Study Title: Operant H-reflex Down-conditioning of Rectus Femoris in Post-stroke Stiff Knee Gait

NCT number: NCT05467774

Document Date: 7/28/2021

SUBJECTS

A total of 7 participants (5 unimpaired [H1-H5, ages 20-26 years, 3 M/ 2 F], 2 post-stroke [S1-S2, 1 M/1 F) were recruited for this study. Each participant was able to stand for 10-minute intervals unassisted, walk on a treadmill for 10-minutes, and provide informed consent. Exclusion criteria included: lower limb musculoskeletal injury (e.g., fractures, sprains, strains, tendonitis, or bursitis), functionally relevant weight-bearing restrictions, vision impairment and must not have taken antispasmodic medication one day prior to the session. All 7 people participated in down-conditioning. The study was approved by the University of Texas Institutional Review Board, and all subjects gave informed consent prior to participation. Both post-stroke individuals had right hemiparesis and the experiment was conducted on the right side.

ACQUISITION AND PROCEDURES

The experimental setup consisted of 8-channel surface electromyography (EMG) sensors (AMT-8, Bortec, Calgary, AL), bipolar electrodes (Ag-AgCl, Noraxon, Scottsdale, AZ) a constant current electrical stimulator (Digitimer DS8R, Hertfordshire, UK), a data acquisition board (NI-PCIe 6321, Austin, TX), a desktop computer, and EPOCS software (**Fig 1**). Additionally, during assessment sessions, stroke subjects walked on an instrumented split-belt force treadmill (Bertec, Columbus, OH), during which the ground reaction forces (GRF) were recorded at 1kHz, and lower limb kinematic data were recorded via inertial motion capture (IMU, Xsens, Enschede, Netherlands) at 60 Hz.

The experiment was conducted over 30 experimental sessions per individual, with 3 sessions per week on non-consecutive days. To prevent diurnal variation in H-reflex magnitude, all training was performed at the same time of the day for each individual. Each experimental session consisted of preparation, femoral nerve navigation, recruitment and stimulation stages. The *preparation stage* started with drawing a grid on the surface of the femoral triangle on the leg. The femoral nerve was identified using an anatomical landmark-based method known as the "four-three finger technique". Surface EMG electrodes were attached over the muscle belly of the rectus femoris (RF), medial hamstring (MH), vastus medialis (VM), and vastus lateralis (VL) based on commonly used anatomical landmarks[31,32]. RF was the primary focus for H-reflex monitoring, MH was monitored for antagonist activity, and VM and VL were monitored to validate the training specificity of RF H-reflex conditioning. EMG activity was amplified, bandpass filtered (3-1,000 Hz), and sampled at 1 kHz.

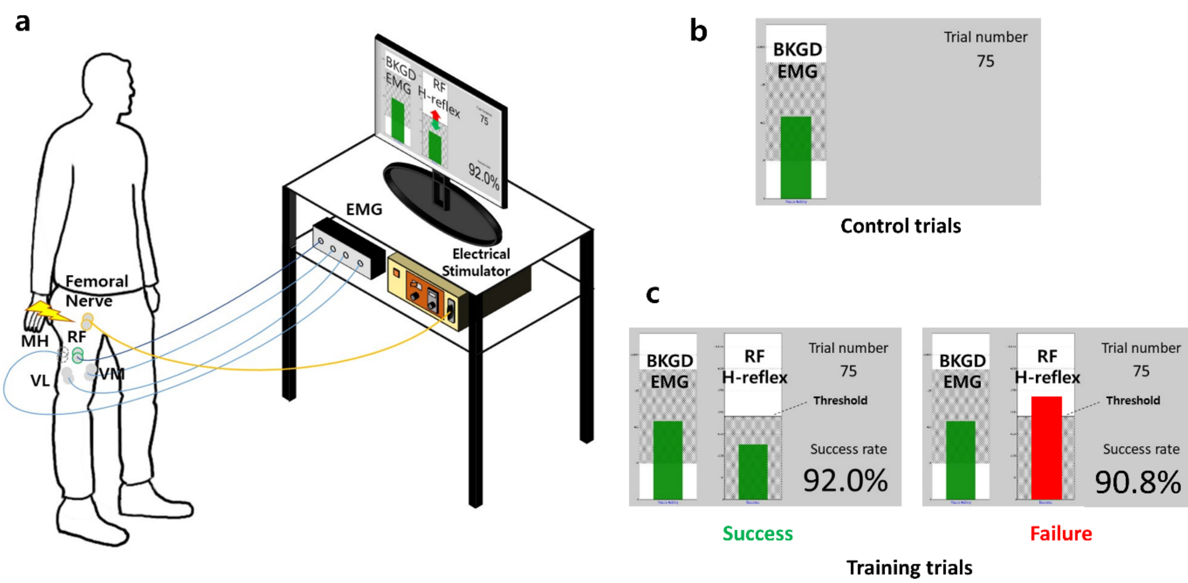

Fig 1 Experimental setup and visual feedback of RF (rectus femoris) operant H-reflex conditioning. (a) Participant with electrodes on the right leg is in the upright standing posture, facing visual feedback from a computer monitor. **(b)** During control/baseline trials, only the

background EMG level of the RF was provided on the left bar graph. If the background EMG level of the RF and its antagonist pair MH (medial hamstrings) was within the shaded region, the bar remained green and the electrical stimulation was delivered. Otherwise, the bar turned red with no stimulation or trial progression. **(c)** During the training trial, RF H-reflex size and cumulative success rate were provided along with background EMG. The bar turned green if the RF H-reflex was below the threshold and turned red if larger than the threshold.

During the *femoral nerve navigation* stage, the optimal stimulation location of the femoral nerve was established via a monopolar probe electrode to each point on the grid drawn on the femoral triangle[34]. Electrical stimulation was delivered as a square stimulus pulse, 1 ms in duration, intensity ranging between 10-20 mA or above depending on the individual, to elicit a reflex response with the subject in quiet standing. We monitored the time-course of the reflex response. The H-reflex was determined as the peak response following the initial stimulus artifact (<3 ms) and M-wave (~10-25 ms), typically within 30-40 ms post-stimulus. We used the following criteria to identify the optimal H-reflex response: 1) a clear biphasic shape, 2) a clear distinction from the M-wave or in case of contamination the biphasic signal lies on the downward slope of the M-wave, and 3) largest signal magnitude of all the candidate spots. After the optimal stimulation spot was located, the monopolar probe was replaced with a disposable Ag/AgCl snap electrode (circular, 1cm diameter) and the anode electrode (square 5x5cm) was placed over the surface of gluteus maximus on the ipsilateral side. To avoid session-to-session variability in electrode locations, the positions of all electrodes were marked using semi-permanent surgical skin marker. After electrodes were placed, a maximum voluntary contraction (MVC) for RF and MH were measured separately. Participants were asked to sit on a chair with their ankle fastened with a belt to the leg of the chair and knee flexed to 90°. The investigator asked the participant to extend or flex their knee with maximum effort for 5-7 s. The participant repeated this three times with 15 s intervals in between. The representative MVC was determined as the mean of three MVC trials.

During the *recruitment* stage, the RF H-reflex recruitment curve was achieved with the subject in a quiet standing posture. Electrical stimulation was delivered at 0.14 Hz only if the background EMG level of RF and MH were within a 10-20% MVC window respectively, representing a slight tonic voluntary activation to avoid altering reflex excitability. To find the maximum M-wave amplitude of the RF, we increased stimulation intensity by 2 mA increments starting at 10 mA until the RF M-wave amplitude remained constant despite increasing current in three successive stimulus intensities, defined as M_{max}. For each stimulus intensity, two H-reflex responses were recorded. The H-reflex size was calculated as the mean of the peak-to-peak amplitudes of the two responses. The corresponding intensity (I_{max}) that elicited maximal representative H-reflex (H_{max}) was used in the following trials. Post-stroke individuals did not require any additional support for standing, however for safety purposes a handrail was placed in front of the individual.

During the *stimulation stage*, also in performed in a standing posture, the femoral nerve was stimulated at I_{max} at 0.14 Hz based on the same 10-20% MVC requirement. The first 6 sessions were baseline sessions with no feedback of RF H-reflex. Each baseline session was composed of 3 runs of 75 trials, where each trial was a single stimulation pulse. The next 24 sessions were training sessions. During training, each session began with 20 control trials with no feedback, followed by 3 runs of 75 trials using the peak-to-peak magnitude of RF H-reflex as visual feedback to the participant (**Fig 1**). During training sessions trials, M-wave size was monitored, and stimulus strength (I_{max}) was minimally adjusted to maintain the predetermined M-wave size. Post-stroke individuals participated in an additional 2 assessment sessions, one before training and one after training sessions. In participant H4, in two of the 30 training sessions, the H-reflex showed overlap with the M-wave. In offline analysis, we used a best decaying exponential fit to obtain the H-reflex estimate as illustrated in our earlier work.

FEEDBACK

As shown in **Fig 1c**, visual feedback consisted of 1) RF and MH background EMG (left bar), 2) RF H-reflex (right bar), 3) trial number, and 4) cumulative success rate. The left bar was green if the background EMG was below activation threshold but was red otherwise. The H-reflex stimulation was delivered only when the background EMG bar was green. The participant was provided with the objective to reduce the height of the right bar, representing H-reflex amplitude, below a given threshold (shaded area in **Fig 1c**). The threshold was updated for each run as the 66^{th} percentile of the H-reflex trials from the previous run. If below the performance threshold, the right bar turned green and cumulative success rate was adjusted accordingly. If above the threshold, the bar turned red, and the success rate fell. Each trial was 7 s duration, with a single stimulation pulse stimulation of 1 ms pulse width followed by feedback for the remainder of the trial. Each session was recorded on a different non-consecutive day, up to 3 times per week. The 6 baseline sessions each consisted of 3 runs of 75 control trials (225 total), while each of 24 training sessions consisted of 20 control trials and 3 runs of 75 training trials (245 total) in standing posture. Subjects were exposed to H-reflex feedback only during the training trials, and no H-reflex feedback was given during the control trials. The participant's score for each run was cumulative success rate ($Scr = \frac{N_{success}}{N_{trial}} \%$) and participants were rewarded in proportion to one's score (i.e., $Reward(\$) = 1.0 + 0.05 * (Scr - 50)$). If the participant was successful in achieving three consecutive success rates that were equal to or higher than 90% for all three runs, they were provided with an extra dollar as bonus, which made the maximum additional compensation amount $10.00. A rest period was provided between each run to minimize fatigue, which could affect muscular responses to stimuli.

CLINICAL ASSESSMENTS

The two individuals with stroke participated in pre and post training assessment sessions. Participants performed a 10 m walk test (10MWT) with instructions to walk with maximum effort. This process was repeated 4 times and the participant's gait kinematics were recorded using inertial motion capture. Next, participants completed the quadriceps pendulum test, which is a non-invasive biomechanical method of evaluating spasticity using gravity to provoke muscle stretch reflexes during passive swinging of the lower limb. Inertial measurement units were attached on top of thigh, shank, and foot to calculate the knee joint angle during the process. This test was repeated 3 times with 30 second rest periods in between. The main outcome of this test was quadriceps reflex threshold angle (QRTA), which was the angle difference from the maximum knee extension position to the first swing excursion, the first transition period of knee flexion to knee extension. Lastly, the participant was asked to walk on an instrumented treadmill while their gait kinematics, ground reaction forces (GRF), and H-reflexes were obtained. During treadmill walking, four gait phases (heel-strike, toe-off, mid-stance, and mid-swing) were detected in real-time using online GRF data using conventional gait analysis[1]. Heel-strike was detected when GRF value exceeded a predetermined force threshold (F_{th}) for the first time (0% gait cycle) and toe-off was detected with GRF dropping below the threshold (F_{th}) (60% gait cycle). Mid-stance was detected as the time point when 30% of step duration, pre-calculated from one's step length and gait speed, had elapsed from the moment of heel-strike. Mid-swing was determined in a similar manner, which was detected at the 80% gait cycle. The H-reflex was elicited and measured only when a desired gait phase was detected and occurred at least 7 s after the last H-reflex trial. Twenty H-reflexes were recorded for each gait phase. A 5 min break after each of four runs was provided to minimize the effect of fatigue on H-reflex. The main outcomes consisted of 1) knee flexion range of motion (RoM), 2) peak knee flexion velocity, and 3) H-reflex amplitude during the four different gait phases.

OUTCOME MEASURES

We normalized the H-reflex measurements. For each session, the H-reflex was normalized with the session's maximum motor response (M-wave, M_{max}), which is the most commonly used method of normalization[40]. To directly compare between session-to-session performances, each session's H-reflex trial was averaged, normalized by M_{max}, and represented as a percentage (%) of the mean H-reflex of 6 baseline sessions. Main outcome measures of each session were the average of 225 peak-to-peak H-reflex responses from the RF and VM.

STATISTICAL ANALYSIS

We investigated the hypothesis that RF H-reflex can be down-conditioned through operant H-reflex conditioning. We first verified normality of the data using a Shapiro-Wilk test. To validate the effect of training, a paired t-test (a<0.05) was used to compare the mean of the 225 normalized H-reflex trials during 6 baseline sessions to that of the last 6 training sessions.

ETHICS APPROVAL

This study was conducted according to the Declaration of Helsinki and had ethical approval from the University of Texas Institutional Review Board. All subjects gave written informed consent prior to data collection.