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RESEARCH ARTICLE

Dose of remote limb ischemic conditioning for enhancing learning in healthy young adults

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Abstract

Remote limb ischemic conditioning (RLIC) is a technique in which tissues distant from the target organ are exposed to brief, sub-lethal bouts of ischemia. The effects of remotely applied ischemic conditioning are systemically transferred to the target organ, and typically manifested as protection from subsequent ischemic injury. Previous studies in our lab have found and confirmed that RLIC enhances learning and retention during motor training on a balance task. The current study tested the effect of RLIC dose (number of cycles) on learning enhancement in young, healthy adults. Forty healthy participants age 18–40 years were randomized to receive 5 cycles of sham conditioning ($n=9$), 3 cycles of RLIC ($n=11$), 4 cycles of RLIC ($n=10$), or 5 cycles of RLIC ($n=10$) using a blood pressure cuff around the upper arm once a day for 7 consecutive weekdays (Days 1–7). Participants concurrently trained on a balance task, bimanual cup stacking task, and a discrete sequence production task on Days 3–7. Change in performance on each of the three tasks was compared across groups. Participants in all four groups improved their performance on each of the three tasks over time. However, RLIC at any dose did not enhance learning on any of the three tasks. While RLIC is safe, inexpensive, and clinically feasible, reproducibility may be challenged by unidentified factors, raising critical challenges to the straightforward translation of RLIC for improving rehabilitation outcomes in individuals recovering from neurological injury.

Keywords Ischemic conditioning · Motor learning · Psychomotor performance

Introduction

Remote limb ischemic conditioning (RLIC) has great potential as an adjunct or combinatorial treatment paired with physical rehabilitation. RLIC is a technique in which tissues distant from the target organ are exposed to brief, sub-lethal bouts of ischemia. The effects of remotely applied ischemic conditioning are systemically transferred to the target organ, usually for protection from subsequent ischemic injury (Dirnagl et al. 2009; Gidday 2006; Iadecola and Anrather 2011; Stetler et al. 2014). An advantage of using remote over local ischemic conditioning is that protection is provided without adding direct stress to the target organ. In non-human vertebrates, and to some degree in humans, the

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benefits of RLIC on cardioprotection and neuroprotection have been described (Ali et al. 2007; Bøtker et al. 2010; Crimi et al. 2013; Gidday 2006; Hougaard et al. 2014; Meng et al. 2012; Ren et al. 2008; Tapuria et al. 2008). It is only recently that studies in our lab have begun to describe and verify an effect of enhanced learning in response to RLIC (Cherry-Allen et al. 2015, 2016). We have previously found that, with respect to motor training on a balance task, RLIC enhances learning and retention in young, healthy adults (Cherry-Allen et al. 2015, 2016). Individuals receiving RLIC had more than a 60% improvement in learning compared to those receiving sham conditioning. Since motor learning is a key component of physical rehabilitation for many patient populations, these findings could have profound implications for neurological rehabilitation.

Although attractive as a clinically feasible and inexpensive technique that can be easily implemented, RLIC may only result in beneficial effects for some tasks but not for others. There are several loosely related animal studies that suggest RLIC may prime the nervous system for learning enhancement (Porter et al. 2012; Rybnikova et al. 2005), but do not provide much information regarding what types of learning tasks may benefit. Our studies have twice shown that, when paired with task-specific training, RLIC can enhance learning on a balance task, but may not extend to cognitive tasks (Cherry-Allen et al. 2015, 2016). It is unclear whether the specific tasks chosen in our previous studies were sufficient to detect enhanced learning due to RLIC or if the benefit simply does not extend to other learning processes. In the current study, we evaluate two more tasks in addition to the previously investigated balance task. One task requires bilateral upper extremity coordination and the other task requires movement sequencing, both of which are important and commonly emphasized aspects of neurorehabilitation training.

The most effective, yet least burdensome RLIC dose to enhance motor learning in humans is unknown. A number of preclinical studies outline recommendations for ischemic conditioning dosing as it applies to cardio- and neuroprotection (Fan et al. 2011; Garcia et al. 2011; Hess et al. 2015; Laskey 2005; Lonborg et al. 2010; Ma et al. 2006; Staat et al. 2005; Thibault et al. 2008). Although controversial (Loukogeorgakis et al. 2007), the amount of muscle mass, or number of involved limbs, does not appear to affect efficacy (Hess et al. 2015). Inflation pressure of the cuff beyond the ischemic threshold had no bearing on human learning enhancement (Cherry-Allen et al. 2015, 2016). Length of cycles ranging from 2 to 5 min have offered relatively similar outcomes for tissue protection, while cycles of 10 min nullify the protection (Hess et al. 2015). Four or five, 5-min cycles are commonly used, since some studies have shown increasing protection up to that amount (Garcia et al. 2011; Hess et al. 2015; Staat et al. 2005; Yang et al. 2007). No further protection is observed from six to eight cycles (Johnsen et al. 2016). Ischemic conditioning dosing as it applies to motor learning has not been fully investigated in animals nor humans; however, our previous studies have shown a benefit of learning at five, 5-min cycles, a dose similar to that used in the protection literature (Cherry-Allen et al. 2015, 2016).

Therefore, the goals of the current investigation were to determine: (1) the effect of RLIC dose (number of cycles) on learning enhancement; and (2) to what other tasks the benefit could be applied. This information is important prior to translation of RLIC into clinical use for two reasons. First, we will gain knowledge regarding the administration of the most effective and least burdensome RLIC dose. Second, we will broaden our knowledge of the specific types of rehabilitation training RLIC could benefit. We hypothesized that as dose of RLIC increased, magnitude of benefit compared to sham conditioning would increase. We further hypothesized that in addition to the balance task, RLIC would enhance learning on two other tasks: a bimanual cup stacking task and a discrete sequence production task.

Methods

Experimental design

This study used a repeated measures design and included eight total visits to examine the combined effect of RLIC dose (number of cycles) and motor training on learning. Procedures for the study were completed between February 2017 and February 2018, were approved by the Human Research Protection Office at Washington University, and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The trial ended when the target sample size was completed. Participants provided written informed consent prior to beginning of the study and were compensated for their time and effort.

Participants

Participants were included if they were between the age of 18 and 40 years (Fig. 1). Exclusion from the study was determined by self-report and occurred if participants had: (1) a history of any neurological condition, attention deficit disorder or attention deficit hyperactive disorder, any balance impairment, or vestibular disorder; (2) a history of sleep apnea which could confound the effects of RLIC (Drager et al. 2010; Yang et al. 2013); (3) a history of lower extremity condition, injury, or surgery which could compromise performance on motor tasks; (4) any extremity soft tissue, orthopedic, or vascular injury (i.e., uncontrolled hypertension, peripheral vascular disease) which may contraindicate RLIC; (5) any cognitive, sensory, or communication

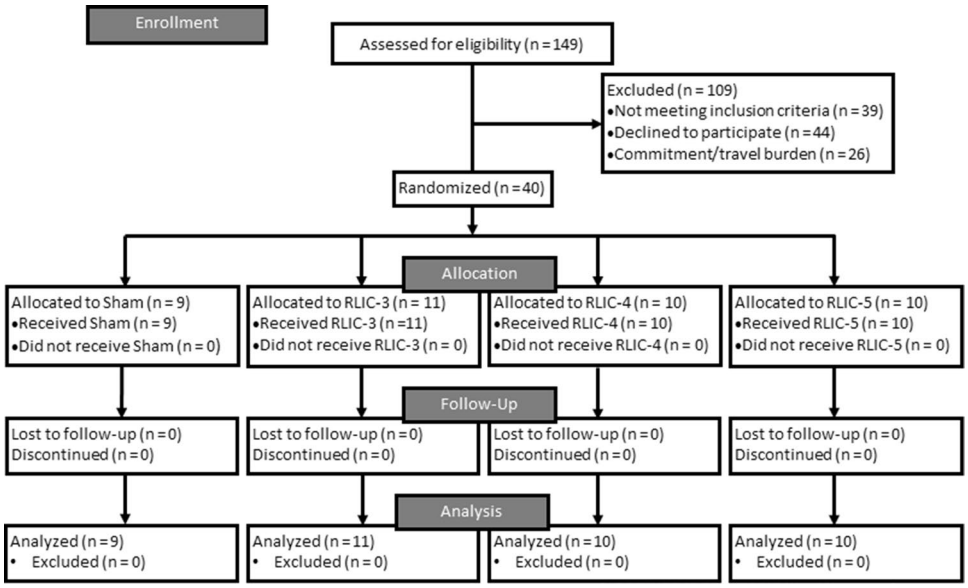

Fig. 1 CONSORT flow diagram. One hundred forty-nine individuals were assessed for eligibility. Of those, 109 individuals were excluded for not meeting inclusion/exclusion criteria, declining to participate/uninterested, or inability to meet time commitment. Forty individuals were allocated to 1 of 4 groups. No participants were lost to follow-up or discontinued the study. Nine individuals in the Sham group, 11 in the RLIC-3 group, 10 in the RLIC-4 group, and 10 in the RLIC-5 group had complete datasets, which were used for analysis. Microsoft Publisher was used to create this figure

problem that would prevent completion of the study; (6) a routine which included current weight lifting or interval training exercise, which could confound the effects of RLIC (Ding et al. 2011; Rahimi et al. 2015; Yarrow et al. 2010); (7) current substance abuse or dependence; (8) current use of medications, such as selective serotonin reuptake inhibitors, that could decrease nervous system excitability (Koch and Gonzalez 2013); and (9) an inability or unwillingness to travel for all study visits. These criteria were established to maintain a relatively homogenous sample and to optimize our ability to detect an effect of RLIC on learning.

Order of experiment

This experiment included seven consecutive weekday visits (D1-D7) and one follow-up visit (FU) which occurred 4 weeks later and assessed retention of learning (Fig. 2a). Information and results of the follow-up assessments are not reported on further due to non-significant findings within the first seven visits. On D1, participants completed written informed consent and provided demographic data, including information related to health and exercise history. Participants engaged in baseline assessments on three motor tasks (balance task, bimanual cup stacking task, and discrete sequence production task; described below). Participants were randomly assigned to one of the four groups: (1) five cycles of sham conditioning (SHAM); (2) three cycles of RLIC (RLIC-3); (3) four cycles of RLIC (RLIC-4); or (4) five cycles of RLIC (RLIC-5). Participants were informed they would receive either sham conditioning or RLIC, but were not aware of the specific conditioning treatment they were receiving. Participants intuitively knew how many

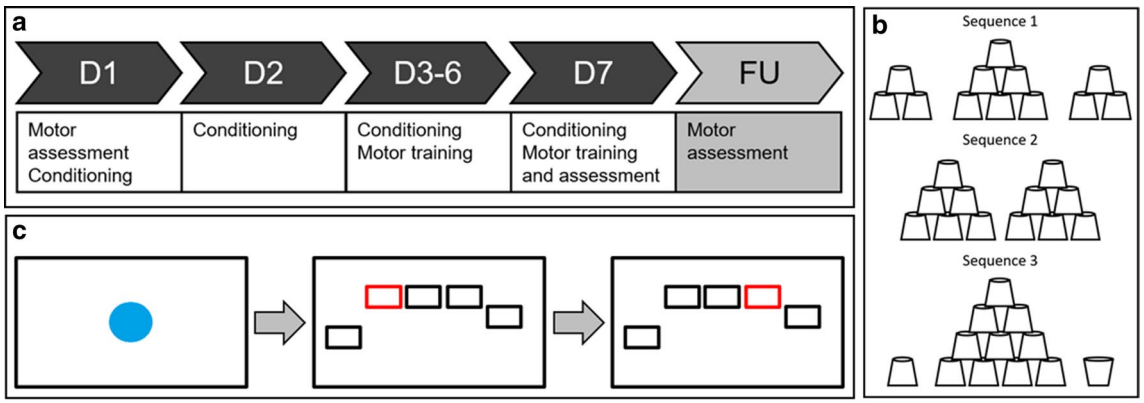

Fig. 2 Experimental design and tasks. **a** Study timeline, b bimanual cup stacking patterns. Participants must stack and unstack cups beginning with sequence 1 (top) and ending with sequence 3 (bottom). **c** Discrete Sequence Production task computer view. Left panel depicts a sequence identity cue, displayed before the first stimulus (middle panel). When the correct and corresponding key is pushed, the next stimulus would immediately appear (right panel). Microsoft Publisher was used to create this figure

cycles were delivered to them, but were not told some groups received different doses and, therefore, could not deduce to which treatment they were assigned. Participant underwent one session of their assigned conditioning treatment at the given dose. On D2, participants completed one session of their assigned conditioning. Days 3–7 participants completed one session of conditioning, followed by training on the three motor tasks. Day 7 also included post assessments of motor performance on all tasks. The order in which the motor task assessments and training were completed was randomized each day.

Remote limb and sham conditioning

All four groups engaged in conditioning which consisted of cyclic inflation and deflation of a blood pressure cuff secured around the non-dominant, upper extremity. The non-dominant arm was used for practical purposes and was determined by self-report. Group randomized occurred using a random number generator in MATLAB. A study team member, not responsible for data collection or participant enrollment, concealed group allocation until intervention was assigned. Inflation pressure for all RLIC groups (RLIC-3, -4, and -5) was 20 mmHg above that day's resting systolic blood pressure. Inflation pressure for the sham conditioning group (SHAM) was 10 mmHg below that day's resting diastolic blood pressure. We chose 10 mmHg below resting diastolic blood pressure to stay consistent with our previous studies (Cherry-Allen et al. 2015, 2016) and because it gives the illusion of cuff inflation, but does not induce arterial occlusion (Groothuis et al. 2003; Lorentsen et al. 1970; Pallares et al. 1994). For all groups, inflations and deflations were each 5 min long, with the total conditioning time equaling 45 min for the SHAM and RLIC-5 groups, 35 min for the RLIC-4 group, and 25 min for the RLIC-3 group.

Initial blood pressure was measured after several minutes of resting quietly, prior to each conditioning session. Ischemia was confirmed by periodically placing a pulse oximeter on a finger of the conditioned hand. A reading of "0" confirmed the presence of ischemia for RLIC groups. For the SHAM group, a pulse oximeter reading similar to preconditioning levels confirmed the absence of unintentional ischemia. Visual inspection of the conditioned arm and hand was performed intermittently during inflations to ensure that the color and temperature was as anticipated. If at any time, pulse oximeter readings or arm and hand temperature/color were not as anticipated for the participants' group assignment, inflation pressure was slowly adjusted up or down appropriately until confirmation of ischemia or non-ischemia was achieved.

Conditioning safety was monitored through measurement of heart rate, blood pressure, and oxygen saturation taken on the non-conditioned arm before, during, and after each session. Average pain across each session was surveyed by self-report using an 11-point Likert pain scale ranging from 0 (no pain) to 10 (worst pain imaginable). There were no significant group differences in pain ratings and, therefore, pain is not discussed further. Conditioning, regardless of group, was terminated if oxygen saturation on the non-conditioned arm fell below 80%, heart rate fell below 40 bpm or rose above 110 bpm, systolic blood pressure fell below 80 mmHg or rose above 150 mmHg, or if pain reached greater than a "6".

Balance task

The primary motor task used a stability platform (Lafayette Instrument, model 16030, Lafayette Instrument, Lafayette, IN). This balance task was selected because our previous work showed a benefit of learning enhancement due to RLIC using the same conditioning plus learning paradigm (Cherry-Allen et al. 2015, 2016). The balance task engages a wide range of brain systems, including vestibular, visual, motor, somatosensory, and cognitive, serving as a simple probe to determine the global motor learning response to RLIC and investigation of the minimum required RLIC dose to observe potential benefits.

Participants were instructed to stand on the stability platform with their feet facing forward. The goal of the task was to keep the platform level for as long as possible during trials of 30 s. Performance for each trial was determined by quantifying the cumulative amount of time that a participant was able to maintain the platform within 3° of horizontal. Participants performed a total of 80 balance trials, which included 5 pre-test trials on D1, 15 trials each visit on D3–D7. The final five trials on D7 were averaged together and served as the post-test assessment of performance on the balance task. Each 30-s trial was separated by 30 s of resting quietly on the platform. For safety, a handrail was positioned in front of the platform such that participants could hold on while climbing on and off the platform, positioning themselves prior to each block, and during rests between each trial. Use of the handrail was not permitted during balance trials.

Participants were provided feedback of their cumulative time within 3° of horizontal following each trial. Prior to each balance session, the participants were reminded of their average cumulative score from the previous visit and encouraged to beat that score. Suggestions regarding balance strategies were not provided; however, participants were encouraged to choose between trying new things or sticking with their current strategy, allowing the participant to select their own techniques based on trial and error (i.e., discovery learning). To maintain consistency, conditioning and balance procedures were performed as closely as possible to our previous work (Cherry-Allen et al. 2015, 2016) and the same stability platform settings were used.

Bimanual cup stacking task

A bimanual, high-speed cup stacking task based on the instructions developed by the World Sport Stacking Association was used to assess learning of upper extremity coordination. This task was chosen because it has been used previously to assess motor learning and coordination in healthy children, young adults, older adults, and individuals with chronic stroke (Granados and Wulf 2007; Lessa and Chiviacowsky 2015; Tretriluxana et al. 2015; Udermann et al. 2004). While rapid cup stacking is not a function trained in rehabilitation, the task demands of grasping, moving, and releasing objects mimics upper limb skills that are trained in rehabilitation settings.

Participants used 12 specialized cups specifically developed for speed stacking (Speed Stacks, Inc., Castle Rock, CO). A non-slip mat (Speed Stacks, Inc., Castle Rock, CO) was placed in front of the participant and a competition timer was secured to latches on the front side of the mat. The timer features touch pads for beginning and stopping timing and a resolution of 0.001 s to ensure precise measurement. Before each trial, cups were stacked together, upside down, inside each other on the mat with a three-cup stack on the participants' left, a six-cup stack in the middle, and a three-cup stack on the right. The task consisted of three sequences of cup stacking and unstacking (Fig. 2b). Once the last sequence was stacked with no mistakes, the participant unstacked all the cups such that the cups were once again in stacks of 3, 6, and 3.

Prior to beginning, participants were instructed on how to perform the task with the following instructions: (1) "perform the task as fast as you can"; (2) "always work form the left to the right, stacking and then unstacking"; (3) "use both hands"; (4) "if an error occurs, fix it and then continue until the cups are placed in the correct pattern"; and (5) "the cups must start and end in the same three stacks". The experimenter then slowly demonstrated the task and allowed the participant to ask any questions. Participants completed one practice trial with the experimenter cuing and instructing the participant as needed.

On D1, participants completed three trials (not including the practice trial). The time to complete each trial, or Movement Time, was recorded and the three trials were averaged together for the baseline performance measurement. On D3–D7, participants completed five trials. All five trials on D7 were averaged together for post-test assessment of performance. Participants completed a total of 28 trials. Prior to performing the task on D4 and D6, the experimenter slowly demonstrated the task for the participant to encourage strategy exploration and learning. No verbal information was provided at any time regarding specific strategy of stacking or unstacking cups outside of the demonstrations and instructions. The experimenter only provided cuing or correction in stacking patterns when the participant was stuck or unaware of their mistake. Laminated cards for each stacking sequence were placed on the table for visual cuing each day. Participants were allowed to view the cards during stacking trials.

Discrete sequence production task

The discrete sequence production (DSP) task was used to measure motor sequence learning. We chose the DSP because it has previously been shown to be a good probe of motor sequence learning (Wymbs and Grafton 2015) and also because the general ability to sequence movements is part of functional training in rehabilitation settings.

The goal of the DSP was to learn six pre-determined sequences of key presses and execute them as quickly and accurately as possible. Each of the six sequences was ten key presses long and remained unchanged throughout the duration of the study. Participants placed the fingers of their right hand, regardless of hand-dominance, on a keyboard such that their thumb was resting on "V", index on "Y", middle on "U", ring on "I", and pinky on "L". The participants were instructed to ignore the specific letters on the keys, as they were not significant to the task.

The DSP task was executed using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). Instructions and stimuli were presented electronically on the testing laptop. On a white background, the laptop screen displayed five black-outlined boxes matching the configuration of the five keys on the keyboard (Fig. 2c). Once the participant was ready to start, he or she would push any key to begin the task. One of the black boxes would turn red (stimulus) and the participant would push the corresponding key on the keyboard as quickly and accurately as possible (response). If the correct key was pressed, the red box turned back to black and a new box turned red. If the incorrect key was pressed, the box would remain red until the participant pushed the correct key. In this fashion, the sequence continued for the remaining key presses. For all sequences, each finger was pressed twice, with no finger pushed twice in a row or every other push. Prior to each sequence, a sequence identity cue (i.e., blue or magenta circle, green or yellow triangle, or orange or black star) was displayed on the screen for 1 s. Each of the six sequences was assigned one of the sequence identity cues. Over time, the participant would learn that a sequence identity cue (e.g., blue circle) was paired with a particular sequence of key presses and would begin to anticipate the red box stimuli for that sequence.

For assessing motor sequential performance at baseline (D1) and post-test (D7), participants completed three blocks of sequences. Two sequences were in each block, with Block 1 consisting of circle sequences, Block 2 consisting of triangle sequences, and Block 3 consisting of star sequences.

Sequences were presented five times each in a random order during their assigned block. Training on the DSP occurred in each visit on D3–D7 and consisted of one large block with all six sequences presented in a random order. Sequences were presented at three pre-determined exposure levels. For extensive training, each circle sequence was presented 80 times during a training session (EXT), for moderate training, triangle sequences were presented 20 times (MOD) each, and for minimum training, star sequences were presented 5 times (MIN) each. A total of 210 sequences were completed during each DSP training session. Over the course of the study, participants completed each circle sequence 410 times, each triangle sequence 110 times, and each star sequence 35 times.

The primary outcome for this task, Movement Time (MT), was defined as the total amount of time taken to complete each sequence. Movement Time was measured by E-Prime and averaged post hoc by training level for each session. Accuracy was defined as the percent of sequences within a training level executed with no errors. Feedback regarding the previous session's MT and accuracy for each training level (EXT, MOD, MIN) was provided to the participants before beginning a new session. If accuracy was low or had dropped, participants were encouraged to slow down to increase accuracy. If accuracy was high, participants were encouraged to keep trying to decrease their MT, while still maintaining accuracy.

Data analysis

Data were securely managed and stored using REDCap database (Harris et al. 2009) and statistical analyses were performed in SPSS Statistics 21 (IBM, Armonk, NY). Sample size was determined using data from previous studies (Cherry-Allen et al. 2015, 2016). Ten participants were needed for each group to achieve at least 80% power to detect mean differences in change in balance performance. Forty total participants allowed us to detect differences on the balance task of at least 3–4 s. Differences less than this are unlikely to be clinically relevant in future target populations. All statistical tests are two-sided and the criterion for statistical significance was set at $\alpha \leq 0.05$. Normality was tested using the Kolmogorov–Smirnov test. In case of non-normally distributed data, we used a repeated measure ANOVA. A repeated measure ANOVA was used to analyze group differences in performance on the balance task and the DSP. The within-subjects factor was performance across time (two levels; pre- and post-assessment). The between-subjects factor was set as conditioning group. For the balance task and the DSP, Mauchly's test of sphericity was performed to test the assumption of sphericity. Sphericity was not satisfied, so a Huynh–Feldt correction was used. A Kruskal–Wallis ANOVA with multiple comparisons was used to determine group differences in change in performance (post-test–pre-test) on the bimanual cup stacking task. The hypothesis that increasing dose of RLIC will result in greater magnitude of learning enhancement would be supported if a significant group by time interaction was found.

Results

Forty adults were enrolled and randomized into the study, with all participants completing the protocol and contributing to data analysis (Fig. 1). There were no significant differences in demographic data between groups (Table 1). No serious adverse events occurred, termination criteria were never met, and no sessions were ended prematurely. Average cuff inflation pressure for the sham conditioning group was 64 ± 10 mmHg, with only two participants' average cuff pressure reaching over 70 mmHg (both 81 mmHg). Average cuff inflation pressure for the RLIC-3, -4, and -5 groups were 138 ± 9, 138 ± 12, and 134 ± 13, respectively. Average oxygen saturation during conditioning for the sham group was $97 \pm 1\%$, a "normal" value confirming ischemia was not induced.

Unexpectedly, RLIC at any dose did not enhance learning on the balance task. All four groups improved balance over time (Fig. 3a, main effect of time, $p < 0.001$), but there were no differences in performance between groups ($p = 0.141$), nor a significant group by time interaction ($p = 0.172$). Average group change in time in balance from pre- to post-assessment ranged from 7 to 11 s, with standard deviations ranging from 4.5 to 5.7 s. Likewise, RLIC did not enhance learning on the bimanual cup stacking task (Fig. 3b). All four groups improved on the cup stacking task over time ($p < 0.001$, for all groups; paired t tests), but there were no differences in performance between groups ($p = 0.169$). All four groups improved on the DSP over time (Fig. 4, main effect of time, EXT: $p < 0.001$; MOD: $p < 0.001$; MIN: $p < 0.001$) with the most improvement on the most practiced sequences (EXT), as expected. There were no differences in performance between groups (EXT: $p = 0.198$; MOD: $p = 0.587$; MIN: $p = 0.810$), nor significant group by time interaction (EXT: $p = 0.501$; MOD: $p = 0.899$; MIN: $p = 0.397$).

Discussion

This study investigated the effect of RLIC dose, as quantified by number of cycles, on learning enhancement of training on three tasks. Our results unexpectedly showed that RLIC at any dose did not enhance learning on any of the three chosen tasks, indicated by the lack of between group differences in performance and group by time interactions. This study was powered to detect differences in balance of 3–4 s, which

Table 1 Demographic data

Participants					
Characteristics	Sham ($n=9$)	RLIC-3 ($n=11$)	RLIC-4 ($n=10$)	RLIC-5 ($n=10$)	Main effect of group* (p)
Age (years)	26.1 ± 1.6	26.2 ± 4.6	25.9 ± 2.9	25.5 ± 3.9	0.972
Female/male	8/1	6/5	7/3	7/3	0.452
Dominant side (R/L)	7/2	9/2	10/0	10/0	0.222
Weight (kg)	77.24 ± 27.87	80.78 ± 16.60	71.21 ± 16.60	68.53 ± 18.34	0.502
Height (m)	1.67 ± 0.11	1.74 ± 0.06	1.72 ± 0.12	1.69 ± 0.09	0.405
BMI (kg/(m m))	27.2 ± 8.1	26.7 ± 5.3	23.9 ± 4.3	24.1 ± 6.6	0.520
Resting systolic (mmHg)	116 ± 12	117 ± 11	116 ± 14	113 ± 14	0.509
Resting diastolic (mmHg)	74 ± 8	75 ± 8	74 ± 10	73 ± 10	0.721
Average pain	1.0 ± 1.0	2.4 ± 1.6	2.3 ± 0.9	1.8 ± 1.2	0.082
Race					0.769
Caucasian	5	6	7	6	
African American	1	2	2	2	
Asian	3	3	1	2	

Values are "n" or "mean ± SD

RLIC remote limb ischemic conditioning, *BMI* body mass index

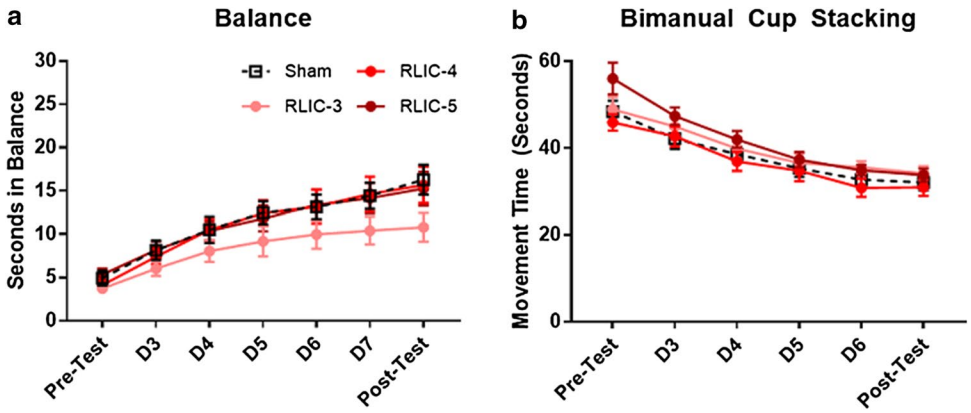

Fig. 3 Balance and bimanual cup stacking. **a** Average number of seconds in balance during 30-s trials for each group. Data points represent the average of 5 trials (pre-test and post-test) or 15 trials (D3-D7). Error bars represent SEM. **b** Average movement time in seconds for each group. Data points represent the average of 3 trials (pre-test) or 5 trials (D3–D6 and post-test). Error bars represent SEM. GraphPad Prism was used to create this figure

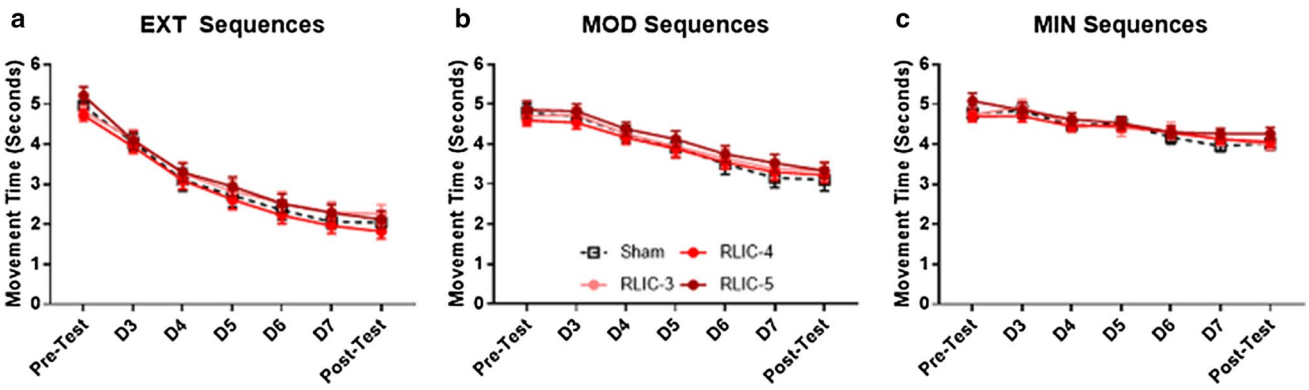

Fig. 4 Discrete sequence production average movement time in seconds per exposure level for each group. **a** Extensive (EXT) sequences, **b** moderate (MOD) sequences, **c** minimum (MIN) sequences. Data points represent the average of 5 trials (pre-test and post-test) or predetermined number of exposures for each training level described above (D3-D7). Error bars represent SEM. GraphPad Prism was used to create this figure

was half of the group difference we have found previously. Inspection of the overlapping group lines in Figs. 3 and 4 illustrate that more subjects would not have led to significant group × time interactions. All three tasks, however, showed significant improvement over time, suggesting that each task was an appropriate probe of learning.

Importantly, these findings contradict our previous results (Cherry-Allen et al. 2015, 2016), in which we observed a robust enhancement of motor learning due to RLIC on the balance task in a participant population similar to that used in the current study (Cherry-Allen et al. 2015, 2016). Average improvement of balance times from the previous studies were 17.6 s and 10.8 s in the RLIC and Sham groups, respectively. For the current study, average group change in balance time from pre- to post- assessment were comparable to the Sham group from our previous studies, confirming that proper replication of procedures was achieved, but without the expected benefit. Unfortunately, hypotheses about the effects of RLIC cycle dose could not be fully evaluated, since no effect was seen at three, four, or five cycles of RLIC. Demographic data of participants, including age, BMI, and sex, were similar across studies (Supplementary Tables 1 and 2) and do not appear to be the source of the resulting discrepancies. Self-reported activity level was somewhat higher in the current study samples and, therefore, we cannot rule out the possibility that coordination or cardiorespiratory fitness was different across studies. It cannot explain, however, why improvement in balance for all groups of the current study is comparable to our previous studies' Sham groups. Since a double-blinded study design was not used in any of our experiments, it is also possible that the previous studies had introduced unintended and unconscious biases. Therefore, it remains unclear at present why conflicting results transpired. Data from our lab (Sutter et al. 2018), however, suggest that the identical RLIC and training protocol may provide a small benefit to learning on the same balance task in individuals without co-morbidities who are aged 40–80 years. Our findings in young health adults and, particularly, the 40–80-year population, begs the question regarding potential responders/non-responders and/or the possibility of a RLIC response ceiling effect in young, healthy individuals. The present data cannot determine whether or not these results would extend into disease populations (Koch et al. 2014).

These negative results cannot be explained by the specific training tasks chosen. All three tasks have been successfully used previously as a probe of learning in various capacities (Cherry-Allen et al. 2015, 2016; Lessa and Chiviacowsky 2015; Taubert et al. 2010; Tretriluxana et al. 2015; Verwey et al. 2010; Wymbs and Grafton 2015). The DSP task is unique in its ability to test learning at different exposure levels and has been used to show that representation of brain activity of the motor system is flexible based on the amount of practice. Examination of Figs. 3 and 4 shows that these three tasks were good probes of learning, with room for more improvement available for all three tasks. Participants, regardless of group, demonstrated changes in behavior over multiple days of training on each of the three tasks. Specifically, for the DSP, training elicited the expected effects in that there was differential benefit and clear separation in the amount of learning for each exposure level.

A potential explanation for our results may be the universal issue of study replication. Successful replication is paramount for the advancement of research; however, only a small percentage of scientific findings may be fully reproducible (Ioannidis 2005; Open Science 2015; Prinz et al. 2011). A large-scale effort to quantify reproducibility in psychological science showed that while a striking 97% of original studies found significant results with alpha levels less than 0.05, only 36% of those replications resulted in significant findings (Open Science 2015). Methodologists have suggested that the high rate of the lack of scientific confirmation may be due to the "convenient, yet ill-founded strategy of claiming conclusive research findings solely on the basis of a single study assessed by formal statistical significance" (Ioannidis 2005). In fact, scientific findings should not be interpreted based on statistical significance alone, as they may also depend on factors such as power and bias, the ratio of true to null relationships among those probed, flexibility in design definition, and outcomes, as well as interest and prejudices (Ioannidis 2005). Regarding our current series of investigations of RLIC's effect on learning enhancement, it is conceivable that some of these factors have come into play or that the previous two investigations happened to randomize high-performing outlier participants to RLIC groups.

Although not evident from the current study, there remains the possibility that RLIC will be useful within neurorehabilitation clinical settings to enhance other activities. The benefits of RLIC on skeletal muscle strengthening, a fundamental ingredient of physical rehabilitation for many patient populations, is currently being explored. Recently, it was shown that one bout of ischemic conditioning administered to individuals with stroke can increase leg muscle strength in the paretic limb by 16.1% (Hyngstrom et al. 2018). Increases of muscle strength were accompanied by an increase in electromyographic activity and a decrease in motor unit recruitment thresholds. Thus, RLIC has the potential to modulate motoneuron activity which in turn could be harnessed to improve muscle strength and function. Further investigation, followed by replication, is merited.

In conclusion, despite two previous studies in our laboratory indicating a benefit of RLIC on learning enhancement, the current investigation was not able to replicate such benefits at the same RLIC dose, as well as at other doses, on any of the chosen tasks. While RLIC is safe, inexpensive,

and clinically feasible, the translation of RLIC to improving rehabilitation outcomes in individuals recovering from neurological injury may be limited by yet-to-be-identified variables, leading us to reconsider our original intent to pair RLIC with motor skill training in any kind of routine manner. In view of the large body of literature suggesting a cardio- and neuro-protective benefit of RLIC, future studies should continue to investigate potential markers of response, mechanisms of action, and potential benefit to enhance other outcome measures, such as strength training. Collectively, this information will eventually aid in determining when RLIC has been successfully administered and fully reveal the breadth of RLIC's utility for improving health outcomes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Ali ZA et al (2007) Remote ischemic preconditioning reduces myocardial and renal injury after elective abdominal aortic aneurysm repair: a randomized controlled trial. Circulation 116:I98-105 https://doi.org/10.1161/circulationaha.106.679167

Bøtker HE et al (2010) Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. Lancet 375:727–734. https://doi.org/10.1016/s0140-6736(09)62001-8

Cherry-Allen KM, Gidday JM, Lee JM, Hershey T, Lang CE (2015) Remote limb ischemic conditioning enhances motor learning in healthy humans. J Neurophysiol 113:3708–3719. https://doi.org/10.1152/jn.01028.2014

Cherry-Allen KM, Gidday JM, Lee JM, Hershey T, Lang CE (2016) Remote limb ischemic conditioning at two cuff inflation pressures yields learning enhancements in healthy adults J Mot Behav. https://doi.org/10.1080/00222895.2016.1204268

Crimi G et al (2013) Remote ischemic post-conditioning of the lower limb during primary percutaneous coronary intervention safely reduces enzymatic infarct size in anterior myocardial infarction: a randomized controlled trial. JACC Cardiovasc Interv 6:1055–1063. https://doi.org/10.1016/j.jcin.2013.05.011

Ding Q, Ying Z, Gomez-Pinilla F (2011) Exercise influences hippocampal plasticity by modulating brain-derived neurotrophic factor processing. Neuroscience 192:773–780. https://doi.org/10.1016/j.neuroscience.2011.06.032

Dirnagl U, Becker K, Meisel A (2009) Preconditioning and tolerance against cerebral ischaemia: from experimental strategies to clinical use. Lancet Neurol 8:398–412. https://doi.org/10.1016/s1474-4422(09)70054-7

Drager LF, Jun JC, Polotsky VY (2010) Metabolic consequences of intermittent hypoxia: relevance to obstructive sleep apnea. Best Pract Res Clin Endocrinol Metab 24:843–851. https://doi.org/10.1016/j.beem.2010.08.011

Fan YY et al (2011) Activation of the central histaminergic system is involved in hypoxia-induced stroke tolerance in adult mice. J Cereb Blood Flow Metab 31:305–314. https://doi.org/10.1038/jcbfm.2010.94

Garcia S et al (2011) Long-term follow-up of patients undergoing postconditioning during ST-elevation myocardial infarction. J Cardiovasc Transl Res 4:92–98. https://doi.org/10.1007/s12265-010-9252-0

Gidday JM (2006) Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci 7:437–448. https://doi.org/10.1038/nrn1927

Granados C, Wulf G (2007) Enhancing motor learning through dyad practice: contributions of observation and dialogue. Res Q Exerc Sport 78:197–203

Groothuis JT, van Vliet L, Kooijman M, Hopman MTE (2003) Venous cuff pressures from 30 mmHg to diastolic pressure are recommended to measure arterial inflow by plethysmography. J Appl Physiol 95:342–347

Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG (2009) Research electronic data capture (redcap)–a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 42:377–381

Hess DC, Blauenfeldt RA, Andersen G, Hougaard KD, Hoda MN, Ding Y, Ji X (2015) Remote ischaemic conditioning-a new paradigm of self-protection in the brain. Nat Rev Neurol 11:698–710. https://doi.org/10.1038/nrneurol.2015.223

Hougaard KD et al (2014) Remote ischemic perconditioning as an adjunct therapy to thrombolysis in patients with acute ischemic stroke: a randomized trial. Stroke 45:159–167. https://doi.org/10.1161/STROKEAHA.113.001346

Hyngstrom A, Murphy S, Nguyen J, Schmit B, Negro F, Gutterman D, Durand M (2018) Ischemic conditioning increases strength and volitional activation of paretic muscle in chronic stroke: a pilot study. J Appl Physiol 124:1140–1147

Iadecola C, Anrather J (2011) Stroke research at a crossroad: asking the brain for directions. Nat Neurosci 14:1363–1368. https://doi.org/10.1038/nn.2953

Ioannidis JP (2005) Why most published research findings are false. PLoS Med 2:e124. https://doi.org/10.1371/journal.pmed.0020124

Johnsen J, Pryds K, Salman R, Lofgren B, Kristiansen SB, Botker HE (2016) The remote ischemic preconditioning algorithm: effect of number of cycles, cycle duration and effector organ mass on efficacy of protection. Basic Res Cardiol 111:10. https://doi.org/10.1007/s00395-016-0529-6

Koch S, Gonzalez N (2013) Preconditioning the human brain: proving the principle in subarachnoid hemorrhage. Stroke 44:1748–1753. https://doi.org/10.1161/STROKEAHA.111.000773

Koch S, Della-Morte D, Dave KR, Sacco RL, Perez-Pinzon MA (2014) Biomarkers for ischemic preconditioning: finding the responders. J Cereb Blood Flow Metab 34:933–941. https://doi.org/10.1038/jcbfm.2014.42

Laskey WK (2005) Brief repetitive balloon occlusions enhance reperfusion during percutaneous coronary intervention for acute myocardial infarction: a pilot study. Catheter Cardiovasc Interv 65:361–367. https://doi.org/10.1002/ccd.20397

Lessa HT, Chiviacowsky S (2015) Self-controlled practice benefits motor learning in older adults. Hum Mov Sci 40:372–380. https://doi.org/10.1016/j.humov.2015.01.013

Lonborg J et al (2010) Cardioprotective effects of ischemic postconditioning in patients treated with primary percutaneous coronary intervention, evaluated by magnetic resonance. Circ Cardiovasc Interv 3:34–41. https://doi.org/10.1161/CIRCINTERVENTIONS.109.905521

Lorentsen E, Hansteen V, Sivertssen E (1970) The influence of venous collecting pressure on measurement of calf blood flow by venous occlusion plethysmography. Angiol Nov 21:661–677

Loukogeorgakis SP et al (2007) Transient limb ischemia induces remote preconditioning and remote postconditioning in humans by a K(ATP)-channel dependent mechanism. Circulation 116:1386–1395. https://doi.org/10.1161/CIRCULATIONAHA.106.653782

Ma XJ, Zhang XH, Li CM, Luo M (2006) Effect of postconditioning on coronary blood flow velocity and endothelial function in patients with acute myocardial infarction. Scand Cardiovasc J 40:327–333. https://doi.org/10.1080/14017430601047864

Meng R et al (2012) Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. Neurology 79:1853–1861

Open Science C (2015) Estimating the reproducibility of psychological science. Science 349:aac4716. https://doi.org/10.1126/science.aac4716

Pallares LC, Deane CR, Baudouin SV, Evans TW (1994) Strain gauge plethysmography and Doppler ultrasound in the measurement of limb blood flow. Eur J Clin Investig Apr 24:279–286

Porter BA et al (2012) Repeatedly pairing vagus nerve stimulation with a movement reorganizes primary motor cortex. Cereb Cortex 22:2365–2374. https://doi.org/10.1093/cercor/bhr316

Prinz F, Schlange T, Asadullah K (2011) Believe it or not: how much can we rely on published data on potential drug targets? Nat Rev Drug Discov 10:712. https://doi.org/10.1038/nrd3439-c1

Rahimi M et al (2015) The effect of high intensity interval training on cardioprotection against ischemia-reperfusion injury in wistar rats. EXCLI J 14:237–246. https://doi.org/10.17179/excli2014-587

Ren C, Gao X, Steinberg GK, Zhao H (2008) Limb remote-preconditioning protects against focal ischemia in rats and contradicts the dogma of therapeutic time windows for preconditioning. Neuroscience 151:1099–1103. https://doi.org/10.1016/j.neuroscience.2007.11.056

Rybnikova E, Vataeva L, Tyulkova E, Gluschenko T, Otellin V, Pelto-Huikko M, Samoilov MO (2005) Mild hypoxia preconditioning prevents impairment of passive avoidance learning and suppression of brain NGFI-A expression induced by severe hypoxia. Behav Brain Res 160:107–114. https://doi.org/10.1016/j.bbr.2004.11.023

Staat P et al (2005) Postconditioning the human heart. Circulation 112:2143–2148. https://doi.org/10.1161/CIRCULATIONAHA.105.558122

Stetler RA et al (2014) Preconditioning provides neuroprotection in models of CNS disease: paradigms and clinical significance. Prog Neurobiol 114:58–83. https://doi.org/10.1016/j.pneurobio.2013.11.005

Sutter EN et al (2018) Remote limb ischemic conditioning and motor learning: evaluation of factors influencing response in older adults. Transl Stroke Res. https://doi.org/10.1007/s12975-018-0653-8

Tapuria N, Kumar Y, Habib MM, Abu Amara M, Seifalian AM, Davidson BR (2008) Remote ischemic preconditioning: a novel protective method from ischemia reperfusion injury—a review. J Surg Res 150:304–330. https://doi.org/10.1016/j.jss.2007.12.747

Taubert M, Draganski B, Anwander A, Muller K, Horstmann A, Villringer A, Ragert P (2010) Dynamic properties of human brain structure: learning-related changes in cortical areas and associated fiber connections. J Neurosci 30:1670–11677

Thibault H et al (2008) Long-term benefit of postconditioning. Circulation 117:1037–1044. https://doi.org/10.1161/CIRCULATIONAHA.107.729780

Tretriluxana J, Taptong J, Chaiyawat P (2015) Dyad training protocol on learning of bimanual cup stacking in individuals with stroke: effects of observation duration. J Med Assoc Thail 98:106–112

Udermann B, Murray S, Mayer J, Sagendorf K (2004) Influence of cup stacking on hand–eye coordination and reaction time of second-grade students. Percept Mot Skills 98:409–414

Verwey WB, Abrahamse EL, de Kleine E (2010) Cognitive processing in new and practiced discrete keying sequences. Front Psychol 1:32. https://doi.org/10.3389/fpsyg.2010.00032

Wymbs NF, Grafton ST (2015) The human motor system supports sequence-specific representations over multiple training-dependent timescales. Cereb Cortex 25:4213–4225. https://doi.org/10.1093/cercor/bhu144

Yang X-C et al (2007) Reduction in myocardial infarct size by postconditioning in patients after percutaneous coronary intervention. J Invasive Cardiology 19:424–430

Yang Q, Wang Y, Feng J, Cao J, Chen B (2013) Intermittent hypoxia from obstructive sleep apnea may cause neuronal impairment and dysfunction in central nervous system: the potential roles played by microglia. Neuropsychiatr Dis Treat 9:1077–1086. https://doi.org/10.2147/NDT.S49868

Yarrow JF, White LJ, McCoy SC, Borst SE (2010) Training augments resistance exercise induced elevation of circulating brain derived neurotrophic factor (BDNF). Neurosci Lett 479:161–165. https://doi.org/10.1016/j.neulet.2010.05.058