

# Effect of tDCS on well-being and autonomic function in professional male players after official soccer matches

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## ABSTRACT

This study aimed to examine the effect of transcranial direct current stimulation (tDCS) used as a recovery strategy, on heart rate (HR) measures and perceived well-being in 12 male professional soccer players. tDCS was applied in the days after official matches targeting the left dorsolateral prefrontal cortex (DLPFC) with 2 mA for 20 min (F3-F4 montage). Participants were randomly assigned to anodal tDCS (a-tDCS) or sham tDCS sessions. Players completed the Well-Being Questionnaire (WBQ) and performed the Submaximal Running Test (SRT) before and after tDCS. HR during exercise (HREx) was determined during the last 30 s of SRT. HR recovery (HRR) was recorded at 60 s after SRT. The HRR index was calculated from the absolute difference between HREx and HRR. A significant increase was observed for WBQ (effect of time;  $p < 0.001$ ;  $\eta_p^2 = 0.417$ ) with no effect for condition or interaction. A decrease in HRR ( $p = 0.014$ ;  $\eta_p^2 = 0.241$ ), and an increase in HRR index were observed ( $p = 0.045$ ;  $\eta_p^2 = 0.168$ ), with no effect for condition or interaction. No change for HREx was evident ( $p > 0.05$ ). These results suggest that a-tDCS over the DLPFC may have a positive effect on enhancing well-being and parasympathetic autonomic markers, which opens up a possibility for testing tDCS as a promising recovery-enhancing strategy targeting the brain in soccer players. The findings suggest that brain areas related to emotional and autonomic control might be involved in these changes with a possible interaction effect of tDCS by placebo-related effects, but more research is needed to verify this effect.

## 1. Introduction

Non-invasive and non-exhaustive measures of fitness-status have been proposed to be used to monitor soccer players' responses to training and competitive load and assess alterations in fitness and symptoms of fatigue [1,2]. One of these approaches is based on psychological monitoring via questionnaires as it seems to be an effective means of assessing soccer players' response to training [3]. For example,

it has been reported that the Well-Being Questionnaire (WBQ) was sensitive to the subtle daily changes in the training load during an intensified training camp in elite male soccer players [3]; and Rabbani et al. [4] demonstrated that the WBQ is a promising tool for tracking match-induced fatigue during the season in professional soccer. Indeed, Thorpe et al. [5] showed that the perceived ratings of wellness should be used as a non-invasive assessment of fatigue status in elite soccer players during the in-season competitive phase. These data strongly suggest that

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the WBQ can be used as a tool for monitoring fatigue and recovery in a professional soccer setting.

The monitoring of heart rate (HR) measures in response to a Submaximal Running Test (SRT) has also been widely adopted and used in research to evaluate fitness-status, fatigue, and recovery in soccer [6–8]. Cardiovascular responses are usually reported as HR during exercise (HReX), with lower values indicative of greater cardiac efficiency and HR recovery (HRR), with faster return to pre-exercise HR indicating a better aerobic fitness [9]. Indeed, HRR measured after the end of the 5-minute submaximal running test has been proposed as a marker of autonomic function and to be useful to monitor changes in a training status in athletes [10]. Given that this method is non-invasive and not influenced by players' subjective assessment, it could be used together with self-reporting (i.e. WBQ) for monitoring fatigue and recovery status. Besides, SRT can easily be incorporated into a training schedule in professional field-based team sports such as soccer [1] as it has shown to be actually a submaximal test, with HReX ranging from 75 to 85% of maximal HR [6]. Therefore, monitoring training status in soccer players using HR measures from SRT combined with the WBQ, could be a valid and useful approach. This could be particularly valuable in a practical setting to monitor players' responses to recovery strategies in soccer.

While these submaximal tests and psychometric measures may aid in the assessment of fitness-status, fatigue, and recovery in soccer, an important issue in soccer training is to figure out efficient recovery-enhancing strategies to be used post-matches and post-training sessions. Many adopted recovery strategies still lack strong evidence (i.e. active recovery, stretching, compression garments, massage, and electrical stimulation) [11] and their applicability in the professional setting still needs to be investigated [12]. Moreover, less is known on the effect of recovery strategies focused on the brain. Although it is unequivocally accepted that central components of fatigue are responsible for some performance-related decrements, limited research has actually examined post-exercise recovery strategies focused on the brain [13]. Among possible alternative methods, non-invasive brain stimulation might be considered, but it has yet to be empirically examined. Non-invasive brain stimulation has been increasingly used by clinicians and neuroscientists to induce changes in the status of the human brain [14]. Notably, transcranial direct current stimulation (tDCS) is a neuromodulatory intervention that may induce excitability changes in the human cortex [15,16]. tDCS has been shown to modulate the autonomic nervous system both at rest and during exercise in athletes [14,17–19]. Okano et al. [14] reported a reduction of HR and ratings of perceived exertion (RPE) at submaximal workloads, and an increased peak power output and time to exhaustion, in cyclists performing a maximum graded exercise cycling test after anodal tDCS (a-tDCS) over the left temporal cortex aiming to modulate the left insular cortex. Indeed, it has been demonstrated that applying a-tDCS over the left dorsolateral prefrontal cortex (DLPFC) induced beneficial and long-lasting effects on vigilance, reaction time, and aspects of mood which are negatively influenced by fatigue in active-duty military subjects [20]. These data suggest that applying a-tDCS over the DLPFC might induce improvements in the well-being perception of elite soccer players, which in turn, could contribute to the recovery process.

Moreover, it should be highlighted that tDCS montage with the anodal electrode over the left and cathodal electrode over the right DLPFC (+F3/-F4 montage) has been shown to produce large amounts of electric current in the anterior insula [21], which is a brain area involved in emotional functions [22] and subjective feelings from the body [23,24]. Limited research has been published on the effects of tDCS on sports performance and fatigue [25]. Recently, Angius et al. [26] provided evidence that a-tDCS over the left DLPFC can improve inhibitory control and endurance cycling performance in healthy individuals. Despite these results, limited research still exists on the effects of a-tDCS over the DLPFC on recovery markers in team sport athletes, and less is known on the effect of a-tDCS over the DLPFC on autonomic indicators (HReX and HRR) and perceived well-being in elite professional soccer

players [14,25,27]. Therefore, this study aimed to examine the effect of tDCS on perceived well-being and HR measures in elite male soccer players after official soccer matches. It was hypothesized that a-tDCS would show greater improvement in perceived well-being while reducing HReX and providing faster HRR during and after the SRT, respectively, compared to sham-tDCS (control) condition.

## 2. Methods

### 2.1. Trial design

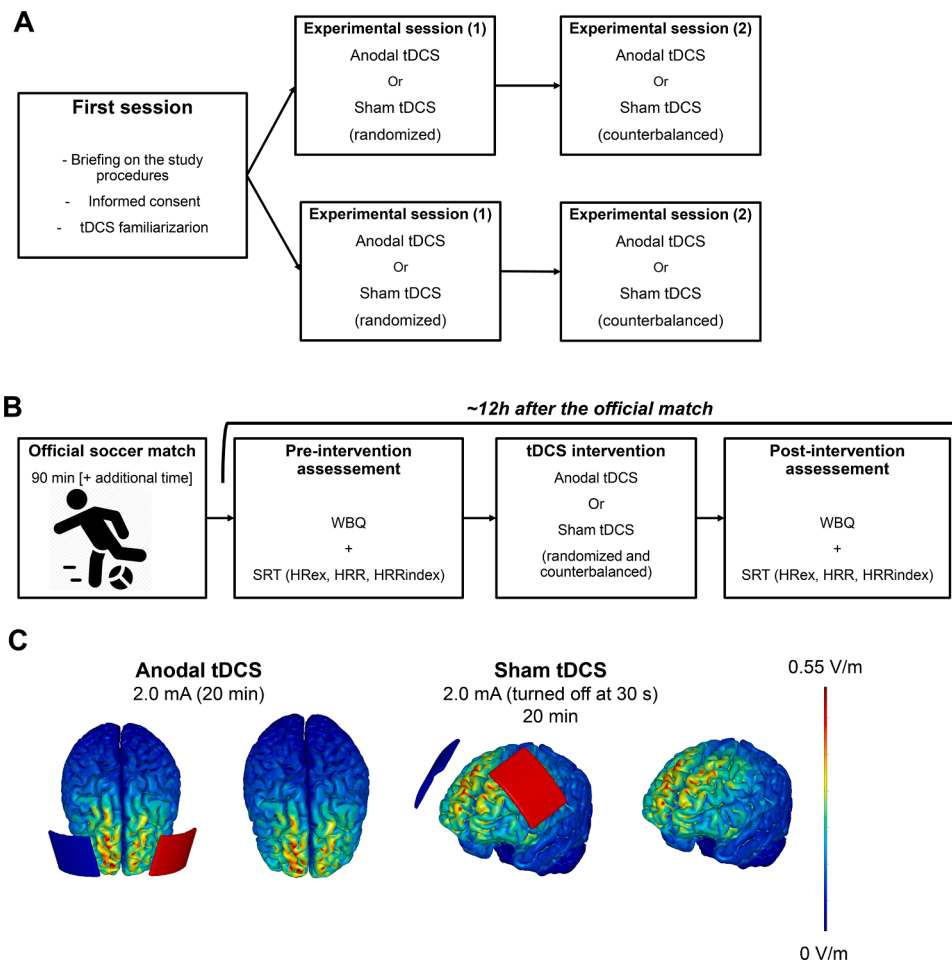
This was a single-center, randomized (1/1 ratio), crossover, double-blinded, sham-controlled trial with a within-subject design to compare the effects of a-tDCS on well-being and HR in elite soccer players. All players were already familiarized with the assessment tools used in the present study as they were part of their habitual monitoring training process. Participants were familiarized with tDCS in the first session, which occurred seven days before the experimental sessions. The experimental sessions occurred in the morning following the official matches (~12–13 h after the end of the matches), played at the home of the assessed team. At baseline, players filled the WBQ and performed the SRT. Next, they received the tDCS condition randomly assigned to that day (a-tDCS or sham). Finally, players again filled the WBQ and performed the SRT. The experimental sessions lasted approximately 60 min (Fig. 1). The study was performed at the team facility, with recruitment and the data acquisition occurring between August 24th, 2019, and January 4th, 2020. The study was approved by the Institutional Research Ethics Committee (CAAE 96440618.3.0000.5391) and it was conducted following the declaration of Helsinki. Informed consent was obtained from all participants before entering the study. However, they were not informed about the use of a placebo condition until the end of the study, when they were fully debriefed.

### 2.2. Participants

A professional Under-20 (U-20) soccer team was selected by convenience with 20 elite male soccer players, naïve to tDCS. All players were full-time professional soccer players. Since we aimed to analyze the effects of tDCS on the outcome measures after official soccer matches in elite male soccer players and that the experiment was performed with one professional team during an official competition, the only inclusion criterion was that the player had to have participated for at least 75% of the match duration preceding the experimental sessions. From the 20 players signed to the team, 8 participants were not included for not attending the inclusion criteria and 12 met the inclusion criterion and were analyzed (age:  $19 \pm 1.0$  years, height:  $178 \pm 8$  cm, body mass:  $71 \pm 7$  kg; Yo-Yo IR1 performance (aerobic power):  $1888 \pm 366$  m). The sample included two central defenders, two fullbacks, three central midfielders, three wide midfielders, and two attackers. Players underwent a thorough medical assessment to verify their health status before participation and were free from illness or injury at the time of the study. In the year that the study was performed the players were competing in the first division of the State and the Brazilian Soccer Championships and international competitions. During the period of the investigation, the assessed team became the vice-champion of the U-20 State Championship and was also vice-champion in an International competition played in South America, which included teams from Europe, Central America and South America. The players' typical training schedule was comprised of 10–12 sessions per week with each session lasting ~90–120 min, and one competitive play per week.

### 2.3. Randomization and concealment

The randomization was performed using an online randomizer (<http://www.randomization.com/>) which creates random permutations of treatments for situations where subjects are to receive all of the



**Fig. 1. Study design.** Official evening soccer match play(A); experimental session in the day after match play(B); the high-resolution computational modeling of the estimated electric field induced by transcranial direct current stimulation with the anodal (red) and cathodal electrode (blue) targeting the left and right dorsolateral prefrontal cortex (F3 and F4), respectively (C). WBQ = Well-Being Questionnaire; SRT = Submaximal Running Test; HReX = Heart rate during the last 30 s of the submaximal running test; HRR = heart rate one minute after the end of the test; HRRindex = the number of heartbeats recovered one minute after the end of the test; tDCS = transcranial direct current stimulation.

treatments in random order. For concealment, the name of the athletes entering the study was listed alphabetically and numbered. The random sequence for the athletes was created using balanced permutations so that five players received a-tDCS in the first and seven players received sham tDCS first. Then, the athletes were crossed over to the opposite condition, so that at the end of the experiment each athlete underwent one session of a-tDCS and one session of sham tDCS. Considering the inclusion criterion (to have participated in at least 75% of the soccer match) and due to logistic constraints, it took four games to complete the sample size of 12 athletes. Note that not all 12 athletes were assessed in the four games. Following the randomization and participation criteria in the games the tDCS intervention was performed as follows: game 1: five players (2 a-tDCS and 3 sham); game 2: five players (3 a-tDCS and 2 sham); game 3: seven players (3 tDCS and 4 sham); game 4: seven players (4 tDCS and 3 sham). One researcher performed the randomization and configured the tDCS device accordingly, other researcher prepared the athlete and applied tDCS (blinded to the condition) and a different researcher assessed outcome measures (blinded to the condition). Besides, participants were not informed that the study included placebo condition neither a priori nor during the study.

#### 2.4. Official matches

The experiments were performed in the morning following the official matches of the U-20 São Paulo State Championship (Brazil), played at the home of the assessed team. The team won all matches played during the study period. During the match, each player wore a 15-Hz global positioning system (GPS) unit coupled with a 100 Hz tri-axial accelerometer (SPI Elite, GPSports, Canberra, Australia) to assess

match running performance parameters. Each unit was harnessed between the shoulder blades and anchored using an undergarment to minimize movement. This unit provides valid and reliable measures of total and high-intensity distance [28]. Match running performance parameters included the total running distance (TRD) and high-speed running distance (HSRD), which considered speeds  $>19.8 \text{ km} \cdot \text{h}^{-1}$ . After each evening match, the players had their dinner and slept at the team facility. Therefore, the players' food intake and sleeping environment were standardized.

#### 2.5. Well-Being questionnaire

The WBQ was used based on the recommendations of Hooper and Mackinnon [29] and applied by McLean et al. [30]. This questionnaire was used to assess general indicators of player wellness, including fatigue, sleep quality, general muscle soreness, stress level, and mood on a five-point scale (ranging from 1 to 5). Following the approach adopted by McLean et al. [30], the overall WBQ score was determined by summing the five scores. The WBQ was used on players' normal training/competition routines so that players were already familiarized with it. Players filled the questionnaires before and after tDCS on the day following the match, with no contact with the other players and with no interference from the researchers.

#### 2.6. Submaximal running test and HR measures

A 5-minute submaximal running test was performed. The intensity of the exercise bout was fixed at  $10 \text{ km} \cdot \text{h}^{-1}$  across 40 m shuttles, following the speed adopted by Aoki et al. [31]. All players were fitted with Polar

monitors (T31 coded transmitter; Polar Electro, Kempele, Finland) for measuring HR during exercise (HREx) and recovery (HRR). For the analysis, the HREx during the last 30 s of the exercise was considered. At the end of the exercise, players stopped within 3 s and sat down, and HRR was recorded at 60 s of recovery. An index of HRR (HRRindex) was calculated by taking the absolute difference between HREx and the HRR. HRRindex represents the interplay between parasympathetic reactivation and sympathetic withdrawal, with the former presenting a faster contribution to the early recovery of heartbeats after exercise [32]. HRRindex also presents good reliability [33].

## 2.7. Transcranial direct current stimulation

tDCS was performed targeting to produce anodal stimulation over the left DLPFC and cathodal stimulation over the right DLPFC (+F3/-F4 montage). The electrode placement used in the present study was based on high-resolution computational modeling of finite element models of volume conduction of electrical current (Fig. 1C). a-tDCS was applied through a pair of electrodes with a current intensity of 2 mA for 20 min with a ramp up and down of 30 s at the beginning and end of the stimulation period using a battery-driven constant electric stimulator (MicroEstim – Genius, NKL, Brusque/Brazil). Two silicone-conductive electrodes (7 × 5 cm, 35 cm<sup>2</sup>; 0.057 mA/cm<sup>2</sup>) involved in a sponge soaked with a saline solution were used for stimulation. The anode electrode was positioned vertically over F3 and the cathode over F4, based on the 10–20 International EEG system. Sham tDCS was applied using the same electrode placement as the anodal tDCS. However, the stimulator was turned off after 30 s of stimulation (with a ramping period of 30 s at the beginning and end of the stimulation), to deceive participants [34]. All participants were naïve to tDCS and were familiarized with tDCS in the first session (seven days before the experimental sessions) to dissipate any concerns related to the intervention (Fig. 1A). The order of the experimental condition was randomized (as described above) and was performed in a double-blinded fashion so that neither the researchers nor the players knew which type of stimulation was applied. Also, different researchers configured the tDCS device, applied tDCS, and assessed the outcome measure. Moreover, the WBQ was filled by players on an individual basis with no interference from the researcher.

## 2.8. Assessment of tDCS-induced sensations and blinding

At the end of each session, participants filled a questionnaire proposed by Fertonani et al. [35] indicating the sensations and the degree of intensity felt during the stimulation. The questionnaire included the following sensations: itching, pain, burning, warmth/heat, pitching metallic/iron taste, fatigue, other (opened question). The degrees were none (0), mild (1), moderate (2), considerable (3), strong (4). Participants also reported when the discomfort began (1 = beginning, 2 = at approximately the middle, 3 = towards the end), when it stopped (1 = stopped quickly; 2 = stopped in the middle; 3 = stopped at the end), and if these sensations affected their exercise performance (0 = not at all; 1 = slightly; 2 = considerably; 3 = much; 4 = very much). To provide an evaluation of the general perceived discomfort induced by tDCS an aggregate variable (referred to as discomfort) was computed as the summation of the strength score recorded for every single sensation so that the discomfort variable ranged from 0 (absence of discomfort) to 28 (maximum discomfort).

## 2.9. Statistical analysis

The normal distribution of the data was assessed and confirmed by Shapiro-Wilk's test. Values are presented as means and standard deviation (SD) or median and interquartile range (IQR) as stated. A Wilcoxon Signed-Rank test was used to compare tDCS-induced sensations. A one-way analysis of variance (ANOVA) was used to compare TRD and HSRD

between the four matches preceding the experimental sessions. A two-way ANOVA with repeated measures was used to compare data of the WBQ (overall and domains scores) and HR (HREx, HRR, and HRRindex). The time (pre- vs. post-intervention) and the conditions (a-tDCS vs. sham-tDCS) was used as factors for comparison. If a significant effect was found, post hoc analyses were conducted using a Bonferroni post hoc test. A  $p \leq 0.05$  was considered significant. The sphericity and homogeneity of the variances were tested and confirmed by the Mauchly and Levene tests, respectively. Partial eta squared ( $\eta_p^2$ ) was used as a measure of effect size (ES) for the ANOVAs and was classified as follows: small (0.0099), medium (0.0588), and large (0.1379) [36]. All analyses were performed using Statistica 13.0 for Windows (StatSoft™, Tulsa, OK, USA).

## 3. Results

### 3.1. Running match performance parameters

There was no difference among matches for both TRD ( $F_{(3, 20)}=0.11$ ;  $p = 0.95$ ) and HSRD ( $F_{(3, 20)}=0.04$ ;  $p = 0.98$ ) for the four official matches performed ~12–13 h before the experimental sessions (Fig. 2).

### 3.2. tDCS-induced sensations and blinding

All 12 participants received the experimental conditions according to the randomization and there was no dropout. There was no serious side or adverse effects reported. The most common sensations reported were itching, burning, and pitching, but only the latter was significantly different between conditions. The location of the sensations was on the head, started at the beginning of the stimulation, and stopped quickly after stimulation (Table 1). Only one participant reported feeling “sleepy/dizzy” during a-tDCS, but no other sensation was reported.

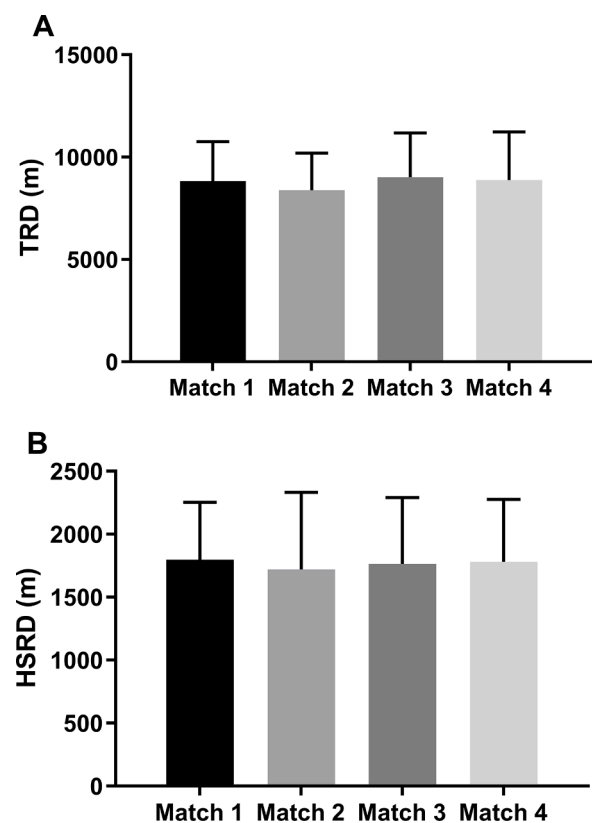


Fig. 2. Total running distance (A) and high-speed (>19.8 km·h<sup>-1</sup>) running distance (B) in male professional soccer players over four official matches ( $n = 12$ ).



**Table 1**Sensations and the general sensation index (discomfort) felt by elite soccer athletes during anodal and sham transcranial direct current stimulation ( $n = 12$ ).

Sensation	a-tDCS Mean $\pm$ SD	Median (IQR)	Subjects n(%)	Sham Mean $\pm$ SD	Median (IQR)	Subjects n(%)	Z	p
Itchiness	1.25 $\pm$ 1.22	1.0 (0.25–1.75)	9 (75%)	1.09 $\pm$ .94	1.0 (0.0–2.0)	8 (73%)	–0.680	.496
Pain	0.33 $\pm$ 0.65	0.0 (0.0–0.75)	3 (25%)	0.27 $\pm$ 0.65	0.0 (0.0–0.0)	2 (18%)	–0.577	.564
Burning	1.08 $\pm$ 0.90	1.0 (0.25–1.75)	9 (75%)	0.91 $\pm$ 1.22	0.0 (0.0–2.00)	5 (45%)	–1.00	.317
Warmth/Heat	0.50 $\pm$ 0.67	0.0 (0.0–1.00)	5 (42%)	0.45 $\pm$ 0.69	0.0 (0.0–1.0)	4 (36%)	.000	1.00
Pinching	1.67 $\pm$ 1.30	1.0 (1.0–3.0)	10 (83%)	1.00 $\pm$ 0.63	1.0 (1.0–1.0)	9 (82%)	–2.01	.045
Iron taste	0.17 $\pm$ 0.39	0.0 (0.0–0.0)	2 (17%)	0.09 $\pm$ 0.30	0.0 (0.0–0.0)	1 (9%)	–0.577	.564
Fatigue	0.67 $\pm$ 0.99	0.0 (0.0–1.00)	5 (42%)	0.64 $\pm$ 0.92	0.0 (0.0–2.0)	4 (36%)	–0.276	.783
Discomfort	5.67 $\pm$ 4.10	5.0 (2.0–10.3)	–	4.08 $\pm$ 3.14	4.0 (2.0–7.0)	–	–1.43	.151
Start	1.17 $\pm$ 0.39	1.0 (1.0–1.0)	–	1.30 $\pm$ 0.68	1.0 (1.0–1.25)	–	–0.45	.655
End	1.33 $\pm$ 0.78	1.0 (1.0–1.0)	–	1.00 $\pm$ 0.00	1.0 (1.0–1.0)	–	–1.41	.157
Affect performance	0.08 $\pm$ 0.29	0.0 (0.0–0.0)	1 (8%)	0.18 $\pm$ 0.41	0.0 (0.0–0.0)	2 (18%)	–0.577	.564

Note: mean  $\pm$  standard deviation; median (interquartile range); Subjects = indicates the number and percentage of subjects who experienced a particular sensation.

Importantly, most individuals (9/12; 75%) reported these sensations did not affect their performance in any tDCS condition. But three individuals reported tDCS sensations “slightly” influenced their subsequent responses in one session (two of them for sham and only one for a-tDCS). Regarding the blinding, the percentage of correct guesses regarding the tDCS condition was 29%. Seven individuals (58.3%) correctly guessed the condition of one of their sessions, however, five out the seven (71.4%) thought they were stimulated in both sessions.

### 3.3. Effect of tDCS on well-being

A significant effect of time ( $F_{(1, 11)}=12.424$ ;  $p = 0.005$ ;  $\eta_p^2=0.530$ ) was observed, with an increase in perceived well-being from pre to post-tDCS. However, there was no effect of tDCS condition ( $F_{(1,11)}=0.316$ ;  $p = 0.585$ ;  $\eta_p^2=0.028$ ) and condition vs time interaction ( $F_{(1,11)}=1.347$ ;  $p = 0.270$ ;  $\eta_p^2=0.109$ ; Table 2). No other significant main effects or interactions were found for WBQ domains (all  $p$ -values  $> 0.07$ ).

### 3.4. Effect of tDCS on measures of HR

For HRex, there was no main effect of time ( $F_{(1,11)}=0.038$ ;  $p = 0.848$ ;  $\eta_p^2=0.001$ ), tDCS condition ( $F_{(1,11)}=0.066$ ;  $p = 0.808$ ;  $\eta_p^2=0.002$ ), or condition vs time interaction ( $F_{(1,11)}=1.565$ ;  $p = 0.224$ ;  $\eta_p^2=0.066$ ; Fig. 3A). A significant effect of time was found for HRR ( $F_{(1, 11)}=7.022$ ;  $p = 0.014$ ;  $\eta_p^2=0.241$ ). There was a decrease in HRR at post-tDCS compared to pre-tDCS. No effect for tDCS condition ( $F_{(1,11)}=0.069$ ;  $p = 0.794$ ;  $\eta_p^2=0.003$ ) or condition vs time interaction ( $F_{(1,11)}=0.829$ ;  $p = 0.372$ ;  $\eta_p^2=0.036$ ; Fig. 3B) was observed for HRR. Similarly, a significant effect of time was found on HRRindex ( $F_{(1, 11)}=4.472$ ;  $p = 0.045$ ;  $\eta_p^2=0.168$ ). An increase from pre-to post-tDCS was observed. compared to pre-tDCS. No effect of tDCS condition ( $F_{(1,11)}=0.177$ ;  $p = 0.677$ ;  $\eta_p^2=0.008$ ) and condition vs time interaction ( $F_{(1,11)}=0.168$ ;  $p = 0.685$ ;  $\eta_p^2=0.007$ ; Fig. 3C) was observed for HRRindex.

**Table 2**

Overall and domain scores of the WBQ before and after tDCS applied in elite male soccer players after official soccer matches ( $n = 12$ ).

Variables	a-tDCS Pre	Post	Sham Pre	Post
Overall WBQ (a.u.) *	14.0 $\pm$ 1.95	16.5 $\pm$ 3.18	14.0 $\pm$ 2.83	15.5 $\pm$ 3.37
WBQ domains				
Fatigue (a.u.)	2.58 $\pm$ 0.67	2.50 $\pm$ 0.52	2.67 $\pm$ 0.78	2.67 $\pm$ 0.49
Sleep (a.u.)	3.92 $\pm$ 0.51	3.92 $\pm$ 0.51	3.58 $\pm$ 0.79	3.58 $\pm$ 0.79
Soreness (a.u.)	2.75 $\pm$ 0.62	2.67 $\pm$ 0.65	2.83 $\pm$ 0.93	2.67 $\pm$ 0.65
Stress (a.u.)	1.75 $\pm$ 0.62	2.25 $\pm$ 0.87	2.00 $\pm$ 0.85	2.17 $\pm$ 1.11
Mood (a.u.)	3.00 $\pm$ 1.04	2.92 $\pm$ 1.08	2.92 $\pm$ 1.08	2.50 $\pm$ 1.24

Note: a-tDCS = anodal transcranial direct current stimulation; a.u. = arbitrary units; N/A = not applicable; WBQ = Well-Being Questionnaire; \* = significantly different from pre ( $p = 0.01$ ).

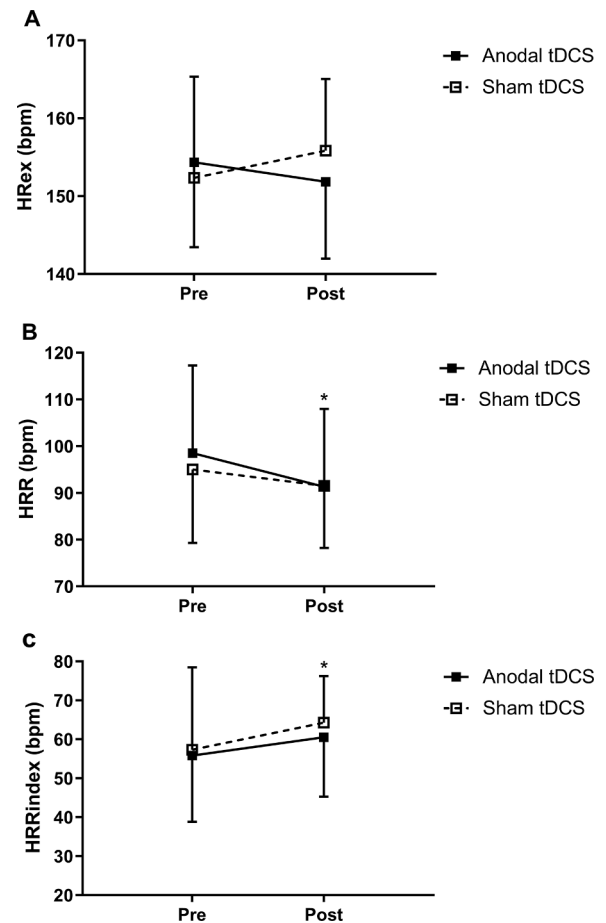


Fig. 3. Heart rate during the last 30 s of the submaximal running test (HRex; A); heart rate one minute after the end of the test (HRR; B); and the number of heartbeats recovered one minute after the end of the test (HRRindex; C), before (pre) and after anodal and sham transcranial direct current stimulation in male professional soccer players ( $n = 12$ ). \* significant differences from pre tDCS ( $p < 0.05$ ).

## 4. Discussion

This study aimed to examine the effect of tDCS-DLPFC on perceived well-being (WBQ) and autonomic (HR-related measures) regulation in professional male soccer players in the following morning after official match play. The main findings of this study were that both a-tDCS and sham-tDCS were associated with positive changes in WBQ, HRR, and HRRindex. However, no effect was observed for HRex, and, most importantly, there was no effect of condition (a-tDCS and sham-tDCS) or

interaction (condition and time-points) for any of the assessed recovery markers. The current findings are unique, novel, and add important findings to the literature. The present results suggest that the placebo effect could have occurred for perceived well-being (i.e., WBQ) and autonomic control markers (i.e., HR measures) and that this effect might be associated with the expectation of using the tDCS intervention. This effect thought as an interaction effect of tDCS by placebo-related effects has been earlier suggested in the non-sports environment [37,38]. Aslaksen et al. [38], for example, conducted a study in which subjects were clustered into three groups. One group receiving a-tDCS (2 mA for 7 min) over the primary motor cortex (M1); one sham-tDCS group, with the same electrode montage but the current, was turned off after 30 s, and a third group in which the electrodes were not even placed onto their head but received the same pain stimulation. The results of this study showed that a-tDCS provided an analgesic effect on high-intensity pain (heat pain 47 °C condition), but similar effects were showed between a-tDCS and placebo regarding the medium and low pain (45 and 43 °C conditions). In the present study, it could be hypothesized that a similar effect to that reported by Aslaksen et al. [38] might have occurred.

Another important element that should be considered in the present study, is the issue of players' expectations on the effect of tDCS. Wager et al. [37] reported that the expectation activates brain structures that have also been shown to be modulated by tDCS and other electrical stimulations [39]. Indeed, the similar results between a-tDCS and sham in the present study might, therefore, be related to the expectation or anticipation of a positive effect of the tDCS, even though the tDCS protocol was only fully explained to players after the end of data acquisition for all team participants. Nevertheless, as the intervention was applied the day after the match, it was impossible to avoid that players would have associated this intervention with a recovery strategy. In a professional setting, it could be reasonable to assume that players would expect receiving a useful recovery strategy, and therefore, assuming that it could improve their post-match recovery. Interestingly, Raglin et al. [40] suggest that in the context of placebos, expectation, and conditioning could influence and interact to affect the placebo effects (or nocebo – negative-) effects. Therefore, concerning the findings of the present study, it cannot be ruled out the occurrence of a high expectation of the players on the positive effects of tDCS application, which in turn, may have impacted the results, regardless of the blinding approach used during the investigation. In fact, sensations reported were similar between conditions, except for pitching, which started at the beginning of a-tDCS/sham and stopped quickly. The discomfort caused by tDCS, which represent the overall sensation was similar between condition, which suggests our blinding was effective. Importantly, these sensations did not impact subsequent responses or performance. It should be noted that only 29% of the guesses on the experimental condition was correct, which was way below chance levels. Moreover, five out the seven (71.4%) individuals who correctly guessed the condition of one of the experimental sessions thought they received a-tDCS in both sessions, which clearly shows they guessed correctly only by chance (50/50).

The effect of tDCS (anodal and sham) on well-being presents an important practical application while extending the knowledge on the use of tDCS as a recovery strategy in professional soccer players. As already pointed out, due to similar results between sham and a-tDCS, the placebo effect might have occurred for the overall WBQ score. The significant changes from pre-to-post in WBQ values, for both a-tDCS and sham, should be highlighted. It is worth mentioning that these changes occurred within a short period (approximately 60 min from pre to post WBQ responses). One could argue that the changes found in well-being in the present study could be due to the SRT performed before tDCS, which might have influenced post-tDCS WBQ responses. However, considering that the WBQ has been demonstrated to present stable values throughout a two-week training camp in high-performance team sport athletes [1], it would not be expected to verify significant increases

in WBQ within 60 min during a recovery session completed 12 h after an official match-play. Thus, we believe that the change found on the WBQ was not due to the SRT previous to tDCS. Furthermore, one could also argue that a priming effect for tDCS could have been created by the exercise performed previously to tDCS (i.e., SRT). Recent studies have demonstrated in both healthy individuals and stroke survivors that exercise of different intensity did not prime the brain for tDCS [41,42]. For instance, Hendy et al. [42] found that a single bout of high-intensity interval training (HIIT) for 20 min performed before a-tDCS over the DLPFC produced no cumulative benefit on cognitive performance, serum BDNF, and the cerebral hemodynamics in healthy adults. Also, Sivaramakrishnan and Madhavan [41] showed no additive priming effects of 20 min of aerobic exercise (i.e., recumbent stepping exercise) performed before a-tDCS over the motor hotspot of the paretic tibialis anterior on corticomotor excitability in stroke survivors. Therefore, increases in WBQ may seem to indicate that the recovery-enhancing protocol (tDCS) affected the perception of players of their well-being.

Other affective (emotional) states and their related cortical areas have also been demonstrated to be affected by placebo. Wager et al. [37], for example, demonstrated, using functional magnetic resonance imaging, that placebo analgesia was related to decreased brain activity in pain-sensitive brain regions, including the thalamus, insula, and anterior cingulate cortex. The results from the study of Wager et al. [37] also showed that the placebo effect was associated with an increase in PFC activity during anticipation of pain. The authors concluded that these findings could provide evidence on the effect of placebo for altering the experience of pain. Interestingly, Lieberman [43] reported that placebo effects have been consistently shown to be associated with reductions in subjective distress, dorsal anterior cingulate cortex activity, or amygdala activity, with rostral anterior cingulate cortex and LPFC activations predicting the magnitude of these reductions. It could be speculated, therefore, that the tDCS montage used in the present study modulated these brain areas, despite being under a-tDCS or sham, increasing the overall feeling of well-being.

A similar placebo effect might have also occurred for HRR responses. There is some evidence from systematic reviews that parameters controlled by the autonomic nervous system (ANS) may be involved in the top-down modulation via placebo interventions [44]. Indeed, Beedie et al. [45] suggested that placebos could be associated with increased activity of the opioid, endocannabinoid, and dopamine neurotransmitter systems, as well as with measurable effects on the autonomic nervous system. The findings of the present study for HRR and HRRindex (proposed as autonomic function markers) are unique and suggest a positive effect for tDCS related to the activation of brain areas involved in autonomic control, regardless of using a-tDCS or sham. The results of the present study also suggest that the placebo effects might be strongest associated with parasympathetic autonomic activity. Brunoni et al. [46] demonstrated that the left insular cortex would be involved in parasympathetic autonomic activity, while the right insular cortex would be associated with the sympathetic autonomic activity. Indeed, Geuter et al. [47] revised numerous studies intending to examine the brain systems underlying placebo effects on pain, autonomic, and immune responses, and they proposed that the ventromedial prefrontal cortex, insula, amygdala, hypothalamus, and periaqueductal gray may be considered as central brain structures underlying placebo effects. In the present study, therefore, the tDCS intervention might lead to alterations in any of these brain areas related to autonomic control, leading, ultimately, to activation in parasympathetic autonomic activity, optimizing the players' recovery.

Despite HR variability (HRV) is the most widely recognized non-invasive measure of cardiac autonomic control, the use of HR parameters as markers of the ANS function has been demonstrated. For example, Schestatsky et al. [48] revising the effects of non-invasive brain stimulation (NIBS) and the ANS, showed that within the selected tDCS studies, the HR was the second most common measure of cardiac autonomic control markers, followed by HRV in the third. However,

Schestatsky et al. [48] found only one study showing ANS alterations indicated by HR, and three showing ANS alterations indicated by HRV. Interestingly, the study that demonstrated a positive effect of tDCS on HR used the DLPFC montage, suggesting that this montage may be useful to affect the ANS and that this change may be examined by using the HR as an ANS marker [48]. Indeed, it is also important to highlight that Schestatsky et al. [48] suggested that a possible explanation for the lack of association between NIBS and ANS could be attributed to the suboptimal use of HR, in particular, performed without challenge to the ANS (e.g., measurement during resting state), contrary to what was performed in the present study, in which HR was measured during and after the end of the SRT. A reduction in HRR related to a scenario involving recovery should be interpreted as an increased player readiness to perform. The opposite effects (increase or no HRR reduction) might suggest an undesirable accumulated fatigue condition. The present results for HRR (and HRRindex) may indicate a positive result of using tDCS (anodal and sham) as a recovery strategy in elite male soccer players, as the findings reveal a favorable outcome associated with the recovery process.

Moreover, increases in the sympathetic activity associated with a decrease in parasympathetic activity cause an increase in HR, and the HRR is regulated by the parasympathetic reactivation and sympathetic withdrawal [10,32,49]. In the literature concerning the use of HReX and HRR in sports, data from many studies have consistently demonstrated the validity and usefulness of adopting such indices to examine ANS function in athletes. For example, Borresen and Lambert [50] indicated that HR may provide a measure of the disturbance in autonomic control in response to endurance training. Also, Daanen et al. [10] concluded in a systematic review that HRR (after the end of the 5-minute SRT – as used in the present study) would be a valuable indicator to monitor the training load and also to monitor the accumulation of fatigue while demonstrating that this index can be used as a marker of autonomic function in athletes. Additionally, Buchheit [51] in a technical report provided evidence that using HR during submaximal exercise is likely the most useful monitoring tool to examine ANS changes from training and competition. It should be mentioned that measures derived from 5-min recordings (almost daily) of resting HRV have also been shown to reflect cardiac parasympathetic activity and could be adopted for monitoring purposes [7]. However, measuring HRV in a sports setting is not free from concerns. For example, Plews et al. [52,53] provided evidence that using daily HRV in athletes could lead to misinterpretation of real change due to training, given its large day-to-day variability, and that the HRV indices could be useful and valid if measured at rest, on the mornings and frequently. In the sports setting, when staff members are selecting the most appropriate measure for intermediate-to-long term training monitoring with athletes, the balance between the sensibility and power of a given measure, and the practical likelihood of implementation, should be considered [51]. Thus, even considering that the resting HRV could be a possible more powerful measure of ANS function, in a 'real-world' of sports it could only be collected occasionally. On the other hand, assessing HReX and HRR using a submaximal exercise, would increase the likelihood of a more frequent assessment, without losing the robustness and the rigor of the measurement. Therefore, taking into account the findings from the literature and the data from the current work, it could be claimed that the present study will not only aid in the advancing of the knowledge on the use of the tDCS (+F3/-F4 montage) as a possible recovery strategy in elite athletes but as we have used the HReX and HRR due to the submaximal running exercise as ANS parameters, it might contribute to the increasing use of these indices in a practical setting and may encourage an evidence-based practice, as the methods used herein in, could be replicated also in a practical setting. Finally, it is worth mentioning that HR measures have been used to measure the effect of tDCS on cardiac autonomic control during exercise in almost all studies involving tDCS and exercise (for some examples, see [14,26,27,54–56]).

Despite the novelty of the present findings, some limitations should

be highlighted to recommend caution in generalizing the results. Even considering that the evaluated players were elite and full professional players, they were all from a single team, and the recovery environment was therefore unique to the analyzed team. Hence, the assessments of multiple teams during an official competition would provide a more complete picture of the effects of tDCS as a recovery-enhancing strategy. It should be noted, however, that the access to elite teams as well as getting permission to perform an intervention in elite athletes during a 'real-world' competition is a difficult task which also limited the sample size. Also, as the present study only includes limited recovery conditions and one tDCS montage, caution is needed when interpreting the present findings. Another limitation was that we analyzed players from different positions (i.e., two central defenders, two fullbacks, three central midfielders, three wide midfielders, and two attackers), which could create a bias on the cardiac autonomic control as it has been demonstrated to differ between positions [57]. Additionally, the measure of HRV at rest associated with the HR measures during exercise could provide a bigger picture of the possible effect of tDCS on cardiac autonomic control. However, due to time and equipment constraints, we could not measure it. Of note, HRR is a valid and reliable measure of the interplay between parasympathetic reactivation and sympathetic withdrawal [32,33]. Finally, a measurement of brain activity was not performed, which would contribute to understanding the possible brain changes subjacent to tDCS intervention. Researchers should consider these aspects when designing future investigations.

## 6. Conclusions

In conclusion, the improved perceived well-being and parasympathetic autonomic responses suggest that brain areas related to emotional and autonomic control might be involved in these changes with a possible interaction effect of tDCS by placebo-related effects. Moreover, the findings also suggest that HRR and HRRindex, together with WBQ, could be used to evaluate the possible short-term changes from recovery induced by tDCS in soccer players.

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## Declaration of Conflicting Interest

A.M., D.G.S.M., L.M., G.U., P.S.B., A.F.B., E.M., T.C., L.M., V.Z., and A.H.O. declare they have no conflict of interest concerning the content of this manuscript. The City University of New York holds patents on brain stimulation with M.B. as an inventor. M.B. has equity in Soterix Medical Inc. M.B. consults, received grants, assigned inventions, and/or serves on the SAB of Boston Scientific, GlaxoSmithKline, Mecta, Halo Neuroscience, X.

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