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THE EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION ON CHRONIC ANKLE INSTABILITY

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ABSTRACT

Purpose: Given maladaptive neuroplasticity following musculoskeletal injury, interventions capable of restoring corticospinal excitability should be considered. We therefore aimed to determine if 4-weeks of anodal transcranial direct current stimulation (aTDCS) with eccentric exercise would improve neural excitability, functional performance, and patient-reported function in individuals with chronic ankle instability (CAI). Methods: Twenty-six individuals with CAI were recruited to undergo 4-weeks of eccentric evetor strengthening. Subjects were randomized into aTDCS (n=13) and sham (n=13) groups, where the aTDCS group received 18-minutes of aTDCS (1.5mA) over the primary motor cortex. Participants were assessed for cortical excitability, dynamic balance & muscle activation, functional performance & strength, and patient-reported function at baseline, week-2, week-4, and week-6. Results: Twenty-two subjects completed the training and test sessions. Cortical excitability (resting motor threshold) to peroneus longus in aTDCS increased from baseline (36.92 ± 11.53) to week-6 (32.91 ± 12.33 , $p=0.024$), while sham increased excitability from baseline (36.67 ± 12.74) to week-2 (27.86 ± 14.69 , $p=0.007$), but decreased at week-4 (35.63 ± 13.10 , $p=0.022$) & week-6 (35.99 ± 13.52 , $p=0.006$). Dynamic balance and muscle activation also improved in the aTDCS group from baseline to week-6 ($p=0.034$). Functional performance on a side-hop test increased in all participants from baseline to week-2 ($p=0.003$). The aTDCS group had decreased perceived disablement from week-2 (18.09 ± 6.41) to week-4 (15.55 ± 4.82 , $p=0.046$), whereas the sham group reported increased disablement from baseline (17.91 ± 4.59) to week-2 (21.00 ± 8.52 , $p=0.047$). Conclusions: Our results provide preliminary evidence that 4-weeks of eccentric training with aTDCS improves cortical excitability, functional performance, and patient-reported

function in individuals with CAI. These data are the first to show the efficacy of non-invasive brain stimulation therapies in patients with musculoskeletal injury, and demonstrates the link between improved neural excitability and functional outcomes.

Key Words: Non-invasive brain stimulation; Ankle rehabilitation; Transcranial magnetic stimulation; Eccentric exercise; Dynamic postural stability.

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INTRODUCTION

Sixty percent of the general population experience ankle sprains that – despite a mild rehabilitation process – leads to re-injury and the formation of chronic ankle instability (CAI) in nearly half of patients experiencing an initial injury (1, 2). CAI is characterized by repeated sensations of rolling and giving-way at the joint following injury (3), and has been linked to decreased health-related quality of life and long-term joint degeneration (4, 5). Given high rates of disablement in patients with CAI, it appears that current impairment-based rehabilitation (i.e. targeting strength, range-of-motion, and balance deficits) are insufficient, highlighting the need for more innovative approaches to rehabilitation.

Recent paradigm shifts in joint instability etiology at both the ankle and knee have identified changes within the central nervous system that alter motor planning, generating movement patterns that predispose individuals for re-injury (6). Specifically, individuals with CAI and those with anterior cruciate ligament (ACL) injury demonstrate decreased excitability of the primary motor cortex (M1), altered somatosensory cortex activation in response to joint loading, and utilize increased activation of visual and planning areas in simple movement execution when compared to uninjured controls (6). Therefore, typical impairment-based rehabilitation programs may be able to restore clinical function as patients develop affordances whereby additional cortical resources are recruited to execute ‘normal’ movement and overcome decreased M1 excitability. However, this cortical spread may contribute to a loss of affordances when additional task constraints are imposed (e.g. dual-tasking, decision making, external distractions) that contribute to a degradation of motion and re-injury (7). Therefore, clinical interventions should address changes in cortical plasticity to enhance M1 excitability and decrease reliance on

extraneous cortical areas in movement execution, in conjunction with impairment-based rehabilitation.

One such intervention capable of positively affecting cortical excitability and thus addressing the maladaptive neuroplasticity following joint instability is anodal transcranial direct current stimulation (aTDCS). This intervention is a form of non-invasive brain stimulation in which a small electrical current (0.6 to 2.0 mA) is applied between areas of the cortex (8). Specifically, aTDCS can be applied with an anode over M1 and cathode over the forehead to modify resting membrane potentials of intracortical neurons, thus decreasing the threshold for membrane depolarization and facilitating long-term potentiation (LTP) (8). Only recently has this intervention been found to be efficacious in targeting the deeper area of the motor cortex that controls the lower limbs (9), with its use described to improve reaction times and balance in the legs (10, 11). Previous investigations into this intervention have generated conflicting results (12), but this could be due to the populations in which these studies are conducted. Often aTDCS is implemented to improve motor function among healthy individuals or those with neurological impairment (e.g. stroke, Parkinson's disease, traumatic brain injury); however, the former may face a ceiling effect towards potential improvements, while the latter typically present with a structural change to the brain, rather than a functional neuroplasticity such as that experienced by patients with joint injury.

A key consideration in the use of aTDCS is selecting a motor task with which to pair it, as its primary use is as an adjuvant therapy to enhance the acquisition of a task (8). In patients with CAI, deficits are most commonly described towards neural control of the peroneus longus – the

primary stabilizer against injurious supination moments (6). Therefore, enhancing neural excitability to this muscle would be the intention of treatment. Recently, emerging evidence in models of ACL injury have described the potential benefits of eccentric exercise in improving neuromuscular function (13). Eccentric exercise allows for heavier loads on the muscle that, aside from contributing to hypertrophy, decreases neural inhibition to target musculature and thus improves excitability (14). Therefore, the combination of neuromodulatory interventions in the form of aTDCS and eccentric exercise of the peroneus longus may increase neural excitability and therefore improve muscle recruitment and function among these individuals.

To date, no published investigations have reported the effects of aTDCS in patients with musculoskeletal injury. Therefore, we aimed to conduct a preliminary investigation into the feasibility and efficacy of 4-weeks of eccentric ankle exercise in conjunction with aTDCS in improving neural excitability, functional performance, and patient-reported outcomes in individuals with CAI. We hypothesized that while eccentric training would contribute to improvements across all participants, the individuals receiving aTDCS could demonstrate earlier, greater, and more durable improvements in outcome variables. The results of this study would provide a foundation on which further clinical trials aimed at modifying joint injury rehabilitation paradigms could be based.

METHODS

Study Design

This study incorporated a longitudinal randomized, single-blinded, and controlled trial intended to explore the feasibility of aTDCS among individuals with CAI. Participants were randomized

into aTDCS and sham groups and completed 4-weeks of eccentric ankle strength training supervised by one of three trained members of the research team. Outcome measures were assessed at baseline, halfway through training (week-2), completion of training (week-4), and retention (week-6). Independent variables of interest included group (aTDCS versus sham) and time (baseline, week-2, week-4, week-6). Dependent variables included cortical excitability to the peroneus longus and tibialis anterior, intracortical inhibition, dynamic balance, muscle activation, functional performance, strength and patient-reported outcomes of ankle and global disablement.

Participants

Twenty-six individuals with CAI and between the ages of 18 to 40 were recruited for this investigation (Table 1). CAI was defined as experiencing an ankle sprain more than 1 year prior to the initiation of the study, with recurrent sensations of rolling or giving-way as defined by a score on the Identification of Functional Ankle Instability (IdFAI) greater than 10 (3, 15). In the case of bilateral instability, the side with the higher IdFAI score was selected. Subjects were recruited from a university community through flyers, class and electronic mail announcements, and website postings. Subjects were excluded if they had an injury that limited them from performing physical activity within the previous three months or had a history of leg fracture or surgery. Additionally, participants met criteria for the safe practice of transcranial magnetic stimulation (TMS), and aTDCS (16). All participants provided Appalachian State University IRB-approved informed consent (18-0237). Participants were asked to refrain from ingesting caffeine and alcohol for 12 hours prior to reporting to the lab for both testing and training sessions. Following the baseline session, subjects were randomly allocated into aTDCS or sham

groups using a block randomization scheme (block size 4-6) using a list randomizer from random.org.

This investigation is a preliminary investigation with no data available within this population to determine the efficacy of aTDCS. Previous aTDCS investigations using long-term training in healthy individuals with similar outcome variables (i.e. cortical excitability, strength) were used to estimate a sample size ($f=0.43$ to 0.55 ; $1-\beta=0.8$; $\alpha=0.05$) (10, 17), with 10 subjects per group identified as achieving sufficient power. Thus, 26 subjects were recruited to account for up to 25 percent attrition.

Assessment of Dependent Variables

Neural excitability was assessed in a Faraday-shielded electrophysiology laboratory using TMS over M1. Prior to each testing session, participants completed a safety questionnaire to confirm no changes to risk factors related to TMS or aTDCS. Participants were instrumented with surface electromyography (EMG) sensors over the tibialis anterior and peroneus longus. The area over each muscle was palpated, shaved if necessary, cleaned with an alcohol pad, and abraded prior to placing the sensor which was connected directly to an amplifier (B&L Engineering, Santa Ana, CA). An elastic wrap was placed around the lower leg to secure the sensors, and elastic cap was placed over the head to allow investigators to identify landmarks for the TMS coil. The order of testing each day was as follows: familiarization with TMS procedures, location of the M1 “hotspot” for the peroneus longus, obtaining a resting stimulus-response curve, and assessment of intracortical inhibition.

After explaining procedures to subjects, a lower extremity magnetic coil connected to a 2.0T stimulator (MagStim 200², Wales, UK) was placed at the vertex of the skull and gradually increasing stimuli were applied until a visible twitch in the ankle was observed. The intensity would then slightly be lowered as the investigator searched a 5cm² area beginning 1cm lateral and anterior to the vertex, until the largest EMG response was observed from the peroneus longus. This location was designated as the hotspot and was used for subsequent testing. A stimulus-response curve was obtained by applying 40-50 pulses of varying intensity, ranging from below the motor threshold until maximal responses were obtained, and plotted using a Levenberg-Marquardt algorithm with a modified Boltzmann equation to determine the resting motor threshold (RMT) ($R^2 \geq 0.75$) (18, 19). Participants were then asked to maintain a 15 percent peroneus longus contraction by pronating the ankle while ten pulses at 110 and 130 percent RMT were applied over the hotspot (20). All stimuli were triggered and time-synchronized EMG data were collected in custom LabVIEW software (National Instruments, Austin, TX) at 2000 Hz.

Outcome variables for neural excitability included the RMT, I_{50} (intensity at peak slope), and slope parameter from stimulus-response curves for each muscle (18, 19), and the cortical silent period from facilitated trials (20). Dependent variables were assessed offline by a trained investigator, blinded to group status, by extracting peak-to-peak motor evoked potential size. The location of the hotspot was also measured using a headband (EASYStrap, Soterix, Inc., New York, NY), so that this location could be identified during training sessions.

Dynamic balance and muscle activation were assessed in a biomechanics laboratory through a hop-to-stabilization as described by Brown et al. (21). Maximal jump height was assessed on a

Vertec (Sports Imports, Hilliard, OH). Participants were instrumented with EMG sensors (Bagnoli-4, Delsys Inc., Boston, MA) over the tibialis anterior, peroneus longus, and soleus as described above. They were then instructed to stand 70cm from the edge of an in-ground force plate (Bertec FP6090-15, Columbus, OH) and hop forward off of both legs to a height of 50 percent of their maximum jump, land with their test limb on the force plate, stabilize as quickly as possible and maintain that stance for 15s. Subjects were provided as many practice trials as necessary until they felt comfortable with the task, and then performed 5 successful trials (defined as jumping to an adequate height, landing on the force plate, and maintaining unipedal stance for 15s). Analog force and EMG data were synchronized and collected in custom LabVIEW software at 1000 Hz.

All data were analyzed offline in separate LabVIEW software. Postural stability indices were calculated in the anteroposterior (APSI), mediolateral (MLSI), and vertical (VSI) planes, as well as a composite score (DPSI) described by Wikstrom et al (22). EMG data were extracted 250ms prior to and 250ms following force plate contact (23). Data were bandpass filtered (20-400 Hz), rectified, and low-pass filtered (10 Hz) to create a complete linear envelope. Muscle activation was normalized to the ensemble peak across all trials for that muscle. Average activation of each muscle was extracted for analysis in two phases: 250ms prior to (Pre) and following (Post) force plate contact.

Functional performance was assessed with a double-leg side-hop test (24). Two lines oriented in the anteroposterior plane were placed 30cm apart. Participants were instructed to hop side-to-

side across the lines, a total of 10 times as fast as possible. One practice trial was allowed, and the time it took to successfully complete the task was extracted for analysis.

Ankle strength was assessed on an isokinetic dynamometer (HUMAC Norm, CSMI, Inc., Stoughton, MA). Participants were seated in a reclined position, with the hip flexed approximately 90 degrees, the knee flexed approximately 45 degrees, the calf supported in a pad, and the shod foot secured in a foot plate. Concentric and eccentric ankle inversion and eversion strength were assessed for five and eight trials at 30 and 90 degrees per second, respectively, on the involved side. The peak torque in each direction and speed were averaged and extracted for analysis.

Patient-reported outcomes utilized in this investigation included the Foot & Ankle Ability Measure (FAAM) ADL and Sport subscales (25), the Tampa Scale for Kinesiophobia (TSK-11) (26), and the Disablement in the Physically Active Questionnaire (DPA) (27). These questionnaires were used to assess ankle-specific general function, ankle-specific sport function, movement fear, and global ratings of perceived disablement, respectively. Questionnaires were collected and managed using REDCap (Research Electronic Data Capture) tools hosted at Appalachian State University (28).

Training

Regardless of group allocation, all participants underwent 10 total sessions of eccentric ankle strength training over four weeks, with sessions allocated such that 5 were completed in the first two weeks, and 5 were completed in the second two weeks. Prior to each session, participants

completed a brief progress questionnaire in REDCap to track changes in risk factors related to TDCS, track potential adverse events, and record perceived soreness at rest and during activities of daily living using a visual analog scale. Training was completed on an isokinetic dynamometer, with participants positioned as described above and pictured in Figure 1. The EASYstrap headset was placed around the head and positioned to locate the measurement site of the hotspot from TMS testing and secure sponge electrodes. The skin and scalp were assessed for irritation or lesions prior to being cleaned with an alcohol pad. Two 5x3cm sponge electrodes (EASYpad, Soterix, Inc., New York, NY) were saturated with 4 to 6mL of saline, and rubber electrodes corresponding to the TDCS anode and cathode were placed within the sponges. The anode sponge was placed at the location of M1, while the cathode sponge was placed over the forehead contralateral to the hotspot. The aTDCS stimulator (1x1 tDCS, Soterix, Inc., New York, NY) elicited a pre-stimulus tickle to familiarize the participant with the sensation and allow the investigators to ensure appropriate impedance. The stimulator was set to provide 1.5mA over 18 minutes (10), and the start button was pressed. For individuals in the sham group, a switch on the stimulator was toggled to sham, which provided a 2-minute ramp up of electricity before discontinuing stimulation.

The eccentric training consisted of 4 sets of 10 repetitions in which the participants moved to 10 degrees of eversion with no resistance, at which point they were required to elicit 60 percent of their maximal eccentric torque as the dynamometer forced them to 20 degrees of inversion. The torque threshold was based on peak eversion eccentric torque from testing sessions and was updated at the 2-week time point. As the dynamometer would only continue moving if sufficient torque were produced, investigators might assist the participants by pushing on the footplate

when the motor stopped and participants could not exert sufficient isometric torque to continue motion. If participants were able to perform all repetitions without investigator assistance, the torque threshold was increased by 10 percent on the next session. Further, if participants were unable to complete sets without investigator assistance, the torque threshold was decreased by 10 percent for subsequent sets that day. Week-2 and week-4 testing sessions would occur between 1 to 4 days following the most recent training session (TDCS: 2.7 ± 1.7 days; Sham: 2.4 ± 1.1 days).

Data Analysis

The primary outcome of interest across dependent variables is the group-by-time interaction effect, with the main effect of time indicating a secondary outcome of interest. Hence, data were assessed using two-way factorial analyses of variance (ANOVAs) with the between-subjects factor of group (2 levels) and within-subjects factor of time (4 levels). For cortical silent period, stimulation intensity (110 or 130 percent RMT) was considered an additional within-subject factor. For muscle activation, phase (pre- or post-landing) was included as a within-subjects factor. For strength measurements, speed (30 or 90 degrees per second) was considered an additional within-subject factor. Cases were assessed with a per protocol analysis. Fisher's least significant difference (LSD) was used post hoc to determine locations of significant differences. Paired eta squared was used as a measure of effect size with 0.01, 0.06 and 0.14 considered small, medium, and large, respectively. An a priori level of significance was set at 0.05.

RESULTS

A total of 22 participants completed training and provided data for this study, with the flow of testing presented in Figure 2. Two individuals dropped from each group, with reasons including loss of interest in training (n=2), did not tolerate TMS as outcome measure (n=1), and a medical issue unrelated to the study (n=1).

Neural Excitability

Neural outcome variables are presented in Table 2. Peroneus longus RMT demonstrated a significant time-by-group interaction effect ($F_{[3,51]}=3.401$; $p=0.025$; $\eta_p^2=0.167$). Fisher's LSD comparisons revealed significant differences in the sham group at week-2 compared to other weeks ($p\leq 0.022$). Week-2 values in the sham group were lower, indicating more excitability, compared to baseline ($p=0.007$), week-4 ($p=0.022$), and week-6 ($p=0.006$). Significant differences were also observed in the aTDCS group where week-6 values were lower than week-2 indicating increased excitability at week-6 relative to week-2 ($p=0.024$). Tibialis anterior RMT revealed neither a significant time-by-group interaction effect ($F_{[3,48]}=1.460$; $p=0.237$; $\eta_p^2=0.084$), nor significant main effects of time or group ($p>0.050$).

Peroneus longus I_{50} demonstrated a significant time-by-group interaction effect ($F_{[3,51]}=5.290$; $p=0.003$; $\eta_p^2=0.237$). Fisher's LSD comparisons revealed significant differences in the sham group at week-2 ($p\leq 0.026$). Significant differences were observed in the sham group, where week-2 values were lower than all other time points, indicating more excitability from baseline, and less excitability at week-4 and week-6 (baseline, $p=0.026$; week-4, $p=0.019$ week-6,

p=0.001). Significant differences were also observed in the aTDCS group, where week-6 values were lower, indicating more excitability, than baseline, (p=0.025) and week-4 (p=0.001).

There was a significant time-by-group interaction effect for the tibialis anterior I_{50} ($F_{[3,48]}=4.538$; $p=0.007$; $\eta_p^2=0.221$). Fisher's LSD comparisons revealed significant differences in the sham group at week-2 compared to week-4 and week-6 ($p\leq 0.044$) and from baseline to week-6 ($p=0.016$). Significant differences were observed in the sham group where values increased to week-2 through the end of the intervention, indicating less excitability (week-4, $p=0.044$; week-6, $p=0.016$). In the aTDCS group, significant differences were also observed where I_{50} decreased indicating more excitability between baseline and week-6, ($p=0.047$).

Slope revealed no significant group-by-time interaction effects for peroneus longus ($F_{[3,51]}=1.200$; $p=0.319$; $\eta_p^2=0.066$) or tibialis anterior ($F_{[3,48]}=0.333$; $p=0.802$; $\eta_p^2=0.020$). No main effects of group or time were observed for either muscle ($p>0.050$).

Cortical silent period demonstrated non-significant time-by-intensity-by-group ($F_{[3,51]}=0.728$; $p=0.540$; $\eta_p^2=0.041$), time-by-intensity ($F_{[3,51]}=0.661$; $p=0.580$; $\eta_p^2=0.037$), intensity-by-group ($F_{[1,17]}=1.141$; $p=0.300$; $\eta_p^2=0.063$), and time-by-group ($F_{[3,51]}=0.765$; $p=0.519$; $\eta_p^2=0.043$) interaction effects for cortical silent period. There was a significant main effect of intensity ($F_{[1,16]}=21.788$; $p<0.001$; $\eta_p^2=0.562$). The silent period length at 130 %RMT was greater than that at 110 %RMT ($p<0.001$). There were no significant main effects of time or group ($p>0.050$).

Dynamic Balance and Muscle Activation

There was a significant time-by-group interaction effect for DPSI ($F_{[3,60]}=2.952$; $p=0.040$; $\eta_p^2=0.129$). Fisher's LSD post hoc comparisons did not reveal significant differences between groups or times. To further investigate this, we conducted a subsequent ANOVA with individual components of APSI, MLSI, and VSI as a within-subjects factor. There were non-significant time-by-direction-by-group ($F_{[6,120]}=1.237$; $p=0.292$; $\eta_p^2=0.058$), time-by-direction ($F_{[6,120]}=0.623$; $p=0.711$; $\eta_p^2=0.030$), and direction-by-group ($F_{[2,40]}=0.755$; $p=0.477$; $\eta_p^2=0.036$) interaction effects for PSI components (APSI, MLSI, VSI). There was a significant time-by-group interaction effect ($F_{[3,60]}=3.087$; $p=0.034$; $\eta_p^2=0.134$). There was also a significant main effect of direction ($F_{[2,40]}=1000.077$; $p<0.001$; $\eta_p^2=0.980$). Fisher's LSD comparisons revealed significant differences in the aTDCS group, where PSI values decreased from baseline to week-6 ($p=0.010$) and week-4 to week-6 ($p=0.026$), indicating better postural stability. Directional effects revealed VSI values higher than MLSI & APSI ($p<0.001$), and APSI values greater than MLSI ($p<0.001$) (Table 3).

Tibialis anterior activation revealed non-significant time-by-phase-by-group ($F_{[3,60]}=1.945$; $p=0.132$; $\eta_p^2=0.089$), time-by-phase ($F_{[3,60]}=1.408$; $p=0.249$; $\eta_p^2=0.066$), and phase-by-group ($F_{[1,20]}=0.077$; $p=0.784$; $\eta_p^2=0.004$) interaction effects. There was a significant time-by-group interaction effect ($F_{[3,60]}=3.524$; $p=0.020$; $\eta_p^2=0.150$). There was a significant main effect of phase ($F_{[1,20]}=91.468$; $p<0.001$; $\eta_p^2=0.821$). Fisher's LSD comparisons revealed significant differences in the sham group, where activation decreased from baseline to all other time points (week-2, $p=0.020$; week-4, $p=0.002$; week-6, $p<0.001$). The sham group also significantly decreased tibialis anterior activation from week-2 to week-6 ($p=0.036$) (Table 3).

There was a significant time-by-phase-by-group interaction effect for peroneus longus activation ($F_{[3,60]}=4.302$; $p=0.008$; $\eta_p^2=0.177$). Fisher's LSD comparisons revealed significant differences in the aTDCS group, where activation increased in the post phase (250ms after landing) from baseline to week-6 ($p=0.044$). Significant differences were also observed in the sham group, where in the pre phase (250ms prior to landing), activation increased from baseline to week-2 ($p=0.049$).

For soleus activation, there were non-significant time-by-phase-by-group ($F_{[3,60]}=1.062$; $p=0.372$; $\eta_p^2=0.050$), time-by-phase ($F_{[3,60]}=0.514$; $p=0.674$; $\eta_p^2=0.025$), phase-by-group ($F_{[1,20]}=0.001$; $p=0.974$; $\eta_p^2=0.000$), and time-by-group ($F_{[3,60]}=0.547$; $p=0.652$; $\eta_p^2=0.027$) interaction effects. There was a significant main effect of phase ($F_{[1,20]}=11.760$; $p=0.003$; $\eta_p^2=0.370$). There were no significant main effects of time or group (Table 3).

Functional Performance

There was a non-significant time-by-group interaction effect for the side hop test ($F_{[3,60]}=0.376$; $p=0.770$; $\eta_p^2=0.018$). There was a significant main effect of time ($F_{[6,24]}=5.272$; $p=0.003$; $\eta_p^2=0.209$); and no significant main effect of group ($F_{[1,20]}=0.619$; $p=0.441$; $\eta_p^2=0.030$). Fisher's LSD comparisons revealed significant differences in both groups where baseline values were greater than values from all subsequent time points (week-2, $p=0.024$ week-4, $p=0.016$; week-6, $p=0.032$) (Table 3).

Strength

No significant time-by-group interaction effects were observed for concentric inversion ($F_{[3,60]}=0.317$; $p=0.813$; $\eta_p^2=0.016$), concentric eversion ($F_{[3,60]}=0.216$; $p=0.885$; $\eta_p^2=0.011$), eccentric inversion ($F_{[3,60]}=0.087$; $p=0.967$; $\eta_p^2=0.004$), or eccentric eversion strength measures ($F_{[3,60]}=0.090$; $p=0.965$; $\eta_p^2=0.005$). Further no significant main effects of time were observed for concentric inversion ($F_{[3,60]}=0.513$; $p=0.675$; $\eta_p^2=0.025$), concentric eversion ($F_{[3,60]}=2.027$; $p=0.120$; $\eta_p^2=0.092$), eccentric inversion ($F_{[3,60]}=0.426$; $p=0.735$; $\eta_p^2=0.021$), or eccentric eversion strength ($F_{[3,60]}=0.318$; $p=0.812$; $\eta_p^2=0.016$).

Patient-Reported Outcome Measures

For the FAAM-ADL, no significant group-by-time interaction effect ($F_{[3,54]}=1.266$; $p=0.295$; $\eta_p^2=0.066$) or main effects of group ($F_{[1,18]}=1.080$; $p=0.312$; $\eta_p^2=0.057$) or time ($F_{[3,54]}=0.991$; $p=0.404$; $\eta_p^2=0.052$) were observed. For the FAAM-Sport, no significant group-by-time interaction effect ($F_{[3,57]}=0.436$; $p=0.728$; $\eta_p^2=0.022$) or main effects of group ($F_{[1,19]}=1.343$; $p=0.261$; $\eta_p^2=0.066$) or time ($F_{[3,57]}=1.444$; $p=0.240$; $\eta_p^2=0.071$) were observed. The TSK similarly displayed no significant group-by-time interaction effect ($F_{[3,60]}=1.387$; $p=0.255$; $\eta_p^2=0.065$) or main effects of group ($F_{[1,20]}=0.028$; $p=0.896$; $\eta_p^2=0.001$) or time ($F_{[3,60]}=1.973$; $p=0.128$; $\eta_p^2=0.090$).

The DPA demonstrated a significant group-by-time interaction effect ($F_{[3,60]}=3.150$; $p=0.031$; $\eta_p^2=0.136$). Fisher's LSD comparisons revealed a significant increase between baseline and week 2 in the sham group ($p=0.047$) with no other differences in that group. The aTDCS group, however, decreased significantly from week-2 to week-4 ($p=0.046$).

DISCUSSION

The study aimed to establish the efficacy and feasibility of implementing aTDCS to improve neural, functional, and perceived outcomes in individuals with CAI. No previously published investigations have described the role of non-invasive brain stimulation therapies in populations with musculoskeletal injury. Our results indicated that the use of aTDCS in conjunction with eccentric training increased M1 excitability, dynamic postural stability, muscle recruitment during a hop-to-stabilization, and decreased perceived disablement, with these improvements most notable at the retention time point (week-6). Functional performance was observed to improve across all participants, suggesting eccentric training was able to improve this outcome measure without the aid of aTDCS. Additionally, improvements in cortical excitability and muscle activation in the sham group were observed during training (week-2), but were not sustained throughout training, suggesting an acute benefit to eccentric training that may be stabilized by the addition of aTDCS. The overall implications of this investigation is that aTDCS with eccentric training may be beneficial for addressing etiological and symptom-based impairments (without directly targeting those impairments) in individuals with CAI.

Neural Adaptation

The underlying rationale for the interventions implemented in this study is that decreased M1 excitability among patients with CAI and ACL injury contributes to cortical spread throughout the execution of movement. Hence, increased excitability of M1 would be the desired outcome to restore typical motor pathways to stabilizing muscles (6). Both groups appeared to have improved neural excitability in response to the intervention, although notably different changes with relation to time were observed. Over the 6-weeks, the aTDCS group demonstrated a

leftward shift of the stimulus response curves for peroneus longus (and tibialis anterior to a lesser extent), as evident through decreased RMT and I_{50} , with no changes to slope (Figure 3). This finding is in line with our a priori hypothesis that aTDCS would enhance LTP-like changes to improve excitability of the corticospinal tract (8). The RMT and I_{50} variables are both tied to resting membrane depolarization thresholds of intracortical neurons, which would be decreased by aTDCS, while the slope parameter correlates with recruitment of additional cortical neurons. CAI has been tied to decreased active and resting motor thresholds to the tibialis anterior and peroneus longus. Although aTDCS has been tied to increased cortical excitability (29), few studies have implemented aTDCS over long-term training for the legs in attempts to change cortical excitability, with tibialis anterior MEP size previously found to increase with 10 sessions of aTDCS in a stroke population (30). Clear differences exist within CAI and stroke populations, as cortical excitability may be less severely affected in CAI, yet stroke may have a limit to excitability improvements secondary to structural changes in cortical tracts (31). Some caution should be urged as increases in excitability were not observed throughout training, but rather only following training; this may be due to the timeframe in which measurements were taken relative to training. Participants were typically tested 1 to 4 days from their most recent training session and therefore may have had levels of residual soreness or fatigue that impacted our ability to detect changes in excitability (32).

A curious effect was observed in the sham group whereby a notable increase in excitability was detected from baseline to week-2 that decreased at all subsequent time points to levels lower than baseline testing (though not significant). It was hypothesized eccentric training would have disinhibitory effects on motor excitability, and this effect at week-2 may be reflective of this

disinhibitory effect (14); however, it remains unclear why the aTDCS group also did not change, and why no changes were observed in the silent period inhibitory measures. Potentially hypotheses drawn from these data could be that the current from aTDCS provided a stabilizing effect against the rapid increase of excitability from eccentric training, which could be possible were the eccentric training contributing to presynaptic modulation of reflexive excitability, which was not measured in the current investigation (33). An alternate hypothesis might have been that soreness, time from previous training session, or effort during training may have been different in this group, facilitating more initial changes; however, post hoc analyses revealed no apparent differences in perceived soreness, time between training sessions, or work done during training sessions across these groups. Because of this effect, we suggest that subsequent investigations incorporate an additional control group to determine if initial increases are due only to eccentric training or represents the role of TDCS in modulating cortical excitability. Similarly, this change to study design could allow a better understanding of the rebound excitability decrease observed in the sham group, which was contrary to our a priori hypothesis.

Functional Changes

Maladaptive neuroplasticity is hypothesized to cause altered movement patterns; thus, correction of neural excitability should improve functional performance, balance, and muscle activation (6). Yet, very few investigations have tied changes in an individual's neural excitability to modified functional status following intervention among those with musculoskeletal injury. Our findings indicated that while all participants improved quickly on side-hop test performance time, group-specific effects were observed in balance performance that may be tied to neural excitability. Like peroneus longus RMT and I_{50} , postural stability indices decreased (indicating improved

balance) from baseline & week-2 to week-6 in the aTDCS group, while no changes were observed in the sham group. Interesting, while an overall interaction effect was observed for DPSI, this variable did not reveal significant differences in post hoc testing, likely due to low power. Rather, these findings became apparent in exploring the effects of the individual components of the DPSI. The mechanism by which the postural stability indices changes seems to be increased and better timing of peroneus longus activation during balance.

Previous investigations have demonstrated improvements in static and dynamic balance in those with CAI, although many of these interventions are tied to balance-training interventions (34). Simple eccentric exercise training in conjunction with aTDCS to increase neural drive to stabilizing muscles appeared to similarly improve dynamic balance, despite no inclusion of balance-specific training. Our findings suggest that the reason for this seems to be improved activation of peroneus longus, specifically immediately after landing, indicating faster muscular recruitment, and activation highest during the timeframe that injury and roll-over events would be most likely to occur (35). Acute interventions have been demonstrated to increase lower limb muscle activation in healthy adults (10), but this is the first study to demonstrate this effect in the lower limb over a long-term intervention.

Divergent effects were observed in the sham group, although these changes mimicked the results observed for cortical excitability. Specifically, while no differences were observed for postural stability indices, the sham group had increased tibialis anterior and peroneus longus activation at week-2 that then subsequently decreased at later time points. These results support the role of cortical excitability in regulating muscle activation in dynamic balance and suggest that eccentric

training alone may have transient effects to improve muscle activation that may not be sustained with prolonged training. It is again unclear why this effect was not seen in the aTDCS group acutely, but we posit the lack of these changes are tied to a stabilizing role of aTDCS on cortical excitability during training.

One final functional change to note was the lack of strength improvements in either group following the eccentric training intervention. Prior interventions using similar eccentric training loads and progressions among those with ACL injury and similar instrumentation (i.e. isokinetic dynamometer) have described strength improvements that corresponded with improved neuromuscular function (13). However, despite multiple neuromuscular factors improving, strength itself did not. One potential explanation for this could be population differences between ACL-injured and CAI populations, with CAI being far more heterogeneous, containing subsets of those with mechanical and/or functional instability, and thus demonstrating more varied evidence of strength deficits (36, 37). Further, the targeted muscles could differentiate these studies, as the peroneus longus is notably smaller and has different architecture than the quadriceps femoris group, and therefore may have a ceiling effect to potential strength adaptations. Additionally, the peroneus longus controls the multiplanar motion of ankle pronation that may have been difficult to target with the rotational axis provided by the isokinetic dynamometer, although prior studies have still induced mild strength increases (38). A recent study has demonstrated ankle strength gains in a CAI population using elastic tubing, which may allow for an individual to more functionally move through pronation-supination (39).

Patient-Reported Outcomes

Given decreased perceived joint-specific and global function, as well as kinesiophobia among those with CAI (4), we studied whether our intervention impacted these patient-reported outcome measures. Subjects demonstrated no statistically significant improvements in ankle-specific function or kinesiophobia, but perceived disablement was decreased in the aTDCS group significantly at week-4 and near-significantly at week-6. This finding is encouraging as it indicates that the incorporation of aTDCS with eccentric training improves perceptions of global function and echoes the timeframe of changes observed for both cortical and balance-based measures. A significant increase in disablement was seen in sham at week-2. This is curious as individuals had increased cortical excitability at this time compared to aTDCS, but may reflect increased disablement relative to the intervention itself (i.e. soreness). While we collected daily soreness logs to determine if training needed to be modified, these logs did not reflect levels of increased soreness.

While no statistically significant differences were observed for ankle-specific function or kinesiophobia, several considerations should be taken. First, despite high IdFAI scores among both groups confirming the presence of CAI, individuals in this study reported generally higher baseline FAAM-ADL and FAAM-Sport scores than previous investigations, potentially limiting the extent to which improvements could be observed on these questionnaires (40). It is important to note that these questionnaires ask about difficulty performing daily living and sport-specific tasks, rather than sensations of giving way. However, with the exception of perceived disablement, patient-reported outcomes trended towards improvements at weeks 4 and 6, while the sham group either slightly improved or stayed constant across all time points. Our a priori

power analyses were based on cortical and functional measures, and therefore it is possible that these values would become significant with an increased sample size.

Limitations

Given the preliminary nature of this study, several limitations should be considered in this investigation that could be addressed in follow-up studies. First, given the curious findings related to the sham group at week-2, we would consider the lack of a true control or comparison to standard of care a potential limitation of this investigation. Further, while participants were blinded to group status, assessors and therapists were not blinded, although assessors were blinded during data reduction efforts. Given the heterogeneity of the CAI population, no control was made for specific baseline impairments, including discriminating the presence of mechanical instability; however, all subjects had some degree of functional instability. Finally, limited preliminary data or previous investigations were available with this population and outcome measures, limiting the utility of our a priori power analyses. Effect sizes are incorporated throughout this manuscript to facilitate power analyses for subsequent investigations.

CONCLUSIONS

Given the link between maladaptive neuroplasticity and poor long-term function after ligamentous injuries, it appears the incorporation of a neuromodulatory therapy such as aTDCS improved cortical excitability and subsequently muscle activation, dynamic postural stability, and perceived disablement following a 4-week intervention. Those receiving eccentric training with sham stimulation appeared to improve cortical excitability and muscle activation during the intervention; however, these changes were transient and not sustained following the intervention.

This is the first investigation describing the role of non-invasive brain stimulation on function in those with musculoskeletal injuries, and provides crucial evidence linking improvements in cortical excitability with improved function. These findings lend support to the hypothesis that addressing neuroplasticity-related changes can be an effective alternate or adjunct therapy to impairment-based rehabilitation. Further investigations utilizing larger cohorts, alternate patient populations, and direct comparison to impairment-based rehabilitation (e.g. balance training) are warranted to better establish the effectiveness of these neuromodulatory interventions and their utility in clinical settings.

ACCEPTED

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List of Figures

FIGURE 1: Participant set-up for training and strength testing. Picture includes EASYstrap and electrode placement for aTDCS.

FIGURE 2: CONSORT diagram of study procedures and number of subjects at each stage of the study.

FIGURE 3: Group changes in peroneus longus stimulus-response curve from baseline to week-6. Curves estimated from group means of stimulus-response curve parameters. *Abbreviations:* MEP, motor evoked potential; %2T, Percent of 2 Tesla stimulator output.

Figure 1



Figure 2

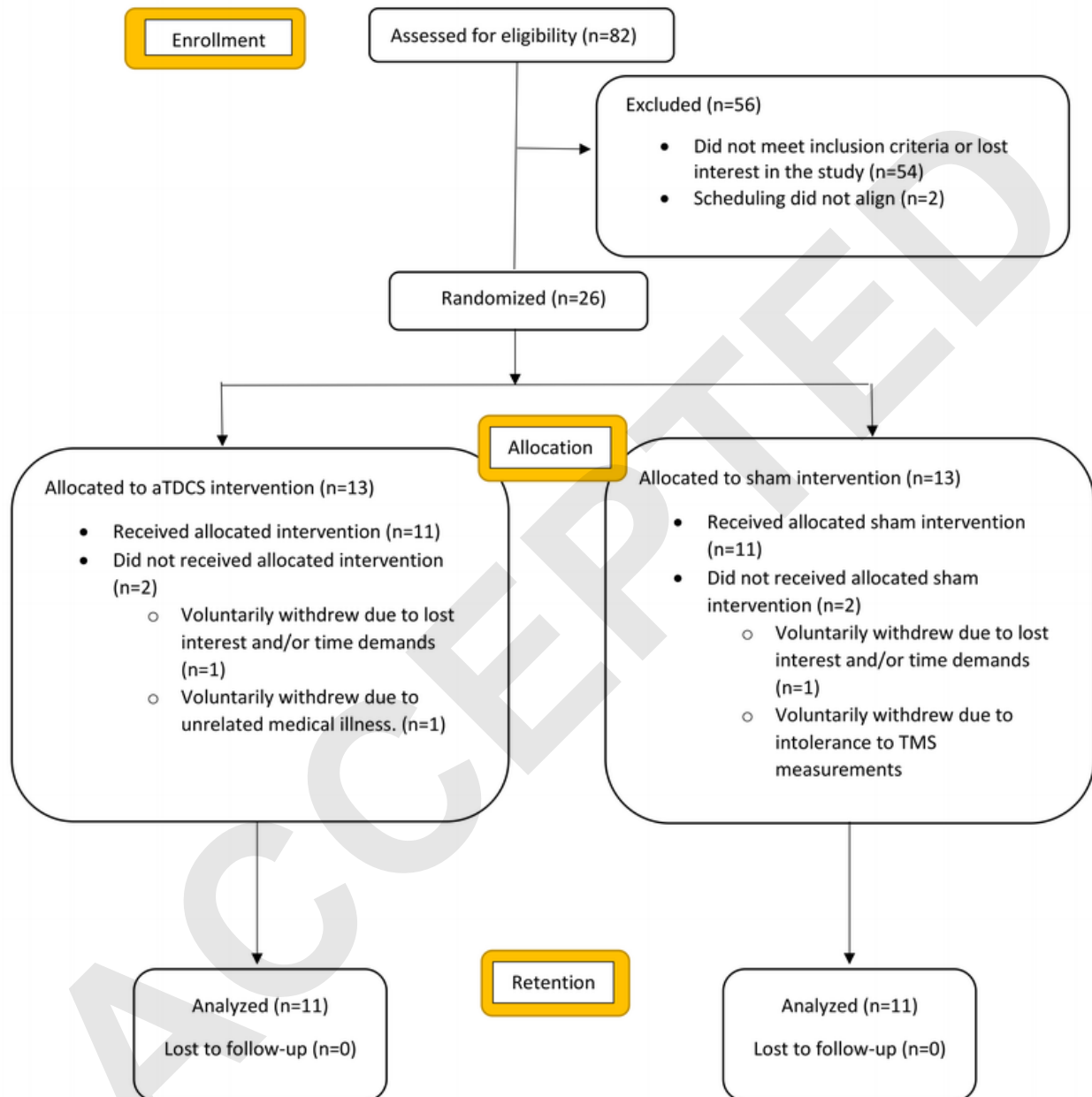


Figure 3

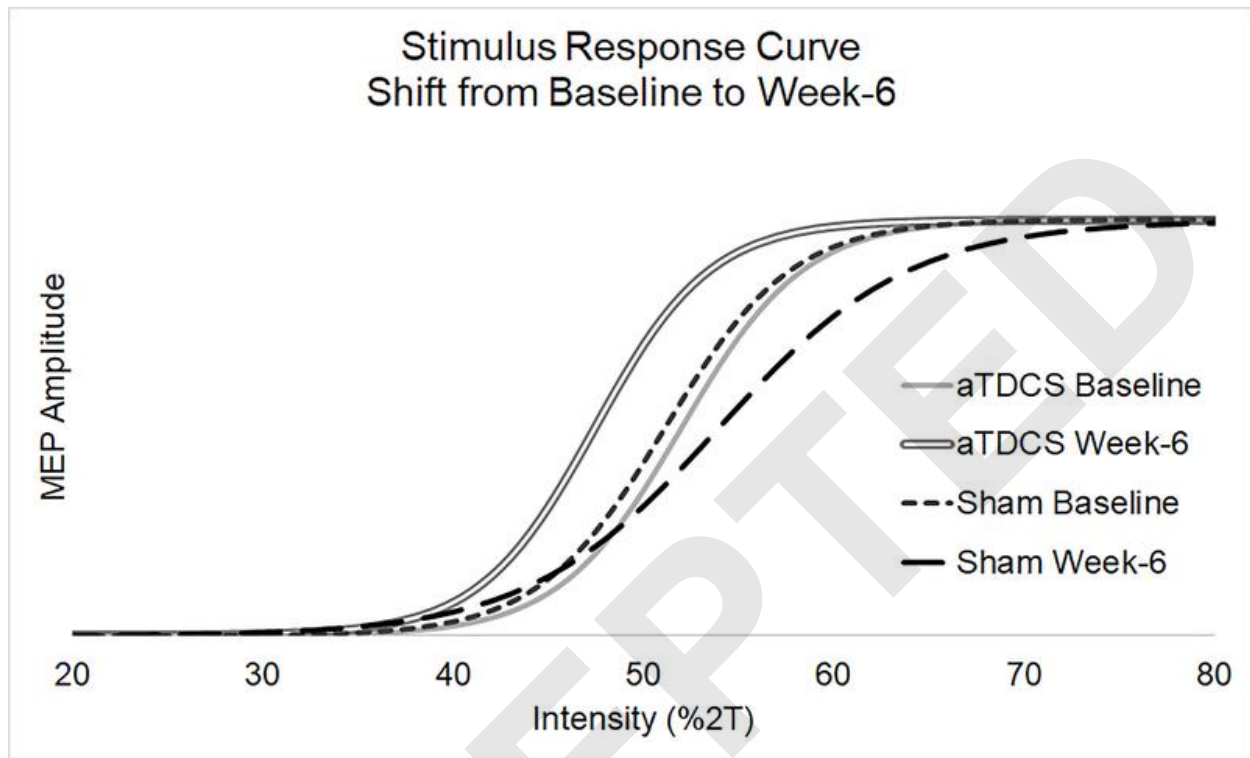


TABLE 1: Means (standard deviations) for group demographics. P-values represent independent sample t-test comparisons between groups. P-value for bilateral symptoms determined from chi-squared analysis.

Abbreviations: IdFAI, Identification of Functional Ankle Instability instrument.

| | aTDCS | Sham | P |
|--------------------------|---------------|---------------|----------|
| N (sex) | 13 (3M/10F) | 13 (6M/7F) | |
| Age (yrs) | 22.2 (2.8) | 22.5 (3.2) | 0.803 |
| Height (cm) | 170.08 | 174.22 | 0.263 |
| Mass (kg) | 71.11 (15.29) | 81.67 (13.22) | 0.073 |
| Baseline IdFAI | 21.46 (5.69) | 22.08 (6.03) | 0.791 |
| Bilateral CAI (n) | 10 | 7 | 0.216 |

TABLE 2: Means (standard deviations) for neural excitability variables. ^a Significant difference from baseline; ^b Significant difference from week-2; ^c significant difference from week-4. *Abbreviations:* PL, peroneus longus; TA, tibialis anterior; RMT, resting motor threshold; CSP₁₁₀, cortical silent period at 110% RMT; CSP₁₃₀, cortical silent period at 130% RMT.

| | aTDCS | | | | Sham | | | | Group-by-Time Effect | |
|--------------------------------|-------------------|-------------------|-------------------|--------------------------------|--------------------|-------------------------------|-------------------------------|-------------------------------|----------------------|-------|
| | Baseline | Week-2 | Week-4 | Week-6 | Baseline | Week-2 | Week-4 | Week-6 | F | P |
| PL RMT (%2T) | 36.92 (11.53) | 39.02 (9.30) | 37.46 (9.22) | 32.91 (12.33) ^a | 36.67 (12.74) | 27.86 (14.69) ^a | 35.63 (13.10) ^b | 35.99 (13.52) ^b | 3.401 | 0.025 |
| TA RMT (%2T) | 38.54 (13.91) | 34.83 (13.63) | 36.55 (6.02) | 32.90 (7.97) | 30.75 (10.20) | 29.41 (13.90) | 36.57 (13.68) | 37.31 (15.76) | 1.460 | 0.237 |
| PL I₅₀ (%2T) | 51.97 (6.47) | 51.35 (9.38) | 55.89 (7.63) | 47.42 (5.633) ^{ac} | 51.11 (11.27) | 45.47 (10.62) ^a | 52.31 (11.30) ^b | 53.91 (12.04) ^b | 5.290 | 0.003 |
| TA I₅₀ (%2T) | 53.42 (6.19) | 54.67 (11.92) | 52.05 (7.33) | 49.26 (5.93) ^a | 49.06 (10.40) | 44.62 (12.96) | 53.08 (8.01) ^b | 54.14 (11.42) ^b | 4.538 | 0.007 |
| PL Slope | 0.31 (0.22) | 0.36 (0.21) | 0.21 (0.08) | 0.33 (0.20) | 0.30 (0.10) | 0.24 (0.14) | 0.25 (0.11) | 0.20 (0.05) | 1.200 | 0.319 |
| TA Slope | 0.38 (0.30) | 0.85 (1.86) | 0.28 (0.14) | 0.43 (0.60) | 0.23 (0.13) | 0.85 (1.94) | 0.33 (0.22) | 0.92 (2.13) | 0.333 | 0.802 |
| CSP₁₁₀ (ms) | 217.60 (79.26) | 212.70 (49.35) | 229.50 (77.31) | 226.60 (82.89) | 249.00 (90.49) | 198.11 (89.27) | 223.33 (79.04) | 237.67 (119.4) | 0.765 | 0.519 |
| CSP₁₃₀ (ms) | 279.40 (88.21) | 291.20 (75.66) | 298.80 (97.22) | 282.30 (106.4) | 346.78 (128.49) | 276.44 (146.92) | 372.89 (98.71) | 334.78 (155.27) | | |

TABLE 3: Means (standard deviations) for balance, muscle activation, functional performance, and patient function. ^a Post hoc tests revealed difference in aTDCS from baseline and week-4 to week-6; ^b significant difference from baseline; ^c significant difference from week-2; ^d significant change from pre to post. *Abbreviations:* DSPI, dynamic postural stability index; APSI, anteroposterior postural stability index; MLSI, mediolateral postural stability index; VSI, vertical postural stability index; TA, tibialis anterior; PL, peroneus longus; SOL, soleus; FAAM, Foot & Ankle Ability Measure; ADL, activity of daily living scale; TSK, tampa scale for kinesiophobia; DPA, disablement in the physically active

| | aTDCS | | | | Sham | | | | Group-by-Time Effect | |
|-----------------------------------|------------------|-----------------------------|------------------------------|------------------------------|------------------|------------------------------|------------------------------|------------------------------|----------------------|--------------------|
| | Baseline | Week-2 | Week-4 | Week-6 | Baseline | Week-2 | Week-4 | Week-6 | F | P |
| Postural Stability Indices | | | | | | | | | | |
| DPSI | 0.50 (0.07) | 0.49 (0.06) | 0.49 (0.04) | 0.47 (0.05) | 0.50 (0.05) | 0.52 (0.07) | 0.51 (0.05) | 0.51 (0.06) | 2.952 | 0.040 |
| APSI | 0.12 (0.04) | 0.11 (0.04) | 0.13 (0.02) | 0.10 (0.05) | 0.12 (0.03) | 0.10 (0.05) | 0.10 (0.05) | 0.11 (0.04) | 3.087 | 0.034 ^a |
| MLSI | 0.04 (0.02) | 0.04 (0.02) | 0.03 (0.01) | 0.04 (0.01) | 0.04 (0.01) | 0.04 (0.01) | 0.04 (0.01) | 0.04 (0.01) | | |
| VSI | 0.48 (0.07) | 0.47 (0.06) | 0.47 (0.04) | 0.46 (0.06) | 0.47 (0.05) | 0.50 (0.07) | 0.49 (0.06) | 0.50 (0.07) | | |
| Muscle Activation | | | | | | | | | | |
| TA Pre (%Max) | 0.31 (0.12) | 0.22 (0.09) | 0.22 (0.08) | 0.26 (0.10) ^b | 0.32 (0.10) | 0.27 (0.10) ^b | 0.23 (0.07) ^b | 0.25 (0.08) ^{bc} | 3.524 | 0.020 |
| TA Post (%Max) | 0.46 (0.16) | 0.47 (0.15) | 0.49 (0.16) | 0.46 (0.16) | 0.58 (0.11) | 0.51 (0.12) ^b | 0.48 (0.09) ^b | 0.46 (0.12) ^{bc} | | |
| PL Pre (%Max) | 0.49 (0.12) | 0.52 (0.12) | 0.48 (0.07) | 0.48 (0.09) | 0.46 (0.12) | 0.55 (0.12) ^a | 0.52 (0.12) | 0.50 (0.11) | 4.302 | 0.008 |
| PL Post (%Max) | 0.51 (0.12) | 0.61 (0.10) ^d | 0.58 (0.14) ^d | 0.60 (0.11) ^{bd} | 0.56 (0.16) | 0.57 (0.12) | 0.57 (0.11) | 0.58 (0.10) | | |
| SOL Pre (%Max) | 0.58 (0.09) | 0.59 (0.13) | 0.63 (0.06) | 0.59 (0.12) | 0.66 (0.12) | 0.61 (0.14) | 0.60 (0.11) | 0.57 (0.14) | 0.547 | 0.652 |
| SOL Post (%Max) | 0.49 (0.16) | 0.47 (0.19) | 0.42 (0.17) | 0.44 (0.21) | 0.51 (0.14) | 0.45 (0.15) | 0.46 (0.16) | 0.44 (0.16) | | |
| Functional Performance | | | | | | | | | | |
| Side Hop Test (s) | 11.22 (4.45) | 9.76 (2.63) ^b | 9.08 (2.20) ^b | 9.19 (2.38) ^b | 11.91 (4.50) | 10.29 (2.44) ^b | 10.38 (3.27) ^b | 10.64 (3.66) ^b | 0.376 | 0.770 |
| Patient-Reported Outcomes | | | | | | | | | | |
| FAAM- ADL (%) | 93.69 (5.33) | 94.52 (5.59) | 95.83 (4.13) | 95.95 (3.64) | 92.74 (7.26) | 91.54 (8.92) | 91.54 (8.06) | 92.86 (7.36) | 1.266 | 0.295 |
| FAAM- Sport (%) | 84.37 (12.88) | 84.38 (13.33) | 88.35 (9.38) | 88.92 (10.67) | 79.37 (18.05) | 78.44 (19.57) | 79.37 (17.50) | 80.93 (15.27) | 0.436 | 0.728 |
| TSK | 32.91 (4.68) | 33.00 (4.90) | 31.91 (5.07) | 29.91 (4.11) | 31.18 (6.82) | 31.36 (7.19) | 32.73 (7.40) | 30.91 (6.86) | 1.387 | 0.255 |
| DPA | 18.09 (5.45) | 18.09 (6.41) | 15.55 ^c (4.82) | 15.45 (5.48) | 17.91 (4.59) | 21.00 ^b (8.52) | 21.09 (8.77) | 20.00 (8.23) | 3.150 | 0.031 |