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Non-Invasive tDCS of the Dorsolateral Prefrontal Cortex Increased the Release of Brain Neurotransmitters and Enzymes

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ABSTRACT

Transcranial direct current stimulation (tDCS) is capable of modulating brain activity significantly and it has emerged as a promising treatment for schizophrenia and pain, and also to inhibit cravings. However, there is a rapid increase on the use of this neuro-stimulation method to enhance cognitive ability in healthy people. The present study investigated the release of neurotransmitters such as dopamine, acetylcholine and serotonin, and enzymes like cytochrome C-oxidase and glucose-6-phosphate dehydrogenase after tDCS on the dorsolateral prefrontal cortex. Thirty two adult male Wistar rats were used for this study. The rats were divided into six groups with each group having six rats with exception of the control and sham groups which had four rats each. Groups T5, T10, T15 and T20 were stimulated for 5, 10, 15 and 20 minutes daily with 2.3 mA consecutively for the period of fourteen days. The animals were sacrificed by cervical dislocation after the experiment and each animal's brain was carefully removed. The prefrontal cortex was dissected and homogenized for enzymes and neurotransmitters assay. The results showed significant increase in the levels of both the brain neurotransmitters and enzymes (except in cytochrome C-oxidase) in the treated groups compared to the control which was duration dependent. This suggests that tDCS does not have any side effect on the activities of brain neurotransmitters and enzymes but could be effective in the treatment of different brain disorders that is due to the decreased levels of brain neurotransmitters and enzymes.

Key words: *tDCS, Prefrontal Cortex, Cerebrum, Stimulator, Neurotransmitters*

INTRODUCTION

Transcranial direct current stimulation (tDCS) is a non-invasive, painless neuro-stimulation treatment that involves the use of constant but yet controlled current delivered to the brain area of interest through the use of electrode to the scalp or forehead. tDCS is a technique that is capable of modulating brain activity significantly and as shown promising effects in the treatment of various diseases such as schizophrenia, neuropathic pain, and also inhibit food cravings (Lefaucher et al. 2017). It was developed to help patients with different type of neurodegenerative diseases which include depression, Parkinson's and Alzheimer's and but not limited to pain treatments. Transcranial direct current stimulation is an emerging non-invasive technique of neurostimulation for treating pain (Dimov et al. 2016). However, there is the rapid and ongoing use of this neuro-stimulation

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method to enhance cognitive ability in healthy people. Experiments done using the tDCS have actually proven that it can make the brain function better. A research carried out at Air Force Research Laboratory provide evidence that tDCS has the ability to augment and enhance multitasking capability in a human operator (Nelson et al. 2016). It was also observed that depressive symptoms respond to tDCS in patients with bipolar disorder (Donde et al. 2017). The use of tDCS is on the rise as it can be applied in the post-operation treatment of neuropathic pain after spinal cord injury (Soler et al. 2010), which makes it essential to explore the importance of the repeated use of tDCS.

The dorsolateral cortex is also known as the dorsolateral prefrontal cortex (DLPFC). It is the area of the prefrontal cortex of the brain of humans and non-human primates which continues developments till adulthood (Olson and Luciana 2008). The dorsolateral cortex lies in the middle frontal gyrus in humans (lateral portion of the Brodman's areas 9 and 46) (Cieslik 2013). The dorsolateral cortex is the end point for the dorsal pathway. The dorsolateral cortex is connected to the orbitofrontal cortex and to a variety of brain areas which include the dorsal caudate nucleus of the basal ganglia, the thalamus, hippocampus, posterior temporal, parietal and occipital areas. Therefore the connections allow the dorsolateral prefrontal cortex to regulate activities of the regions (Collins and Nelson 2001), such as cognitive functions, working memory and planning which can all be called executive functions (Monsell, 2003). Previous researches show the effect of tDCS applied over the DLPFC in the enhancement of working memory (Fregni et al. 2005). Findings show that anodal tDCS over the right DLPFC may prevent working memory impairment induced by acute stress (Bentivoglio et al. 2012).

Stimulation of the primary motor cortex and dorsolateral prefrontal cortex (DLPFC) reduce the perception of pain. Researches show that anodal tDCS of the DLPFC could decrease the perception of unpleasantness and reduces emotional discomfort or pain. Findings also show that tDCS involve a cascade of events at the cellular and molecular levels and is associated with glutamergic, GABAergic, dopaminergic, serotonin and cholinergic activity modulation (Brunoni et al. 2012).

Another evidence suggests that tDCS interact with various cerebral neurotransmitter systems and is mediated by dopamine, acetylcholine, serotonin or GABA (Brunoni et al. 2012). This study was aimed at investigating if tDCS which is being used in the treatment of neurodegenerative disorders of the brain could have any possible side effects on the activities and the release of neurotransmitters of the dorsoteral prefrontal cortex.

MATERIALS AND METHODS

A total of thirty two adult male Wistar rats (*Rattus norvegicus*) weighing between 120-170 g were used for this experiment. The rats were assigned into 6 groups with 6 animals in all the groups except the control and sham groups which had 4 rats each. Animal were housed in clean plastic cages which was laid with sawdust under natural light and at room temperature. Animals in all groups were fed normal laboratory rat chow and had easy access to water *ad libitium*. The groups (Table 1) were labeled as Control, Sham, T5, T10, T15 and T20. Groups T5, T10, T15, T20 were electrically stimulated using the same volts but with duration of 5, 10, 15 and 20 minutes respectively.

The transcranial direction current stimulation device

Table 1: Experimental Animal Grouping and Treatment

Groups	Treatment
Control	Control group which was not given any stimulation
Sham	Control group was given a sham (stimulation for 30 seconds) for 14 days
T5	Stimulation of the dorsolateral cortex with 2.3 mA for 5 minutes was done daily for 14 days
T10	Stimulation of the dorsolateral cortex with 2.3 mA for 10 minutes was daily for 14 days
T15	Stimulation of the dorsolateral cortex with 2.3 mA for 15 minutes was done daily for 14 days
T20	Stimulation of the dorsolateral cortex with 2.3 mA for 20 minutes was done daily for 14 days

T5 (were stimulated for 5 minutes), T10 (were stimulated for 10 minutes), T15 (were stimulated for 15 minutes), T20 (were stimulated for 20 minutes), mA: Milliampere

used in the experiment was the Brain Stimulator tDCS travel model V2.0, which was ordered from the Brain Stimulator Company, based in California, United States of America. The electrodes were adjusted to fit the rat model.

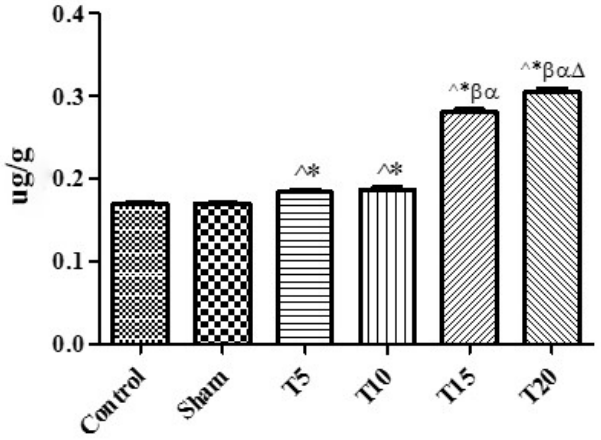

Figure 1: Acetylcholine levels (µg/g) of the groups after tDCS for 14 days. Values are mean ± SEM of Mean; *=significantly different from Control; β=significantly different from T5; α=significant different for T10; Δ=significantly different from T15; p values (p<0.05). Sham (tDCS for 30 seconds), Control (no tDCS), T5 (tDCS for 5 minutes), T10 (tDCS for 10 minutes), T15 (tDCS for 15 minutes), T20 (tDCS for 20 minutes).

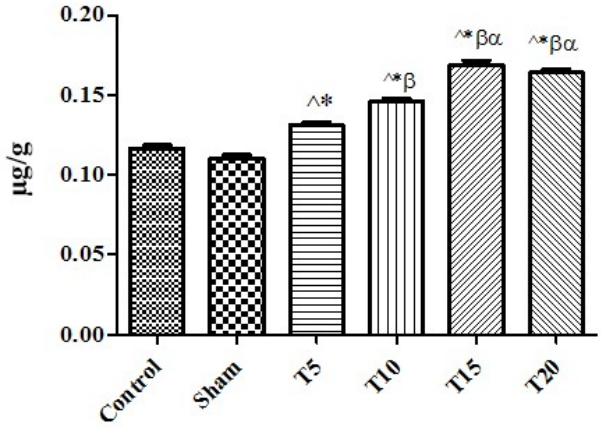

Figure 2: Dopamine levels (µg/g) of the groups after tDCS for 14 days. Values are mean ± SEM of Mean; *=significantly different from Control; β=significantly different from T5; α =significant different for T10; p values (p < 0.05). Sham (tDCS for 30 seconds), Control (no tDCS), T5 (tDCS for 5 minutes), T10 (tDCS for 10 minutes), T15 (tDCS for 15 minutes), T20 (tDCS for 20 minutes)

The fur medial to the ears of the experimental animals was shaved to ensure the electrode-scalp contact. The brain was stimulated using the Brain Stimulator; the electrodes were placed on both sides, medial to the ears and the electrodes were held in place with tape. The voltage and time span used were selected based on a research carried out on the reversal of chronic stress-induced pain by transcranial direct current stimulation on animal model (Adachi et al. 2012).

On the 14th day, two hours after stimulation the experimental animals were sacrificed by cervical dislocation. With the fur around the head shaved as a result of the experiment the skin was then flapped back carefully, a pair of scissors was then carefully used to slide along the inner surface of the skull, this was done to prevent damage to the brain. A rongeur was then used to peel the skull away from the brain. A spatula was then used to severe olfactory bulbs and nerve connections at the anterior and underside location of the brain. Then the brain was carefully removed and placed on a sterile board and then weighed. The section of the cerebral cortex was cut and placed in a plain bottle containing phosphate buffer and was later homogenized for enzymes and neurotransmitters assay. The neurotransmitters and enzymes assayed for in this study were dopamine, acetylcholine, serotonin, cytochrome-C- oxidase and glucose-6-phosphate. Tissues were excised and placed in 30g/dm^3 sucrose and then homogenized at a low temperature. Colorimetric analysis method was used for the assay. Data were analyzed using descriptive and inferential statistics. The results were presented as mean ± SEM. P-value < 0.05 was considered significant.

Ethical Clearance

The research study protocol was approved by Babcock University Health Research Ethical Committee (BUHREC) and the ethical clearance was given along with an assigned BUHREC no: 651/16. Also, the animal experiments conform to BUHREC standards.

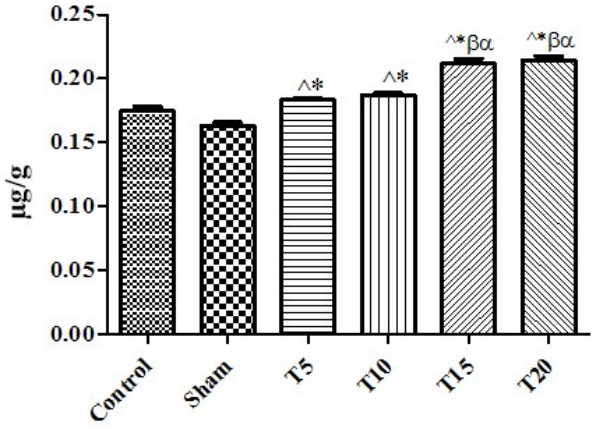

Figure 3: Serotonin levels (µg/g) of the groups after tDCS for 14 days. Values are mean ± SEM of Mean; *=significantly different from Control; β=significantly different from T5; α=significant different for T10; p values (p < 0.05). Sham (tDCS for 30 seconds), Control (no tDCS), T5 (tDCS for 5 minutes), T10 (tDCS for 10 minutes), T15 (tDCS for 15 minutes), T20 (tDCS for 20 minutes).

RESULTS

Acetylcholine

There was significant ($p < 0.05$) increase in the acetycholine (Figure 1) between groups sham (0.1697 ± 0.00), control (0.1697 ± 0.00) and other treated groups T5, T10, T15 and T20. There was significant ($p < 0.05$) increase in the acetylcholine level in groups T15 (0.2808 ± 0.00) and T20 (0.3045 ± 0.00) compared to groups T5 (0.1835 ± 0.00) and T10 (0.1867 ± 0.00). Acetylcholine level in group T20 was significantly higher than T15.

Dopamine

The dopamine level (Figure 2) in Sham (0.1103 ± 0.00) was significantly ($p < 0.05$) lower compared to other treated groups and the Control (0.1167 ± 0.00). The dopamine level was significantly ($p < 0.05$) higher in groups T5 (0.1310 ± 0.00), T10 (0.1460 ± 0.00), T15 (0.1688 ± 0.00) and T20 (0.1640 ± 0.00) compared to group Sham. The dopamine level was significantly ($p < 0.05$) higher in groups T10, T15 and T20 compared to group T5, and also the dopamine level was significantly ($p < 0.05$) higher in groups T15 and T20 when compared to group T10.

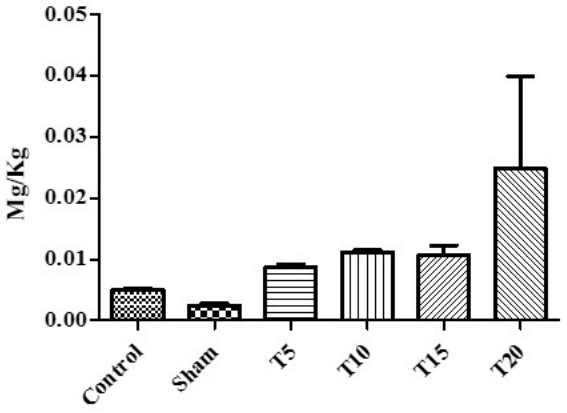

Figure 4: Cytochrome-C-Oxidase level (mg/kg) of the groups after tDCS for 14 days. Values are mean ± SEM of Mean; p values ($p<0.05$). Sham (tDCS for 30 seconds), Control (no tDCS), T5 (tDCS for 5 minutes), T10 (tDCS for 10 minutes), T15 (tDCS for 15 minutes), T20 (tDCS for 20 minutes).

Serotonin

The serotonin level (Figure 3) in Sham (0.1632 ± 0.00) was significantly ($p < 0.05$) lower compared to other treated groups and the Control (0.1747 ± 0.00). The serotonin level was significantly ($p < 0.05$) higher in groups T5 (0.1832 ± 0.00), T10 (0.1867 ± 0.00), T15 (0.2118 ± 0.00) and T20 (0.2142 ± 0.00) compared to the control group. The dopamine level was significantly ($p < 0.05$) higher in groups T10, T15 and T20 compared to group T5. The dopamine level was also significantly ($p < 0.05$) higher in groups T15 and T20 compared to T10.

Cytochrome-C-Oxidase

There was no significant difference across all groups (Figure 4). However, there was observable increase in group T5 (0.008667 ± 0.00), T10 (0.01117 ± 0.00), T15 (0.01067 ± 0.00), T20 (0.02483 ± 0.01) was lower compared to the Sham (0.025 ± 0.00). The cytochrome-C-oxidase level in control group (0.050 ± 0.00) was higher when compared to the Sham. There was a slight increase in T20 compared with the control and the rest of the treated group.

Glucose-6-Phosphate Dehydrogenase

The G6PDH level (Figure 5) in Sham (0.01183 ± 0.00) was significantly ($p < 0.05$) lower compared to the treated groups T5 (0.01983 ± 0.00), T10 (0.0185 ± 0.00), T15 (0.02317 ± 0.00) and T20 (0.0180 ± 0.00). The G6PDH level in the control group was significantly ($p < 0.05$) lower than groups T5 and T15. There is no significance ($p < 0.05$) difference between Sham and the control group (0.01367 ± 0.00)

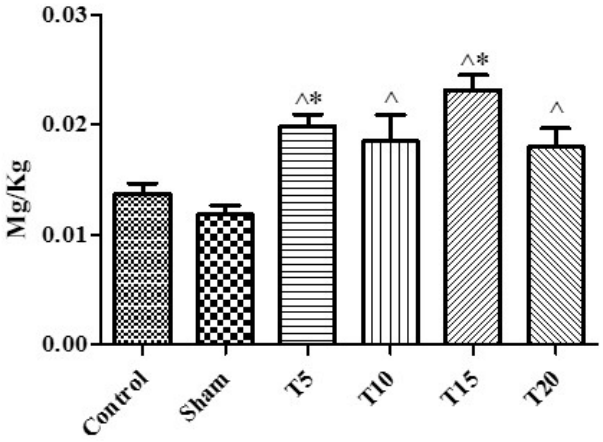

Figure 5: Glucose 6 phosphate levels (mg/kg) of the groups after tDCS for 14 days. Values are mean ± SEM of Mean; ^=significantly different from Sham; *=significantly different from the control; p values ($p<0.05$). Sham (tDCS for 30 seconds), Control (no tDCS), T5 (tDCS for 5 minutes), T10 (tDCS for 10minutes), T15 (tDCS for 15 minutes), T20 (tDCS for 20 minutes).

DISCUSSION

Transcranial direct current stimulation of the DLPFC results in a shift in the excitability which occur during and after stimulation (Nitsche et al. 2008). Research in enhancing working memory is definitely one of the major things that can be done with the use of the tDCS. Anodal tDCS applied on the DLPFC facilitates working memory processes (Fregni et al. 2005). Findings show that anodal tDCS over the right DLPFC may prevent working memory impairment induced by acute stress (Bogdanov et al 2017). Stimulation of the primary motor cortex and

dorsolateral prefrontal cortex (DLPFC) reduces the perception of pain. Researches show that anodal tDCS of the DLPFC could decrease the perception of unpleasantness and reduces emotional discomfort or pain. This study was aimed at investigating the possible side effects of tDCS on the activities and the release of neurotransmitters of the dorsolateral prefrontal cortex.

Acetylcholine is a neurotransmitter produced in acetylcholinergic neurons to send signals to other cells. Acetylcholine has functions in both peripheral and central nervous systems, where it plays important roles in skeletal muscle movement, regulation of smooth and cardiac muscles, as well as in arousal, attention and cognitive processes (Kuo et al. 2008). Researches show that the neurotransmitter acetylcholine is essential for working memory function of the DLPFC (Yang et al. 2013). In the cerebral cortex of the brain, acetylcholine may work to indirectly inhibit or stimulate certain mental states. Acetylcholine plays an important role in the mood regulation and there are ongoing researches on the relationship between acetylcholine and depression. Deficiency in acetylcholine can lead to excessive fatigue, severe anxiety, memory loss and mood swings (Sparing et al. 2008). The level of acetylcholine was significantly higher in the treated groups compared to the Sham group and Control at the end of the stimulation period. The particular result supports the use of tDCS in the treatment of acetylcholine deficiency which indirectly leads to the treatment and control of fatigue, anxiety, memory loss and mood swings. There is also the possibility of the treatment of the memory loss symptoms of Alzheimer's disease using tDCS, as acetylcholine level decrease is one of the causes of memory loss in Alzheimer's disease (Kihara and Shimohama 2004; Yu et al. 2014).

Dopamine is a neurotransmitter that helps control the brain's reward and pleasure centres. The symptoms of low dopamine levels can include insomnia, memory loss and depression (Brown and Gershon 1993). The deficiency of dopamine is seen in Parkinson's disease (Gruner et al. 2010), attention deficit hyperactivity disorder (Volkow et al. 2009) and chronic neuropathic pain (Wood 2008). People with low dopamine activity may be prone to addiction as addictive drugs increase dopamine neuronal activity. The level of dopamine was significant higher in the treated groups compared to the Sham and Control, and this is in accordance with Tanaka et al. (2013) that tDCS has a direct and/or indirect effect on the dopaminergic system in the rat basal ganglia. There is therefore the possibility of the use of tDCS in the treatment of Parkinson's, pain and attention deficit hyperactivity disorder.

Serotonin is a monoamine neurotransmitter, and it is found in the gastrointestinal tract, blood platelets and in the central nervous system (Young 2007). Serotonin performs a number of functions in the body, which include mood regulation, social behaviour, sleep, memory, learning and appetite (Di-Giovanni et al. 2008). Low levels of serotonin can lead to depression, migraine, poor cognitive function, insomnia and digestive disorders among others. The level of serotonin was significantly higher in the treated groups compared to the control group, and this is in accordance with (Nitsche et al. 2009; Kuo et al. 2008) that tDCS can aid in the treatment of diseases such as depression and other neuropsychiatric diseases. Serotonergic reinforcement may enhance facilitatory effects and thereby increase the efficacy of these tools.

Cytochrome-C-oxidase is the terminal oxidase of the mitochondrial electron transport chain (Srinivasan and Narayan 2012). The alteration of the level of cytochrome-C-oxidase is usually observed in genetic defects (Zee and Glerum 2006). Cytochrome-C-oxidase dysfunction is also related to oxidative stress. There was no significant difference across all groups but there was an observable increase in the treated groups when compared to Sham. There is the possibility of the use of tDCS in the raising of the cytochrome-C-oxidase level.

Glucose-6-phosphate dehydrogenase (G-6-PDH) is a cytosolic enzyme that catalyzes the chemical reaction. G-6-PDH deficiency is a genetic disorder that occurs most often in males and the most common medical problem associated with G-6-PDH deficiency is haemolytic anaemia, which occurs when red blood cells are destroyed faster than the body can replace them (Cappellini and Fiorelli 2008). There was a significant increase in the level of G-6-PDH in the treated groups compared to the Sham and Control groups. There was no significant difference in the level of G-6-PDH in groups sham and control. There is a relationship between G-6-PDH deficiency and Alzheimer's disease (Ulusu 2015), therefore there is the possibility of the treatment of Alzheimer's and G-6-PDH deficiency using anodal tDCS.

The present study showed the varying effects of brain stimulation on the activities of both the enzymes and neurotransmitters of the brain and it was observed that the more the duration of stimulation the higher the activities of the enzymes and neurotransmitters. These indicate that the stimulation of the brain is more promising in disorders that are caused as a result of deficiencies in brain neurotransmitters and enzymes.

Conclusion

In conclusion, this present study showed tDCS duration of 10, 15 and 20 minutes had significant differences compared to the control group, which infers the duration dependent action, and that tDCS could be effective in the treatment of different disorders such as Parkinson's, Alzheimer's, epilepsy, anxiety, G-6-PDH deficiency and depression among others.

Conflict of Interest
None declared.

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None.

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REFERENCES

Adachi, L. N. S., Caumo, W., Laste, G., Medeiros, L. F., Rozisky, J. R., De Souza, A., Fregni, F. and Torres, L. S. (2012) Reversal of chronic stress-induced pain by transcranial direct current stimulation (tDCS) in an animal model. Brain Research. 1489:17-26. Bentivoglio, A. R., Fasano, A., Piano, C., Soleti, F., Daniele, A., Zinno, M., Piccininni, C., De Simone, C., Policicchio, D., Tufo, T., Meglio, M. and Cioni, B. (2012) Unilateral extradural motor cortex stimulation is safe and improves Parkinson disease at 1 year. Neurosurgery. 71(4):815-825.

Bogdanov, M., Ruff, C. C. and Schwabe, L. (2017) Transcranial stimulation over the dorsolateral prefrontal cortex increases the impact of past expenses on decision-making. Cerebral Cortex. 27(2):1094-1102

Brown, A. S. and Gershon, S. (1993) Dopamine and depression. Journal of Neural Transmission General Seciont. 91(2-3):75-109.

Brunoni, A. R., Nitsche, M. A. and Bolognini, N. (2012) Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. Brain Stimulation. 5:175-195

Cappellini, M. D. and Fiorelli, G. (2008) Glucose 6 phosphate dehydrogenase deficiency. Lancet. 371 (9606):64-74

Cieslik, E. (2013) Is there “one” DLPFC in cognitive action control? Evidence for heterogeneity from co-activation- based Parcellation. Cerebral Cortex. 23(11):2677-2689.

Collins, M. L. and Nelson, C. A. (2001) Handbook of Developmental Cognitive Neuroscience. (2nd ed.). Cambridge, Mass. [u.a.]: The MIT Press..

Di-Giovanni, G., Di-Matteo, V., Pierucci, M., and Esposito, E. (2008) Serotonin-dopamine interaction: electrophysiological evidence. Progress in Brain Research. 172:45-71.

Dimov, L. F., Franciosi, A. C., Campos, A. C. P., Brunoni, A. R. and Pagano, R. L. (2016) Top-down effect of direct current stimulation on the nociceptive response of rats. PloS ONE. 11(4).

Donde, C., Amad, A., Nieto, I., Brunoni, A. R., Neufeld, N. H., Bellivier, F., Poulet, E. and Geoffroy, P. A. (2017) Transcranial direct current stimulation (tDCS) for bipolar depression: a systematic review and meta-analysis. Progress in Neuropsychopharm-acology and Biological Psychiatry.1;78:123-131. Doi:10.1016/j.pnpbp.2017.0 5.021.

Fregni, F., Boggio, P. S., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., Marcolin, M. A., Rigonatti, S. P., Silva, M. T., Paulus, W. and Pascual-Leone, A. (2005) Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Experimental Brain Research. 166(1):23-30.

Gruner, U., Eggers, C., Ameli, M., Sarfeld, A. S., Fink, G. R. and Nowak, D. A. (2010) 1 Hz rTMS preconditioned by tDCS over the primary motor cortex in Parkinson’s disease: effects on bradykinesia of arm and hand. Journal of Neuro-Transmission. 117:207-216.

Kihara, T. and Shimohama, S. (2004) Alzheimer’s disease and acetylcholine receptors. Acta Neurobiologiae Experimentalis. 64(1):99-105.

Kuo, M. F., Paulus, W. and Nitsche, M. A. (2008) Boosting focally-induced plasticity by dopamine. Cerebral Cortex.18:648-651.

Lefaucher, J. P., Antal A., Ayache, S. S., Benninger, D. H., Brunelin, J., Cogiamanian F., et al. (2017) Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clinical Neurophysiology. 128(1):56-92. Doi:10.1016/j.clinph. 2016.10.087.

Monsell, S. (2003) Task switching. Trends in Cognitive Sciences. 7(3):134-140.

Nelson, J., Mckinley, R. A., Phillips, C., McIntire, L., Goodyear, C., Kreiner, A. and Monforton, L. (2016) The effects of transcranial direct current stimulation (tDCS) on multitasking throughput capacity. Frontiers in Human Neuroscience. 10:589. Doi:10.3389/fnhum. 2016.00589

Nitsche, M. A., L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., Paulus, W., Hummel, F., Boggio, P. S., Fregni, F., Pascual-Leone A. (2008) Transcranial direct current stimulation: state of the art 2008. Brain Stimulation. 1(3):206-223.

Nitsche, M. A., Kuo, M. F., Karrasch, R., Wachter, B. Liebetanz, D. and Paulus, W. (2009) Serotonin affects transcranial direct current-induced neuroplasticity in humans. Biological Psychiatry. 66: 503-508.

Olson, E. and Luciana, M. M. (2008) The development of prefrontal cortex functions in adolescence: theoretical models and a possible dissociation of dorsal versus ventral subregions. In Nelson, C. A. and Luciana M. M. (eds.), The Handbook of Developmental Cognitive Neuroscience (2nd ed.). London :MIT Press. Pp. 575-591.

Soler, M. D., Kumru, H., Pelayo, R., Vidal, J., Tormos, J. M., Fregni, F., Navarro, X., Pascual-Leone, A. (2010) Effectiveness of transcranial direct current stimulation and visual illusion on neuropathic pain in spinal cord injury. Brain. 133(9):2565-2577. Doi:10.1093/brain/awq184.

Sparing, R. and Mottaghy, F. M. (2008) Noninvasive brain stimulation with transcranial magnetic or direct

current stimulation (TMS/tDCS) from insights into human memory to therapy of its dysfunction. Methods. 44(4):329-337.

Srinivasan, S. and Narayan, G. A. (2012) Cytochrome C oxidase dysfunction in oxidative stress. Free Radical Biology and Medicine. 53:1252-1263.

Ulusu, N. N. (2015) Glucose-6-phosphate dehydrogenase deficiency and Alzheimer's disease: partners in crime? The hypothesis. Medical Hypothesis. 85:219-223.

Volkow, N. D., Wang, G. J., Kollins, S. H., Wigal, T. L., Newcorn, J. H., and Telang. F., (2009) Evaluating dopamine reward pathway in ADHD: Clinical implication. JAMA Neurology. 302:1084-1091

Wood, P. B. (2008). Role of central dopamine in pain and analgesia. Expert Review of Neurotherapeutics. 8(5):781-797.

Yang, Y., Paspalas, C. D., Jin, L. E, Picciotto, M. R. Arnsten, A. F. T. and Wang, M. (2013) Nicotonic a7 receptors enhance NMDA cognitive circuits in dorsolateral prefrontal cortex. Proceedings of National Academy of Sciences. 110(29):12078-12083.

Young, N. S. (2007) How to increase serotonin in the human brain without drugs. Journal of Psychiatry Neuroscience. 32(6):394-399.

Yu, S. H., Park, S. D., and Sim, K. C. (2014) The effects of tDCS on cognition and neurologic recovery of rats with Alzheimer's disease. Journal Physical Therapy Science. 26(2): 247-249.

Zee, J. M. and Glerum, D. M. (2006). Defects in cytochrome oxidase assembly in humans: lessons from yeast. Biochemistry and Cell Biology. 84(6): 859-869.