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Cumulative benefits of frontal transcranial direct current stimulation on visuospatial working memory training and skill learning in rats

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ABSTRACT

Transcranial direct current stimulation (tDCS) of the prefrontal cortex, which non-invasively alters cortical activity, has been established to affect executive functions in humans. We hypothesized that changes in excitability by tDCS, found to improve cognitive functions dependent on moderate prefrontal cortex activity, would operate similarly in animals as in humans. To verify this we performed experiments using a rat behavioral model of visuospatial working memory and skill learning paired with tDCS of the frontal cortex. The effect of anodal/cathodal tDCS was examined in three sessions using the allothetic place avoidance alternation task (APAAT) and later re-examined without stimulation. Stimulation had no measurable short term effect on on-going place avoidance learning. However, in the follow-up session on day 21 the rats previously treated with cathodal tDCS showed significantly more efficient place avoidance and skill retention in comparison to the controls. This demonstrates a long-term benefit of diminished excitability by frontal tDCS when paired with training on working memory and skill learning in a novel task. The presented behavioral model provides a tool to evaluate the underlying mechanisms of how tDCS modulates neural network function to support successful behavior.

1. Introduction

The importance of the prefrontal cortex (PFC) for encoding and retrieval of spatial working memory is well documented (Churchwell, Morris, Musso, & Kesner, 2010; Wang & Morris, 2010). Depending on phase and classification, in humans and rats memory requires a circuitry involving hippocampal and neocortical structures (Winocur, Moscovitch, & Bontempi, 2010). In rats, their disconnection has been shown to disrupt spatial working memory (Wang & Cai, 2006). Whereas their interaction supports dynamic use of spatial information for navigation and allows for optimization and switching of strategy according to the degree of demands and the context (Rich & Shapiro, 2009).

Working memory joins multiple cognitive functions for successful completion of daily activity in both humans and animals. It supports temporary storage of recent (short-term memory) and remote (long-term memory) information to allow for its manipulation in order to resolve a current task (Repovs & Baddeley, 2006). Impaired working memory due to chronic stress is mediated by altered dopaminergic activity in the PFC of rats (Mizoguchi et al., 2000). On the contrary, working memory improves with training which induces associated changes in brain activity patterns and dopamine receptor density in the prefrontal cortex of humans (Klingberg, 2010). These improvements are attributable to cognitive skill learning, which is also prefrontal dependent. This form of learning fosters performance increments through task repetition and development of task-related rules, procedures and strategies (Beauchamp, Dagher, Aston, & Doyon, 2003).

Transcranial direct current stimulation (tDCS) is a non-invasive tool that transiently alters neuronal activity of targeted brain regions with effects outlasting the period of stimulation (Nitsche et al., 2007). The physical parameters of tDCS such as current density, stimulation site, electrode size, duration of stimulation and current polarity have diverse effects (Nitsche et al., 2008). Many of the known neurophysiological and associated functional changes induced by tDCS are derived from the human primary motor cortex (M1) and are expressed by altered motor-evoked potentials (MEPs) and motor learning (Nitsche et al., 2007). In humans, the long lasting

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effects of tDCS on M1 (both anodal ($tDCS_a$) and cathodal ($tDCS_c$)) were suppressed after NMDA-receptor blockade indicating dependence of stimulation on glutamatergic activity (Liebetanz, Nitsche, Tergau, & Paulus, 2002). Skill learning in humans is strengthened by anodal tDCS of M1 paired with training (Galea & Celnik, 2009). Enhancement of motor skill acquisition by $tDCS_a$ seems to relate to brain-derived neurotrophic factor (BDNF) as polymorphism in humans is associated with altered proclivity to tDCS-induced benefits on skill learning (Fritsch et al., 2010). In humans, BDNF polymorphism, known to influence synaptic plasticity, has been related to responsiveness to tDCS-induced plasticity of MEPs changes (Antal et al., 2010).

Studies involving mice showed that tDCS of M1 changed the excitability of the motor pathway detected by MEPs in a polarity-specific manner consistent with findings humans (Cambiaghi et al., 2010). In rats, tDCS has been used to evaluate the propagation velocity of cortical spreading depression (Liebetanz et al., 2006a), the anticonvulsive effect in a rat cortical model of epilepsy (Liebetanz et al., 2006b) and the safety limits of cathodal tDCS (Liebetanz et al., 2009). Electrophysiological data from rat hippocampal slices show that dc stimulation modulates neuronal activity and alters both excitability and network function in both the short and long-term (Bikson et al., 2004). Significantly increased fMRI signal intensities in the frontal cortex and nucleus accumbens of rats after anodal tDCS suggest that frontal tDCS induces neuronal activation of these regions (Takano et al., 2011).

There is an inverted U-shaped relationship between prefrontal-based cognitive functions and levels of catecholamines, with moderate levels of activity in prefrontal networks sustaining optimal working memory function (Arnsten, 2009). Excitability changes induced by tDCS allow evaluation of a causal link between prefrontal activity and performance in a particular cognitive task. In healthy humans learning phase-dependent enhancement of working memory and planning (Tower of London task) was found after tDCS stimulation (Dockery, Hueckel-Weng, Birbaumer, & Plewnia, 2009). Here, cathodal tDCS when applied during acquisition and early consolidation improved performance. In contrast, anodal tDCS enhanced performance when applied in the later sessions. These early tDCS effects during training were associated with long-term differences found at the follow-up a year later under sham tDCS. However while the available data suggest that constant current stimulation influences brain activity, the cellular and molecular mechanisms need further investigation.

An animal model allows the possibility to evaluate changes in higher brain function (memory) in addition to the underlying mechanisms. Here we used transcranial direct current stimulation of the frontal cortex in order to measure effects on cognitive function in the rat. A modified version of the active place avoidance test, known as the Allothetic Place Avoidance Alternation task (APAAT), was used to incorporate working memory with increased task load and avoidance strategy formation (Dockery & Wesierska, 2010). We hypothesized that in healthy rats, in a novel task with continually novel task conditions, frontal cathodal stimulation would benefit place avoidance performance via moderated PFC activity.

2. Materials and methods

2.1. Animals

Both stages of the experiment were conducted in accordance with the regulations of the Polish Communities Council for the care and use of laboratory animals and the European Community Directive for the ethical use of experimental animals. Forty-one naïve, male Long Evans rats (250–325 g at the start of the experiment), grouped as 29 tDCS-treated ($n = 15$ anodal, $n = 14$ cathodal) and 12 control rats (previously reported in Dockery & Wesierska, 2010), were employed for this study. The rats used for these experiments were housed in standard conditions, with food and water available *ad libitum* and normal light-dark cycles (light from 8:00 to 20:00). The animals were handled for 4 days prior to experiment onset. The animals were closely observed during stimulation and the experiments to determine possible abnormal behavior related to the stimulation.

2.2. Transcranial direct current stimulation (Supplementary Fig. 1)

In our between subject design, rats received either cathodal or anodal tDCS, or no tDCS treatment. The investigator responsible for the behavioral testing was blind to the treatment type. tDCS was administered prior to behavioral training over a 30 min. period using a battery-driven, constant current stimulator (model: CX 6650, Rolf Schneider Electronics, Gleichen, Germany) with a 200 μA current run between an epicranial electrode (3.5 mm^2) fixed over the frontal cortex and a counter electrode comprised of a conventional rubber-plate electrode (10.5 cm^2; Physiomed Elektromedizin AG, Schnaittach, Germany) that was strapped to the back by a latex jacket fixed on the shoulders.

2.2.1. Epicranial electrode connector assembly: Placement, construction and implantation of a prefabricated electrode connector unit (Supplementary Fig. 2)

In an effort to replicate the setup used in human tDCS studies, the current was applied transcranially over the rat frontal cortex. As reported in Liebetanz et al. (2006b, 2009), the stimulation electrode was anchored onto the cranium using dental acrylic (Duracryl Plus, Spofa Dental A Kerr Company, Jicin, Czech Republic) to fix and cover a defined, and small surface area over the rat frontal cortex: the anterior cingulate and premotor cortices, and the medial edge of the primary motor cortex (1.5 mm anterior to bregma; 1.5 mm right or left of the sagittal fissure) (Paxinos & Watson, 2007). The epicranial electrode was composed of two parts: a gold pin which was fitted to be plugged into a plastic cannula. This was fixed onto the skull in a surgical procedure 1 week before the experiment began. Rats were anesthetized with isoflorine (from 3% to 1.5% isoflurane mixed with oxygen). The cranium was fixed using a stereotaxic apparatus (Stoelting Company, Wood Dale, Illinois, United States).

For stimulation, the cannula was filled with saline solution (0.9% NaCl) to conduct the current via the contact area on the skull. This unipolar arrangement limits the total current penetrating the skull and prevents currents from bypassing due to limited space for two juxtaposed epicranial electrodes. For the counter electrode, a wet sponge was placed between the rubber-plate electrode and the back of the rat to decrease impedance. $tDCS_a$ was performed with the anode above the frontal cortex and the cathode above the shoulders. The montage was reversed for $tDCS_c$.

2.3. Place avoidance apparatus

The place avoidance apparatus was described previously (Wesierska, Adamska, & Malinowska, 2009), so briefly stated it was located in a 3 × 4 m dimly lit room in which visual landmarks marked the walls. The arena was comprised of an elevated (80 cm), flat, metal disk (diameter 80 cm), with a lip on the periphery (1 cm). The place-avoidance system (Bio-Signal Group, Brooklyn, New York) was employed for data collection and analysis. Infrared light-emitting diodes (LED) fixed between the shoulders on a latex harness and the arena periphery allowed for tracking the position of rats at 20 ms intervals using an infrared-sensitive camera connected to a computer system (Wesierska, Dockery, & Fenton, 2005). Shocks were delivered via a cable connected to a low-impedance, electrode that was clipped to the back of the rat and

grounded via the high-impedance from contact between the arena surface and the rat's feet. A moderate response to the shock (e.g. – not freezing or escaping from the arena) was evoked by adjusting the current amplitude (0.2–0.5 mA) for each rat. The arena rotated in one direction at a speed of 1 rpm. The computer system tracked the trajectory of the rats and delivered a constant-current (50 Hz, 0.5 s) when triggered by an entrance into a 45° to-be-avoided sector. If the rat did not escape from the sector, shock was repeated every 1.5 s. The experiment was monitored in an adjacent room in which the computer-controlled recording equipment was located (Fig. 1).

2.4. Behavioral procedures (Fig. 1)

For application of active tDCS, the rats were taken to a separate room and underwent tDCS delivered epi-cranially to the left or right frontal cortex. For habituation, following exposure to the stimulation procedure (no current administered), the rats were first placed on the rotating arena without an active shock sector for 5 min. The subsequent day was the first training day (D1) in which avoidance training began with a 5 min habituation session (ha, rotating arena, no shock), followed directly by a 10–15 min training session (tr1, tr2, rotating arena, shock). The rats completed training after 10 min if they showed continuous avoidance for a minimum of 90 s. If they did not perform according to this criteria before the end of 10 min, they underwent an additional 5 min of training. Following the training session, the rats were removed from the arena and underwent a 5 min break in a cage next to the experiment room. Immediately following, the rats were returned to the rotating arena for 5 min and tested for retrieval (ts) with the original shock sector inactivated. During Stage 1, rats underwent 3 days of training and testing sessions on the rotating arena in a dimly lit room. The to-be-avoided sector was defined by room-frame coordinates and was changed each day in the following random order: D1 – Northwest, D2 – Northeast and D3 – Southwest. Cues from the rotating dry arena were not useful according to this condition. During Stage 2, as a follow-up to test long-term influences of stimulation on memory and skill learning, in a fourth session on D21 the rats underwent habituation, then trained and tested with a novel sector (D21 – Southeast). Unlike for Stage 1, here there was no active tDCS stimulation; however the rats were also put in the separate room prior to training under the same conditions without tDCS. The independent variables included the number of entrances in the shock sector (ENTR), the maximum time spent avoiding the shock sector (s) (T_{max}) and the number of shocks per entrance (SH/ENTR).

2.5. Data analysis

Based on the above measures for Stage 1 the effect for across session performance was performed by a three-way ANOVA (group ($tDCS_a$, $tDCS_c$, control) × day (D1, D2, D3) × condition (ha, tr1, tr2, ts): 3 × 3 × 4; with repeated measures on the last factors) followed by a Tukey HSD multiple comparison post-hoc test.

For Stage 2, on D21, a two-way ANOVA (group ($tDCS_a$, $tDCS_c$, control) × condition (ha, tr1, tr2, ts): 3 × 4; with repeated measures

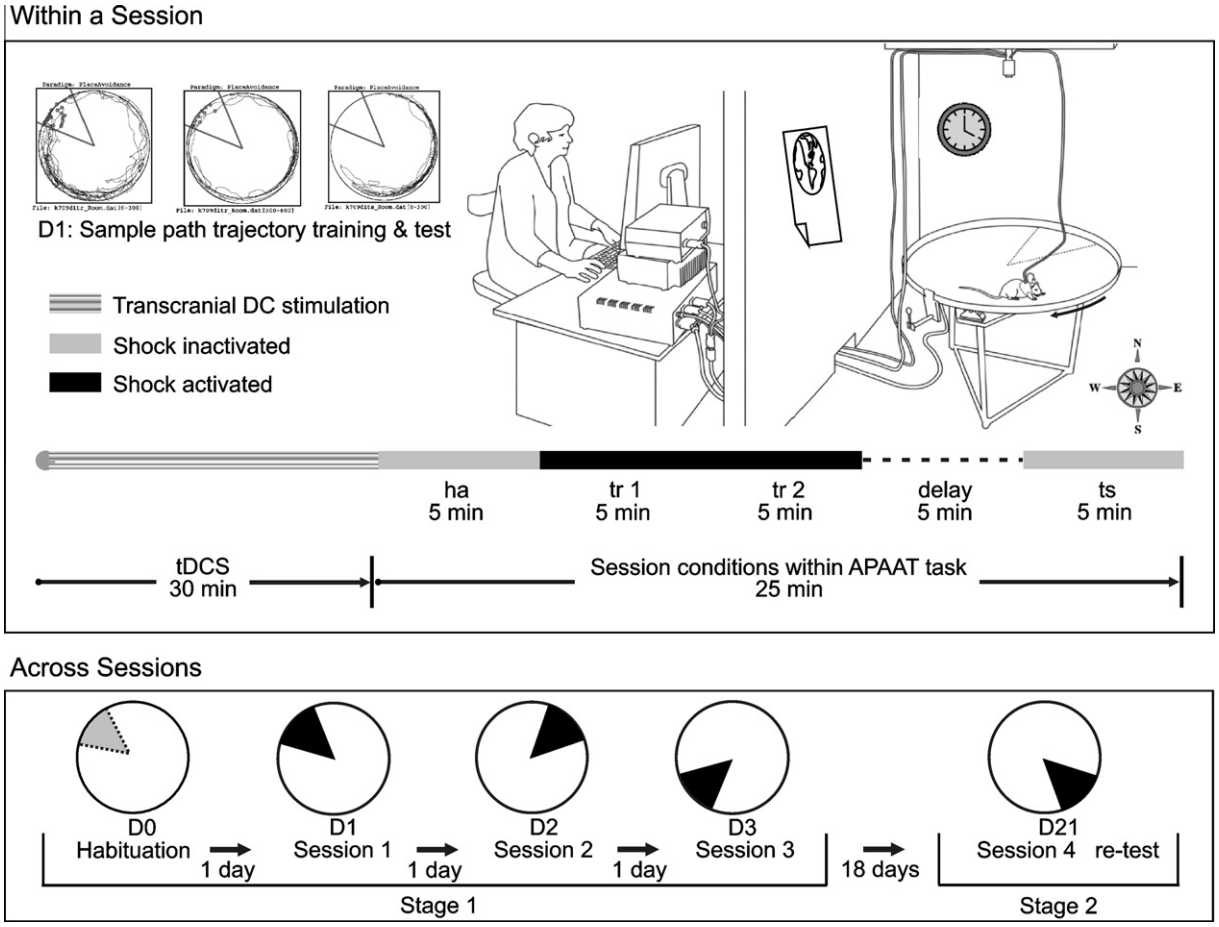

Fig. 1. Experimental design of transcranial direct current stimulation and the allothetic place avoidance alternation task (APAAT) *adapted from* Dockery and Wesierska (2010). Within a session rats underwent a habituation interval (ha), two 5-min training intervals (tr1, tr2), a 5-min delay, followed by a retrieval test (ts). Across sessions, rats underwent a habituation day (D0), followed by a training period across three sessions (D1–D3). The location of the to-be-avoided sector was changed for every session. For the re-test session (D21), no tDCS was applied and the location of the to-be-avoided sector was again changed in order to test the robustness of the rats' skill to avoid.

on the last factor) was performed with a Tukey HSD test for multiple comparisons. Significance was accepted at the $P < 0.05$ level. Statistical analysis was performed with STATISTICA 7.1. Averages ± SEM are reported.

3. Results

3.1. Offline effects of tDCS on working memory and skill learning during Stage 1 (D1, D2, D3) – across session comparison including conditions (ha, tr1, tr2 and ts)

Rats from the $tDCS_a$, $tDCS_c$ and control groups behaved similarly in the three consecutive APAAT sessions as confirmed by an insignificant group effect for number of entrances (ENTR) (F 2, 38 = 0.54; $P = 0.58$) and maximum time avoided (T_{max}) (F 2, 38 = 0.20; $P = 0.81$) (Fig. 2; see also Supplementary Fig. 3 Stage 1). As expected, comparison of the various behavioral conditions (ha, tr1, tr2, ts) resulted in significant differences in place avoidance performance for both ENTR (F 3, 114 = 83.54; $P < 0.0000$; ha > tr1 > tr2 = ts $P = 0.0004$) and Tmax (F 3, 141 = 60.16; $P < 0.0000$; ha < tr1 < tr2 = ts; $P < 0.003$). Rats avoided the place during the avoidance training (tr1, tr2), when shock was presented. They also avoided during the retrieval test (ts), in contrast to the habituation (ha), even though the shock was inactive for both ha and ts conditions. This was confirmed by a decreased number of entrances and increased maximum time avoided mainly during tr2 and ts. Hence, rats from all groups resolved the working memory test well. However they avoided better on D2 than D1 and D3 (ENTR: F 2, 76 = 5.41; $P < 0.006$; D1 > D2 < D3, $P < 0.004$; T_{max}: F 2, 76 = 6.28; $P < 0.003$; D1 < D2 > D3; $P < 0.002$).

Significant day by condition interactions for ENTR (F 6, 228 = 4.17; $P < 0.0005$) and for the T_{max} (F 6, 228 = 2.51; $P < 0.02$) indicated a non-static working memory process in the APAAT. The ENTR for tr2 was similar for all days and lower than during ha of the same days ($P < 0.001$). However, for the memory retrieval (ts) the number of ENTR was comparable on D1 and D2, when rats were newly learning to avoid the shock sector, which showed fewer entrances than on D3 ($P < 0.0005$), when rats had already learned that there was no active shock for the ts. The T_{max} showed a similar pattern of results for ha and tr1 during Stage 1. However, on D3 for the ts condition, when avoidance is no longer necessary, the T_{max} was shorter than on D1 when rats were the most naïve to the procedure ($P < 0.03$). On D2 when the increased task load is presumably counter-balanced by experience, rats showed the highest maximum avoidance time of the shock sector during tr2 than on other days ($P < 0.001$).

Anodal and cathodal tDCS stimulation had no effect on skill learning in the place avoidance test, presented as the ratio of shocks per entrance (SH/ENTR) for Stage 1 (F 2, 38 = 2.56, $P = 0.09$) (Fig. 3; see also Supplementary Fig. 4 Stage 1). The SH/ENTR ratio changed across conditions (F 3, 114 = 39.39;

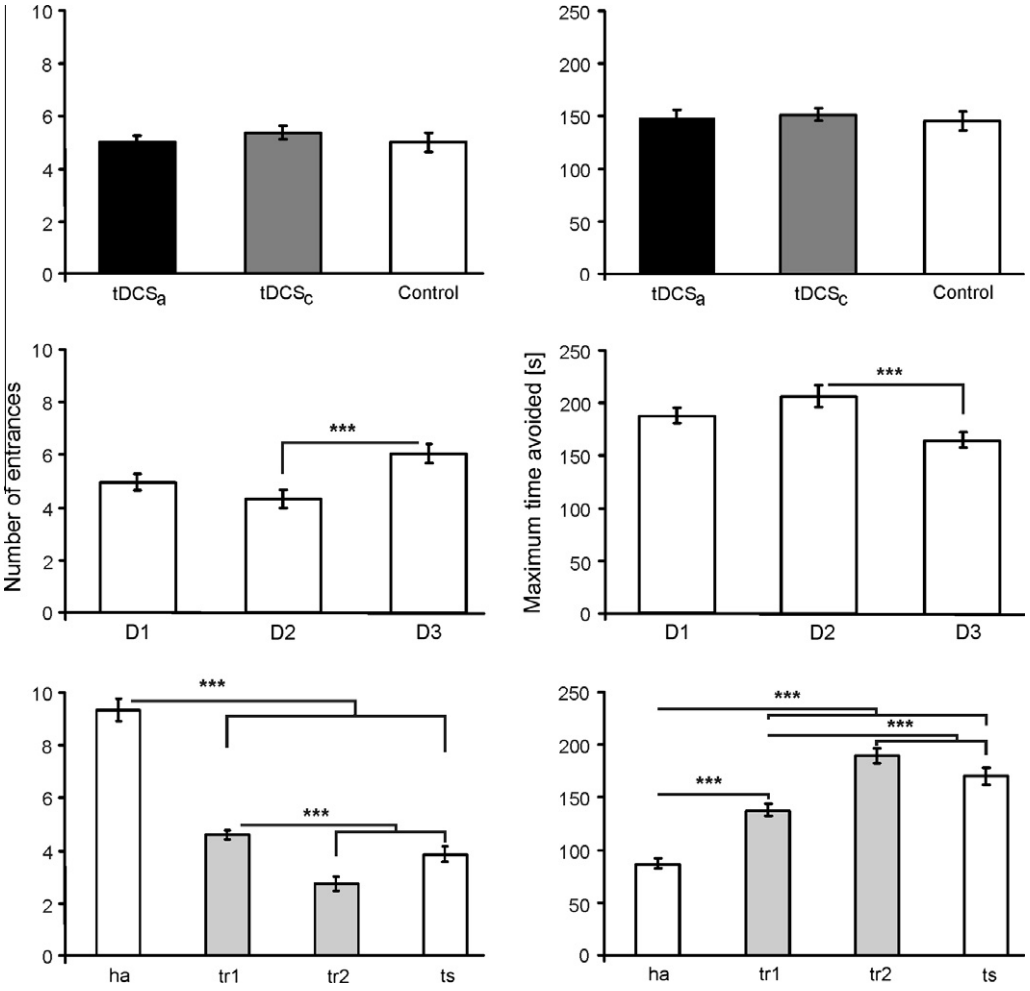

Fig. 2. Working memory. Working memory in the place avoidance task is presented as the number of entrances (ENTR; panel on left) and the maximum time avoided (s) (T_{max}; panel on right) in reference to the sector to-be-avoided. Values are presented as grand averages ± SEM according to group, day and condition. The post hoc results, marked by asterisks, were equivalent to: $^{***}P < 0.001$. For conditions (third row), the bars filled with grey indicate an active shock sector.

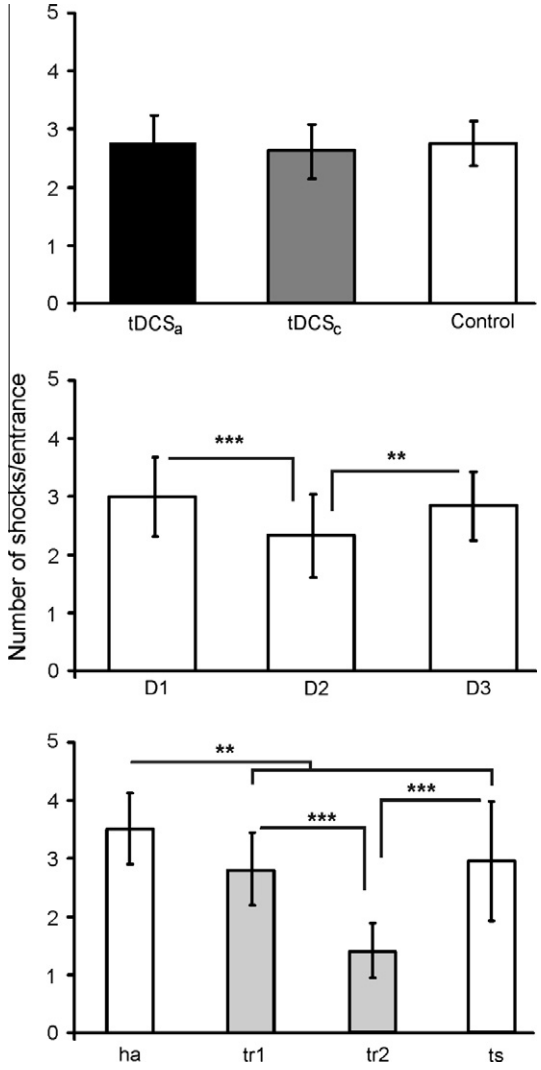

Fig. 3. Skill learning. Skill learning in the place avoidance task is presented as the ratio of shocks per entrance (SH/ENTR) into the sector to-be-avoided. Values are presented as grand averages ± SEM according to group, day and condition. The post hoc results, marked by asterisks, were equivalent to: $^{**}P < 0.01$ and $^{***}P < 0.001$. For conditions (third row), the bars filled with grey indicate an active shock sector.

$P < 1 \times 10^{-16}$; ha > t1 > t2 < ts = t1; $P < 0.01$) and was at a similarly high level during tr1 and ts than for tr2, but at a lower level than during habituation. Skill learning for Stage 1 differed depending on day (F 2, 76 = 7.50; $P < 0.001$; D1 > D2 < D3; $P < 0.002$) and was at a similarly high level during the initial presentation to the new procedure (D1) as when rats were already familiar with it (D3), than on D2.

3.2. Long-term effects of offline tDCS paired with working memory training during Stage 2 (D21) – within session comparison

The long-term effects of stimulation and working memory training were tested without stimulation 20 days after the first exposure to the APAAT paradigm preceded by anodal or cathodal tDCS. On D21 significant group effects were found for both the number of ENTR (F 2, 37 = 3.61; $P < 0.036$; $tDCS_a = tDCS_c < Contr$, $P < 0.07$) and T_{max} (F 2, 37 = 4.76; $P < 0.014$; $tDCS_a < tDCS_c > Contr$, $P < 0.02$) (Fig. 4). Although rats previously treated with $tDCS_a$ or $tDCS_c$ presented similar performance in the APAAT, rats from the $tDCS_c$ group made significantly fewer entrances with a longer maximum avoidance time than the control rats (Supplementary Fig. 3 Stage 2). A significant effect of conditions was found for ENTR (F 3, 111 = 8.23; $P < 0.0000$; ha > tr1 > tr2 = ts, $P < 0.006$) and T_{max} (F 3, 111 = 10.00; $P < 0.0000$; ha < tr1 < tr2 > ts, $P < 0.01$). Rats made a similar number of entrances during tr2 and ts, which was lower than during tr1 and ha. The maximum time avoided was longer during tr2 than during the other conditions.

During Stage 2 the SH/ENTR ratio also significantly differed for group (F 2, 38 = 4.67; $P < 0.015$; $tDCS_a = tDCS_c$, $tDCS_c < Contr$, $P < 0.02$) and condition (F 3, 114 = 29.00; $P < 5 \times 10^{-13}$; ha = ts > tr1 > tr1, $P < 0.0001$) (Fig. 4; see also Supplementary Fig. 4 Stage 2). For the long-term follow-up session, rats previously stimulated by $tDCS_a$ and $tDCS_c$ showed a similar level of SH/ENTR ratio. However for $tDCS_c$ rats this ratio was lower than for the control group. The SH/ENTR ratio presented by rats during tr1 and tr2 (active shock) was lower than during ha and ts (inactive shock).

4. Discussion

To date, polarity dependent effects of tDCS on performance in cognitive tasks has only been studied in humans. We investigated the effects of anodal and cathodal transcranial direct current stimulation of the frontal cortex on rat performance in spatial working memory and skill learning in the Allothetic Place Avoidance Alternation Task. In the present study, we found that cathodal and anodal stimulation applied before working memory training have no acute effects on performance. In contrast, benefits from cathodal stimulation appeared on day 21 (without stimulation). Our results show a long-term effect of acute stimulation when combined with task performance. Rats which had undergone three training days preceded by cathodal stimulation performed better when tested after 18 days in spatial working memory and skill learning than rats from the control group (Figs. 2 and 3). Even though the shock sector location was novel, these rats showed a decreased number of entrances and a significantly longer maximum avoidance time (Fig. 4). Retention of the learned skill to avoid was robust for the cathodal group as on D21 they showed a lower ratio of shocks per entrance than the control group.

In a place avoidance paradigm for working memory, recent memory is engaged by the necessity to remember the location of the to-be-avoided sector which is alternated daily. This is then tested by the number of entrances which decreases in a later phase through acquisition of place avoidance (Cimadevilla, Kaminsky, Fenton, & Bures, 2000). Consequently, long-term skill learning of place avoidance is expressed by an increased maximum avoidance time, a decreased number of shocks per entrances within a session, and a maintained level of avoidance or increased avoidance strategies across sessions. Due to the daily alternation of the shock sector location, working memory was expressed within- and between sessions during training and the retrieval test conditions. The complexity of task demands increases as new learning of the novel place occurs while past representations must be extinguished/inhibited. At the same time retention of the skill to avoid must be transferred from session to session, and sustained.

Parameters of tDCS stimulation in the present experiment followed those used by the protocol of Liebentanz et al. (2006b, 2009). Importantly, these parameters were chosen to ensure the safety limits of the stimulation. Considering that tDCS has poor spatial resolution on brain tissue it is important to be critical about the precision with which tDCS can target the rat frontal cortex. While with our tDCS parameters we cannot assure that the peak-induced electric field magnitude was focally concentrated in the prefrontal cortex, the behavioral changes we observed compared to controls were polarity specific and did not yield observable changes in motor or peripheral nervous system function (e.g. normal gait and breathing). The results suggest that rats provide a useful model of human prefrontal cortex function. Paired with tDCS,

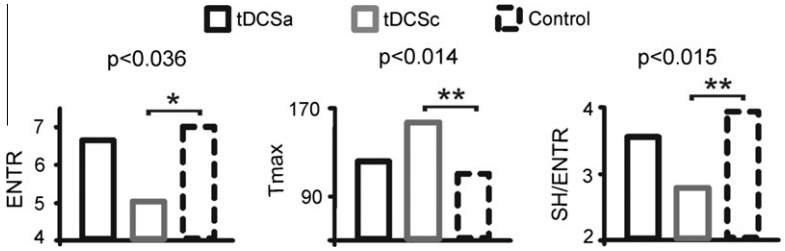

Fig. 4. Grand averages for Stage 2 – D21. The grand averages for groups during Stage 2 were significant (p-values indicated) for the variables number of entrances (ENTR), maximum time avoided (T_{max}) and number of shocks per entrance (SH/ENTR). The post hoc results, marked by asterisks, were as follows: *ENTR $P < 0.07$, **T_{max} $P < 0.02$, and **SH/ENTR $P < 0.02$. The unfilled columns reflect that during day 21 no stimulation was administered and the key describes the pre-treatment of each group during Stage 1. Values are presented as averages ± SEM.

this rat behavioral model provides a viable possibility to study learning and memory at both behavioral and cellular levels. In our experiments an electrode conducting constant current was located above part of the frontal cortex involving the premotor and dorsal anterior cingulate cortices belonging to the rat's prefrontal cortex. This localization is associated with the established role of the prefrontal cortex which is a structure crucial for working memory. The structural and functional characteristics of the rat prefrontal cortex are considered homologs to the medial and orbital, and dorsolateral prefrontal cortices of primates (Uylings, Groenewegen, & Kolb, 2003). In the monkey, damage of the prefrontal cortex affected visuospatial working memory performance (Curtis & D'Esposito, 2004). A pharmacological lesion study in rats showed that the prefrontal–hippocampal circuit is important in spatial working memory (Wang & Cai, 2006). Visuospatial working memory performance in rats is impaired after damage to both the hippocampus (Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002) and the prefrontal cortex (Curtis & D'Esposito, 2004). Temporary hippocampal blockade in the rat impaired performance in the visuospatial-based place avoidance test (Cimadevilla, Wesierska, Fenton, & Bures, 2001).

To date most studies involving tDCS measured the effects on cortical excitability. It was found that anodal current depolarized, whereas cathodal current hyperpolarized neurons of the motor cortex in the cat (Purpura & McMurtry, 1965). In rodents and humans anodal stimulation of M1 facilitated motor-evoked potentials, whereas cathodal stimulation suppressed them (Cambiaghi et al., 2010; Nitsche & Paulus, 2000). Following anodal stimulation of the rat frontal cortex fMRI signals increased which suggests that tDCS induced neuronal activation of this region (Takano et al., 2011). Polarity dependent changes in cerebral blood flow show increases with anodal and decreases with cathodal tDCS (Wachter et al., 2011).

There is no direct evidence that anodal and cathodal tDCS have the same excitability effects in the frontal cortex as has been established in the motor cortex (Stagg & Nitsche, 2011). Stimulation does not modulate excitability changes in different brain areas in a homogenous manner. Homeostatic metaplasticity, reflected by seemingly paradoxical tDCS effects on consequent behavioral training, is well documented for the motor cortex. This indicates that the history and current state of a particular cortical area matter in how tDCS modulates excitability (Ridding & Ziemann, 2010). Our results may reflect homeostatic metaplasticity in the PFC. While the results of our study only provide indirect evidence of common CNS effects of frontal tDCS in rats and humans (Dockery et al., 2009), it is informative and valuable due to the paucity of research on tDCS in rats. However, the results should be interpreted cautiously, and further basic research is needed to elucidate the significance of the findings reported here.

In our experiment, tDCS was not concurrent with, but preceded training. This was due to the ambulatory nature of the task and the need for a static stimulatory set-up. It is known that the effects of tDCS outlast the stimulation dependent on the current intensity applied, and that even when not immediately coupled with the task or a measurement (e.g. in an "on-line" approach) the effects of 100 μA persist for a 30 min post-stimulation period (Wachter et al., 2011). Due to our offline approach, we expected the effects to be less pronounced over the short-term and instead more robust over the long term related to a cumulative build up of tDCS-induced plasticity changes paired with training effects (Stagg & Nitsche, 2011). Cumulative effects of tDCS repeated daily have been found to yield benefits on human motor function in stroke patients (Boggio et al., 2007). Further, long-term effects of tDCS during training in a cognitive task, specifically planning/visuospatial working memory, have been found in healthy humans (Dockery et al., 2009). Thus we tested for retention of the learned skill to avoid as a possible long-term effect of tDCS on APAAT performance (Stage 2). As a result, in association with cathodal tDCS pretreatment, we found skill improvement measured by the shock to entrance ratio on Day 21.

There are other important distinctions between the study design involving humans compared to the design employed in this rat experiment (Dockery et al., 2009). Both an on- and off-line tDCS approach was used in the human study, and the participants underwent a cross-over design as opposed to the between subject design used here. Even though the number of sessions was the same, for humans the sessions occurred with 1-week intervals, indicating a longer time span of training, whereas the rats had daily sessions. For humans there were not new conditions introduced in every session so that the novelty of the task decreased consistently across sessions. For rats, the task to avoid was only novel at the beginning but they still had to learn new shock sector locations daily which increased the task load as the training went on (see D2 vs. D3). The optimal performance on D2, with worse performance on D1 (naïve state) and D3 (higher load state) is reminiscent of an inverted-U shaped relationship between frontal activity and performance (Arnsten, 2009).

Functional changes in prefrontal brain activity, driven by training-induced plasticity, have been observed in human visuospatial working memory (Klingberg, 2010). Since interactions between both the neocortex and the hippocampus are needed for spatial working memory, the temporal dynamics of their contributions should be considered. One model suggests that fast learning in the hippocampus supports the synaptic changes in the neocortex related to slow learning (Lynch, 2004). In this model maintenance of short-term memory storage is secured by the hippocampus while the neocortex governs long-term storage. This suggests that frontal tDCS could yield differential contributions on memory processing, not only dependent on polarity of stimulation, but also on which phase of learning and memory is currently activated.

In our study, the rat's frontal cortex was stimulated before the training over 3 days which included acquisition of the cognitive skill to avoid and of the new shock sector location each day. The

prefrontal cortex is especially important under novel tasks and complex behavioral conditions in which its role is to inhibit task-irrelevant information while relying on excitatory activity to maintain attention and working memory (Knight, Staines, Swick, & Chao, 1999). A dissociation exists on a cellular level between working memory involving novel stimuli from that with familiar stimuli (Hasselmo & Stern, 2006). Working memory involving novel stimuli relies not only on the PFC but also on temporal lobe structures. From this model, it follows that increased activity of the PFC could increase the inhibitory control needed to suppress distracters which would be critical for training. This increase occurs naturally as a result of arousal or novelty (Feenstra, Botterblom, & van Uum, 1995). However, excessive levels of excitation due to "overstimulation" by both novelty (endogenous activation) and increased excitability by anodal tDCS (exogenous stimulation) may be disadvantageous to working memory and skill learning.

For spatial working memory formation NMDA receptor-dependent LTP/LTD mechanisms in prefrontal circuitry are required ⁎Otani, 2004). Also NMDA-dependent plasticity occurs in the PFC during memory consolidation and reconsolidation (Akirav & Maroun, 2006). An optimal level of activation of PFC neurons is necessary for sustained enhancement of hippocampal–prefrontal LTP (Jay et al., 2004). Due to the need for moderate activity in both the prefrontal and hippocampal structures, cathodal tDCS may optimize the inherent prefrontal activation pattern of a healthy rat under the novel and highly demanding training conditions in our visuospatial working memory test. Specifically in regard to cathodal tDCS after effects, the exogenous down-regulation of the glutamatergic neuronal activity (Stagg et al., 2009), may counteract high endogenous activation of excitatory PFC neurons due to the novel and difficult working memory task. This could result in optimal activation of NMDAr leading to increased intracellular Ca+ which could support long-term changes in learning and memory (Dash, Moore, Kobori, & Runyan, 2007). A theoretical framework for tDCS-induced PFC activity changes by which functional gains in working memory and learning may result is presented in Fig. 5.

Pharmacological approaches to elucidate mechanisms of the long-lasting after-effects of tDCS have been studied (Liebetanz et al., 2002; Nitsche et al., 2003; Siebner et al., 2004). It was found that motor cortex excitability increased after anodal tDCS and that the effects were glutamatergic-dependent and involved membrane mechanisms. On the contrary, the after-effects of $tDCS_c$ of M1 on the central and peripheral nervous systems have been found to be due to non-synaptic mechanisms of action (Ardolino, Bossi, Barbieri, & Priori, 2005). More recent evidence suggests that anodal tDCS results in a reduction of GABA concentration, while cathodal stimulation decreases glutamate concentrations, in correlation with reduced GABA levels, measured by magnetic resonance spectroscopy in the sensorimotor cortex (Stagg et al., 2009). Considering the long term effects of the applied paradigm, in which the re-test was separated by a long break between training (early learning), the involvement of adult neurogenesis cannot be excluded (Deng, Aimone, & Gage, 2010).

In our study, offline cathodal tDCS of the frontal cortex seems to promote benefits in working memory and skill learning which manifest over a longer time period (on D21). This may emphasize the importance of the frontal cortex for encoding and recall of task-specific information. In humans during early training when endogenous PFC activity is high, paradoxical effects of cathodal tDCS of the left PFC on visuospatial working memory were found to result in faster reaction times at equal or better accuracy levels (Dockery et al., 2009).

The notion of a "behavioral tagging process" accounts for how transient short-term memory can become input specific, and shows that long-term memory (LTM) requires both the novel experience and occurrence within a critical time window (Ballarini, Moncada, Martinez, Alen, & Viola, 2009). Weak tetanization has been shown to reinforce induced early-LTP; however this is modulated by many factors. This occurred with novelty-exposure before and directly after weak tetanization (Straube, Korz, & Frey, 2003), and 15 min after weak tetanization with performance in a complex spatial task (Uzakov, Frey, & Korz, 2005) and with consequent stress-exposure (swim test) (Korz & Frey, 2003). In this regard, it is possible that in our cognitive paradigm, tDCS acts as a "cognitive modulator" and may promote the transformation of short-term memory to long-term memory by influencing the potency of synaptic and behavioral tagging.

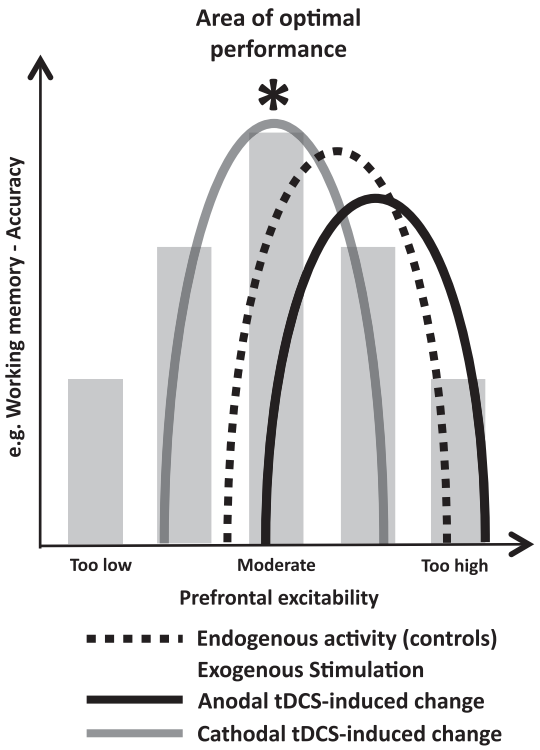

Fig. 5. A theoretical framework for tDCS-induced prefrontal cortex (PFC) excitability changes by which functional gains occur in working memory. The grey bars represent working memory performance (*y*-axis) at various PFC excitability levels (*x*-axis). The curves express performance under the tDCS stimulation polarities and for controls. In the case of a novel, difficult task, the endogenous PFC activity would be high and remain high as long as the task was difficult. With decreased neuronal excitation by cathodal tDCS (grey line), the high endogenous PFC activity associated with baseline performance (controls, dotted black line) would be brought into the moderate range which provides optimal physiological conditions to support learning and memory processes. Conversely performance associated with the expected excitability increase by anodal tDCS (solid black line) would drive PFC excitability towards an excessive range. While this difference may not yet be visible on the behavioral level during early learning/training, the consequences of stimulation on cell function may emerge in later performance.

5. Conclusions

The results of this study suggest that tDCS paired with visuospatial working memory training in the APAAT provides a tool to understand the mechanisms of transiently induced activity changes which underlie improvements in learning and memory function. Prospective studies can test how tDCS optimizes learning and memory in relation to the interaction of neural activity and experience-dependent plasticity at other levels in the adult brain. The current knowledge of tDCS effects in rats is highly limited, thus further investigation of anodal and cathodal tDCS effects on the rat frontal cortex are needed.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nlm.2011.06.018.

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