in Figure 2D.

Following previous work (Mortimer et al., 1981; Pruszynski et al., 2011a), responses were categorized by their latency (Fig. 2D) as short-latency stretch reflex (R1; 20–50 ms), long-latency stretch reflex (R2; 50–100 ms), and voluntary reflex response (>100 ms). Within the R1 and R2 windows, the percentage increase of the area under the curve relative to the background was used as a measure of reflex amplitude. The significance of changes in reflex amplitude were assessed using t tests on the single sweep measures of the area under the curve above background, with a threshold of $p < 0.05$.

Results

Figure 2D illustrates an example result from one subject, in which the wearable device was programmed to deliver electrical stimuli to the biceps muscle 10 ms before a click (Fig. 1C, protocol). The background level of EMG was very similar for recordings made before and after the wearable device intervention (Fig. 2D, red vs black traces), confirming the efficiency of the method of sweep selection described in Materials and Methods (Fig. 2C). Although very similar for the R1 component, the long-latency stretch reflex (R2) was noticeably enhanced in the later recording. Figure 2F presents measures of the area under the curve for each response window; there was a significant increase in the R2 response.

Figure 3 presents group results for recordings from the biceps muscle. These have been separated into R1 (Fig. 3A,B) and R2 (Fig. 3C,D) components. Figure 3, A and C, shows mean reflex amplitudes, calculated across all subjects participating in a given wearable device protocol (as defined in Fig. 1C–F). The R2 component showed a significant increase ($p < 0.0002$, paired t test), on average from 176% to 263% of baseline (an increase of 49%) for the condition where the electrical stimulus preceded the click by 10 ms. There was a significant decrease ($p < 0.005$, paired t test), on average from 239% to 165% of baseline (a decrease of 31%) when the click preceded the electrical stimulus by 25 ms (Fig. 3C). The direction of these effects was as predicted on the
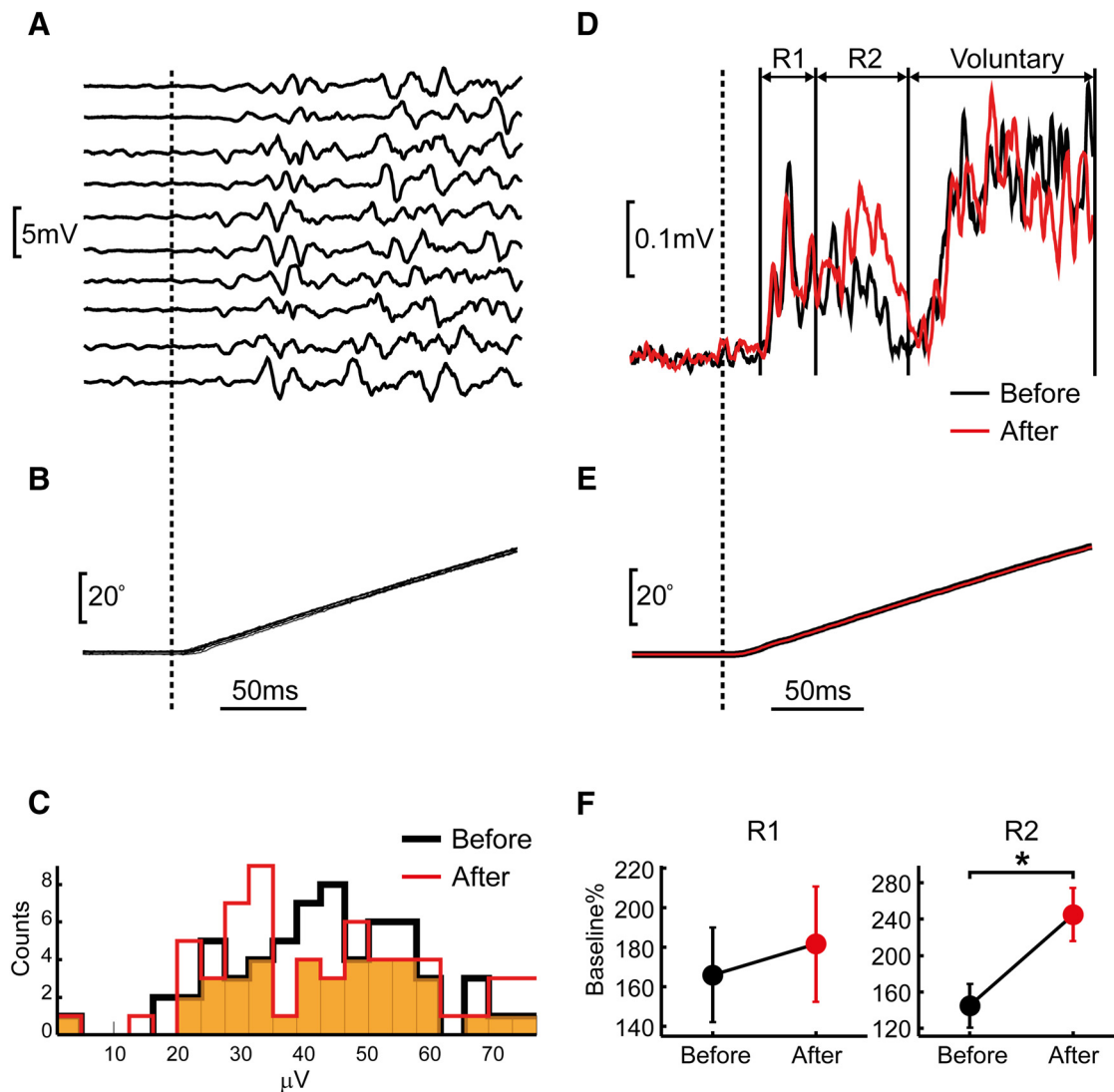
A

D

B

E

C

F

Figure 2. Example result in a single subject. **A**, Raw biceps EMG recording after 10 perturbations. Dotted line indicates the perturbation onset. **B**, Overlaid single sweeps (thin lines) and average (thick line) of elbow angle during the perturbation for the same experiment as in **A**. **C**, Distribution histograms of mean background EMG in the biceps muscle in one subject, for recordings before and after the wearable device stimulation. Orange shading marks the minimum value of each bin. **D**, Average rectified EMG in the biceps muscle following perturbation onset (dotted line), for recordings before and after wearable device stimulation (Fig. 1C, protocol Bi-10ms-C). Average has been compiled using the sweep selection procedure described in the text and illustrated in **C**, such that baseline EMG is matched. Short-latency (R1), long-latency (R2), and voluntary response epochs are marked. **E**, Average elbow angle for the same experiment as in **C**; traces for measurements before and after wearable device stimulation are overlaid, but are barely distinguishable. **F**, Measurements of the area under the curve as a percentage of baseline EMG for the experiment shown in **D**. The R2 response was significantly facilitated (*$p < 0.05$). For all panels, black traces refer to measurements before wearable device stimulation, and red traces refer to those after.

basis of spike timing-dependent plasticity (Fig. 1C,D). There was no significant difference in the size of the control reflex (before wearable device stimulation) between these two experiments. A small but significant decrease (by 17%; $p < 0.02$, paired t test) was also seen in the R1 reflex for the second protocol. The control protocols, where either electrical or auditory stimuli were given alone, produced no significant changes ($p > 0.05$, paired t test).

It is now well recognized that plasticity protocols often lead to substantial heterogeneity in response across subjects (Wiethoff et al., 2014); some of this may be determined by genetic factors (Cheeran et al., 2008). Accordingly, Figure 3, B and D, examines at the single-subject level how many showed significant changes. Each bar shows the number of subjects with significant increases, significant decreases. or no significant change for a given protocol and response window. For protocols with no significant change in average response, the pattern across subjects tended to be in-

consistent, with some increases and decreases seen. However, for R2 reflex following paired stimulation, a clear pattern emerged that supported the group-averaged data. When the electrical stimuli preceded the click, 15 of 25 subjects showed a significant rise in R2, compared with only 2 of 25 subjects showing a decrease. By contrast, when the click preceded the electrical stimulus, 24 of 33 subjects showed a drop in R2 amplitude, but only 5 of 33 subjects showed a rise. To see counts as extreme as 15 of 25 and 24 of 33 subjects is highly unlikely by chance ($p < 10^{-17}$, based on the binomial distribution, with $n = 25$ and $n = 33$ respectively and success probability $p(\text{hit}) = 0.025$). Similar results were obtained if we considered simply whether reflexes increased or decreased, regardless of whether these changes were significant for an individual subject (electrical stimulus before click, 23 of 25 subjects showed increased R2; click before electrical stimulus, 26 of 33 subjects showed de-
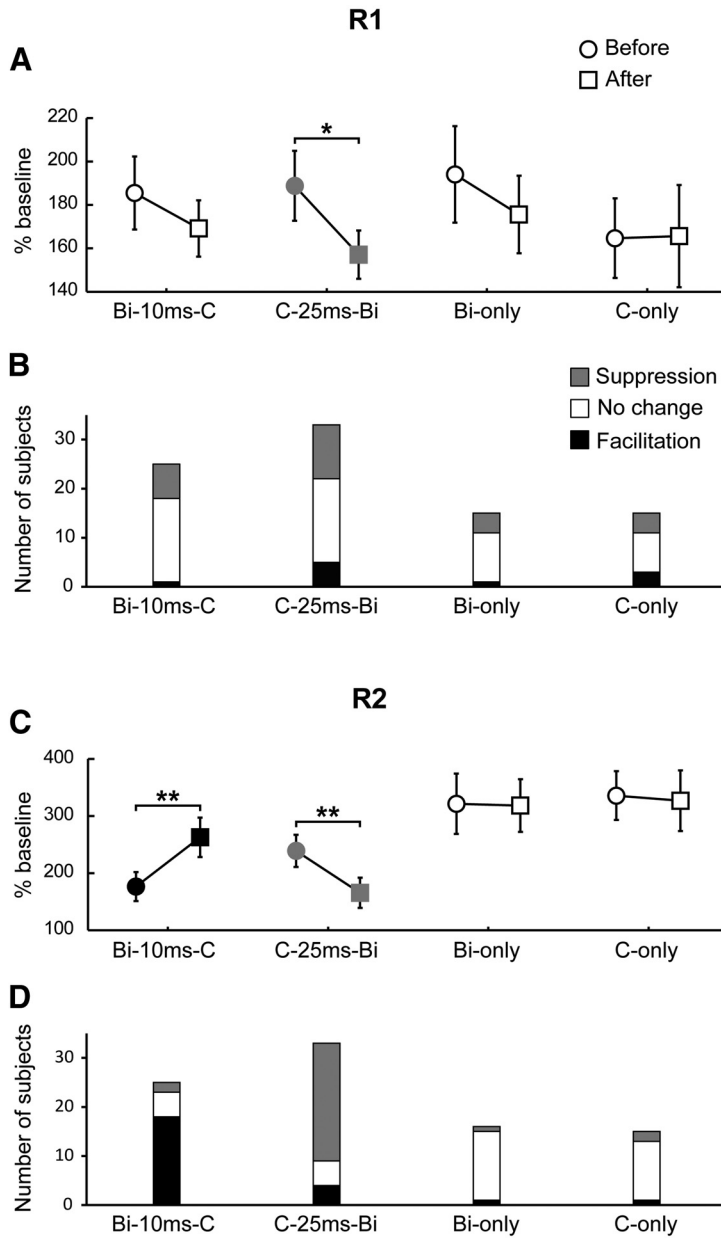
Figure 3. Group results in the biceps muscle. **A, C,** Comparison of mean reflex size (expressed as a percentage of baseline EMG activity) before (circles) and after (squares) wearable device stimulation, for the four stimulation protocols illustrated in Figure 1C–F. *$p < 0.05$; **$p < 0.005$. **B, D,** Stacked bar plots showing the number of subjects with significant ($p < 0.05$) reflex facilitation (black), suppression (gray), or no change (white). **A** and **B** relate to the short-latency (R1) reflex component; **C** and **D** relate to the long-latency (R2) component. Bi-10ms-C, Biceps stimulation 10 ms before the click; C-25ms-Bi, click 25 ms before biceps stimulation; Bi-only, biceps stimulation alone; C-only, click stimulation alone. Error bars show standard errors of the mean.

creased R2; both $p < 0.002$ based on binomial distribution with $p(\text{hit}) = 0.5$.

The wearable device protocols examined involved electrical stimulation of the biceps muscle; however, stretch reflex recordings were made from both biceps and brachioradialis, which are both elbow flexors in the arm posture tested. This allowed us to examine the extent to which changes in reflex amplitude were specific to the stimulated muscle or might spread to anatomical agonists. Figure 4 presents the results for the brachioradialis muscle, in a format similar to those shown in Figure 3. No significant changes were seen in any of the group averages (Fig. 4A,C). At the single-subject level, similar

numbers of subjects in a given protocol showed significant increases or decreases (Fig. 4B,D), suggesting that this reflected noise fluctuations in amplitude measurement rather than consistent plastic changes.

Discussion
Pathways contributing to the long-latency stretch reflex
Following the discovery of the LLSR (Hammond, 1954), considerable research focused on the pathways responsible. This reached a consensus by the early 1990s that the LLSR in muscles acting on the digits or wrist was mediated largely by Group Ia muscle afferents traversing a transcortical pathway (Matthews, 1991). However, this did not exclude other contributions. For example, some continued to argue that cutaneous afferents play a dominant role (Corden et al., 2000); earlier studies suggested that the LLSR was a spinal reflex mediated by slower conducting Group II afferents (Matthews, 1984), although subsequent results did not support this (Matthews, 1989). For muscles acting around the elbow or shoulder, motor cortical recordings in monkeys reveal evidence for some transcortical contribution (Evarts and Tanji, 1976; Pruszynski et al., 2011b). Transcranial magnetic brain stimuli delivered over motor cortex and timed to coincide with the LLSR are facilitated, also suggesting a transcortical contribution (Pruszynski et al., 2011b). However, evidence from patients with motor disorders suggests that the transcortical route for the LLSR may be less important in elbow muscles compared with the hand (Thilmann et al., 1991; Fellows et al., 1996). The tonic vibration reflex, which may relate to the LLSR, relies on the brainstem reticular formation (Gillies et al., 1971). Even for finger perturbations, we have recently shown that the reticular formation probably contributes to the LLSR (Soteropoulos et al., 2012). It therefore seems probable that the reticular formation contributes to LLSR in more proximal muscles as well (Kurtzer, 2015), and could even be the dominant pathway. Such evidence caused us to measure changes in the LLSR following elbow perturbations after a paired stimulus protocol designed to target reticulospinal output. Our finding that the LLSR exhibits plastic changes, in a manner consistent with our predictions of how paired stimuli should modify reticulospinal output, is consistent with a reticulospinal role in elbow muscle LLSR.

Spike timing-dependent plasticity
Several features of our results suggest that we were able to induce spike timing-dependent plastic changes. First, the control conditions that delivered either electrical stimulation of biceps alone or clicks alone failed to generate consistent changes in the reflex

measures. This not only controls for the effects of the unpaired stimuli, but also provides confidence that there were no consistent changes in the reflexes between the morning and evening assessments, caused, for example, by diurnal rhythms or fatigue. Second, it is striking that shifting the relative timing of the two stimuli by only 35 ms should have had such a profound effect, reversing an average facilitation in biceps R2 reflex to a suppression. Finally, plastic changes were seen only in the biceps muscle, which was stimulated, and not in the closely related agonist brachioradialis. This seems to fulfil the "specificity" criterion of long-term potentiation whereby effects are limited to the stimulated site, although we cannot rule out the alternative possibility that only pathways targeting the biceps are capable of showing these changes.

The stimulus timings were chosen based on the expected delays to generate activity within the reticular formation. In monkeys, we know that reticular responses to clicks have and onset latency of ~7 ms (Fisher et al., 2012). Although the human head is larger, most of this delay relates to central processing rather than axonal conduction, so that it is likely to be only slightly longer in humans. Electrical stimulation of the human median nerve at the wrist produces EEG responses attributed to the medial lemniscus, with a latency of ~14 ms (Taylor and Black, 1984); effects should reach the reticular formation shortly afterward. We estimate the distance between the biceps motor point and the wrist as 350–400 mm; using a fast afferent conduction velocity of 85 m/s, this would imply a reduction of 4–5 ms in latencies to account for the more proximal stimulus site. Synaptic potentials in the reticular formation should thus start at ~10 ms after the biceps electrical stimulus. Stimulating the biceps motor point 10 ms before the click should therefore place the earliest synaptic potentials from afferent input at ~7 ms before the action potential burst generated by the click. This timing is therefore appropriate to generate potentiation of the synaptic inputs.

When reversing the timings, we must also take account of the duration of the action potential burst; neural firing can continue for up to 25 ms after the click (Fisher et al., 2012). Placing the biceps stimulus 25 ms after the click should, therefore, position the synaptic potentials from afferent input at ~10 ms after the end of the action potential burst; this should be appropriate to depress the synaptic inputs.

It is impossible to be certain of the site of the plastic changes that we have measured in the LLSR, but the success of the chosen timings argues that some modification of synapses may have occurred within the brainstem itself. Other possibilities are within
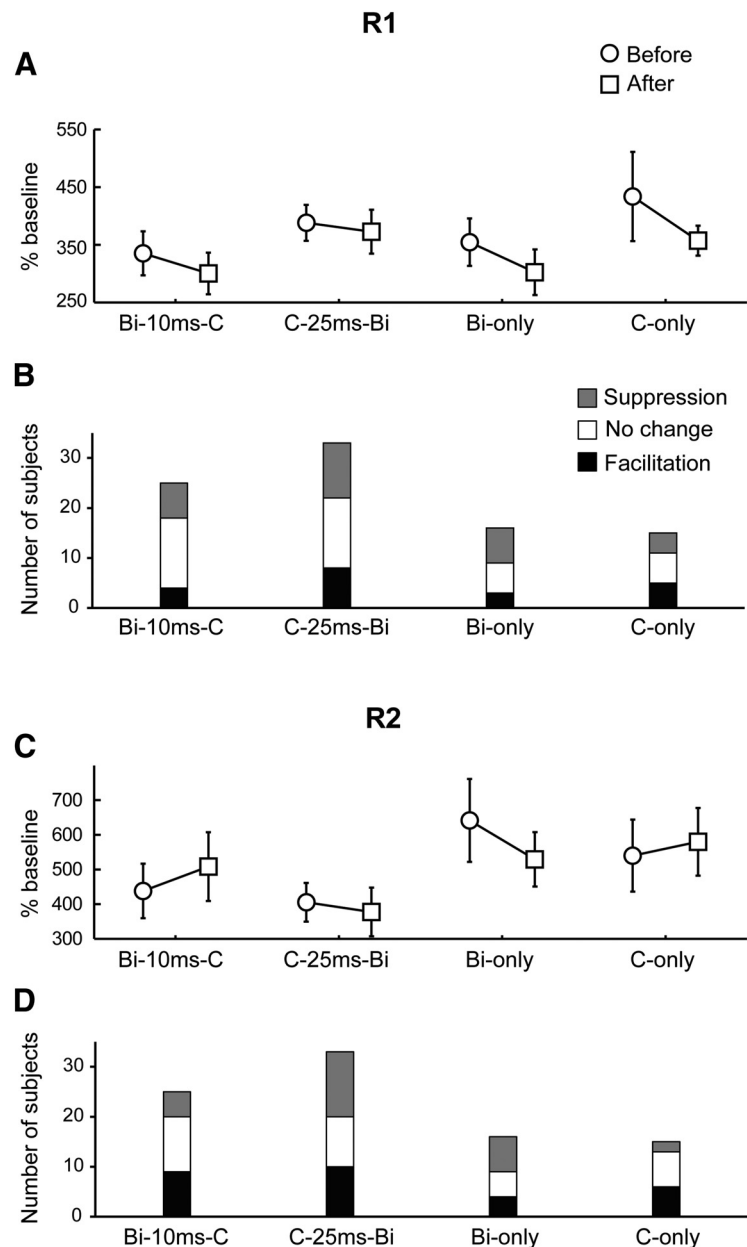

Figure 4. Group results in the brachioradialis muscle. **A**, **C**, Comparison of mean reflex size (expressed as a percentage of baseline EMG activity) before (circles) and after (squares) wearable device stimulation for the four stimulation protocols illustrated in Figure 1**C–F**. There were no significant differences ($p > 0.05$). **B**, **D**, Stacked bar plots showing the number of subjects with significant ($p < 0.05$) reflex facilitation (black), suppression (gray), or no change (white). **A** and **B** relate to the short-latency (R1) reflex component; **C** and **D** relate to the long-latency (R2) component. Bi-10ms-C, Biceps stimulation 10 ms before the click; C-25ms-Bi, click 25 ms before biceps stimulation; Bi-only, biceps stimulation alone; C-only, click stimulation alone. Error bars show standard error of the mean.

spinal cord interneurons, or within the cortex, but the anatomy of the conduction delays conspires to make these less likely. If a spinal cord interneuron discharged following a click-elicited reticulospinal burst, this would be at least 3–4 ms later than the burst onset in the brainstem. This estimate is based on the fact that central motor conduction time from M1 to cervical enlargement in human is ~7 ms (Jaiser et al., 2015). The brainstem is approximately halfway along this path, and fast reticulospinal and corticospinal fibers have a similar conduction velocity (Riddle et al., 2009). By contrast, an afferent volley following the biceps stimulus would arrive at the cervical enlargement 3–4 ms earlier than at the brainstem. The interval between the responses

then increases from the estimate of 7 ms at the brainstem estimated above, to ~14 ms at the spinal cord; this is less likely to drive plastic changes. For the cortex, the responses to biceps stimulation will be delayed by ~6 ms relative to the brainstem, but those following the auditory click by substantially more: the earliest cortical auditory-evoked potential occurs with 50 ms latency (Farrell et al., 1980). The responses in the brainstem appear best timed to generate spike timing-dependent changes, although it is impossible to rule out a contribution from other centers.

A small suppression was seen in the R1 reflex when the biceps stimulus followed the click (Fig. 3A), indicating that changes within spinal circuits may also have played a role in our results. Human subjects, monkeys, and rats can all learn to increase or decrease the size of an H reflex (the electrical analog of the R1 reflex) if appropriately rewarded (Thompson and Wolpaw, 2014). In monkeys subjected to repeated reflex testing, but with no attempt at up- or down-conditioning, there is a progressive reduction of the R1 reflex (Meyer-Lohmann et al., 1986). In the rat, reflex conditioning depends on the corticospinal tract and sensorimotor cortex, but not other descending pathways (Chen and Wolpaw, 1997, 2002; Chen et al., 2006a,b). Down-conditioning leads to increases in identifiable GABAergic terminals in the spinal cord (Wang et al., 2006), but is dependent on an intact cerebellum (Chen and Wolpaw, 2005); therefore, spinal plasticity seems to be guided and maintained by supraspinal pathways. It is likely that conceptually similar processes are occurring here, although whether the same central structures and descending pathways that contribute to reflex conditioning in the rat are responsible in this case remains to be determined.

The LLSR is known to change depending on the behavioral context; this appears flexibly to integrate the known biomechanics of the limb (Kurtzer et al., 2008). It is unclear how the plastic changes that we have seen would interact with these task-dependent changes. It is also unclear how long plastic changes would last, and whether they could be prolonged by applying the stimulus pairing for longer than the ~7 h that we tested in this report. All of these questions remain to be addressed in future studies. However, this report marks the first demonstration of plasticity in the LLSR induced with paired stimulus paradigms, and may indicate that brainstem as well as corticospinal descending systems can undergo plastic changes. Previous work has shown that rehabilitation after spinal cord injury can be enhanced by up- or down-conditioning of spinal reflexes (Chen et al., 2006c; Thompson et al., 2013). We hope that the novel protocol introduced here may open up new possibilities for enhancing rehabilitation during recovery from stroke or spinal cord injury, in which we have shown that brainstem pathways play an important role (Zaaimi et al., 2012).

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