Training of a discrete motor skill in humans is accompanied by increased excitability of the fastest corticospinal connections at movement onset

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Key points

- The primary motor cortex (M1) is fundamentally important for the acquisition of skilled motor behaviours.
- We tested the excitability changes of distinct M1 circuits at movement onset with TMS H-reflex conditioning.
- Human subjects trained a discrete spatiotemporal motor skill. Practice was associated with reduced kinematic variability and improved motor performance.
- Performance improvements were paralleled by task-specific excitability increases of the fastest corticospinal connections at infragranular layer 5b of M1. No task-related changes in excitability were observed at supragranular layers.
- Excitability changes in the fastest corticospinal connections were not directly related to changes in motor performance.

Abstract The primary motor cortex (M1) is fundamentally important for the acquisition of skilled motor behaviours. Recent advances in imaging and electrophysiological techniques have improved our understanding of M1 neural circuit modulation in rodents and non-human primates during motor learning. However, little remains known about the learning-related changes of distinct elements in the human brain. In this study, we tested excitability changes of different neural circuits (infragranular and supragranular layers) in the M1 of human subjects who underwent training in a discrete spatiotemporal motor skill. Excitability modulations were assessed by recording H-reflex facilitation from transcranial magnetic stimulation at movement onset. Motor practice improved the consistency of movements and was accompanied by an excitability increase of the fastest corticospinal connections during the initial stages of motor practice. No such excitability changes were observed for training in a simple motor skill and circuits at supragranular layers of M1. Notably, changes in excitability were not associated with changes...
inability to learn new motor skills (Kawai et al. 2015; Hwang et al. 2019). Notably, neural elements within M1 may undergo distinct changes during skill learning (Papale & Hooks, 2018). The infragranular layers of M1 contain corticospinal neurons that form long-range connections to spinal motoneurons (Oswald et al. 2013). These pathways influence the neural drive to the muscles and have been associated with movement production and several kinematic and muscular parameters (Omrami et al. 2017). The fastest direct corticospinal connections (corticomotoneuronal neurons) are essential for performing skilled hand and independent digit movements (Lemon, 2008). Neural elements at supragranular layers in M1 primarily form intracortical connections, including short-range connections to corticospinal neurons (Weiler et al. 2008; Hooks et al. 2011). Neurons at supragranular layers receive inputs from other cortical brain areas such as somatosensory and premotor areas (Huber et al. 2017). These are considered important for processing task-relevant sensory inputs and conveying this information to pyramidal and/or extrapyramidal motor pathways (Kurz & Leukel, 2019). Functional and structural plasticity at infra- and supragranular layers of M1 have been shown to accompany motor performance improvements in rodents (Peters et al. 2014, 2017a; Chen et al. 2015; Li et al. 2017; Papale & Hooks, 2018). In humans, knowledge regarding the adaptations at different M1 circuits to motor learning remains very limited, largely due to methodological constraints.

In the present study, we tested the excitability changes of distinct M1 elements during motor skill training using a non-invasive electrophysiological method. This method involves transcranial magnetic stimulation (TMS) and peripheral nerve stimulation (PNS) and was developed more than three decades ago (Cowan et al. 1986; Nielsen et al. 1993). Recently, the method has been improved following findings in macaques, which suggested that the first corticospinal wave from TMS is composed of two different components (Kurz et al. 2019). The first component originates from transsynaptic activation of corticospinal neurons in the deep layers of M1, while the second component also reflects the recruitment of additional corticospinal neurons from TMS inputs at superficial layers. Experiments in humans in which the TMS H-reflex conditioning technique was used indicated that excitability changes of different laminar circuits can also be probed in human subjects (Kurz et al. 2019).

Here, we assessed the excitability changes of different laminar circuits in response to training in two different motor tasks in humans. We hypothesised that training in a demanding spatiotemporal motor skill will result in excitability changes at infra- and supragranular layers, while the execution of a simple movement requiring no such explicit spatial and temporal precision will be accompanied by no neural changes. Since previous studies rarely demonstrated a link between TMS measures and behavioural changes during motor learning (Carson et al. 2016), we further hypothesised that the excitability changes will not be associated with motor performance changes.

Methods

Ethical approval

The present study conformed to the standards set by the Declaration of Helsinki (latest revision in Fortaleza (Brazil), 2013), except for registration of the study in a database. The study was approved by the local ethics committee of Albert Ludwigs University of Freiburg (approval number 327/18). All subjects provided written informed consent for the procedures performed in the study. Participants had no contraindications to TMS (Rossi et al. 2009).

Participants

Thirty-five healthy subjects participated in the study. Nineteen subjects (mean age: 24.42 ± 4.3 years, 12 females) trained in a spatiotemporal motor skill (Task 1) and sixteen subjects (mean: 24.94 ± 5.03 years, 11 females) performed a simple motor task (Task 2). All subjects were naive to

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the purpose of the study and the motor tasks. Handedness was assessed by the Edinburgh Handedness Questionnaire (Task 1: 76.05 ± 42.12, 1 left-handed; Task 2: 52.81 ± 68.87, 3 left-handed) (Oldfield, 1971). We included only subjects in whom H-reflexes in the M. flexor carpi radialis (FCR) could be elicited and H-reflex onsets could be determined (see section headed Peripheral nerve stimulation).

Surface electromyography and kinematics

Surface electromyography (EMG) (EISA, Pfitec Biomedical Systems, Endingen, Germany) was recorded from the FCR and the M. extensor carpi radialis (ECR) of the left arm. The left arm was used because we assumed that participants will have worse baseline performance with their non-dominant hand, thus maximising the dynamic learning range (Shmuelof et al. 2012). Bipolar surface EMG electrodes (Blue sensor P, Ambu, Bad Nauheim, Germany) were placed over the muscle bellies (electrode distance: 2 cm) and one common ground electrode was placed at the caput ulnae. Impedances were kept below 5 kΩ. Signals were pre-amplified (100×), further amplified (2×), bandpass filtered (10–1300 Hz) and stored on a computer for offline analyses. A robotic manipulandum with a built-in goniometer recorded the angular position of the wrist. All data were sampled at 10 kHz.

Motor tasks

Subjects sat comfortably on a custom-built laboratory seat approximately 40 cm in front of a computer screen (21-inch LCD monitor with 60 Hz frame rate and 1920 × 1200 pixel resolution). The left forearm rested in a neutral position in a splint on a wooden board mounted on the left side of the chair. The forearm was stabilised with Velcro straps to avoid changes in elbow and shoulder joint angles. The left hand was attached to the handle of a robotic manipulandum. Subjects could move the handle of the robotic manipulandum by performing wrist flexion movements (Fig. 1A). The angular position of the wrist was illustrated as a white cursor on a black computer screen (Fig. 1B). At the start of each trial, the wrist was positioned in a neutral position with the white cursor shown on the left side of the computer screen.

In Task 1, subjects practised a spatiotemporal motor task (Fig. 1B, upper part). For this purpose, a small green target (110 pixels width) was illustrated on the right side of the computer screen. The aim was to place the cursor into the target. There were no restrictions on movement speed for Task 2.

In both tasks, flexion movements could be self-initiated once the subjects were ready (in a time window of 2 s from the display of the target). Subjects were told to plan the required force in advance and make no adjustments of the cursor end position once they finished the movement. At the end of each movement, the robotic manipulandum pushed the wrist back to the neutral starting position. During this period, subjects observed a black screen. A new trial (with the start of the visual scene on the screen) began every 5 s.

A movement was considered correct if at least half of the cursor was placed within the target field (Task 1 and Task 2) and the mean movement speed was within the specified range (only Task 1). Both tasks aimed to achieve as many correct movements as possible.

Figure 1. Behavioural setup

A, motor training encompassed discrete wrist flexion movements that were performed with a robotic manipulandum. B, subjects had to practise a spatiotemporal visuomotor skill (Task 1, upper panel) or a simple spatial visuomotor skill (Task 2, lower panel). In both tasks, the angular wrist position was illustrated as a white cursor on a computer screen. In Task 1, subjects had to move the cursor with a pre-defined movement speed into a narrow green target. In Task 2, subjects had to move the cursor into a wide green target field without any speed constraints. C, the angular wrist position was indirectly analysed based on the cursor motions on the screen. Movement speed was calculated as the amplitude of the cursor movement (in pixels) divided by the movement time (in ms). Movement speed was visually presented to the subjects after each trial. [Colour figure can be viewed at wileyonlinelibrary.com]
Mean movement speed was calculated after each trial by dividing the movement amplitude (in pixels) by the movement time (in ms) (Fig. 1C). In both tasks, subjects received online feedback regarding the cursor position. Furthermore, subjects received offline feedback about the end position of the cursor and the mean movement speed after each trial. The horizontal position of the target was identical in all trials. Prior to both tasks, subjects were familiarised with the setup by performing 10 self-paced wrist flexion movements with visual online and offline feedback without displaying any targets. The target sizes (Task 1 and Task 2) and ranges of movement speed that were considered correct (only Task 1) were determined in pilot experiments ($n=9$; results from these pilot experiments are not reported in this paper). These measurements showed robust performance improvements after training Task 1, while Task 2 did not lead to any performance changes. The setup and online calculations were performed using MATLAB (The MathWorks, Natick, Massachusetts, United States, R2019a).

Peripheral nerve stimulation

FCR H-reflexes were elicited by stimulating the nervus medianus of the left arm just above the elbow joint. For this purpose, we used a constant current stimulator (DS7a, Digitimer, Hertfordshire, UK) that produced square wave-pulses of 0.2 ms in duration. A bipolar electrode configuration was applied. A graphite-coated rubber pad of $2 \times 5$ cm was used as anode and placed proximal to the olecranon. The best stimulation position for the cathode was determined during a search procedure, which involved moving a custom-made round pad (1 cm diameter) on the skin surface at the medial area of the os humeri just above the elbow joint. The optimal position was defined as the site where low stimulation intensities (5–30 mA, monophasic pulse) elicited no or minimal M-wave, while H-reflex sizes remained constant. Furthermore, H-reflexes had to be clearly distinguishable from the M-wave so that H-reflex onsets could be determined. We also ensured that no H-reflexes were elicited in the antagonistic ECR muscle. We attached a self-adhesive cathode (Blue sensor P, Ambu) at the optimal position, and this electrode was utilised for the remainder of the experiment. At the beginning of each experiment, maximal M-waves ($M_{\text{max}}$) and H-reflexes were recorded, and the required stimulation intensity for TMS-conditioned H-reflex measurements was calculated.

Transcranial magnetic stimulation

Single-pulse TMS was applied over the right motor area of the wrist muscles using a Magstim 2002 stimulator with a BiStim unit (Magstim, Whitland, UK) and a figure-of-eight coil (50 mm). The stimulation position was determined during a mapping procedure at the beginning of each experiment. We recorded motor evoked potentials (MEPs) in the EMG of the FCR at several positions and stimulation intensities. The optimal position was defined as the site where TMS elicited clear MEPs at the lowest possible stimulation intensity. This optimal position was targeted during the entire experiment with the help of aBrainsight TMS navigation system (Brainsight 2, Rogue Research, Montreal, Canada). A stand (Manfrotto Magic Arm, Lino Manfrotto & Co, Cassola, Italy) stabilised the coil on the subjects’ head. The coil was held tangentially on the scalp at an angle of 45° to the mid-sagittal plane. The current direction was posterior-anterior. Resting motor threshold ($\text{rMT}$) was defined as the minimum required percentage stimulator output to evoke MEPs of at least 50 $\mu$V in at least three out of five subsequent stimulations at a certain intensity (Rossini et al. 1994). During all experiments, the TMS intensity was set to 115% of rMT to evoke early I-waves and no D-waves (Niemann et al. 2018).

TMS H-reflex conditioning

TMS H-reflex conditioning was applied to test distinct M1 circuits. The method was performed in accordance with previous studies (Kurz & Leukel, 2019; Kurz et al. 2019; Wiegel et al. 2020).

The method aims to segregate corticospinal waves that are elicited through TMS (Di Lazzaro & Ziemann, 2013) by additionally applying PNS (H-reflex stimulation). Distinct corticospinal waves coincide with the afferent volley induced by PNS at the spinal motoneurons. The delay between TMS and PNS can be arbitrarily adjusted with high temporal precision (in steps of 0.1 ms in our study). Detailed explanations and illustrations of the TMS H-reflex conditioning method can be found in previous publications (Kurz & Leukel, 2019; Kurz et al. 2019; Wiegel et al. 2020).

The delay between TMS and PNS, at which the fastest TMS-induced corticospinal waves lead to a change in the recruitment of spinal motoneurons, is called early facilitation. This delay is denoted as an early facilitation delay (EFD) of 0 ms. An additional delay between TMS and PNS of 0.6 ms relative to EFD 0 ms is accordingly denoted as EFD +0.6 ms. In the present study, EFDs of 0 ms and +0.6 ms were used to test motoneuron recruitment from two parts of the first indirect corticospinal wave (II-wave). In a previous study, we demonstrated that EFD 0 ms and EFD +0.6 ms are informative of changes in the excitability of circuits at infragranular (EFD 0 ms) and supragranular layers (EFD +0.6 ms) (Kurz et al. 2019).

We performed a two-step procedure at the beginning of each experiment according to (Kurz et al. 2019) to determine the individual EFD 0 ms delay between TMS 

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and PNS. First, delays from $-5$ ms to $-2$ ms were tested in 0.5 ms steps (negative delays indicate that PNS was applied prior to TMS). We recorded 12 repetitions for each delay and the unconditioned H-reflex ($12 \times 10$ conditions) in a block design. The stimuli with different delays were randomly tested. After this first session, we statistically determined the individual EFD 0 ms by calculating uncorrected paired Student’s $t$ tests between all delay conditions and the unconditioned H-reflex. The first more positive delay (starting at $-5$ ms) that showed a significant increase in H-reflex size ($P < 0.05$) was denoted as the first EFD 0 ms estimate (Fig. 2A). To increase the robustness of this procedure, the following two more positive delays also had to be significantly increased in comparison to the unconditioned H-reflex. In the second step, we tested delays between the first EFD 0 ms estimate and delays 0.8 ms more negative to this delay in steps of 0.1 ms. Again, we recorded 12 repetitions for each condition in a block design with the stimuli applied randomly. The aforementioned statistical approach was then performed to determine the final EFD 0 ms with 0.1 ms precision (Fig. 2B). The mean delay between TMS and PNS for EFD 0 ms was $-3.41$ ms (standard deviation: 0.52 ms) and $-3.39$ ms (standard deviation: 0.57 ms) in subjects that trained for Task 1 and Task 2, respectively (Fig. 2C).

The delay for EFD $+0.6$ ms was calculated according to the individual delay for EFD 0 ms (delay between TMS and PNS for EFD 0 ms, adding 0.6 ms). The interval between stimulations in both preparation procedures was 4 s to avoid changes in the post-activation depression of the H-reflex (Crone & Nielsen, 1989). The intensity for PNS was set so that H-reflexes had sizes between 15 and 25% of $M_{\text{max}}$ (Crone et al. 1990).

**Experimental design and electrophysiological testing**

Subjects were allocated to one of the two training groups (Task 1 or Task 2). The training phase in both tasks consisted of $6 \times 30$ trials in a block design. One block lasted 3 min and there was a 2-min resting period between blocks. Thus, the entire training session lasted approximately 30 min. During practice blocks, we applied TMS H-reflex conditioning. We tested H-reflex facilitation at EFD 0 ms and EFD $+0.6$ ms. Additionally, unconditioned H-reflexes were recorded by applying PNS. Thus, there were three conditions: EFD 0 ms, EFD $+0.6$ ms, and the unconditioned H-reflex. Within a block, testing of the conditions was performed randomly ($10 \times$ EFD 0 ms, $10 \times$ EFD 0.6 ms, $10 \times$ unconditioned H-reflexes). The size of the unconditioned H-reflex was matched across blocks by adjustments made to the stimulation intensity. Stimulations were applied during all movements to assess performance-related excitability changes, and these were always triggered by the online EMG signal of the FCR (Fig. 3). Stimulations were elicited when the rectified EMG signal exceeded 3 standard deviations of the baseline EMG with a 20 ms offset. We used a real-time signal processing system (STIMULI; Pfitec Biomedical Systems, Endingen, Germany) for this purpose.

Before and after completion of the training phase, all subjects (i.e. subjects that trained in Task 1 and Task 2) performed 10 trials in Task 1 (‘pre’ and ‘post’ tests). These pre and post tests were only performed in Task 1 since we did not expect any performance changes in Task 2. No electrophysiological stimulations were applied during these pre- and post-training phase movements.

![Figure 2. Determining EFD 0 ms](https://www.journalofphysiology.com/content/3489)

**Figure 2. Determining EFD 0 ms**

A, EFD 0 ms was individually determined for each subject via a two-step procedure. In the first step, delays between PNS and TMS were tested from $-5$ ms to $-2$ ms in steps of 0.5 ms. H-reflex facilitation was calculated as the percentage change in H-reflex magnitude with respect to the unconditioned H-reflex. Data from one subject are shown, for whom the first EFD 0 ms estimate was $-3$ ms. $R$, in the second step, we tested all delays between the interval of the first EFD 0 ms estimate ($-3$ ms in the illustrated example) and delays that preceded this interval by up to 0.8 ms ($-3.8$ ms in the illustrated example). The first significant increase in H-reflex facilitation was denoted as the final EFD 0 ms ($-3.4$ ms in the illustrated example). Note that only every second x-axis tick value is presented. C, histogram showing the delay between TMS and PNS for EFD 0 ms for all subjects in Task 1 and Task 2. [Colour figure can be viewed at wileyonlinelibrary.com]
Data analysis

The onset of the H-reflex was visually determined from superimposed unconditioned H-reflexes and the averaged unconditioned H-reflex (Fig. 4A). The mean H-reflex onset was 16.62 ms (standard deviation: 1.04 ms) in subjects that trained for Task 1 and 16.53 ms (standard deviation: 0.97 ms) in subjects that trained for Task 2 (Fig. 4B). EMG values were corrected for EMG offsets by shifting the EMG signal so that the H-reflex onset value was set to zero. This is important because the EMG offset (y-position) may change during the experimental protocol and thus bias the experimental results. H-reflex magnitudes were calculated to express H-reflex facilitation at EFD 0 ms and EFD +0.6 ms. As seen in Fig. 4C, H-reflex magnitudes are larger at EFD 0 ms and EFD +0.6 ms than for unconditioned H-reflexes. H-reflex magnitudes were analysed by calculating the root mean square values (RMS) from the initial 0.5 ms of the H-reflex from the unrectified EMG (Fig. 4D). This analysis ensures that H-reflex magnitudes only include the earliest monosynaptic components of the reflex. Mean H-reflex magnitude was calculated by taking the average from all repetitions of a condition. Mean H-reflex facilitation was expressed as the percentage change of H-reflex magnitudes at EFD 0 ms and EFD +0.6 ms relative to the mean unconditioned H-reflex multiplied by 100.

Trials in which H-reflexes could not be differentiated from the background EMG activity were discarded from the analyses. Notably, this occurred in subjects with small H-reflex amplitudes and large trial-to-trial H-reflex amplitude variability. Furthermore, all trials in which stimulation was not applied at movement initiation were excluded from the analyses. This was the case when subjects activated their FCR before the trial started. Consequently, the H-reflexes of training block 6 from one subject (Task 1) could not be included in the analyses since PNS did not elicit any unconditioned H-reflexes. On average, 9.3% of all trials (standard deviation = 7.1%) had to be excluded from the final analysis. Notably, the exclusion of several trials (>15% <30%) occurred for some subjects (n = 6). We investigated the influence of...
those subjects on the results in additional analyses (see Results section).

The pre-stimulus muscle activity of the agonistic FCR and antagonistic ECR was assessed by calculating the RMS of the 20 ms time window prior to stimulation. Performance in both tasks was assessed as the number of correct trials divided by the total number of trials and expressed as a percentage (e.g. 15/30 = 50%).

Movement kinematics were calculated based on the goniometer signal. For this purpose, we calculated the first derivative of the goniometer signal of each trial. These goniometer traces were smoothed with a moving average of 2000 samples and filtered with a 3rd order Butterworth low-pass filter (20 Hz). Next, we used the filtered signal to calculate the start and end points of movements by determining the maximum movement speed. Start and end points were defined as the sample when the signal exceeded 15% (start) or dropped below 15% (end) of its respective maximum (Wiegel et al. 2020). Subsequently, we defined movement time as the time between the start and end point. Movement amplitude was defined as the difference in wrist angle (in deg) between the start and end position. Mean movement speed was subsequently calculated as movement time divided by the movement amplitude. Maximum movement speed was calculated from the first derivative of the goniometer signal. For all kinematic parameters, within-block motor variability was calculated using the coefficient of variation (CV).

### Statistics

In the first step of the analysis, all data were visually inspected. Descriptive statistics for H-reflexes and behavioural measures were then examined for each EFD. Kolmogorov-Smirnov tests were calculated to test for the normal distribution of the data. Levene’s test was used to test for the homogeneity of variances. Due to violations in normality, EMG data were log-transformed for all further analyses.

To test the effect of the factors Time (six training blocks) and Task (Task 1 and Task 2) on our dependent measures (unconditioned H-reflexes, H-reflex facilitation and behavioural measures), we used a linear mixed model design. The factors Time (time-varying) and Task (time-constant) were entered as fixed effects into the model. As random effects, we included the intercepts for subjects. Subsequently, we compared the Akaike’s information criterion (AIC) between models with distinct covariance structures that were selected based on visual inspection of the data (diagonal, ante dependence, autoregressive with heterogeneous variances and unstructured). The best fit was selected based on the AIC, with lower values indicating a better fit (Crawley, 2013). For all models, the restricted maximum likelihood estimation method was used. Post hoc t tests were performed to test single comparisons between all training blocks (15 comparisons). We then used the Benjamini-Hochberg procedure to correct the significance level and control for Type I error for the post hoc t tests (Benjamini & Hochberg, 1995). Thus, only statistical results from pairwise comparisons that survived this corrector were considered significant. A detailed list of all post hoc comparisons can be found in the statistical summary document. Uncorrected P values are presented in the Results section.

Bivariate correlations were computed between changes in H-reflex facilitation and changes in motor performance using Pearson’s coefficient. Changes in performance were defined as the slope of the regression lines across training blocks in individual subjects.

A significance level of P < 0.05 was assumed. The data in the Results section are presented as mean values, with the standard deviation (SD) presented in parenthesis. Note that data in the figures are shown in the non-log-transformed form (Drummond & Tom, 2011). All statistical analyses were performed using SPSS software 24 (SPSS, Chicago, IL, USA).

### Results

#### Motor performance

The performance measure was analysed across training in Task 1 and Task 2 to test the effect of motor practice. The statistical analyses revealed a significant main effect for Time (F = 9.51, [5,42.7], P < 0.001), Task (F = 449.57, [1,35.5], P < 0.001) and a significant Time × Task interaction (F = 6.09, [5,42.7], P < 0.001). Post hoc pairwise comparisons between all training blocks within Task 1 and Task 2 were calculated to assess block-to-block performance changes (Fig. 5A). In Task 1, performance increased steadily from 28.95% (14.06%) in block 1 to 47.54% (11.39%) in block 6. Performance increased most prominently from block 2 (31.23% (9.69%)) to block 3 (40.35% (8.71%)) (t = −3.13, P = 0.006). In all subsequent training blocks (block 4: 43.68% (13.96%), block 5: 47.19% (13.21%) and block 6), performance was significantly better than in blocks 1 and 2 (all P < 0.01). In Task 2, nearly optimal performance was achieved in block 1 with 89.58% (6.76%), while relatively small performance changes were observed throughout training (block 2: 92.5% (8.56%); block 3: 93.13% (8.47%); block 4: 94.58% (4.69%); block 5: 92.5% (6.48%); block 6: 93.33% (7.79%)).

Since TMS H-reflex conditioning was applied in all trials during practice, we were interested in whether the performance changes are also evident in trials with no electrophysiological stimulation. Indeed, subjects that practised Task 1 exhibited substantial performance improvements in ‘no stimulation trials’ from pre-test
to post-test \((t = -3.52, \ P = 0.002)\) (Fig. 5B). To test whether subjects that practised Task 2 also improved performance in a spatiotemporal motor skill, we also tested their performance in Task 1 pre- and post-motor practice. Although subjects that practised Task 2 improved their performance in Task 1, this improvement was not significant \((t = -2.11, \ P = 0.05)\). Thus, training in a spatiotemporal motor skill (Task 1) led to continuous significant performance improvements, while training in a simple motor task (Task 2) lead to non-systematic changes in motor performance.

**Kinematics**

The results from the statistical analyses are presented in Table 1. Raw traces from the goniometer signals from practice in block 1 and block 6 are shown in Fig. 6A for Task 1 (upper panel) and Task 2 (lower panel). The time courses of mean kinematic parameters are shown in the left panels of Fig. 6B–E, while variability assessed with the coefficient of variation (CV) is shown in the right panels of Fig. 6B–E.

In summary, training in Task 1 resulted in less variable spatiotemporal wrist flexion movements (Fig. 6A, upper panel), while training in Task 2 did not (Fig. 6A, lower panel). All subjects performed movements with a movement amplitude of 36–42°. Subjects who trained in Task 1 performed movements with slightly greater movement amplitude than subjects who trained in Task 2 (on average by approx. 0.5–1°) (Fig. 6B, left panel).

In both motor tasks, movements in later blocks were lower in amplitude (about 0.2–0.3°) compared to earlier blocks. In most cases, mean and maximum movement speeds were 70–90°/s and 120–160°/s, respectively, and did not significantly differ between tasks and training blocks (Fig. 6C and D, left panels). Movement time ranged between 400 and 600 ms, and was greater by 20–30 ms in Task 1 than in Task 2, on average (Fig. 6E, left panel). The variability of all kinematic parameters (coefficient of variation) decreased from training block 1 to block 6 in subjects that practised Task 1 (in all kinematic variables from 0.1/0.2 to <0.05/0.1, Fig. 6B–E, right panels). In contrast, subjects who performed Task 2 exhibited only minor changes in movement variability estimates (mostly ranging from 0.1 to 0.2). Some of these subjects showed substantial non-systematic fluctuations.

**Electrophysiological testing**

**Pre-stimulus electromyography.** Table 2 summarises the statistical results of the analyses. The time courses of pre-stimulus activity for the FCR and ECR from one exemplary subject are presented in Fig. 7A and D, respectively.

The pre-stimulus activity of the FCR varied mostly between 0.04 and 0.1 mV, while the pre-stimulus activity of the ECR did not exceed background EMG values (0.01–0.02 mV). Analyses of the pre-stimulus activity of the FCR at EFD 0 ms yielded a significant main effect of Time. The amount of pre-stimulus FCR EMG activity

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**Figure 5. Performance during motor training**

*Significant paired \(t\) tests after correction for multiple comparisons. \(B\), mean (large circles and bars) and individual (small circles) performance in Task 1 for ‘no stimulation trials’. These motor tests were performed before training (pre) and after training (post) by all subjects. Note that x-axis values were adjusted to more clearly present the data. [Colour figure can be viewed at wileyonlinelibrary.com]
slightly decreased in the time course of the experiment in both Task 1 and Task 2. All other comparisons yielded no significant differences between tasks and training blocks as well as also no significant interaction effect of both factors (Fig. 7B and C for FCR and Fig. 7E and F for ECR). These results indicate that there were no magnitude differences in the neural drive to the muscles at the time of the TMS and PNS stimulations between both motor tasks.

**H-reflex facilitation during the acquisition of a discrete motor skill.** H-reflex facilitation showed considerable intra-individual variability in both Task 1 and Task 2 (Fig. 8). For EFD 0 ms, the statistical analyses yielded no significant effect for the factors Time ($F = 0.58_{[5,41.9]}$, $P = 0.72$) and Task ($F = 0.01_{[1,34.6]}$, $P = 0.76$); however, a significant interaction was observed between Time $\times$ Task ($F = 2.74_{[5,41.9]}$, $P = 0.03$) (Fig. 8A). For EFD +0.6 ms, the analyses revealed no significant effects for the factors Time ($F = 1.69_{[5,42.1]}$, $P = 0.16$) and Task ($F = 0.01_{[1,34.3]}$, $P = 0.94$) as well as no significant interaction between Time $\times$ Task ($F = 0.44_{[5,42.1]}$, $P = 0.82$) (Fig. 8B). The significant interaction of the factors Time $\times$ Task for EFD 0 ms indicates differences in the time course of H-reflex facilitation across training for Task 1 and Task 2. Student's $t$ tests were subsequently performed to investigate these differences in detail. Between-group unpaired $t$ tests suggest that H-reflex facilitation at EFD 0 ms was not significantly different between Task 1 and Task 2 in any of the training blocks ($all \, P > 0.05$). For Task 1, within-group paired Student’s $t$ tests revealed that H-reflex facilitation at EFD 0 ms increased significantly from 112.99% (16.5%) in block 1 to 131.22% (22.52%) in block 3 ($t = 3.56$, $P = 0.002$). Although this early excitability increase was present in subsequent training blocks, the $P$ values did not survive correction of the significance level. For Task 2, H-reflex facilitation at EFD 0 ms did not significantly differ between any of the training blocks ($all \, P > 0.05$). Visual inspection and descriptive statistics also pointed towards increased facilitation at EFD +0.6 ms after the initial training blocks. However, the ANOVA results yielded no significant interaction effects and we did not compute post hoc comparisons accordingly. Analyses of unconditioned H-reflexes yielded no effects of Time ($F = 0.73_{[5,33]}$, $P = 0.61$), Task ($F = 0.87_{[1,33]}$, $P = 0.36$) and no interaction between the factors ($F = 0.69_{[5,33]}$, $P = 0.64$).

In summary, these results indicate a specific increase in H-reflex facilitation at EFD 0 ms after the first few training blocks in subjects that trained in Task 1.

As seen in Fig. 8, we observed high fluctuations in H-reflex facilitation within and between subjects. Two additional analyses were performed to increase the robustness of the present results. First, we investigated the influence of subjects in whom H-reflex responses were highly variable and sometimes absent. We repeated the same analyses but excluded all subjects for whom we had to exclude more than 15% of all trials ($n = 6$ subjects, four subjects practise Task 1 and two subjects practicing Task 2). The 15% threshold corresponds to the average number of excluded trials plus one SD and left 29 subjects with an average of 6.7% (4.1%) of their trials excluded. The analyses with this subset of subjects yielded the same results as the previous analyses. There was no significant effect of Time ($F = 0.83_{[5,33.6]}$, $P = 0.54$) and Task ($F = 0.01_{[1,28.9]}$, $P = 0.95$), yet a significant

### Table 1. Linear mixed model results for kinematics

| Coefficient of variation (CV) | Amplitude $F_{[\text{DF, error}]} (P)$ | Mean speed $F_{[\text{DF, error}]} (P)$ | Max. speed $F_{[\text{DF, error}]} (P)$ | Time $F_{[\text{DF, error}]} (P)$ |
|-----------------------------|---------------------------------------|---------------------------------------|---------------------------------------|----------------------------------|
| Mean Time                   | 2.86$_{[5,61.1]}$ (0.02)              | 0.38$_{[5,42.3]}$ (0.86)              | 0.54$_{[5,35.4]}$ (0.74)              | 1.23$_{[5,48.2]}$ (0.31)          |
| Mean Task                   | 7.34$_{[1,31.9]}$ (0.01)              | 2.48$_{[5,20.42]}$ (0.13)             | 4.04$_{[1,40.4]}$ (0.05)              | 4.46$_{[1,32.4]}$ (0.04)          |
| Mean Time $\times$ Task     | 1.36$_{[5,61.1]}$ (0.25)              | 1.99$_{[5,42.3]}$ (0.1)               | 2.14$_{[5,35.4]}$ (0.08)              | 1.15$_{[5,48.2]}$ (0.35)          |
| Coefficient of variation (CV) | 7.74$_{[5,41]}$ (0.01)               | 4.91$_{[5,42.5]}$ (0.001)             | 2.69$_{[5,51.5]}$ (0.03)              | 3.8$_{[5,49.7]}$ (0.005)          |
| Coefficient of variation (CV) | 12.19$_{[1,36]}$ (0.001)             | 5.88$_{[1,34.8]}$ (0.02)              | 4.74$_{[1,33.8]}$ (0.04)              | 0.45$_{[1,35.9]}$ (0.5)           |
| Coefficient of variation (CV) | 1.35$_{[5,41]}$ (0.26)               | 3.12$_{[5,42.5]}$ (0.02)              | 2.6$_{[5,51.5]}$ (0.04)               | 2.18$_{[5,49.7]}$ (0.07)          |

### Table 2. Linear mixed model results for pre-stimulus electromyography

| Task          | EFD 0 ms $F_{[\text{DF, error}]} (P)$ | EFD +0.6 ms $F_{[\text{DF, error}]} (P)$ |
|---------------|--------------------------------------|----------------------------------------|
| **FCR**       |                                      |                                        |
| Time          | 3.31$_{[5,44.1]}$ (0.01)              | 1.66$_{[5,56.2]}$ (0.16)              |
| Task          | 1.99$_{[1,33]}$ (0.17)                | 1.24$_{[1,33]}$ (0.27)                |
| Time $\times$ Task | 0.49$_{[5,44.1]}$ (0.78)      | 0.69$_{[5,56.2]}$ (0.64)              |
| **ECR**       |                                      |                                        |
| Time          | 0.39$_{[5,44.8]}$ (0.85)              | 0.91$_{[5,44.4]}$ (0.42)              |
| Task          | 2.32$_{[1,33.1]}$ (0.14)              | 2.04$_{[1,33.1]}$ (0.16)              |
| Time $\times$ Task | 0.78$_{[5,44.8]}$ (0.57)      | 0.81$_{[5,44.4]}$ (0.55)              |
interaction between Time × Task ($F = 3.49_{[5,33.8]}, P = 0.01$) for EFD 0 ms. H-reflex facilitation at EFD 0 ms remained significantly greater in block 3 than in block 1 ($t = 4.42, P = 0.001$). The analyses for EFD +0.6 ms still yielded no significant main and interaction effects (Time: $F = 2.11_{[5,33.8]}, P = 0.08$; Task: $F = 0.01_{[1,27.7]}, P = 0.97$; Time × Task: $F = 0.45_{[5,33.8]}, P = 0.81$). Second, high variability may also be caused by the relatively low number of stimulation repetitions ($n = 10$) in each training block. Due to this variability in H-reflex responses, we recorded

Figure 6. Kinematic parameters during motor training
A, single goniometer traces during training in block 1 (left side) and block 6 (right side) from Task 1 (upper panel) and Task 2 (lower panel). Data are aligned to movement onset (0 ms). The angular position (in degrees (°)) from movement start to movement end is shown. The colour code (yellow to blue in Task 1, yellow to red in Task 2) indicate the trial number within a training block. B, mean and single-subject data of movement amplitude averages (left panels) and coefficients of variation (right panel) for Task 1 ($n = 19$, blue) and Task 2 ($n = 16$, red). C, mean and single-subject data of movement speed averages (left panels) and coefficients of variation (right panel) for Task 1 ($n = 19$, blue) and Task 2 ($n = 16$, red). D, mean and single-subject data of maximum movement speed averages (left panels) and coefficients of variation (right panel) for Task 1 ($n = 19$, blue) and Task 2 ($n = 16$, red). E, mean and single-subject data of movement time averages (left panels) and coefficients of variation (right panel) for Task 1 ($n = 19$, blue) and Task 2 ($n = 16$, red). *Significant paired t tests after correction for multiple comparisons. [Colour figure can be viewed at wileyonlinelibrary.com]
between 18 and 20 repetitions per condition in previous studies (Kurz & Leukel, 2019; Kurz et al. 2019). Thus, we pooled all H-reflex data (including all subjects) from block 1 and block 2 (early training stage), block 3 and block 4 (middle training stage) and block 5 and block 6 (late training stage) (Fig. 9). For this new design, the findings parallel those observed in the previous analyses. The statistical model for EFD 0 ms showed no significant effect for the factors Time ($F = 0.34[2,39.6], P = 0.71$) and Task ($F = 0.2[1,29.36], P = 0.656$) but a significant Time $\times$ Task interaction ($F = 4.86[2,39.6], P = 0.01$) (Fig. 9A). Pairwise comparisons revealed that H-reflex facilitation at EFD 0 ms increased significantly from the early to middle training stages in Task 1 ($t = 2.64, P = 0.017$). All other comparisons were not significant for EFD 0 ms in Task 1 and Task 2. Although H-reflex facilitation at EFD +0.6 ms also increased, the analyses revealed no significant effect for the factor Time ($F = 1.25[2,37.9], P = 0.29$) and Task ($F = 0.01[1,33.7], P = 0.94$) as well as no significant interaction between Time and Task ($F = 1.29[2,37.9], P = 0.29$) (Fig. 9B). In summary, both additional analyses left the initial results unchanged, referring to a specific increase in H-reflex facilitation for EFD 0 ms in the early phases of motor practice.

### Association between changes in performance and changes in H-reflex facilitation.

The association between changes in H-reflex facilitation and changes in performance in Task 1 were assessed using Pearson’s bivariate correlation analyses. For both variables (H-reflex facilitation and performance), we decided to use the pooled H-reflex responses rather than changes between

![Figure 7. Pre-stimulus EMG of FCR and ECR](image-url)

A, mean rectified EMG time course of the FCR prior to electrophysiological testing (at 0 ms). The shaded area indicates the SD. Data from one subject that performed Task 1 in the first training block are shown. B, mean and single-subject data of the pre-stimulus FCR EMG in Task 1 ($n = 19$, blue) and Task 2 ($n = 16$, red) for stimulations at EFD 0 ms. C, same as in B but for EFD +0.6 ms. D, mean rectified EMG time course of the ECR prior to electrophysiological testing (at 0 ms). The shaded area indicates the SD. Shown are data from the same subject as in A. E, same as in B but for the ECR. F, same as in C but for the ECR. Note: for the ECR, y-axis scales differ between Task 1 and Task 2. [Colour figure can be viewed at wileyonlinelibrary.com]
single blocks since these would have been more heavily influenced by the large variability observed in the data. Changes in H-reflex facilitation were defined as the percentage changes from the early to the middle training stage. The corresponding changes were correlated with the slope of the individual regression line from the performance outcome (% correct movements) assessing the gain in performance over time. To capture different training phases, regression lines were separately calculated from block 1 to block 4 (early performance gains), block 3 to block 6 (late performance gains) and block 1 to block 6 (whole training gains).

In summary, the analyses revealed no significant correlations between changes in H-reflex facilitation and changes in performance. There was no significant correlation between changes in H-reflex facilitation at EFD 0 ms and performance improvements (early performance gains: $r = 0, P = 1$; late performance gains: 

![Figure 8. H-reflex facilitation in the time course of motor practice (six-block design)](image)

![Figure 9. H-reflex facilitation in the time course of motor practice (three-stage design)](image)
$r = 0.19, P = 0.44$; whole training gains: $r = -0.16, P = 0.5$). Likewise, there was no significant correlation for EFD +0.6 ms (early performance gains: $r = -0.11, P = 0.64$; late performance gains: $r = 0.15, P = 0.53$; whole training gains: $r = -0.25, P = 0.31$). The results suggest that changes in H-reflex facilitation observed in the early training stages of motor practice were not associated with performance changes in any of the training phases.

**Discussion**

M1 is an important brain region for learning skilled motor behaviours. Recent rodent studies support this notion by demonstrating that the inactivation or lesioning of M1 results in the inability to learn new motor skills (Kawai et al. 2015; Hwang et al. 2019). However, these studies also show that control of a motor skill becomes independent of M1 after long-term training. These findings suggest a putative role of M1 in the initial phase of acquiring a new motor skill. Therefore, in the present study, we focused on the initial stage of motor practice and tested the excitability modulations of M1 elements in different layers (EFD 0 ms and EFD +0.6 ms) during practice. Motor skill practice consisted of training discrete spatiotemporal wrist flexion movements (Task 1) and analogous non-specific wrist flexion movements (Task 2).

Excitability changes at infra- and supragranular layers in M1 were measured with TMS H-reflex conditioning. Probing was performed at movement onset (shortly after EMG onset) to capture movement-related excitability changes. Motor practice in Task 1 resulted in the reduced variability of movement kinematics and higher motor performance. Motor practice in Task 2 resulted in non-systematic changes in performance and kinematics. H-reflex facilitation at EFD 0 ms significantly increased in the early stages of training for Task 1, while it did not significantly change in Task 2. Notably, H-reflex facilitation at EFD +0.6 ms was not systematically modulated in both tasks. Changes in H-reflex facilitation at EFD 0 ms in Task 1 were not associated with changes in performance. Therefore, the results indicate task-specific modulations of fast-conducting corticospinal connections (EFD 0 ms) in the early stages of motor skill acquisition.

The fastest corticospinal connections are likely to be engaged in wrist flexion movements requiring fine-coordinated muscle activity patterns (Lemon, 2008, 2019). Excitability of the corticospinal pathway and the firing rates of direct corticospinal connections particularly increased at the time of movement onset (Chen et al. 1998; Soteropoulos, 2018). The activity of direct corticospinal neurons during movement was further related to intrinsic muscle parameters such as EMG activity or force (Griffin et al. 2008; Omrani et al. 2017). Since our subjects had to produce accurate force profiles – and thus well-timed muscle activity patterns – to move the robotic manipulandum with a constant speed, it is likely that direct corticospinal connections contributed to the initiation of the wrist flexion movements performed in this study.

Training of wrist flexion movements led to performance improvements and the reduced variability of spatiotemporal kinematics (Fig. 5). These behavioural changes were accompanied by increased excitability of the fastest corticospinal connections. This excitability increase may reflect the integration of additional corticospinal connections in the early stages of motor learning. Previous studies in rodents and monkeys reported increased variability and expansion of M1 activity in the early stages of motor learning (Kargo & Nitz, 2003; Mandelblat-Cerf et al. 2009; Peters et al. 2014). Supporting evidence for the increased recruitment of corticospinal connections during motor skill acquisition comes from human studies that demonstrated changes in several MEP measures from TMS after a single session of motor practice – but not in later training sessions (Rosenkranz et al. 2007). The exploration and redundancy of task-related corticospinal activity may serve to find possible new network states and ultimately drive motor learning (Rokni et al. 2007).

The excitability increase observed in our study may also point to a learning-related restructuring at layer 5b. Li et al. (2017) reported the restructuring of layer 5b neurons in rats, which became more temporally aligned with the movement and better encoded the performed actions during motor skill practice (Li et al. 2017). However, Li et al. (2017) also noted that the destination of the connections observed in their study was uncertain and may not be the spinal cord.

The changes occurring at the fastest-conducting corticospinal connections in the early stages of motor learning may be followed by the reorganisation and fine-tuning of M1 activity in later stages of skill learning in humans. In rodents, long-term motor practice has demonstrated dynamic relationships between corticospinal activity and movements (Peters et al. 2017a). Moreover, the development of less variable and more correlated M1 activity has been observed at supragranular layer neurons in rodents after longer motor training intervals (Peters et al. 2014; Hwang et al. 2019). This finding in rodents may also explain why we did not observe any significant task-specific excitability changes at the supragranular layer circuits of M1. Evaluation of this issue may require longer training durations, which could be the subject of future research.

Notably, the statistical analyses revealed significant excitability modulations (Time × Task interaction) for EFD 0 ms but not for EFD +0.6 ms. The latter finding is surprising. At EFD +0.6 ms, excitability changes occurring at EFD 0 ms should also be reflected because all preceding corticospinal volleys are integrated. One possible reason for the finding could relate to the statistical analysis. While H-reflex facilitation at EFD 0 ms increased only
in Task 1 (and not in Task 2), H-reflex facilitation at EFD +0.6 ms increased in both tasks. This means that, at EFD 0.6 ms, there was no contrast between H-reflex facilitation in Task 1 and Task 2 as there was at EFD 0 ms. Our results do not support a link between changes in the excitability of the fastest corticospinal connections (and more superficial M1 circuits) and changes in movement parameters – a result supported by recent animal (Peters et al. 2017a) and human studies (Carson et al. 2016). The relationship between the activity of corticospinal neurons and movements remains dynamic throughout motor learning (Peters et al. 2017a). Moreover, the activity of a given muscle can be driven by distinct activity patterns in M1 and corticospinal neurons and dependent on the behavioural context (Kakei et al. 1999; Davidson et al. 2007; Griffin et al. 2015). Although the activity/excitability of corticospinal neurons may be able to predict intrinsic muscle-related parameters, correlations with extrinsic parameters such as kinematics or motor performance have rarely been reported (Omrani et al. 2017). Also, we believe that the excitability measure of the population of neurons that we targeted with TMS may be too unspecific to capture possible associations with behavioural outcomes. Indeed, human TMS studies have rarely demonstrated significant correlations between measures of corticospinal excitability and motor performance (Carson et al. 2016).

This study has several limitations. First, we could only record a small number of unconditioned and conditioned H-reflexes per training block. This small number of trials was associated with large individual fluctuations in H-reflex facilitation. Thus, we attempted to minimise this disadvantage by performing two additional analyses (excluding a subset of subjects with variable H-reflex responses and pooling H-reflex responses). Moreover, due to the time constraint of recording at more than one probing instant, we cannot answer the question of whether the increase in excitability of the fastest corticospinal connections is similar or different at other time points during the movement. Third, the electrophysiological method interfered with the ‘natural’ activity of M1 and altered the natural EMG pattern. Learning may thus relate to coping with the stimulation as well as physical practice. Lastly, we note that the contributions of different M1 elements to motor learning depend on the characteristics of the motor skill. For example, a previous study indicated that after learning a sequential motor task, skill representations may emerge not in M1 but rather in premotor or sensory brain areas (Yokoi et al. 2018). Therefore, it is conceivable that motor skills that do not require changes in movement quality per se (but rather changes in timing or sequencing of movements) may involve the observed M1 elements differently or even not at all (Krakauer et al. 2019).

In conclusion, using recent advances in non-invasive human electrophysiological testing of different M1 elements, we have demonstrated that the excitability of the fastest corticospinal connections increased during the acquisition of a spatiotemporal motor skill. The results support the notion of increased recruitment and/or reorganisation of the fastest corticospinal connections during early motor skill learning in human subjects, which might be crucial for performance changes.

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Additional information

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.
Competing interests

The authors declare no conflicts of interest.

Author contributions

P.W. and C.L. conceived and designed research; P.W. performed experiments; P.W. analysed data; P.W. and C.L. interpreted results of experiments; P.W. prepared figures; P.W. drafted manuscript; P.W. and C.L. edited and revised manuscript; P.W. and C.L. approved final version of manuscript. Both authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

corticospinal, motor cortex, motor learning, neural circuits, sensorimotor control

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document

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