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Acquisition of a Simple Motor Skill: Task-Dependent Adaptation and Long-Term Changes in the Human Soleus Stretch Reflex

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Abstract

Changing the H-reflex through operant conditioning leads to CNS multi-site plasticity and can affect previously learned skills. In order to further understand the mechanisms of this plasticity, we operantly conditioned the initial (M1) component of the soleus stretch reflex. Unlike the H-reflex, the stretch reflex is affected by fusimotor control, comprises several bursts of activity resulting from temporally dispersed afferent inputs, and may activate spinal motoneurons via several different spinal and supraspinal pathways.

Neurologically normal participants completed six baseline sessions and 24 operant conditioning sessions in which they were encouraged to increase (M1up) or decrease (M1down) M1 size. Five of eight M1up participants significantly increased M1; the final M1 size of those 5 participants was 143±15% (mean±SE) of the baseline value. All eight M1down participants significantly decreased M1; their final M1 size was 62±6% of baseline. Similar to the previous H-reflex conditioning studies, conditioned reflex change consisted of within-session task-dependent adaptation and across-session long-term change. Task-dependent adaptation was evident in conditioning session 1 with M1up and by session 4 with M1down. Long-term change was evident by session 10 with M1up and session 16 with M1down. Task-dependent adaptation was greater with M1up than with the previous H-reflex up-conditioning. This may reflect adaptive changes in the muscle spindle sensitivity, which affects the stretch reflex but not the H-reflex. Because the stretch reflex is related to motor function more directly than the H-reflex, M1 conditioning may provide a valuable tool for exploring the functional impact of reflex conditioning and its potential therapeutic applications.

New & Noteworthy

Since the activity of stretch reflex pathways contributes to locomotion, changing it through training may improve locomotor rehabilitation in people with CNS disorders. Here we show for the first time that people can change the size of soleus spinal stretch reflex through operant conditioning. Conditioned stretch reflex change is the sum of task-dependent adaptation and long-term change, consistent with H-reflex conditioning yet different from it in the composition and amount of the two components.

Introduction

In animals and humans, an operant conditioning protocol can increase or decrease the size of the Hoffman reflex (H-reflex), which is produced by a wholly spinal pathway (Wolpaw, 1987; Chen and Wolpaw, 1995; Carp et al., 2006; Thompson et al., 2009). Acquisition of this simple skill can alter the pattern of muscle activation during walking in people and animals with spinal cord injury (Chen et al., 2011; 2014; Thompson and Wolpaw, 2015). Accumulating evidence suggests induction of complex plasticity at many sites within the nervous system. Some of these changes clearly underlie the new skill of a larger or smaller H-reflex while others are likely to be compensatory changes that prevent the plasticity responsible for the new skill from interfering with pre-existing behaviors (Wolpaw and Lee, 1989; Wolpaw, 2010).

The H-reflex is elicited by weak electrical stimulation of the muscle nerve; it is often viewed as an electrical analogue of the spinal stretch reflex, which can be increased or decreased by operant conditioning (Wolpaw et al., 1983c; Evatt et al., 1989). Stretch reflex conditioning has several advantages as a research model. First, the H-reflex is elicited mainly by synchronous activation of primary afferent (group-Ia and large group-II) fibers, thus the afferent volley is minimally dispersed when it arrives at the spinal motoneurons (Burke et al., 1983). In contrast, the stretch reflex can be elicited by a rapid joint rotation, which produces a temporally dispersed activation of afferent fibers, the same afferent may even fire several times (Matthews, 1972). The afferents are activated in a manner similar to that occurring during natural movement (e.g., the stretch reflex is less affected by presynaptic inhibition than is the H-reflex (Morita et al., 1998)). Second, unlike the H-reflex, the stretch reflex is affected by muscle spindle sensitivity, and thus by changes in gamma drive (Matthews, 1972; Arris and Henneman, 1980; Matthews1981). This provides a stretch reflex conditioning protocol an additional mechanism for changing reflex size. Third, unlike the H-reflex, the stretch reflex comprises several successive peaks of excitation. The first (M1) is generated mainly by group Ia afferents. Later peaks (M2 and M3) have contributions from Group Ib and II afferents (M2;(Schieppati and Nardone, 1997; Dietz, 1998; Schieppati and Nardone, 1999; Dietz and Duysens, 2000; Grey et al., 2001; Sinkjaer et al., 2004; af Klint et al., 2010)) and from transcortical pathways (M3; (Marsden et al., 1973; 1977; Capaday et al., 1991; Palmer and Ashby, 1992; Petersen et al., 1998; Mrachacz-Kersting et al., 2006)). The pathways responsible for these later peaks contribute to normal movement. Thus, the impact of M1 conditioning on these pathways should also be monitored.

The present study set out to demonstrate the feasibility of operant conditioning of the soleus M1 stretch reflex, to characterize the time course of M1 stretch reflex changes in conditioning responders, and to compare those to the previous soleus H-reflex conditioning. It introduces a novel stretch reflex conditioning protocol for the human soleus muscle based on the H-reflex conditioning protocol of (Thompson et al., 2009). Each conditioning session began with a set of control trials in which feedback

64 was not provided. Thus, in contrast to previous stretch reflex conditioning studies in monkeys and
65 humans (Wolpaw et al., 1983b; Wolpaw and O'Keefe, 1984; Evatt et al., 1989; Wolf and Segal, 1996),
66 this study aimed to differentiate the task-dependent adaptation in reflex size that occurs within each
67 conditioning session from the long-term change that develops over many sessions and affects pathway
68 function outside of the conditioning paradigm. While task dependent adaptation is attributable to rapid
69 plasticity in the cortex (Thompson et al., 2009), long-term change reflects plasticity in the spinal cord
70 (Wolpaw, 1997; 2007). This study also assesses the effects of M1 conditioning on the M2 component of
71 the stretch reflex. The results and their differences from H-reflex conditioning provide new insights into
72 the mechanisms and wider effects of spinal reflex conditioning.

Materials and Methods

Participants: Fourteen participants (8 women, 6 men; ages 19-35 years) provided written informed consent for the study. Three participated in both up and down-conditioning protocols, with at least six months between the two protocols. Since up- and down-conditioning are physiologically different phenomena (Carp and Wolpaw, 1994; 1995; Carp et al., 2001a; 2001b; Wolpaw and Chen, 2001), the first direction of conditioning would not affect the second direction of conditioning (Thompson et al., 2009). All participants were free of any known physical or neurological disorders. Approval for the study was provided by the scientific ethics committee for Nordjylland (Reference Number: N-20120044). The study was performed in accordance with the Declaration of Helsinki.

Operant conditioning study overview: After attending a familiarization session (see ***Familiarization session*** below), each participant completed 6 baseline sessions and 24 up-conditioning or 24 down-conditioning sessions that occurred at a pace of 3 times per week. Figure 1 shows the operant conditioning session schedule and the set-up for stretch reflex elicitation. In each session, after preparing for the soleus and tibialis anterior (TA) EMG recording (see the section below), the soleus H-reflex/M-wave recruitment curve was obtained during natural standing with a stable level of soleus and TA background EMG activity. Then, 245 trials of stretch reflexes were elicited while the sitting participant produced approximately 10% maximum voluntary contraction (MVC) level of soleus EMG activity and no activation of the TA with the right lower leg fixed on the custom-made apparatus (Figure 1B) (see *Session protocol* below). In baseline sessions, all 245 reflexes were elicited without any feedback on reflex size. In contrast, in each conditioning session, the first 20 reflexes were elicited without any feedback on reflex size, and then 225 conditioning reflexes were elicited. In these 225 conditioning trials, the participant was asked to increase (up-conditioning) or decrease (down-conditioning) the size of M1 reflex with the aid of visual feedback, which showed after each perturbation whether the resulting reflex was larger (for up-conditioning) or smaller (for down-conditioning) than a criterion value. Soleus and TA background EMG levels were kept stable throughout data collection. In order to avoid session-to-session variability in the location of electrodes, the positions of all electrodes were mapped in relation to landmarks on the skin (e.g., moles or scars) during the familiarization session. To prevent the potential diurnal variation in reflex size from affecting the results, each participant's sessions always occurred at the same time of day (i.e., within the same 3-hr time window). A typical baseline or conditioning session took about 1.5 hour.

[Figure 1 near here]

EMG recording: Electromyographic (EMG) activity was recorded with custom-made amplifiers and surface Ag/AgCl electrodes (Medicotest 720-01-K) placed over the belly of the right soleus (SOL) and tibialis anterior (TA) muscles in accordance with the recommendations of (Cram and Criswell, 2011) to optimize recording from these muscles and avoid contamination from other muscles. EMG activity was

amplified using custom made EMG amplifiers, filtered at 20 Hz- 2kHz, and digitized (2 kHz) with scientific software Mr. Kick II 2.3 (Knud Larsen, Center for Sensory-Motor Interaction, Aalborg University, Denmark) and stored for later off-line analysis.

Familiarization session: All participants attended one familiarization session in which the experimental procedures were explained and implemented. This was to ensure that participants were comfortable with the electrical stimuli and perturbations that would elicit a stretch reflex (Figure 1C). During this session, the participants were asked to perform the maximal isometric voluntary SOL contraction (MVC) while standing. The instructions were to rise up on the toes as rapidly as possible and to hold this for 1 s. This was repeated twice and the best effort (quantified by the rectified SOL EMG amplitude) was defined as the MVC. Absolute soleus EMG amplitude range for 5-15% MVC (i.e., centering 10% MVC) level determined during the familiarization session was used for all stretch reflex measurements in the familiarization session and in all subsequent sessions. During stretch reflex trials, participants were asked to tonically activate the right SOL to produce this pre-set level of absolute EMG activity while sitting in a custom-made apparatus chair (Figure 1B) with their right TA silent. EMG electrode locations were mapped in relation to permanent marks on the skin (e.g., moles and scars) so that they could be maintained the same throughout the rest of the study for each participant.

Custom joint-rotation device and stretch reflex elicitation: During all stretch reflex measurements, participants were seated in a custom-made chair that was fixed to the floor, with their knee joint flexed at approximately 60˚ (Figure 1B). The right foot was fixed to a servo-controlled electrical actuator, such that the anatomical ankle axis of rotation was closely aligned with the fulcrum of the actuator and the foot rested on a footplate. This position minimized both hip and knee movement, ensuring that the movement of the actuator was transmitted solely to the ankle joint. This knee position also minimized the possible influence of gastrocnemius activity on the soleus stretch reflex; the gastrocnemius muscle is biarticular with two heads arising from just above the femoral condyles, and act both to flex the knee joint and to plantarflex the ankle joint. The left foot was placed on a custom-made plate that extended from the actuator such that the left leg was in the same starting position as the right leg. The angular position of the actuator was monitored with an angular displacement transducer (Transtek DC ADT series 600). To elicit the stretch reflex, 6˚ of dorsiflexion rotation was applied at 175˚/s with randomly varying intervals of 5-7 s, when the participants had maintained a background SOL contraction of 5-15% MVC for at least 2 s.

Session protocol: In each of the baseline and conditioning sessions, first, an H-reflex/M-wave recruitment curve was measured. Then, 245 stretch reflex trials were performed. Exact session procedures are described here.

After EMG electrode placement, an H-reflex/M-wave recruitment curve was obtained while the participants stood upright and provided the pre-set level (i.e., 5-15% of MVC level, determined during

the familiarization session) of background activation in SOL. Using an isolated stimulator (Noxitest IES 230), monopolar stimulation of the tibial nerve of the right leg was produced with the cathode (PALs Platinum round electrode, model 879100, 3.2 cm diameter, Axelgaard Man. Co. Ltd.) in the popliteal fossa and the anode (PALs Platinum rectangular electrode, model 895340, 7.5 x 10 cm, Axelgaard Man. Co. Ltd.) on the anterior aspect of the knee at the level of the patella. The cathode location was adjusted to maximize the soleus M-wave. Stimuli were delivered every 5–7 s if the background level of SOL activation had been maintained at the pre-set level (i.e., 5-15% MVC) for at least 2 s. Stimulus intensity was increased in 5-mA increments, with three to four stimuli at each level, until an M-wave [with the size >50 uV] was observed; this was deemed the motor threshold (MT). Stimulus intensity continued to increase until M-wave peak-to-peak amplitude plateaued; this was defined as M_{max}. Then, the scientific software Mr. Kick II 2.3 (Knud Larsen, Center for Sensory-Motor Interaction, Aalborg University, Denmark) was used to control the output of the stimulator such that 10 different stimulation intensities (up to that producing M_{max}) were applied randomly with three stimuli at each intensity. Stimuli were delivered every 5–7 s only if the background level of activation had been maintained at the required level for at least 2 s. The peak-to-peak values of the H-reflex and M-wave for each trial was extracted and the recruitment curves were constructed. Typically, the same range of stimulus intensities were used for all baseline and conditioning sessions. The same stimulus location was maintained throughout the study for each participant.

Next, the soleus stretch reflexes were elicited in one block of 20 control trials followed by three blocks of 75 control trials (in a baseline session) or 75 conditioning trials (in a conditioning session). During control trials, no feedback was provided as to M1 size, and participants were not asked to increase or decrease it. During conditioning trials (i.e., three sets of 75 trials in each of 24 conditioning sessions), participants were asked to either increase (M1up) or decrease (M1down) the size of the M1 component of the stretch reflex. Immediate visual feedback was provided indicating whether the trial was a success (i.e., whether M1 size was above (M1up) or below (M1down) a size criterion (Figure 1D).

Visual feedback: Visual feedback provided to the participant is essentially the same as the one used in the previous H-reflex conditioning studies (Thompson et al., 2009; 2013; Makihara et al., 2014), except that the feedback targeted the M1 response, instead of the H-reflex.

A screen ~1.5 m in front of the participant provided visual feedback on the ongoing SOL EMG activity level (left) and the size of the M1 component of the SOL stretch reflex (right), which occurred typically 39±2 ms after the onset of perturbation (Figure 1C). The background EMG panel (Figure 1D) was the same for both control and conditioning trials. The shaded area of the background EMG panel represented the target window (i.e., corresponding to the 5-15%MVC range, determined during the familiarization session), within which the SOL EMG activity had to be maintained prior to reflex elicitation. The bar

indicated the SOL EMG level in real-time and was updated every 100 ms; it was green if the EMG level stayed within the shaded area and red if it did not.

The M1 panel (Figure 1D) differed between control trials, during which the participant was not asked to modify M1 size, and conditioning trials, during which the participant was asked to increase (M1up) or decrease (M1down) M1 size. During control trials, the shaded area of the M1 panel indicating the range of M1 sizes that satisfy the reward criterion, was set as large as possible so that all trials with various M1 sizes would be registered as "success". During conditioning trials, this shaded area covered only the upper (for M1up) or lower (for M1down) portion of the panel; with M1up, the bottom border of the shade represented the reward criterion, while with M1down the top border represented the reward criterion. For how the reward criterion was calculated, see the paragraph below. When M1 size satisfied the criterion (i.e., when the top of the bar (i.e., M1 size) got in the shaded area), the bar was green, indicating success; when M1 size did not satisfy the criterion (i.e., the top of the bar got out of the shaded area), the bar was red, indicating failure. This feedback appeared 200 ms after the imposed ankle rotation began.

As described in previous studies (Thompson et al., 2009; 2013; Makihara et al., 2014), the reward criterion level was based on the average reflex size for the previous block of trials. Thus, in each conditioning session, the criterion level for the first block of 75 conditioning trials was based on the immediately preceding block of 20 control trials, and the criterion levels for the second and third blocks of conditioning trials were based on the immediately preceding block of 75 conditioning trials. The criterion level was calculated such that if M1 sizes for the new block were similar to those for the previous block, 50–60% of the trials would be successful (Chen and Wolpaw, 1995; Thompson et al., 2009). The thick horizontal line represents the average M1 size for the six baseline sessions. Thus, the participants also received information as to their current performance in relation to their average initial M1 size. The percentage of successful trials within the current block was displayed at the bottom of the screen and updated after each trial, while the number of completed trials was shown at the top of the screen.

Data analysis: To calculate M1 size for each participant's session, M1 size was defined as a root mean square (RMS) value of the rectified SOL EMG in the M1 window minus an RMS value for 100 ms of pre-perturbation period. For each participant, the M1 window was determined as a 10-ms window including the M1 peak (the first peak response that occurred around 45 ms after perturbation onset) by visual inspection (e.g., Figure 1C). Then, the average size was calculated for the 20 within-session control trials, each block of 75 trials, and for all three blocks of 75 trials together. Values were expressed as a percentage of their average values for the six baseline sessions. The size of M2, the second set of peak responses that occur around 60 ms post perturbation onset, was also calculated in a similar way.

The M2 window was typically around 58-68 ms after perturbation onset, whereas a typical M1 window was around 42-52 ms (see Figure 1C).

To determine for each participant if the conditioning (M1up or M1down) had been successful, the average M1 size for the three 75-trial blocks of conditioning trials in the last six conditioning sessions (sessions 19-24) were compared to that for the three blocks of 75 control trials of the six baselines by a single-tail *t*-test. A significant change in M1 size ($p<0.05$) in the direction of conditioning (i.e., increased for M1up, decreased for M1down) defined successful conditioning.

Regardless of whether the data are from a baseline session or a conditioning session, for each participant, M1 sizes from all three 75-trial blocks were averaged together and called "conditioned M1," and M1 sizes from the 20 control trials were averaged together and called "control M1." The final effect of the protocol on the conditioned M1 size was calculated by averaging the M1 size for the three 75-trial blocks of conditioning sessions 22–24 and expressing the value as a percentage of the average M1 size for the three 75-trial blocks of the six baseline sessions. The final effect on the control M1 size was calculated by averaging the M1 size for the 20 control trials of conditioning sessions 22–24 and expressing the value as a percentage of the average M1 size for the first 20 trials of the six baseline sessions. To assess the time course of changes, a repeated-measures ANOVA was used to evaluate conditioned and control M1 sizes across successive 6-session bins (i.e., baseline sessions 1–6 and conditioning sessions 1– 6, 7–12, 13–18, and 19 –24). Comparable procedures were used to assess the impact of the conditioning protocol on the M2 component of the stretch reflex. This procedure was chosen over characterizing the learning via a function, based on the inter-session variability of the reflexes.

In order to assess the session-to-session variability in EMG recording condition, the peak-to-peak M_{max} and the peak-to-peak H_{max} were calculated from the recruitment curve measured at the beginning of each session. To assess the stability of background EMG activity across sessions, the SOL and tibialis anterior background EMG were calculated for each session, along with M1 and M2 sizes. These values were evaluated with a repeated-measures ANOVA, in the same way as the time course evaluation for M1 and M2 changes.

Results

Stability of the M_{max}, H_{max}, and background EMG

In order to ensure that M1 changes over sessions were not due to inter-session differences in electrode placements, we measured the M_{max} at the beginning of every session. Across all M1up participants, the M_{max} averaged 7.7±0.2 (SE) mV during the baseline sessions and 7.7±0.2 mV during the conditioning sessions. One-way repeated measures ANOVA revealed no significant difference across the sessions ($F_{(4,28)} = 0.91$, $p = 0.47$). Similarly, the M1down group showed no significant difference across sessions ($F_{(4,28)} = 1.01$, $p = 0.42$; M_{max} baseline sessions: 6.5±0.8 mV; conditioning sessions: 6.5±0.8 mV). H_{max} did not change significantly across sessions for either the M1up ($F_{(4,28)} = 0.899$, $p = 0.48$) or the M1down group ($F_{(4,28)} = 0.47$, $p = 0.76$); it averaged 3.3±0.2 mV for M1up and 2.8±0.4 mV for M1down during the baseline sessions and 3.5±0.2 mV for M1up and 2.8±0.2mV for M1down during the conditioning sessions. SOL background EMG also remained stable throughout the study; $F_{(4,28)} = 0.335$, $p = 0.852$ for the M1up group; $F_{(4,28)} = 0.338$, $p = 0.850$ for the M1down group, by one-way repeated measures ANOVA. TA background EMG remained at resting level (i.e., <5 μV) in both groups throughout the study. Overall, SOL M_{max} and H_{max}, and SOL and tibialis anterior background EMG values all remained within ±10% of the baseline values throughout the study. Stability of these values in the M1up and M1down groups of participants in whom conditioning was successful is displayed in Figure 2 and summarized in Table 1.

[Figure 2 and Table 1 near here]

M1 and M2 stability in the baseline sessions

All participants completed six baseline sessions in each of which 245 control reflexes were elicited. There was no significant difference in average M1 size between the initial 20 control trials and the subsequent 225 control trials in either the M1up or M1down group (all participants included, two-tailed paired t-test, $p = 0.99$ for each group). M1 size did not differ significantly among the three 75-trial blocks nor across the baseline sessions (block × session interaction, $p = 0.13$ (M1Up) and $p = 0.99$ (M1down)).

There was no significant difference in average M2 size between the initial 20 trials and the subsequent 225 trials in either the M1up or M1down (two-tailed paired t-test, $p = 0.99$ for both types of training). M2 size did not differ significantly among the three 75-trial blocks nor across the baseline sessions (block × session interaction, $p = 0.41$ (M1Up group) and $p = 0.47$ (M1down group)).

The effect of conditioning on the size of the conditioned M1 reflex

As noted above, for each session, the data for the three 75-trial blocks were combined to calculate the average conditioned M1 reflex size, which is referred to as “the conditioned M1 size” and is expressed

as a percentage of the participant's average M1 size for the three 75-trial blocks of the six baseline sessions. Across all participants (i.e., N=8 for M1up and N=8 for M1 down) there was a significant change between the baseline session and the last six conditioning sessions (paired t-test, one-tailed, $p = 0.02$ and $p < 0.001$ for M1up and M1 down respectively). By the analysis described in Methods, conditioning was successful in five of eight M1up participants and in all eight M1down participants. In the other three M1up participants, M1 size did not change significantly. Of the three participants who completed both the M1up and M1down protocols, two were successful in both while one was unsuccessful in M1up conditioning and subsequently (>6 months later) was successful in M1down conditioning.) Figure 3A shows the final conditioned M1 sizes (defined as the average conditioned M1 sizes for sessions 22-24) for the eight M1up and the eight M1down participants. The filled symbols represent the successful and the open symbols the unsuccessful participants. Since the main aim of this study was to characterize the time course of M1 and M2 changes in the responders of M1 conditioning, the rest of this presentation focuses primarily on the data from the 13 successful participants (5 M1up and 8 M1 down).

[Figure 3 near here]

The bottom panels of Figure 4 show the average rectified SOL EMG data of the three 75-trial blocks for one baseline session and the last conditioning session in single participants. The top panels show the ankle angle during these stretch reflex trials. Figure 5A summarizes the time course of M1 size changes for the successfully conditioned participants of both groups; each symbol indicates the group average (±SE) for each session. The conditioned M1 size increased in the M1up group ($F_{(4,16)} = 4.46$, $p = 0.013$; and decreased in the M1down group ($F_{(4,28)} = 18.79$, $p < 0.001$) (top panel). The final conditioned M1 size was 143±15SE % for the M1up group and 62±6% for the M1down group. To aid in assessing the time course of M1 changes, the time course of H-reflex changes using data from (Thompson et al., 2009) is displayed in Figure 5B.

[Figure 4 and 5 near here]

The effect of conditioning on the size of the control M1 reflex

As defined in Methods, the M1 reflex obtained in the first 20 control trials of the control sessions and the 20 control trials of the conditioning sessions is referred to as the "control M1." The middle panel of Figure 5A shows the time course of control M1 size changes across six baseline and 24 conditioning sessions for the M1up and M1down participants; each symbol represents the group mean (±SE) for each session, expressed as a percent of its baseline value. The control M1 size increased in the M1up group ($F_{(4,16)} = 3.09$, $p = 0.046$; and decreased in the M1down group ($F_{(4,28)} = 6.72$, $p = 0.001$). The final control M1 size was 126±18(SE)% of baseline for the M1up group, and 75±8% of baseline for the M1down group.

306 It appears that the control M1 requires more time to change, compared to the conditioned M1 reflex. For
307 the M1up group, the conditioned M1 size was consistently above baseline values from conditioning
308 Session 1 on, while the control M1 did not exceed the baseline value until Session 10. For the M1down
309 group, the conditioned M1 was consistently below the baseline by Session 4 while it was not until
310 Session 16 that the control M1 was consistently below baseline. Although the onset was delayed, the
311 control M1 change was obvious towards the end. Figure 6 shows a typical example of control M1
312 change with M1down conditioning.

313 [Figure 6 near here]

314 **Within-session task-dependent adaptation in the M1 reflex**

315 To quantify the task-dependent change in the M1 reflex from control trials to conditioning trials, for
316 each session, the control M1 size was subtracted from its conditioned M1 size (the bottom panel, Figure
317 5A). A within-session difference in the correct direction (i.e., positive for the M1up group and negative
318 for the M1down group) is referred to as task-dependent adaptation (Thompson et al., 2009). It appears in
319 session 1 for M1up participants and by session 4 for M1down participants, and it remains about the same
320 over the remaining sessions. Its average final (sessions 22-24) values are 24±9SE % and -15±4% for the
321 M1up and M1down groups, respectively. Notably, the amount of task-dependent adaptation with up-
322 conditioning was larger with M1up than with HRup ($p=0.01$ by unpaired t-test for sessions 1-24). Figure
323 7 shows an example of within-session task dependent adaptation in a typical M1up participant.

324 [Figure 7 near here]

325 Table 1 summarizes for the M1up and M1down participants the changes in control and conditioned M1
326 reflexes and their within-session difference (i.e., task-dependent adaptation) across the conditioning
327 sessions grouped into four 6-session bins (C1-6, C7-12, C13-18 and C19-24). To delineate the
328 similarities and differences between M1 conditioning and H-reflex conditioning, the data from this study
329 are presented with the previous H-reflex conditioning data (Thompson et al., 2009). Significant
330 differences from the average of the six baseline sessions are indicated by asterisks. Table 1 verifies the
331 control/conditioned M1 differences in the onset of the impact of conditioning. For the M1up group the
332 within-session difference is significant from the first bin of 6 sessions on, while the conditioned M1 is
333 significantly different from the second bin of 6 sessions and the control M1 is not significantly different
334 until the fourth (and final) bin. For the M1down group, the within-session difference is significant from
335 the third 6-session bin, the conditioned M1 is significantly smaller from the second 6-session bin and the
336 control M1 is not significantly different until the fourth bin.

337 **The effect of M1 conditioning on the size of the M2 reflex**

338 Similar to the M1 size calculation, the M2 values from the three 75-trial blocks were combined to
339 calculate the average M2 size for each conditioning session; this is referred to as "the conditioned M2"

size and expressed as a percentage of the participant's average M2 size across the six baseline sessions. M1 conditioning changed M2 size in the direction of M1 conditioning in 4 of the 8 M1up participants and in 5 of the 8 M1down participants. For the whole group, the final conditioned M2 did not change significantly in the successful M1up group (N=5, p = 0.28, by paired t-test) or successful M1down group (N=8, p = 0.16), (Figure 3B). Final conditioned M2 size averaged 120±32% for the successful M1up and 89±10% for the successful M1down group, respectively. Final control M2 size was 118±32% for the successful M1up and 99±5% for the successful M1down group, respectively. The final M2 within-session change was 2±14% and -10±9% for the M1up and M1down groups, respectively. Table 2 summarizes the changes in conditioned M2, control M2, as well as M2 within-session change across the four 6-session bins of conditioning sessions (C1-6, C7-12, C13-18 and C19-24).

[Table 2 near here]

Discussion

This is, to our knowledge, the first demonstration of operant conditioning of the M1 component of the human soleus stretch reflex. The reflex changed without alterations in background EMG, initial muscle length, or imposed perturbation. M1up conditioning was successful in five of eight participants and M1down conditioning in all eight participants. M1 size increased to 143±15% of its initial value in the successful M1up participants and decreased to 62±6% in the M1down participants. These success rates are similar to those for soleus H-reflex and biceps stretch reflex conditioning in animals (Wolpaw, 1983; Wolpaw et al., 1983a; Wolpaw, 1987; Chen and Wolpaw, 1995; Carp et al., 2005) and humans (Evatt et al., 1989; Wolf and Segal, 1996; Thompson et al., 2009).

The results are particularly notable in two respects. First, they confirm the two-phase acquisition of an operantly conditioned spinal reflex increase or decrease, hypothesized in 1984 (Wolpaw and O'Keefe, 1984) and first documented in 2009 (Thompson et al., 2009). Second, they assess the impact of M1 conditioning on the M2 component of the stretch reflex. Because the stretch reflex is more directly related to motor function than the H-reflex, both of these contributions illuminate the implications of spinal reflex conditioning for understanding normal motor function and for developing protocols that can address the reflex abnormalities associated with spinal cord injury, stroke, or other chronic disorders.

Task-dependent adaptation and long-term change in M1

As in soleus H-reflex conditioning (Thompson et al., 2009), the data reveal a two-phase phenomenon; task-dependent adaptation within conditioning sessions (phase 1) and long-term change across conditioning sessions (phase 2). However, the time courses of these two phases differed from those found for the soleus H-reflex (Thompson et al., 2009). The task-dependent adaptation for a conditioning session was defined as the conditioned M1 size (M1 size for the 225 conditioning trials) minus the control M1 size (M1 size for the 20 control trials). Thus, it shows the immediate effect of asking the participant to increase (or decrease) M1 and providing immediate feedback as to whether the size criterion was met. In contrast, long-term change was indicated by the increase (or decrease) in the control M1 size over conditioning sessions. Thus, it assesses the persistent effect of the conditioning sessions.

In the present results, task-dependent adaptation first appeared in conditioning session one (M1up) or four (M1down) and remained stable over the remaining sessions. Long-term change appeared in session 10 (M1up) or session 16 (M1down) and grew gradually over the remaining sessions. The difference between the M1up and M1down groups in the onset of task specific adaptation and long-term change (also seen for soleus H-reflex conditioning (Thompson et al., 2009)) is further evidence that up-conditioning and down-conditioning are not mirror images of each other but have different mechanisms (Wolpaw, 2007; Thompson et al., 2009). The development of the two phases over the course of up- and

down-conditioning with M1 or H-reflex conditioning (Thompson et al., 2009) are summarized in Table 1. M1up conditioning produces greater task-dependent adaptation and less long-term change (although insignificant) than does H-reflex up-conditioning; in contrast, M1down conditioning produces comparable task-dependent adaptation and greater (although insignificant) long-term change compared to H-reflex down-conditioning. Overall, for both task-dependent adaptation and long-term change, the time courses of development are similar for M1 and H-reflex conditioning.

How do we interpret the differences and similarities between M1 and H-reflex conditioning? The H-reflex is referred to as the electrical analog of the M1 stretch reflex. A principal difference is that the H-reflex bypasses the muscle spindle while M1 is affected by the sensitivity of the spindle and thus by γ-motoneuron activity. This may constitute an extra degree of freedom available for participants as they learn to change M1 size. Muscle spindle excitation is affected by initial muscle length (Matthews, 1972) and muscle background activity (Marsden et al., 1976; 1983). Both of these variables were maintained stable throughout the study; thus, it is unlikely that they contributed to the changes. It is possible that alterations in γ-motoneuron activity contributed to M1 change, as suggested for monkey biceps M1 conditioning (Wolpaw and O'Keefe, 1984).

Another potential site of change is the Ia-synapse, known to be modulated by presynaptic inhibition (Eccles et al., 1962; Stein, 1995). This inhibition is influenced by corticospinal, reticulospinal and vestibulospinal pathways (Iles, 1996; Meunier and Pierrot-Deseilligny, 1998; Pierrot-Deseilligny and Burke, 2012; Baldissera et al., 1981) and is task-dependently modulated (Hultborn et al., 1987; Hultborn and Meunier, 1987; Stein and Capaday, 1988; Stein, 1995; Côté and Gossard, 2003). Morita et al. (Morita *et al.*, 1998) reported evidence that the stretch reflex is less sensitive to presynaptic inhibition than the H-reflex; this is not consistent with the present finding that the conditioned M1 change is greater than the H-reflex change. In the present study, M1up produced a larger within-session M1 increase (task-dependent adaptation), for which the most plausible mechanism is a change in presynaptic inhibition (Capaday and Stein, 1987). Indeed, a release of presynaptic inhibition at the segmental level has been implicated to be a mechanism responsible for the increase in H-reflex and stretch reflex size during the Jendrassik maneuver (Dowman and Wolpaw, 1988; Zehr and Stein, 1999). However, in the current study, participants were instructed to maintain the upper body (including facial muscles) relaxed throughout all the reflex trials. Thus, changes in presynaptic inhibition via the Jendrasski maneuver are unlikely to have occurred here. In addition to presynaptic inhibition, animal studies, which would capture long-term physiological and/or anatomical changes, provide substantial evidence of altered motoneuron properties, in GABAergic terminals on motoneurons, and possibly in oligosynaptic afferent pathways (Wolpaw, 1997; 2010; Thompson and Wolpaw, 2014). Further studies are needed to identify the mechanisms underlying M1 and H-reflex conditioning and the extent to which they differ.

The effect on the M2 component of the soleus stretch reflex

For M1up and M1down groups, the changes in M1 did not have large or consistent effects on M2 size. This differs from stretch reflex conditioning in the biceps brachii M1, which produced significant changes in M2 (Wolf et al., 1995). Since the M2 of upper limb muscles is likely generated through transcortical pathways (Crago et al., 1976; Marsden et al., 1977; Rothwell et al., 1986; Goodin et al., 1990; Capaday et al., 1991; Thilmann et al., 1991; Palmer and Ashby, 1992), it might be more comparable to the M3 for lower limb muscles (Petersen et al., 1998; Mrachacz-Kersting et al., 2006). (Note that the M3 was not measurable in the present study in which the conditioning protocol was administered in sitting participants.) The M2 of the soleus muscle is thought to be largely mediated by group Ib and/or group II afferents (Schieppati and Nardone, 1997; Dietz, 1998; Schieppati and Nardone, 1999; Dietz and Duysens, 2000; Grey et al., 2001; Sinkjaer et al., 2004; af Klint et al., 2010). Thus, the soleus M2 is not comparable to the M2 of the biceps brachii.

A possible explanation why M2 did not change consistently, is a potentially mixed origin of M2. Because the distinction between M1 and M2 is based on their latencies, there is a possibility that delayed Ia excitation of motoneurons may contribute to M2, in addition to the excitation from Ib and/or group II afferents. With M1up conditioning, such delayed Ia excitation could decrease, as those motoneurons could start firing in the M1 time window. This explanation seems feasible in some, but not all participants, however. Another explanation is related to the functional relevance of M2 during the sitting task in the current study. It is unlikely that the M1 or M2 in the soleus would have significant function in the sitting posture, thus changes in M2 would not necessarily reflect systematic changes (compensatory or reactive plasticity (Wolpaw, 2010)). Future studies should condition the soleus stretch reflex during more functional tasks, such as standing as has been done for the soleus H-reflex conditioning (Thompson et al., 2009; Makihara et al., 2014).

Functional Implications

Several types of afferents generate the stretch reflex; Group Ia and II afferents arising from muscle spindles, Group Ib afferents from Golgi tendon organs, and cutaneous afferents. Providing perturbations such as the ankle joint rotations used here, is one way to probe these pathways and quantify their role during tasks such as walking. These perturbations activate the afferents in a manner similar to what occurs during normal behaviour: they are dispersed in time and the same afferents may be activated several times. Additionally, and unlike the H-reflex, the stretch reflex size is affected by the sensitivity of the muscle spindle, and thus by descending gamma drive. Conditioning of the stretch reflex thereby provides a more natural paradigm into the adaptations of the underlying circuitry.

Appropriate soleus H-reflex conditioning leads to a return to a more normal gait pattern in spinal cord injured animals and humans (Chen et al., 2006; Manella et al., 2013; Thompson et al., 2013). This favourable effect on locomotion results from the H-reflex changes triggering much wider beneficial plasticity (Thompson et al., 2013; Thompson and Wolpaw, 2014). While the H-reflex depends mainly on

455 the Ia afferent pathway, Group Ib and II afferents appear to play a prominent role in generating soleus
456 locomotor activity in humans (Schieppati and Nardone, 1997; Dietz, 1998; Schieppati and Nardone,
457 1999; Dietz and Duysens, 2000; Grey et al., 2001; Sinkjaer et al., 2004; af Klint et al., 2010). Thus,
458 operant conditioning of the soleus stretch reflex, which engages these other pathways, might prove to be
459 a more efficient and/or effective therapeutic approach than H-reflex conditioning.

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610

Table 1: Soleus and tibialis anterior background EMG during stretch reflex trials and the soleus M_{max} and H_{max} values during standing for each of successive 6-session blocks.

	Group	B1-6	C1-C6	C7-C12	C13-C18	C19-C24
Soleus EMG (μV)	M1up	15.8±1.7	15.8±1.4	15.2±1.6	14.9±1.7	15.6±1.8
	M1down	16.4±2.8	16.4±2.7	15.7±2.6	16.3±2.8	16.1±2.5
TA EMG (μV)	M1up	3.6±0.4	4.2±0.2	3.9±0.3	3.8±0.4	4.0±0.5
	M1down	3.6±0.3	3.9±0.3	3.8±0.4	3.8±0.4	3.7±0.4
Soleus M_{max} (mV)	M1up	8.1±0.6	8.2±0.7	8.5±0.6	8.4±0.7	8.4±0.7
	M1down	6.7±0.9	6.7±0.9	6.7±0.9	6.6±0.9	6.7±0.9
Soleus H_{max} (mV)	M1up	3.9±0.5	4.1±0.6	4.3±0.5	4.3±0.6	4.3±0.7
	M1down	2.7±0.4	2.7±0.6	2.7±0.6	2.6±0.5	2.6±0.5

All values are mean ± SE for successful M1up or M1down participants, and are expressed as percentage of baseline values. None of the values from conditioning sessions are significantly different from the values from baseline sessions.

Table 2: M1 reflex values for all successful M1up and M1down participants compared to H-reflex values for all successful HRup and HRdown participants from the study by Thompson et al. (2009) for each of successive 6-session blocks.

	Group	C1-C6 (%)	C7-C12 (%)	C13-C18 (%)	C19-C24 (%)
Conditioned reflex	M1up	116.9±6.8*	136.8±12.7*	145.7±22.4*	141.2±17.2*
	HRup	115.6±6.2	122.4±5.5*	127.6±7.5*	137.3±8.6*
Control reflex	M1up	96.6±4.3	109.5±5.6	118.4±11.2*	117.2±10.9*
	HRup	106.4±6.0	106.7±3.7	116.5±4.9	128.2±5.4*
Within-session change	M1up	20.3±6.2*	27.2±8.5*	27.3±12.5*	24.0±7.9*
	HRup	9.2±5.4	15.6±4.1*	11.6±4.2*	-12.4±6.0*
Conditioned reflex	M1down	92.9±5.1	84.9±6.2*	74.4±5.9*	62.1±7.1*
	HRdown	93.4±4.0	81.7±4.4*	75.1±4.9*	72.3±5.3*
Control reflex	M1down	100.0±5.8	96.8±4.2	89.2±6.5	77.5±5.5*
	HRdown	97.1±1.8	95.5±2.8	90.5±4.5	86.7±5.9*
Within-session change	M1down	-7.5±3.8	-11.9±5.2	-14.8±5.0*	-15.3±4.0*
	HRdown	-3.8±3.2	-13.9±3.2*	-14.4±5.1*	-14.4±6.3*

Values represent the average ± SE and are expressed as percentage of baseline values.
* Significant differences from the six baseline sessions ($p < 0.05$, LSD *post hoc* after repeated measures ANOVA).
* H-reflex values are from Thompson et al., (2009).

Table 3: M2 stretch reflex values for all successful M2up and M2down participants for each of successive 6-session blocks.

	Group	C1-C6 (%)	C7-C12 (%)	C13-C18 (%)	C19-C24 (%)
Conditioned M2 reflex	M1up	119.4±17.5	126.3±30.1	126.8±28.2	122.7±23.6
	M1down	110.4±7.7	104.8±11.5	97.5±9.3	89.9±9.6
Control M2 reflex	M1up	111.2±14.6	129.1±30.9	139±40.2	124.4±26.3
	M1down	111.4±3.9	110.5±7.7	100.1±8.3	97.4±6.5
Within-session change	M1up	8.2±9.3	-2.8±12.4	-12.2±16.5	-1.7±11.6
	M1down	-1.0±5.7	-5.7±4.8	-2.6±8.3	-7.5±6.8

Values represent the average ± SE and are expressed as percentage of baseline values.
* Significant differences from the six baseline sessions ($p < 0.05$).

Figure Legends

Figure 1: (A) Study session schedule. Six baseline sessions are followed by 24 conditioning sessions, all at pace of 3 sessions per week. **(B)** The stretch reflex pedal. Participants are seated comfortably with both feet on separate foot plates. **(C)** The upper panel shows the change in ankle angle in degrees, the lower panel depicts the activity of the soleus muscle following a single imposed dorsiflexion perturbation. Several peaks may be seen labelled as M1 and M2. The grey shaded area visualizes the time window for the M1 for which the participants received feedback during the conditioning sessions. **(D)** Visual feedback. The feedback on the screen is comprised of two parts, the background EMG and the stretch reflex size both shown as bars. The shaded area in the left panel represents the pre-set range for the soleus background activity, which must be maintained for at least 2s by the participant for a stretch reflex trial to occur. The shaded area in the right panel represents the targeted range for the size of M1 component. During control trials, this right shaded area is set as large as possible since the participant is not training to modify the M1 size. During M1up conditioning trials, this shaded area appears in the upper half (i.e., a criterion level and above), based on the baseline sessions. In contrast, during M1down conditioning trials, this area appears in the lower half (i.e., a criterion level and below). Immediately after a stretch reflex trial occurs (i.e., 200 ms after perturbation onset), a vertical bar reflecting M1 size is displayed. When the bar height falls within the shaded area, the participant had a successful conditioning trial and the bar is depicted as green. If the bar height falls out of the shaded area, the bar becomes red and the trial is registered as an unsuccessful trial. This provides immediate feedback on M1 size to the participant for each single trial performed.

Figure 2: Soleus and TA background EMG and the soleus M_{max} and H_{max} values for all baseline and conditioning sessions in M1up **(A)** and M1down **(B)** participants in whom conditioning was successful. Each set of a symbol and error bars represents the average (±SE) value for successfully conditioned participants. N=5 for M1up **(A)** and N=8 for M1down **(B)**. Circles are for the soleus background EMG amplitude (in μV), squares are for the TA background EMG (in μV), diamonds are for the M_{max} (in mV), and crosses are for the H_{max} (in mV).

Figure 3: (A) The final conditioned M1 size for individual participants. The filled symbols represented successful participants in whom the average conditioned M1 for conditioning sessions 19-24 was significantly increased (5 of 8 M1up participants, upward triangles) or decreased (8 of 8 M1down participants, downward triangles) compared to the average baseline M1. The open symbols show the three unsuccessful participants (i.e., 3 of 8 M1up participants). **(B)** The final conditioned M2 size for individual participants. As for (A), the filled symbols represented the successful participants in whom the average conditioned M1 for conditioning sessions 19-24 was significantly increased (5 of 8 M1up participants, upward triangles) or decreased (8 of 8 M1down participants, downward triangles) compared to the average baseline M1.

Figure 4: Average conditioned and control stretch reflexes for a representative participant of the M1up **(A)** and M1down **(B)** group. The upper panels show the change in ankle angle in degrees, the lower panels depict the average rectified SOL EMG data of the three 75-trial blocks for one baseline session and the last conditioning session in single participants. The dotted black traces represent data from a single baseline session while the red (M1up) and blue (M1down) traces represent data from the last conditioning session.

Figure 5: Average (±SE) M1 sizes and H-reflex sizes (from Thompson et al., 2009) for all successful M1up/HRup (red upward triangle) and M1down/HRdown (blue downward triangle) participants for all baseline and conditioning sessions. **(A)** upper panel: Average conditioned M1 size, middle panel: Average control M1 size and lower panel: within sessions change (average conditioned minus control M1 size). **(B)** upper panel: Average conditioned H-reflex size, middle panel: Average control H-reflex size and lower panel: within sessions change (average conditioned minus control H-reflex size). The vertical dotted line separates the baseline from the conditioning sessions.

Figure 6: Control M1 change with M1down conditioning. Control SOL EMG during one baseline session (black trace) and the final conditioning session (blue trace) for n=1. Each trace is the average of 20 trials.

685 **Figure 7:** Within-session task dependent adaptation with M1up conditioning. Control SOL EMG during
686 the control trials (black trace, the first 20 trials where no visual feedback is provided) and the
687 conditioning trials (red trace, the three blocks of 75 trials where feedback is provided in relation to the
688 size of the M1 response) for n=1.