Development/Plasticity/Repair

Pairing Transcranial Magnetic Stimulation and Loud Sounds Produces Plastic Changes in Motor Output

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Most current methods for neuromodulation target the cortex. Approaches for inducing plasticity in subcortical motor pathways, such as the reticulospinal tract, could help to boost recovery after damage (e.g., stroke). In this study, we paired loud acoustic stimulation (LAS) with transcranial magnetic stimulation (TMS) over the motor cortex in male and female healthy humans. LAS activates the reticular formation; TMS activates descending systems, including corticoreticular fibers. Two hundred paired stimuli were used, with 50 ms interstimulus interval at which LAS suppresses TMS responses. Before and after stimulus pairing, responses in the contralateral biceps muscle to TMS alone were measured. Ten, 20, and 30 min after stimulus pairing ended, TMS responses were enhanced, indicating the induction of LTP. No long-term changes were seen in control experiments which used 200 unpaired TMS or LAS, indicating the importance of associative stimulation. Following paired stimulation, no changes were seen in responses to direct corticospinal stimulation at the level of the medulla, or in the extent of reaction time shortening by a loud sound (StartReact effect), suggesting that plasticity did not occur in corticospinal or reticulospinal synapses. Direct measurements in female monkeys undergoing a similar paired protocol revealed no enhancement of corticospinal volleys after paired stimulation, suggesting no changes occurred in intracortical connections. The most likely substrate for the plastic changes, consistent with all our measurements, is an increase in the efficacy of corticoreticular connections. This new protocol may find utility, as it seems to target different motor circuits compared with other available paradigms.

Key words: **LTP; reticulospinal; spike timing-dependent plasticity; startle**

Significance Statement

Induction of plasticity by neurostimulation protocols may be promising to enhance functional recovery after damage such as following stroke, but current protocols mainly target cortical circuits. In this study, we developed a novel paradigm which may generate long-term changes in connections between cortex and brainstem. This could provide an additional tool to modulate and improve recovery.

Introduction

The primary motor cortex and its corticospinal outputs form the major neural control system for generation of voluntary movements in primates, such as humans (Porter and Lemon, 1993). However, subcortical circuits, such as the brainstem and spinal cord, also play an important role. This may be especially important following damage to the cortex, such as after stroke, when subcortical systems can compensate and thereby mediate some functional recovery (Zaaimi et al., 2012, 2018; Tohyama et al., 2017).

Various neural stimulation approaches have been developed to induce synaptic plasticity in the motor system (e.g., Nitsche and Paulus, 2000; Stefan et al., 2000; Ridding and Uy, 2003; Huang et al., 2005; Foysal and Baker, 2020); these could provide a way to boost connections in surviving circuits and enhance recovery in patients recovering from damage. However, to date, such approaches have not entered routine clinical practice, as they provide inconsistent benefits (Rothwell, 2016). Importantly, most previous protocols to induce plasticity targeted the motor cortex and corticospinal tract. New methods capable of generating long-term changes in a more diverse range of motor pathways might give better options to improve recovery, possibly by allowing individualized treatment based on the specific deficits.

In this laboratory, we recently devised one such protocol, which paired loud auditory clicks with weak electrical stimuli given to a muscle (Foysal et al., 2016). Loud clicks are known to activate reticulospinal cells (Fisher et al., 2012), probably via both cochlear (Irvine and Jackson, 1983) and vestibular (Peterson and

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Abzug, 1975; Rosengren et al., 2010) afferents, and the reticular formation also receives powerful somatosensory inputs (Leiras et al., 2010). LTP or LTD of motor output could be generated depending on whether synaptic inputs from the muscle stimulation arrived at the brainstem before or after action potentials generated by the click, in accordance with the principles of spike timing-dependent plasticity (Markram et al., 1997). We subsequently found changes in a variety of noninvasive measures consistent with a subcortical substrate for plasticity in this protocol (Germann and Baker, 2021), and showed that applying such paired stimuli to stroke patients could produce significant improvement in hand function (Choudhury et al., 2020).

One alternative promising stimulus to target reticulospinal systems is a loud auditory stimulus (LAS), capable of evoking the startle reflex. Such stimuli are typically 500-1000 Hz tone bursts lasting ~50 ms; this contrasts with much briefer clicks (0.1 ms) which do not elicit startle. The neural substrate for the startle reflex involves the nucleus reticularis pontis caudalis (Leitner et al., 1980; Davis et al., 1982; Brown et al., 1991) and its reticulospinal projection (Delwaide and Schepens, 1995). LAS can also dramatically shorten voluntary reaction times, a phenomenon known as the StartReact effect (Valls-Sole et al., 1999, 2008; Carlsen et al., 2004; Rothwell, 2006). This has been deployed by many authors as a measure of the size of reticulospinal inputs to a given motoneuron pool (Carlsen et al., 2009; Honeycutt et al., 2013; Choudhury et al., 2019; Sangari and Perez, 2019; Tapia et al., 2022; Baker and Perez, 2017).

Another approach that efficiently stimulates the reticulospinal tract is to activate the motor cortex with transcranial magnetic stimulation (TMS). This can stimulate corticoreticular projections (Fisher et al., 2012) which activate reticulospinal cells transsynaptically both ipsilateral and contralateral to the stimulated hemisphere (Fisher et al., 2021). Many corticoreticular projections are collaterals of corticospinal axons (Keizer and Kuypers, 1989).

In this study, we investigated the plastic changes generated by consistently pairing LAS with TMS. This protocol produced an enhanced motor output in healthy human volunteers, although the details of the changes differed from those seen following our previous approach involving clicks and peripheral stimuli. Results in monkey also suggested that the new method may be targeting a different set of synapses, and could be of benefit in different circumstances, or be used to augment changes produced by preexisting protocols.

Materials and Methods

Main study in human subjects

Participants

In total, 120 right-handed, healthy volunteers (18-35 years old, 51 females) participated in the study (30 participants, 20 females in Experiment 1; 30 participants, 21 females in Experiment 2; 30 participants, 21 females in Experiment 3; 15 participants, 10 females in Experiment 4 and 15 participants, 8 females in Experiment 5). Some participants took part in several experiments, in which case each session was separated by at least 7 d. All subjects gave written informed consent to the experimental procedures, which were approved by the local ethics committee of the Newcastle University Faculty of Medical Sciences. The study was performed in accordance with the guidelines established in the Declaration of Helsinki, except that the study was not preregistered in a database.

EMG recordings

EMG was recorded from the right biceps muscle through surface electrodes (Kendall H59P, Covidien) secured on the skin over the muscle belly. EMG signals were amplified and filtered (bandwidth 30-2000 Hz) with a bioamplifier (D360 8-Channel Patient Amplifier, Digitimer) and then converted to digital data with a sampling rate of 5 kHz (CED Micro 1401 with Spike2 software, Cambridge Electronic Design) and stored on a computer for offline analysis.

TMS

Transcranial magnetic stimuli were applied using a figure-of-eight coil through a Magstim 200 magnetic stimulator with a monophasic current waveform. We determined the optimal position for eliciting a motor-evoked potential (MEP) in the biceps muscle (hotspot) by moving the coil, with the handle pointing backwards and 45° away from the midline, in small steps along the arm representation of M1. The hotspot was defined as the region where the largest MEP in the biceps muscle could be evoked with the minimum intensity (Rothwell et al., 1999). In all experiments, the magnetic coil was held to induce electrical currents that flowed perpendicular to the presumed line of the central sulcus in a posterior–anterior direction. Active motor threshold was defined as the minimal stimulus intensity needed to produce a visible MEP in at least 5 of 10 consecutive trials in the tonically activated biceps. A TMS intensity of 130% active motor threshold was used to collect all MEPs. To ensure a stable coil position during the experiment, the site of stimulation was marked in a Brainsight neuronavigation system (Rogue Research), which allowed online navigation. A Polaris Vicra camera (Northern Digital) was used to track the coil.

Experimental paradigm (Experiments 1-3)

Figure 1 illustrates the experimental paradigm. Subjects were first seated with both arms relaxed and their forearms resting on their lap. Subjects were then asked to perform a biceps curl by pushing with their right arm up against a table in front of them while keeping their left arm relaxed. Visual feedback of rectified and smoothed EMG activity from the biceps muscle was provided to the subjects, involving a series of colored bars on a computer screen which illuminated in sequence as stronger contractions were made. This system was first calibrated to the subject's individual maximum voluntary contraction; all subsequent TMS measurements were made at 130% active motor threshold, while the subject aimed for a consistent background contraction level, which was set between 5% and 10% of maximum voluntary contraction for a given subject.

Baseline. Twenty MEPs were recorded while subjects performed the controlled isometric biceps contraction. TMS pulses were applied with an interstimulus interval of 10-12.5 s. After baseline, subjects rested for 15 s.

Intervention. In total, subjects received 200 stimuli. The intertrial interval varied between 10 and 12.5 s so that the timing of stimulation was unpredictable. Stimuli were given while subjects performed the controlled isometric biceps contraction. To avoid fatigue, subjects received 20 blocks of 10 stimuli, with 15 s breaks in between each block.

For Experiment 1, subjects received 200 stimuli pairs. A loud acoustic stimulus (LAS, 500 Hz, 120 dB, 50 ms duration) was presented 50 ms before the TMS pulse through two audio speakers located on a table ~100 cm in front of the subject. The interval of 50 ms was chosen because previous work suggests that LAS produces a subcortical facilitation (Rossignol and Jones, 1976; Rudell and Eberle, 1985; Nakashima et al., 1994; Delwaide and Schepens, 1995; Tapia et al., 2022) and a cortical suppression (Furubayashi et al., 2000; Kuhn et al., 2004; Tazoe and Perez, 2017) around this time. We hypothesized that the converging activation of corticofugal fibers by TMS with subcortical activation by the LAS would induce plasticity, possibly by spike timing-dependent mechanisms. For Experiment 2, subjects received 200 loud acoustic stimuli without any TMS. For Experiment 3, subjects received 200 TMS pulses, without any loud acoustic stimuli.

Assessments. Identical to the baseline, 20 MEPs were recorded while subjects performed a controlled isometric biceps contraction. TMS pulses were applied with an intertrial interval of 10-12.5 s. This assessment was repeated 4 times in total: immediately after the intervention (0 min) and starting 10, 20, and 30 min after the end of the intervention.

Cervicomedullary junction stimulation (Experiment 4)

As a way of controlling for spinal excitability, we also measured cervicomedullary MEPs (CMEPs) in the biceps brachii, as they are thought predominantly to reflect corticospinal tract responses unaffected by changes

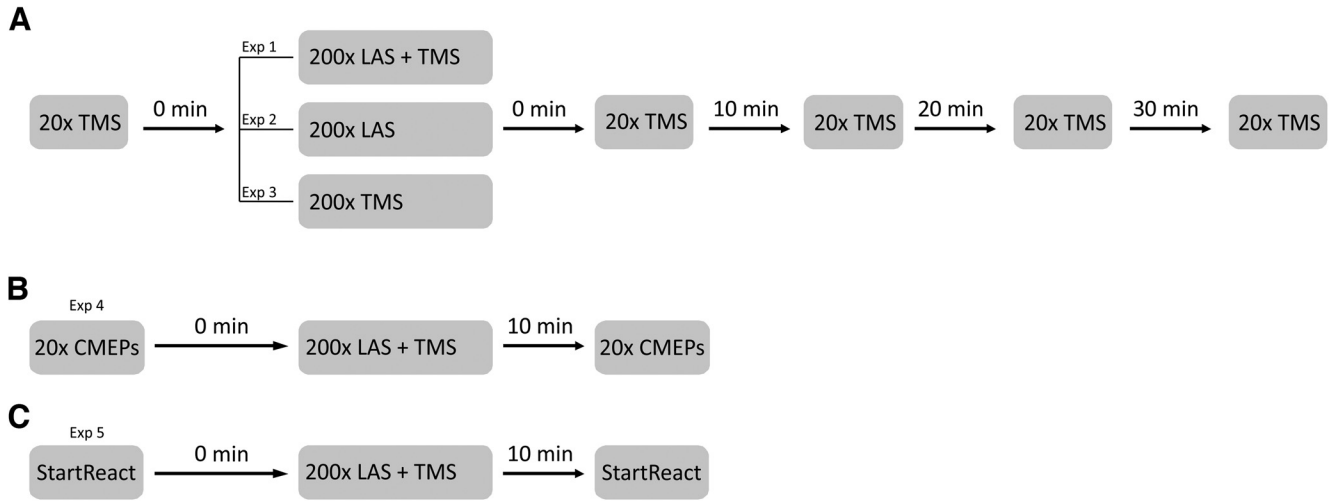

Figure 1. Experimental paradigm. ***A***, Experiments 1-3. Each group received 20 single pulse TMS before, immediately after, and 10, 20, and 30 min after the intervention. The three groups received three different interventions, consisting of paired stimulation of LAS with TMS (Experiment 1), LAS alone (Experiment 2), or TMS alone (Experiment 3). ***B***, Experiment 4. Twenty CMEPs were recorded immediately before and 10 min after the intervention. The intervention consisted of paired stimulation of LAS with TMS (same as Experiment 1). ***C***, Experiment 5. Participants performed the StartReact assessment immediately before and 10 min after the intervention. The intervention consisted of paired stimulation of an acoustic startle sound with TMS (same as Experiment 1).

in cortical excitability (McNeil et al., 2013). CMEPs were elicited via electrical stimulation of the corticospinal tract at brainstem level. Adhesive surface electrodes (Neuroline 720 00-S/25, Ambu) were fixed to the skin over the mastoid processes, and current was passed between them (0.1 ms duration, 80-200 mA; model DS7AH, Digitimer) with the cathode on the left side. The stimulation intensity was adjusted to elicit a CMEP amplitude of 1 mV with an active isometric contraction of 5%-10% maximum voluntary contraction. Twenty CMEPs were recorded before and 10 min after the intervention. The intervention consisted of the same 200 stimuli pairs as in Experiment 1 (Fig. 1*B*).

StartReact (Experiment 5)

The StartReact response was examined using a previously tested paradigm (Baker and Perez, 2017). During testing, subjects held their right arm relaxed, with their forearm supinated and resting on their lap. Subjects were asked to observe a red light-emitting diode (LED) located ~100 cm in front of them. When the LED was illuminated, participants were asked to flex their forearm up as fast as possible. Visual reaction time (VRT) was measured as the time from cue to onset of the EMG burst in the biceps muscle after the LED presentation. In some trials, the LED was presented with either a quiet acoustic stimulus (80 dB, 500 Hz, 50 ms) or a startling acoustic stimulus (120 dB, 500 Hz, 50 ms). Subjects were presented with five consecutive LAS, without performing the task, to familiarize them with the startling cue. The time delay between the presentation of the quiet acoustic stimulus and the onset of the EMG response was referred as the visual-auditory reaction time (VART), whereas the time between the LAS and the EMG onset was defined as the visual-startle reaction time (VSRT).

StartReact responses were recorded before and 10 min after the intervention (Fig. 1*C*). The intervention consisted of the same 200 stimuli pairs as in Experiment 1. In each task, 20 responses were recorded in each condition (VRT, VART, and VSRT) in a pseudorandomized order with an intertrial interval between 5 and 6 s.

Data analysis

Data were analyzed using MATLAB (R2017a, The MathWorks). EMG traces were full-wave rectified and then averaged. MEP amplitude was measured as the area under the curve (AUC) of this average. Onset and offset latencies for this measurement were chosen interactively by the experimenter for each subject; we have found this more reliable than using an automated criterion for latency determination (e.g., the point where the average exceeds 2× SD of baseline), which can often miss small early components of the response. A similar approach has been used by other studies previously (Rossini et al., 1992; Collins et al., 2017a, b; Collins and Button, 2018; Cantone et al., 2019). AUC was normalized to the baseline measurement made before the intervention, by expressing it as a percentage ([assessment MEP × 100]/baseline MEP). All statistics were performed using IBM SPSS Statistics for Windows, version 24. Sphericity was tested with Mauchly's test of sphericity. When sphericity could not be assumed, the Greenhouse–Geisser correction statistic was used.

To compare effects across Experiments 1-3, a two-way mixed ANOVA with between-subject factor CONDITION and within-subject factor TIME was performed. A Tukey Honest Significant Difference Test was used to assess the significance of differences between pairs of group means *post hoc*. Unpaired *t* tests were used to compare individual time points between groups *post hoc*. Only baseline and pre- and post-time points were included in the analysis.

For comparison of the early and late component of the EMG responses, data from 10, 20, and 30 min after the intervention were averaged together, as those were the time points that showed a significant facilitation. Everything from the beginning of the MEP up to the second peak on the rectified EMG traces was defined as the earlier component of the response, with the remaining MEP classified as the later component.

A two-way mixed ANOVA with between-subject factor CONDITION and within-subject TIME was performed on background EMG from Experiments 1-3. Background EMG was defined as mean rectified EMG 150 to 50 ms before TMS stimulation.

For Experiments 1-3, one-way repeated-measures ANOVAs were performed to determine the effect of TIME (before, during, and 0, 10, 20, and 30 min after intervention) on MEP AUC. Only baseline and pre- and post-time points were included in the analysis. For Experiment 4, a one-way repeated-measures ANOVA was performed to determine the effect of TIME (before and after) on CMEP AUC.

For Experiment 5, a two-way repeated-measures ANOVA was performed to determine the effect of SOUND (VRT, VART, and VSRT) and TIME (before and after intervention) on reaction time. An automated program identified the reaction time, defined as the time point where mean rectified EMG signals exceeded 7 SD of the mean EMG measured 200 ms before each stimulus presentation; every trial was inspected visually, and erroneous activity onset times (caused, e.g., by electrical noise artifacts) were manually corrected.

Paired *t* tests were used to compare individual data points *post hoc* and reported with effect size Cohen's *d*. The Benjamini–Hochberg procedure was used to correct for multiple comparisons (Benjamini and Hochberg, 1995). The significance level was set at $p < 0.05$, and group data are presented as mean ± SD in the text.

Binomial tests were performed to determine whether the number of subjects showing a certain change (increase or decrease) were more than

expected by chance based on a binomial distribution, with N = number of subjects and $p = 0.5$. Two-tailed binomial tests were used. Overall, 30 subjects were included in the final analysis for Experiments 1-3 and 15 subjects for Experiments 4 and 5.

Additional study in monkey

The indirect measurements made in humans were supplemented with data from 2 macaque monkeys, in which direct recordings of volleys from the spinal cord were possible.

All animal experiments were conducted under authority of appropriate licenses from the UK Home Office and were approved by the Animal Welfare and Ethical Review Board of Newcastle University. Two adult female macaques (Monkey O: weight 7.75 kg, age 6 years 9 months; Monkey V: weight 7.64 kg, age 7 years) were trained on a variety of grasp tasks for another, unrelated study, and were then surgically implanted with a titanium headpiece to allow head fixation. The headpiece incorporated chambers that allowed access to craniotomies over right M1 and the bilateral reticular formation. In the same surgery, EMG electrodes were implanted in hand and forearm muscles on the left side, and the wires tunneled subcutaneously to a connector on the head. A subsequent brief surgery implanted electrodes for stimulation in the pyramidal tract (PT) at the medulla, as we have previously reported (Baker et al., 1999). Single-unit recordings were made during task performance for the main study, lasting 8 months in Monkey V and 15 months in Monkey O. A further surgery then implanted a recording chamber over the cervical enlargement of the spinal cord, involving fusing vertebrae from C4-T2 (Perlmutter et al., 1998; Riddle and Baker, 2010; Williams et al., 2010), after which neural recordings of spinal activity during task performance were made. All surgical implants were performed with aseptic technique and under full general anesthesia (sedation with 10 mg/kg ketamine IM; maintenance with sevoflurane [1.9%-2.6%] in 100% O_2 with continuous IV infusion of alfentanil, 0.4 μg/kg/h). Monitoring during surgery included pulse oximetry, capnography, noninvasive blood pressure, heart rate, and core and peripheral temperature. Intravenous fluids were given (infusion rate, including drug infusions 5-10 ml/kg/h). The airway was protected with a tracheal catheter, and positive pressure ventilation used. A continuous IV infusion of methylprednisolone (5.4 mg/kg/h) reduced edema. The animal was kept warm with a thermostatically controlled heating blanket, and also a supply of warm air. Postoperative analgesics (buprenorphine 20-30 μg/kg, meloxicam, 0.2 mg/kg), dexamethasone (0.25 mg/kg), and prophylactic antibiotics (Monkey V: 12.5 mg/kg Synulox SC; Monkey O: 2 × 50 mg Synulox orally) were given.

For the present study, measurements were made once from each monkey in the conscious state, when all parts of the main experiment in that animal had been completed. Because both animals had metal headpieces, and the head fixation device in our primate chairs uses large metal blocks, it was not feasible to use TMS to activate the motor cortex. Fortunately, the dural surface over M1 was exposed in a recording chamber already in these animals, allowing us instead to stimulate epidurally. Cathodal stimulation of the cortical surface produces similar descending volleys to TMS (Patton and Amassian, 1954; Rosenthal et al., 1967; Edgley et al., 1990). In Monkey V, at the conclusion of recordings from the M1 chamber, a fine wire (75 μm stainless steel, insulated with Teflon, catalog #FE6215, Advent Research Materials) was bared for a few millimeters at its tip, and this tip was placed on the dura overlying M1 within the recording chamber. The chamber was then sealed with dental acrylic, allowing M1 to be stimulated subsequently simply by connecting to the implanted wire. In Monkey O, the chamber was opened and a silver ball electrode was temporarily placed on the dura to allow M1 stimulation. On the day of the study, the animal entered the primate chair, and the head and spinal chamber were stabilized. An electrode with 32 contacts (0.1 mm intercontact spacing, U probe, Plexon) was penetrated through the spinal dura targeting the lateral funiculus. Stimulation through the M1 electrode was then conducted (Monkey O, 10 mA, biphasic pulses, 0.2 ms per phase, cathodal stimulus first; Monkey V, 10 mA, 0.4 ms cathodal pulse; both with model 2100 isolated stimulator, AM Systems). Clear D and I wave volleys were visible on the spinal recordings.

A block of stimuli was given to assess EMG and spinal responses; this formed a standardized assessment, which was repeated throughout the study. In Monkey O, a block comprised 20 stimuli to M1 at a minimum interstimulus of 10 s. In Monkey V, a block comprised 50 stimuli to M1, and 50 stimuli to the chronically implanted PT electrode (train of two 500 μA stimuli, biphasic pulses, 0.1 ms per phase, 3 ms between stimuli in the train, also model 2100 stimulator, AM Systems). Stimuli to M1 and the PT were alternated, with minimum interstimulus interval 2.5 s. Stimuli were only given if the rectified EMG remained lower than a preselected amplitude, chosen based on recording noise, for 200 ms; this ensured that the animal was always at rest when stimuli were delivered. The EMG used for this purpose was flexor digitorum superficialis in Monkey O, and first dorsal interosseous (1DI) in Monkey V. There was a strong correlation between muscles, such that, if one muscle was at rest, it was a good indicator that the monkey was sitting quietly.

Two assessment blocks were given, beginning 0 and 5 min after the start of the experiment. The intervention started 10 min after the start of the study, in which M1 stimulation as above was paired with LAS. The LAS was generated using the same system and with the same input waveform as for the human subjects (50 ms duration, 500 Hz, 120 dB SPL); M1 stimulation was given at the end of the sound. The interstimulus interval was 10 s; 200 paired stimuli were given. The activity level of the animal was not used to gate these stimuli, but in practice the monkey also sat quietly for this part. After the paired stimulation intervention, seven further blocks of assessment stimuli were given starting 0-30 min after the end of the intervention, in 5 min steps.

The analysis of EMG responses proceeded as for the data from human subjects, with measurement of the AUC of averages of full-wave rectified signals. Clear responses were seen in both animals from the 1DI muscle; this reflects the location of the M1 stimulus over the hand representation, which had been the target of our earlier experiments in these animals. Results from 1DI are therefore presented here. Averages of the spinal recordings were used to identify the profile of the D and I wave volleys with depth; good recordings were seen over the first half of the array, corresponding to the expected location of the dorsolateral funiculus. The probe contact with the largest volleys in the baseline measurements was used in all subsequent analysis. Individual components of the response were identified based on their latency, as described in previous work (Edgley et al., 1990). The amplitude was measured from onset to peak, except for the D wave in Monkey O, which was measured from peak to offset because the stimulus artifact prevented accurate visualization of the onset. For both EMG and volleys, measurements were made from all available single sweeps at one time point. The mean of these values is mathematically the same as making the same measurement from the average; the SD was used as an assessment of variability. Measures were expressed as a percentage of those in the baseline period; t tests on the single sweep values were used to assess the significance of any change relative to the baseline. This involved seven independent comparisons; correction for multiple comparisons used the Benjamini–Hochberg procedure, as for the human studies.

Results

Experiment 1: effects of paired stimulation

In this group, subjects ($N = 30$) received 200 stimulus pairs of a loud acoustic stimulus 50 ms before the TMS pulse. Figure 2*A* illustrates average traces of MEPs elicited by TMS in the biceps muscle from a representative subject. Note how MEP size is drastically suppressed during the intervention and then shows a small but consistent increase after.

Group data for the paired condition is shown in Figure 3*A*; results during the intervention period (point marked "Startle+ TMS") are shown for completeness but were not included in any of the statistical comparisons. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated ($\chi^2_{(9)} = 66.196$, $p < 0.001$); therefore, a Greenhouse–Geisser correction was applied ($\varepsilon = 0.555$). A one-way repeated-measures ANOVA showed that there was a significant change in MEP AUC over time ($F_{(2.220,\ 64.379)} = 4.762$, $p = 0.010$, partial $\eta^2 = 0.141$). MEP AUC showed no change during the intervention ($t_{(29)} =$

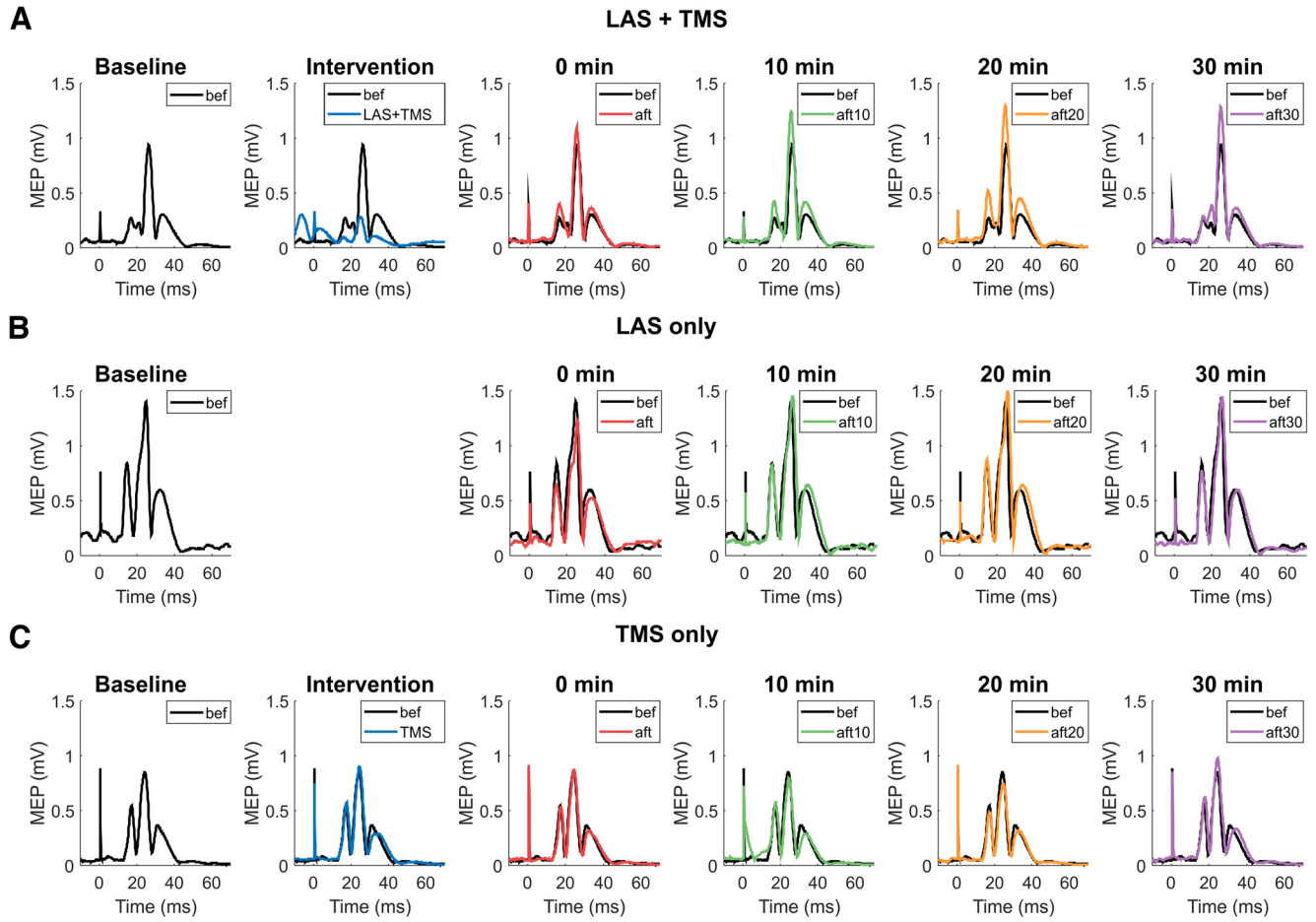

Figure 2. Single subject examples. Average traces of MEPs elicited by TMS in the biceps muscle from a representative subject for Experiment 1 (***A***), Experiment 2 (***B***), and Experiment 3 (***C***). Waveforms represent the average of 20 sweeps of rectified EMG, except for waveforms during the intervention (blue traces), which represent the average of 200 sweeps. Traces for each time point (colored traces) are overlaid with baseline average (black trace) for comparison.

-1.503, $p = 0.144$, $d = -0.2777$), although more subjects than expected by chance based on a binomial distribution showed a decreased MEP size during the intervention ($p < 0.001$; Fig. 3*A*, right). There was also no change in MEP AUC immediately after the intervention ($t_{(29)} = 1.276$, $p = 0.212$, $d = 0.2359$). However, 10, 20, and 30 min after receiving the paired stimulation, MEP AUC was significantly increased compared with baseline ($t_{(29)} = 2.372$, $p = 0.025$, $d = 0.4389$, $t_{(29)} = 2.612$, $p = 0.014$, $d = 0.4842$, and $t_{(29)} = 2.933$, $p = 0.006$, $d = 0.5424$). Importantly, this increase was seen in a significant majority of subjects (more than expected by chance based on a binomial distribution; $p = 0.005$, $p = 0.001$, $p < 0.001$; Fig. 3*A*, right).

Interestingly, when looking at the averaged EMG responses combined across the 10, 20, and 30 min time points after the intervention (Fig. 4*A*; these were the time points with significant facilitation), it appeared that the later part of the response showed a more pronounced facilitation. When quantifying the AUC for the early and late component of the EMG response separately (Fig. 4*B*), it was revealed that the early component was significantly facilitated compared with baseline ($t_{(29)} = 2.598$, $p = 0.015$, $d = 0.474$), as was the later component ($t_{(29)} = 2.869$, $p = 0.008$, $d = 0.524$), but the later part was significantly more facilitated than the earlier part ($t_{(29)} = 2.740$, $p = 0.010$, $d = 0.500$).

Experiment 2: effects of loud acoustic stimulus alone

As a control experiment, subjects ($N = 30$) received 200 LAS without any TMS during the intervention period. Figure 2*B* illustrates average traces of MEPs elicited by TMS in the biceps muscle from a representative subject. There was no MEP to illustrate during the intervention, as subjects did not receive any TMS. Participants were, however, still instructed to maintain a controlled isometric contraction, equal to Experiment 1.

Average MEP AUC across the group are shown in Figure 3*B*. The assumption of sphericity was violated, as assessed by Mauchly's test of sphericity ($\chi^2_{(9)} = 33.691$, $p < 0.001$). Therefore, a Greenhouse–Geisser correction was applied ($\varepsilon = 0.679$).

There was no significant change in MEP AUC across time points, as shown by a one-way repeated-measures ANOVA ($F_{(2.714,78.707)} = 0.541$, $p = 0.638$, partial $\eta^2 = 0.018$). Furthermore, similar numbers of subjects showed an increase or decrease in MEP size ($p = 0.042$, $p = 0.099$, $p = 0.856$, $p = 1$, binomial tests, threshold for significance $p < 0.0125$ using Benjamini–Hochberg correction; Fig. 3*B*, right). We can therefore conclude that the effects observed during Experiment 1 are not likely simply to be because of LAS.

Experiment 3: effects of TMS alone

In a second control experiment, subjects ($N = 30$) received 200 single pulse TMS, without any LAS during the intervention period. Figure 2*C* illustrates average traces of MEPs elicited by TMS in the biceps muscle from a representative subject

Average MEP AUCs across the group are shown in Figure 3*C*. Again, results for the intervention period ("TMS only") are plotted

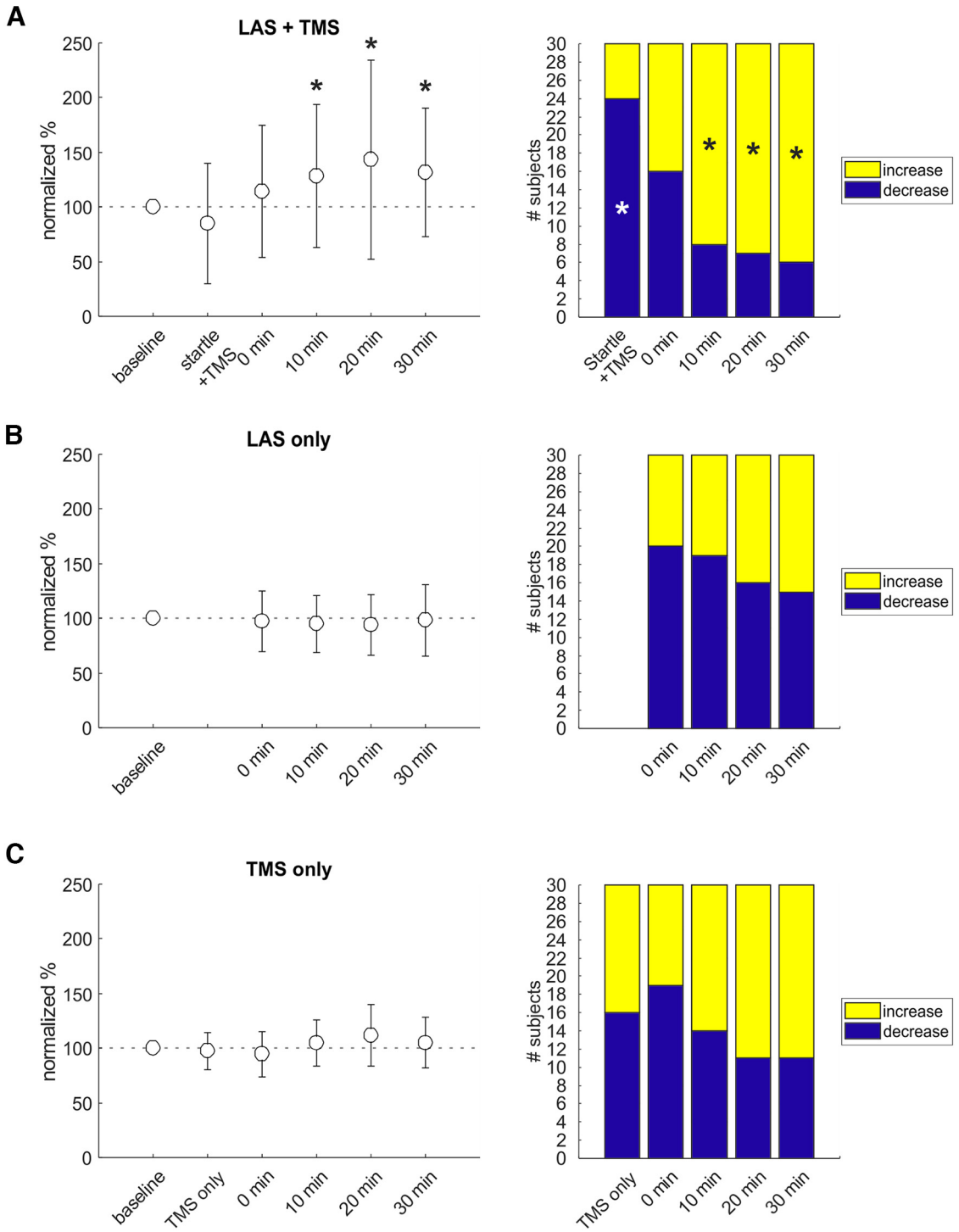

Figure 3. Group data. Group results for Experiment 1 (***A***) Experiment 2 (***B***), and Experiment 3 (***C***). Left, Mean MEP AUC for each time point during the experiment, normalized as a percentage of the baseline. *$p < 0.05$. Error bars indicate SDs. Right, Number of subjects showing either an increase (yellow) or decrease (blue) in MEP AUC compared with baseline. Asterisks indicate proportions significantly different from the 50% expected by chance, based on a binomial distribution.

on Figure 3*C* for completeness but were not included in any statistical comparisons. The assumption of sphericity was met, as assessed by Mauchly's test of sphericity ($\chi^2_{(9)} = 10.769$, $p = 0.293$). A one-way repeated measures ANOVA showed a significant effect of time ($F_{(4,116)} = 4.180$, $p = 0.003$, partial $\eta^2 = 0.126$). However, again, there was no significant majority (more than expected by chance based on a binomial distribution) of subjects showing either an increase or decrease in MEP AUC ($p = 0.855$, $p = 0.099$, $p = 0.856$, $p = 0.099$, $p = 0.099$; Fig. 3*C*, right). *Post hoc t* tests confirmed that MEP AUC was not significantly different from baseline at any point during ($t_{(29)} = -0.765$, $p = 0.450$, $d = -0.1355$) or after the intervention ($t_{(29)} = -1.395$, $p = 0.174$, $d = -0.2508$, $t_{(29)} = 1.225$, $p = 0.231$, $d = 0.2205$, $t_{(29)} = 2.247$, $p = 0.032$, $d = 0.4098$ and $t_{(29)} = 1.145$, $p = 0.261$, $d = 0.2072$; threshold for significance $p < 0.01$ using Benjamini–Hochberg correction for multiple

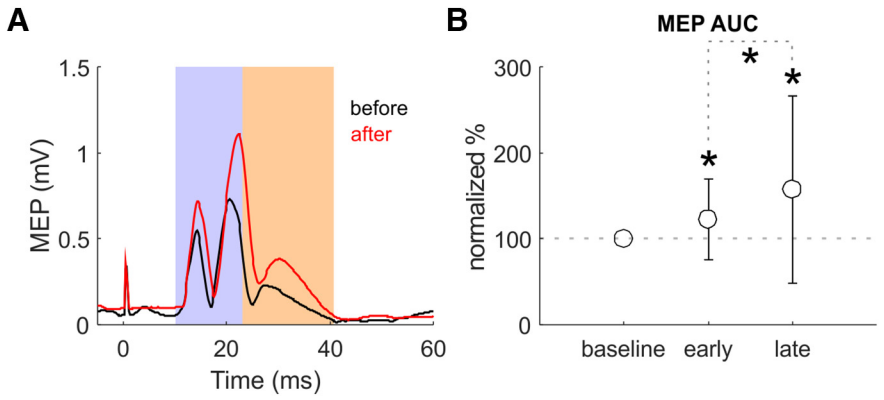

Figure 4. Comparison of early and late MEP components, after paired LAS+TMS. ***A***, Example traces of a single subject, averaged across MEPs at baseline (black) and MEPs elicited 10, 20, and 30 min after the LAS+TMS intervention (red). Waveforms represent the average of 20 sweeps (baseline) or 60 sweeps (after). ***B***, Mean MEP AUC, normalized to baseline, of the early and late MEP component. MEPs elicited 10, 20, and 30 min after LAS+TMS pairing were averaged together. *$p < 0.05$. Error bars indicate SDs.

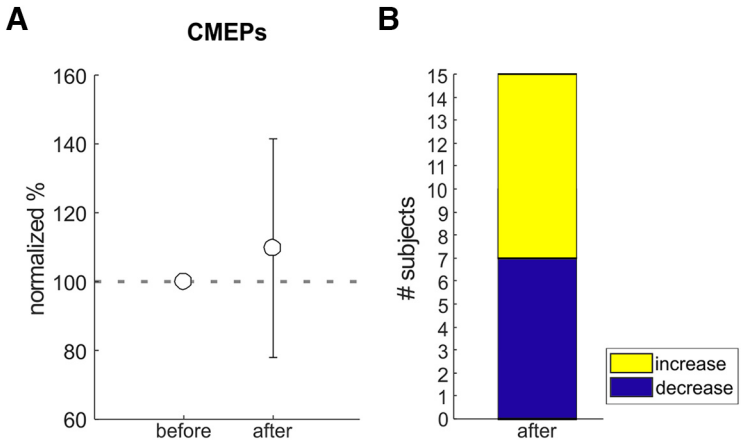

Figure 5. CMEP group results. ***A***, Mean MEP AUC normalized to the baseline. ***B***, Number of subjects ($N = 15$) showing either an increase (yellow) or decrease (blue) in CMEP AUC compared with baseline.

comparisons to give overall $p < 0.05$). It is therefore unlikely that the effects seen in Experiment 1 are because of prolonged TMS.

Comparison of results from Experiments 1-3

In order to compare effects across Experiments 1-3 (paired stimulation, loud acoustic stimulus alone, and TMS alone), a two-way mixed ANOVA was performed. The assumption of sphericity was not met, as assessed by Mauchly's test of sphericity ($\chi^2_{(9)} = 101.228$, $p < 0.001$) and a Greenhouse–Geisser correction was applied ($\varepsilon = 0.667$). The results of the mixed ANOVA confirmed that there was a significant main effect of TIME ($F_{(2.666,\ 231.983)} = 5.183$, $p = 0.003$, partial $\eta^2 = 0.056$) and CONDITION ($F_{(2,87)} = 5.525$, $p = 0.006$, partial $\eta^2 = 0.113$), as well as their interaction ($F_{(5.333,\ 231.983)} = 3.568$, $p = 0.003$, partial $\eta^2 = 0.076$) on MEP AUC. A Tukey *post hoc* test revealed that mean MEP AUC was significantly increased after paired stimulation compared with the loud acoustic stimulus alone (mean difference 26.498, 95% CI = [6.6117, 46.3839], $p = 0.006$) or TMS alone (mean difference 20.3052, 95% CI = [0.4191, 40.1913], $p = 0.044$). There was no statistically significant difference between the LAS only and TMS only condition (mean difference 6.1926, 95% CI = [−26.0787, 13.6935], $p = 0.739$).

Unpaired *t* tests were used *post hoc* to compare significant time points (10, 20, and 30 min after intervention) across groups. This revealed that after paired stimulation MEP AUC was significantly increased at 10, 20, and 30 min ($t_{(58)} = 2.582$, $p = 0.012$, $d = 0.667$, $t_{(58)} = 2.845$, $p = 0.006$, $d = 0.735$, $t_{(58)} = 2.690$, $p = 0.009$, $d = 0.695$) compared with LAS, alone as well as 30 min after the intervention compared with TMS alone ($t_{(58)} = 2.311$, $p = 0.024$, $d = 0.597$), while this difference failed to reach significance after 10 or 20 min ($t_{(58)} = 1.875$, $p = 0.066$, $t_{(58)} = 1.831$, $p = 0.072$).

Participants were instructed to control their contraction level throughout Experiments 1-3, using visual feedback of biceps EMG level on a computer screen. Statistical analysis confirmed that background EMG during voluntary contraction was consistent across TIME ($F_{(3.950,\ 343.640)} = 1.257$, $p = 0.287$) and CONDITION ($F_{(2,87)} = 1.823$, $p = 0.168$), with no interaction ($F_{(7.900,\ 343.640)} = 1.222$, $p = 0.285$), as assessed by a two-way mixed ANOVA.

Baseline MEP amplitudes were similar across the three groups (paired stimulation 10.6 ± 5.2, LAS only 10.9 ± 6.2, TMS only 11.5 ± 8.2; mV.ms; mean ± SD).

Experiment 4: CMEPs

To test for changes in spinal excitability, CMEPs were measured before and after the intervention (LAS paired with TMS, as in Experiment 1). Figure 5*A* shows the group data ($N = 15$ participants), with mean MEP AUC normalized to the baseline. There was no significant change in CMEP AUC after the intervention, as shown by a one-way repeated-measures ANOVA ($F_{(1,14)} = 1.905$, $p = 0.189$, partial $\eta^2 = 0.120$). Similar numbers of individual subjects showed an increase or a decrease in CMEP AUC after the intervention (Fig. 5*B*).

Experiment 5: StartReact

Figure 6*A* illustrates the mean reaction times before and 10 min after the intervention. Participants ($N = 15$) received the same intervention (LAS paired with TMS) as in Experiment 1.

The assumption of sphericity was met, as assessed by Mauchly's test of sphericity ($\chi^2_{(2)} = 4.237$, $p = 0.115$). A two-way repeated-measures ANOVA was performed to test the effects of SOUND (VRT, VART, and VSRT) and TIME (before, after). There was no statistically significant two-way interaction between SOUND and TIME ($F_{(2,28)} = 0.530$, $p = 0.595$) and no significant main effect of TIME ($F_{(1,14)} = 4.080$, $p = 0.063$). The main effect of SOUND showed a statistically significant difference in reaction time between trials ($F_{(2,28)} = 189.894$, $p < 0.001$).

Post hoc testing showed that there was a significant *StartReact* effect (difference in VART vs VSRT) before ($t_{(14)} = 4.530$, $p < 0.001$, $d = 1.053$) and after ($t_{(14)} = 3.533$, $p = 0.003$, $d = 0.601$) the intervention. However, *StartReact* before the intervention did not differ from *StartReact* after ($t_{(14)} = -0.670$, $p = 0.514$; Fig. 6*B*).

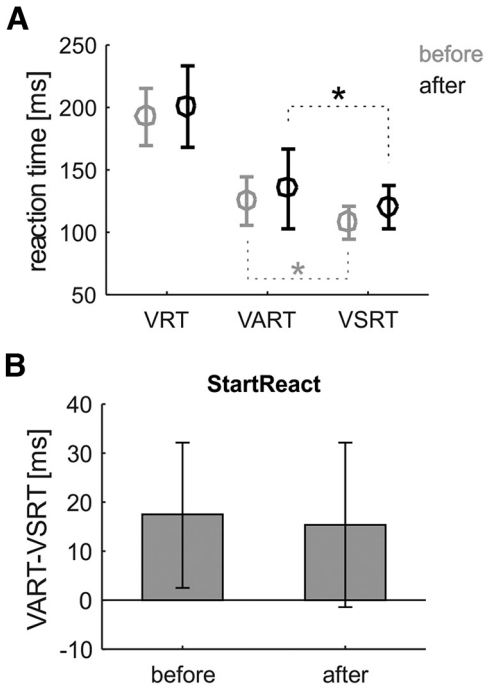

Figure 6. StartReact group results. ***A***, Mean VRT, VART, and VSRT before (gray) and 10 min after (black) the intervention. The StartReact effect was significant both before and after the intervention (*). Error bars indicate SDs. ***B***, StartReact effect (difference in VART vs VSRT) before and 10 min after the intervention. Error bars indicate SDs.

Experiment in monkey

Noninvasive measurements in human subjects necessarily allow only indirect conclusions about the pathways involved. In this study, we were fortunate to be able to test the paired stimulation protocol also in implanted macaque monkeys, where invasive recordings of spinal volleys could be measured alongside EMG. Figure 7 shows the results from these monkey experiments.

The core result observed in humans was largely replicated in both monkeys. Following pairing of M1 stimulation with loud sound, the EMG response of the 1DI muscle was elevated immediately and remained significantly higher than baseline for >15 min (Fig. 7*A*) in Monkey O. In Monkey V, there was also a rise in the EMG response, although this was delayed after the stimulus pairing. The increases at 15 and 20 min after the intervention were significant at the individual level but failed to pass the correction for multiple comparisons, so this should be considered a trend rather than a definitive effect (Fig. 7*B*). These increases were, however, not accompanied by comparable increases in the size of descending volleys at the spinal level. In Monkey O, the D wave volley was initially suppressed but recovered to its baseline amplitude by 15 min after the stimulus pairing. Both I1 and I2 volleys were significantly suppressed at all time points after the intervention; the I3 volley was also suppressed, although this did not develop immediately, but showed a significant reduction only after 10 min (Fig. 7*C*). In Monkey V, both D and I waves were significantly reduced for almost at all time points measured (Fig. 7*D*).

In Monkey V, we also stimulated the PT directly during each assessment block. We would expect the extent of activation to remain constant, since this stimulation of the corticospinal tract in the medulla acts on axons distant from the initial segment, and hence is unaffected by cortical excitability. Instead, there was a steady decline in the size of this direct volley after the intervention (Fig. 7*E*). It is possible that this reflects a change in the recording conditions, for example, a progressive accumulation of fluid around the spinal recording electrode which would shunt signals and reduce their amplitude. However, it was notable that the size of these (presumed artifactual) changes was comparable to those seen in the D wave from M1. The I waves from M1 showed an earlier and greater decrease than the D wave following PT stimulation, suggesting that there was a small genuine suppression of corticospinal output. None of the volley recordings, from either animal, was consistent with LTP of corticospinal output, suggesting that the increase in EMG responses likely had a subcortical origin.

Further insight came from examination of the EMG responses to the PT stimulation in Monkey V, which are shown in Figure 8. Measurement of the AUC for this response indicated a significant facilitation, which lasted until 30 min after the intervention (Fig. 8*A*). However, the averaged traces of the stimulus-evoked activity revealed more complexity. The earliest part of the response seemed to show little change, whereas after the intervention there was a substantial facilitation in the later response component (Fig. 8*B*). This was further quantified by measuring the AUC for each part separately. The early component did not change significantly after the intervention (Fig. 8*C*), but there were robust and strong increases in the later component (Fig. 8*D*). In setting up for this experiment, we found that a single stimulus to the PT produced only a weak response in the 1DI muscle with the monkey at rest, and therefore chose to deliver a train of two stimuli. It is well known that stimulus trains can enhance transmission over multiple synapses by temporal facilitation (Riddle et al., 2009). The finding in monkey that the earliest part of the muscle response to PT stimulation did not change after the intervention is comparable to the finding in humans with CMEPs, where only a single stimulus was used. The changes in the later, presumed oligosynaptic parts of the PT response agree with a subcortical basis for the plasticity.

Discussion

The current study demonstrated that repeated exposure to paired LAS and TMS generates a MEP facilitation, which could last for at least 30 min after the stimulus pairing (Fig. 3*A*). This facilitation was not observed in either control group (Experiments 2 and 3; Fig. 3*B*,*C*), indicating that stimulus pairing was required. Interestingly, there was no increase in MEP amplitude immediately after the end of the pairing; it took time to develop; this was also seen in one of the monkeys (Fig. 7*B*). A similar delayed enhancement of responses has been reported using other plasticity protocols (Taylor and Martin, 2009), with some evidence that delay may depend on age (Fujiyama et al., 2014).

Timing of stimuli in a paired associative stimulation (PAS) protocol is critical for facilitating plastic change (Stefan et al., 2002; Mrachacz-Kersting et al., 2007; Murakami et al., 2008; Kumpulainen et al., 2012). Here we used a 50 ms ISI between the onset of the auditory stimulus and M1 stimulus. Loud sounds facilitate the H reflex at ISIs of ≥50 ms (Rossignol and Jones, 1976; Rudell and Eberle, 1985; Nakashima et al., 1994; Delwaide and Schepens, 1995), indicating an increase in motoneuronal excitability. The suppression of TMS-evoked MEPs at an interval of 50 ms, as reported previously by others (Furubayashi et al., 2000; Kuhn et al., 2004; Tazoe and Perez, 2017), must therefore reflect a cortical suppression superimposed on a smaller spinal facilitation (Germann and Baker, 2021). The cortical suppression most likely results from activation of the reticular formation following the loud sound (Hammond, 1973; Leitner et al., 1980; Davis et al., 1982; Fisher et al., 2012; Tapia et al., 2022), and

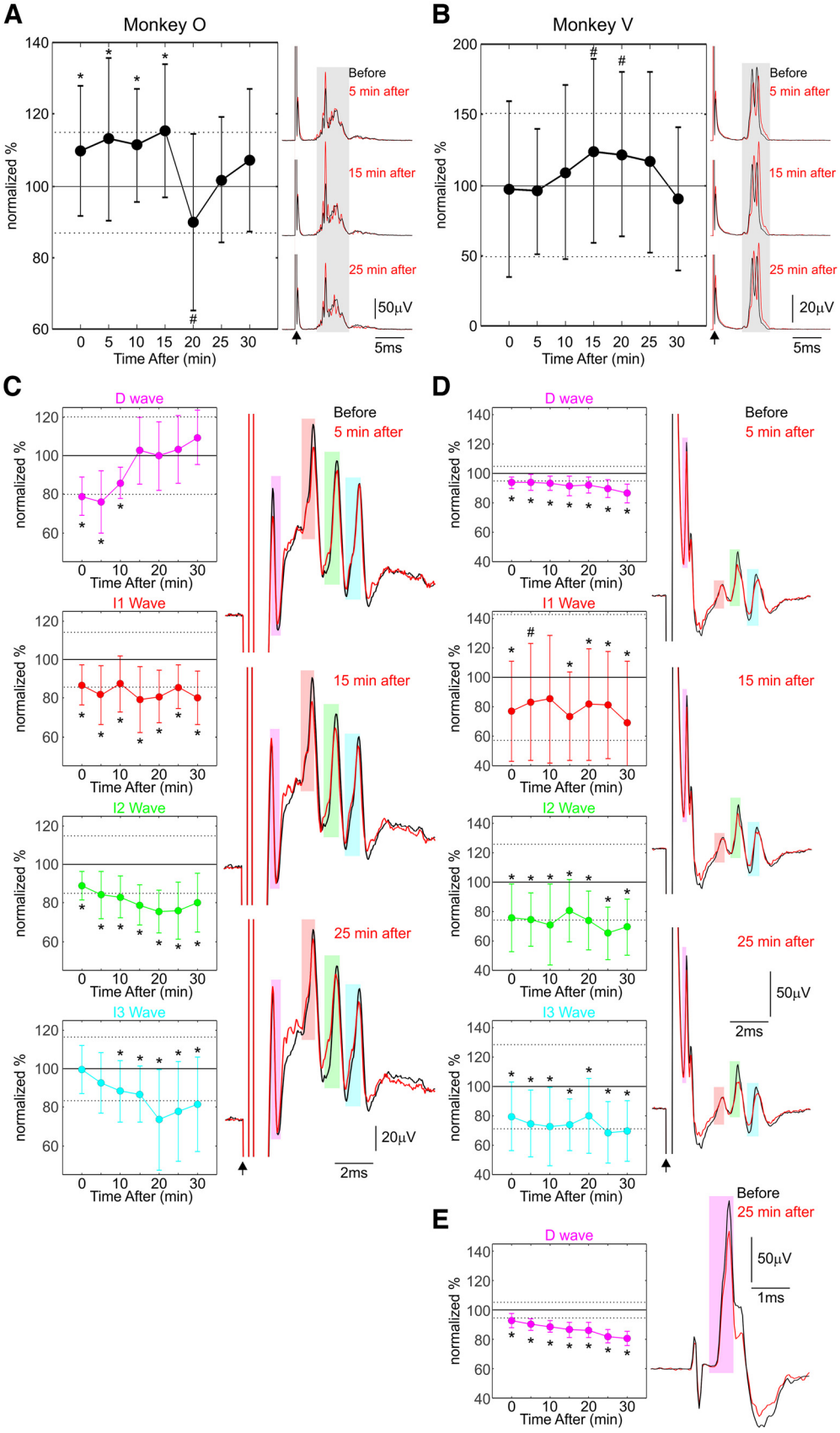

Figure 7. Measurements in monkey. ***A***, Change in MEP in the 1DI muscle after M1 stimulation as a percentage of baseline, at different time points after paired M1-LAS stimulation, for Monkey O. Example traces are illustrated on the right in red, with the baseline response superimposed on all in black for comparison. Gray shading represents the region used to measure the MEP AUC. ***B***, Same as in ***A***, but for Monkey V. ***C***, Changes in the amplitude of ***D***, I1, I2, and I3 volleys measured in the spinal cord following M1 stimulation, at the same time points after paired M1-LAS stimulation as in ***A***, for Monkey O. Right, Example volleys (in red), with the baseline measurement overlain (in black) for comparison. Colored shading represents the regions used to

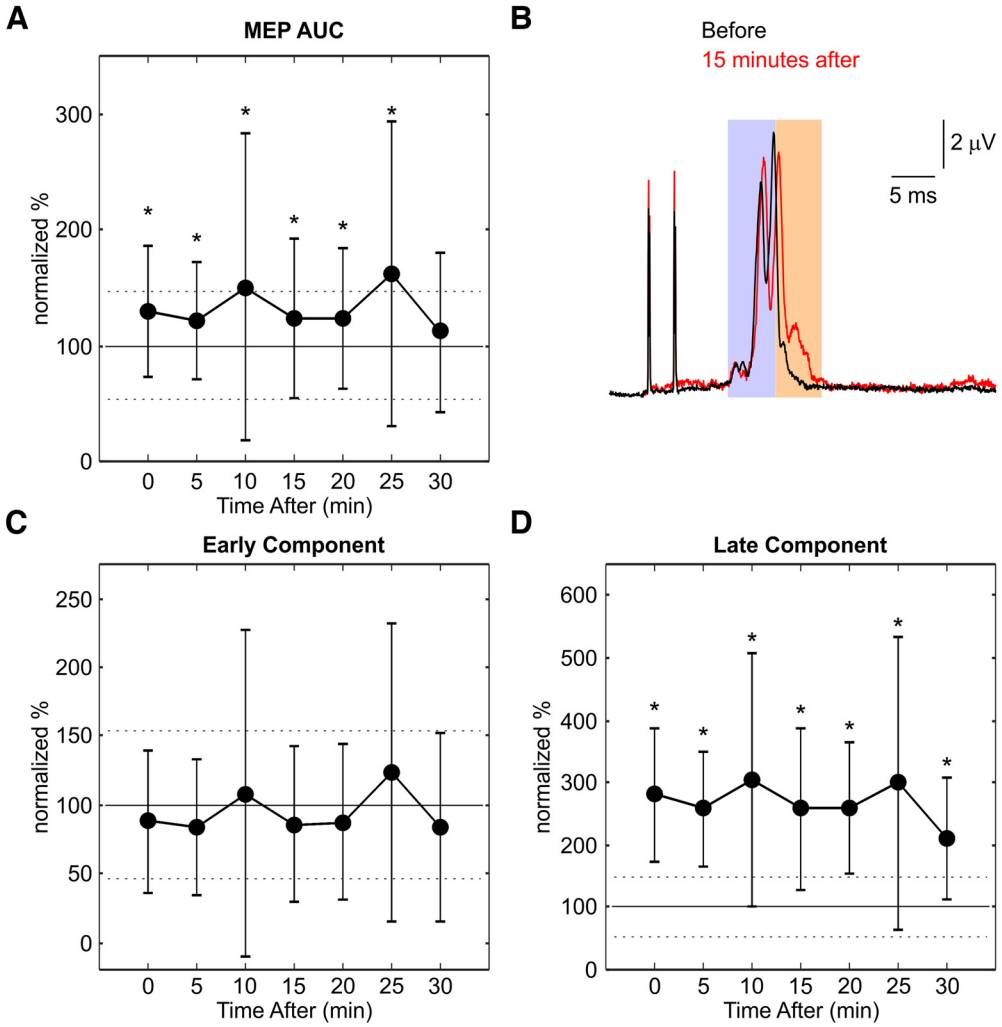

Figure 8. MEP responses to direct PT stimulation in Monkey V. ***A***, Change in AUC of MEP in the 1DI muscle as a percentage of baseline, as a function of time after paired M1-LAS stimulation. ***B***, Example response, at 15 min time point (red), with the baseline response overlain in black for comparison. ***C***, Same as in ***A***, but for the early response component shaded purple in ***B***. ***D***, For the late component, shaded orange in ***B***. ***A***, ***C***, ***D***, $*p < 0.05$, significant change relative to baseline (*t* test corrected for multiple comparisons by the Benjamini–Hochberg procedure). Error bars indicate SD. Horizontal black lines indicate 100% corresponding to the baseline. Dotted lines indicate the SD of this baseline measurement. Points are the average of 50 sweeps, except for baseline measures, which were compiled from 100 sweeps.

subsequent activation of cortical interneurons via reticulothalamic projections (Paré et al., 1988; Steriade et al., 1988).

Changes in MEP size may be influenced by effects at many possible levels of the motor system (see schematic of Fig. 9). To identify the likely sites of plastic changes, we used the same PAS protocol used in Experiment 1 to measure changes in CMEPs and the StartReact effect in humans, and also made direct measurements of descending volleys in monkey.

A single cortical stimulus evokes multiple descending volleys to the spinal cord: the initial direct (D) activation of the corticospinal tract is followed by subsequent indirect (I) waves (Patton and Amassian, 1954; Rosenthal et al., 1967; Stoney et al., 1968; Jankowska et al., 1975). The size of both D and I waves is affected by the level of cortical excitability (Baker et al., 1995; Di Lazzaro et al., 1998). By contrast, because stimulation at the cervicomedullary junction activates the corticospinal tract at the brainstem level (Ugawa et al., 1991; Maertens de Noordhout et al., 1992), the size of the descending volley should be unaffected by the state of the cortex. In the biceps muscle, this stimulation can evoke responses consistent with both monosynaptic (Fig. 9*c*) (Petersen et al., 2002) and oligosynaptic transmission (Fig. 9*d*,*e*) (Nakajima et al., 2017).

We found no change in CMEP amplitude after paired stimulation compared with baseline (Fig. 5). Although CMEPs were measured at only one time point (10 min after the intervention), this was a time at which MEPs were significantly facilitated in Experiment 1 (Fig. 3*A*). The lack of changes in CMEPs therefore suggests that the plastic changes in MEPs were not mediated at corticospinal synapses (Fig. 9*c*,*d*). Consistent with this, there

←

measure the amplitude of each volley, with the same color used to plot the graphs on the left. ***D***, Same as in ***C***, but for Monkey V. ***E***, Change in D wave volley elicited by direct PT stimulation at the medulla, in Monkey V, after paired M1-LAS stimulation. Right, Plot represents an example trace, from 25 min after paired stimulation (red), with the baseline overlain for comparison (black). Shaded region represents the latencies over which the volley amplitude was measured. Note the different time base compared with ***C***, ***D***. In all plots of responses compared with baseline: $*p < 0.05$, significant change (*t* test) which passed the Benjamini–Hochberg correction for multiple comparisons. #Change with which it did not pass this correction, and hence must be considered a trend. Error bars indicate SD. Horizontal black lines indicate 100% corresponding to the baseline. Dotted lines indicate the SD of this baseline measurement. Points are the average of 20 sweeps for Monkey O, and 50 sweeps for Monkey V, except for baseline measures, which were compiled from 40 and 100 sweeps, respectively.

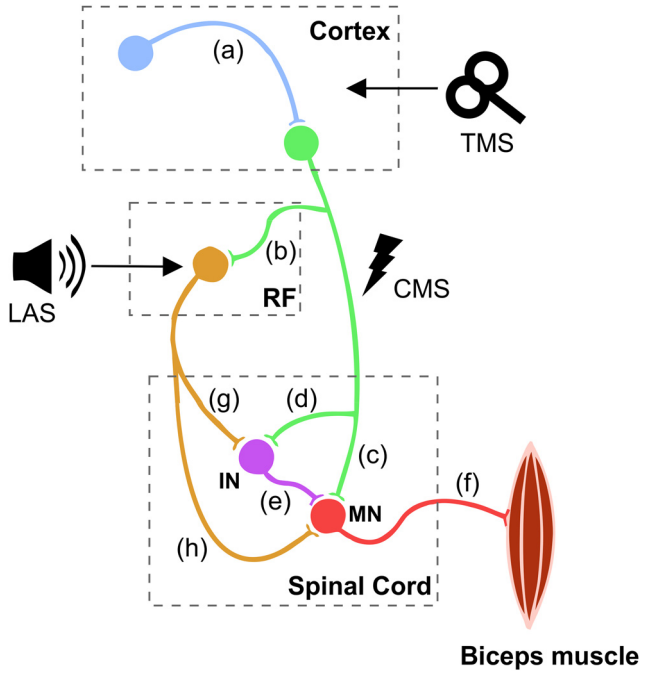

Figure 9. Schematic showing simplified pathways. RF, Reticular formation; IN, spinal cord interneuron; MN, motoneuron; CMS, cervicomedullary stimulation. (*a*) Intracortical connections, (*b*) corticoreticular connections, (*c*) cortico-motoneuronal synapses, (*d*) corticospinal projections to interneurons, (*e*) interneuron projections to motoneurons, (*f*) motoneuron projection to muscle, (*g*) reticulospinal projections to interneurons, and (*h*) reticulospinal projections to motoneurons.

were no changes in the earliest component of EMG responses following PT stimulation in monkey (Fig. 8), which are likely to reflect the most direct corticospinal pathways.

The reticulospinal tract is another possible site for the plasticity, especially since reticular formation neurons are known to be powerfully activated by loud sounds (Lingenhohl and Friauf, 1992) and play a pivotal role in the startle reflex (Davis et al., 1982). The RST makes both monosynaptic and disynaptic connections with upper limb motoneurons (Fig. 9*e*,*g*,*h*) (Riddle et al., 2009). StartReact seems to reflect the speeding up of motoneuron recruitment by enhanced RST activity produced by the LAS (Tapia et al., 2022); the StartReact effect remains intact or is enhanced in patients with damaged corticospinal tract (Honeycutt and Perreault, 2012; Nonnekes et al., 2014; Choudhury et al., 2019).

In Experiment 5, we observed a notable StartReact effect (difference in VART vs VSRT, Fig. 6*A*). However, the size of this difference did not change after the paired stimulation (Fig. 6*B*). If the plasticity increases in MEPs were underpinned by changes in reticulospinal connections (Fig. 9*e*,*g*,*h*), we would expect to see a change in the StartReact effect, as we recently reported using a different plasticity protocol designed to affect the RST (Germann and Baker, 2021). Since such changes did not occur, we conclude that the increased MEP size after paired stimulation was unlikely to be generated by modification of reticulospinal synapses.

A further way in which the MEP amplitude could show long-term changes is if there were increases in the excitability of either motoneurons or interneurons in the spinal cord, as this would lead to larger responses to the same synaptic input. However, such changes would also produce increases in CMEPs and the StartReact effect, which were not seen. Changes in motoneurons should also produce parallel changes in all parts of responses, rather than the selective increase in late components, which was seen in both monkey and human. We therefore rule out postsynaptic excitability changes as contributing to the plasticity which we have observed.

One explanation for our findings in humans is that there was potentiation of intracortical connections (Fig. 9*a*), which would lead to increased I wave volleys following TMS. Indeed, many previous studies have used a situation where MEPs change, but CMEPs do not, to argue for a cortical basis for the observed effect. However, at the 50 ms interstimulus interval used, the cortex was suppressed, meaning that, during the paired stimulation, MEPs were actually reduced in amplitude (Figs. 2*A*, 3*A*), as previously reported by other investigators (Furubayashi et al., 2000; Kuhn et al., 2004; Tazoe and Perez, 2017). It seems counterintuitive that a conditioning stimulus that reduces cortical excitability should lead to LTP of intracortical circuits. However, it should be noted that, in classic PAS, peripheral nerve stimulation is delivered at an interval that suppresses subsequent MEPs (Tokimura et al., 2000) but produces a long-lasting facilitation (Stefan et al., 2000); this form of PAS is typically accepted as generating cortical changes. Direct measurements of volleys evoked from cortical stimulation in monkey revealed an LTD (Fig. 7*A*–*D*), although a similar decrease in amplitude was also seen in the volley after stimulation of corticospinal axons at the medulla (Fig. 7*E*). This suggests that the decrease may have been an artifact of slowly changing recording conditions, since the volley evoked from direct axonal stimulation should be fixed. Nevertheless, the results in monkey certainly do not provide any evidence for an increase in cortical output after the paired stimulation, allowing us to rule out changes in the cortex as underlying the increased MEPs.

One remaining substrate for the observed plasticity, which could be consistent with our experimental findings, is the corticoreticular connection (Fig. 9*b*). It is known that M1 stimulation can powerfully activate corticoreticular fibers (Fisher et al., 2012, 2021), which form an extensive divergent and convergent network linking both primary and premotor areas bilaterally to the reticular nuclei (Fregosi et al., 2017; Darling et al., 2018; Fisher et al., 2021). If the LAS increased the excitability of reticulospinal cells, they would be more likely to respond to corticoreticular inputs, generating reliable postsynaptic spiking just after the corticoreticular presynaptic input, and thereby fulfilling the requirements for LTP by mechanisms of spike timing-dependent plasticity (Markram et al., 1997; Bi and Poo, 2001). We previously demonstrated that stimulation of the corticospinal tract can generate a reticulospinal volley by trans-synaptic activation of the reticular formation (Fisher et al., 2015); many corticoreticular axons are collaterals of corticospinal fibers (Keizer and Kuypers, 1989). Plastic changes at corticoreticular synapses would therefore lead to an increased reticulospinal output after M1 stimulation. It is known that, in primate cervical spinal cord, both motoneurons (Riddle et al., 2009) and interneurons (Riddle and Baker, 2010) receive extensive convergence from corticospinal and reticulospinal tracts (as is shown schematically in Fig. 9). Enhanced reticulospinal inputs would therefore very likely lead to larger MEPs. This circuit would explain why there was a selective effect on different components of the MEP. While the earliest part of the MEP originates from fast and direct corticospinal connections to motoneurons, later components could reflect more indirect pathways, including (but not limited to) cortico-reticulospinal projections.

Importantly, it appears that the method described here acts on a different circuit from another paradigm which we recently introduced, also with the aim of targeting subcortical systems

(Foysal et al., 2016; Germann and Baker, 2021). That paradigm paired loud auditory click sounds with electrical stimulation over a muscle (to activate low threshold afferents), and generated plastic changes in the StartReact effect, but not in MEPs elicited (as here) with the TMS coil oriented to induce current in a posterior-anterior direction. By contrast, with the present protocol, we generated changes in MEPs, but not in the StartReact effect. Stimulus-evoked plasticity is of great interest because it may allow the strengthening of residual motor pathways after damage (e.g., following stroke), and thereby boost functional recovery. Several approaches already exist to target the corticospinal system, but subcortical systems, such as spinal cord interneurons (Mazevet et al., 2003; Tohyama et al., 2017) and the reticulospinal tract, also play an important role in recovery. After corticospinal damage, spontaneous recovery leads to strengthening of reticulospinal connections (Zaaimi et al., 2012). The firing rate of reticular cells also increases, suggesting that there is strengthening of corticoreticular inputs (Zaaimi et al., 2018). Developing a diverse range of noninvasive stimulation protocols which can target these different circuits may allow us to enhance the components of change that already occur spontaneously, and thereby improve functional recovery.

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