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The Effect of tDCS on CD-1 Mouse Behavior Post Induced Sensorimotor Cortex Injury

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Abstract

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The functional topography of the motor cortex has been shown to be modifiable by transcranial direct current stimulation (tDCS). tDCS works by directing electrical currents into the brain which induces alterations in neuroplastic cortical excitability. This experiment's aim was to test the effect of tDCS on recovery of skilled locomotion, recovery of balance, and recovery of grip strength after bilateral electrolytic lesions to sensorimotor cortices in mice. Tests employed in this experiment included the ladder test, grip test, and a balance pole test. We were able to show that 4 days of tDCS post brain injury in mice produced improvements in skilled locomotion, recovery of balance, and recovery of grip strength in mice.

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Introduction

Using Transcranial Direct Current Stimulation (tDCS), which uses low-level, constant electrical currents to stimulate specific areas of the brain, to test the changes in the motor cortex of mice is useful in discovering exactly how neuroplastic changes occur following a motor cortex injury (Dasilva, Volz, Bikson, Fregni, 2011). In theory, tDCS should benefit the ability of mice with this form of injury to function because the functional topography of the motor cortex is modifiable by tDCS, pharmacologic treatment, and behavioral modification (Randolph, et al., 2001). If this hypothesis was proved correct, the clinical use of tDCS in modifying intact cortical tissue is supported. The use of tDCS in rodents has already been shown to increase dopamine production (Tanaka, Takano, Tanaka, Hanakawa… & Honda, 2013), improve orientation in cases of rat-model Parkinson's (Li, Tian, Qian, Yu & Jiang, 2011), decrease epilepsy-related convulsive behavior (Liebetanz, Klinker, Hering, Koch… & Tergau, 2006; Kamida, Kong, Eshima & Fujiki, 2013), reduce pain and related stress (Spezia-Adachi, Caumo, Laste, Fernandes-Medeiros… & Torres, 2012), improving skill- and task-related memory and associative learning (Dockery, Liebetanz, Birbaumer, Malinowska, Wesierska, 2011; Márquez-Ruiz, Leal-Campanario, Sánchez-Campusano, Molaee-Ardekani… & Delgado-García, 2012), and possibly improve cortical density (Faraji, Gomez-Palacio-Schjetnan, Luczak & Metz, 2013).

tDCS works by directing electrical currents into the brain which induces alterations in neuroplastic cortical excitability. This is accomplished by inducing NMDA- and calcium-dependent changes, increasing the calcium level in the targeted area after a period of twenty-four hours with a stable level until seventy-two hours following application. tDCS has been demonstrated to successfully modulate subcortical firing activity, increasing facilitation of the area affected by tDCS. Therefore, tDCS was shown to have a positive effect on improving brain

activity by affecting stable, lasting change in the biochemistry of areas targeted by the therapy (Bolzoni, et al., 2013; Islam, et al., 1995; Nitsche & Paulus, 2000; Nitsche & Paulus, 2001; Stefan et al., 2000 & Wolters, et al., 2003). Taking this into consideration, the use of tDCS in improving the function of mice in performing a series of pre-determined tasks was supported.

This experiment's aim was to test the effect of tDCS on recovery of skilled locomotion, recovery of balance, and recovery of grip strength after brain injury in mice. A group of mice received a craniotomy for this experiment, and were then divided between a control group, which received sham treatments, and an experimental group. The craniotomy control group involved twenty-minute sessions for four consecutive days starting two weeks post-injury. The hypothesis of this study is that applying tDCS would improve recover of balance, skilled locomotion, and grip strength after bilateral motor cortex injury in mice. Following craniotomy and the application of tDCS, the recovery of motor skills was tested to prove this hypothesis. Tests employed included the ladder test, grip test, and a balance pole test. The distance traveled on the ladder, number of falls experienced, ability to maintain balance, and forelimb placement on the ladder were all measured.

Methods

Anesthesia

The mice were anesthetized with a 100mg/kg Ketamine and 10mg/kg Xylazine solution. The dose adjusted as needed. The injury site was disinfected with an iodine solution.

Craniotomy

Two small craniotomies were performed over the sensorimotor cortices. We induced bilateral electro-lesions using monopolar DC stimulation. A needle electrode was inserted into the sensorimotor cortex at two depths: 0.5 mm and then at 2 mm. Current was turned on for 20 seconds at each depth. Bone wax was used to close the craniotomies. The animals were then placed over a heat pad.

Electrode Hub Implantation

2-4 days post craniotomy, a hole was drilled and a small steel rod was placed traversing through the cranium. The hub of a needle from a syringe was cut and attached with dental cement to surround the implanted rod, and act as the docking site for the active electrode during stimulation. The mice were placed on a heating pad post implantation.

tDCS

tDCS was delivered using the ActivaDose iontophoresis delivery unit, distributed by ActivaTek Inc. The active electrode was screwed on to the 0.9% NaCl solution filled hub on the mouse head. The reference electrode was firmly attached, but not impeding circulation, to a 0.9% NaCl solution soaked cloth wrapped around the proximal tail of the mouse.The experimental group received 20 minutes of stimulation at 0.3mA for 4 consecutive days, starting 2 weeks post injury. The same procedure was followed for the control group, except the unit was never turned on, and no stimulation was administered

Image 1: tDCS application to CD-1 mouse.

The Pole Balance Experiment

For the balance pole test, mice were placed on a 80 cm long pole, with a 1cm diameter. The pole was marked with red tape every 10cm. The goal was to have the mouse cross the entire pole, reaching the 80 cm mark. Each mouse (4 stimulated, 3 sham) walked up the pole three times and down the pole three times for a total of 6 trials. The mice's velocity, the distance traveled in comparison to the length of the pole, and the number of falls was assessed.

The number of falls out of the total 6 trials for each mouse was calculated in order to determine each individual mouse's fall ratio (no. of falls/ total trials). Then, a total percentage of falls was calculated as a whole for the sham vs. the stimulated group in order to compare the two. A "fall" was defined by if the mouse did not reach the 80 cm mark and fell off the pole. "No fall" was defined if the mouse crossed 80 cm reaching the 80 cm mark without falling off the pole.

The aim of the speed (cm/sec) portion of the balance pole experiment was to assess the velocity the mouse traveled, which was calculated by the distance it took the mouse to travel (in cm) divided by the time it took the mouse to fall off the pole or reach the end. This was known as the speed index. An average speed index was calculated for the sham walking up the pole, the sham walking down the pole, the stimulated walking up the pole and the stimulated walking down the pole. A final average speed index was calculated comparing the sham vs. the stimulated group.

Lastly, the purpose of the distance (distance traveled/ total distance in cm) segment of the study was to find the distance each mouse traveled over the total 6 trials (up the pole 3 times and down the pole 3 times). The length of the pole was 80 cm. It was documented in each trial, how far the mouse traveled on the pole before falling off. The distance the mouse traveled in each trial was added together and combined to attain the total distance that he/she traveled. The total distance of the pole (80 cm x 6 trials= 480 cm) was 480 cm, and the total distance that the mouse traveled was divided by the total distance of the pole (480 cm). This gave a ratio or percentage that the mouse traveled in comparison to the total distance of the pole.

The Grip Strength Experiment

A device consisting of a wool wire ball attached to a transducer by a string was created in our lab to test grip strength. Mice were tested on the device prior to injury to establish a baseline, two times after injury and before stimulation, and three times after injury and stimulation. For the test, each mouse was held by its mid/base of the tail and lowered to allow it to grasp the wire ball. The wire ball was stabilized by a piece of paper held underneath it to allow the mice to get a firm grip. Once the mouse had a grip on the wire ball, it was pulled

upwards by an examiner until it released the wire ball. In order to control for the direction and force of the examiner's pull on the mouse's tail, the examiner's elbow was stabilized on a Styrofoam box in front of the grip strength measuring device.

8 trials were conducted for each mouse. In order to reduce error, trials in which the mouse grasped the string attached to the wire were eliminated. Additionally, using Labchart Software, the trials were analyzed to find the four top grip forces generated for each mouse for further analysis. Only the top four grip forces were chosen to eliminate the error for when the mouse did not have a firm grip on the wire ball device or when the examiner pulled the mouse with too much force. One examiner was used for all trials in order to eliminate another potential source of error.

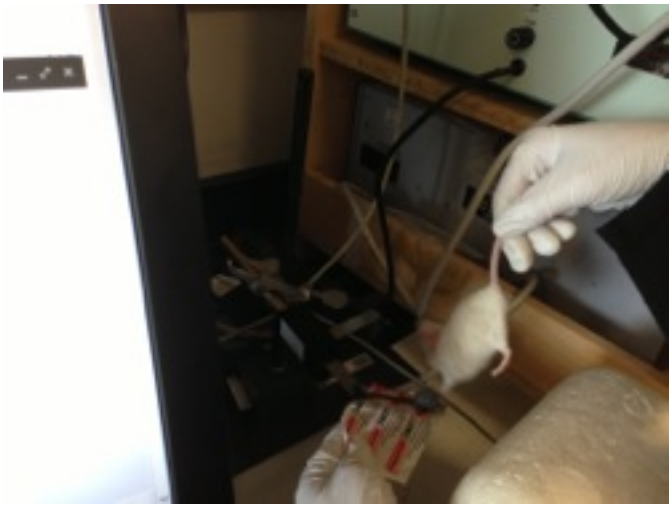

Image 2: The grip strength experiment.

The Ladder Experiment

The horizontal ladder was made of two pieces of plexiglass which stood on small wooden blocks, and were attached with metal bars that served as the steps of the ladder. The ladder was 117cm long, with holes drilled horizontally across every 2.5 cm. The metal bars were spread

across randomly, never skipping more than one hole, with a total of 32 bars. One month post injury, the mice were tested on the ladder. The mice were placed at one end, and had to traverse the length of the horizontal ladder and back while being recorded. The recordings were analyzed at 10-20% speed for front extremity placement onto the bars. The possible outcomes were divided into 3 categories; grab, place, and miss.

Image 3: The ladder test.

	Action Performed
Grab	wrap digit(s) around the bar
Place	did not wrap digit(s) around the bar/tapped bar before grab
Miss	paw fell below horizontal line of the bars

Table 1: The ladder test forelimb placement guidelines.

Results

The Pole Balance Experiment

Fall (no. of falls/ total trials)

For the sham group there was a total of 18 falls out of 18 trials (100 %) for the combined three sham mice. For the stimulated group there was a total of 5 falls out of 24 trials (42%) for the combined four mice. The results show that the stimulated group's number of falls was significantly less than that of the sham group.

SHAM	STIMULATED
MOUSE 2: 6 OUT OF 6	MOUSE 1: 2 OUT OF 6
MOUSE 6: 6 OUT OF 6	MOUSE 3: 0 OUT OF 6
MOUSE 7: 6 OUT OF 6	MOUSE 4: 3 OUT OF 6
	MOUSE 5: 0 OUT OF 6
PERCENTAGE: 100 %	**PERCENTAGE:** 42%

Table 2: Balance pole falls sham and stimulated.

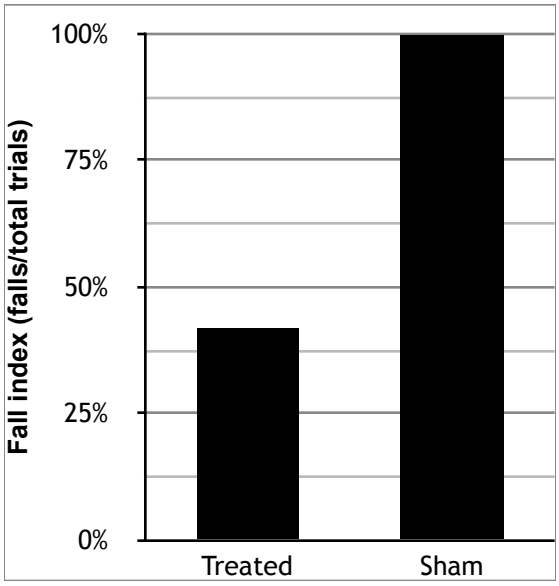

Chart 1: Balance pole falls sham and stimulated.

Distance (distance traveled/ total distance in cm)

The results indicate that the stimulated mice traveled 94% of the total pole length, whereas the sham group only traveled 28% of the total pole length.

SHAM	STIMULATED
MOUSE 2: 80 OVER 480	MOUSE 1: 440 OVER 480
MOUSE 6: 220 OVER 480	MOUSE 3: 480 OVER 480
MOUSE 7: 110 OVER 480	MOUSE 4: 400 OVER 480
	MOUSE 5: 480 OVER 480
Percentage: 28%	**Percentage:** 94%

Table 3: Balance pole experiment distance traveled, sham and stimulated.

Speed

The results indicate that the sham group's speed was significantly less than the stimulated group.

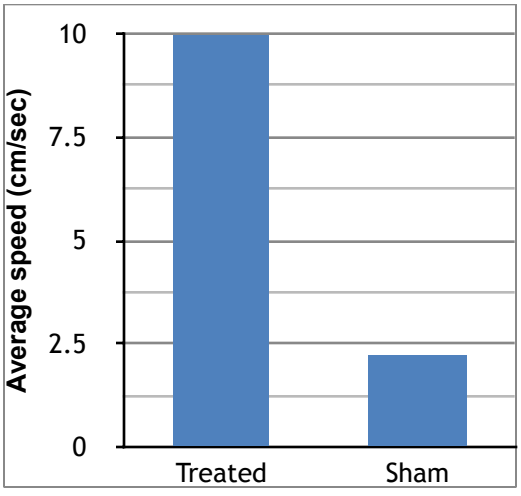

Chart 2: Balance pole experiment average speed, sham and stimulated.

SHAM (cm/sec)	STIMULATED (cm/sec)
1.529556	9.9338473
2.947231	14.811694
2.1994363	5.8390783
	9.2480942
AVG: 2.2254078	**AVG:** 9.9581789

FINAL AVG SPEED INDEX -STIMULATED: 9.9581789 cm/sec

FINAL AVG SPEED INDEX -SHAM: 2.2254078 cm/sec

Table 4: Balance pole experiment average speed, sham and stimulated.

The Grip Strength Experiment

The four mice treated with stimulation (1, 3, 4, and 5), all increased their grip strength on average from 6.36 to 20.75 after treatment as compared to their strength pre-treatment. Two of the sham mice (6 and 7) on average decreased their grip strength, -2.58 and -1.84 respectively, two weeks post injury with no treatment as compared to their strength immediately following injury. One sham mouse (2) showed a minimal improvement of grip strength with no treatment of 4.15.

The grip strength results of the treated and sham mice are shown on the graph below at baseline (before injury), after injury (pre-treatment), and post treatment or sham. The star on the graph at one week post treatment indicates that there is a significant difference in grip strength

between the mice treated by stimulation and the mice treated by the sham.

			Treated			Sham		
Before injury	9/16/2013	Not tested	91.00	81.02	65.44	87.33	93.92	84.44
After injury I	9/21/2013	57.58	67.91	67.84	57.02	65.52	75.62	70.99
After injury II	9/23/2013	53.97	55.78	65.89	44.63	47.72	89.60	68.34
AVG After Injury		55.77	61.84	66.87	50.83	56.62	82.61	69.67
After stim I	9/29/2013							
After stim II	10/5/2013	79.51	76.79	75.26	71.07	58.30	84.77	74.47
After stim III	10/12/2013	72.42	79.73	71.19	72.07	49.77	76.77	73.16
AVG After Stim		75.96	78.26	73.23	71.57	54.04	80.77	73.82
Difference		20.19	16.42	6.36	20.75	-2.58	-1.84	4.15

Table 5: Grip strength test results, sham and stimulated.

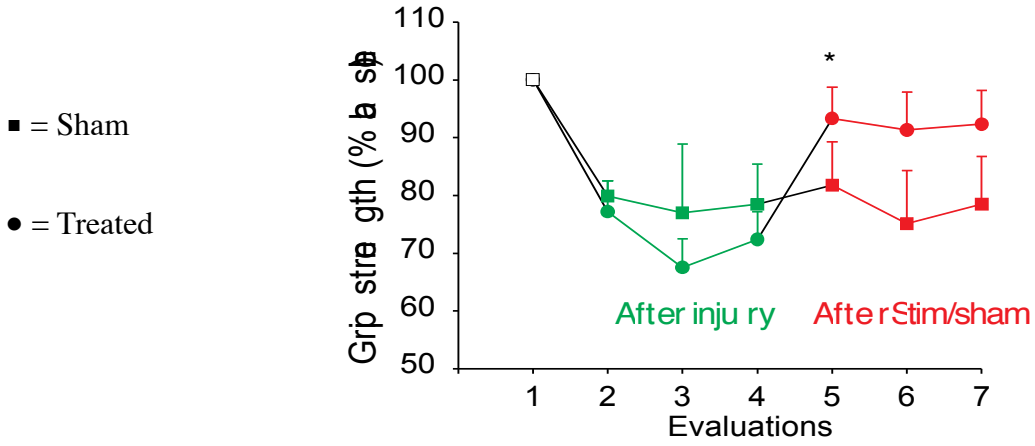

Figure 1: Effects of dc stimulation on grip strength.

The Ladder Experiment

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups for grab (P = 0.071). The difference in the mean values of the two groups is greater than would be expected by chance, and there is a statistically

significant difference between the input groups for placement (P = 0.024). The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.434).

mouse		grab	place	miss	total
1	L	22	6	0	28
	R	18	5	1	24
3	L	22	5	0	27
	R	24	4	1	29
4	L	19	9	1	29
	R	22	7	1	30
5	L	22	6	1	29
	R	19	6	0	25

Table 6: Ladder test forelimb position results for stimulated mice.

mouse		grab	place	miss	total
2	L	15	13	1	29
	R	15	11	1	27
6	L	19	8	2	29
	R	19	7	0	26
7	L	20	7	0	27
	R	19	9	0	28
1r	L	20	6	0	26
	R	20	7	0	27
2r	L	20	5	0	25
	R	20	8	0	28

Table 7: Ladder test forelimb position results for sham mice.

place**:**

t-test

Group Name	N	Missing	Mean	Std Dev	SEM
Stimulated	8	0	0.217	0.0491	0.0174
Sham	10	0	0.296	0.0771	0.0244

t = -2.503 with 16 degrees of freedom. (P = 0.024)

grab**:**

t-test

Group Name	N	Missing	Mean	Std Dev	SEM
Stimulated	8	0	0.761	0.0538	0.0190
sham	10	0	0.690	0.0913	0.0289

t = 1.934 with 16 degrees of freedom. (P = 0.071)

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
Stimulated	8	0	0.976	0.966	1.000
sham	10	0	1.000	0.966	1.000

Mann-Whitney U Statistic= 31.500

T = 67.500 n(small)= 8 n(big)= 10 (P = 0.434)

Table 8: Statistical analysis of ladder test forelimb position sham and stimulated.

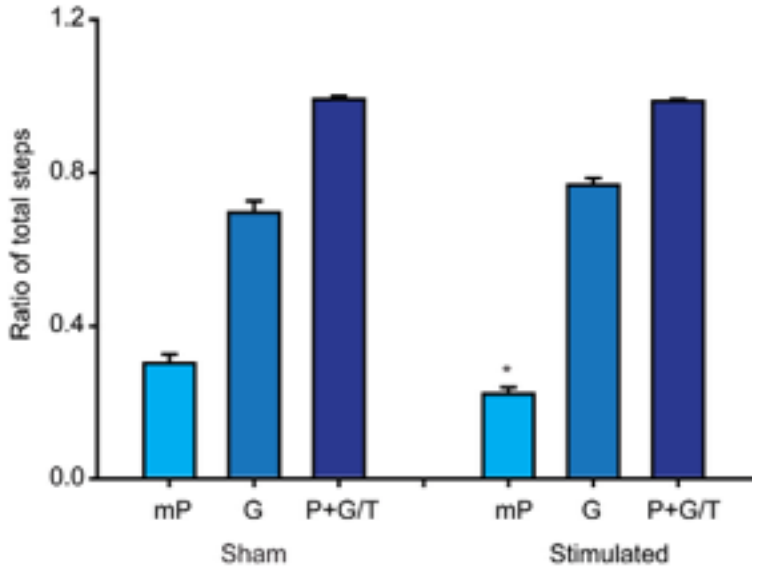

Chart 3: Statistical analysis of ladder test forelimb position sham and stimulated.

Discussion

tDCS has been shown to induce changes in cortical excitability and lead to plastic changes. While our experiment cannot determine exactly what changes took place, we were able to demonstrate that change did occur. tDCS treated CD-1 mice demonstrated significantly improved performance on the pole and grip experiments.

The ladder experiment demonstrated a significant decrease in the amount "placement" steps, however no significance was found between the two groups with the correct "grab" stepping. This may be due to the parameters set on how step category was determined. More precise parameters, with more categories for types of step, may yield different results. We can also see that there was more correct forelimb stepping (grab) in the stimulated group, however the results were not significant which is most likely due to the small sample size. A baseline measurement should also have been taken for the ladder experiments, and is one of the limitations of this study.

Further research can help us determine which parameters of this methodology would produce the most significant results. We would be interested in knowing how much stimulation, and for what period of time, would produce the most gains in our subjects. We would further like to know if these advances in treated subjects are permanent, or if the the sham groups would eventually reach the same level of abilities.

References

Dockery, C. A., Liebetanz, D., Birbaumer, N., Malinowska, M., & Wesierska, M. J. (2011). Cumulative benefits of frontal transcranial direct current stimulation on visuospatial working memory training and skill learning in rats. Neurobiology of learning and memory, 96(3), 452-460.

Faraji, J., Gomez-Palacio-Schjetnan, A., Luczak, A., & Metz, G. A. (2013). Beyond the silence: Bilateral somatosensory stimulation enhances skilled movement quality and neural density in intact behaving rats. Behavioural brain research, 253, 78-89.

Islam N, Aftabuddin M, Moriwaki A, Hattori Y & Hori Y (1995). Increase in the calcium level following anodal polarization in the rat brain. Brain Res 684, 206–208.

Kamida, T., Kong, S., Eshima, N., & Fujiki, M. (2013). Cathodal transcranial direct current stimulation affects seizures and cognition in fully amygdala-kindled rats. Neurological research.

Li, Y., Tian, X., Qian, L., Yu, X., & Jiang, W. (2011, August). Anodal transcranial direct current stimulation relieves the unilateral bias of a rat model of Parkinson's disease. In Engineering in Medicine and Biology Society, EMBC, 2011 Annual International Conference of the IEEE (pp. 765-768). IEEE.

Liebetanz, D., Klinker, F., Hering, D., Koch, R., Nitsche, M. A., Potschka, H., Löscher, Paulus & Tergau, F. (2006). Anticonvulsant Effects of Transcranial Direct−current Stimulation (tDCS) in the Rat Cortical Ramp Model of Focal Epilepsy.Epilepsia, 47(7), 1216-1224.

Márquez-Ruiz, J., Leal-Campanario, R., Sánchez-Campusano, R., Molaee-Ardekani, B., Wendling, F., Miranda, P. C., Ruffini, G., Gruart, A. & Delgado-García, J. M. (2012). Transcranial direct-current stimulation modulates synaptic mechanisms involved in

associative learning in behaving rabbits. Proceedings of the National Academy of Sciences, 109(17), 6710-6715.

Nitsche M & Paulus W (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. JPhysiol 527 , 633–639.

Nitsche M & Paulus W (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57, 1899–1901.

Spezia-Adachi, L. N., Caumo, W., Laste, G., Fernandes-Medeiros, L., Ripoll-Rozisky, J., de Souza, A., Fregni, F. & Torres, I. L. (2012). Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. Brain research.

Stefan K, Kunesch E, Cohen LG, Benecke R & Classen J (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123, 572–584.

Tanaka, T., Takano, Y., Tanaka, S., Hironaka, N., Kobayashi, K., Hanakawa, T., Watanabe, K. & Honda, M. (2013). Transcranial direct-current stimulation increases extracellular dopamine levels in the rat striatum. Frontiers in systems neuroscience, 7.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E,Stefan K, Cohen L, Benecke R & Classen J (2003). A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. J Neurophysiol 89, 2339–2345