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Investigating the Effects of Combining Treadmill Training with Trans-spinal Direct Current Stimulation on Motor Skill Recovery After Spinal Cord Injury

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ABSTRACT

INVESTIGATING THE EFFECTS OF COMBINING TREADMILL TRAINING WITH TRANS-SPINAL DIRECT CURRENT STIMULATION ON MOTOR SKILL RECOVERY AFTER SPINAL CORD INJURY

by

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This study assessed the effect of treadmill training with or without trans-spinal direct current stimulation on motor recovery following a T13/L1 spinal cord hemi section in CD-1 mice. Mice all received the same spinal cord injury, which resulted in left hind limb paralysis, and were assigned to one of three groups. Control animals received no treatment. Treadmill only mice were treated with 5 sessions of treadmill training. Treadmill + stimulation animals were treated with concurrent treadmill training and trans-spinal direct current stimulation. Progress was assessed by analyzing each subject's steps on a horizontal ladder test, which the animals performed both pre-treatment and post-treatment. Data suggests that the combination of treadmill training and trans-spinal direct current stimulation has a significant effect on recovery compared to no treatment. However, limitations were present that will be rectified during further studies to accurately assess the effect of these treatments on the ladder task performance.

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INTRODUCTION

The Hebbian concept (Hebb, D. O., 1949) states that a persistent activation of neurons creates a lasting cellular change in its connection to interconnected cells, strengthening the association. When a pre-synaptic cell fires repeatedly, the postsynaptic cell forms a more efficient pathway for future activation of the postsynaptic neuron. The concept is more commonly known as “neurons that fire together wire together.” This theory offers some hypothetical insight into behavioral learning in animals and how synaptic transmission is modulated through repeated stimuli, such as an electrical current.

Synaptic plasticity, as theorized by the Hebbian model of learning, is the idea that the amount of neurotransmitters released into the synapse is directly affected by the frequency of interconnected neuronal firing. That is to say, when associated neurons fire more often, the pre-synaptic neuron increases the amount of neurotransmitters being released from the terminal axon. The postsynaptic neuron also forms more neurotransmitter receptors for chemical uptake. This combined effect leads to an increase in excitability between the paired neurons (Gerrow, K., & Triller, A. 2010). The opposite holds true in that the quantity of neurotransmitters released and sites for uptake decrease based on disuse of the paired axons. Direct current stimulation can excite or inhibit a neuronal pathway based on the charge being delivered. When a nerve is stimulated with the anode, this will excite it; when stimulated with the cathode, this will inhibit it (Ahmed, Z., 2010).

This synaptic transmission has been shown to be further modified by temporal aspects of neuronal coding. The concept of spike timing dependent plasticity (STDP) postulates that the synaptic strength is strongly influenced by the timing of the pre and postsynaptic action potentials. Long-term effects on plasticity within the synapse is strengthened when the pre-synaptic spike arrives earlier than when the postsynaptic neuron is activated (Song, S. & Abbott, L. F., 2001). The dependency on temporal and frequency aspects of associating neurons are important in regards to regeneration of the corticospinal tract when utilizing electrical stimulation. A better understanding of the mechanics of neurological learning can greatly aid in healing spinal cords post injury in which neurons are severed and new associations must be formed.

Using this theory, it is possible that neuromuscular connections can be strengthened by selective stimulation with attention to the polarity used. Ahmed (2010) demonstrated this in mice by placing the

positive electrode on the motor cortex and the negative electrode on a muscle, leading to an enhanced ability to elicit a muscle contraction via cortical control. This was demonstrated not only in normal animals, but also in animals with a spinal cord injury, giving promise to the idea that electrical stimulation may be a tool used to repair function in patients with spinal cord injuries. Ahmed further explored trans-spinal direct current stimulation by now modulating these muscle contractions (Ahmed, Z., 2011). Cortically elicited twitches were depressed during a-tsDC and enhanced during c-tsDC. This study proves ts-DC to be a neurostimulation tool that can modulate spinal cord excitability and corticospinal transmission.

In many neurological disorders connectivity between the cortex and spinal cord is compromised. Through this application of DC current, spinal neurons were recruited in an orderly fashion, maintaining the normal order of motor neuron recruitment, which is essential to control speed and force of muscle contractions. The ability of this method to interact with cortical activity and help direct it has much potential for clinical use. Very importantly, this distinguishes it from drug-induced experiments and gives research another valid and exciting avenue to explore.

The application of charge has been shown to alter neural tissue on an anatomical level. In a study performed by Borgens & Bohnert (1997), changes were seen in damaged axons and axon branches after a 1-month long treatment with a negative voltage stimulator. Re-growth does occur even without electrical stimulation; however, growth of these projections appeared to be more organized than projections that grew without electrical stimulation (Borgens, R.B., & Bohnert, D.M., 1997). This indicates the possibility of axonal regeneration if future research is performed and the technique is perfected.

It is important that these benefits be applicable on a functional level in order for them to have any real use in clinical practice. Some functional benefits were seen using an animal model in Carmel et. al (2010), where rats with spinal cord injuries had their corticospinal tracts electrically stimulated. Rats with these injuries showed deficits in their ability to place their paws correctly to walk across a ladder; the results of the study showed that injured rats who were stimulated made less errors in the placement of their paws and were therefore able to perform the functional activity with more success than injured rats

who did not receive the stimulation treatment (Carmel, J. B., Berrol, L. J., Brus-Ramer, M., & Martin, J. H., 2010).

Numerous studies have been conducted on animals regarding spinal cord injury in relation to humans. These studies have shown evidence that humans have a great capacity for neural reorganization and there is neuroplasticity of the central nervous for task specific training, such as gait. Seeing that gait is such a fundamental goal to patients with a spinal cord injury, these findings help to provide changes in treatments and plans of care.

Trans-spinal direct current stimulation has been shown to have substantial, long-term effects on the excitability of the spinal cord. It is capable of modifying both single and multi-joint movements (Ahmed, 2013). Depending on the direction of the current flow, it may be used to increase or decrease spinal cord neuronal excitability, making it a possible treatment choice for both hypo- and hypertonia; these effects have been shown in mice (Ahmed, 2014). By way of the connections between the spinal cord and the cortex, along with its non-invasive quality, it is theorized that trans-spinal stimulation can one day be routinely used to facilitate recovery following central nervous system injury.

The usefulness of treadmill training in recovery following spinal cord injury in animals has also been demonstrated previously. Compared with untrained mice, mice that were on a treadmill training exercise program following spinal cord hemi section showed decreased muscle wasting and increased amounts of axonal re-growth (Goldshmit et al., 2008). In Heng & Leon (2009), rats that completed a daily treadmill training program showed more normal steppage patterns than untrained rats.

While the positive effects of each treatment have been separately shown, the present study seeks to combine the therapeutic effects of trans-spinal direct current stimulation and treadmill training in spinal cord injured mice in the hopes that their separate benefits will be augmented by their simultaneous use.

Safely and successfully demonstrating the usefulness of this combined treatment in an animal model may open the door to future studies using human subjects. There are over 10,000 new cases of human spinal cord injuries each year in the United States, and many of these injuries occur in individuals younger than 30 years old (Hamid & Rayek, 2008). Many of these patients go on to develop health

problems related to lack of physical activity in the chronic stages of their injuries, including obesity, diabetes, and cardiovascular disorders (Myers et al., 2007). Preserving the motor function of individuals suffering from spinal cord injuries may help maintain their health and well being over the subsequent years of their life.

METHODS

Subjects

Adult CD-1 mice (n=18; weight, 30-40 g) were used for this study. Protocol was designed in accordance with National Institutes of Health guidelines for the care and use of laboratory animals. The protocol was approved by the Institutional Animal Care and Use Committee of the College of Staten Island. Animals were housed under a 12:12 hour light: dark cycle and provided with food and water ad libitum.

Mice were assigned to one of three groups. Control animals (n=6) received a spinal cord injury but did not get any further treatment. Treadmill only (n=7) mice received an injury and were then treated with treadmill training only, as described below. Treadmill + stimulation animals (n=5) received an injury and were then treated with concurrent treadmill training and trans-spinal direct current stimulation, as described below.

Electrodes

The spinal DCS electrodes (Figure 1) consisted of a small stainless-steel plate (1.5-mm width; 3-mm length; 50-μm thickness) that was sandwiched between silicone rubber (178-μm thickness) and soft cotton-wick fabric (0.5-mm thickness). The three layers were bonded together using silicone adhesive and left overnight to dry before use. The final DCS electrode was 10 mm wide and 15 mm long.

Surgical Procedure

As in Ahmed & Wieraszko (2012), animals were deeply anesthetized using ketamine-xylazine (90/10 mg/kg ip). A laminectomy was performed to expose the junction of the T13 and L1 spinal cord. A right-sided hemi section was performed using an angled microsurgical probe.

For mice receiving stimulation, incisions were made at the skin covering the dorso-lumbar spinal column and the skin covering the cervical region. The tsDCS electrode was sutured fabric side down to

the fascia of the spine to cover the area from the T13 to the L6 vertebral level. The reference electrode was placed fabric side up toward the lateral abdominal skin.

The wounds were sutured closed following the injury and/or electrode placement, and animals were allowed to recover on a heating pad set to 37 degrees Celsius.

Testing Procedure

Pre-Exposure to Treadmill and Ladder

Subjects were exposed to the treadmill (Figure 2) and ladder (Figure 3) prior to formal testing. Each subject was placed on the treadmill for one ten-minute training session with each lane's top door closed. The first five minutes were spent at 15 cm/s and the last five minutes were spent at 20 cm/s. Shocks at an amplitude of 0.6 mA were delivered if a subject did not keep adequate pace and slid to the end of the lane, where a shock bar was present.

Subjects were placed on the ladder three times consecutively on the same day as the above initial treadmill training for familiarization purposes. The ladder consisted of rungs in a random pattern spaced 1 inch apart at minimum and 2 inches apart at maximum. The ladder was elevated approximately 1 foot from the tabletop. The sides of the ladder consisted of two clear plexiglass panels (1 m long). The ladder measured 5 cm wide between the plexiglass.

Subjects were placed by hand onto the first rung. Gentle prodding by hand was used as necessary to move the subjects across the ladder if they arrested their forward movement for longer than 5 seconds.

Following injury and/or electrode placement, subjects were tested weekly on the ladder, three trials per session, to assess for quantity of limb placement errors made. They were allowed to recover in their home cages for two weeks before any further testing began.

Control Group

Control animals underwent ladder testing for once a week, three trials per session, for 8 weeks. Each trial was recorded using a digital camcorder so that data could be analyzed at a later date. Each week, the rung pattern was randomly changed so that the subjects did not learn one particular pattern. No other treatment was provided for this group.

Treadmill Only Treatment Group

Following recovery, mice assigned to the treadmill only (i.e. no stimulation) group were run for five consecutive days on the treadmill at a speed of 20 cm/s. As during initial exposure to the treadmill, shocks were delivered at an amplitude of 0.6 mA if the animal failed to keep pace and slid to the back of the lane. Following this treatment, ladder testing as described for the control group continued for an additional 8 weeks post-treatment.

Treadmill + Stimulation Group

Following recovery, mice assigned to the treadmill and stimulation group were run on the treadmill under the same conditions as the treadmill only group while tsDCS (Figure 4) was simultaneously delivered. Current was delivered at 0.8 mA for the entire twenty minutes the animal spent on the treadmill. Following this treatment, ladder testing as described for the control group continued for an additional 8 weeks post-treatment.

RESULTS

Video data was analyzed for each ladder testing session. A tally of the total number of steps taken on the intact right hind limb was taken to be used as a reference measure. Tallies for the number of completely missed steps on the left hind limb and misplaced steps on the left hind limb were also recorded. A completely missed step was recorded when the animal either a) completely missed the rung it attempted to place the left hind limb on, b) dragged the left hind limb without any attempt at placement, or c) placed the left hind limb but demonstrated no push-off from the rung. A misplaced step was recorded when the animal demonstrated poor left hind limb placement on the rung (grabbing with the toes/nails or the very proximal aspect of the foot, for example with some push-off. A maximum of one error was recorded for each step the animal took with its intact right hind limb. If the animal demonstrated proper foot placement with push-off on the left hind limb, no error was recorded.

From the above, three percent-errors were calculated with the total number of steps on the right used as the reference measure. The percentage of steps on the left that were missed and percentage of steps on the left that were misplaced were each recorded. The total number of errors (missed steps plus missed placements) was also calculated.

As seen in Table 1, the percent error for the number of missteps for the treatment group receiving treadmill training and stimulation decreased the most over the sessions. Alternately, the percent error for the number of misplacements for the same treatment group showed the greatest increase over the sessions (Table 2). This combination shows an improvement in the quality of performance on the ladder test overall for this treatment group.

The combination of treadmill training and stimulation was significantly greater than that of treadmill training alone on both the number of misplacements and missteps (Kruskal-Wallis ANOVA). Post-hoc tests also revealed a significance of the combination of treadmill training and stimulation on the number of misplacements and missteps compared with the control group ($P < 0.05$). However, the total number of errors was not found to have any significance ($P = 0.309$).

DISCUSSION

This study offered a first look at the effects of combined treadmill training and tsDC stimulation. As the results show, there was a significant difference between the control and treadmill+stim groups in both miss-steps and complete misses. Although the number of missteps was significantly higher in the treadmill+stim group compared to the control group, this offered evidence to the effectiveness of the intervention on recovery. A greater number of missteps, when paired with the significantly lower number of complete misses, showed an improvement of functional movement.

Although there were significant differences between the two groups, we believe some main effect interactions were masked by complications in the procedures. The most prevalent issue was our main outcome measure, the horizontal ladder test. The minimum space between rungs was 1 inch. This is in contrast to the horizontal ladder test described by Metz & Whishaw (2009), which had a minimum distance of 1 cm between rungs. The ladder test described was also used in a rat model. Although it was stated to be applicable to a mouse model as well, it is important to note the difference in average size between mice and rats. The combination of smaller sized animals with larger distance between rungs may result in a task too difficult to accurately measure effect size.

Another extraneous variable, which may affect results, is the strategy each mouse used to traverse the ladder. Each subject completed the ladder test at a different speed, with some mice moving

slowly and making pauses and others moving across as quickly as possible. It's possible that these differences can have an impact on the quality of gait unrelated to the injury or treatment received.

Yet another variable with the current ladder test is the limited number of steps that can be recorded and analyzed. This issue can be rectified in future studies using a circular ladder, which has no defined start and end point. A motorized circular ladder also addresses the previous limitation of individual mouse speed and strategy; it has the advantage of having a set number of rotations per minute, limiting the speed of the subjects. The use of a circular ladder results in an unlimited number of steps to be recorded and analyzed.

Another limitation is the delivery of stimulation. Upon removal of the electrodes from one subject, it appeared to have minimal wear, implying that a poor connection to the stimulation unit existed during delivery of stimulation. Further, in a number of subjects, the leads came loose during the time spent on the treadmill. This required resetting of the unit, resulting in a pause in delivery of the current. This led the researchers to fail to be able to confidently quantify the amount of stimulation each subject actually received. This issue is planned to be rectified in future studies with more secure electrode placements.

Secondary to the issues with electrodes, the stimulation-only group was excluded from the study. The limited number of subjects who completed the study with intact electrodes were assigned to the stimulation+treadmill group. This allowed for a proper sample size when looking for a main effect.

Despite some limitations, there was still a significant improvement when comparing groups. This offers some promising evidence to continue research into the effects of treadmill training and tsDC stimulation in the spinal cord injury population.

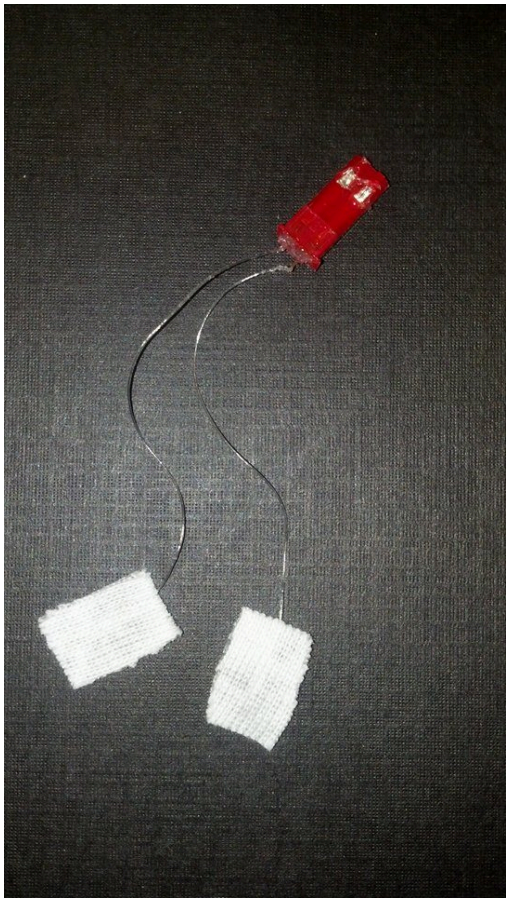

Figure 1. Electrodes

Figure 2. Treadmill

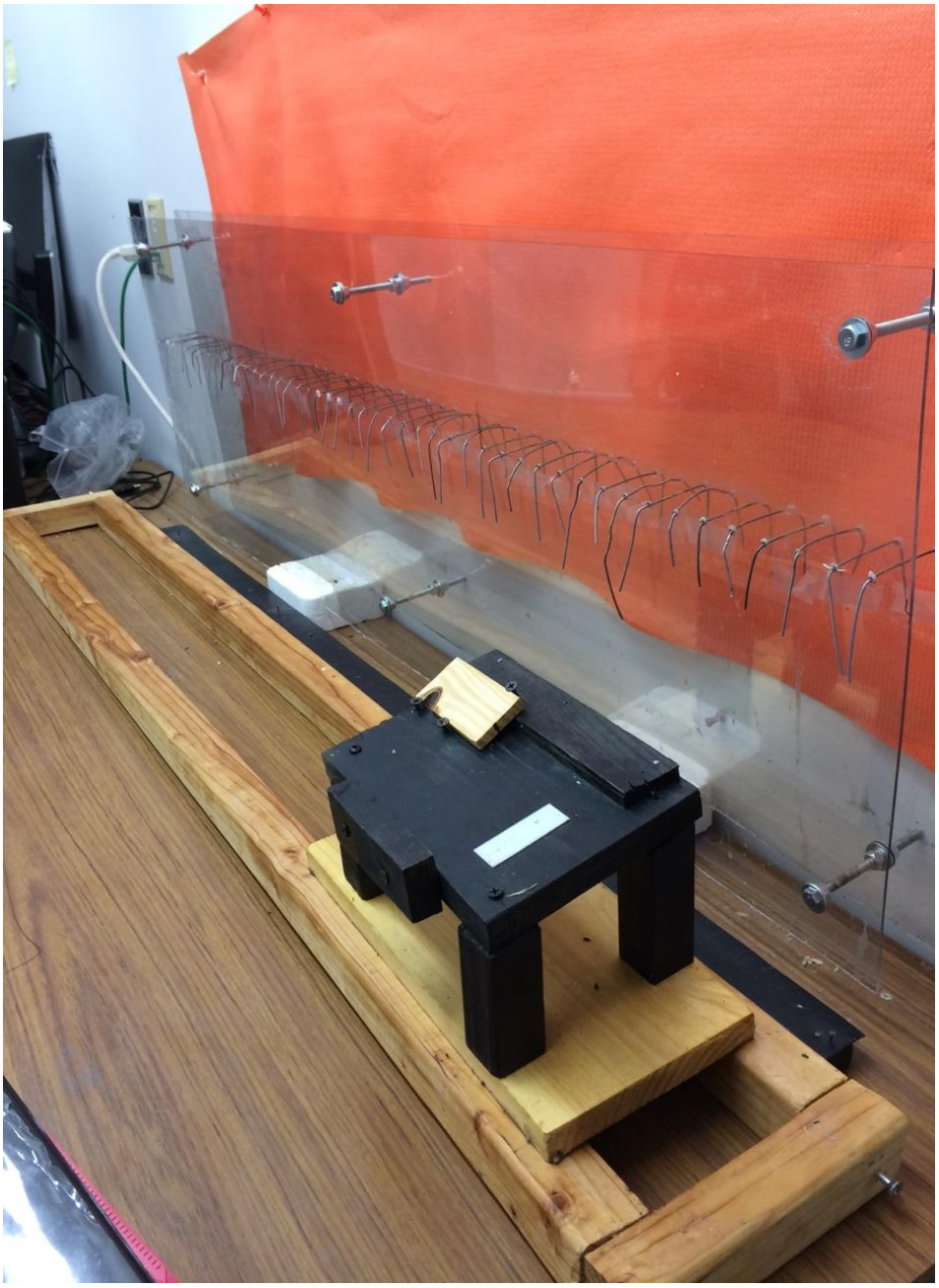

Figure 3. Horizontal Ladder

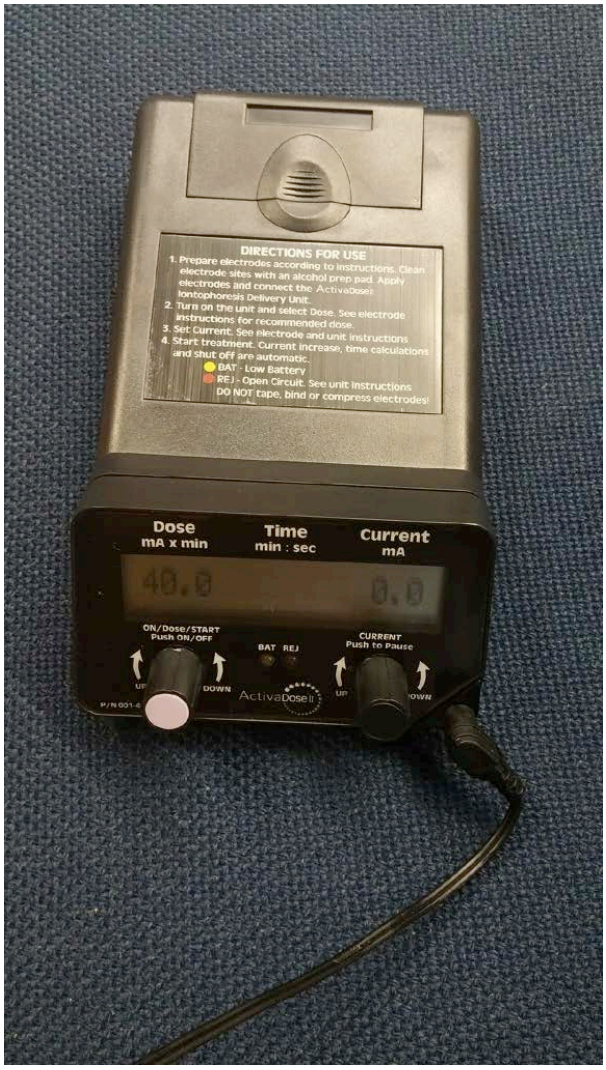

Figure 4. Iontophoresis Stimulation Machine

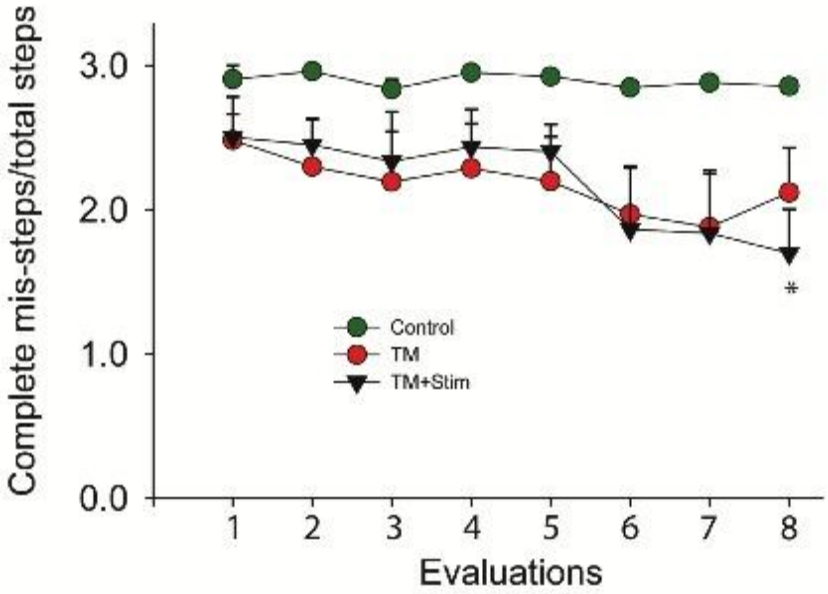

Table 1. The effects of TM and TM+Stim on the number of complete miss-steps.

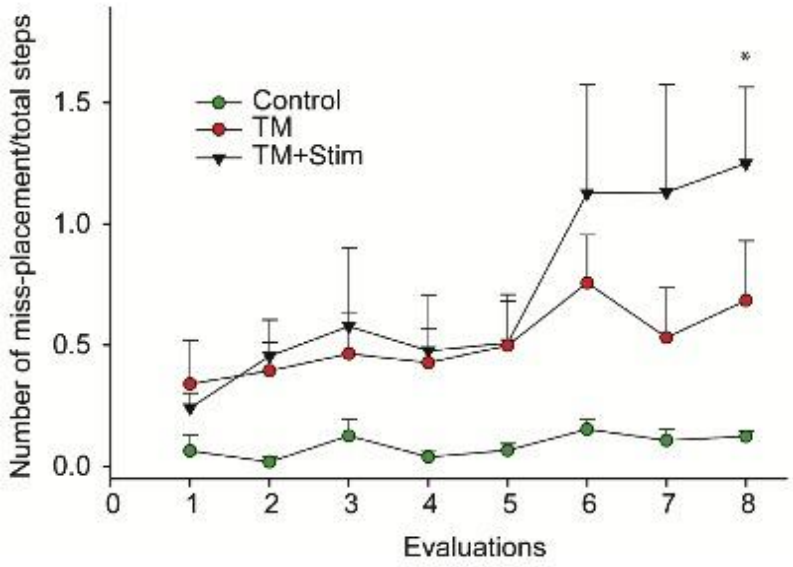

Table 2. The effects of TM and TM+Stim on the number of miss-placements.

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