Cognitive and physiological assessment of prefrontal cortex neuromodulation in low and high risk gambling

University of East London

Elena Gomis-Vicent

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Abstract

Gambling disorder (GD) is the most widely studied behavioural addiction (BA), however there is still an unmet need for more effective treatment strategies. With the aim to improve the understanding of transcranial direct current stimulation (tDCS) as a potential treatment intervention for GD, four experiments were conducted using different protocols and participant samples to measure neuromodulation effects during gambling-related task performance. In Experiments I and II, the effects of tDCS were investigated in low impulsive (LI) and high impulsive (HI) participants. Different high definition (HD) tDCS montages were used to target right dorsolateral prefrontal cortex (rDLPFC) and ventromedial prefrontal cortex (vmPFC), brain areas associated with decision-making and reward processing, respectively. Results revealed effects of tDCS on gambling task performance, but no difference on tDCS effects between rDLPFC and vmPFC targets, or between participant groups. In Experiment III, the potential cumulative effects of rDLPFC tDCS combined with cognitive behavioural therapy (CBT) were investigated across eight sessions, in two patients diagnosed with GD. The intervention combining tDCS and CBT resulted in reductions in gambling severity and cravings, but this effect was also seen in the sham tDCS case. In Experiment IV, physiological data, including electrodermal activity (EDA), electrocardiogram (ECG) and electroencephalogram (EEG), was used to investigate rDLPFC tDCS effects on the autonomous nervous system (ANS), in LI and HI gamblers. Results showed that real stimulation was associated with increased sympathetic activation compared with sham, which was higher during gambling-related wins compared with losses, and in HI compared with LI. There were significant correlations between gambling severity, cognitive outcomes and physiological variables, which helped to identify biological markers associated with GD. These results helped refine the knowledge of specific cognitive and physiological underpinnings of reward processing in different participant samples, and contributed to the development of novel treatment interventions for GD.

Declaration

I declare that this work has been composed entirely by myself, and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where explicitly stated otherwise by reference or acknowledgment, the work presented is entirely my own.

Elena Gomis-Vicent

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Abbreviations

ACC	Anterior Cingulate Cortex
ADHD	Attention Deficit Hyperactivity Disorder
ANS	Autonomous Nervous System
ASRS	Attention Deficit Hyperactivity Disorder Self Report Scale
AUD	Alcohol Use Disorder
AUDIT	Alcohol Use Disorder Identification Test
BA	Behavioural Addiction
BDNF	Brain Derived Neurotrophic Factor
CANTAB	Cambridge Neuropsychological Test Automated Battery
CBF	Cerebral Blood Flow
CGT	Cambridge Gambling Task
DA	Delay Aversion
DLPFC	Dorsolateral Prefrontal Cortex
ECG	Electrocardiogram
EDA	Electrodermal Activity
EEG	Electroencephalogram
FOBT	Fixed Odd Betting Terminals
GD	Gambling Disorder
GRCS	Gambling Related Cognitions Scale
G-SAS	Gambling Symptom Assessment Scale
HD	High Definition
HF	High Frequency
HI	High Impulsive
HR	Heart Rate
HRV	Heart Rate Variability
IST	Information Sampling Task
lDLPFC	Left Dorsolateral Prefrontal Cortex
LF	Low Frequency
LI	Low Impulsive
LTD	Long Term Depression
LTP	Long Term Potentiation
MBIs	Mindfulness Based Interventions

MI	Motivational Interviewing
NAc	Nucleus Accumbens
NIBS	Non-Invasive Brain Stimulation
NPGC	National Problem Gambling Clinic
NU	Negative Urgency
OFC	Orbitofrontal Cortex
PFC	Prefrontal Cortex
PGSI	Problem Gambling Severity Index
PG-YBOCS	Pathological Gambling Yale Brown Obsessive Compulsive Scale
PNS	Parasympathetic Nervous System
P (correct)	Probability of Correct
QDM	Quality of Decision-making
rDLPFC	Right Dorsolateral Prefrontal Cortex
RT	Risk-Taking
rTMS	Repetitive Transcranial Magnetic Stimulation
SCC	Subgenual Cingulate Cortex
SCL	Skin Conductance Level
SCRs	Skin Conductance Responses
SMH	Somatic Marker Hypothesis
SNS	Sympathetic Nervous System
SPSS	Statistical Package for Social Sciences
SST	Stop Signal Task
SSRT	Stop Signal Reaction Time
SUD	Substance Use Disorder
tDCS	Transcranial Direct Current Stimulation
UPPS-P	Urgency Premeditation Perseverance Sensation Seeking Scale
VAS	Visual Analogue Scale
VMPFC	Ventromedial Prefrontal Cortex
VTA	Ventral Tegmental Area

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1 Chapter 1. General Introduction

1.1 Classification and prevalence of gambling disorder

Addictions are among the most predominant psychiatric disorders. From the clinical perspective, losing control over specific behaviours can lead to neglect of personal physical and mental health (Thege, Hodgins, & Wild, 2016). The classification of behavioural addictions (BAs) has been continuously debated, however the consensus that BAs are similar to substance use disorders (SUDs) has been growing since the inclusion of gambling disorder (GD) in the category substance-related and addictive disorders, in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) in 2013 (American Psychiatric Association, 2013). BAs show a wide range of similarities with SUDs, including symptoms such as craving, tolerance and withdrawal, comorbidities, genetic factors and brain alterations (Clark, 2014).

In particular, GD is characterised by persistent recurrent and maladaptive gambling behaviour that disrupts personal, social and professional life, and has been associated with higher morbidity and mortality rates (Fong, 2005), and high risk of suicide (Wardle, John, Dymond, & McManus, 2020). The impact of excessive gambling represents an increasing public health concern (Shaffer & Korn, 2002), with prevalence rates during the past 12 months worldwide ranging from (0.12–5.8%), and from (0.7–6.5%) during lifetime (Calado & Griffiths, 2016). In particular, GD prevalence in Great Britain was estimated to be 0.4% as assessed by the DSM-V. The risk for developing GD is affected by genetic and environmental contributors, which seem to be associated with different stages on the progression from initiation to addiction. Particularly, the initiation in gambling appears to be mediated by environmental experiences that constitute risk factors for developing GD (Shaffer et al., 2004), which include childhood maltreatment (Felsher, Derevensky, & Gupta, 2010), parental gambling behaviour (Schreiber, Odlaug, Kim, & Grant, 2009), and availability of gambling (Welte, Wieczorek, Barnes, Tidwell, & Hoffman, 2004).

1.2 Gambling features

Gambling usually comprises an element of consideration of something valuable, an element of risk in the form of chance, and an element of prize, which is typically money or something of financial value

(Derevensky & Griffiths, 2019). However, while gambling represents a recreational activity for the majority of people, it can become a very serious behavioural disorder for others (Ferland, Fournier, Ladouceur, Brochu, & Pâquet, 2008). Particularly, some forms of gambling are more addictive than others, with fixed odd betting terminals (FOBT) and gaming machine gambling being associated with higher gambling severity (Ronzitti et al., 2016). Research into game characteristics found that sounds and flashing lights can contribute to arousal and become a form of reward. Nonetheless, more important than the rewards per se, the reinforcement schedule, especially random distributions, promote persistence to continue playing (King, Delfabbro, & Griffiths, 2010).

In addition, games that offer a high frequency of rewards create a wining sensation, even though the pay-out is lower than the amount wagered, which is known as "loss disguised as win". Games that include "near misses" features, which show an unsuccessful outcome near to a designated win, create the sensation to be nearly winning, rather than constantly losing (King et al., 2010; Zack, Featherstone, Mathewson, & Fletcher, 2014). Moreover, providing the player with a choice, such as pressing a stop button in slot machines, produces an illusion of control that increases the confidence to win, even when the event is understood to be random (Harrigan, Collins, Dixon, & Fugelsang, 2010). The characteristics of gaming and gambling products have a powerful influence on people´s behaviours, and contribute to the maintenance of GD.

In the past few years, internet gambling has introduced the possibility to gamble at any time and location, involving large stakes with instant feedback and access to unlimited products. In addition, the inclusion of gambling within gaming is concerning, as gambling features have been embedded into video games (sometimes recommended for children as young as 3 +), which include high pay out rates that could create a sense of confidence in young people when gambling. Furthermore, the introduction of loot boxes brought forward the use of real money to buy virtual items or bundles that provide a selection of prizes by chance. With the increased availability of gambling linked to technological advances in recent years, the severity of GD has become more evident, as the urgency to develop treatment strategies for GD (Derevensky & Griffiths, 2019; Gainsbury, 2015).

1.3 Comorbidities

GD is highly comorbid with other psychiatric disorders, such as attention deficit hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD) and substance use disorder (SUD), among

others (Dell'Osso, Allen, & Hollander, 2005). Particularly, alcohol use disorder (AUD) is one of the most frequent comorbidities of GD, which has been shown to influence gambling episodes by decreasing self-control and increasing risk-taking (Baron & Dickerson, 1999; Zois et al., 2014), and has been linked to poorer response to GD treatment (Jiménez-Murcia et al., 2016). The diagnosis and treatment in individuals with co-occurring pathologies can be complicated by the interaction between the disorders, in which one condition might masked others, or in which behaviours could appear alternating with each other (Freimuth et al., 2008).

A cross-sectional study found that around 95% of responders that had GD during lifetime, also met the criteria of one or more other psychiatric disorders. Particularly, in almost 75% of the cases, at least one of the comorbid conditions began at an earlier age than GD, suggesting that some disorders might represent risk factors for developing GD, while GD might be a risk factor for developing other conditions. This study also found that although around 50% of responders with lifetime GD received some form of treatment (often to treat emotional problems or SUDs), none of the interventions were directed to treat GD (Kessler et al., 2008). It is crucial that treatment interventions are adapted to offer integrated strategies to tackle comorbid disorders successfully (Konkolÿ et al., 2016). Individual variation in GD has produced discrepancies in the literature (Singer, Anselme, Robinson, & Vezina, 2014), which highlights the complexity of finding adequate treatment protocols for each particular case. Therefore, there is an urgent need to investigate cognitive and physiological characteristics associated with GD in order to develop individualised treatment interventions.

1.4 Treatment strategies

The most common treatment interventions for GD include: gamblers anonymous (GA), which consist of mutual aid groups that share experiences and support each other in relation to these shared gambling problems (Choi et al., 2017); cognitive behavioural therapy (CBT), which integrates cognitive therapies that focus on changing cognitive distortions and erroneous beliefs, and includes behavioural interventions that attempt to identify triggers that might cause loss of control over gambling, and help determine how to regulate related behaviours; and motivational interviewing (MI), which addresses patients engagement with treatment and guide them to pursue healthier choices. Treatment interventions can be delivered in different setups: self-directed through workbooks or internet; remotely via telephone calls; or in person, which can be individual or in groups, and comprises between 1-20 sessions. Nonetheless, there are no officially validated treatment interventions for GD;

although CBT has been the form of treatment that showed to be most effective in GD (Petry, Ginley, & Rash, 2017).

Furthermore, pharmacological treatment has been prescribed to patients with severe gambling problems that seem not to benefit from therapy interventions. Double blind studies demonstrated the efficacy of the opioid antagonist naltrexone to improve GD severity (Hloch, Mladěnka, Doseděl, Adriani, & Zoratto, 2017). However, no drug has been officially approved for treating GD (Menchon, Mestre-Bach, Steward, Fernández-Aranda, & Jiménez-Murcia, 2018). Alternative novel interventions include: mindfulness based interventions (MBIs), which have been shown to reduce gambling-related symptomatology (Melero Ventola, Yela, Crego, & Cortés-Rodríguez, 2020); virtual reality, which seems to be a promising tool to investigate and treat substance use disorders (SUDs) and behavioural addictions (BAs; Segawa et al., 2020); and non-invasive brain stimulation (NIBS), which has shown certain efficacy to modulate addictive behaviours, including craving in SUDs and GD (Martinotti, 2019). Nevertheless, more research is clearly needed to identify specific protocols to target particular risk-factors associated with specific disorders, and to create individualised approaches for different types of patients.

1.5 Reward circuitry

To better understand the neurobiology of GD and its relationship with other conditions, it is essential to find more objective brain-based traits that serve as concrete markers of the underlying etiology of GD (Grant, Odlaug, & Chamberlain, 2016). The brain reward system evolved to promote essential behaviours such as feeding, sexual behaviour and social interactions (Gardner, 2011). In particular, the mesolimbic reward circuit consists of a dopaminergic pathway linking the ventral tegmental area (VTA) to the nucleus accumbens (NAc; see Figure 1-1).

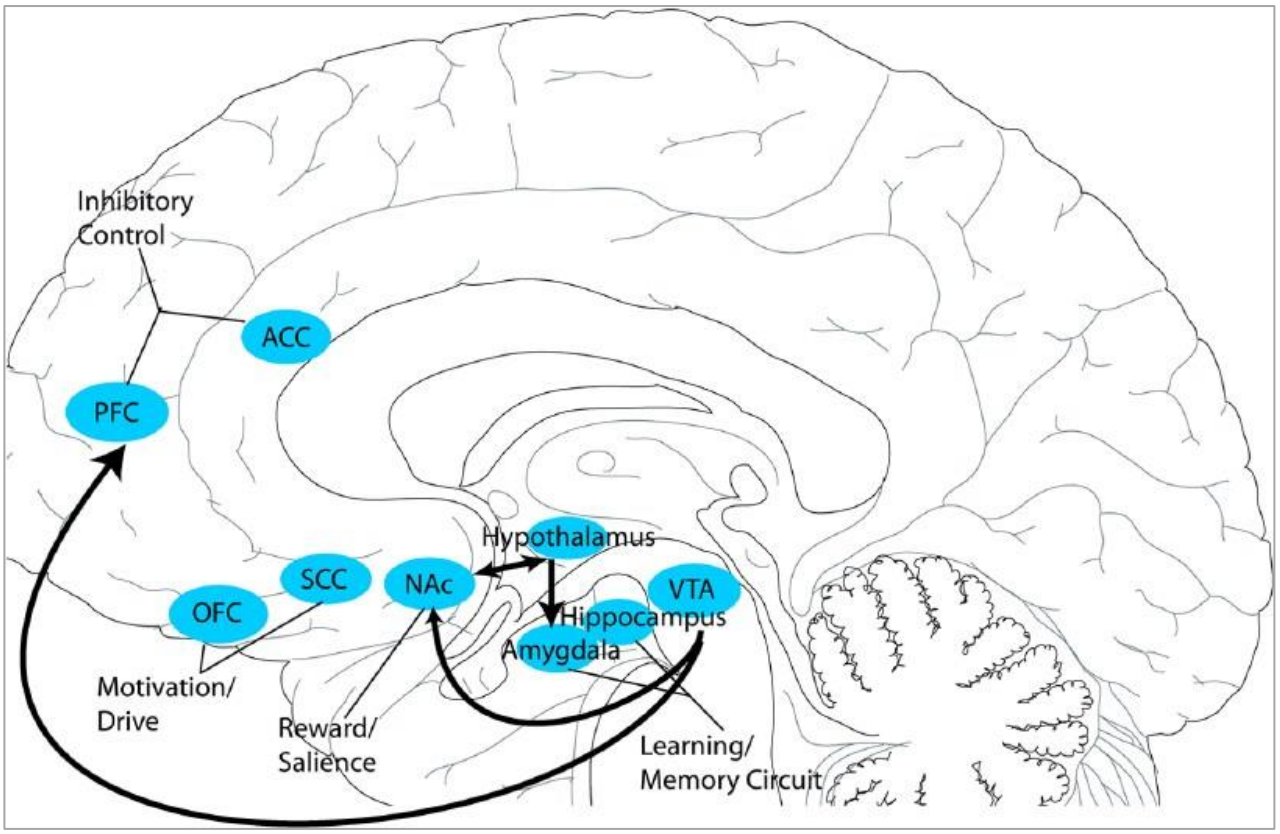

Figure 1-1. Reward system. Hypothalamic-mesocorticolimbic pathways involving reward, motivation, cognitive control and learning circuits (the arrows represent the direction of signal transmission). Inhibitory control has been associated with dorsal and medial prefrontal cortex (PFC) and the anterior cingulate cortex (ACC). Motivation and drive are associated with the orbitofrontal cortex (OFC) and the subgenual cingulate cortex (SCC). Reward and salience are associated with ventral tegmental area (VTA) and nucleus accumbens (NAc). Learning and memory are associated with the amygdala and hippocampus (Lee, Elias, & Lozano, 2018).

The function of NAc is related to the association of a specific value or desire to a stimuli, and has been shown to have a crucial role in reinforcing addictive behaviours (Berridge & Kringelbach, 2015; Koob & Volkow, 2010). The mesocortical pathway involves dopaminergic connections between VTA to the PFC, and is implicated in cognitive control, motivation and regulation of emotional responses (McClure, Laibson, Loewenstein, & Cohen, 2004). The VTA is a region composed largely by dopaminergic neurons that project to the NAc and to the PFC, but also to other areas, including the amygdala and hippocampus, which are associated with emotion and memory, respectively (Koob & Volkow, 2010). Dopaminergic projections from VTA to NAc can increase reward behaviour, whereas projections from VTA to the PFC have been associated with aversion (Cooper, Robison, & Mazei-Robison, 2017; Lammel, Lim, Ran, Huang, & Betley, 2012). External influences, such as drugs or monetary reward cues, have a direct influence on the reward pathways (Limbrick-Oldfield et al., 2017), and can compromise the natural function of this system (Quester & Romanczuk-Seiferth, 2015; Volkow, Wang, Fowler, Tomasi, & Baler, 2011).

The PFC has an essential role in emotion regulation and executive functions, including inhibition control (Brevet-Aeby, Brunelin, Iceta, Padovan, & Poulet, 2016), decision-making (Hämmerer, Bonaiuto, Klein-Flügge, Bikson, & Bestmann, 2016), impulsivity (Korponay et al., 2017), cognitive flexibility (Jansen et al., 2015), and monetary reward processing (Balodis et al., 2013). The orbitofrontal cortex (OFC) seems to be involved in punishment sensitivity (Cooper et al., 2017), and together with the subgenual cingulate cortex (SCC), is implicated in motivation and drive regulation, whereas the anterior cingulate cortex (ACC) is associated with interoception and inhibitory control (Volkow & Baler, 2014). Impairment of regions of the PFC that are involved in top-down regulation can result in the inability to control specific behaviours despite negative consequences (Zilverstand, Huang, Alia-Klein, & Goldstein, 2018). Specifically, these regions have been linked to dysfunctions in gambling-related decision-making and GD (Goudriaan, De Ruiter, Van Den Brink, Oosterlaan, & Veltman, 2010; Murch & Clark, 2016; Potenza et al., 2003).

As a key structure in the reward system, the amygdala has been shown to be closely related to anticipation of reward and cravings (Koob & Volkow, 2010). Cravings represent an intense desire for the reward stimuli (Volkow & Baler, 2014), and are one of the crucial mechanisms involved in addiction (Kober et al., 2016) that have been investigated as a potential target to modify addictive behaviours (Coles, Kozak, & George, 2018; Zack et al., 2016). Particularly, the amygdala is anatomically and functionally connected with ventromedial PFC (vmPFC; Zhang et al., 2014).

Interactions between the amygdala and vmPFC are mediated by the dorsolateral PFC (DLPFC). DLPFC is crucial for decision-making considering different sources of information, whereas vmPFC is more relevant in decisions based on reward values (Reuter et al., 2005; Zare-Sadeghi, Oghabian, Zare-Bidoky, Batouli, & Ekhtiari, 2019). Functional connectivity between DLPFC and vmPFC has been associated with increased self-control (Hare, Hakimi, & Rangel, 2014). In particular, increased activity in DLPFC was shown during attempts of self-control and reducing cravings (Shahbabaie et al., 2014). The inability to avoid particular addictive behaviours might arise from disruption in functional brain circuits. Top-down regulation, reflected by the cognitive control over the temptation for the reward stimuli, seems to be in part controlled by the DLPFC and vmPFC network. Therefore, investigating the role of these areas in gambling-related decision-making could provide insights into the mechanisms underlying addiction and GD (Fecteau, Pascual-Leone, et al., 2007; Gay et al., 2017; Potenza et al., 2003; Zare-Sadeghi et al., 2019).

1.6 Cognitive characteristics of gambling disorder

Individual differences in personality traits might help to identify risks for developing GD, and to disentangle specific characteristics of co-occurring disorders. In particular, GD has been widely associated with impulsivity, cognitive distortions and risk-taking behaviour. Impulsivity can be defined as a multidimensional construct, characterised by the tendency to rapid, poorly considered and disinhibited actions, despite negative consequences (Evenden, 1999). Impulsive decision-making might increase the acceptance of erroneous beliefs during gambling (Michalczuk, Bowden-Jones, Verdejo-Garcia, & Clark, 2011). These erroneous beliefs, known as cognitive distortions, imply for example that gamblers are susceptible to assume superstitions or rituals driven by an illusion of control, or have the sensation that can predict the outcome of a bet, and thus, fail to appreciate the random characteristics of the game (Raylu & Oei, 2004).

High impulsivity has been shown to precede general risky behaviours and gambling participation in college students (Cyders & Smith, 2008). In addition, pre-existing negative affect and inhibitory control deficits related with elevated impulsivity, have been suggested to be risk-factors associated with GD (Lobo & Kennedy, 2009; Slutske, Avshalom Caspi, Moffitt, & Poulton, 2005; Slutske et al., 2001). This is illustrated in a developmental work, which found that impulsivity predicted depressive symptoms and gambling behaviour, however once emerged, a mutual influence between the disorders

contributed to the maintenance of both pathologies (Dussault, Brendgen, Vitaro, Wanner, & Tremblay, 2011).

Moreover, higher delay discounting rates (preference for small immediate rewards rather than larger but more delayed rewards) were associated with GD compared with healthy controls, and in gamblers with comorbid SUDs compared with gamblers without comorbidity (Petry, 2001). Individuals with GD, with and without comorbid disorders, showed deficits in decision-making (Zois et al., 2014), which might also affect at-risk gamblers (Ioannidis, Hook, Wickham, Grant, & Chamberlain, 2019). In fact, it could be possible that cognitive deficits appear in people at risk before developing GD, or alternatively, that the progression of the disorder influences the emergence of these deficits (Hodgins, Stea, & Grant, 2011). Studies investigating GD symptomatology after periods of abstinence would provide insights into the nature of the alterations produced by the disorder. Nevertheless, whether some factors precede the development of GD, or if they appear as a consequence of it, is a question that is still under investigation (Rash, Weinstock, & Patten, 2016).

1.7 Physiological characteristics of gambling disorder

The behavioural manifestation of cognitive alterations in GD has a basis in functional brain changes involved in self-regulation (Goudriaan, Oosterlaan, De Beurs, & Van Den Brink, 2004). Neurotransmitter systems contribute to arousal, impulse control, reward processing and cravings (Leeman & Potenza, 2012a). Dopamine is one of the most commonly studied neurotransmitters in addiction, given that it has the function to mediate incentive motivation that promotes survival behaviours, and that addictive substances can hijack this circuitry developing a similar drive to pursue drugs, despite adverse consequences. GD is associated with dysfunctions in the dopamine system, which codes reward anticipation and outcome evaluation (Linnet, 2014). The association between dopamine and GD is emphasised by research revealing that dopaminergic medication prescribed to Parkinson´s disease patients can lead to the development of GD (Bhattacharjee, 2018; Dodd et al., 2005; Kelley, Duker, & Chiu, 2012). Nevertheless, dopamine activation in the brain can also occur by engaging in certain behaviours without a chemical agent hijacking the neural circuitry (Zack, George, & Clark, 2020). In this way, reward uncertainty activates dopaminergic systems during gambling, similarly to chronic exposure to drugs (Zack et al., 2014). Interestingly, dopamine is released during gambling episodes in response to unpredictable rewards, rather than by the reward

per se. Therefore, the inability to predict reward outcomes might determine the motivation to gamble (Anselme & Robinson, 2013).

In fact, reward uncertainty is a core part of the definition of gambling, which consists of wagering something of value on an event with an uncertain outcome (Derevensky & Griffiths, 2019). The definition of gambling involves other aspects of human and non-human animal behaviours. In ecological systems there is a natural order of gambling, of taking potential risks (with health, hierarchy, mate access etc.) in order to seek psychosocial, health and environmental gains, and improve survival. Nonetheless, while the dopaminergic function to keep the motivation to continue searching for food in unpredictable situations might have been an evolutionary advantage in nature, (in which most environments are probabilistic), in gambling, most games are entirely random, and this raises some problems for cognition (Clark, 2014). Usually people try to make sense of the environment, however in events involving chance, the lack of causality explaining specific outcomes results in the assumption of non-existent contingencies between independent events to predict future consequences, and these assumptions are expressed in form of cognitive distortions (Ladouceur, 2004), which were described in section 1.6 of this chapter.

Furthermore, the distinction between reward anticipation (defined as dopaminergic processes occurring before receiving the reward outcome), and the reward response, (defined as dopaminergic processes occurring after receiving the reward outcome), may have relevant implications to understand dopaminergic alterations in GD (Linnet, 2014). Similarly, cues associated with rewards following an unpredictable reward delivery, may induce dopamine activation and reinforce learning and repetition of specific behaviours, akin to the effects which drug cues produce in SUDs (Clark, Boileau, & Zack, 2018). Therefore, dopaminergic coding of uncertainty could account for neurobiological dysfunctions in GD, and the persistent gambling behaviour despite continuous losses (Linnet, 2020).

Altered dopaminergic activity might lead to increased affective reactions and impaired decision-making. According to the Somatic Marker Hypothesis (SMH), decision-making is based on the integration of emotions and affective information associated with reward and punishment events (Damasio, 1996). This hypothesis suggests that unconscious physical responses in the body, known as “somatic markers”, guide behaviour before conscious awareness. Somatic markers represent

feelings generated by secondary emotions that have been connected by learning processes, and help predict future scenarios. Heart rate (HR) and electrodermal activity (EDA) are somatic markers, which are regulated by the autonomous nervous system (ANS). The ANS can be separated into sympathetic and parasympathetic branches that are activated in a complementary way. The sympathetic nervous system (SNS) produces physiological changes associated with "flight or fight" responses to stress, including increased cardiac response and vasoconstriction, whereas the parasympathetic nervous system (PNS) is responsible of maintaining processes that are inhibited during stress, such as normal maintenance of internal organs or growth, associated with relaxation (Diamond & Cribbet, 2013).

Skin conductance responses (SCRs), as a form of EDA, and HR, change by exposure to emotional experiences, but can also be induced by activation of neural pathways, for example recalling past experiences, or thinking of a hypothetical situation. Research suggested that reduced SCRs might be associated with high impulsivity and altered dopaminergic activation in GD (Peterson et al., 2010a), and that SCRs could affect decision-making by altering dopaminergic transmission (Sevy et al., 2006). Therefore, somatic markers facilitate intuitive decision-making, and inadequate signalling of emotional responses to specific cues might produce the inability to make advantageous decisions. Indeed, patients with ventromedial prefrontal cortex (vmPFC) damage and individuals with SUDs showed decreased generation of somatic markers during gambling task performance (Bechara, Damasio, Tranel, & Damasio, 1997). The inability to inhibit gambling behaviour in individuals with GD might be due to reduced somatic responses to risky choices (Blaszczynski & Nower, 2002; Sharpe, 2002). Therefore, investigating somatic markers can reveal emotional states and personality traits, and the consideration of different autonomic measures can provide varied information. While EDA can be used to quantify aspects of emotional arousal, HR is more sensitive to the valence of emotional response and attentional and cognitive processing (Sohn, Sokhadze, & Watanuki, 2001). Therefore, investigating both components simultaneously might provide more detailed information about the sympathetic and parasympathetic balance in each individual (Angioletti, Siri, Meucci, Pezzoli, & Balconi, 2019).

Individual differences in ANS functioning reflect stress and emotion regulation capability, and therefore, are associated with social behaviour. The neurovisceral integration model suggests that PNS activity is associated with emotional self-regulation, which is fundamental for maintaining social relationships (Diamond & Cribbet, 2013). This model accounts for reciprocal relationships between autonomic function, cognitive performance and emotions, and suggests that increased activity in the

prefrontal cortex (PFC) is associated with increased parasympathetic and decreased sympathetic activity (Thayer & Lane, 2000). A recent study provided anatomical substrates for this model, showing that heart rate variability (HRV) served as an index of the integrative neural network, involving amygdala, dorsal and medial PFC and anterior cingulate cortex, which processed affective, cognitive and physiological responses towards goal-directed behaviour (Wei, Chen, & Wu, 2018). Consistently with the model, GD individuals have shown reduced autonomic activity, indicated by decreased SCRs and lower HR, and poorer gambling task performance, compared with healthy controls (Goudriaan, Oosterlaan, de Beurs, & van den Brink, 2006).

Dysfunctions in PFC and subcortical networks projecting to the PFC have been widely associated with addiction (Goudriaan et al., 2004; Rash et al., 2016). Autonomic activity has been shown to modulate the cingulate anterior cortex, vmPFC (Nagai, Critchley, Featherstone, Trimble, & Dolan, 2004) and dorsolateral prefrontal cortex (DLPFC) activity. Particularly, during reward delay the activity in orbitofrontal cortex was modulated by uncertainty, however, DLPFC activity was modulated by anticipatory arousal (Critchley, Mathias, & Dolan, 2001). This suggests that altered SCRs and HR in GD might inform about abnormal functioning of these brain areas (Goudriaan et al., 2006). The dorsal PFC has a central role in how PFC exerts control over behaviour, whereas the medial PFC is closely associated with limbic structures for long-term memory and processing of affect and motivation (Miller & Cohen, 2001). In particular, gamblers showed decreased dorsal PFC and medial PFC activation during control inhibition (Goudriaan et al., 2010; Potenza et al., 2003; Ruiter et al., 2009). Prefrontal control circuit alterations may underlie vulnerability to gambling cues, and reduced control over craving and negative emotions (Spagnolo, Gómez Pérez, Terraneo, Gallimberti, & Bonci, 2018).

Corticostriatal-limbic activity alterations have been associated with GD (Reuter et al., 2005), showing that decreased ventral striatum and ventromedial prefrontal cortex (vmPFC) activations during simulated gambling, might contribute to cognitive processes characterised by impaired impulse control in GD (Potenza, 2014). Increased sensation seeking was associated with dopamine activation in the striatum, which supports the idea that high impulsivity constitutes a risk-factor to develop addictive behaviours (Gjedde, Kumakura, Cumming, Linnet, & Møller, 2010). Therefore, dysfunctional neural networks, associated with genetic or developmental deficits, can destabilise the interaction between brain circuits, increasing the vulnerability to develop addiction (Volkow, Wang, Tomasi, & Baler, 2013). The investigation of cognitive and physiological characteristics associated

with the ANS functioning during gambling, might help understand better individual differences in GD, and therefore help identify particular risk-factors and biomarkers associated with GD, and support the development of more effective treatment interventions.

2 Chapter 2. Methods to investigate gambling disorder

2.1 Transcranial direct current stimulation

2.1.1 Mechanisms

The field of non-invasive brain stimulation (NIBS) englobes a wide range of interventional technologies designed to modulate activity within the nervous system, with the aim to achieve a therapeutic effect that modify pathological disorders (Bashir & Yoo, 2016). Transcranial direct current stimulation (tDCS) is a NIBS technique that delivers a very weak electric current to the scalp, increasing or decreasing cortical excitability, depending on the polarity of the stimulation. Generally, anodal stimulation increases cortical excitability by depolarising neurons, whereas cathodal stimulation decreases cortical excitability by hyperpolarising neurons (Nitsche & Paulus, 2000). However, the general polarity-specific effects of tDCS can invert due to the neuronal orientation, with axon orientation determining whether the current is excitatory or inhibitory (Kabakov, Muller, Pascual-Leone, Jensen, & Rotenberg, 2012).

The immediate effects of tDCS consist of changes of resting membrane potential and the modulation of neurons excitability. In addition, secondary after-effects of tDCS include long term potentiation (LTP) and depression (LTD) that induce long lasting effects in the nervous system. The effects of tDCS on plasticity are not only seen in the targeted area, but in the connected network, in which neuronal cells and non-neuronal cells, such as glia and vascular systems are also altered. The effects of tDCS have also been studied in relation to Glutamate and GABA mechanisms, including neuromodulators such as dopamine, serotonin and acetylcholine that are involved in neuroplasticity alterations, which are particularly relevant for the study of psychiatric disorders (Jamil & Nitsche, 2017). However, the basic physiological mechanisms of tDCS are still not well understood, and this constitutes a fundamental step towards creating potential clinical neuromodulation interventions.

Beyond the polarity dependent effects of the tDCS current, the stimulation intensity (or dose) has also been shown to alter the outcomes of neuromodulation studies. Higher anodal tDCS intensities have been associated with increases of cerebral blood flow (CBF), whereas higher cathodal tDCS intensities were associated with decreases of CBF (Jamil et al., 2020). In theoretical models or strictly controlled environments, such us in-vitro studies, it could be assumed that the current flow intensity in the brain increases linearly with the current applied. However, in the living brain the complexity of

these interactions grows. The electric field varies with individual anatomy, and the effects of the current are brain state dependent and can change according to the measurements employed (Esmaeilpoura et al., 2018). In fact, research revealed that cortical excitability after both, anodal and cathodal tDCS, showed a non-linear relationship with the current intensities delivered (Benwell, Learmonth, Miniussi, Harvey, & Thut, 2015; Jamil et al., 2017). However, further research is needed to better understand tDCS dose-response mechanisms, which is essential to create individualised neuromodulation interventions.

The majority of tDCS research has focused on investigating behavioural effects of the stimulation simply by placing electrodes over a target area and assuming that brain function will be modified, however the underlying neural mechanisms have often not been assessed. Research should also consider issues including tDCS intensity, the physics of current flow, and the relationships between cortical activity and behavioural performance (Giordano et al., 2017). Particularly, therapeutic interventions that aim to induce long-term behavioural changes should be able to demonstrate functional changes in the cortex, which would occur through modulation of synaptic plasticity (Stagg & Nitsche, 2011). The experimental manipulation of neuronal excitability with tDCS over specific neural circuits could be used to investigate causal relationships between brain activity and behaviour. This might inform about the physiological characteristics associated with typical and dysfunctional cognitive functioning, helping ultimately to identify potential biomarkers of gambling disorder (GD).

2.1.2 Specificity

Anatomical specificity of tDCS results from the accuracy of driving the electrical current to the brain target of interest, whereas functional specificity depends on ongoing activity of the targeted neuronal networks, with tDCS acting preferably on brain circuits that are already activated (Bikson & Rahman, 2013). Anatomical specificity of tDCS can be improved by the use of high definition (HD) montages involving multiple electrodes, and employing computational modelling approaches that investigate the most effective protocol, including intensity, direction of the current and electrode placement, to achieve a more focal effect on the desired brain target (Ruffini et al., 2013). However, the effects of tDCS depend also on the existent brain activity during stimulation. The nervous system is a dynamic entity, and therefore neuromodulation effects depend on brain state and neural plasticity (Bashir & Yoo, 2016). Brain state manipulations could be used as a functional priming of specific neural circuits (Silvanto, Bona, & Cattaneo, 2017). Recent research has shown that tDCS over DLPFC induced an increase of dopamine in the striatum, involving the reward-motivation network (Fonteneau et al.,

2018). Therefore, if tDCS can modulate dopamine signalling associated with motivational processes, coupling the stimulation with relevant cognitive tasks, such as cue-reactivity tasks in addiction (Dinur-Klein et al., 2014), might facilitate inherent learning processes that could help improve the functional specificity of potential treatment interventions (Bikson & Rahman, 2013; Spagnolo, Montemitro, Pettorruso, Martinotti, & Di Giannantonio, 2020). In fact, tDCS focality has been shown to be higher when combined with cognitive tasks (Lapenta, Minati, Fregni, & Boggio, 2013).

2.1.3 Potential therapeutic interventions

In recent years, research has been investigating tDCS as a potential neuromodulation intervention to restore the altered brain circuitry in psychiatric disorders, including GD (Dunlop, Hanlon, & Downar, 2017; Lefaucheur et al., 2017; Martinotti et al., 2018). As an emergent procedure, tDCS has certain advantages over other treatment approaches, such as therapy or pharmacological interventions. To begin with, tDCS is a non-expensive (Bashir & Yoo, 2016) and safe technique (Bikson et al., 2016; Lefaucheur et al., 2016), with varied capabilities, including the modulation of cognitive processes (Ouellet et al., 2015), adjusting neurophysiological brain circuitry (Hone-Blanchet, Edden, & Fecteau, 2016) and reduction of addiction severity and cravings (Martinotti et al., 2018), with substantially fewer associated adverse events compared with pharmacological medications (Kampman & Jarvis, 2015; Yip & Potenza, 2014). In addition, NIBS can be used in combination with other treatment interventions. Given that pharmacological medications, therapeutic interventions and cognitive tasks affect brain activity, coupling them with NIBS might help identify individual variability factors, and contribute to improve clinical treatment outcomes. Nevertheless, further research is needed to establish the most effective protocols to create clinical interventions for specific disorders, but the prospect of combining pharmacological and therapy interventions with NIBS, seems to be a promising approach for treating psychiatric disorders, including addiction (Spagnolo et al., 2020).

Various therapeutic interventions have been coupled with tDCS to guide functional specificity. For example, physical therapy coupled with tDCS has shown to promote recovery after brain injury (Edwards et al., 2009), and cognitive behavioural therapy (CBT) has also been shown to enhance modulatory effects of prefrontal stimulation (Tan, Hizli Sayar, Önen Ünsalver, Arat, & Karamustafalioğlu, 2015). Therefore, clinical interventions for GD might benefit from the combination of NIBS with CBT, which could offer further control of neural activity on the engaged brain network (Sathappan, Luber, & Lisanby, 2019). Studies should also consider that each brain area is involved in multiple tasks, therefore, even if the anatomical specificity of tDCS is high, the

functional effects of tDCS might not be specific, but instead, be associated with enhancement and inhibition of different cognitive processes simultaneously. Therefore, it is crucial to investigate the basis of tDCS specificity to develop protocols capable of targeting specific risk-factors associated with particular disorders (Bikson & Rahman, 2013).

Beyond tDCS specificity that depends on the protocols used, and that also changes according to individual variability factors, such as patients anatomy and comorbidities (Bashir & Yoo, 2016), another aspect that seems to affect the results of neuromodulation interventions is the number of stimulation sessions. Multiple session interventions seem to produce an accumulative effect of the stimulation (Schluter, Daams, van Holst, & Goudriaan, 2018; Ulam et al., 2015), showing increasing tDCS effects after each session (Boggio et al., 2009). The frequency of stimulation can led also to different cumulative effects of tDCS, with daily stimulation showing increased changes in cortical excitability compared with stimulation every two days (Alonzo, Brassil, Taylor, Martin, & Loo, 2012).

2.1.4 Research in gambling disorder

Few studies have investigated the use of NIBS in behavioural addictions (Gomis-Vicent, Thoma, Turner, Hill, & Pascual-Leone, 2019). Specifically, a recent systematic review found 11 studies investigating NIBS effects in GD (Zucchella, Mantovani, Federico, Lugoboni, & Tamburin, 2020), from which only four were conducted using tDCS (Dickler et al., 2018; Martinotti et al., 2018; Martinotti, et al., 2019; Soyata et al., 2018). All NIBS studies targeted DLPFC, and one targeted medial PFC (Zack et al., 2016). The selection of the tDCS target was based on the interest of studying altered functions in GD, involving the cognitive control circuit, which includes the medial PFC, the DLPFC, orbital and vmPFC and the anterior cingulate cortex (Van Holst, Van Den Brink, Veltman, & Goudriaan, 2010). The cognitive control circuit provides flexibility to adapt to the changing environment by producing task processing strategies, attentional allocation and managing information interferences, and inhibition of inappropriate responses (Huster, Enriquez-Geppert, Lavallee, Falkenstein, & Herrmann, 2013). In particular, tDCS studies targeted DLPFC bilaterally, with the intention to modulate simultaneously cravings with anodal tDCS over the left side, and impulsivity, with anodal tDCS over the right side (Boggio et al., 2010; Fecteau, Knoch, et al., 2007; Martinotti et al., 2018). However, the evidence on the use of tDCS in GD is still very limited and heterogeneous, with no consistency in protocols and outcome measures employed across studies (Zucchella et al., 2020).

2.1.5 Effects on physiological measures

Research started to use electroencephalogram (EEG) measures to disclose possible tDCS effects on spontaneous cortical activity (Mangia, Pirini, & Cappello, 2014), and studies with varied protocols revealed different outcomes. For example, studies using the same stimulation target have produced varied results, with anodal tDCS over left DLPFC being associated with a decrease of delta power in one study (Keeser et al., 2011), but producing an increase of frequencies below 15 Hz (including delta band) in another study (Boonstra, Nikolin, Meisener, Martin, & Loo, 2016). Additionally, since the first reports in the 1960s about the capability of tDCS to modulate autonomic control (Bindman, Lippold, & Redfearn, 1964; Costain, Redfean, & Lippold, 1964), remarkably, very few studies have continued to investigate this (Clancy, Johnson, Raw, Deuchars, & Deuchars, 2014). NIBS could be applied to investigate the ANS function, and at the same time, ANS measures could inform about neurophysiological mechanisms underlying NIBS (Schestatsky, Simis, Freeman, Pascual-Leone, & Fregni, 2013). Research protocols and results in this field are heterogeneous too, showing that tDCS over the motor cortex increased sympathetic activation, indexed by heart rate variability (HRV) measures (Clancy et al., 2014), and that tDCS over right inferior frontal cortex decreased skin conductance responses (SCRs), indicative of sympathetic activation (Herrmann et al., 2018). Similarly, tDCS over DLPFC produced an increase in parasympathetic arousal by increasing high frequency (HF) HRV power, while no affecting sympathetic activation (Boonstra et al., 2016). In addition, NIBS research using repetitive transcranial magnetic stimulation (rTMS) in GD found no variations in clinical outcomes, but rTMS effects on autonomic measures, showing a decrease in diastolic blood pressure (Zack et al., 2016).

The results of these studies highlight the potential impact of investigating the capability of NIBS to study cortical activity and autonomic control mechanisms, and to directly modulate the ANS and associated cognitive processes. Furthermore, these studies emphasise the significance of using physiological measures to quantify the effects of NIBS interventions, and that relaying only on the use of clinical or behavioural outcomes might not allow to detect specific effects on the underlying brain circuits (Gomis-Vicent et al., 2019). Many questions remain to be investigated, such as optimal stimulation protocols, including selection of anatomical and functional targets, duration, intensity and direction of the stimulation, and generally, how to adapt neuromodulation procedures to account for inter-individual differences.

2.2 Physiological measures

Autonomic arousal influences decision-making and motivational behaviour (Bechara et al., 1997), features that have been linked to addiction and GD (Peterson et al., 2010b; Schutter & Van Honk, 2005; Worhunsky, Potenza, & Rogers, 2017). Previous experiments have shown cortical and subcortical effects on sympathetic arousal control (Critchley et al., 2001; Patron, Mennella, Messerotti Benvenuti, & Thayer, 2019; Zhang et al., 2014). For example, prefrontal cortex EEG activation has been associated with autonomic control of heart rate (HR) at rest (Patron et al., 2019), and vmPFC functional activation lead to a decrease of skin conductance (Zhang et al., 2014). Dysfunctional alterations on cortical plasticity have been shown to reduce the capability of PFC to provide executive control over addictive behaviours (Kalivas & Volkow, 2005). In particular, GD has been associated with altered serum brain derived neurotrophic factor (BDNF), indicative of synaptic plasticity (Choi et al., 2016; Geisel, Banas, Hellweg, & Müller, 2012). Individual variability factors, such as cortical plasticity, have been shown to influence the response to NIBS (Cheeran et al., 2008), and in turn, NIBS has the capability to induce plasticity like effects (Huang et al., 2017). Therefore, altered cortical plasticity in participants with GD might result in different responsiveness to tDCS.

Cortical excitability can be modulated with tDCS, which holds promise as a means to improve addiction interventions. However, a fundamental question that needs to be investigated is the existent variability in tDCS results, which are to a large extent subject to participant individual differences. Individual variability factors that influence the response to tDCS can be investigated through the analysis of autonomic responses, including electrodermal activity (EDA) and cardiovascular function, indicative of sympathetic/parasympathetic balance (Clancy et al., 2014; Feeser, Prehn, Kazzer, Mungee, & Bajbouj, 2014; Santarnecchi et al., 2014). Neuroimaging techniques, such as EEG, could also help to identify individual differences that might affect the response to interventions, and to quantify the physiological effects of tDCS (Accornero et al., 2014; Keeser et al., 2011; Ulam et al., 2015). Therefore, research exploring a more complete understanding on the individual cognitive and physiological characteristics associated with different types of gamblers, and the capabilities of tDCS to directly modulate autonomous nervous system (ANS) processes, might contribute to the development of specific neuromodulation protocols as potential individualised treatment interventions for GD.

2.2.1 Electrodermal activity

Electrodermal activity (EDA) reflects autonomic changes in the electrical properties of the skin (Boucsein, 2012), and has been proposed to be one of the most reliable measures of changes in emotional and cognitive states in association with sympathetic activity, without parasympathetic activity signal interferences (Braithwaite et al., 2015). Specifically, EDA can be used to measure attentional processing and emotional responses that might happen unconsciously (Nagai et al., 2004). Skin conductance responses are generated by sweat secretion, and are measured by applying an electrical potential between two points that result in a current flow. Skin conductance is measured in micro Siemens (μS), and refers to the capability of the skin to conduct electricity when a direct constant voltage is applied externally (Figner & Murphy, 2011). EDA complex includes two main components: the tonic skin conductance level (SCL), which is a measure of slower background activity and general changes of arousal, and the skin conductance responses (SCRs), which constitute the phasic component that changes more rapidly after exposure to specific stimulus (Braithwaite et al., 2015).

The majority of physiological research on gambling has measured average changes on tonic arousal over periods of several minutes. However, the identification of arousal responses to specific gambling features, such as reward anticipation and outcome phases or responses to wins and losses, requires the use of phasic measures (Lole, Gonsalvez, Barry, & Blaszczynski, 2014). The physiological responses during gambling are affected by several processes. Gambling trials usually involve a decision phase, during which participants are presented with a choice; an anticipation phase, during which participants wait to receive the reward outcome; and the outcome phase, during which participants receive the reward outcome. Research investigating separately these processes might reveal insights into the underlying mechanisms of gambling behaviour more accurately, helping to identify target measures to investigate in GD (Agren, Millroth, Andersson, Ridzén, & Björkstrand, 2019).

2.2.2 Electrocardiogram

An electrocardiogram (ECG) measures cardiovascular responses, including heart rate variability (HRV), which reflects the changes in time intervals between adjacent heart beats, and is generated by brain-heart interactions and ANS processes. HRV levels are associated with executive functions and emotional processing (Luque-Casado, Perales, Cárdenas, & Sanabria, 2016; Wei et al., 2018). In clinical terms, HRV frequency-domain measurements estimate the distribution of absolute and

relative power into frequency bands, including low frequency (LF) band from (0.04-0.15 Hz) and high frequency (HF) band from (0.15-0.40 Hz). The LF/HF ratio can be used to estimate the balance between sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). The LF band is typically measured during a minimum period of two minutes, and is assumed to be produced by sympathetic-parasympathetic activity with a dominant sympathetic component. The HF band is typically measured during a minimum period of one minute, and is associated with parasympathetic activity. Therefore a high LF/HF ratio will reflect sympathetic dominance (Park et al., 2019; Shaffer & Ginsberg, 2017), which can be due to an increase of sympathetic activity, and/or a decrease of parasympathetic activity (Clancy et al., 2014). Absolute power is measured in milliseconds (ms) squared divided by cycles per second (ms^2/Hz). Relative power is calculated dividing the absolute power for a specific frequency band by the summed absolute power of LF and HF bands, and is expressed in percentage of total HRV or in normal units (nu). In terms of the recording length for HRV, short term measurements have been widely used in research and are based on five minutes of data (Berntson et al., 1997; Report, 1996).

Heart rate (HR) is measured in beats per minute (bpm). Faster HR results from lower time between successive heartbeats, and consequently lower HRV. Inversely, slower HR is associated with longer times between heartbeats, which increases the opportunities for the inter beat interval to vary, and therefore results in higher HRV (McCraty & Shaffer, 2015). HR can be used to measure phasic responses, for example showing that HR responses during risky choices vary depending on the probability of winning or losing (Studer & Clark, 2011). The investigation of physiological factors underlying individual variance has clinical relevance to develop addiction interventions (Studer, Scheibehenne, & Clark, 2016). HRV has previously been used in research as a predictor of behavioural results (Pappens et al., 2014), and is widely used measure of physiological response to stress and changes in mental load (Luque-Casado et al., 2016). The type of information that EDA and cardiovascular measures can provide is diverse. While EDA activity indicates emotional arousal, HR is more sensitive to the valence of the emotional response, attention and cognitive processing (Sohn et al., 2001). Therefore, research using simultaneously both types of physiological measures may provide more precise information about individual physiological responses associated with GD (Angioletti et al., 2019).

2.2.3 Electroencephalogram

Electroencephalography is a neuroimaging technique that measures the electrical activity of underlying neurons. The neurons membrane maintain a voltage gradient due to differences in charged ions of sodium, potassium, chloride and calcium. When the voltage changes significantly due to the movement of ions across the membrane channels, an action potential is generated, which electrical activity can be measured and displayed as a brain wave. This pulse is transferred from one neuron to another neuron across a connection known as synapse (Zhang, 2019). Electroencephalogram (EEG) measures current flows during synaptic excitation in the cortex. EEG can measure electrical activity only from large populations of active neurons, as the current has to pass through different neuronal layers, skull and skin. This is done by electrodes placed on the scalp that amplify massively the electrical signals detected (Teplan, 2002). Therefore, EEG power represents the sum of neurons firing synchronously, which might reflect cortical information processing performance (Kanda, Anghinah, Smidth, & Silva, 2009). EEG has a high temporal resolution, and this together with its non-invasive characteristics, makes it a widely used method to study brain activity during affective responses (Rajamanickam Yuvaraj et al., 2014).

Quantitative EEG involves power spectral analysis of frequency band waves, such as delta (1–4 Hz), theta (4–8 Hz), alpha (8– 12 Hz) and beta (12–30 Hz). Previous research suggested that slow waves power (delta and theta bands) is associated with low cortical arousal, whereas beta power is associated with higher excitability of the central nervous system (Kim, Choi, Lee, & Kim, 2018). A recent review investigating EEG spectral analysis in different psychiatric disorders, including addiction, found a high variability on EEG data reported in research. The majority of studies investigated absolute power, which measures the amplitude of electrical activity in microvolts (μV). In addition, some studies included also relative power, usually calculated by dividing the power of each band by the sum of power across all bands. Although absolute power has been more widely used, relative power results were more consistent between studies, so the consideration of both measures would allow to produce more reliable interpretations. In addition, results were reported for broad cortical regions or individual channels, and using eyes opened or eyes closed conditions.

The review found that across all psychiatric disorders, lower frequency bands (delta and theta) were generally associated with power increases, whereas decreases in power dominated alpha band, and particularly beta band was associated with either increases or decreases of power, compared with healthy controls. Overall, psychiatric disorders were associated with decreased theta/beta ratio. In

addition, results showed that correlations between band power and symptomatology were not specific of any disorder, however this might be due to the overlap of similar symptoms and comorbidities across different disorders (Newson & Thiagarajan, 2019). Therefore, further research is needed to better understand individual differences in cortical excitability, and whether specific EEG frequency bands are associated with GD and different responsiveness to tDCS.

2.3 Main aims of the research project

With the current GD prevalence rates ranging from (0.7–6.5%) during lifetime worldwide (Calado & Griffiths, 2016), which represents an increasing public health concern (Shaffer & Korn, 2002), there is an urgent need to develop treatment strategies for GD (Paglieri et al., 2014). Cognitive behavioural therapy (CBT) has become the most widely used treatment intervention for GD (Menchon et al., 2018), however novel methodologies such as tDCS, might offer improved treatment opportunities for GD. Research has shown that there is not a unique mechanism explaining how all neuromodulation interventions act (Bikson & Rahman, 2013; Spagnolo et al., 2020). While tDCS might be used as a tool to investigate how behaviours arise from underlying neuronal circuitry, and ultimately be used as an intervention to modulate these mechanisms, multiple influencing factors should be examined. These include the investigation of neural networks targeted and specific stimulation protocols, short and long term effects, electrical current interactions with individual characteristics, comorbid disorders and simultaneous therapeutic treatment interactions (Bashir & Yoo, 2016). With such complexity involving numerous interrelated factors, it is fundamental to quantify the effects of the stimulation with methods that allow the measurement of functional and anatomical changes, such as neuroimaging and physiological techniques, in combination with cognitive measurements, to allow a more complete understanding of neuromodulation effects.

To investigate some of these questions, four experiments were conducted throughout the project. In Experiments I and II, a two session crossover design was used to investigate tDCS effects over different brain areas in non-gambler and gambler participants. Furthermore, in Experiment III, the cumulative effects of tDCS combined with cognitive behavioural therapy (CBT) across eight sessions were investigated in two patients diagnosed with GD. Lastly, in Experiment IV, the effects of tDCS on the autonomous nervous system (ANS) were investigated with a two session crossover design, in low and high risk gamblers. An overview of the aims and methodology used across experiments is represented in Figure 2-1.

The main aims of the project were:

- To investigate the effects of tDCS on different participant samples involving non-gamblers, at risk gamblers and individuals with GD (Experiments I, II, III and IV).
- To investigate the effects of tDCS during gambling task-performance using protocols designed to target different brain areas associated with GD (Experiments I and II).
- To investigate the cumulative effects of tDCS across sessions combined with CBT (Experiment III).
- To investigate the effects of tDCS on cortical excitability and the ANS (Experiment IV).
- To investigate potential risk-factors associated with GD by exploring different cognitive and physiological responses associated with gambling behaviour in different participant samples (involving low risk and high risk gamblers) during different gambling phases (anticipation, outcome) and reward valence (wins, losses; Experiment IV).

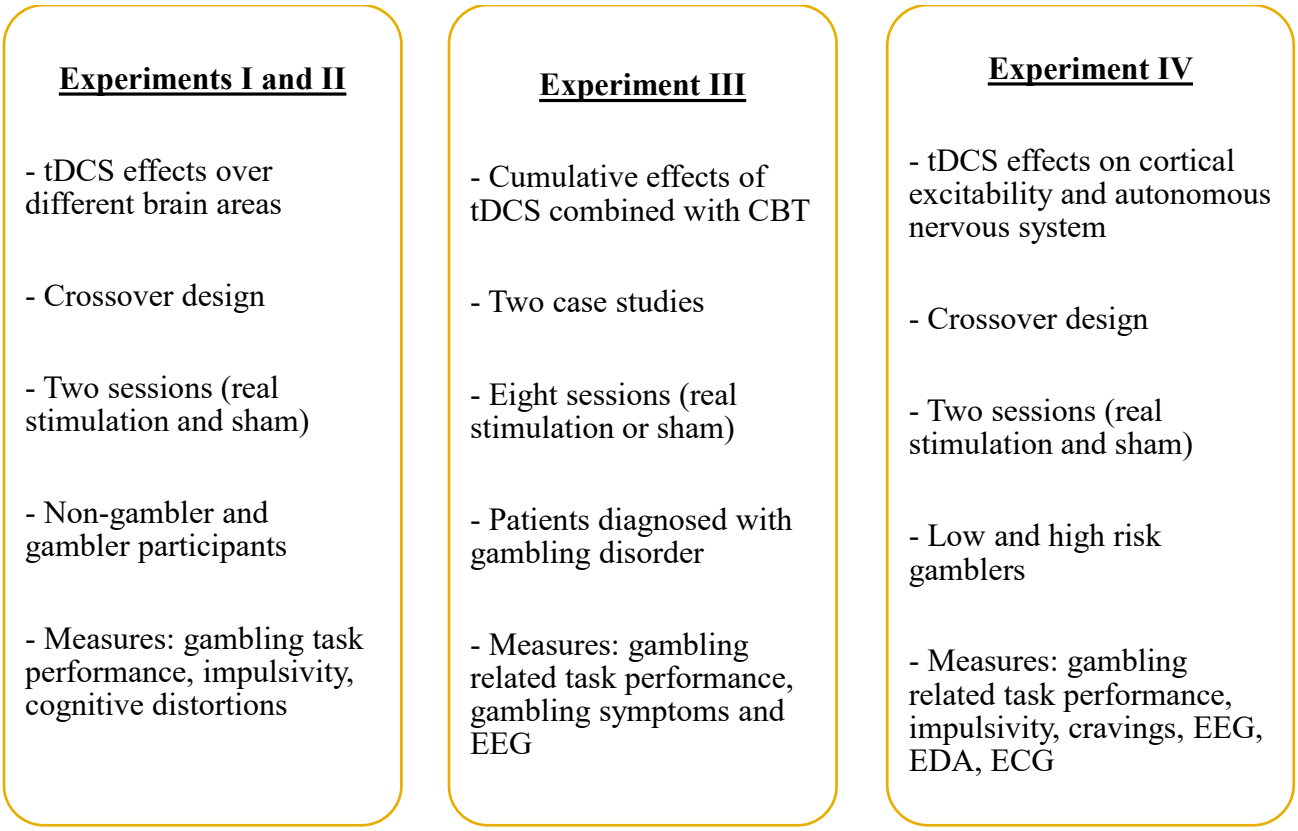

Figure 2-1. General overview of the research project. Simplified summary of aims and methodology used in the experiments.

3 Chapter 3. Experiments I and II: effects of tDCS montages designed to target rDLPFC and vmPFC on gambling task performance

3.1 Summary

Two experiments were conducted to investigate the effects of high definition (HD) tDCS montages with a ring configuration (1x4), on different brain areas. Non-gambler and gambler participants were divided according to their self-reported impulsivity levels into two groups: high impulsive (HI) and low impulsive (LI). In Experiment I, two tDCS montages were designed to target right dorsolateral prefrontal cortex (rDLPFC) and ventromedial prefrontal cortex (vmPFC), brain areas associated in general with decision-making and reward processing, respectively. Results showed significant tDCS effects on gambling task performance, but no difference between tDCS effects depending on brain target or participant groups. Particularly, tDCS was associated with higher quality of decision-making (QDM) and risk-taking (RT) behaviour, but did not affect delay aversion (DA). In Experiment II, the tDCS montage to target rDLPFC was modified by increasing the distance between the anode and the return electrodes, to investigate potential focality differential effects of tDCS between both brain regions. In this case, the tDCS montage designed for vmPFC used in Experiment I was compared with a new montage designed to target rDLPFC. Results in Experiment II replicated the findings from Experiment I. In conclusion, the significant tDCS effects found on cognitive-task performance, together with the lack of significant differences identified on tDCS effects between both brain areas, emphasised the need for further research investigating neuromodulation effects over this circuitry in combination with neuroimaging techniques.

3.2 Introduction

Gambling disorder (GD) has been associated with specific personality traits, including deficits in decision-making and increased impulsivity, RT behaviour, DA, negative affect and cognitive distortions (Cyders & Smith, 2008; Lobo & Kennedy, 2009; Michalczuk et al., 2011; Petry, 2001; Slutske et al., 2005; Zois et al., 2014). Cognitive alterations can be linked to functional brain changes (Goudriaan et al., 2004). In particular, decreased activation during inhibition control was associated with decreased dorsal prefrontal cortex (PFC) and medial PFC, in gamblers (Goudriaan et al., 2010; Potenza et al., 2003; Ruiter et al., 2009). The capability of PFC to exert control over addictive behaviours is affected by alterations on cortical plasticity (Kalivas & Volkow, 2005), and particularly,

GD has shown altered synaptic plasticity in previous research (Choi et al., 2016; Geisel, Banas, Hellweg, & Müller, 2012). Addictive behaviours have been associated with dysfunctions on dorsolateral prefrontal cortex (DLPFC) and ventromedial prefrontal cortex (vmPFC) areas in various studies (Coles et al., 2018; Cyders et al., 2014; Genauck et al., 2017; Park et al., 2010). The role of these areas was associated with the expression of personality characteristics, such as impulsivity and RT behaviour (Brevet-Aeby et al., 2016; Fauth-Bühler, Mann, & Potenza, 2017; Verdejo-García, Lawrence, & Clark, 2008). In fact, similar decision-making processes might underlie delay discounting and RT, which are associated with subjective value of reward (Brevet-Aeby et al., 2016). Broadly, DLPFC has been linked to cognitive functions, such as decision-making, whereas vmPFC has been associated with reward processing (Koenigs & Grafman, 2009)[1].

Transcranial direct current stimulation (tDCS) can be used for the manipulation of cortical excitability of specific brain networks associated with GD. This might inform about individual differences and risk-factors that could be targeted with future neuromodulation interventions. However, there is a high variability in tDCS results across studies, in part due to individual variability, the heterogeneity of methodologies used, and in general, the lack of understanding of which protocols are more effective to target specific symptoms. Individual variability factors, such as cortical plasticity, can influence the particular response to NIBS (Cheeran et al., 2008). Regarding the methodologies used, high definition (HD) tDCS montages, involving multiple electrodes, can be used to improve tDCS anatomical specificity by avoiding shunting of electrical current to broader brain areas, and therefore help to investigate neuromodulation effects of tDCS on specific cortical regions (Villamar et al., 2013). Specifically, electrode distance and size of electrodes have been shown to affect stimulation focality (Moliadze, Antal, & Paulus, 2010; Nitsche et al., 2007). Configurations of HD tDCS montages can be modified to improve stimulation targets (Dmochowski, Datta, Bikson, Su, & Parra, 2011). In particular, the 4x1 ring configuration uses a central electrode placed over the brain target of interest, and which determines the polarity of the stimulation, surrounded by four return electrodes. The radius between the central electrode and the return electrodes limit the area of stimulation, resulting in higher target focality, compared with traditional bipolar montages (Datta et al., 2009). In addition, tDCS acts preferably over brain areas that are already activated. Therefore, coupling tDCS with cognitive tasks designed to investigate gambling-related behaviours, such as the Cambridge gambling task (CGT), might improve functional specificity by facilitating tDCS neuromodulation

[1] Prefrontal cortex subdivisions (rDLPFC and vmPFC) functions were discussed in more detail in the general introduction in Chapter 1.

effects over relevant cognitive processes associated with GD (Bikson & Rahman, 2013; Dinur-Klein et al., 2014; Lapenta et al., 2013).

Most non-invasive brain stimulation (NIBS) studies in addiction that showed reduction of symptoms (e.g. impulsivity, cravings) used montages designed to target DLPFC (Coles et al., 2018). Specifically, all the studies using NIBS in GD targeted DLPFC, and one study also targeted medial PFC (Zack et al., 2016; Zucchella et al., 2020). Apart from NIBS research in GD, other tDCS studies found that anodal tDCS over rDLPFC was associated with a reduction of RT behaviour in a sample of impulsive individuals (Gilmore, Dickmann, Nelson, Lamberty, & Lim, 2018), and also in samples of healthy volunteers, who chose the safest options more often under anodal tDCS compared with sham, indicative of more advantageous decision-making performance associated with tDCS (Fecteau, Knoch, et al., 2007; Gorini, Lucchiari, Russell-Edu, & Pravettoni, 2014).

Brain imaging research also identified a dysregulation of the reward-circuitry related to vmPFC in both substance use disorders (Konova et al., 2019) and behavioural addictions (Yoon et al., 2017). Particularly in GD, cognitive alterations were identified consistently in relation to risk-reward decision-making, associated with vmPFC, rather than DLPFC (Potenza, 2014). More recently, tDCS research has started to investigate ventral PFC areas. Particularly, a reduction of negative emotions and mind-wandering was associated with tDCS stimulation of medial PFC, and right ventrolateral PFC (Abend et al., 2019; Bertossi, Peccenini, Solmi, Avenanti, & Ciaramelli, 2017; Vergallito, Riva, Pisoni, & Romero Lauro, 2018). Decision-making and impulse control were improved after tDCS over orbitofrontal cortex (OFC; Ouellet et al., 2015). Moreover, while impulsive behaviour was reduced after anodal stimulation of vmPFC, cathodal stimulation was associated with increased impulsivity levels (Manuel, Murray, & Piguet, 2019). Evidence revealed functional connectivity between both areas during reward-based decisions (Hare et al., 2014). Higher connectivity between rDLPFC and vmPFC was shown when fairness and self-interest were in conflict in healthy individuals, demonstrating the role of this circuit on decision-making and reward processing (Baumgartner, Götte, Gügler, & Fehr, 2012). Therefore, further exploration of the potential effects of neuromodulation on these areas is necessary to better understand their specific contribution to dysfunctional behaviours in GD. Interventions could be directed to decreasing impulsive reward circuit activity, or to increasing the executive control circuit. Most interventions have focused on the latter, however evidence shows support for investigating both strategies (Hanlon, Dowdle, & Scott Henderson, 2018).

The main objectives of Experiments I and II[2] were to investigate whether different HD tDCS montages designed to target right DLPFC (rDLPFC) and vmPFC would be associated with different gambling-related cognitive outcomes, and to investigate whether different addiction-related participant characteristics would be a dependent factor of the effects of tDCS on gambling task performance. Specifically, it was hypothesised that tDCS real stimulation would be associated with improved decision-making and reduced RT and DA compared with sham. It was also hypothesised that tDCS over rDLPFC would be related to increases in decision-making, whereas stimulation of vmPFC would be more linked to RT and DA reduction, compared with sham. Lastly, it was hypothesised that individuals with higher GD severity and impulsivity levels would show poorer cognitive outcomes, including decreased decision-making and increased RT and DA.

3.3 Methods

3.3.1 Participants

In Experiment I, 24 participants were recruited, and 40 participants in Experiment II. Recruitment was carried out through advertisements within the University of East London and participants were compensated for their time with shopping vouchers. Participants were allocated into two groups according to the tDCS montage that they were tested with: rDLPFC (n=12, mean age 27.92 ± 4.48 years) and vmPFC (n=12, mean age 26.92 ± 3.26 years) in Experiment I, and rDLPFC (n=19, mean age 24.32 ± 6.71 years) and vmPFC (n=21, mean age 26.86 ± 7.35 years) in Experiment II. Furthermore, considering participants impulsivity levels measured with the negative urgency (NU) trait of the urgency premeditation perseverance sensation seeking scale (UPPS-P NU), two groups were created using NU trait scores median split (low impulsive (LI, n=12, 28 ± 3.27 years, and high impulsive (HI), n=12, 26.83 ± 4.45 years) in Experiment I, and (LI, n=21, 27.62 ± 7.61 years and HI, n=19, 23.47 ± 5.90 years) in Experiment II. In Experiment I, UPPS-P NU scores range by group was (LI=15-26; HI=27-40), and (LI=13-26; HI=27-40) in Experiment II. Participants were screened for the study inclusion criteria. This included male or females between 18-65 years who could speak and read English, capable of giving informed consent**,** and not having any of the exclusion criteria (based on non-invasive brain stimulation safety recommendations).

[2] Experiments I and II are presented together in the same chapter because the same rational, methodology and outcome measures were used in both experiments, and the results are interrelated and complement each other. Therefore, it was considered that this format might simplify the presentation and understanding of the experiments and avoid repetition.

Exclusion criteria

a. History or evidence of chronic or residual neurological disease.

b. A pacemaker.

c. Metal implants in head or neck area (e.g. postoperative clips after intracerebral aneurysm; arterial aneurysm in the vascular system, implantation of an artificial hearing aid).

d. Intracerebral ischemia/history of bleeding.

e. Prior evidence of epileptic seizures, history of epilepsy.

f. History of head injury with loss of consciousness.

g. Any serious medical conditions (disease of the internal organs).

h. Pregnancy or breast-feeding.

The sample size was planned based on previous studies using similar tDCS with protocols involving a two session crossover design, in different addictions, including: smoking with samples of 20 participants (Fecteau et al., 2014), and 24 participants (Fregni et al., 2008) and alcohol use disorder with a sample of 13 participants (Boggio et al., 2008).

The original plan was to group participants according to their gambling severity scores using the South Oaks gambling screen (SOGS), however, the number of participants who classified as disordered gamblers was low. It was therefore decided to group participants according to their impulsivity scores using the UPPS-P NU, which helped increase group sizes and strengthened the analyses. Impulsivity has been broadly linked to addictive behaviours (Clark, Robbins, Ersche, & Sahakian, 2006; Leeman & Potenza, 2012b) and in particular, the NU trait has been shown to be strongly associated with GD (Albein-Urios, Martinez-González, Lozano, Clark, & Verdejo-García, 2012; Cyders et al., 2007; Michalczuk et al., 2011; Mick et al., 2016; Navas et al., 2017). Specifically, the NU trait of impulsivity has been described as a risk-factor factor influencing the development and maintenance of addictive behaviours (Boothby, Kim, Romanow, Hodgins, & McGrath, 2017; Mallorquí-Bagué et al., 2018; Rømer Thomsen et al., 2018). In addition, grouping participants by impulsivity levels is supported by previous research that created different impulsive groups with GD participants (Lee et al., 2017a),

and substance use disorder (SUDs) participants, who were grouped using median split of impulsivity scores (Tziortzis, Mahoney, Kalechstein, Newton, & La Garza, 2011).

3.3.2 Materials

A tDCS Starstim 8 tDCS device (Neuroelectrics, Barcelona) was used with Ag/AgCl Pistim electrodes with a circular contact area of π cm², filled with Signagel conductive saline gel. The Cambridge Neuropsychological Test Automated Battery (CANTAB) Cambridge Gambling Task (CGT) was used to measure quality of decision-making (QDM), risk-taking (RT) and delay aversion (DA). The UPPS-P was used as a measure of self-reported impulsivity. Gambling severity was measured with the SOGS. The questionnaire Gambling Related Cognition Scale (GRCS) was used to measure cognitive distortions. To control for alcohol dependence, participants completed the Severity of Alcohol Dependence Questionnaire (SADQ).

- The South Oaks gambling screen (SOGS; Lesieur & Blume, 1987): it is a 20-item self-report questionnaire based on Diagnostic and Statistical Manual (DSM)-III criteria to screen for life-time pathological gambling. It has shown to have high validity and internal consistency reliability. Some examples of the questions are: 'have you ever claimed to be winning money gambling but weren´t really? in fact, you lost?'; 'have you ever felt guilty about the way you gamble?'; 'have you ever borrowed money from someone and not paid them back as a result of gambling?'.

- The Urgency Premeditation Perseverance Sensation Seeking scale (UPPS-P; Cyders et al. 2007): it is a 59-item self-report questionnaire using a Likert scale, from 1 (I agree strongly) to 4 (I disagree strongly), to assess five impulsivity subscales, including: urgency (inability to inhibit action impulses, especially in a negative motivational state despite long-term consequences); premeditation (inability to anticipate the consequences of one's actions); perseverance (inability to continue with boring or difficult tasks); sensation seeking (tendency to seek novel situations). Some examples of subscales questions are: negative urgency (e.g. 'sometimes when I feel bad, I can't seem to stop what I am doing even though it is making me feel worse'); positive urgency (e.g. 'when over joyed, I feel like I can't stop myself from going overboard'); (lack of) premeditation (e.g. 'I usually make up my mind through careful reasoning'); (lack of) perseverance (e.g. 'I finish what I start'); and sensation seeking (e.g. 'I would enjoy the sensation of skiing very fast down a high mountain slope').

- The gambling related cognitions scale (GRCS; Raylu & Oei, 2004): it is a 23-item self-report questionnaire that uses a seven-point Likert scale from 1 (strongly disagree) to 7 (strongly agree) to assess five subscales: predictive control (e.g. 'losses when gambling are bound to be followed by a series of wins'); illusion of control (e.g. 'I have specific rituals and behaviours that increase my chances of winning'); interpretive bias (e.g. 'relating my winnings to my skill and ability makes me continue gambling'); gambling expectancies (e.g. 'gambling makes things seem better'); and inability to stop gambling (e.g. 'I'm not strong enough to stop gambling').

- The severity of alcohol dependence questionnaire (SADQ; Stockwell, Hodgson, Edwards, Taylor, & Rankin, 1979): it is a self-report questionnaire divided into five sections: physical symptoms of withdrawal ('my hands shake first thing in the morning'); affective symptoms of withdrawal ('I am frightened of meeting people first thing in the morning'); craving and withdrawal-relief drinking ('I like to have a morning drink'); typical daily consumption ('I drink more than a quarter of a bottle of spirits per day (4 doubles or 1 bottle of wine or 4 pints of beer'); and rapidity of reinstatement of symptoms after a period of abstinence ('my body would shake'). For all questions, the respondents were instructed to focus on their most recent period of heavy drinking. All withdrawal symptom items refer to how the respondent felt when waking up, because this is the most common time for such symptoms to occur. Each item has its own frequency scale ranging from 'almost never' to 'nearly always' and, in addition, the items themselves were designed to cover a range of severity of symptomatology.

- The Cambridge gambling task (CGT; Zois et al., 2014): it is a cognitive task that measures decision-making, impulsivity and RT behaviour. On each trial, the participants are presented with a row of 10 boxes coloured red and blue, in different ratios of red:blue boxes (9:1, 8:2, 7:3, 6:4), which represent the risk conditions, where the ratio 6:4 constitutes the most risky condition. The participants were asked to make a probability judgement followed by a bet. First, participants chose under which colour a token is most likely hidden, and after that, they could place a bet at one of five levels: 5%, 25%, 50%, 75% or 95% of their total points available. In the assessed stages, participants started with 100 points and selected a proportion of these points to bet on their decision. The bet was offered under two different conditions: in the ascending condition, the amount of points that participants can bet appeared in the screen starting at 5% of their points, followed by increasing steps up to 95%; in the descending condition, bets started at 95% with

step-down to 5% of their current points. A number on the screen displayed the current bet value in points, which either incrementally increased or decreased. One of the key features of the CGT is that it allows to dissociate risk-taking from impulsivity. This is because in the ascending condition, the participant who seeks to make a risky bet has to wait for it to appear on the screen, however, in the descending condition, riskier bets would not require to wait. The variables measured were: QDM, which is the proportion of trials where the majority colour was selected (sometimes referred as the proportion of rational responses); RT, which measures the proportion of points bet when the participant chose the most likely option to win; and DA, as a measure of impulsivity, is the difference in risk-taking behaviour between descending and ascending conditions. The CGT is represented in Figure 3-1.

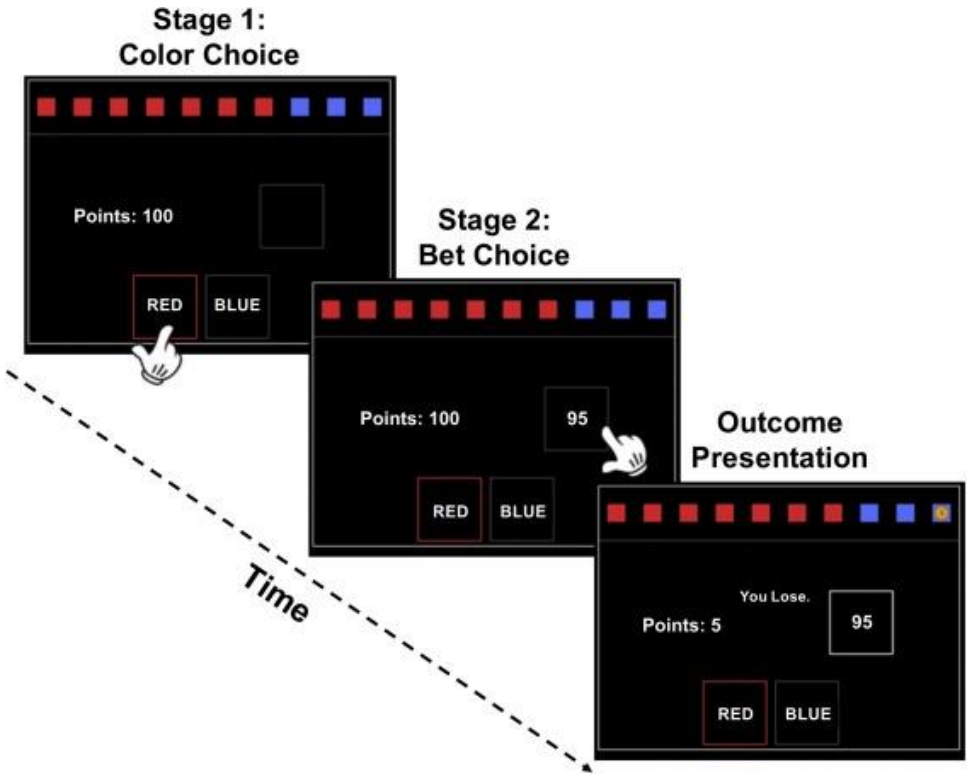

Figure 3-1. Cambridge gambling task (CGT) stages. The participants were asked to guess under which colour a token was most likely hidden. First, they selected the colour on the bottom of the screen and subsequently a bet appeared in ascending or descending order with proportions 5%, 25%, 50%, 75% and 95% of their current points. The bet was shown as the number of points that can be gained or lost (rather than the proportion itself), which participants must select, as the box shows the changing bet (Romeu, Haines, Ahn, Busemeyer, & Vassileva, 2020).

3.3.3 Procedure

Both experiments were conducted to investigate the effects of transcranial direct current stimulation (tDCS) using high definition (HD) montages designed to target two different brain areas: right dorsolateral prefrontal cortex (rDLPFC) and ventromedial prefrontal cortex (vmPFC). Two groups of participants were created depending on the montage with which they received tDCS (rDLPFC, n=12; vmPFC n=12) in Experiment I, and (DLPFC, n=19; vmPFC n=21) in Experiment II. Moreover, to understand the possible influence of addiction-related personality characteristics on the effects of tDCS in gambling task performance, a separate analysis was conducted grouping participants by their impulsivity levels.

The experiments involved two sessions with crossover design, in which each participant received tDCS in a counterbalanced order (real stimulation and sham) one week apart, using a single blind mode (the participant was not aware of the tDCS condition). Participant allocation to each group was not random in Experiment I because the original plan was to compare the effects of tDCS in both brain areas throughout two different experiments (the first using a rDLPFC montage and the second using a vmPFC montage). However, after a reconsideration, it was decided to change the approach to ensure having more data informing about the potential effects of both montages on gambling task performance, before continuing with the following experiment. Consequently, two participant groups were created in each experiment according to the tDCS montage that was used with each participant. Therefore, the first nine participants were tested with the rDLPFC montage, the next nine participants with the vmPFC montage, and consecutively each new participant was allocated to a group with a different montage to the previous participant, which continued in the following experiment. In Experiment II, it was decided to create a new montage designed to target rDLPFC, which was compared against the same vmPFC used in Experiment I. Therefore, three different tDCS montages were used: one to target vmPFC used in both experiments, and two different montages to target rDLPFC (the details of the montages are explained in the next section 3.3.4 of this chapter).

Each testing session had a duration of one hour, and in addition, the setting up and cleaning of the equipment took around 40 minutes. A total of 64 sessions were conducted, including both experiments. Before participation in the study, written informed consent was obtained, which was approved by the University of East London Research Ethics Committee (UREC_161731, see

Appendix D). All experimental procedures were conducted following the Declaration of Helsinki. On each session, participants completed a series of questionnaires to measure individual gambling-related characteristics (SOGS, UPPS-P, GRCS and SADQ), and after that the CGT was administered during tDCS stimulation. All participants completed the CGT ascending condition first (Zois et al., 2014) because it was demonstrated that ascending/descending conditions order did not influence CGT performance (Lawrence, Luty, Bogdan, Sahakian, & Clark, 2009).

3.3.4 Transcranial direct current stimulation (tDCS) high definition (HD) montages

Experiments were conducted using different tDCS HD montages (1 x 4, anode-returns) targeting rDLPFC and vmPFC with an intensity of 1.5 milliamps (mA) and a ramp up and ramp down of 30 seconds. In the active session, participants received 20 minutes of real stimulation, whereas in the sham session, there was no electrical current delivered between the ramping up and ramping down. The montage designed to target vmPFC was the same in both experiments (anode: Fpz, returns: F7, F3, F4, F8), and it was compared against montages designed to target rDLPFC: (anode: F4, returns: F8, C4, Fz, Fp2) in Experiment I, and (anode: F4, returns: Fpz, F3, Cz, T8) in Experiment II. The electrode positions followed the international 10-10 EEG system with the central Cz position aligned to the vertex of the head, and are represented in Figure 3-2**.**

The same montage was used to target vmPFC in both experiments because in vmPFC the targeted area is not very accessible, and there were no alternative electrode positions that would fit well a HD montage that maintained the four return electrodes at a similar distance from the anode (Fpz), without separating them more than it was intended to. However, rDLPFC target is more accessible, so two different montages were designed with different return positions and the same anode (F4). In Experiment I, the return electrodes were closer to the anode, whereas in Experiment II the location of the return electrodes was separated slightly, with the intention to increase the relative amount of current traveling through the scalp to the targeted brain area (Bikson, Datta, Rahman, & Scatturo, 2010; Datta, Elwassif, Battaglia, & Bikson, 2008).

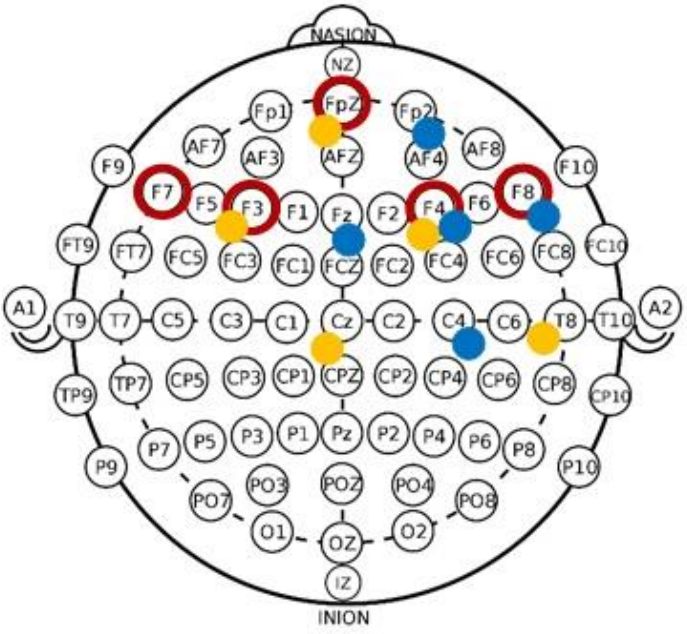

Figure 3-2. Transcranial direct current stimulation (tDCS) high definition (HD) montages in Experiment I and Experiment II. Electrode positions were based on the International EEG 10-10 system. The circles in red represent the electrode positions of the montage designed to target vmPFC (anode: Fpz, returns: F7, F3, F4, F8) in both experiments. Blue dots indicate electrode positions of the rDLPFC montage in Experiment I (anode: F4, returns: F8, C4, Fz, Fp2), and the yellow dots indicate electrode positions of the rDLPFC montage in Experiment II (anode: F4, returns: Fpz, F3, Cz, T8). Montage tDCS effects for vmPFC (red) and rDLPFC (blue) were compared in Experiment I, and montage tDCS effects for vmPFC (red) and rDLPFC (yellow) were compared in Experiment II.

3.3.5 Analysis plan in Experiments I and II

The statistical analysis was conducted with IBM SPSS (Statistical Package of the Social Sciences, Version 23, SPSS Inc., Chicago, IL). Significance threshold was set at $\alpha = 0.05$. Demographic data (age/gender) were analysed using t-tests and Chi-square tests. Data was checked for skewness, kurtosis and normality. Non-normally distributed data according to Kolmogorov-Smirnov test was transformed using logarithmic transformation, or arcsine transformation for variables expressed as a proportion. When sphericity was not satisfied, Greenhouse-Geisser correction was employed.

The effects of tDCS session order on CGT performance were analysed with a repeated measures ANOVA with between participants factor being session order, with two levels (real stimulation first or sham first). To take into account the interaction of the tDCS stimulation condition with the order of the session, when session order was significant, session order was used as a covariate, and a repeated measures mixed factor ANCOVA was employed. Significant group interactions were analysed using independent sample t-tests (2-tailed), and significant tDCS condition interactions were analysed with paired sample t-tests (2-tailed).

The variable QDM was analysed with a 2 x 2 x 2 x 4 mixed factor repeated measures ANOVA, with within-participants factors being tDCS, with two levels (real stimulation and sham), condition, with two levels (ascending and descending) and risk conditions (box ratio), with four levels (6:4, 7:3, 8:2, 9:1), and group as the between-participants factor (HI and LI, or rDLPFC and vmPFC). RT was analysed using a 2 x 2 x 2 x 4 mixed-factor repeated measures ANCOVA, with within-participants factors condition, with two levels (ascending and descending), tDCS, with two levels (real stimulation and sham) and box ratio, with four levels (6:4, 7:3, 8:2, 9:1), and group as the between-participants factor (HI and LI, or rDLPFC and vmPFC), and session order as a covariate. DA was analysed using a 2 x 2 x 4 mixed factor repeated measures ANOVA with within-participants factor tDCS, with two levels (real stimulation and sham), box ratio, with four levels (6:4, 7:3, 8:2, 9:1), and group as the between-participants factor (LI and HI, or rDLPFC and vmPFC).

3.4 Results

3.4.1 Results in Experiment I

Demographic data and questionnaires are reported in Table 3-1, including groups divided by impulsivity levels (LI and HI) and by tDCS target (rDLPFC and vmPFC). Independent sample t-test for age differences between LI (M=28 ± 3.27) and HI (M=26.83 ± 4.45) were not significant (t (22) = .535, p = .472). Chi-square tests were not significant for gender interactions with UPPS-NU group (X^2 (1) = .178, p=.673) and for education level interactions with UPPS-P NU group (X^2 (1) = .178, p=.673). For the participants grouping by tDCS target, independent t-tests for age differences between rDLPFC (M=27.92) and vmPFC (M=26.92) were not significant (t (22) = .625, p=.538). Chi-square tests revealed no inter-group differences for gender interactions with tDCS target groups (X^2 (1) = .178, p=.673) and no differences for education level interactions with tDCS target group (X^2 (1) = .178, p=.673). Independent sample t-test analysis for UPPS-P total score differences between LI and HI was significant (t (22) = -2.865, p=.009), but non-significant between rDLPFC and vmPFC groups. No other measures including SADQ, GRCS and SOGS showed significant differences between groups. Data that was not normally distributed was log transformed (GRCS, SADQ, SOGS) and arcsine transformed in the case of QDM.

Table 3-1. *Demographics and participant characteristics by group in Experiment I.*

	LI (n=12)	HI (n=12)	Test Statistic	rDLPFC (n=12)	vmPFC (n=12)	Test Statistic
Age in years	28.00 (3.27)	26.83 (4.45)	t (22) = .732, p=.472	27.92 (4.48)	26.92 (3.26)	t (22) = .625, p=.538
Gender						
Females	5	4	X^2 (1) = .178, p=.673	4	5	X^2 (1) = .178, p=.673
Males	7	8		8	7	
Education						
Primary	-	-		-	-	
Secondary	8	7	X^2 (1) = .178, p=.673	8	7	X^2 (1) = .178, p=.673
High	4	5		4	5	
SOGS	1.17 (3.16)	1.00 (1.60)	t (22) = .163, p=.872	0.25 (0.62)	1.92 (3.26)	t (22) = -1.740, p=.096
UPPS-P Total	24.43 (1.77)	27.97 (4.19)	t (22) = -2.475, p=.021*	26.40 (2.46)	26.00 (4.62)	t (22) = -.663, p=.514
GRCS Total	34.33 (12.50)	40.58 (18.90)	t (22) = -.917, p=.369	35.17 (13.82)	39.75 (18.23)	t (22) = -.663, p=.514
SADQ	4.17 (6.81)	8.25 (6.74)	t (22) = -1.476, p=.154	4.33 (5.10)	8.08 (8.21)	t (22) = 1.345, p=.192

Notes: SOGS, South Oaks gambling screen; UPPS-P, urgency, premeditation, perseveration, sensation seeking scale; GRCS, gambling related cognitions scale; SADQ, severity of alcohol dependence questionnaire. Participant groups are divided by impulsivity (high: HI; low: LI) and by tDCS target area (right dorsolateral prefrontal cortex (rDLPFC) and ventromedial prefrontal cortex (vmPFC)). Values represent mean and inside the parenthesis standard deviation, except in gender and education, in which values represent number of participants (* $p < .05$).

In Experiment I there were no significant differences of tDCS effects between montages designed to target rDLPFC and vmPFC in any of the variables, including QDM (F (1, 22) =.001, p= .998, ηp^2 = .001), RT (F (1, 22) =.878, p= .359, ηp^2 = .038) and DA (F (1, 22) =.148, p= .704, ηp^2 = .007). Experiment results are reported below using participant groups split by impulsivity levels. Groups were created using median split of NU scores (median = 26.5). Therefore participants scoring 26 or lower in NU were allocated to LI group, and participants scoring 27 or higher in NU were allocated to the HI group. The distribution of UPPS-P NU scores across participants is presented in Figure 3-3.

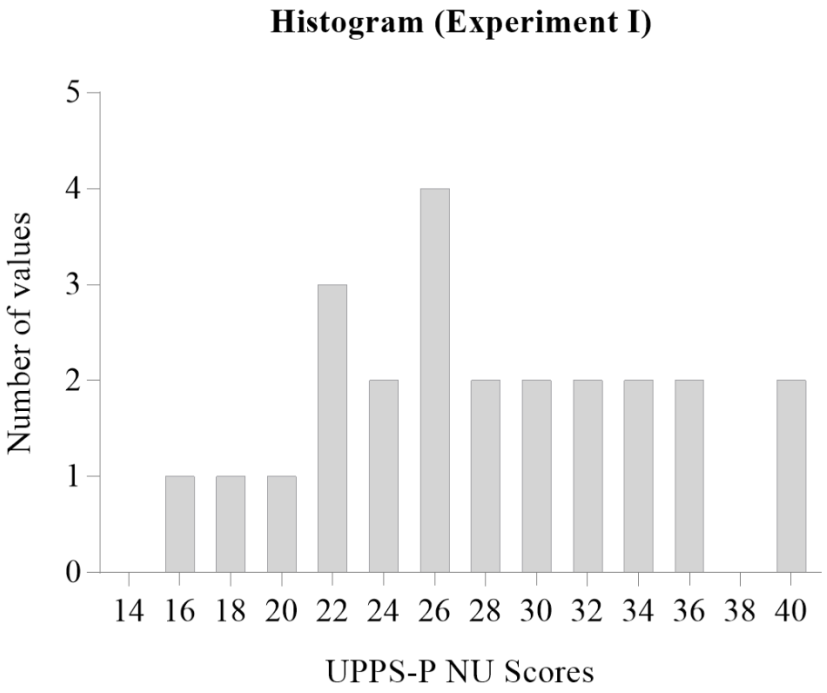

Figure 3-3. Histogram in Experiment I. Distribution of the negative urgency (NU) scores across participants from the urgency, premeditation, perseverance and sensation seeking scale (UPPS-P).

There were significant main effects of tDCS in QDM (F (1, 22) = 4.788, p= .040, ηp^2 = .179), with real stimulation enhancing decision-making compared to sham, and in RT, in which real stimulation was associated with higher RT compared with sham (F (1, 21) = 10.072, p= .004, ηp^2 = .179). In RT, there were also main effects of task condition (F (1, 21) = 7.719, p= .011, ηp^2 = .338), showing that RT was lower in ascending condition compared to descending condition, and a main effect of risk (F (1, 21) = 6.633, p= .001, ηp^2 = .240), showing that RT was lower as risk conditions increased. There was an interaction of tDCS x session order, showing that RT was higher in the second session (F (1, 21) = 14.685, p= .001, ηp^2 = .269), and also risk condition x group interactions (F (3, 63) = 2.942, p= .040, ηp^2 =.123) represented in Figure 3-4. Independent samples t-test to compare the interaction with groups, showed that HI was associated with significantly lower RT in real stimulation ascending lowest risk condition (9:1) compared with LI (t (22) = 2.583, p=.017), however in descending highest risk condition (6:4), HI had higher RT compared with LI, but this difference was not significant (t (22) = -1.760, p=.092). There were no significant differences between the groups in sham condition. No significant differences were found in the variable DA (F (1, 22) =.588, p= .451, ηp^2 = .026).

In addition, 2-tailed Pearson´s correlation indicated that UPPS-P NU correlated positively with GRCS subscale inability to stop gambling, DA, and with RT in the highest risk condition, but negatively with RT in the lowest risk condition. Gambling severity measured with SOGS correlated positively with alcohol dependence measured with SADQ, with GRCS total, and with the subscales gambling expectancies, and interpretive bias. No other correlations were significant (all p > .005). Correlation results are presented in Table 3-2.

Table 3-2. *Correlations between questionnaires and task scores with UPPS-P NU and SOGS in Experiment I.*

	UPPS-P NU		SOGS	
	r	p	r	p
UPPS-P NU	-	-	.135	.529
UPPS-P total	.728	.001 **	.071	.743
SOGS	.135	.529	-	-
GRCS total	.113	.598	-.451	.027 *
SADQ	.351	.092	.682	.001 **
GRCS inability to stop gambling	.410	.047 *	.371	.075
GRCS illusion of control	.044	.838	.318	.130
GRCS predictive control	-.013	.950	.252	.234
GRCS gambling expectancies	.044	.838	.451	.027 *
GRCS interpretative bias	.150	.484	-.502	.012 *
DA	.428	.037 *	.106	.622
QDM	.067	.755	.116	.590
RT in CGT 9:1	-.454	.026 *	.178	.405
RT in CGT 8:2	-.312	.138	-.089	.678
RT in CGT 7:3	-.324	.122	-.009	.678
RT in CGT 6:4	.471	.020 *	.027	.899

Note: UPPS-P NU, negative urgency trait of urgency, premeditation, perseverance and sensation seeking scale; SOGS, South Oaks gambling screen; GRCS, gambling related cognition scale; SADQ, severity of alcohol dependence questionnaire; DA, delay aversion; QDM, quality of decision-making; RT, risk taking (* $p < .005$; ** $p \leq .001$).

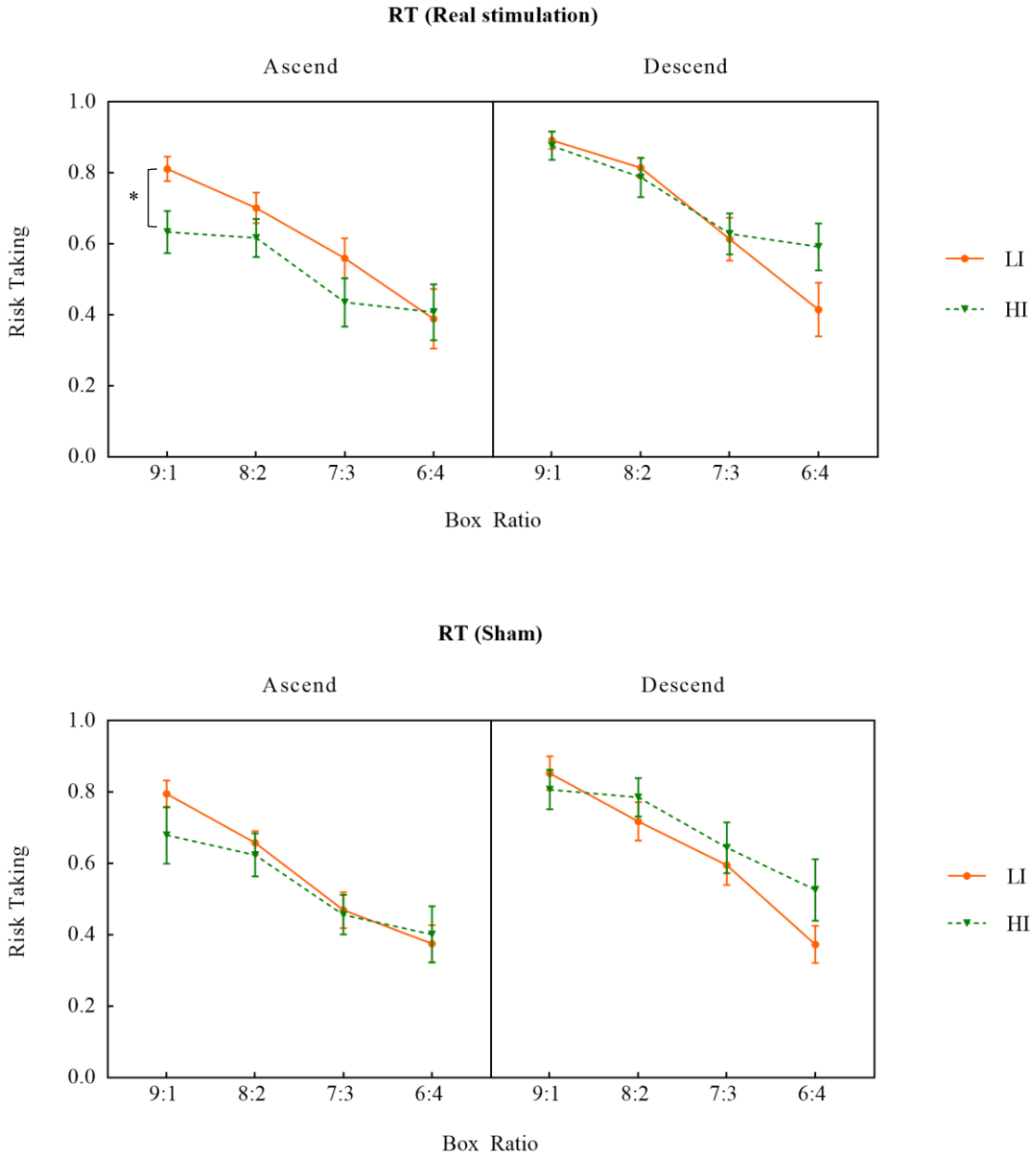

Figure 3-4. Cambridge gambling task (CGT) groups interactions in Experiment I. Risk-taking (RT) in ascending and descending task conditions across different risk conditions, represented by box ratios (9:1, 8:2, 7:3 and 6:4) in low impulsive (LI) and high impulsive (HI) groups, during tDCS real stimulation and sham. Data represents mean and SEM (* $p < .05$).

3.4.2 Results in Experiment II

Demographic data and questionnaires are reported in Table 3-3, including groups divided by impulsivity levels (LI and HI) and by tDCS target (rDLPFC and vmPFC). Independent t-test to analyse age differences between LI (M=27.62 ± 7.61) and HI (M=23.47 ± 5.90) groups, were not significant (t (38) = -1.138, p=.262). Chi-square tests for gender interactions with UPPS-P NU group were significant (X^2 (1) = 5.414, p=.020), and not significant for education level interaction with UPPS-P NU group (X^2 (1) = .018, p=.894). For the participants grouping by tDCS target, independent t-tests analysis for age differences between rDLPFC (M=27.92) and vmPFC (M=26.92) were not significant (t (22) = .625, p=.538). Chi-square tests revealed no inter-group differences for gender interactions with tDCS target grouping (X^2 (1) = .178, p=.673), and no interactions for education level and tDCS target grouping (X^2 (1) = .658, p=.451). Independent t-tests analysis for UPPS-P total score differences between LI and HI was significant (t (22) = -2.865, p=.009), and non-significant between rDLPFC and vmPFC groups. No other measures including SOGS, GRCS and SADQ showed significant differences between groups. Kurtosis, skewness and normality was assessed and data that was not normally distributed according to Kolmogorov-Smirnov test was log transformed (DA, GRCS, SADQ, SOGS) or arcsine transformed (QDM).

Table 3-3. *Demographics and participant characteristics by group in Experiment II.*

	LI (n=21)	HI (n=19)	Test Statistic	rDLPFC (n=19)	vmPFC (n=21)	Test Statistic
Age in years	27.62 (7.61)	23.47 (5.90)	t (38) = 1.911, p=.064	24.32 (6.71)	26.86 (7.35)	t (38) = -1.138, p=.262
Gender						
Females	9	15	X^2 (1) = 5.414, p=.020*	10	14	X^2 (1) = .819, p=.366
Males	12	4		9	7	
Education						
Primary	-	-		-	-	
Secondary	18	16	X^2 (1) = .018, p=.894	17	17	X^2 (1) = .658, p=.451
High	3	3		2	4	
SOGS	0.90 (1.64)	1.58 (2.48)	t (38) = -1.024, p=.313	1.42 (1.74)	1.05 (2.38)	t (38) = .562, p=.578
UPPS-P	23.54 (3.84)	28.30 (2.24)	t (38) = -4.727, p=.001*	26.8 (3.50)	24.90 (4.21)	t (38) = 1.539, p=.132
GRCS	40.47 (17.66)	62.05 (26.51)	t (38) = -3.056, p=.004*	62.11 (25.49)	40.43 (18.92)	t (38) = .408, p=.012*
SADQ	4.33 (3.26)	5.16 (4.39)	t (38) = -.679, p=.501	5.21 (3.90)	4.29 (3.77)	t (38) = .821, p=.417

SOGS, South Oaks gambling screen; UPPS-P, urgency, premeditation, perseveration, sensation seeking scale; GRCS, gambling related cognitions scale; SADQ, severity of alcohol dependence questionnaire. Participant groups are divided by impulsivity (high: HI; low: LI) and by tDCS target area (right dorsolateral prefrontal cortex (rDLPFC) and ventromedial prefrontal cortex (vmPFC)). Values represent mean and inside the parenthesis standard deviation, except in gender and education, in which values represent number of participants (* $p < .05$).

In Experiment II there were no significant differences of tDCS effects between montages designed to target rDLPFC and vmPFC in any of the variables, including QDM ($F (1, 38) = .907$, $p = .347$, $\eta p^2 = .023$), RT ($F (1, 38) = .001$, $p = .996$, $\eta p^2 = .001$) and DA ($F (1, 38) = .034$, $p = .855$, $\eta p^2 = .001$). Experiment results are reported using participant groups split by impulsivity levels. Groups were created using median split of NU scores (median = 26.5). Therefore participants scoring 26 or lower in NU were allocated to LI group, and participants scoring 27 or higher in NU were allocated to the HI group. The distribution of UPPS-P NU scores across participants is presented in Figure 3-5.

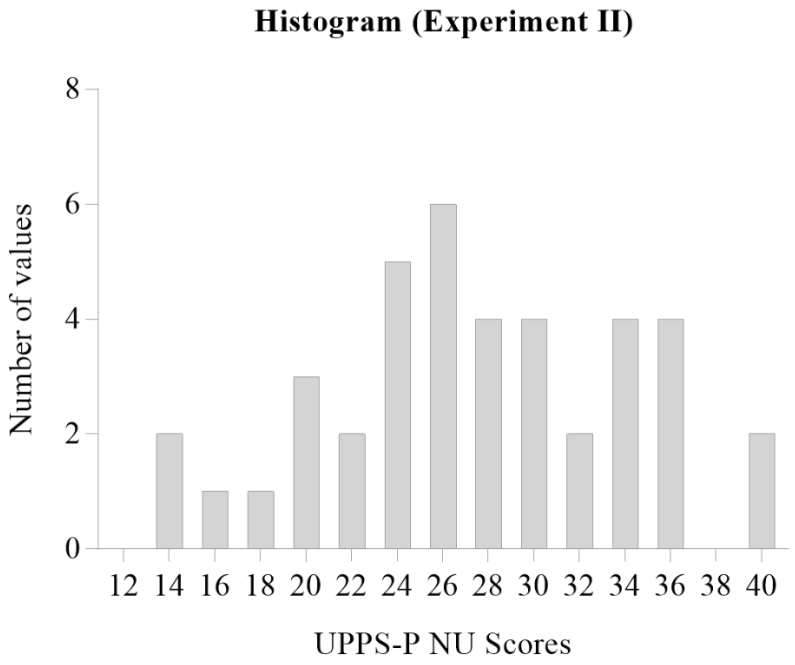

Figure 3-5. Histogram in Experiment II. Distribution of the negative urgency (NU) scores across participants from the urgency, premeditation, perseverance and sensation seeking scale (UPPS-P).

There was a main effect of task condition (F (1, 38) = 7.183, p=.011, ηp^2 = .159), indicating that QDM was lower in ascending condition. Also, a main effect of risk (F (3, 114) = 4.484, p=.015, ηp^2 = .106), showed that QDM was lowest in the highest risk condition and highest in the lowest risk condition. There was an interaction tDCS x risk condition (F (3, 114) = 3.519, p=.032, ηp^2 = .085), showing that in the sham condition the difference in QDM between the lowest (9:1) and the highest (6:4) risk conditions (with higher QDM in the lowest risk condition), was significant in both ascending (t (39) = 2.922, p=.006), and descending conditions (t (39) = 2.282, p=.028), however, in real stimulation there were no significant differences between risk conditions (Figure 3-6). In multivariate analysis there was an interaction risk condition x group (F (3, 36) = 3.830, p=.018, ηp^2 = .242), indicating that LI showed higher QDM compared with HI in the risk condition 8:2 in real stimulation (t (38) = 2.569, p=.014).

There was also a main effect of tDCS in RT (F (1, 37) = 12.458, p=.001, ηp^2 = .252), showing that RT was higher in real stimulation compared with sham, a main effect of condition (F (3, 114) = 5.285, p=.027, ηp^2 = .125), indicating that RT was lower in ascending condition, and a main effect of risk (F (3, 111) = 3.802, p=.030, ηp^2 = .093), showing that RT was lower as risk conditions increased. There were also interactions between tDCS x session order (F (1, 37) = 14.960, p=.001, ηp^2 = .288), indicating that RT was higher in the second session of tDCS. An interaction condition x groups (F (1, 37) = 5.252, p=.028, ηp^2 = .124), an interaction risk x groups (F (3, 111) = 6.255, p=.004, ηp^2 = .145) and a three way interaction tDCS x condition x risk (F (3, 111) = 3.174, p=.030, ηp^2 = .085), represented in Figure 3-7 and Figure 3-8.

Independent samples t-test to compare group interactions, showed that compared with HI, LI showed significantly lower RT in real stimulation descending highest risk conditions 7:3 (t (38) = -2.282, p=.028), and 6:4 (t (38) = -3.244, p=.002), and in sham descending condition 6:4 (t (38) = -2.609, p=.013). Paired samples t-test to compare tDCS interactions, showed that RT was higher in descending conditions compared with ascending conditions in real stimulation 9:1 (t (39) = -3.128, p=.004), 8:2 (t (39) = -3.196, p=.003), 7:3 (t (39) = -2.225, p=.032), and in sham 9:1 (t (39) = -5.723, p=.001), 8:2 (t (39) = -4.421, p=.001), 7:3 (t (39) = -4.443, p=.001) and 6:4 (t (39) = -4.867, p=.001), and that tDCS real stimulation was associated with significantly higher RT in ascending highest risk condition (6:4) compared with sham (t (39) = 2.025, p=.050).

In addition, 2-tailed Pearson´s correlation indicated that UPPS-P NU correlated positively with gambling severity measured with SOGS, GRCS subscales gambling expectancies, illusion of control, predictive control, inability to stop gambling, interpretive bias and total GRCS. Also, UPPS-P NU correlated positively with RT in the highest risk condition (6:4). Gambling severity (SOGS) correlated positively with UPPS-P NU, as mentioned previously, and UPPS-P total, and with GRCS subscales inability to stop gambling, interpretive bias and GRCS total, and with RT risk conditions 7:3 and 6:4. In addition, SOGS correlated negatively with QDM. Correlations are represented in Table 3-4.

Table 3-4. *Correlations between questionnaires and task scores with UPPS-P NU and SOGS in Experiment II.*

	UPPS-P NU		**SOGS**	
	r	p	r	p
UPPS-P NU	-	-	.347	.028 *
UPPS-P total	.810	.001 **	335	.035 *
SOGS	.347	.028 *	-	-
SADQ	.083	.612	.008	.961
GRCS total	.448	.004 *	.392	.012 *
GRCS inability to stop gambling	.434	.005 *	.645	.001 **
GRCS illusion of control	.325	.041 *	.252	.117
GRCS predictive control	.350	.027 *	.153	.347
GRCS gambling expectancies	.418	.007 *	.276	.084
GRCS interpretative bias	.397	.011 *	.440	.004 *
DA	.136	.403	-.146	.368
QDM	-.201	.213	-.329	.038 *
RT in CGT condition 9:1	-.003	.985	.189	.242
RT in CGT condition 8:2	-.061	.707	.166	.305
RT in CGT condition 7:3	.215	.182	.312	.050 *
RT in CGT condition 6:4	.348	.028 *	.527	.001 **

Note: UPPS-P NU, negative urgency trait of urgency, premeditation, perseverance and sensation seeking scale; SOGS, South Oaks gambling screen; SADQ, severity of alcohol dependence questionnaire; GRCS, gambling related cognition scale; DA, delay aversion; QDM, quality of decision-making; RT, risk taking (* $p < .005$; ** $p \leq .001$).

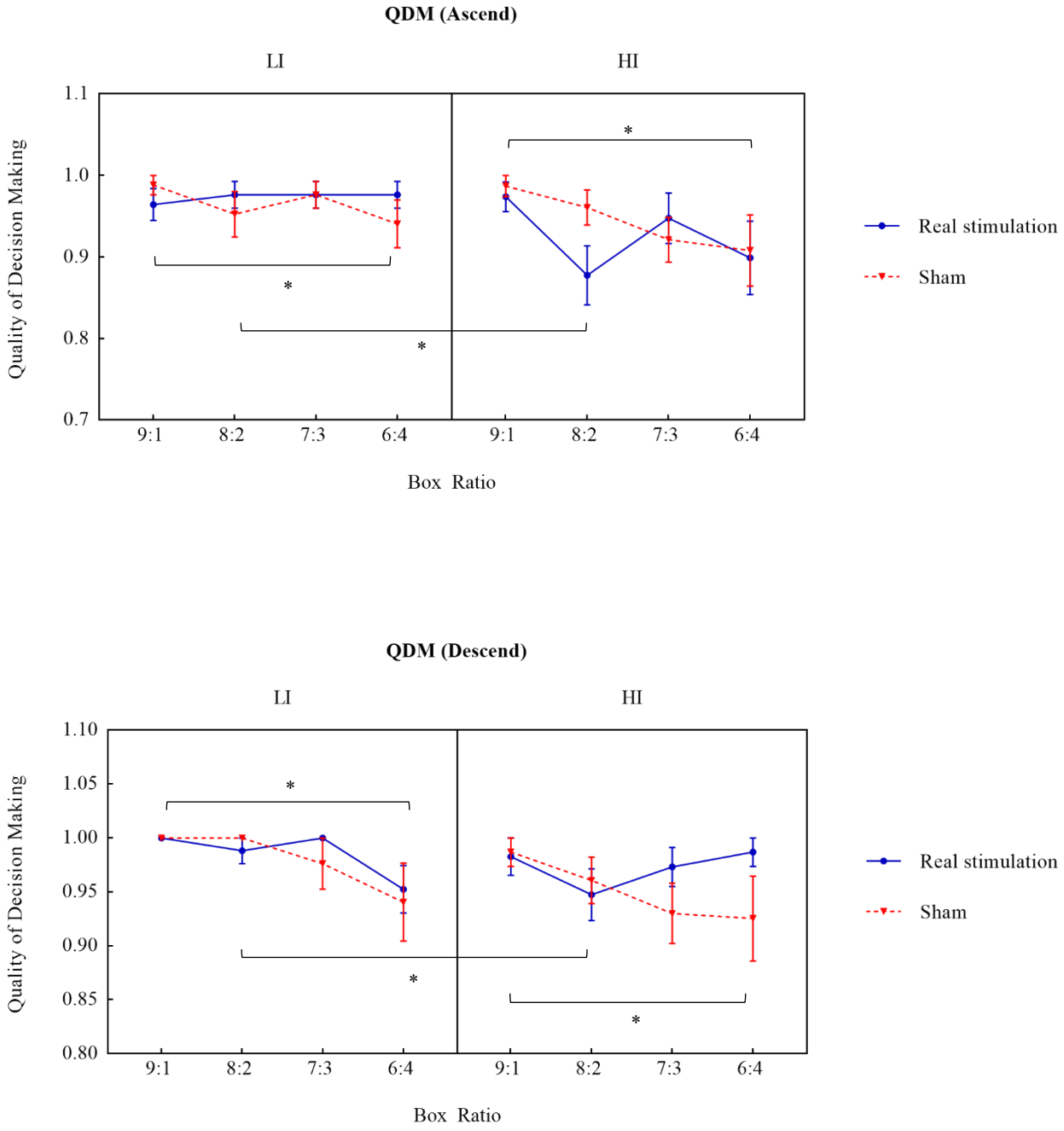

Figure 3-6. Cambridge gambling task (CGT) quality of decision-making (QDM) interactions with tDCS in Experiment II. QDM in tDCS real stimulation and sham across different risk conditions, represented by box ratios (9:1, 8:2, 7:3 and 6:4), for low impulsive (LI) and high impulsive (HI) groups in ascending and descending task conditions. Significant differences were found between sham lowest (9:1) and highest (6:4) risk conditions, and between LI and HI in 8:2 in real stimulation. Data represents mean and SEM (* $p < .05$).

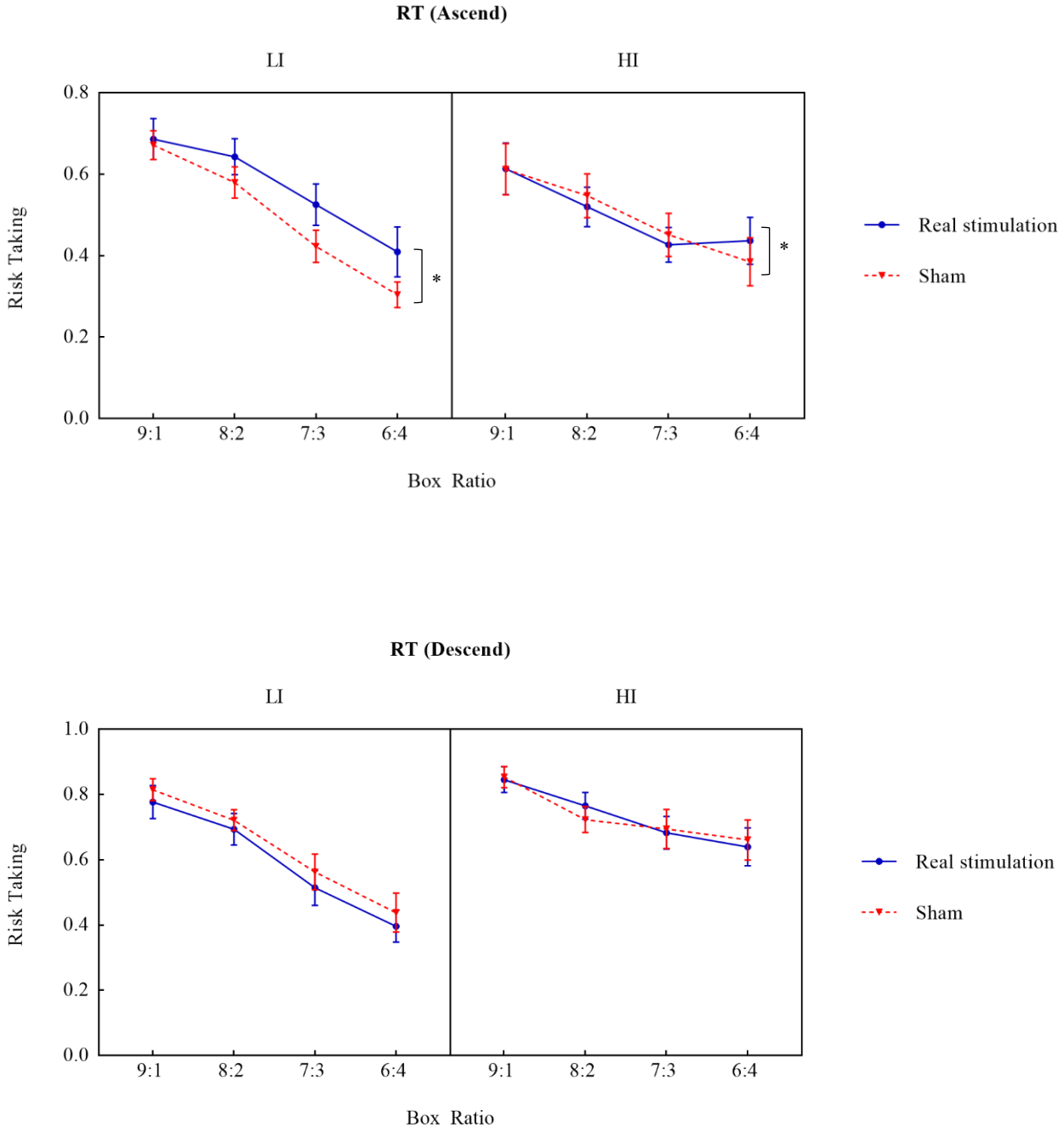

Figure 3-7. Cambridge gambling task (CGT) risk-taking (RT) tDCS interactions in Experiment II. RT in tDCS real stimulation and sham across different risk conditions, represented by box ratios (9:1, 8:2, 7:3 and 6:4), for low impulsive (LI) and high impulsive (HI) groups, in ascending and descending task conditions. Data represents mean and SEM (* $p \leq .05$).

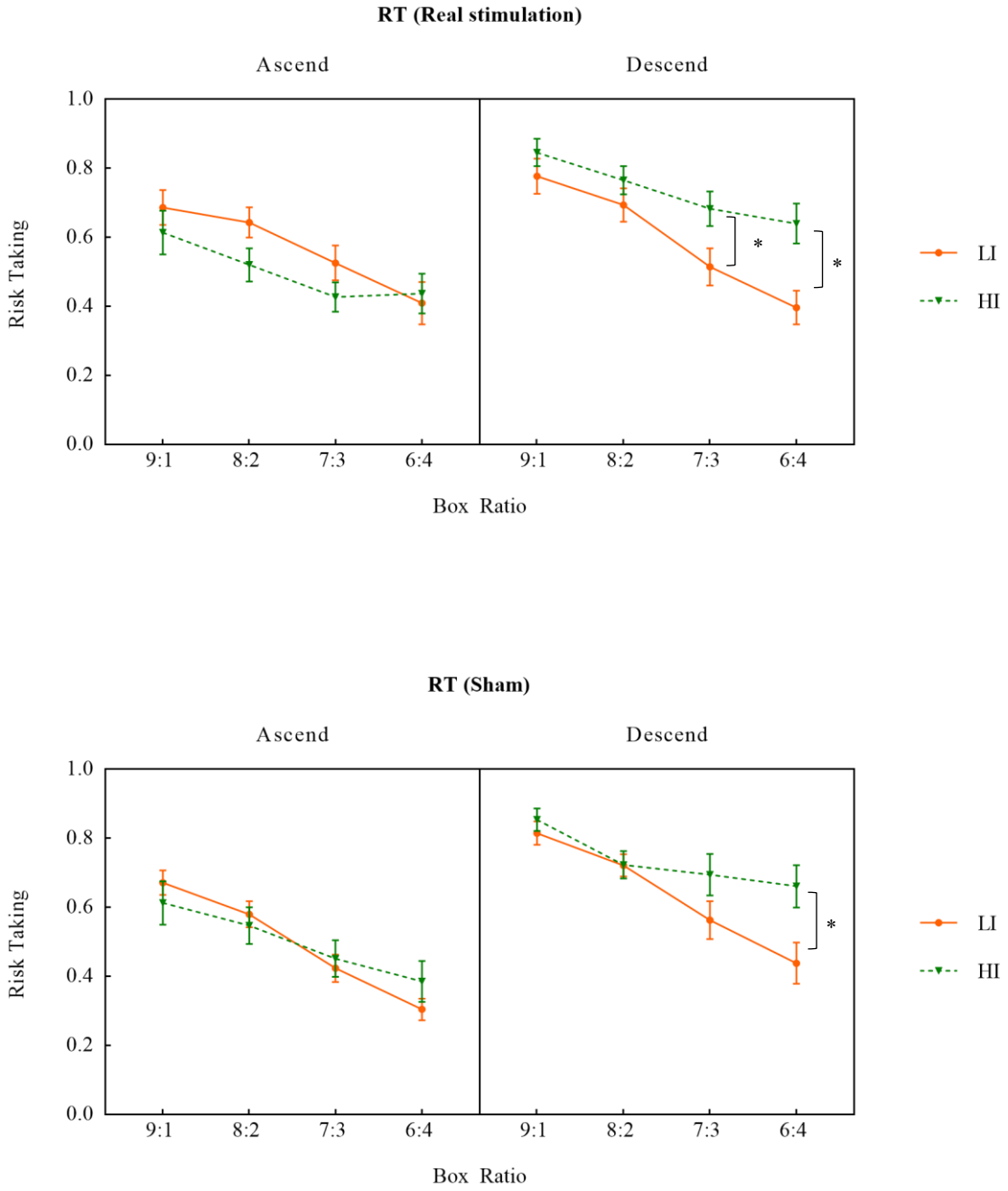

Figure 3-8. Cambridge gambling task (CGT) risk-taking (RT) interactions with groups in Experiment II. RT in ascending and descending task conditions, across different risk conditions, represented by box ratios (9:1, 8:2, 7:3 and 6:4), for low impulsive (LI) and high impulsive (HI) groups, in tDCS real stimulation and sham. Data represents mean and SEM (* $p < .05$).

3.5 Discussion

These two experiments investigated the effects of different HD tDCS montages (1 x 4, anode-returns) designed to target rDLPFC and vmPFC on gambling task performance, and whether addiction-related participant characteristics, particularly, the impulsivity trait of negative urgency (NU), was related to the effects of tDCS. Two experiments were conducted using the same methodology but different tDCS montages and different sample sizes (24 and 40 participants, respectively). In both experiments it was used the same tDCS montage designed to target vmPFC, and this was compared against two different montages designed to target rDLPFC. In Experiment I, the rDLPFC montage was designed with the return electrodes positioned closer to the anode, but in Experiment II, the return electrodes were positioned with slightly more distance to the anode, with the aim to increase the amount of current traveling through the scalp to the target area. Self-reported impulsivity measured with the UPPS-P NU was used to create two groups of participants (LI and HI), to investigate whether potential tDCS effects on gambling task performance varied across participants with different impulsivity levels. Results showed significant effects of tDCS on quality of decision-making (QDM) and risk-taking (RT), as well as different gambling task performance between LI and HI participants, but no difference on tDCS effects between rDLPFC and vmPFC areas or impulsivity participant groups.

Both experiments revealed similar results on the Cambridge Gambling Task (CGT) performance: results showed that real stimulation enhanced QDM and increased RT compared with sham, but there were no tDCS effects on DA. Both experiments revealed that tDCS effects did not differ between LI and HI participants, or between montages designed to target rDLPFC and vmPFC. Results also showed that RT decreased with increasing risk conditions, and that RT was higher in descending condition compared with ascending condition. The fact that RT was higher in the descending condition might indicate that participants betting choice was driven more by impulsivity rather than by a deliberate intention to increase the bet itself. This interpretation is based on the characteristics of the CGT (Fauth-Bühler et al., 2017), which allows for differentiation between impulsive choice and RT behaviour. In the ascending condition, participants need to wait for higher bets to appear on the screen, however, in the descending condition the highest bet appears first, and participants have to wait to choose a smaller bet until it appears on the screen. Nevertheless, even though this argument could be sustained due to the task characteristics, it has to be noted that there were no effects on DA, which measures the difference in RT between descending and ascending conditions, as a behavioural impulsivity measure. However, if impulsivity did not influence bet choice, in principle it could be expected to not find significant differences between RT in ascending and descending conditions,

contrary to what it was found. Therefore, there is some evidence to support that participants bet choice was driven by impulsivity, but results are not strong to confirm the argument.

There were significant differences in RT across different risk conditions between LI and HI, as well as a session order effect in both experiments, showing that RT was higher in the second testing session, perhaps due to participants getting used to the experimental procedure, and therefore feeling more eager to take risks. In both experiments there were positive correlations between UPPS-P NU and RT in the highest risk condition (6:4), between UPPS-P NU and GRCS subscale inability to stop gambling, and between gambling severity (SOGS) and GRCS total score and subscales inability to stop gambling and interpretive bias.

In contrast, there were also outcomes that varied across the experiments. In Experiment I, LI showed higher RT in the lowest risk condition (9:1) compared with HI, and in Experiment II, HI showed higher RT in the highest risk condition (6:4) compared with LI. Although these two outcomes are not exactly the same, they are not conflicting either. In addition, significant interaction effects were found with tDCS in QDM and RT in Experiment II that were not present in Experiment I. Accordingly, Experiment II revealed that QDM was higher in the lowest risk condition (9:1) compared with the highest risk condition (6:4) in sham, but no significant differences in QDM across risk conditions were found in real stimulation. This result could be explained considering also the effect of tDCS on QDM found in both experiments (which associated real stimulation with higher QDM). If tDCS real stimulation helped increase QDM, especially in the highest risk conditions compared with sham[3], this would result in a more stable task performance between risk conditions, and therefore explain the lack of significant differences in QDM between the lowest and highest risk conditions in real stimulation, but in sham, as it was found. Nevertheless, this argument is just an assumption that could help explain the results, but a more reliable interpretation cannot be asserted because of the lack of statistical evidence supporting the statement.

Moreover, RT was higher in the ascending highest risk condition (6:4) in real stimulation compared with sham. This effect of tDCS on RT in Experiment II, together with the results found in both experiments, showing that increased RT was associated with real stimulation, and that independently of the stimulation participants showed higher RT in descending condition (which could be an

[3] Paired t-tests to compare tDCS effects on QDM in different risk conditions were not significant.

indication of impulsive behaviour, as discussed above), could indicate that real stimulation might increase RT behaviour, rather than modulating impulsive choice, which would be consistent with the fact that there were no tDCS effects on DA.

Besides the significant correlations found in both experiments mentioned above, in Experiment I, it was identified a positive correlation between UPPS-P NU and DA, and a negative correlation between UPPS-P NU and RT in the lowest risk condition (9:1). In addition, SOGS correlated positively with SADQ and GRCS subscale gambling expectancies. In Experiment II, UPPS-P NU correlated positively with gambling severity (SOGS) and with GRCS total score and subscales of gambling expectancies, illusion of control, predictive control and interpretive bias, whereas SOGS correlated negatively with QDM. The correlations revealed in these experiments are consistent with previous research showing that CGT variables were mediated by impulsivity in GD, with irrational choices being negatively associated with impulsivity scores (Zois et al., 2014). In a similar way, other studies also showed that impulsivity was correlated positively with the level of gambling cognitive distortions measured with the GRCS (Michalczuk et al., 2011), and with gambling severity measured with SOGS (Steel & Blaszczynski, 1998), which is consistent with these results.

The association of impulsivity with gambling-related variables that was found, is in line with previous research that suggested that impulsivity could be a potential marker to target in addiction, and specifically in GD (Leeman & Potenza, 2012b; Verdejo-García et al., 2008). There were more correlations between variables in Experiment II, as well as interaction effects that were not present in Experiment I. This could be explained by the difference in sample size between the experiments. Having 40 participants in Experiment II, compared with the smaller sample of 24 participants in Experiment I, could have facilitated finding significant interactions and more correlations between variables in the second experiment. In any case, the different interactions found in the two experiments suggested similar interpretations of the results, and correlations between variables followed the same direction in both experiments.

These results showed that real stimulation over dorsal and ventral areas of PFC improved QDM compared with sham, which is in line with research showing that tDCS over orbitofrontal cortex (OFC), was associated with improved decision-making (Ouellet et al., 2015) and that bilateral DLPFC tDCS increased advantageous decision-making in GD (Soyata et al., 2018). In addition, these

experiments revealed that real stimulation increased RT behaviour. This result is inconsistent with previous research showing that right DLPFC tDCS was associated with decreased RT in a sample of healthy participants (Ota, Shinya, & Kudo, 2019) and in a clinical impulsive sample (Gilmore et al., 2018). Nonetheless, these results are consistent with research showing that anodal tDCS over DLPFC was associated with increased RT in cannabis users (Lapenta, Marques, Rego, Comfort, & Boggio, 2018), and studies that showed that tDCS over left DLPFC increased RT behaviour in cocaine dependent users (Gorini et al., 2014), and that bilateral tDCS over DLPFC was associated with participants choosing more risky options in real stimulation compared with sham, regardless of the DLPFC side of the stimulation (Ye et al., 2015).

The laterality of tDCS stimulation has been a subject of debate, and results showing that unilateral anodal tDCS over DLPFC did not change RT behaviour, but a decrease of RT was found when cathodal modulation of the contralateral DLPFC anodal stimulation was applied (Fecteau, Pascual-Leone, et al., 2007), highlight the importance of investigating the effects of different types of tDCS montages designed to target different brain areas, to better understand the mechanisms of tDCS on brain circuitries. Also, research has shown conflicting effects of tDCS depending on participant clinical characteristics: while real stimulation compared with sham was associated with less risky behaviours in a sample of healthy participants, chronic marijuana users risky behaviour was higher with both left and right DLPFC tDCS (Boggio et al., 2010). Unlike previous research that identified different tDCS effects depending on participant clinical characteristics, in these experiments tDCS effects did not vary between participants with different impulsivity levels.

In addition, there were no different effects of tDCS between rDLPFC and vmPFC targets, however previous studies showed that risky decision-making performance changed when comparing dorsal and ventral areas of PFC, indicating that right anodal/left cathodal stimulation of DLPFC increased preference of risk, whereas right anodal/left cathodal stimulation of OFC decreased preference of ambiguity (Yang, Gao, Shi, Ye, & Chen, 2017). More research investigating the effects of tDCS in different types of participants and exploring tDCS mechanisms in the brain, will help to establish the most effective protocols to target specific cognitive functions, in order to better understand the potential use of neuromodulation to create more individualised treatment interventions.

These experiments have several limitations. Both experiments were conducted using a single blind design (participants were not aware of the experimental condition - real stimulation or sham), however double or triple blind designs (neither the participant, the researcher conducting the testing session nor the researcher analysing the data are aware of the experimental condition), would be more robust to ensure that results are less likely to be biased. Results identified a gender interaction with groups in Experiment II, showing a higher number of females in the high impulsive group. Matching groups for gender in future studies is recommended given that GD has been associated with gender-related differences in motivations to gamble and also in the problematic consequences of gambling (Potenza et al., 2001). In addition, due to the changes of design during recruitment discussed in section 3.3.3 of this chapter, participants allocation to groups in Experiment I was not randomised, which reduced the strength of the experimental procedure.

Moreover, the design of the tDCS HD montages was done simply by positioning the anode on the electrode location that was identified with the brain area of interest: F4 for rDLPFC and Fpz for vmPFC (Zheng et al., 2016), with the four return electrodes creating a ring at a similar distance around the anode. However, using computational models could help improve tDCS focality, by identifying the most effective electrode positions and current intensity to be delivered through each electrode, to reach the targeted brain area more accurately (Ruffini et al., 2013). In addition, only behavioural outcomes were employed to measure the effects of tDCS. With this approach, it could only be speculated that the electrical current reached the desired brain areas, based on previous studies using similar protocols. However, combining tDCS with neuroimaging and physiological methodologies would provide more reliable information about the functional effects of neuromodulation that might not be identified using only behavioural measures (Gomis-Vicent et al., 2019). Lastly, the experiments followed a two session crossover design to investigate the short term effects of real stimulation compared with sham, however the effects of tDCS have been shown to accumulate across different sessions (Boggio et al., 2009). Therefore, conducting multiple session studies might help to identify long term effects of the stimulation that could potentially be useful to create tDCS clinical interventions.

4 Chapter 4. Experiment III: Clinical trial at the National Problem Gambling Clinic (NPGC)

4.1 Summary

A clinical trial was conducted to investigate the cumulative effects of tDCS in combination with cognitive behavioural therapy (CBT) across eight weekly sessions. The experiment was designed as a randomised control trial, however due to recruitment complications data was analysed as single-participant studies. Two patients diagnosed with gambling disorder (GD) were recruited at the National Problem Gambling Clinic (NPGC). Each patient was allocated to one tDCS condition (real stimulation and sham) and treatment progression was assessed across sessions. There were limitations resulted from the change of analysis approach, and it was not possible to investigate the efficacy of tDCS alone, or to distinguish between the effects of the tDCS procedure (which involved cognitive task performance), and the effects of the CBT. Nevertheless, results showed that in both cases, gambling severity and cravings were reduced at the end of the intervention. In addition, there were significant correlations between gambling severity and electroencephalogram (EEG) power in both cases.

Specifically, in the patient receiving tDCS real stimulation, there was a high variability in EEG results across sessions, which could be explained in part by the modifications in the experimental protocol caused by schedule changes in the CBT treatment. This, complicated establishing reliable conclusions about the intervention progression. Nevertheless, tDCS appeared to induce a short term increase of mean frequency power in the majority of sessions, however no clear direction of long term effects was found. In the patient allocated to sham tDCS, more consistent interpretations of the electrophysiological results were suggested. Findings indicated that short and long term increases of EEG power could be explained by concurrent stop signal task (SST) performance during the tDCS, while potential cumulative effects of CBT could account for the long term reduction of EEG power found at the end of the intervention. Moreover, in this case there were significant correlations between gambling severity, cognitive task performance and mean frequency power, suggesting that these electrophysiological measures could potentially be used in future research as biomarkers to assess changes in cognitive states, and as targets for neuromodulation studies in GD. Overall, contributions derived from this experiment to research in this field include the methodological implications raised from the complications experimented when designing and conducting the clinical trial, such as the

clinical sites availability or the CBT format allocation to patients with specific characteristics, which may be useful to consider prior conducting future clinical studies.

4.2 Introduction

4.2.1 Clinical settings

The clinical and neurobiological component of GD was not formally recognised until the changes made in the DSM-V, in 2013 (Clark, 2014). This was a fundamental step towards creating clinical treatment interventions for GD. Research investigating the prevalence of GD identified that gambling problems affect 0.7–6.5% of the population during lifetime worldwide (Calado & Griffiths, 2016). GD has been associated with increased mortality, suicidality and psychiatric comorbid disorders (Karlsson & Håkansson, 2018), and only 7-12% of people with GD seek treatment (Slutske, 2006), which is in part explained by the limited availability of services offering interventions for GD (Petry et al., 2017). Furthermore, treatment providers are usually not trained to recognise behavioural addictions (BA). Especially, when patients seek treatment for comorbid addiction and psychiatric disorders, BA are often underestimated, and when co-occurring addictions and mental health disorders are not identified and treated concurrently, the effectiveness of treatment interventions could be compromised (Freimuth et al., 2008).

Interventions addressing maladaptive thought and behavioural patterns have shown benefits for both GD and substance use disorders (SUDs). However, long term effects of these interventions are not so clear (Petry et al., 2017). Current treatment available for GD consist mainly on therapy interventions, which have been shown to decrease GD symptoms, and seem especially useful for patients with comorbid disorders. In particular, cognitive behavioural therapy (CBT), is the most common psychological intervention for GD (Menchon et al., 2018). CBT focuses on altering cognitive components of gambling, such as cognitive distortions, and behavioural aspects like promoting alternative responses to problematic behaviour (Grant & Chamberlain, 2020). There is no pharmacological treatment that has been officially approved for GD, however previous studies have shown that GD without comorbidities seem to benefit from naltrexone (Hloch et al., 2017). Novel intervention approaches such as tDCS might offer improved opportunities for treating GD. However, further research is needed to identify neurocognitive targets more accurately and long term effects of neuromodulation, which might help developing individualised treatment interventions that account for the clinical complexity of GD.

Only 11 studies have investigated the effects of non-invasive brain stimulation (NIBS) in GD (Zucchella et al., 2020), and two of those consisted of case report studies (Martinotti et al., 2018; Pettorruso et al., 2019). Four studies were conducted using tDCS, and all targeted the DLPFC. These studies involved tDCS stimulation during two sessions with a crossover design (Dickler et al., 2018), three sessions (Soyata et al., 2018) and five sessions (Martinotti, 2019), following a design with parallel assignment, and a case report, investigating tDCS stimulation twice a day for 10 days, followed by stimulation once a week for three months, and consecutively once every two weeks for another three months (Martinotti et al., 2018). All studies, reported reduction of gambling behaviours, and related symptoms, including cravings. In addition, the combination of NIBS with other therapeutic interventions has been shown to increase functional specificity of neuromodulation, and improve the outcome of the treatment (Edwards et al., 2009; Spagnolo et al., 2020; Tan et al., 2015).

Individual variability factors that affect NIBS responsiveness might be identified through the analysis of physiological responses, such as electroencephalogram (EEG). Specific patterns of EEG have been shown to be associated with addiction and GD, including increases of low frequency bands and reduced high frequency bands (Lee et al., 2017a; Newson & Thiagarajan, 2019). Furthermore, low cortical arousal has been associated with increased power of slow waves (delta and theta bands), whereas increased power in fast waves (alpha and beta) was associated with higher excitability of the nervous system (Kim et al., 2018). As a tool to modulate cortical excitability, tDCS over the DLPFC showed that real stimulation increased beta frequency power measured in the mid-frontal region (Song, Shin, & Yun, 2014), and reduced left frontal delta absolute power (Keeser et al., 2011). EEG might provide an improved understanding of neuromodulation effects on the nervous system, and help identify potential risk-factors to target with tDCS (Al-Kaysi et al., 2017). Therefore, psychophysiological research investigating the use of tDCS coupled with therapy interventions, might support the development of novel clinical strategies for treating GD.

The main aim of this experiment was to investigate the cumulative effects of multiple sessions of tDCS coupled with CBT in patients with GD. Specifically, whether tDCS could help to improve the outcomes of the CBT intervention. It was hypothesised that GD symptomatology would improve across sessions in both real stimulation and sham conditions (due to the CBT effects), and that real stimulation condition would be associated with further improvements on treatment outcomes compared with sham.

In terms of the cognitive task performance (which could also improve due to the CBT treatment), it was hypothesised that real stimulation would be associated with an increased improvement of cognitive performance compared with sham. This would include also the tasks that were performed before tDCS in each session, due to the potential cumulative effect of the intervention. In Cambridge gambling task (CGT), it would be expected to find higher quality of decision-making (QDM), lower risk-taking (RT) and lower delay aversion (DA)[4], and larger differences between the groups in higher risk conditions of the task. In the information sampling task (IST), higher number of boxes opened, probability of correct response (P (correct)) at point of decision and total correct trials. In SST lower stop signal task reaction time (SSRT), higher total correct stop trials and total correct go trials. In addition, it was expected to find a short term effect of tDCS, indexed by a reduction of EEG slow waves (delta and theta) and an increase of EEG fast waves (alpha and beta), after each session, in larger extent during real stimulation compared with sham. Lastly, it was hypothesised to find correlations between electrophysiological and clinical outcomes.

4.2.2 Clinical research designs

Randomised controlled trials (RCTs) are robust research designs that allow to identify the effects associated with an intervention, with the intention to probe its efficacy. However, the resources required to implement RCTs involve substantial amounts of funding, time, and employees, as well as large sample sizes of participants that meet specific inclusion criteria, which beyond complicating recruitment procedures, also limit the generalisation of the results to patients with the particular characteristics that made them suitable to take part in the study. Although RCTs are strong designs to investigate novel treatment interventions, often are difficult to implement. Besides these complications, conducting only RCTs could have other disadvantages, like inclining the development of interventions towards some patients, while not addressing the needs of others. In addition, RCTs results are often presented as group means, and therefore might not represent any individual participant. As an alternative, other study designs, such as single participant studies, can offer scientific base to investigate the effects of treatment interventions in specific circumstances (Lobo, Moeyaert, Cunha, & Babik, 2017).

[4] Although in Experiments I and II results showed an increase of RT and no changes of DA after tDCS targeting both rDLPFC and vmPFC, it was hypothesised that tDCS might reduce RT and DA due to the changes in the HD montage used in this experiment, the different sample characteristics, and based on previous literature discussed in the Introduction section of Experiments I and II.

There are different types of single participant designs. The case report or case study/series involves the participation of a single subject, but there is not a purposeful manipulation of an independent variable (participants are observed across time without experimental manipulation of the intervention), there are not necessarily repeated measures, and is usually presented in a narrative way. In contrast, single-case studies are stronger designs that are presented numerically or graphically, the dependent variables (outcome measures) are recorded repeatedly for individual participants across time, and there are different levels (phases) of an intervention (independent variable) that is systematically manipulated to allow hypothesis testing. One phase is used as a baseline so each participant serves as their own control. Single-case studies allow establishing causal inferences for experimental assessment of intervention effects, and although this design has been often undervalued in comparison with RCTs, there are advantages and disadvantages to both types of design (Lobo et al., 2017).

4.2.3 Clinical trial settings at the UK National Problem Gambling Clinic

Until September 2019, the UK National Problem Gambling Clinic (NPGC) was the only NHS clinic in UK dedicated to treat GD. This highlights the limited availability of public services offered in relation to the increasing GD-related public health concerns during the past years. The clinical trial design planned to be conducted at the NPGC consisted of a RCT with two groups (real stimulation and sham), that attended eight sessions of tDCS combined with the treatment offered at the clinic. Recruitment at the NPGC was sustained for 11 months after the NHS Health Research Authority (HRA) ethical approval was obtained, which application process took one year to complete.

Recruiting participants was a much more complicated process than originally anticipated. The clinical trial inclusion criteria was one of the most important factors that made recruitment challenging. Most patients that were referred to the clinic had a complicated profile, with severe GD and comorbid substance use disorders (SUDs) and mental health conditions that were reason for exclusion of the study. In addition, the design of the clinical trial planned for this project implied the combination of tDCS with a specific form of treatment delivered at the clinic, to investigate whether tDCS could help improve current treatment interventions. The form of treatment provided at the clinic to be combined with tDCS was conveyed in collaboration with the psychiatrist and psychologists working at the clinic. Treatment forms available at the clinic that were not adequate for the combination with tDCS

were: remote CBT (participants needed to attend the sessions in person to receive tDCS, therefore, patients allocated to remote treatment were not considered); group CBT (there was only one tDCS device dedicated to the clinical trial, consequently, it was not possible to test all participants allocated to each group having to combine both interventions, which consisted of delivering tDCS just before the CBT session. Also, offering the opportunity to participate in the trial to only one person of the group could have contributed negatively to the whole group dynamics, and could have influenced treatment outcomes. Therefore, group CBT patients were not considered for the trial); and pharmacological medication that was prescribed to severe cases of GD (receiving pharmacological medication was a reason for exclusion of the study, due to the lack of data that informed of possible interactions between tDCS and the chemical components of the medication). Therefore, individual CBT was the form of treatment that was more appropriate for the combination with tDCS in the clinical trial. In turn, this was also an added complication that limited the number of potential participants suitable for the study, especially because the patients that were allocated to individual CBT, often had co-occurring mental health disorders (which was part of the exclusion criteria).

In addition, even when patients met the inclusion criteria, and agreed to take part in the study, the limited availability of the services was another hurdle, because patients usually had to wait for around three months to start treatment, and sometimes by the end of the waiting period they had changed their mind, could not get enough time off work to come for the extra time to take part in the research, or even decided to drop the CBT treatment at the clinic. In an effort to improve recruitment rates, the inclusion criteria was broadened to avoid excluding patients with common comorbid psychiatric disorders such as anxiety, obsessive compulsive disorder or depression (if patients were not currently taking medication). However, this did not result in any increase of participants recruited, because even though the number of potential participants was higher, these patients were usually taking medication or not agreeing to take part in the research. Therefore, as part of the PhD project, there was an essential limit of time and resources that could be dedicated to pursue completing an RCT, and with the recruitment complications described, it was decided to terminate the trial with very little data collected.

4.3 Methods

4.3.1 Participants

Two patients that attended eight sessions at the NPGC were recruited. Recruitment was carried out through NPGC staff who assessed NHS patients that were referred to the clinic to receive treatment for GD. NPGC staff screened that potential participants met the study inclusion criteria, involving male or females between 18-65 years diagnosed with GD based on the Problem Gambling Severity Index (PGSI), who could speak and read English, capable of giving informed consent**,** and not having any of the exclusion criteria (for exclusion criteria details see section 3.3.1 in Chapter 3). In addition, patients had to be allocated to a specific type of treatment at the clinic, which consisted on attending individual CBT sessions. Participants were compensated for their time with shopping vouchers. One participant was allocated to the real stimulation condition (male, 44 years) and the other participant (male, 46 years) was allocated to sham condition.

4.3.2 Materials

This experiment was conducted with a tDCS Starstim 8 tDCS device (Neuroelectrics, Barcelona) and Ag/AgCl NG Geltrode electrodes with a contact area of 1 cm^2 that were filled with Signagel conductive saline gel. EEG resting state was recorded with 8 Ag/AgCl electrodes (Neuroelectrics, Starstim 8) and spectral analysis was conducted with the software LabChart 8 (ADInstruments) for each electrode individually. The CANTAB battery of tasks was used to measure gambling-related behavioural performance, including the Cambridge gambling task (CGT), information sampling task (IST) and stop signal task (SST). The CGT was used as an end of the intervention assessment measuring quality of decision-making (QDM), risk-taking (RT) and delay aversion (DA). IST was used as a priming task and to assess reflection-impulsivity, measuring the variables mean probability of making a correct decision (Mean P (correct)) and number of boxes opened per trial. The SST was used to measure inhibition control during tDCS, through the variables total correct on go and stop trials, and stop signal reaction time (SSRT). The Yale-Brown obsessive compulsive scale (PG-YBOCS) was used to measure gambling severity, whereas the gambling symptom assessment scale (G-SAS) was used to measure gambling symptoms, and the visual analogue scale (VAS) to measure gambling cravings. PG-YBOCS and VAS allowed to assess changes of gambling severity and cravings across sessions. The tDCS events sham questionnaire was used to control for participants sensations associated to tDCS. For more detailed information about the CANTAB CGT, see section 3.3.2 in Chapter 3.

- Yale-Brown Obsessive Compulsive Scale adapted for Pathological Gambling (PG-YBOCS; (Pallanti, 2005)[5]: it is a 10-item questionnaire that measures gambling severity. The scores range from 0 to 4 in each question, and the total score ranges from 0 to 40. The questions 1 to 5 assess urges and thoughts associated with gambling disorder, and the rest of the questions assess the behavioural component of the disorder. Gambling severity is higher with higher PG-YBOCS scores.

- Gambling Symptom Assessment Scale (G-SAS; Kim, Grant, Adson, & Shin, 2001): it is a 12-item scale to measure gambling symptoms. Each of the 12 questions has a score ranging from 0 to 4 based on the last week. It is useful to measure changes during treatment. The total score ranges from 0 to 48. The symptoms severity is higher with higher G-SAS scores.

- Visual Analogue Scale (VAS; Sauvaget et al., 2018): a VAS consists of a horizontal line of length 10 cm, where the left side corresponds to the lowest score and the right side to the highest score (total range from 0 to 10). The participant draws a mark on the line indicating a level that best represents their gambling craving/urge to gamble at the current time. Higher VAS scores indicate higher levels of craving/urge.

- Events sham questionnaire (Brunoni et al., 2011; Reckow et al., 2018): this questionnaire is widely used to control for participants awareness of the stimulation sensations to control the blinding procedure, and to inform about possible adverse events that could be associated with non-invasive brain stimulation methodologies.

- Information sampling task (IST; Clark, Roiser, Robbins, & Sahakian, 2009): this task was administered on a touch-sensitive monitor. Participants were presented with a 5x5 array of grey boxes on the screen and two larger coloured panels below these boxes. Participants were instructed that they were playing a game for points, which they could win by making a correct decision about which colour was in the majority under of the 25 boxes. They had to select the grey boxes one at a time,

[5] In Experiments I and II, gambling severity was measured with the South Oaks gambling screen (SOGS) because this was a commonly used questionnaire in previous gambling studies (Goudriaan et al., 2004; Zilberman, Yadid, Efrati, Neumark, & Rassovsky, 2018). However, in Experiment III, the PG-YBOCS was chosen to measure gambling severity because unlike SOGS, PG-YBOCS can capture changes of gambling symptoms across sessions.

these then opened up immediately to reveal one of the two colours shown at the bottom of the screen. Once a box had been selected, it remained open. When the participants made their decision about which colour was in the majority, they selected the panel of that colour at the bottom of the screen to indicate their choice. The task was presented in two different conditions, fixed and decreased. In the fixed condition, participants could win 100 points for a correct response, independently of how many boxes they opened. However, in the decreased condition, 250 were available at the start, and decreased 10 points with each box opened. In this condition there is a conflict between the level of certainty and the reward available. In both conditions, incorrect responses result in a deduction of 100 points. The IST is represented in Figure 4-1. The variables measured with this task include total correct trials, mean number of boxes opened and the probability of making a correct response (P(correct)), which is related to the levels of uncertainty tolerated during decision-making.

- The Stop Signal Task (SST; Logan, Cowan, & Davis, 1984; Ouellet et al., 2015): this task measures response inhibition or impulse control and was administered on a touch-sensitive monitor. Participants are presented by go and stop trials. On go trials, stimuli represented by arrows pointing to the right or to the left are displayed randomly, and participants are required to match the direction of the arrow by selecting one of two options on the bottom of the screen. On stop trials, the arrows are immediately followed by an auditory signal, which indicated that participants have to refrain from responding. The SST is represented in Figure 4-2. The difference between go reaction time and stop signal delay represents the time available to execute the act of control. Therefore, the response can be inhibited only if the act of control finishes before the go response. The stop signal reaction time (SSRT) represents the time required to prevent a planned response.

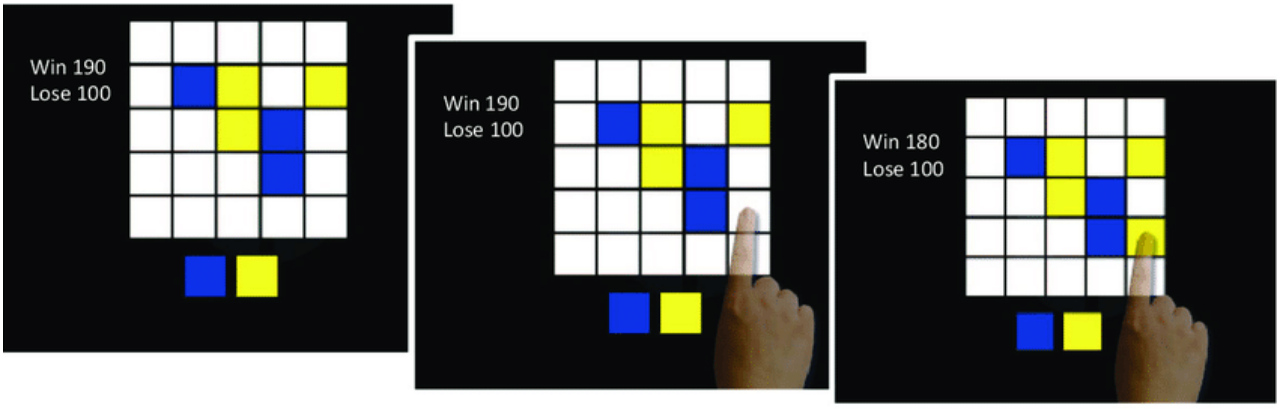

Figure 4-1. Information sampling task (IST). Participants were presented with a 5x5 array of grey boxes on the screen and two larger coloured panels below these boxes. Participants could win points by making a correct decision about which colour was in the majority under the 25 boxes. They selected the grey boxes one at a time to reveal one of the two colours shown at the bottom of the screen. When participants made their decision about which colour was in the majority, they selected the panel of that colour at the bottom of the screen. The decreased condition is represented, in which 10 points are taken off for each boxed opened from the total points available to win (Banca et al., 2016).

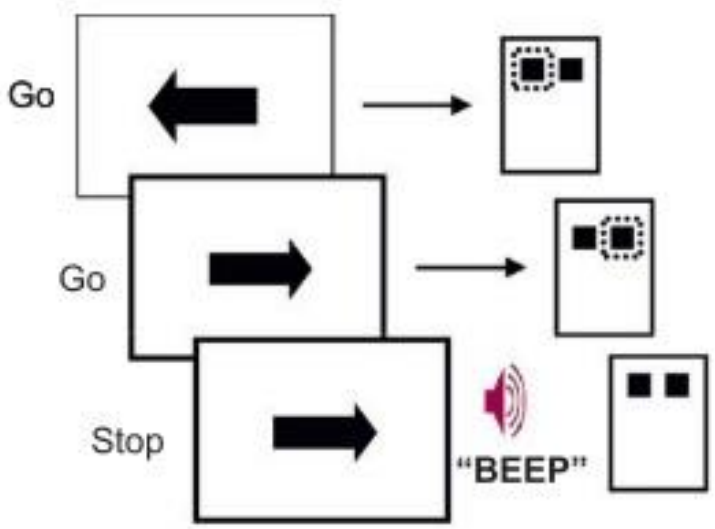

Figure 4-2. Stop signal task (SST). The SST paradigm requires the intentional inhibition of a voluntary speed response. Participants had to respond to a go stimulus represented by an arrow, by selecting one of two options, depending on the direction in which the arrow pointed to. If an audio tone was displayed, participants had to withhold making that response (Ouellet et al., 2015).

4.3.3 Procedure

Each participant received 20 minutes of tDCS in each session during eight weeks (eight sessions). Each session had a duration of one hour, with the setting up and cleaning of the equipment taking around 40 minutes more. A total of 16 sessions were conducted. Before participation in the study, written informed consent was obtained, which was approved by the University of East London Research Ethics Committee (UREC_171823) and by the NHS Health Research Authority (HRA) Research Ethics Committee (NHS IRAS_241677, REC_18/LO/1454) included in Appendix D. All experimental procedures were conducted following the General Data Protection Regulation (GDPR).

In session one and session eight, participants completed the G-SAS and the CGT, before the tDCS/EEG protocol. These two tests were only performed in the first and last sessions to avoid repetition, and to assess the final outcome of the treatment. In addition, to measure intervention effects across sessions, participants completed in every session the PG-YBOCS, the VAS, and the IST, before the tDCS/EEG protocol, and the SST during tDCS.

Electroencephalogram (EEG) resting state was recorded with eyes open while participants remained still looking at a fixed mark on the screen for five minutes before and after tDCS, to inform about the neurophysiological effects of the intervention. The IST was used before tDCS as a priming task, with the aim to help activate the brain areas involved in gambling-related cognitive functions of interest to be targeted with tDCS. This should help to increase the effects of neuromodulation by facilitating reaching the membrane action potential during real stimulation (Colombo, Bartesaghi, Simonelli, & Antonietti, 2015; Fertonani, Brambilla, Cotelli, & Miniussi, 2014; Fujiyama et al., 2017). IST ascending and descending conditions were administered in a counterbalanced order across sessions, to introduce some variation, trying to lessen as much as possible the practice effects, given that the task was used in all sessions. At the end of the last session participants completed the tDCS events sham questionnaire to assess participants sensations related to the stimulation, and awareness of the stimulation condition they had been allocated to.

4.3.4 Transcranial direct current stimulation high definition montage modelling

A computational model created by Neuroelectrics (STIMWEAVER SPR0122), was used to identify the most effective tDCS montage to target the rDLPFC (Ruffini et al., 2013). Optimization parameters

were performed with total injected current of 4 mA and a maximum current per electrode of 1.8 mA[6]. The montage selected involved the use of six electrodes for tDCS stimulation (with eight electrodes being used for EEG recording of resting state before and after tDCS), using an excitatory electric field in the target area of 0.25 V/m.

The standard safety constraints were applied with maximal injected current into the brain at any given time being below 2 mA. In the target map, the rDLPFC was identified as Broadmann area 46 on the right hemisphere, which was selected to receive the excitation, and no stimulation was directed to the rest of the cortex. The optimised montage included electrodes with currents: Cz (-200 uA), AF8 (909 uA), AF4 (1268 uA), Fpz (-1692 uA), F4 (2018 uA) and FC6 (-1303 uA). The tDCS montage is represented in Figure 4-3.

[6] In Experiments I and II the current employed was 1.5 mA given that this was a commonly used parameter in previous tDCS studies (Lefaucheur et al., 2016). However, in this experiment a computational model was used, resulting in the recommendation to use a maximum current of 1.8 mA per electrode, to reach the desired brain target more effectively.

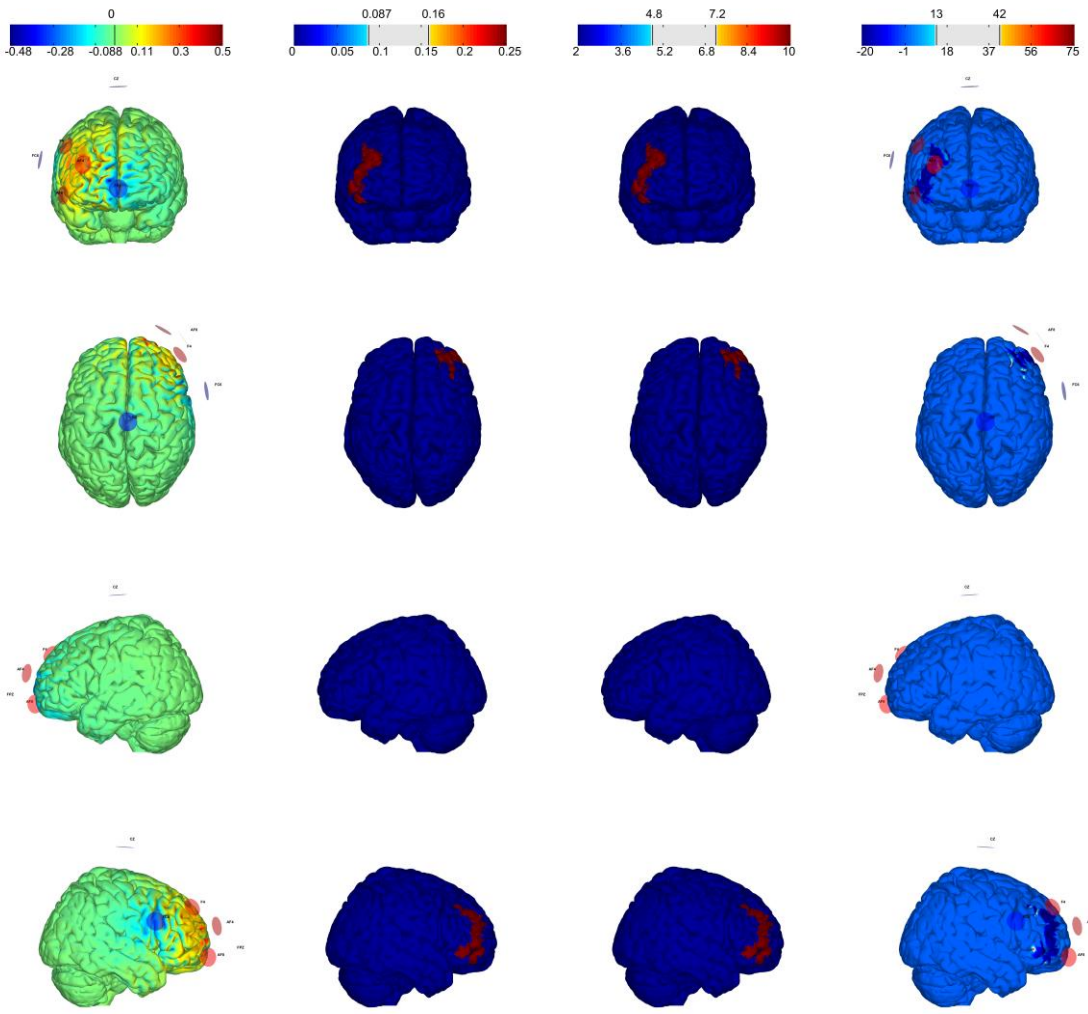

Figure 4-3. Optimized transcranial direct current stimulation montage. I Max = 1:7mA, 6 electrodes montage. From left to right: normal component of the E-field E_n *(V=m)*, target E-field*. (V=m)*, target weight and ERNI *($mV^2=m^2$)* for grey matter. (Neuroelectrics, Barcelona).

4.3.5 Electroencephalogram (EEG) data processing

EEG resting state eyes open was recorded for five minutes pre-tDCS stimulation and five minutes post-tDCS stimulation, with 8 Ag/Ag electrodes with frontal locations (Cz, Fpz, F4, AF8, AF4, FC6, F3, AF7). EEG was sampled at 500 Hz and channels were referenced to the right mastoid. Resting state EEG was used for spectral analysis for each electrode individually. Recordings were segmented into two second epochs that were fast Fourier transformed (FFT) with a Hamming window with 25% overlap, and filtered with a 50 Hz filter applied online, and offline digital filters (0,1 Hz high pass filter, followed by a 40 Hz low pass filter). EEG data was visually inspected and automatic artefact detection (threshold of 100 μV) was used to identify and reject epochs containing muscle and eye blink artefacts. Each session had a minimum of 70 artefact free epochs, with a total minimum duration of one minute. Mean frequency band was calculated, as well as the absolute and relative power was measured in four frequency bands delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-30 Hz) and theta/beta ratio for each electrode in each EEG recording (pre/post tDCS), with the aim to compare tDCS effects in each session.

4.3.6 Analysis plan

The clinical trial design originally planned at the NPGC consisted of two randomised controlled groups with parallel assignment with a duration of eight sessions. Given the unsuccessful recruitment situation described in section 4.3.1 of this chapter, data that was collected during the clinical trial was analysed according to single participant designs to investigate treatment progression. The data was analysed following case reports designs, as in previous studies where repeated sessions of tDCS and rTMS, respectively, were applied to a single participant with GD (Martinotti et al., 2018; Pettorruso et al., 2019), and obsessive compulsive disorder (Palm et al., 2017), with variables being analysed as simple percentage change in scores across sessions. Although data could be analysed according case report designs, access to patient personal clinical data was not approved for this trial, and therefore it was not possible to include an assessment of clinical outcomes, as usual in case reports. Data was analysed by measuring the percentage change in questionnaire and task scores between session one (S_1) and the other sessions (S_{2-8}) with the formula: $[(S_{2-8}-S_1)/ S_1] \times 100$.

To strengthen data analysis, a part from presenting results as two separate case reports, an adjusted analysis for single-case studies (Meiron et al., 2018) was also conducted. The current study design would be comparable to the simplest variant of the single-case study designs “AB”. The AB design

consists of data collected from several observations during a baseline phase (denoted "A"), prior to implementing a treatment phase (denoted "B"), with the aim to provide a standard (the baseline), to which to compare the effects of the treatment, with recommended minimum of five data points in each phase (Ellis, 1999; Lobo et al., 2017). As mentioned, due to this study not being originally planned as a single-case design, the analysis plan had to be adjusted to fit the existing data, which implied that measures had to be directly compared within baselines and within intervention periods (except for the EEG data that could be compared as usual), and that two outcome variables (CGT, G-SAS) had fewer observation points than the recommended in single-case studies.

The measures that were taken before the intervention took place (A) were G-SAS, CGT, PG-YBOCS, VAS, IST and EEG1 (pre-tDCS), in session one. During the intervention phase (B) the SST was measured, and EEG2 (post-tDCS) was recorded. All these variables were measured again in session eight, after the participant received weekly sessions of tDCS (real stimulation or sham) and CBT sessions. Considering that the effects of tDCS have been shown to accumulate across sessions (Boggio et al., 2009; Jansen et al., 2013; Mondino et al., 2018), those variables were compared between sessions to investigate the changes during the intervention, given that the variables (PG-YBOCS, VAS, IST and EEG1) were measured prior the tDCS in all sessions, as well as (SST and EEG2) during and after tDCS, therefore having eight observation points in each phase. In addition, the change of EEG frequency power before and after tDCS was assessed in each session (in this case EEG1 was A, and EEG2 was B), having eight observation points in each phase, and being able to compare A vs B. To evaluate the final intervention outcome, sessions one and eight were directly compared between (A in session one (A S_1) vs A S_8) and (B S_1 vs B S_8). A graphical representation of the variables comparisons is presented in Figure 4-4. Lastly, correlations between variables were investigated with the aim to find potential behavioural and physiological markers that indicated intervention response.

For statistical analysis, IBM SPSS (Statistical Package of the Social Sciences, Version 23, SPSS Inc., Chicago, IL) was used. Significance threshold was set at $\alpha = 0.05$. Data was checked for skewness, kurtosis and normality. Non-normally distributed data according to one sample Kolmogorov-Smirnov test was transformed using logarithmic transformation (SST – total correct go trials, total correct stop trials and total correct go and stop trials), and arc sin transformation in the case of QDM. G-SAS was analysed using Wilcoxon Signed Ranks Test, and VAS was analysed with one sample Kolmogorov-Smirnov when data was not normally distributed. When sphericity was not satisfied we employed Greenhouse-Geisser correction.

The single case design statistical analysis was conducted using one way repeated measures ANOVA, with within factors session, with eight levels (except VAS that was analysed with one sample t-test). Paired t-tests were used (Meiron et al., 2018; Rojo et al., 2011) to compare scores between session one and session eight to assess the final outcome of the treatment. Questionnaire subscales and task conditions had to be collapsed in order to have enough data points to perform the statistical analysis. CGT variables (QDM, RT and DA) were analysed collapsing risk conditions and ascending/descending conditions. G-SAS total score was used adding all questionnaire items. VAS had only one data point per session, therefore sessions were collapsed together. PG-YBOCS total score was used adding the subscales urges and behaviours. IST variables (probability of correct response (P (Correct)), number of boxes opened and discrimination errors) scores were collapsed across fixed and decreased conditions. SST variables (stop signal reaction time (SSRT), total correct in go and stop trials, total correct in go trials and total correct in stop trials) scores were collapsed across the blocks of the task.

To investigate the EEG mean frequency power, the theta/beta ratio as well as the absolute and relative power were analysed for each frequency band separately and for each electrode separately (Keeser et al., 2011). Relative power was calculated as the percentage of the sum of the four frequency bands in absolute power (Rajamanickam Yuvaraj et al., 2014). Absolute and relative power results are included in Appendix B. To investigate the differences in EEG mean frequency power and theta/beta ratio before and after tDCS across sessions, a 2 x 8 x 8 repeated measures ANOVA with within factors tDCS time (pre-tDCS or post-tDCS), session (1, 2, 3, 4, 5, 6, 7 and 8) and electrode position (Cz, AF8, AF4, Fz, F4, FC6, F3 and AF7) was conducted. To assess the final outcome of the intervention, EEG frequency power results were compared between session one and session eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. To compare the effects of tDCS on EEG frequency power before and after the stimulation within each session, paired t-test (2-tailed) were conducted, considering within session comparisons only in sessions one and eight. A graphical representation of the EEG frequency power comparisons is presented in Figure 4-4.

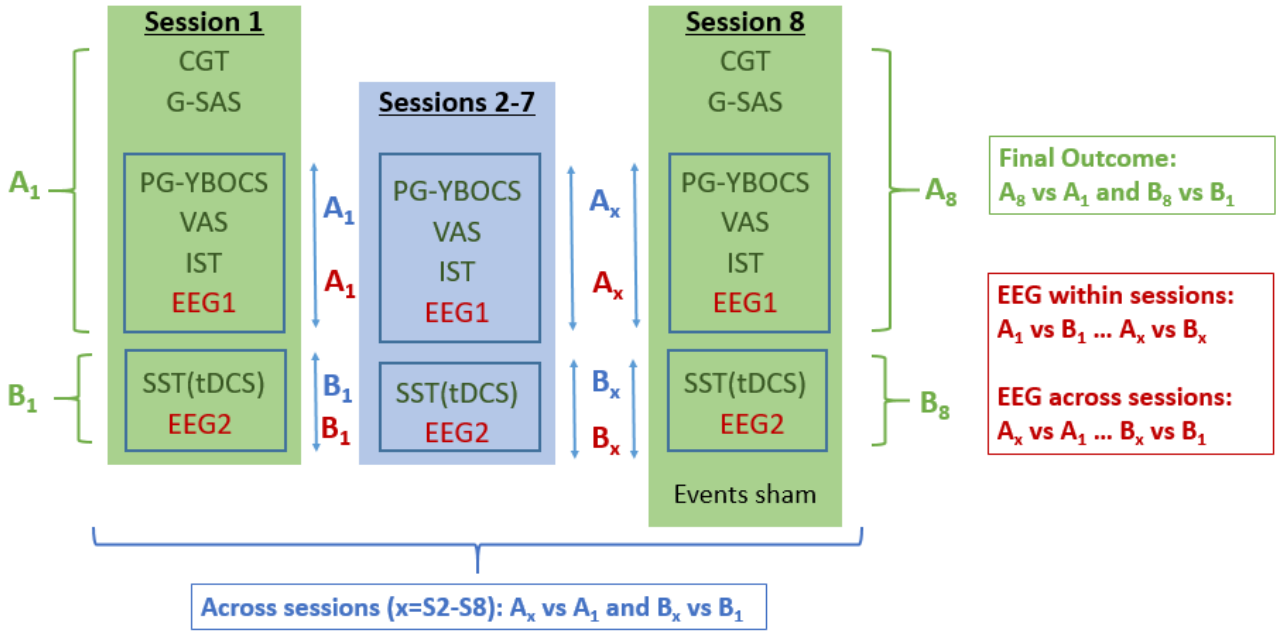

Figure 4-4. Experimental protocol at the National Problem Gambling Clinic. Questionnaires and tasks administration across sessions. In sessions one and eight participants completed the Cambridge gambling task (CGT) and the gambling symptoms assessment scale (G-SAS). In all sessions (one to eight) participants completed the Yale Brown obsessive compulsive scale for pathological gambling (PG-YBOCS), the visual analogue scale (VAS), the information sampling task (IST) and the stop signal task (SST). Electroencephalogram (EEG) resting state was recorded before (EEG1) and after (EEG2) tDCS. Variables A represent the measurements taken before the intervention using tDCS, and variables B represent the measurements taken during and after the intervention using tDCS. Analysis plan for single case studies involved comparing variable outcomes across sessions (blue colour) as well as comparing variables between the first and the last session to assess the final outcome of the intervention, in both, the tests (green colour) and the EEG (red colour).

4.4 Results

4.4.1 Participant allocated to tDCS sham condition

4.4.1.1 Self-report measures and cognitive task performance

The percentage change of questionnaires and tasks scores across all sessions is presented in Table 4-1. Results on percentage change in scores at the end of the treatment (session eight) compared with session one, indicated that there was a reduction of 44% in gambling severity measured with PG-YBOCS, a reduction of 86% in cravings measured with VAS, and a reduction of 38% of gambling symptoms measured with G-SAS. In addition, CGT variables showed a reduction of 64% of DA, an increase of 3% of QDM, and an increase of 11% of RT. Moreover, IST scores showed a reduction of mean number of boxes opened of 10%, a reduction of mean P (Correct) of 18% and no change of scores in total correct responses. In SST there was an increase of 10% of the total correct stop and go trials, an increase of 100% of the total correct stop trials, no change of total correct go trials and a decrease of 16% in SSRT.

One sample t-test results revealed significant differences between VAS scores across sessions ($t(7) = 2.949$, $p = .021$). One way repeated measures ANOVA results revealed significant differences across sessions in PG-YBOCS total scores ($F(7, 42) = 11.364$, $p = .001$, $\eta p2 = .654$) and in SSRT ($F(7, 7) = 11.650$, $p = .002$, $\eta p2 = .921$). There were no significant differences in the other variables from IST and SST. IST condition order (fixed, decreased) was not significant for any of the variables. GCT variables and GSAS data were collected only in the first and last session, therefore, they were analysed with t-tests. Paired samples t-test (2-tailed) analysis to assess the final outcomes of the eight week intervention by comparing test scores between session one and session eight are presented in Table 4-1, and showed a significant reduction of total PG-YBOCS ($t(9) = 3.498$, $p = .007$), from the first session. In addition, G-SAS was assessed with Wilcoxon signed-ranks test and showed a significant reduction of gambling symptoms ($Z = -2.547$, $p = .011$).

CGT variables showed a significant reduction of DA ($t(3) = 10.312$, $p = .002$), but not significant changes in QDM or RT. Reflection impulsivity measured with IST showed no significant changes in the number of boxes opened or the mean probability of making a correct decision. SST scores across sessions showed a significant increase of the total correct stop and go trials ($t(2) = -4.741$, $p = .042$)

and a significant decrease in SSRT (t (1) =22.196, p =.029) from the first to the last session. The IST variable total correct, and SST variables total correct stop trials and total correct go trials, could not be assessed with t-tests, because the standard error of the difference was 0, and therefore, the statistic t could not be computed. PG-YBOCS, VAS, SSRT and SST total correct stop and go scores across sessions are represented in Figure 4-5. In addition, the participant completed the tDCS events sham questionnaire at the end of the last session, revealing that the patient thought was allocated to real stimulation, marking the certainty in four, in a scale of one to five.

Table 4-1. *Questionnaires and tasks scores across sessions in participant allocated to sham condition. Percentage change from session 1 (S_1): [(S_{1-8}-S_1)/ S_1] x 100, and final intervention outcome statisctics (S_1 vs S_8).*

Questionnaires and tasks	Sessions								Test Statistic (S_1 vs S_8)
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	
Total PGYBOCS	25 -	25 (0%)	21 (-16%)	15 (-40%)	9 (-64%)	37 (+48%)	15 (-40%)	14 (-44%)	t (9) = 3.498, p =.007 *
VAS	7 -	7 (0%)	2 (-71%)	1 (-85%)	0 (-100%)	6 (-14%)	1 (-86%)	1 (-86%)	[a] t (7) = 2.949, p =.021 *
G-SAS	39 -	-	-	-	-	-	-	24 (-38%)	Z = -2.547, p = .011 *
CGT DA	0.45 -	-	-	-	-	-	-	0.16 (-64%)	t (3) = 10.312, p =.002 *
CGT QDM	0.97 -	-	-	-	-	-	-	1 (+3 %)	t (7) = -1.000, p =.351
CGT RT	0.68 -	-	-	-	-	-	-	0.76 (+11%)	t (7) = -1.419, p =.199
IST Mean number boxes opened	9.8 -	7.7 (-21%)	8.8 (-10%)	10.45 (+7%)	7.2 (-26%)	8.55 (-12%)	9.25 (-6%)	8.85 (-10%)	t (1) = 3.800, p =.164
IST Mean probability correct	0.77 -	0.67 (-12%)	0.72 (-6%)	0.77 (0%)	0.70 (-9%)	0.72 (-6.5%)	0.74 (-4%)	0.76 (-1%)	t (1) = -0.11, p =.993
IST Total Correct	16 -	17 (+6%)	16 (0%)	15 (-6%)	4 (-12%)	16 (0%)	16 (0%)	16 (0%)	Not Computed **
SST Total correct stop and go	40 -	42 (+5%)	43 (+7%)	42 (+5%)	41 (+2)	41 (+2)	44 (+10%)	44 (+10%)	t (2) = -4.741, p =.042 *
SST Total correct stop	4 -	6 (50%)	7 (+75%)	6 (+50%)	5 (+25%)	7 (75%)	9 (+125%)	8 (+100%)	Not computed **
SSRT (milliseconds)	175 -	165 (-5%)	145 (-17%)	151 (-13%)	113 (-35%)	127 (-27%)	139 (-21%)	146 (-16%)	t (1) =22.196, p =.029 *

Note: PG-YBOCS, Yale Brown obsessive compulsive scale for pathological gambling; VAS, visual analogue scale; G-SAS, gambling symptoms assessment scale; CGT, Cambridge gambling task; DA, delay aversion; QDM, quality of decision-making; RT, risk-taking; IST, information sampling task; SST, stop signal task; SSRT, stop signal reaction time. Data represents test scores and inside the parenthesis percentage change from session 1 [(S_{1-8}-S_1)/ S_1] x 100. (* $p < .05$; ** the t cannot be computed because the standard error of the difference is 0). [(a)] VAS analysis involved all sessions.

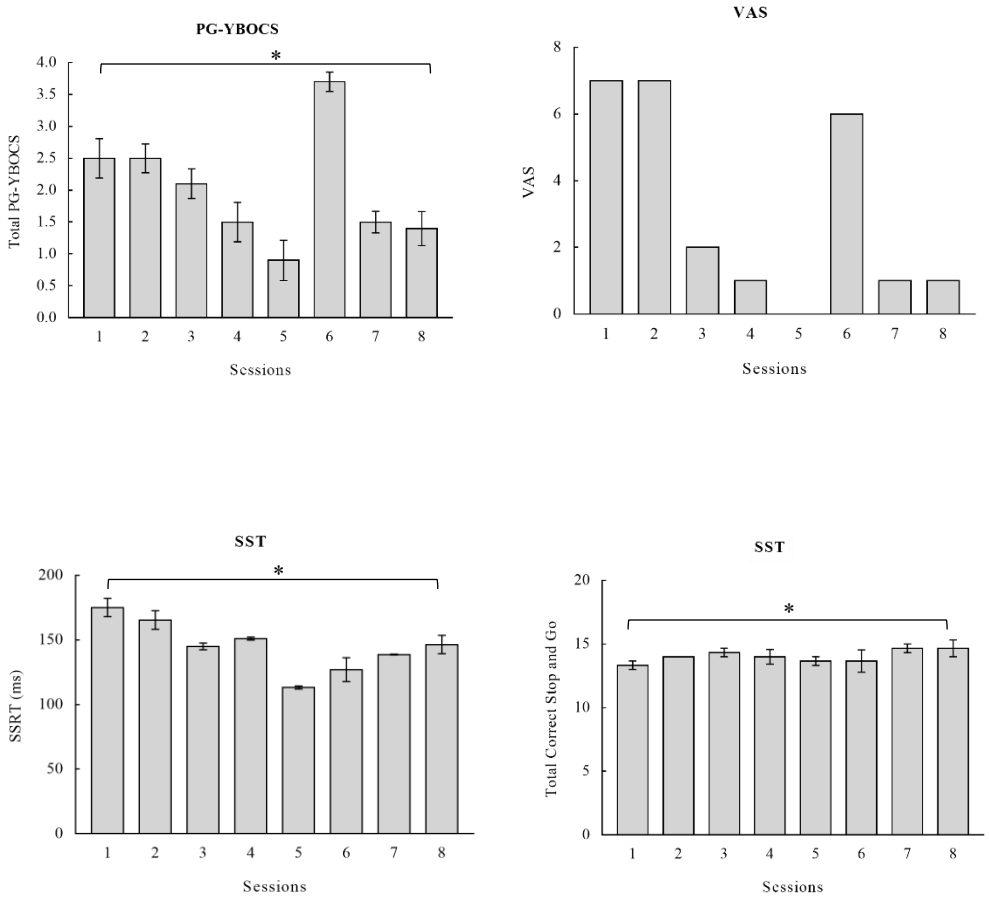

Figure 4-5. Questionnaires and stop signal task (SST) performance across sessions in the participant allocated to sham condition. Scores across sessions for gambling severity measured with the Yale Brown obsessive compulsive scale for pathological gambling (PG-YBOCS), gambling cravings measured with the visual analogue scale (VAS), stop signal task reaction time (SSRT) and total correct stop and go trials. Data represent mean and SEM, except in VAS in which each bar represents one data point (* $p < .05$).

4.4.1.2 Theta/beta ratio:

There were no significant results in theta/beta ratio across sessions ($F(1.039, 73.752) = 1.909$, $p = .171$, $\eta p^2 = .026$), electrodes ($F(1, 71) = 1.456$, $p = .231$, $\eta p^2 = .020$) or tDCS time ($F(1.084, 76.973) = 1.786$, $p = .185$, $\eta p^2 = .025$).

4.4.1.3 Mean frequency power:

One way repeated measures ANOVA analysis of mean frequency power revealed a significant main effect of session ($F(5.300, 370.972) = 17.179$, $p = .001$, $\eta p^2 = .197$), showing that session four had the highest mean frequency power in the range of beta frequency, followed by the rest of the sessions in the range of alpha frequency. There was also a main effect of tDCS time ($F(1, 70) = 12.531$, $p = .001$, $\eta p^2 = .152$), showing that mean frequency power was lower in EEG1 (pre-tDCS) compared with EEG2 (post-tDCS), and a main effect of electrode ($F(3.697, 258.789) = 275.477$, $p = .001$, $\eta p^2 = .797$) indicating that electrodes from the highest to the lowest mean frequency power were AF7, F3, AF8, F4, FC6, Cz, AF4 and Fpz. In addition, there was an interaction session x electrode ($F(16.434, 1150.389) = 49.576$, $p = .001$, $\eta p^2 = .415$), showing that session four had the highest mean frequency power in electrodes F4, AF7 and AF8.

There was also an interaction tDCS time x electrode ($F(3.243, 227.021) = 16.594$, $p = .001$, $\eta p^2 = .192$), showing that in all electrodes except for Cz, higher mean frequency power was associated with EEG2 compared with EEG1. A three way interaction session x tDCS time x electrode ($F(17.457, 1222.023) = 30.267$, $p = .001$, $\eta p^2 = .302$), showed that in EEG1 the electrode with highest mean frequency power was AF7, however in EEG2 the electrode with highest mean frequency power was F4. Also, the session with highest frequency power by electrode in EEG1 was session seven in AF7, session five in AF8, F3, Cz, AF4 and Fpz, and session four in F4 and FC6. In EEG2, session four showed the highest mean frequency power in all electrodes except for F3, in which session five showed the highest mean frequency power. Mean frequency power values across sessions and electrode positions are represented in Figure 4-6.

To assess the final outcome of the intervention, EEG mean frequency power results were compared between session one and session eight with paired t-tests for each electrode position and each frequency band separately. Results showed that EEG1 mean power frequency in session one was

significantly higher than EEG1 mean frequency power in session eight, for electrodes AF8 (t (117) =6.641, p =.001), Fpz (t (117) =4.007, p =.001) and FC6 (t (117) =3.533, p =.001). EEG2 mean frequency power in session one was significantly lower than EEG2 mean frequency power in session eight for all electrodes: Cz (t (116) = -4.550, p =.001), AF8 (t (116) = -2.422, p =.017), AF4 (t (116) = -3.211, p =.002), Fpz (t (116) = -3.428, p =.001), F4 (t (116) = -5.450, p =.001), FC6 (t (116) = -6.763, p =.001), F3 (t (116) = -4.251, p =.001) and AF7 (t (116) = -2.565, p =.012).

To compare the effects of tDCS on EEG mean frequency power before and after the stimulation within each session, considering sessions one and eight, paired t-test revealed that mean frequency power was lower in EEG1 than in EEG2 in session one for electrodes AF8 (t (120) = 2.358, p =.020), Fpz (t (120) = 3.440, p =.001) and F3 (t (120) = 2.229, p =.028), and in session eight for all electrodes except F3: Cz (t (116) = -3.080, p =.003), AF8 (t (116) = -5.848, p =.001), AF4 (t (116) = -3.865, p =.001), Fpz (t (116) = -3.435, p =.001), F4 (t (116) = -4.291, p =.001), FC6 (t (116) = -8.943, p =.001) and AF7 (t (116) = -3.273, p =.001).

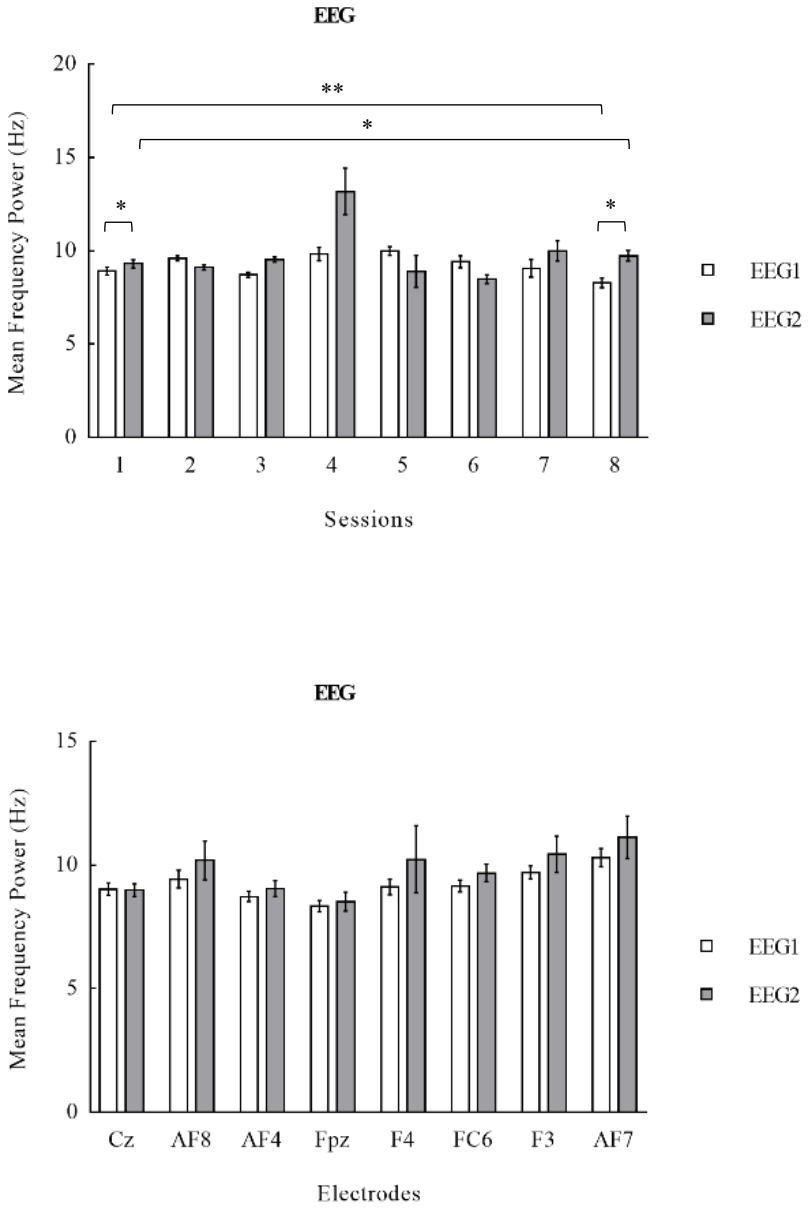

Figure 4-6. Electroencephalogram (EEG) mean frequency power in sham condition. Mean frequency power (Hz) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2) across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found only in electrodes AF8, Fpz and FC6. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found only in electrodes AF8, Fpz and F3, however in session eight EEG1 and EEG2 were significantly different in all electrodes except F3.

4.4.1.4 Correlations

To investigate the relationship between gambling severity and the rest of the variables, 2-tailed Pearson's correlation indicated that gambling severity measured with PG-YBOCS correlated positively with VAS for gambling cravings (r =.835, n = 8, p =.010) and with EEG2 theta/beta ratio in F3 (r = .948, n = 8, p =.001). In addition, PG-YBOCS correlated negatively with SST total correct stop and go trials (r = -.737, n = 8, p =.037), total correct stop trials (r = -.715, n = 8, p =.046), total correct go trials (r = -.771, n = 8, p =.025). Correlations with CGT could not be computed due to the lack of enough data points. Other significant correlations with EEG variables were included in Appendix A.

Having found all significant correlations between gambling severity and EEG variables, specifically in the electrode position F3, during the post-tDCS recordings (EEG2), these variables were further explored with the aim to find physiological markers that could inform about individuals gambling severity, gambling-related behaviours and symptomatology. Results showed that VAS gambling cravings correlated positively with theta/beta ratio in F3 (r = .739, n = 8, p =.036). In addition, IST total correct correlated positively with EEG2 in F3 with theta/beta ratio (r = .719, n = 8, p =.045).

4.4.2 Participant allocated to tDCS real stimulation condition

4.4.2.1 Self-report measures and cognitive task performance

The percentage change of questionnaires and tasks scores across all sessions is presented in Table 4-2. Results on percentage change in scores at the end of the treatment (session eight) compared with session one, indicated that there was a reduction of 66% in gambling severity measured with PG-YBOCS, a reduction of 71% in cravings measured with VAS, and a reduction of 6% of gambling symptoms measured with G-SAS. In addition, CGT variables showed an increase of 21% of DA, no changes in QDM, and an increase of 6% of RT. Moreover, IST scores showed an increase of mean number of boxes opened of 46%, an increase of mean P (Correct) of 8% and an increase in total correct responses of 6%. In SST, there was an increase of 11% of the total correct stop and go trials, an increase of 200% of the total correct stop trials, no change of total correct go trials, and an increase of 2% in SSRT. One sample Kolmogorov-Smirnov test results revealed significant differences between VAS scores across sessions (D (8) = .436, p =.001). One way repeated measures ANOVA results revealed significant differences across sessions in PG-YBOCS total scores (F (7, 63) = 37.807, p= .001, ηp^2 = .808). There were differences across sessions in IST number of boxes opened per trial (F (7, 7) = 10.817, p= .012, ηp^2 = .866), mean P (correct; F (7, 7) = 7.643, p= .008, ηp^2 = .884), but no significant differences were found in total correct. IST condition order (fixed, decreased; all effects p > .05). In SST, there were significant differences across sessions in SSRT (F (7, 7) = 27.391, p= .004, ηp2 = .965). There were no significant differences in the total correct go and total correct stop in SST (all effects p > .05). GCT variables and GSAS data were collected only in the first and last session, therefore, they were analysed with t-tests.

Paired samples t-test (2-tailed) analysis to assess the final outcomes of the eight week intervention by comparing test scores between session one and session eight are presented in Table 4-2, showing a significant reduction of PG-YBOCS (t (9) = 3.674, p =.005) from the first session. No other variables showed significant differences in scores between session one and session eight. All questionnaires and tasks scores across sessions, as well as final outcome statistics are represented in Figure 4-7. In addition, the participant completed the tDCS events sham questionnaire at the end of the last session, revealing that the participant thought was allocated to real stimulation, marking the certainty in three, in a scale of one to five.

Table 4-2.*Questionnaires and tasks scores across sessions in participant allocated to real stimulation condition. Percentage change from session 1: [(S_{1-8}-S_1)/ S_1] x 100, and final intervention outcome statisctics (S_1 vs S_8).*

Questionnaires and tasks	Sessions								Test Statistic (S_1 vs S_8)
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	
Total PGYBOCS	18 -	3 (-83%)	3 (-83%)	26 (+44%)	3 (-83%)	14 (-22%)	1 (-94%)	6 (-66%)	t (9) = 3.674, p =.005 *
VAS	7 -	2 (-71%)	2 (-71%)	4 (-42%)	2 (-71%)	2 (-71%)	2 (-71%)	2 (-71%)	[a] D (8) =.436, p =.001 *
G-SAS	25	-	-	-	-	-	-	19 (-6%)	Z = -1.980, p = .059
CGT DA	0.19	-	-	-	-	-	-	0.23 (+21 %)	t (3) = -.595, p =.594
CGT QDM	1.00	-	-	-	-	-	-	1.00 (0%)	Not Computed **
CGT RT	0.53	-	-	-	-	-	-	0.56 (+6%)	t (7) = -1.177, p =.278
IST Mean number boxes opened	7.6 -	8.5 (+12%)	10.3 (+36%)	13.1 (+72%)	10.2 (+35%)	10.7 (+41%)	14.8 (+95%)	11.1 (+46%)	t (1) = -1.392, p =.397
IST Mean probability correct	0.72 -	0.73 (+1%)	0.80 (+11%)	0.83 (+15%)	0.80 (+11%)	0.79 (+10%)	0.90 (+25%)	0.78 (+8%)	t (1) = -1.000, p =.500
IST Total Correct	16 -	16 (0%)	18 (+12%)	19 (+19%)	17 (+6%)	18 (+12%)	20 (+25%)	17 (+6%)	t (1) = -.200, p =.874
SST Total correct stop and go	37 -	40 (+8%)	41 (+11%)	44 (+19%)	43 (+16%)	39 (+5%)	41 (+11%)	41 (+11%)	t (2) = -1.000, p =.423
SST Total correct stop	2 -	6 (+200%)	5 (+150%)	8 (+300%)	7 (+250%)	4 (+100%)	6 (+200%)	6 (+200%)	t (1) = -1.000, p =.500
SSRT (milliseconds)	201 -	237 (+17%)	197 (-2%)	216 (+8%)	220 (+9%)	250 (+24%)	252 (+25%)	205 (+2%)	t (1) =-1.616, p =.353

Note: PG-YBOCS, Yale Brown obsessive compulsive scale for pathological gambling; VAS, visual analogue scale; G-SAS, gambling symptoms assessment scale; CGT, Cambridge gambling task; DA, delay aversion; QDM, quality of decision-making; RT, risk-taking; IST, information sampling task; SST, stop signal task; SSRT, stop signal reaction time. Data represents test scores and inside the parenthesis percentage change from session 1 [(S_{1-8}-S_1/S_1)] x 100. (* p ≤ .05; ** the t cannot be computed because the standard error of the difference is 0). [(a)] VAS analysis involved all sessions.

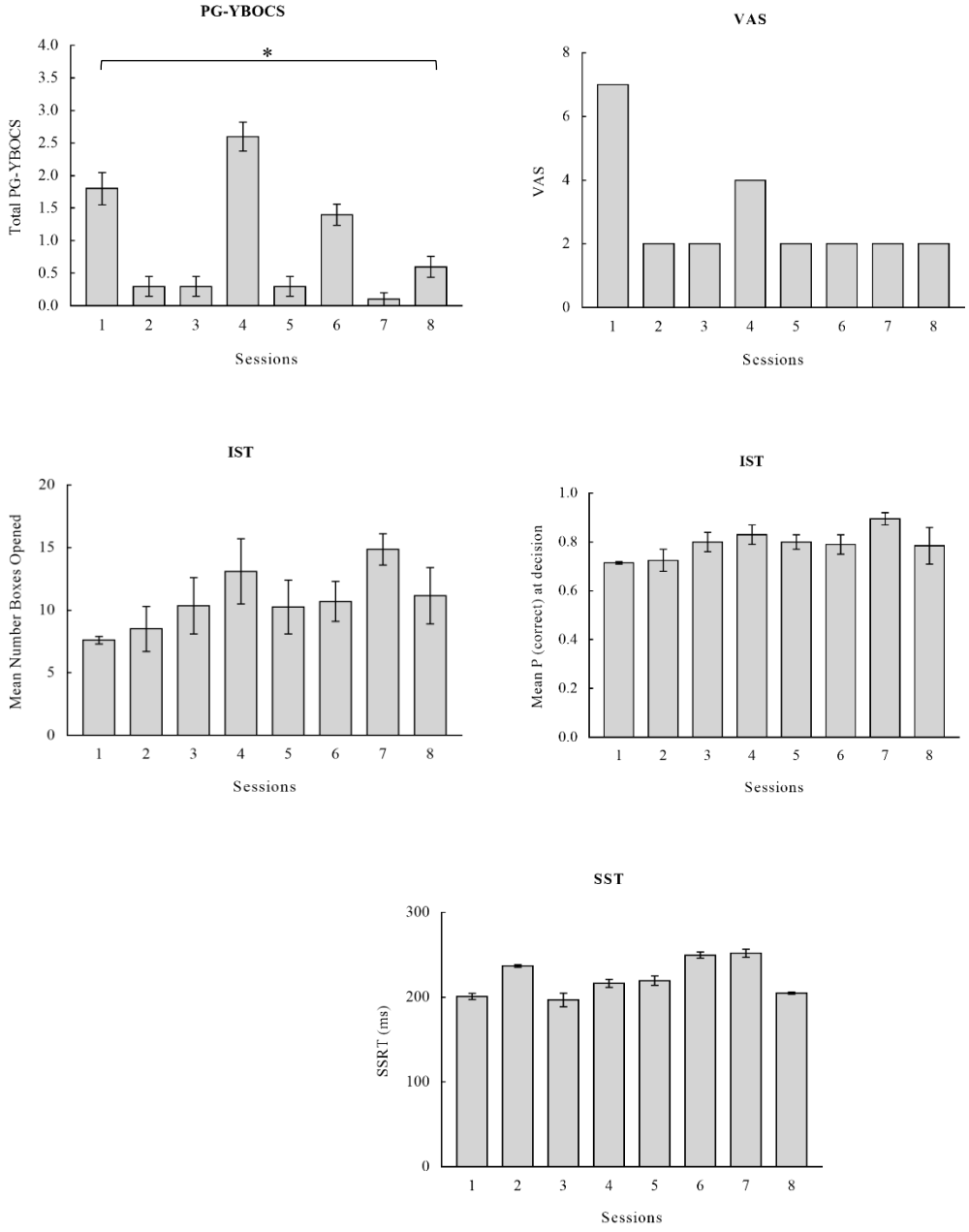

Figure 4-7. Questionnaires and stop signal task (SST) performance across sessions in participant allocated to real stimulation condition. Scores across sessions for gambling severity measured with the Yale Brown obsessive compulsive scale for pathological gambling (PG-YBOCS), gambling cravings measured with the visual analogue scale (VAS), information sampling task (IST) variables of mean number of boxes opened and mean probability correct (mean P (correct)) at point of the decision and stop signal task (SST) reaction time (SSRT). Data represent mean and SEM, except in VAS, in which each bar represents one data point (* $p \leq .05$).

4.4.2.2 Theta/beta ratio:

There were no significant results in theta/beta ratio across sessions (F (1.007, 98.678) = 1.862, p= .175, ηp^2 = .019), electrodes (F (1.043, 102.257) = 2.240, p= .137, ηp^2 = .022) or tDCS time (F (1, 98) = 2.630, p= .108, ηp^2 = .026).

4.4.2.3 Mean frequency power:

One way repeated measures ANOVA analysis of mean frequency power revealed a significant main effect of session (F (5.994, 587.403) = 99.409, p= .001, ηp^2 = .504), showing that session four followed by session seven had the highest mean frequency power in the range of beta frequency, and the lowest mean frequency power was found in session eight, in the range of delta frequency. There was also a main effect of tDCS time (F (1, 98) = 202.135, p= .001, ηp^2 = .673), showing that mean frequency power was lower in EEG1 (pre-tDCS) compared with EEG2 (post-tDCS), and a main effect of electrode (F (3.697, 258.789) = 345.346, p= .001, ηp^2 = .779) indicating that the electrode with slightly higher mean frequency power was FC6 reaching alpha frequency, but the rest had all similar mean frequency power in the range of theta.

In addition, there was an interaction sessions x tDCS time (F (6.009, 588.911) = 20.890, p= .001, ηp^2 = .176), showing that sessions five and eight had similar mean frequency power in both EEG1 and EEG2, but in the rest of the sessions EEG1 was lower than EEG2. An interaction session x electrode (F (16.982, 1664.283) = 25.608, p= .001, ηp^2 = .207), showed that session four had the highest mean frequency power in all electrodes, being the highest FC6, followed by session seven, three, one, five, six and eight. There was also an interaction tDCS time x electrode (F (16.881, 1654.322) = 14.830, p= .001, ηp^2 = .949), showing that in all electrodes higher mean frequency power was associated with EEG2 compared with EEG1. A three way interaction session x tDCS time x electrode (F (16.881, 1654.322) = 14.830, p= .001, ηp^2 = .131), showed that in all electrodes there was lower mean frequency power in EEG1 compared with EEG2, but the greatest difference between EEG1 and EEG2 was found in electrode FC6, showing similar differences in each session between EEG1 and EEG2 in mean frequency power, with the smallest difference being in session eight. Mean frequency power values across sessions and electrode positions are represented in Figure 4-8.

To assess the final outcome of the intervention, EEG mean frequency power results were compared between session one and session eight with paired t-tests for each electrode position and each frequency band separately. Results showed that EEG1 mean power frequency in session one was significantly higher than EEG1 mean power frequency in session eight, in all electrodes except in Fpz and AF7: Cz (t (100) = 3.263, p =.002), AF8 (t (100) = 2.564, p =.002), AF4 (t (117) =4.104, p =.012), F4 (t (100) = 3.108, p =.002), FC6 (t (100) = 3.621, p =.001) and F3 (t (100) = 4.117, p =.001). EEG2 mean frequency power in session one was significantly higher than EEG2 mean frequency power in session eight, in all electrodes: Cz (t (110) = 10.157, p =.001), AF8 (t (110) = 7.526, p =.001), AF4 (t (110) = 7.493, p =.001), Fpz (t (110) = 5.671, p =.001), F4 (t (110) = 9.019, p =.001), FC6 (t (110) = 11.475, p =.001), F3 (t (110) = 6.416, p =.001) and AF7 (t (110) = 2.329, p =.022).

To compare the effects of tDCS on EEG mean frequency power before and after the stimulation within each session, considering sessions one and eight, paired t-test revealed that mean frequency power was lower in EEG1 than in EEG2 in session one in all electrodes: Cz (t (118) = -9.231, p =.001), AF8 (t (118) = -5.936, p =.001), AF4 (t (118) = -4.538, p =.001), Fpz (t (118) = -3.689, p =.001), F4 (t (118) = -6.965, p =.001), FC6 (t (118) = -8.205, p =.001), F3 (t (118) = -2.768, p =.007), and AF7 (t (118) = -3.120, p =.002), however in session eight there were no significant differences between EEG1 and EEG2 mean frequency power.

4.4.2.4 Correlations

To investigate the relationship between gambling severity and the rest of the variables, 2-tailed Pearson's correlation indicated no significant correlations with the main variables studied. However, there were positive correlations between gambling severity measured with PG-YBOCS and EEG1 relative power across different electrode positions and frequency bands (included in Appendix A).

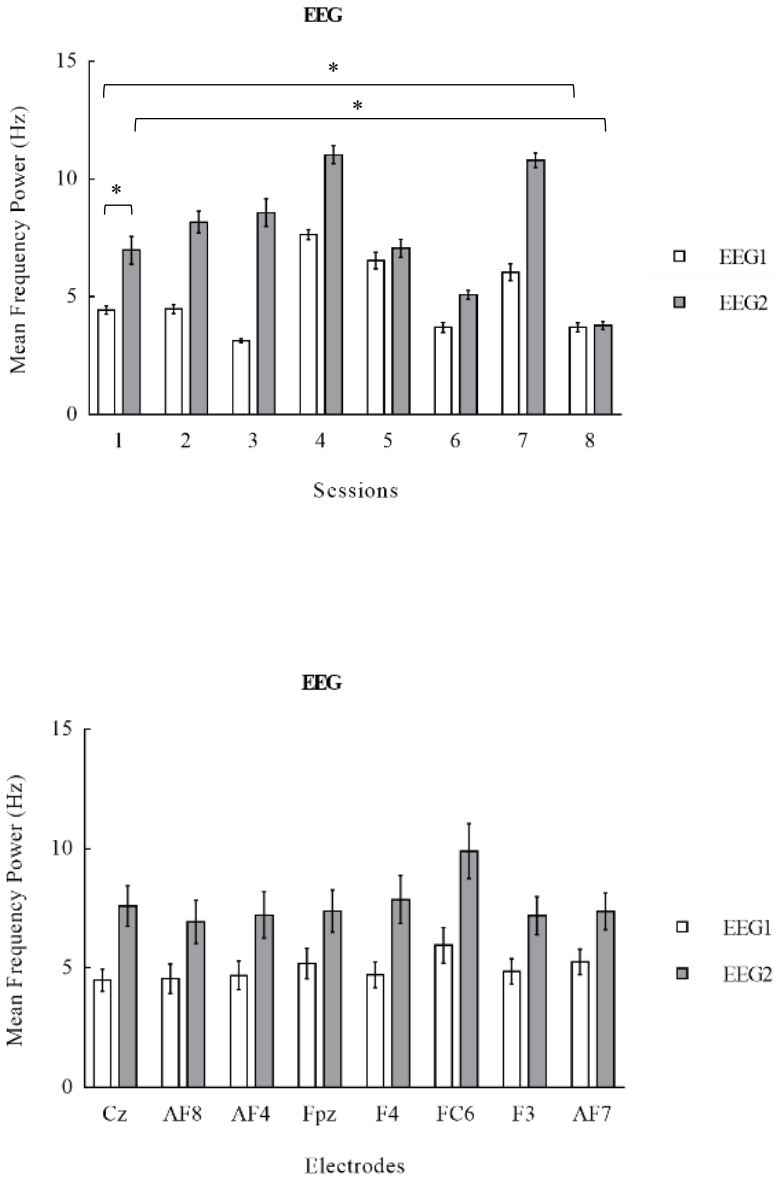

Figure 4-8. Electroencephalogram (EEG) mean frequency power in real stimulation condition. Mean frequency power (Hz) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2) across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes except in Fpz and AF7. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found in all electrodes, however in session eight there were no significant differences between EEG1 and EEG2 mean frequency power.

4.5 Discussion

This experiment was originally designed as an RCT to study the effects of tDCS across sessions in combination with the CBT treatment offered at the NPGC, with the aim to investigate the potential use of this particular intervention to improve current treatment approaches for GD. The trial planned followed a longitudinal controlled design with two groups, real stimulation and sham, with parallel assignment. The data was collected according to the RCT design, however, the analysis plan had to be adapted to fit a single-participant design, due to the recruitment complications described in section 4.3.1 of this chapter. The data collected consisted of gambling symptomatology self-report measures, cognitive behavioural tasks and EEG resting state, from two participants who attended eight sessions of tDCS combined with CBT.

The participants were assigned to two different tDCS groups (one participant received real stimulation, and the other participant received sham). Data was analysed according to two different methodologies usually employed to analyse single-participant designs: percentage change of scores across sessions was calculated as in case report studies, and statistical analysis was performed, based on single-case study analysis methods. However, given that data was collected following the RCT design, both, the case report and the single-case study analysis approaches employed had to be adapted to fit the existing data, as described in section 4.3.6 of this chapter. Results revealed significant effects of the intervention combining the tDCS procedure with CBT on gambling severity, cravings, cognitive task performance, and EEG power frequency, in both participants.

4.5.1 Participant allocated to sham condition

In the participant allocated to sham condition, results showed significant differences across sessions in gambling severity measured with PG-YBOCS, gambling cravings measured with VAS, and inhibitory control measured with SSRT (which indicates de ability to stop a prominent response). To assess the final outcome of the intervention, session eight was compared against session one, revealing that there were statistically significant changes, which were also quantified using percentage change of scores: a decrease in PG-YBOCS, G-SAS, DA and SSRT, and an increase in SST total correct trials. VAS statistics could only be calculated across sessions due to a lack of enough data points, which showed significant differences across sessions, and in addition, percentage change between the last and the first session revealed a reduction in gambling cravings. Across sessions, it was identified that session five had the lowest scores in self-report measures PG-YBOCS and VAS. Similarly,

session five in the cognitive behavioural tasks variables show the lowest scores in: IST mean number of boxes opened and total correct, and SST total correct stop and go, total correct stop and SSRT[7]. The final outcome of the intervention showed that the sham tDCS procedure combined with CBT was associated with a significant improvement of GD severity and symptomatology, including cravings, as well as behavioural impulsivity, and the ability to inhibit prominent responses. Another GD case study using a longitudinal intervention with real stimulation tDCS over bilateral DLPFC reported similar improvements, showing a reduction of 62.5% of PG-YBOCS, a reduction of 100% of VAS for gambling cravings, a reduction of 69.2% in G-SAS and a reduction of 23.6% of self-reported impulsivity (Martinotti et al., 2018).

The effectiveness of tDCS to improve addiction symptomatology is still unclear (Trojak et al., 2017). Literature reviews identified a medium size effect in favour of active tDCS compared with sham to reduce cravings (Jansen et al., 2013), however, beyond cravings modulation, research also found no differences between active and sham groups in cigarette consumption, suggesting that real tDCS might not be more effective than sham to reduce addictive behaviours (Mondino et al., 2018). The effectiveness of tDCS to treat addictive behaviours needs to be further investigated (Coles et al., 2018).

Overall, mean frequency power was highest around left DLPFC (lDLPFC), and lowest around vmPFC[8]. In particular, in EEG1 (which was recorded just after the participant completed the IST, and before receiving tDCS sham), mean frequency power was highest around left DLPFC, whereas in EEG2 (recorded after the participant received tDCS sham coupled with SST performance), the highest mean frequency power was found in right DLPFC (rDLPFC), which is the target of the stimulation protocol. This switch of activity from left to right PFC, could be interpreted as an effect of SST performance, assuming that sham tDCS had no effects on cortical excitability. Given that the SST requires participant to inhibit a prominent response, these results would be consistent with research showing that suppression of the rDLPFC (but not the lDPFC) by repetitive transcranial direct current stimulation (rTMS) reduced inhibitory control, demonstrating the specific role of rDLFC in inhibition

[7] Statistical analysis comparing each of the eight sessions against the rest was not performed (paired comparisons were conducted only between the first and the last session), and therefore, the results discussed about any other specific session, refer simply to the variables values observed. The statistical analysis was more focused on assessing the final outcome of the intervention, and to compare each session against the others (involving between and within sessions analysis), would extend the work load and amount of data reported excessively, considering the limitations associated to the adapted analysis methodologies used, the time available for the project and the limited space to report the results. Nevertheless, this will be considered to further explore the data in the future.

[8] Electrodes were not compared against each other, these results reflect the main effect found in the variable electrode.

(Knoch et al., 2006). In addition, these results are consistent with research that associated higher activity in rDLPFC due to executive functions related task performance, compared with lDPFC (Cerqueira, Almeida, & Sousa, 2008).

However, the absence of physiological effects on the brain associated with sham tDCS is still under debate (Neri et al., 2020). Therefore, an alternative explanation of these results would be that the electrical current delivered during the ramp up and ramp down of the sham condition, contributed to increase the cortical activity around the stimulation target in rDLPFC. Furthermore, this possibility, could have an added psychological influence, due to the fact that the participant was confident that received real stimulation condition. These interpretations would be supported by previous research that showed influences on cortical excitability produced by participants motivations and expectations (Brangioni et al., 2018; Rabipour, Wu, Davidson, & Iacoboni, 2018).

Results showed significant differences in mean frequency power, across sessions, between the first and the last session, and within session one and session eight. Mean frequency power was the highest in EEG2 during session four, whereas in EEG1, sessions four and five showed the highest mean frequency power. Interestingly, the participant showed the lowest gambling severity, cravings and SSRT in session five. These results would be consistent by research showing a negative association between high impulsive patients with GD and EEG power (Lee et al., 2017a).

In particular, examining separately EEG recordings before and after the tDCS (EEG1 and EEG2, respectively), and considering sessions one and eight in each frequency band, results revealed significant short term effects of the intervention, showing that in both sessions, one and eight, there was an increase of mean frequency power after tDCS coupled with SST. In addition, results revealed significant long term effects of the intervention, showing that in session eight, mean frequency power decreased in EEG1, but increased in EEG2, compared with session one. Assuming that tDCS sham had no effects, the short term increase of mean frequency power could be explained by task performance effects on cortical excitability (Gill, Shah-basak, & Hamilton, 2015; Newson & Thiagarajan, 2019). Moreover, the reduction of EEG1 mean frequency power in session eight could be a result of the CBT treatment effects across sessions, whereas the increase of EEG2 in session eight, could be interpreted as a cognitive enhancement due to recurrent practice on SST performance (Manuel, Bernasconi, & Spierer, 2013).

Previous research showed a reduction of EEG mean frequency power after both real stimulation and sham tDCS (Boonstra et al., 2016). However, they did not coupled tDCS with any cognitive task, as it was done in the current experiment. Therefore, the inconsistent results showing an increase mean frequency power in sham tDCS in this experiment, might be explained by the task related effect produced by the SST (Gill et al., 2015; Newson & Thiagarajan, 2019). Another study employing cognitive tasks found improved cognitive performance after sham but not after real stimulation (Nikolin, Boonstra, Loo, & Martin, 2017). Neuromodulation interventions have been associated with improvements in both real stimulation, but also in sham conditions (Ulam et al., 2015), which have raised the debate about the applicability of sham as a control condition in tDCS research (Boonstra et al., 2016). In fact, a study found that there was a placebo effect caused by sham stimulation, showing effects on EEG features that were not shown under a non-stimulation condition (Petersen & Puthusserypady, 2019). Therefore, it is not possible to rule out a possible placebo effect that could account for the results found in this experiment.

In addition, correlation analysis showed that gambling severity correlated negatively with SST variables total correct stop and go trials, total correct stop trials and total correct go trials, as well as with EEG2 mean power frequency in left DLPFC. An additional assessment of the neurophysiological variables that correlated with gambling severity was conducted, and correlation analysis between EEG variables in left DLPFC and gambling related behavioural and self-report measures revealed that theta/beta ratio correlated positively with cravings and with IST total correct. Additional relevant correlations from this analysis were found, and are presented in Appendix A, including negative correlations between gambling severity and beta relative power in left DLPFC, and a negative correlation between beta relative power with cravings, and between absolute power in all frequency bands with SSRT.

Previous research showed that decreased theta/beta ratio has been associated with several psychiatric disorders including addition (Newson & Thiagarajan, 2019), and with decreased inhibition control measured with SSRT (Lansbergen, Schutter, & Kenemans, 2007). Previous studies also found a negative correlation between theta/beta ratio and reward and punishment-related reversal learning (Schutte, Kenemans, & Schutter, 2017), and considering that reversal learning has been associated with gambling problems (Jara-rizzo, Navas, Rodas, & Perales, 2020), those findings would be

consistent with the positive correlation between gambling severity and theta/beta ratio found in this experiment.

4.5.2 Participant allocated to real stimulation condition

In the participant allocated to real stimulation condition, results showed significant differences across sessions in gambling severity measured with PG-YBOCS, reflection impulsivity measured with IST, in particular results showed differences in IST variables number of boxes opened per trial, probability of a correct decision, and in addition, there were significant differences in in the SST variable SSRT. VAS statistics could only be calculated across sessions due to a lack of enough data points, which showed significant differences across sessions, and also, percentage change between the last and the first session revealed a reduction of 71% in gambling cravings. To assess the final outcome of the intervention, session eight was compared against session one, revealing that there were statistically significant changes only in PG-YBOCS, which showed a reduction of 66%. In addition, at the end of the intervention, the participant thought that received tDCS real stimulation. These results are consistent with the findings from the case study mentioned previously, showing a reduction of 62.5% of PG-YBOCS, and a reduction of 100% of VAS for gambling cravings after a longitudinal intervention with real stimulation tDCS over DLPFC in GD (Martinotti et al., 2018).

EEG frequency power analysis results showed significant differences in mean power frequency across sessions, between the first and the last session, and within session one and session eight. The highest mean frequency power was seen in sessions four and seven, and the lowest in session eight[9]. Interestingly, gambling severity was the highest in session four, and the mean number of boxes opened in IST (therefore less impulsive decision-making) was higher in sessions four and seven. These results could indicate a possible positive association between EEG mean frequency power and gambling severity, and a negative association between EEG mean frequency power and behavioural impulsivity. However, it would be in part inconsistent (regarding the impulsivity component) with previous research showing that high impulsive patients with GD showed decreased EEG power, but consistent considering GD severity outcomes in previous results (Lee et al., 2017a).

[9]Sessions were not compared against each other. These results represent the main effect found in the variable session.

In particular, examining separately EEG recordings before and after the tDCS (EEG1 and EEG2, respectively), and considering sessions one and eight in each frequency band, results revealed short term effects of the intervention in session one, showing that in mean frequency power increased from EEG1 to EEG2, however in session eight, there were no significant differences between mean frequency power in EEG1 and EEG2. Results revealed also long term effects of the intervention, showing that mean frequency power significantly decreased from session one to session eight in both EEG1 and EEG2. Comparing only session eight against session one, the long term effects of the intervention seemed to produce a general decrease of mean frequency power, however, tDCS effects within each session varied. In general, there was a short term increase of mean frequency power associated with tDCS in all sessions, except for session five and session eight[10], and high variability on mean frequency power across sessions. Moreover, no significant correlations were found considering mean frequency power and theta/beta ratio. Nonetheless, significant correlations between gambling severity and EEG relative power have been included in Appendix A.

Differences between neuromodulation effects across sessions have been described also in previous research, showing that the reduction of cravings increased after each tDCS session, except for the last stimulation session (Boggio et al., 2009). In addition, other studies showed certain variability on tDCS results, revealing that stimulation over left DLPFC was associated with a decrease of mean frequency power (Boonstra et al., 2016), but also with an increase of mean frequency power (Accornero et al., 2014). Overall, the heterogeneity of results might reflect inter-individual variability, while intra-individual variability could account for differences found across sessions in the present study (Newson & Thiagarajan, 2019).

To hold these results, it is essential to consider the particular experimental conditions that did not follow the planned procedure: the participant had a break of one weak between session two and session three, in which the CBT treatment was delivered remotely without the combined tDCS protocol. In session six, the participant received two sessions of CBT after the tDCS procedure, and in sessions seven and eight only the tDCS procedure was administered, without the following CBT

[10] Statistical analysis comparing each of the eight sessions against the rest was not performed (paired comparisons were conducted only between the first and the last session), and therefore, the results discussed about any other specific session, refer simply to the variables values observed. The statistical analysis was more focused on assessing the final outcome of the intervention, and to compare each session against the others (involving between and within sessions analysis), would extend the work load and amount of data reported excessively, considering the limitations associated to the adapted analysis methodologies used, the time available for the project and the limited space to report the results. Nevertheless, this will be considered to further explore the data in the future.

session. In addition, the participant was tested during hot days in the summer season, in a room without controlled temperature, after traveling for four hours to attend the treatment at the clinic. There were considerable amounts of sweat on the scalp that probably affected the EEG data, which would explain the high amount of artefacts that could not be completely removed after data processing, and the extremely high absolute power values, especially in low EEG frequencies (Thompson, Steffert, Ros, Leach, & Gruzelier, 2008). Under these conditions, unfortunately it is not possible to draw reliable conclusions about the effectiveness of the intervention, however data from each session might provide some information about the mechanisms of tDCS.

4.5.3 General conclusions and limitations

Results revealed that the interventions combining tDCS (real stimulation and sham) and cognitive behavioural therapy (CBT) reduced gambling severity, cravings and modulated cortical excitability, in both patients. Particularly, EEG results showed short and long term effects of the intervention on cortical excitability. In the patient receiving tDCS real stimulation, there were further alterations on the experimental procedure due to changes of schedule in the CGT treatment, which seem to match with a high variability in EEG results across sessions. Although tDCS seemed to produce a short term increase of mean frequency power in the majority of sessions, no clear direction of long term effects was found.

The electrophysiological findings in the patient allocated to sham tDCS appeared to have more consistent interpretations. In particular, concurrent SST performance could have explained both, the short term and long term changes in EEG power. There was an increase of mean frequency power from EEG1 to EEG2 after the patient received sham tDCS coupled with the SST, and also an increase of mean frequency power associated with EEG2 in session eight compared with session one. Therefore, SST effects could explain the short and long term effects found after tDCS, whereas cumulative effects of CBT could account for a reduction of mean frequency power in EEG1 at the end of the intervention. This interpretation would be consistent with the distribution of EEG power found in the PFC, indicating that the highest mean frequency power changed from left DLPFC during EEG1 to right DLPFC during EEG2, after having performed the SST. This would be based on previous results that showed a crucial response inhibition role associated with right but not left DLPFC (Knoch et al., 2006).

In addition, in the patient allocated to sham, gambling severity correlated with behavioural measures, and EEG variables. Particularly, gambling severity correlated negatively with SST total correct trials and with EEG2 mean frequency power in left DLPFC. In addition, theta/beta ratio in left DLPFC correlated positively with cravings and IST total correct. The significant correlations found between these variables are particularly relevant for neuromodulation research in GD, given that these electrophysiological measures could be used in future research as biomarkers to assess changes in cognitive states, and as targets for neuromodulation studies.

In this experiment, some of the limitations highlighted in previous experiments were addressed: a triple-blinded design (in which neither the participant, nor the researcher conducting the experiment and the data analysis were aware of the stimulation condition) was used to ensure that data analysis was less likely to be biased; a longitudinal design was conducted to investigate the potential cumulative effects of tDCS; a computational model was used to create the most effective tDCS montage to target the brain area of interest more accurately; and neurophysiological data (EEG) was used to quantify the effects of the intervention.

Nevertheless, even though previous limitations were tackled, additional limitations arose in this experiment, given that the original design planned as an RCT had to be adapted to analyse data following single-participant designs. Therefore, the results obtained cannot be used to establish conclusions about the effectiveness of the intervention combining tDCS and CBT. The results are from single cases, with unavoidable differences in testing protocols with the originally planned RCT, and so it is not possible to reliably infer the validity of any observed qualitative differences or similarities between the cases. Therefore, results obtained cannot be used to draw conclusions about the effectiveness of the intervention combining tDCS and CBT, and the particular adaptations prevent from contrasting the findings consistently against previous studies results.

Especially, in the participant allocated to real stimulation condition (with whom the testing procedure did not follow the planned protocol, and multiple artefacts contaminated the EEG data), results obtained are not reliable to interpret the intervention outcomes congruently. However, in the participant allocated to sham condition (with whom experimental testing was carried out as planned), results revealed associations between self-report measures, behavioural outcomes and

neurophysiological data that support further investigation of these variables to study potential biological markers that could be used to assess the effectiveness of treatment interventions.

5 Chapter 5. Experiment IV: physiological mechanisms associated with transcranial direct current stimulation during gambling-related task performance

5.1 Summary

This experiment investigated the effects of tDCS on the autonomous nervous system (ANS) during different reward phases in gamblers grouped according to their self-reported impulsivity levels. Results showed that tDCS real stimulation was associated with an increase of sympathetic activation and changes in cortical excitability compared with tDCS sham. The findings suggested also that changes of cortical excitability could have been explained by task-related effects, which seemed to have a higher influence in low impulsive (LI) gamblers compared with high impulsive (HI) gamblers. Inter-individual differences were identified during gambling-related task performance through heart rate variability (HRV), heart rate (HR) and skin conductance responses (SCRs). Particularly, increased sympathetic activation was found in HI compared with LI, and during wins compared with losses in a gambling task. Results showed positive correlations between gambling severity and cravings, EDA and HR, and negative correlations between gambling severity and EEG outcomes. In addition, EDA baseline correlated with different variables measuring reward response and cortical excitability, which could indicate the potential usefulness of EDA as a biomarker in future studies in GD. In conclusion, results demonstrated the role of impulsivity in gambling behaviour, and provided some evidence of the capability of tDCS to modulate ANS and cortical excitability. However, results should be carefully interpreted considering that the blinding of tDCS conditions was not effective, and participants awareness of the stimulation could have also explained the increase of sympathetic activation, rather than this being directly caused by tDCS.

5.2 Introduction

Altered physiological responses to positive and negative reward outcomes have been associated with the development and maintenance of GD (Blaszczynski & Nower, 2002; Sharpe, 2002). The prefrontal cortex (PFC) modulates subcortical pathways that regulate the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). The sympathetic nervous system is associated with increased activation of internal glands and organs, whereas the parasympathetic

nervous system inhibits this activity. In particular, addiction has been related with increased sympathetic dominance (Huang, 2017). PFC activity has been associated with changes in heart rate variability (HRV), which is associated with sympathetic and parasympathetic tone, and might serve as an index to inform about emotional regulation through the interactions with the brain.

The neuro-visceral integration model, suggests that increased activity in the PFC is associated with increased parasympathetic and decreased sympathetic activity (Park et al., 2019; Thayer & Lane, 2000). In line with this model, previous research showed that HRV can be modulated with tDCS applied over the PFC (Montenegro et al., 2011), and through cognitive task performance (Luque-Casado et al., 2016), as well as with the combination of tDCS and cognitive task performance (Nikolin et al., 2017). One of the most widely studied characteristics of addiction, and that has been shown to be modulated with NIBS, is inhibitory control (Jacobson, Javitt, & Lavidor, 2011; Verdejo-García et al., 2008). Particularly, the neuro-visceral integration model describes inhibitory processes as negative feedback mechanisms that allow the interruption of ongoing behaviours. When these inhibitory processes are impaired, disinhibited behaviours in the form of positive feedback might arise (Thayer & Lane, 2000).

Research using NIBS and cognitive tasks (the SST) in GD has demonstrated that activity in the motor cortex explained individual differences in response inhibition (Chowdhury, Livesey, Blaszczynski, & Harris, 2018). In addition, tDCS has been shown to increase HF HRV and reduce LF HRV, indicating a potential enhancing effect of parasympathetic activity, and reduction of sympathetic activity associated with real stimulation (Montenegro et al., 2011). Therefore, the prospect of developing NIBS protocols that could help regulate the ANS, could offer promising opportunities for the treatment of addiction and GD.

Physiological responses, including heart rate (HR) and electrodermal activity (EDA), have been associated with GD (Clark, Crooks, Clarke, Aitken, & Dunn, 2012; Peterson et al., 2010a). EDA captures autonomic changes in electrical properties of the skin, and has two main components: the skin conductance level (SCL), which is a slow tonic background component, and a fast phasic component, the skin conductance responses (SCRs), that result from sympathetic neuronal activity. EDA has been described as the only autonomic physiological variable not contaminated by parasympathetic activity, which can be used to measure emotional and cognitive states (Braithwaite

et al., 2015). Previous studies showed that gamblers using their own money during a "real life" gambling scenario had increased HR (Meyer et al., 2000). In particular, HR was predicted by the outcomes during play, in which winning was necessary to maintain HR elevated (Coventry & Hudson, 2001).

Furthermore, the severity of gambling problems was associated with increased EDA, and smaller HR inter-beat interval (Ulrich, Ambach, & Hewig, 2016a). However, different studies have found contrasting results regarding EDA levels associated with reward outcomes and GD. While higher EDA was associated with increased bet sizes, being higher in losses than in wins in a sample of healthy participants (Studer & Clark, 2011), comparing a sample of healthy participants and participants with GD, SCRs to wins were lower for GD participants, but there were no differences in SCRs related to losses between the groups. More research will help to clarify EDA-related sensitivity to reward in different populations.

The somatic marker hypothesis (Damasio, 1996), suggests that unconscious physiological states developed through pleasurable or adverse experiences, and referred to as somatic markers (such as HR and SCRs), have a direct influence on consequent behaviours. Research has shown that in GD individuals these markers are weaker, and when dealing with risky situations, SCRs are lower when comparing with healthy individuals (Goudriaan et al., 2006). Previous studies showed that NIBS can modulate EDA and HRV (Feeser et al., 2014; Morales-Quezada et al., 2014; Wang et al., 2016), showing that tDCS real stimulation was associated with a decrease of SCRs (Herrmann et al., 2018), and therefore reduced sympathetic arousal. Therefore, considering the somatic marker hypothesis, research investigating the particular physiological characteristics in different types of participants during reward and punishment outcomes, could inform the development of NIBS protocols that might help to control specific physiological features that influence risky behaviours during gambling.

Electroencephalogram (EEG) resting state has also been used to study neuro-correlates of cognition and behaviour in relation to other physiological outcomes. Increased theta/beta ratio has been associated with reduced cortical inhibition (Lansbergen et al., 2007), poor reversal learning (Schutte et al., 2017*)* and risky decision-making (Massar, Rossi, Schutter, & Kenemans, 2012). Low cortical arousal has been associated with increased power of slow waves (delta and theta bands), whereas increased power in fast waves (alpha and beta) has been associated with higher excitability of the

nervous system (Kim et al., 2018). Decreased delta power was associated with decreased HR, and with higher HF HRV component, indicating increased parasympathetic activity (Patron et al., 2019).

Research has suggested that GD participants with higher impulsivity levels would show increased delta and theta bands and decreased alpha and beta bands, compared with lower impulsive participants (Lee et al., 2017b). In addition, research showed that gambling severity correlated positively with theta activity (Dymond et al., 2014). Moreover, studies using tDCS over the DLPFC showed that real stimulation increased beta frequency power (Song et al., 2014), and reduced left frontal delta absolute power (Keeser et al., 2011). Investigating the relationship between EEG resting state and gambling-related physiological and cognitive characteristics, and the effects of tDCS on specific frequency bands on the PFC, could inform about potential biological markers associated with different types of gamblers, and therefore help develop more individualised treatment interventions.

Therefore, physiological assessment of tDCS delivered during gambling-related task performance to participants with different gambling severity and impulsivity levels, might help explain specific neurophysiological and behavioural features associated with low risk and high risk gambling, and help to understand the underlying mechanisms of tDCS on the PFC and the ANS.

In this experiment, physiological measures, including ECG, EDA and resting-state EEG were recorded while participants completed gambling-related cognitive tasks on a computer and received tDCS (real stimulation and sham), in a mixed factor crossover design. It was hypothesised that real stimulation would be associated with improved task performance compared with sham: in IST, higher number of boxes opened, P (correct) at point of decision and total correct trials. In SST, lower SSRT, higher total correct stop trials and total correct go trials. CGT was performed before tDCS, therefore, results expected would be dependent of the participant groups and task-conditions (lower QDM, higher RT and higher DA in HI compared with LI, and larger differences between the groups in higher risk conditions of the task)[11].

[11] Specific predictions about cognitive performance are included also in this chapter (although the current tasks were performed already in earlier experiments), because the procedure and participant samples are different across experiments. In addition, although previous experiments showed an increase of RT and mixed results on DA after tDCS, it was hypothesised that tDCS might reduce RT and DA due to the different sample characteristics, and based on more extensive literature results discussed in the Introduction section of Experiments I and II.

In EDA, it was expected to find decreased SCL in real stimulation, which would be higher during task performance than at rest. Higher SCRs would be associated with task-related wins compared with losses. Nevertheless, EDA responses might depend on the participant characteristics, and considering that previous research suggested that the altered brain reward network in behavioural addictions might be linked to higher sensitivity to positive reinforcement (Gomis-Vicent et al., 2019), it was hypothesised that participants with higher impulsivity levels and gambling severity, might show higher SCRs associated to wins than to losses, whereas participants with lower impulsivity levels and gambling severity might show increased SCRs associated with losses.

Similarly, in ECG, it was expected that HR would be higher in wins than in losses. In addition, tDCS real stimulation would help to shift HRV towards parasympathetic dominance, determined by decreased LF and increased HF components. It was therefore predicted that cognitive and physiological measures would differ between participant groups divided by impulsivity levels, with poorer task performance associated with HI participants compared with LI participants. Higher sympathetic activity, increased EDA, LF/HF ratio, HR and delta and theta bands, but decreased alpha and beta bands, would be associated with HI compared with LI. Similarly, correlations would be expected to be positive between gambling severity and poorer task performance, sympathetic activity (determined by higher LF, HR and EDA), and with EEG slow waves activation. Therefore, the main goals of this experiment were to investigate the physiological responses associated with gambling-related cognitive and behavioural variables, to explore whether participants with more severe gambling problems and impulsivity levels would show sympathetic dominance and lower cortical arousal, and if tDCS could help shifting to increased parasympathetic activity and cortical activation, compared with sham.

5.3 Methods

5.3.1 Participants

In this experiment, 17 participants were recruited through online advertisements on research participation websites, social media and advertisements posted at the University of East London. Participants received two counterbalanced sessions of tDCS (real stimulation and sham) one week apart, and were compensated for their time with shopping vouchers. All participants were screened for exclusion criteria following tDCS safety recommendations and the Problem Gambling Severity

Index (PGSI). For tDCS exclusion criteria details please see section 3.3.1 in Chapter 3. Two participants were classified as low-risk gamblers (PGSI=1-2), four participants as moderate-risk gamblers (PGSI= 3-7) and 11 participants were classified as high-risk gamblers (PGSI=+8). Using this data, a median split of UPPS-P NU scores was carried out to create two groups of participants: LI (n= 8, mean age = 38 ± 4.7 years) and HI (n = 9, mean age = 35.6 ± 3.6 years).

Participants groups were created using impulsivity self-report scores for different reasons: in Experiments I and II participant groups were also created using UPPS-NU median split, and therefore, continuing to do so consistently, would allow a better understanding of the results across experiments in the project. In addition, multiple regression analysis revealed that the impulsivity trait of negative urgency (UPPS-P NU) was a significant predictor of PGSI ($F (1, 15) = 10.829$, $p = .005$, $R^2 = .419$). Lastly, using UPPS-P NU median split allowed sample size to be more balanced between groups than using PGSI scores, which should strengthen data analysis. The sample size was based on studies employing similar methodologies and outcome measures: in a tDCS study, it was calculated that the number of participants to detect a difference in HRV of 40% with a power of 0.8 at $\alpha=0.05$ was 8 participants per group, recruiting 17 participants in total (Clancy et al., 2014); studies combining tDCS and EEG, had sample sizes of 6 participants (Leite et al., 2017), 10 participants (Keeser et al., 2011) and 20 participants (Boonstra et al., 2016).

5.3.2 Materials

Details of the tDCS and EEG device and electrodes used in this experiment are described in section 4.3.2 in Chapter 4. Data collection and analysis of EDA and ECG, as well as EEG spectral analysis, was conducted with Powerlab 26T and the software LabChart 8 (ADInstruments, Australia), including the HRV analysis module. The CANTAB battery of tasks was used to measure gambling-related behavioural performance_including the CGT, IST and SST. The CGT was used as a priming task, measuring QDM, RT and DA. IST was used to assess reflection-impulsivity during tDCS, measuring the variables mean probability of making a correct decision (Mean P (correct)) and number of boxes opened per trial. The SST was used to measure inhibition control during tDCS, through the variables total correct on go and stop trials, and stop signal reaction time (SSRT).

The PGSI was used to measure gambling severity, whereas GRCS was used to measure gambling symptoms, UPPS-P was used to measure self-reported impulsivity and VAS was used to measure

gambling cravings. In addition, alcohol use disorders identification test (AUDIT) was used to control for alcohol dependence, and the adult ADHD self-report scale (ASRS) to control for comorbid attention deficit hyperactivity disorder (ADHD). AUDIT and ASRS are often used in GD research (Chowdhury et al., 2018) to control for comorbid alcohol use and ADHD symptomatology, as both could influence the outcomes measured with the cognitive tasks. Lastly, the tDCS events sham questionnaire was used to control for participants sensations associated to tDCS. For more detailed information about the CGT, GRCS and UPPS-P, please refer to section 3.3.2 in Chapter 3; for VAS, IST, SST and events sham questionnaire, refer to section 4.3.2, in Chapter 4.

- Problem Gambling Severity Index (PGSI; Ferris & Wynne, 2001)[12]: it is a nine question self-report scale that was developed specifically to measure problem gambling in the general population, identifying different subgroups of problem gamblers with different levels of risk status (no risk (scores = 0), low risk (scores = 1-2), moderate risk (scores = 3-7), and high risk (scores = 8 or higher)). Participants were asked about the frequency (never/sometimes/most of the time/almost always) they have experienced specific situations in the past year (e.g., have you bet more than you could really afford to lose?).

- Alcohol Use Disorders Identification Test (AUDIT; Reinert & Allen, 2002): it is a 10-item scale designed to assess three conceptual domains: alcohol intake (items 1–3), dependence (items 4–6), and adverse consequences (items 7–10). The score is obtained by adding the values associated with the various response alternatives. Scores range from 0 to 40.

- Adult ADHD Self-Report Scale (ASRS-v.1.1; Daigre et al., 2009): it is a self-report scale to assess symptoms of ADHD based on the DSM-IV symptom criteria. It is comprised of two parts: part A (6 questions) and part B (12 questions). Positive scores start from 4 or more in part A. Part B is not used for diagnostic purposes, but these items provide insight into the frequency of symptoms[13].

[12] The PGSI was chosen to measure gambling severity in this experiment (instead of the questionnaires SOGS and PG-YBOCS that were used in previous experiments), because the PGSI was designed to be used also in non-clinical settings (Orford, Wardle, Griffiths, Sproston, & Erens, 2010), and therefore was more adequate for the expected participant sample characteristics.

[13] ASRS was not used as a diagnostic tool, but rather to control for ADHD traits comorbidity.

5.3.3 Procedure

The experiment involved two sessions with crossover design, in which each participant received tDCS in a counterbalanced order (real stimulation and sham) one week apart, using a triple blind mode (neither the participant nor the researcher conducting the experiment and analysing the data were aware of the tDCS condition). Each testing session had a duration of one and a half hours, and in addition, the setting up and cleaning of the equipment took around 40 minutes. A total of 34 sessions were conducted in this experiment. Before participation in the study, written informed consent was obtained, which was approved by the University of East London Research Ethics Committee (UREC_161731, see Appendix D). All experimental procedures were conducted following the General Data Protection Regulation (GDPR).

On each session, EDA and ECG were recorded from the beginning to the end of the experimental procedure. Participants completed a series of questionnaires to measure individual gambling-related characteristics (PGSI and UPPS-P in the first session, and AUDIT and ASRS in the second session). After that, participants completed the CGT, which was followed by a recording of five minutes of EEG resting state with eyes opened, then participants completed the IST and SST while receiving 20 minutes of tDCS, which was followed by five minutes of EEG resting state with eyes opened, and lastly participants completed the VAS for gambling cravings and the events sham questionnaire to assess the blinding procedure. The procedure is represented in Figure 5-1.

The CGT was used before tDCS as a priming task, with the aim to help activate the brain areas involved in gambling-related cognitive functions that were targeted with tDCS, to increase the effects of neuromodulation by facilitating reaching the membrane action potential during real stimulation (Colombo et al., 2015; Fertonani et al., 2014; Fujiyama et al., 2017). All participants completed the CGT ascending condition first (Zois et al., 2014), because it was demonstrated that ascending/descending conditions order did not influence CGT performance (Lawrence, Luty, Bogdan, Sahakian, & Clark, 2009). Similarly, all participants completed the IST fixed condition first, given that previous research did not find significant differences on task performance depending on condition order (Clark et al., 2006).

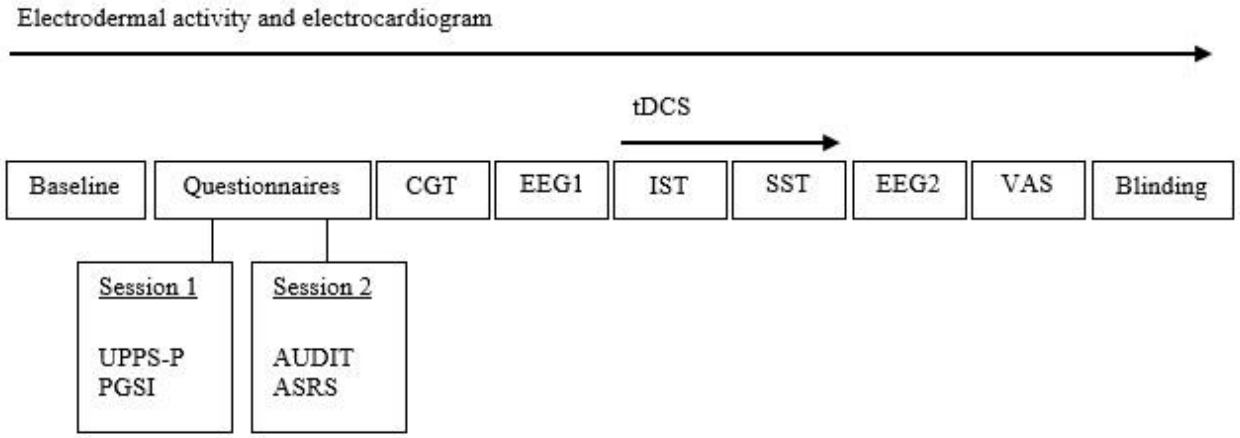

Figure 5-1. Experimental procedure in Experiment IV. Electrodermal activity and electrocardiogram were recorded from the baseline for two minutes and continued for the entire session. Questionnaires completed in the first session included the urgency premeditation, sensation-seeking scale (UPPS-P) and problem gambling severity index (PGSI), whereas the alcohol use disorder identification test (AUDIT) and attention deficit hyperactivity disorder self-report scale (ASRS) were completed in the second session, with counterbalanced order in each session. Participants completed the Cambridge gambling task (CGT), before receiving transcranial direct current stimulation (tDCS) for 20 minutes while performing the information sampling task (IST) and the stop signal task (SST). Before and after the tDCS, five minutes of electroencephalogram (EEG) resting state were recorded (EEG1 and EEG2, respectively). After the tDCS the visual analogue scale (VAS) was completed to assess cravings and the events sham questionnaire to assess the blinding procedure.

5.3.4 Transcranial direct current stimulation high definition montage modelling

A computational model created by Neuroelectrics (STIMWEAVER SPR0122), was used to identify the most effective tDCS montage to target the rDLPFC (Ruffini et al., 2013). The details of this montage were described in section 4.3.4 in Chapter 4[14].

5.3.5 Physiological data collection and processing

5.3.5.1 Electroencephalogram (EEG) data processing

EEG data was processed as specified in section 4.3.5 in Chapter 4.

5.3.5.2 Electrodermal activity (EDA) and electrocardiogram (ECG) data collection

EDA and ECG were sampled with Powerlab 26T software LabChart 8 (ADInstruments) at 1000 samples/second. Skin conductance was measured from the non-dominant hand using two stainless steel bipolar finger electrodes attached to the distal phalanges of the index and middle finger. The channel range was set at 2 V, and an anti-alias fitter was used to remove signal component frequencies > 0.5 of the sampling frequency. Electrodes were attached for around five minutes prior to beginning of the task to allow EDA levels to stabilize (Boucsein et al., 2012; Braithwaite et al., 2015). ECG was recorded with three Ag/Ag-Cl disposable electrodes placed according to the Einthoven II lead (ground electrode below the left collarbone, negative electrode below the right collarbone and positive electrode on the left side below the rib cage). The range was set at 20 mV, and was sampled with a high pass filter of 0.5 Hz to minimise baseline drift, and a mains filter to suppress electrical interference (Nikolin et al., 2017; Zimmerman & Thompson, 2016).

EDA and ECG baseline were recorded for two minutes before the experiment tasks started and during the entire session. Events occurring during tasks performance were logged in LabChart manually by the researcher, through inserting pre-set comments that indicated the section of the experimental session. These events are referred to as conditioned stimuli (CS), and include the different cognitive tasks conditions (ascending/descending, fixed/decreased), CGT risk-conditions (9:1, 8:2, 7:3, 6:4), and performance outcomes (whether the participant won or lost in each trial). The rate of change of

[14] In Experiments I and II the current employed was 1.5 mA given that this was a commonly used parameter in previous tDCS studies (Lefaucheur et al., 2016). However, in this experiment a computational model was used, resulting in the recommendation to use a maximum current of 1.8 mA per electrode, to reach the desired brain target more effectively.

the physiological measures should be shorter than the speed of the researcher´s reaction time to insert the pre-set comments (pressing a key on the computer). Nevertheless, as cognitive tasks and LabChart software were not interfaced as is common in other studies, it was assumed that there was a certain delay (around 0.5 seconds) on the presentation of stimuli in the recordings that was taken into account for the data analysis.

5.3.5.3 Electrodermal activity (EDA) data processing

EDA captures autonomic changes in electrical properties of the skin, and has two main components: the skin conductance level (SCL), which is a slow tonic background component, and a fast phasic component, the skin conductance responses (SCRs). LabChart software applies an automatic zeroing to the signal, therefore comparisons between participants are acceptable despite inter-individual differences in SCL. Two different ways to obtain the SCL were used in this experiment: measurements of SCL were taken from periods outside the SCRs signal, and measurements of local SCL were taken just before each SCR (Braithwaite et al., 2015). The average SCL was calculated from the baseline resting periods, from data segmented into one second epochs. To obtain the SCL outside the SCRs periods, the signal amplitude in micro Siemens (μS) was measured from three rest periods: the baseline (two minutes), and the EEG periods before and after tDCS stimulation (EEG1 and EEG2) (Braithwaite et al., 2015; Pappens et al., 2014).

SCL was expected to change as a result of the tDCS and tasks performance, and specifically, it was expected that changes might be more detectable during the start of the resting period, rather than during the whole EEG duration (five minutes). Therefore, it was decided to divide the EDA recording during the EEG periods into two parts that were called “EEGa” and “EEGb”, which corresponded to the first half and second half of the recording, respectively. In addition, the local SCL during the risk conditions in the CGT was obtained by measuring the signal amplitude of the two seconds preceding each SCR (which corresponded with the CS associated with each CGT risk condition). Local SCL can be calculated from segments shorter than one second preceding the SCRs (Braithwaite et al., 2015), however in this experiment two seconds were a more reliable measure to take into account the delay caused by logging manually the presentation of the stimuli.

To obtain the SCRs, the SCL signal was filtered with a high pass filter at 0.05 Hz, to eliminate the downward drift and allow identification of SCRs (Figner & Murphy, 2011; Ulrich, Ambach, & Hewig,

2016b). The minimum threshold to consider the classification of SCRs was set to 0.03 μS (Braithwaite et al., 2015). SCRs were calculated as the maximum positive change in a time window (0-5 seconds) from the CS, with a minimum inter-trial interval of six seconds (Denburg, Recknor, Bechara, & Tranel, 2006; Figner & Murphy, 2011). A broader time window was used as in (Ulrich et al., 2016b) compared to the most commonly used (1-4s) in EDA research, due to the delay in the presentation of the stimulus.

The SCRs were divided into reward anticipatory responses and feedback responses: the amplitude of the signal was measured for two seconds before of the CS to obtain the reward anticipatory response. To obtain the reward feedback response, the amplitude of the five seconds after the CS associated with task outcomes won and lost was measured. Anticipatory responses correspond to the two second period before the participant received the reward outcome, and feedback responses correspond to the five second period after the participant received the reward outcome. The relationship between anticipatory and feedback responses was analysed during participants task-performance. Similarly, other studies calculated the anticipatory response using the time between the end of the five seconds reward CS interval and the next CS (Miu, Heilman, & Houser, 2008).

5.3.5.4 Electrocardiogram (ECG) data processing

The LabChart HRV analysis module software (ADInstruments) was used to obtain measures of heart rate variability (HRV) frequency domain, and automatically remove artefacts and ectopic beats. In addition, visual analysis was performed to ensure that all beats were classified correctly before the analysis. The frequency domain measures were analysed, including low frequency (LF = 0.04-0.15 Hz), high frequency (HF = 0.15-0.40 Hz) and the (LF/HF) ratio. The heart rate (HR) was calculated from the ECG in beats per minute (bpm). The R-R interval considered normal was set between 500 ms and 1200 ms, except for one participant that had to be changed between 500 ms and 1300 ms due to having slower HR (lower than 50 bpm in rest periods). ECG data was divided into five minutes segments (except for the baseline that was two minutes long) according to short-term recording metrics norms (Shaffer & Ginsberg, 2017), and to ensure that HRV reflected similar levels of task stress-related load in each variable.

Total power measured in ms^2/Hz, as well as low frequency (LF) and high frequency (HF) power measured in normal units (nu) and the LF/HF ratio, were measured during rest periods and tasks

performance, with a minimum of two minutes recording period in each section. In addition, during task performance, HR was measured using the mean, minimum and maximum HR during the five seconds after the conditioned stimuli (risk conditions and wins/losses). HRV frequency measures cannot be obtained during task performance conditions (risk, wins and losses) because it is required a minimum epoch length of one minute. HRV frequency measures were analysed separately for tasks performance and rest periods (as well as during the whole session), with the aim to obtain more information about the association of HRV measures within each particular phase of the experiment and to create a more comparable outcome between tasks and rest periods.

5.3.6 Analysis plan

Data was analysed with IBM SPSS (Statistical Package of the Social Sciences, Version 23, SPSS Inc., Chicago, IL). Significance threshold was set at $\alpha = 0.05$. Demographic data (age/gender) were analysed using t-tests and Chi-square tests. Data was checked for skewness, kurtosis and normality. Non-normally distributed data according to Kolmogorov-Smirnov test was transformed using logarithmic transformation (SCL and SCRs; IST variables mean probability correct and discrimination errors; SST variables stop signal reaction time (SSRT) and total correct responses), and arc sin transformation in the case of QDM. When sphericity was not satisfied Greenhouse-Geisser correction was used.

The effects of tDCS condition order between sessions on CGT performance were analysed with a repeated measures ANOVA with between participants factor being session order, with two levels (real stimulation first or sham first). To take into account the interaction of the tDCS stimulation condition with the order of the session when session order was significant (in QDM and RT), session order was used as a covariate and a repeated measures mixed factor ANCOVA was conducted. tDCS session order was not significant for any of the other tasks variables or any of the physiological measures. Group interactions were analysed using independent sample t-tests (2-tailed), and tDCS condition interactions were analysed using paired sample t-tests (2-tailed). Pearson´s correlation analysis was conducted to investigate the relationships between gambling severity and self-report measures, behavioural outcomes and physiological variables.

5.3.6.1 Blinding procedure

The blinding procedure was assessed with the events sham questionnaire, using non-parametric McNemar test (2-tailed) for paired samples with two categorical end points (guess correct or guess not correct) for variables tDCS real stimulation and sham (Du Prel, Röhrig, Hommel, & Blettner, 2010; Nikolin et al., 2017).

5.3.6.2 CANTAB tasks performance

The CGT variable QDM was analysed with a 2 x 2 x 2 x 4 mixed-factor repeated measures ANCOVA, within-participants factors being tDCS with two levels (real stimulation and sham), condition with two levels (ascending and descending) and risk conditions (box ratio) with four levels (6:4, 7:3, 8:2, 9:1), and the between-participants factor groups (HI and LI), and session order as a covariate. RT was analysed using a 2 x 2 x 2 x 4 mixed-factor repeated measures ANCOVA with within-participants factors being condition with two levels (ascending and descending), tDCS with two levels (real stimulation and sham) and box ratio with four levels (6:4, 7:3, 8:2, 9:1), and the between-participants factor group (HI and LI), and session order as a covariate. DA was analysed using a 2 x 2 x 4 mixed factor repeated measures ANOVA with within-participants factor tDCS with two levels (real stimulation and sham), box ratio with four levels (6:4, 7:3, 8:2, 9:1), and between-participants factor group (LI and HI).

IST variables (mean P (correct), total correct and mean number of boxes opened) were analysed using a 2 x 2 x 2 mixed factor repeated measures ANOVA, with within-participants factor tDCS with two levels (real stimulation and sham), task condition with two levels (fixed and decreased) and between-participants factor group (LI and HI).

SST variables were analysed using a 2 x 2 mixed factor repeated measures ANOVA, with within-participants factors tDCS with two levels (real stimulation and sham) and between-participants factor group (LI and HI).

5.3.6.3 EEG resting state

To investigate the differences in EEG power before and after tDCS, a 2 x 2 x 2 x 8 mixed factor ANOVA was conducted, with within factors tDCS (real stimulation and sham), time (EEG1 (pre-tDCS) and EEG2 (post-tDCS)) and electrode (Cz, Fpz, F4, AF8, AF4, FC6, F3 and AF7) and between factor group (LI and HI).

5.3.6.4 Electrodermal activity (EDA)

To investigate the SCL during the entire session (including rest phases and cognitive tasks performance), a 2 x 2 x 8 repeated measures ANOVA was conducted, with within participant factors tDCS (stimulation or sham) and period (baseline, CGT ascendant, CGT descendent, EEG1, IST fixed, IST decreased, SST and EEG2) and between participant factor group (LI and HI). To investigate SCL during the rest periods EEG1 (pre-tDCS) and EEG2 (post-tDCS), including the mentioned subdivisions of the EEG (EEGa and EEGb), a 2 x 2 x 2 mixed factor repeated measures ANOVA was conducted with within participant factors tDCS (stimulation or sham) and time (pre-tDCS and post-tDCS), and between participant factor group (LI or HI).

To investigate the local SCL during CGT performance, a 2 x 2 x 2 x 4 mixed factor repeated measures ANOVA was conducted, with within participant factors tDCS (stimulation or sham), condition (ascending and descending) and risk (9:1, 8:2, 7:3 and 6:4), and between participant factor group (LI and HI). To investigate SCRs (involving anticipatory and feedback responses) in CGT, a 2 x 2 x 2 x 2 x 2 mixed factor repeated measures ANOVA with within participant factors tDCS (stimulation and sham), condition (ascending and descending), outcome (wins and losses) and response (anticipatory and feedback) and between participant factor group (LI and HI). In IST, SCRs were analysed with a 2 x 2 x 2 x 2 x 2 mixed factor repeated measures ANOVA with within participant factors tDCS (stimulation and sham), condition (fixed and decreased), outcome (wins and losses) and response (anticipatory and feedback[15]), and between participant factor group (LI and HI). In SST, SCRs were analysed with a 2 x 2 x 2 x 2 mixed factor repeated measures ANOVA with within participant factors tDCS (stimulation and sham), outcome (wins and losses) and response (anticipatory and feedback), and between participant factor group (LI and HI).

[15] Reminder: anticipatory responses correspond to the two second period before the participant received the reward outcome. Feedback responses correspond to the five second period after the participant received the reward outcome.

5.3.6.5 Electrocardiogram (ECG)

To investigate HRV frequency measures during the entire session, including rest phases and cognitive tasks performance, a 2 x 2 x 8 mixed factor repeated measures ANOVA was conducted, with within participant factors tDCS with two levels (stimulation and sham) and period with eight levels (baseline, CGT ascendant, CGT descendent, EEG1, IST ascendant, IST descendent, SST and EEG2) and between participant factor group (LI and HI).

To investigate HRV before and after tDCS (EEG1 and EEG2), a 2 x 2 x 2 repeated measures ANOVA was conducted, with within participant factors tDCS with two levels (stimulation and sham) and tDCS time with two levels (EEG1 and EEG2), and between participant factor group with two levels (LI and HI). To investigate HRV during task performance periods, a 2 x 2 x 5 mixed factor repeated measures ANOVA was conducted, with within participant factors tDCS with two levels (stimulation and sham) and period with five levels (CGT ascendant, CGT descendent, IST ascendant, IST descendent and SST) and between participant factor UPPS group with two levels (LI and HI). To investigate heart rate (HR) during tasks performance periods, a 2 x 2 x 5 mixed factor repeated measures ANOVA was conducted, with within participant factors tDCS with two levels (stimulation and sham) and period with five levels (CGT ascendant, CGT descendent, IST ascendant, IST descendent and SST) and between participant factor UPPS group with two levels (LI and HI).

To investigate HR during risk conditions in the CGT, a 2 x 2 x 2 x 4 mixed factor repeated measures ANOVA was conducted, with within participant factors tDCS with two levels (stimulation and sham), condition with two levels (ascending and descending) and risk with four levels (9:1, 8:2, 7:3 and 6:4), and between participant factor group with two levels (LI and HI). To investigate HR during CGT and IST reward outcomes, a 2 x 2 x 2 x 2 mixed factor repeated measures ANOVA was conducted, with within participant factors tDCS with two levels (stimulation and sham), condition with two levels (ascending and descending in CGT; or fixed decreased in IST) and outcome with two levels (wins and losses), and between participant factor group (LI and HI). Finally, to investigate HR during SST performance, a 2 x 2 x 2 repeated measures ANOVA was conducted with within participant factors tDCS with two levels (stimulation and sham) and outcome with two levels (wins or losses), and between participant factor group with two levels (LI and HI).

5.4 Results

5.4.1 Demographics and questionnaires

Demographics and questionnaire results by group are presented in Table 5-1. Results revealed that groups did not differ in age, gender, alcohol dependence, ADHD symptomatology, gambling severity or gambling cravings. Groups were created using median split of NU scores (median = 32). Therefore participants scoring 31 or lower in NU were allocated to LI group, and participants scoring 32 or higher in NU were allocated to the HI group. The distribution of UPPS-P NU scores across participants is presented in Figure 5-2. Independent sample t-test (2-tailed) analysis for age differences between LI (mean age 38 ± 4.70 years) and HI (mean age 35.6 ± 3.60) were not significant (t (15) =.523, p = .608). Chi-square tests were not significant for gender interactions with groups (X^2 (1) = .275, p = .600) and not significant for education level interaction with groups (X^2 (2) = 1.733, p = .420). UPPS-P total average score was not significantly different between LI and HI (t (15) = -1.474, p = .161). There were no significant differences between LI and HI in alcohol consumption (t (15) = .973, p = .346) or ADHD symptoms (t (15) = -.002, p = .998). There were no significant differences between LI and HI in VAS after real stimulation (t (15) = -1.520, p = .149), or sham (t (15) = -.374, p = .713). In addition, there were no significant differences in VAS between real stimulation and sham (t (16) = -.523, p = 608). The blinding procedure was assessed with the events sham questionnaire, showing that 100% of participants in the real stimulation session were able to guess the tDCS condition, whereas when participants received sham, 65% of participants guessed correctly the tDCS condition. A McNemar test (2-tailed) for paired samples with two categorical end points (correct or not correct), showed that when participants received real stimulation, were able to guess significantly better the tDCS condition than when they received sham (p = .001). No adverse events were reported.

Table 5-1. *Demographics and participant characteristics by group in Experiment IV.*

	LI (n=8)	**HI (n=9)**	**Test Statistic**
Age in years	38.00 (4.70)	35.60 (3.60)	t (15) =.523, p = .608
Gender			
Females	1	2	X^2 (1) = .275, p = .600
Males	7	7	
Education			
Primary	1	1	
Secondary	2	5	X^2 (2) = 1.733, p = .420
Higher	5	3	
PGSI	6.37 (4.53)	11.11 (5.35)	t (15) = -1.955, p = .069
UPPS-P Total	27.67 (2.29)	30.42 (4.79)	t (15) = -1.474, p = .161
AUDIT	4.30 (1.40)	2.60 (1.40)	t (15) = .973, p = .346
ASRS	28.30 (3.50)	42.40 (4.40)	t (15) = -.002, p = .998
VAS (Real stimulation)	2.75 (2.37)	4.78 (3.03)	t (15) = -1.520, p = .149
VAS (Sham)	3.87 (2.80)	4.45 (3.40)	t (15) = -.374, p = .713

Notes: PGSI, problem gambling severity index; UPPS-P, urgency, premeditation, perseveration, sensation seeking scale; AUDIT, alcohol use disorder identification test; ASRS, attention deficit hyperactivity disorder (ADHD) self-report scale; VAS, visual analogue scale for cravings. Participant groups are divided by impulsivity (low: LI; high: HI). Values represent mean and inside the parenthesis standard deviation except in gender and education, in which values represent number of participants.

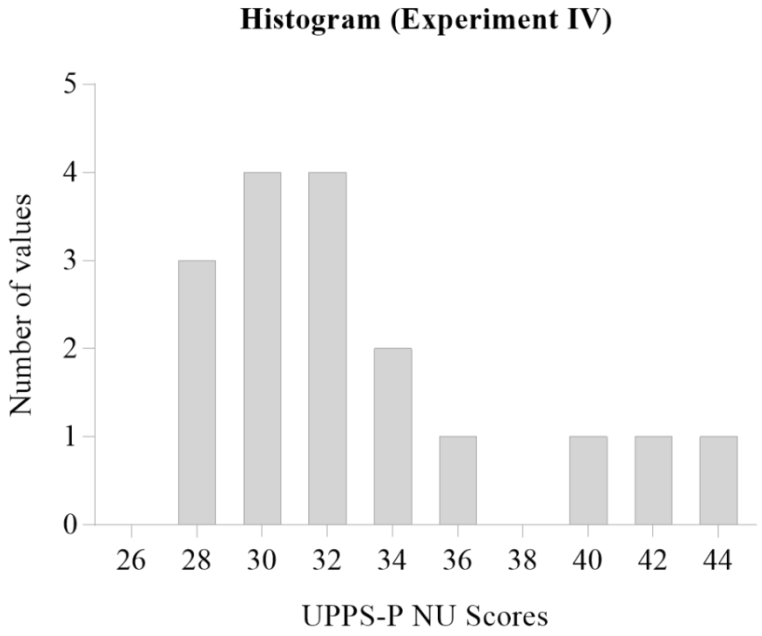

Figure 5-2. Histogram in Experiment IV. Distribution of the negative urgency (NU) scores across participants from the urgency, premeditation, perseverance and sensation seeking scale (UPPS-P).

5.4.2 CANTAB cognitive tasks

CGT results[16] in QDM revealed a significant main effect of condition ($F (1, 14) = 4.823$, $p = .045$, $\eta p^2 = .256$), showing that QDM was lower in ascending condition compared with descending condition. There were no main effects of tDCS or risk (all effects $p > .05$). However, there were interactions between tDCS x session order ($F (1, 14) = 5.742$, $p = .031$, $\eta p^2 = .291$), showing that in real stimulation, QDM was higher when the first session was real stimulation, and QDM was lower when the second session was real stimulation, however in sham, there was no difference in session order ($p > .05$). An interaction tDCS x condition ($F (1, 14) = 5.939$, $p = .029$, $\eta p^2 = .298$), showed that real stimulation was associated with lower QDM in both ascending and descending conditions. In real stimulation, ascending condition had higher QDM than descending condition, whereas in sham, descending condition was associated with higher QDM than ascending condition. A four way interaction tDCS x condition x risk x group ($F (3, 42) = 3.480$, $p = .024$, $\eta p^2 = .199$), represented in Figure 5-3, showed that in real stimulation, both tasks conditions had similar QDM across risk conditions, and that the largest difference in QDM between LI and HI was found in descending highest risk condition (6:4). However, in sham, descending condition was associated with higher QDM, compared with ascending condition.

In RT, there was a main effect of tDCS ($F (1, 15) = 35.591$, $p = .001$, $\eta p^2 = .704$), showing that real stimulation was associated with lower RT compared with sham, and a main effect of risk ($F (3, 45) = 24.987$, $p = .001$, $\eta p^2 = .625$), showing that RT decreased with increasing risk conditions. There was an interaction condition x session order ($F (1, 15) = 6.321$, $p = .024$, $\eta p^2 = .296$), showing that descending condition was associated with higher RT when real stimulation was delivered the first session, but with lower RT when real stimulation was delivered in the second session. RT in ascending condition was similar in both real stimulation and sham. There were no significant main effect of tDCS or risk in DA (all effects $p > .05$).

In IST, there was a main effect of tDCS in total correct ($F (1, 15) = 14.131$, $p = .002$, $\eta p^2 = .485$), showing that tDCS was associated with higher total correct trials in real stimulation compared with sham. In mean P (correct) there was a main effect of tDCS ($F (1, 14) = 112.284$, $p = .001$, $\eta p^2 = .889$), showing that real stimulation was associated with lower mean P (correct) compared with sham. There

[16] Although the CGT is performed before tDCS, and therefore potential effects of tDCS will be interpreted as a session effect, it was decided to use the same terminology as with the other variables to refer to the factor analysed (tDCS condition, rather than session), for simplicity, and to retain as much information as possible that could be related to each session directly or indirectly.

was also a main effect of condition (F (1, 15) = 35.591, p =.001, ηp^2 =.704), showing that mean P (correct) was lower in fixed condition. An interaction tDCS x condition (F (1, 14) = 326.238, p =.001, ηp^2 =.959) represented in Figure 5-4, showed that in real stimulation mean P (correct) was similar between fixed and decreased conditions, however in sham, fixed condition was associated with lower mean P (correct) than decreased condition. There were no significant differences in mean number of boxes opened (p > .05). In SST, there was a main effect of tDCS in total correct stop trials (F (1, 14) = 6.535, p =.023, ηp^2 =.318), showing that total correct stop trials was lower in real stimulation compared with sham. There were no significant differences in SSRT or total correct go trials. Paired t-tests to compare significant interactions with tDCS conditions showed no significant differences between real stimulation and sham in any of the variables (all effects p > .05). Independent t-test to compare group interactions showed no significant differences between LI and HI participants in any of the variables (all effects p > .05).

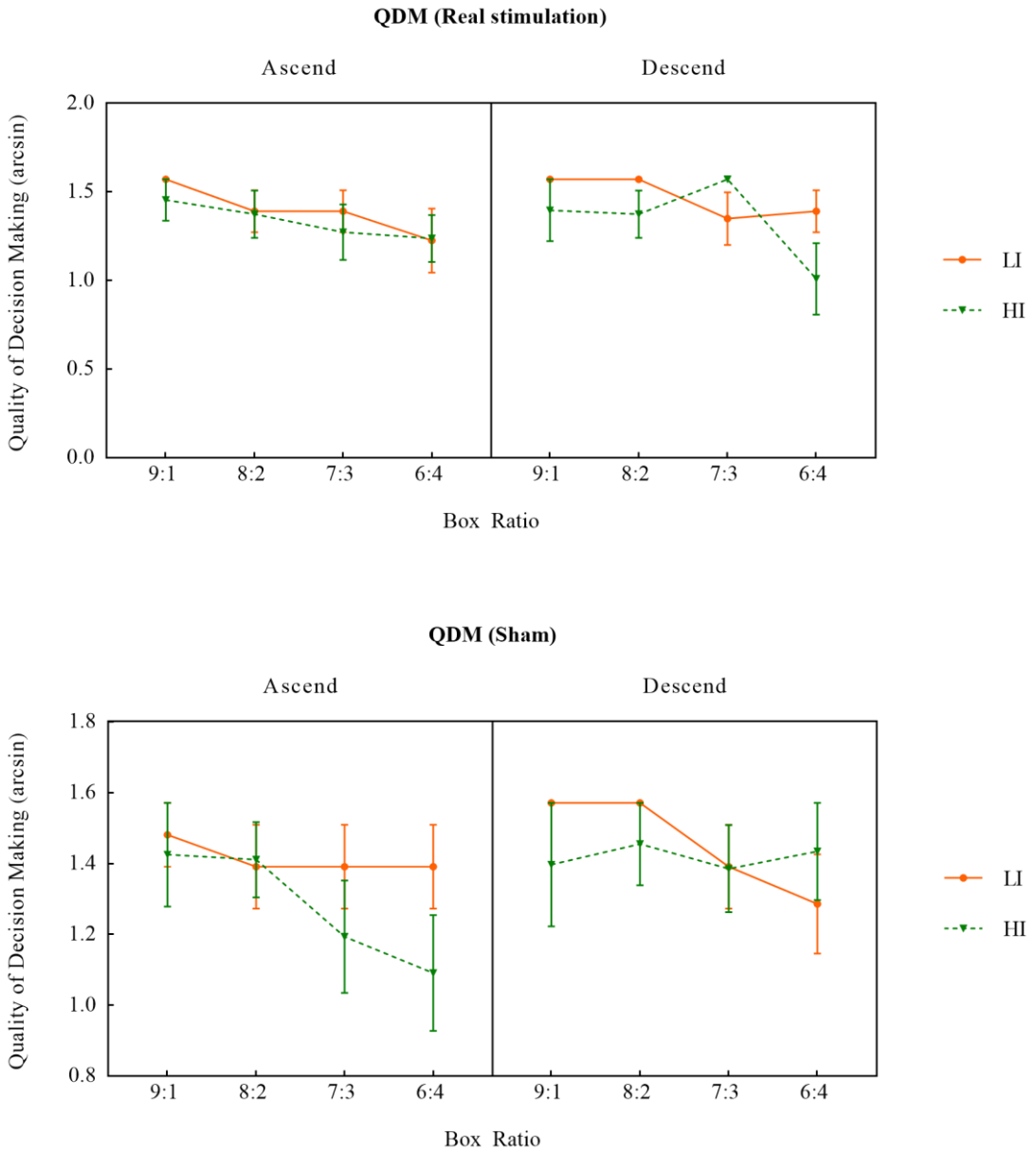

Figure 5-3. Quality of decision-making (QDM) in Experiment IV. Cambridge gambling task (CGT) variable QDM across risk conditions represented by box ratios (9:1, 8:2, 7:3 and 6:4) in low impulsive (LI) and high impulsive (HI) groups in tDCS conditions real stimulation and sham. Data represents mean and SEM.

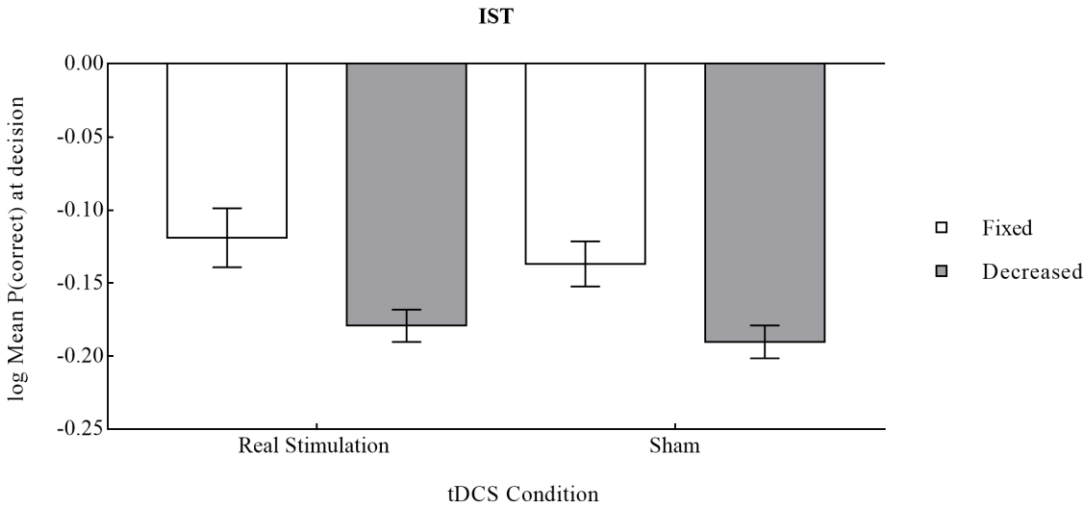

Figure 5-4. Mean probability correct (P (correct) at point of decision in Experiment IV. Information sampling task (IST) variable mean P (correct) at decision during fixed and decreased conditions in tDCS real stimulation and sham. Data represents mean and SEM.

5.4.3 Electrodermal activity (EDA)

5.4.3.1 Skin conductance level during the whole session (including rest periods and cognitive tasks performance)

Results showed a main effect of period (F (7,105) = 19.147, p =.001, ηp^2 =.561), indicating that from the lowest to the highest levels, SCL was lowest in the rest phases EEG1, baseline and EEG2, followed by the IST decreased and fixed conditions, and SCL was the highest in the SST, CGT ascending and CGT descending conditions. There were no significant main effects of tDCS (p > .05). However, there was a significant interaction tDCS x period (F (1, 15) = 5.377, p =.035, ηp^2 =.264), showing that SCL was lower in real stimulation compared with sham in the baseline and IST ascending condition. In the rest of the phases of the experiment, SCL was higher in real stimulation compared with sham. There was also an interaction tDCS x period x group (F (1,15) = 5.754, p =.030, ηp^2 =.277) which is represented in Figure 5-5, showing that in real stimulation: LI had lower SCL compared with HI in all periods of the experiment except for the IST descending condition, in which SCL was slightly lower in HI. In the rest of the periods (baseline and EEG phases), SCL was lower in both groups in comparison with the tasks periods. Specifically, in LI, EEG2 showed the highest SCL, whereas the lowest SCL was shown in the baseline and EEG1. In HI, the lowest SCL was found also in EEG1, however, SCL in baseline and EEG2 were similar.

Independent sample t-tests to compare SCL differences between groups during baseline and task performance revealed that SCL was significantly higher in HI compared with LI in real stimulation periods: in the baseline (t (15) = -2.829, p = .013), EEG1a (t (15) = -2.207, p = .043), EEG2 (t (15) = -2.393, p = .030) and EEG2b (t (15) = -2.667, p = .018), and in sham periods: CGT descendent condition (t (15) = -2.326, p = .034), EEG1 (t (15) = -2.533, p = .023), EEG1a (t (15) = -2.506, p = .024), EEG1b (t (15) = -2.461, p = .026), IST fixed condition (t (15) = -2.398, p = .010), and IST decreased condition (t (15) = -2.545, p = .022), EEG2 (t (15) = -2.196, p = .044), and EEG2b (t (15) = -2.351, p = .033). Paired t-test to compare tDCS interactions showed the only significant difference between real stimulation and sham was found in IST fixed condition (t (16) = 2.341, p = .033), indicating that real stimulation was associated with higher SCL than sham.

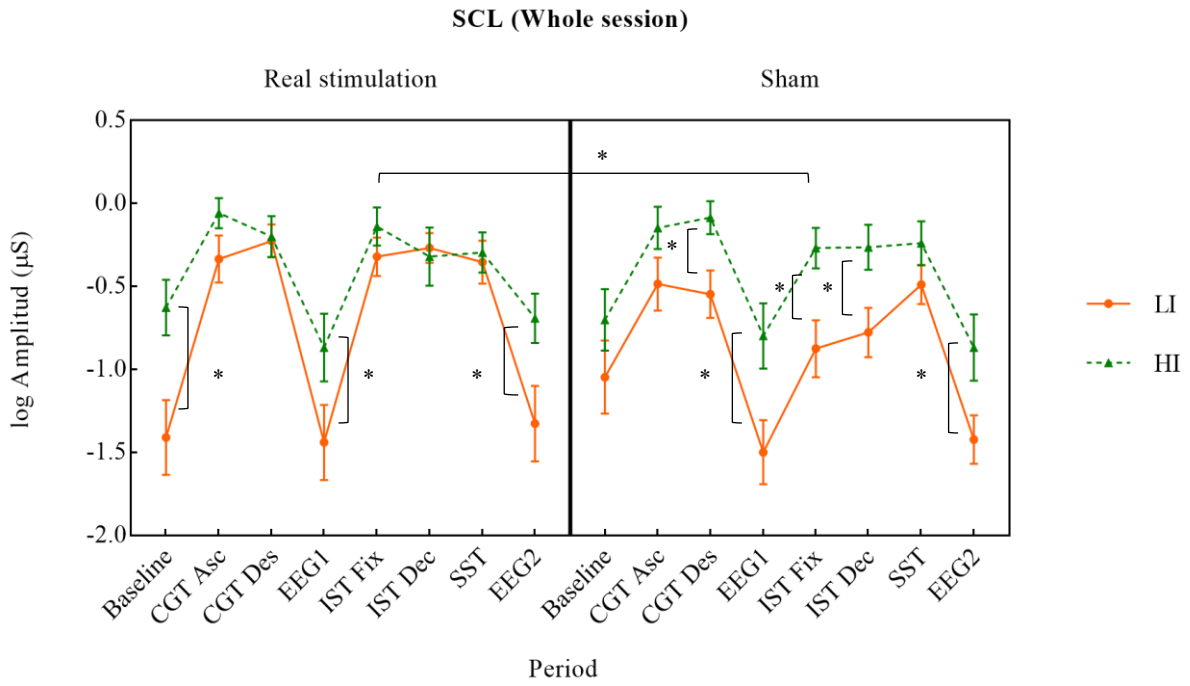

Figure 5-5. Skin conductance level (SCL) during the whole session in Experiment IV. SCL log amplitude (µS) in low impulsive (LI) and high impulsive (HI) participants, during tDCS real stimulation and sham conditions, across rest periods (baseline, pre-tDCS electroencephalogram (EEG1) and post-tDCS (EEG2)) and tasks performance periods (Cambridge gambling task (CGT) in ascending (asc) and descending (des) conditions; information sampling task (IST) in fixed (fix) and decreased (dec) conditions; and stop signal task (SST). Data represents mean and SEM (* $p < .05$).

5.4.3.2 Skin conductance level during pre-tDCS and post-tDCS rest periods

Results showed no significant differences in tDCS time ($p > .05$) in SCL between pre-tDCS (EEG1) and post-tDCS (EEG2) conditions. However, SCL during EEG1 and EEG2 was also analysed subdividing the EEG recordings into two parts (EEGa and EEGb, for first and second half of the recordings, respectively). EEGb showed no significant main effects of tDCS time, nor significant interactions (all effects $p > .05$). However, in EEGa, there was a main effect of tDCS ($F (1, 15) = 14.063$, $p = .002$, $\eta p^2 = .484$) indicating that SCL was lower in condition real stimulation compared with sham. A main effect of tDCS time ($F (1, 15) = 69.110$, $p = .001$, $\eta p^2 = .822$), showed that SCL was lower in EEG1 compared with EEG2. An interaction tDCS x tDCS time ($F (1, 15) = 19.994$, $p = .001$, $\eta p^2 = .571$), showing that both real stimulation and sham conditions had similar SCL in EEG1. In EEG2, SCL increased compared with EEG1, however the increase in real stimulation condition was lower compared with the increase in sham condition.

There was also an interaction time x group ($F (1, 15) = 9.679$, $p = .007$, $\eta p^2 = .392$), showing that HI had higher SCL compared with LI in both EEG1 and EEG2, and that the difference between the groups was lower in EEG2, compared with EEG1. In EEGb, there was an interaction tDCS x tDCS time x group ($F (1, 15) = 4.930$, $p = .042$, $\eta p^2 = .247$), showing that LI had lower SCL than HI in both tDCS conditions. Also, in real stimulation, LI had similar SCL in EEG1 and EEG2, and HI had higher SCL in EEG2 compared with EEG1, but in sham both groups LI and HI, had higher SCL in EEG2 compared with EEG1. The largest difference between the groups was found in EEG2 in real stimulation, but in EEG1 in sham.

To analyse SCL interactions found between EEG1 and EEG2 periods, paired samples t-tests showed that SCL was significantly lower in EEG1 compared with EEG2 ($t (16) = -2.409$, $p = .028$) and in EEG1a compared with EEG2a ($t (16) = -3.807$, $p = .002$) in real stimulation, but not significantly different in EEGb in real stimulation, or in any of the EEG subdivisions in sham. Independent sample (2-tailed) t-tests to analyse SCL interactions with groups revealed that SCL was higher in HI compared with LI in real stimulation, in EEG1a ($t (15) = -2.207$, $p = .028$), in EEG2 ($t (15) = -2.393$, $p = .028$) and EEG2b ($t (15) = -2.682$, $p = .017$), as well as in sham, in EEG1 ($t (15) = -2.533$, $p = .023$), EEG1a ($t (15) = -2.506$, $p = .024$), EEG1b ($t (15) = -2.461$, $p = .026$), EEG2 ($t (15) = -2.196$, $p = .044$) and EEG2b ($t (15) = -2.294$, $p = .037$). EEG1a results are represented in Figure 5-6.

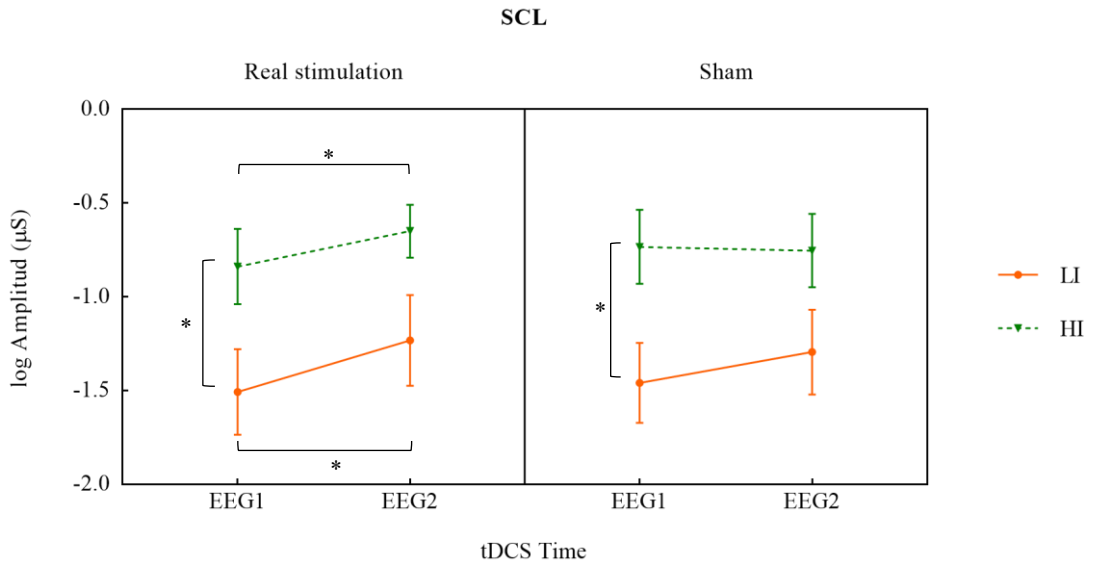

Figure 5-6. Skin conductance level (SCL) before and after tDCS. SCL log amplitude (µS) during the first half of the five minutes electroencephalogram (EEGa) recordings during pre-tDCS (EEG1) and post-tDCS (EEG2), in tDCS conditions real stimulation and sham with low impulsive (LI) and high impulsive (HI) participants. Data represents mean and SEM (* $p < .05$).

5.4.3.3 Local skin conductance level during Cambridge gambling task risk conditions

Results showed that main effects were not significant for tDCS, tDCS time, risk or condition (all effects $p > .05$). However, there was a significant three way interaction tDCS x condition x group ($F(1, 15) = 6.551$, $p = .022$, $\eta p^2 = .304$), indicating that in real stimulation, LI had lower SCL in CGT ascending condition compared with descending condition, however, HI had higher SCL in ascending condition compared with descending condition. In sham, LI had no different SCL between both task conditions, but HI had slightly higher SCL in descending condition. In both tDCS conditions, HI showed higher SCL than LI, but their levels were closer in real stimulation descending condition. Moreover, a three way interaction tDCS x risk x group ($F(1, 15) = 3.732$, $p = .018$, $\eta p^2 = .199$), showed that in real stimulation, SCL decreased with increasing CGT risk conditions in LI, however in HI, SCL increased with increasing risk conditions, with the largest difference between groups found in the most risky condition (6:4), and the smallest difference between groups in the lowest risk condition (9:1). In sham, LI showed higher SCL in the 6:4 compared with 9:1, however in HI, 6:4 was associated with similar SCL than 9:1. In all conditions, SCL was higher in HI than in LI.

Independent t-tests (2-tailed) to investigate the differences in CGT SCL between groups showed that in real stimulation, LI had significantly lower SCL in ascending 6:4 ($t(15) = -2.643$, $p = .018$) compared with HI, however in sham, LI had lower SCL in the ascending 9:1 ($t(15) = -2.388$, $p = .031$) and descending 8:2 ($t(15) = -2.292$, $p = .037$) and 6:4 ($t(15) = -2.541$, $p = .023$), compared with HI. Paired t-tests to investigate differences in tDCS condition showed no significant results. These results are represented in Figure 5-7.

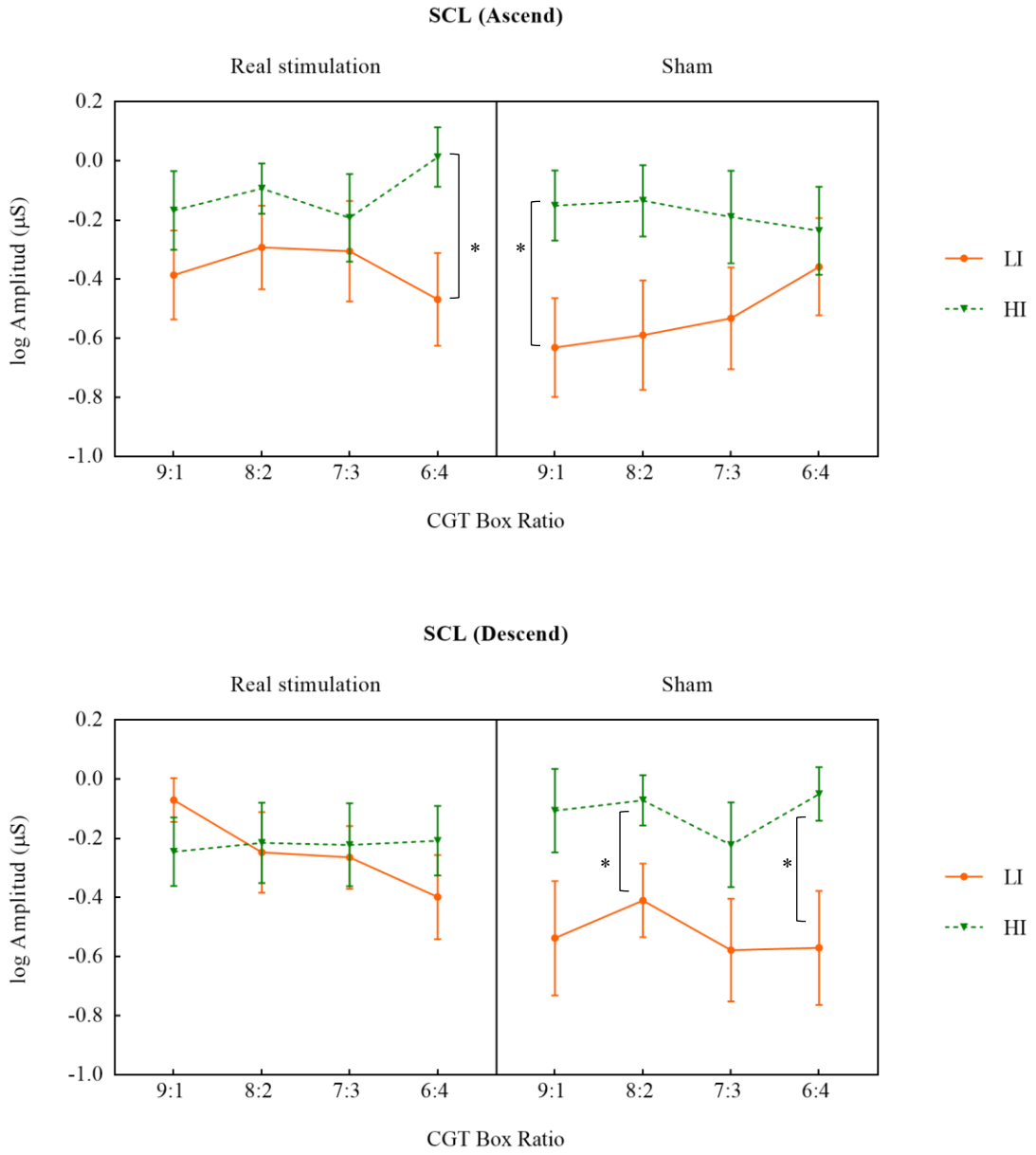

Figure 5-7. Skin conductance level (SCL) during different risk conditions (box ratio) in Experiment IV. SCL amplitude (µS) in low impulsive (LI) and high impulsive (HI) participants during tDCS real stimulation and sham conditions, across Cambridge gambling task (CGT) risk conditions represented by box ratios (9:1, 8:2, 7:3 and 6:4) in ascending and descending task conditions. Data represents mean and SEM (* $p < .05$).

5.4.3.4 Skin conductance responses (SCRs) during task reward outcomes (wins and losses)

Cambridge gambling task (CGT)

Results showed a main effect of outcome ($F (1, 15) = 5.667$, $p = .031$, $\eta p^2 = .274$), indicating that SCRs were higher in wins than in losses. There was a main effect of response ($F (1, 15) = 43.923$, $p = .001$, $\eta p^2 = .745$), showing that SCRs were higher in feedback response compared with anticipatory response. However, there were no main effects of tDCS ($p > .05$). A three way interaction tDCS x outcome x group ($F (1, 15) = 5.082$, $p = .040$, $\eta p^2 = .253$), showed that in both sessions, HI showed higher SCRs compared with LI, and in both groups SCRs were higher in wins than loses, however in the session in which real stimulation was delivered, SCRs were higher in wins than in losses in HI, but had similar levels between wins and losses in LI. In the session with sham tDCS, SCRs were more similar between wins and losses in HI, with greater difference between wins and losses found in LI. There was also an interaction outcome x response ($F (1, 15) = 11.345$, $p = .004$, $\eta p2 = .431$), indicating that there was a larger difference between anticipatory response and feedback response in losses compared to wins.

Independent t-tests (2-talied) to investigate the differences in CGT SCRs between groups showed that in sham condition, LI had lower feedback SCRs associated to wins in ascending condition compared with HI ($t (15) = -2.336$, $p = .034$). In addition, LI had lower anticipatory SCRs ($t (15) = -2.495$, $p = .025$) and feedback response ($t (15) = -2.382$, $p = .031$) associated with losses compared with HI. To investigate the differences in SCRs between tDCS conditions, paired t-tests revealed no significant differences between real stimulation and sham. To investigate the interactions outcome x response, paired t-tests were conducted revealing significant differences between anticipatory and feedback SCRs in real stimulation in ascending wins ($t (16) = -5.475$, $p = .001$), and losses ($t (16) = -4.102$, $p = .001$) and descending wins ($t (16) = -5.449$, $p = .001$) and losses ($t (16) = -4.111$, $p = .001$). In sham condition, differences between anticipatory and feedback response were found in CGT ascending wins ($t (16) = -4.477$, $p = .001$), and losses ($t (16) = -5.042$, $p = .001$), descending wins ($t (16) = -5.037$, $p = .001$) and losses ($t (16) = -5.247$, $p = .001$). In real stimulation condition, differences in SCRs between wins and losses in CGT ascending anticipatory responses ($t (16) = 2.335$, $p = .033$), showing higher anticipatory responses associated with wins than with losses. In sham condition differences between wins and losses in CGT descending anticipatory responses ($t (16) = 2.489$, $p = .024$). These results are represented in Figure 5-8.

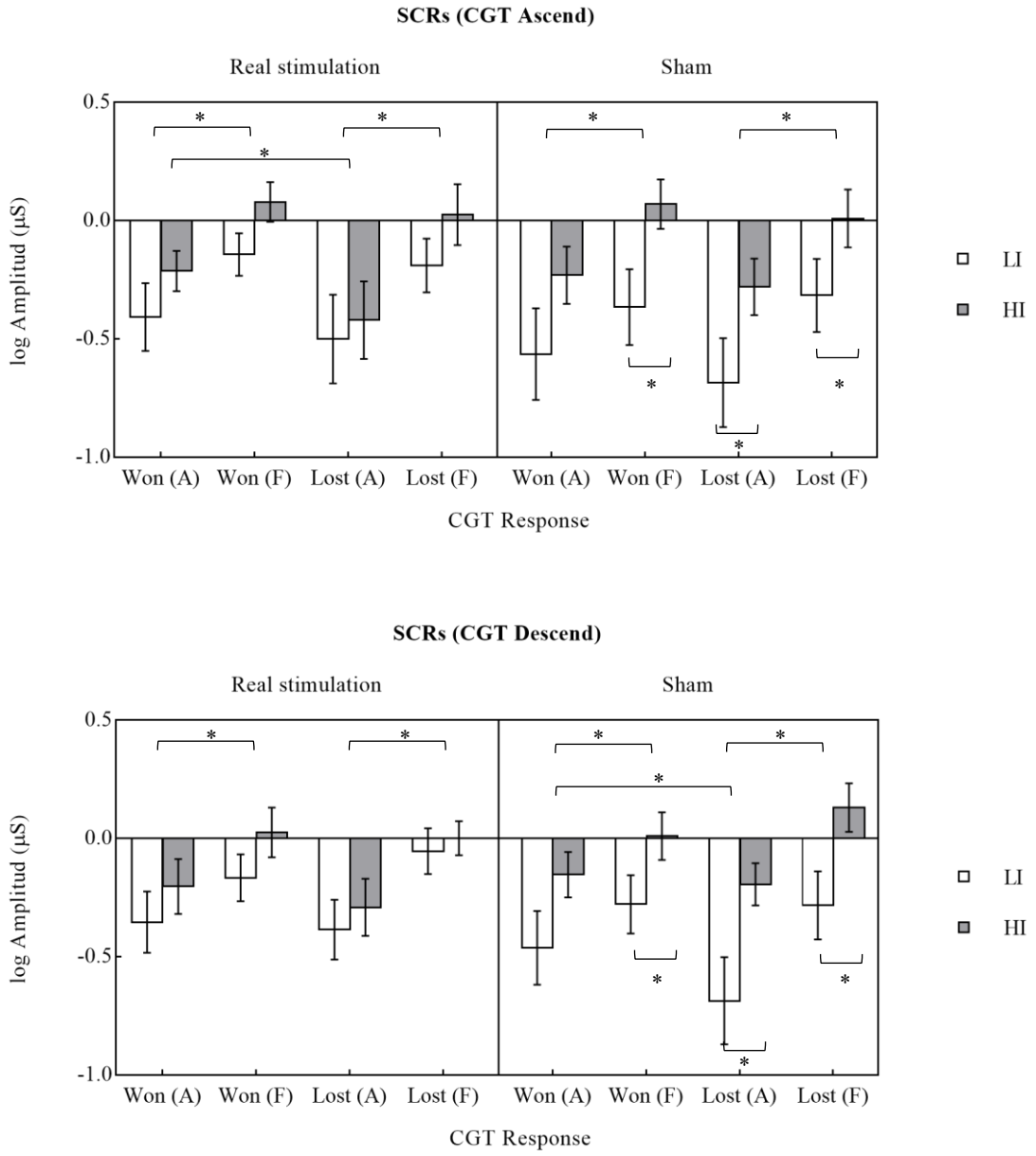

Figure 5-8. Skin conductance responses (SCRs) during Cambridge gambling task (CGT) performance in Experiment IV. SCRs log amplitude (µS), including reward anticipatory (A) and feedback (F) responses, when low impulsive (LI) and high impulsive (HI) participants won or lost each trial in the CGT, during tDCS real stimulation and sham and ascending and descending tasks conditions. Data represents mean and SEM (* $p < .05$).

Information sampling task (IST)

Results showed a main effect of response (F (1, 15) = 36.090, p =.001, ηp^2 =.706), indicating that the SCRs were higher in feedback responses than in anticipatory responses. However there were no significant main effects of tDCS or outcome (all effects p > .05). In addition, there was an interaction tDCS x response (F (1, 15) = 5.106, p =.039, ηp^2 =.254), showing that SCRs were higher in real stimulation than in sham, and that the difference between anticipatory responses and feedback responses was larger in sham compared with real stimulation. There was a four way interaction tDCS x response x condition x groups (F (1, 15) = 5.660, p =.031, ηp^2 =.274), showing that HI had higher SCRs than LI. In real stimulation, SCRs were higher in ascending condition in HI, but lower compared with descending condition in LI, however in sham condition, both groups had similar SCRs between ascending and descending task conditions, with higher SCRs in HI compared with LI. The anticipatory responses had lower amplitude than feedback responses, however the difference between them seem to be greater in sham compared to real stimulation.

To investigate groups differences in SCRs in IST independent t-tests (2-tailed) analysis showed that, in fixed condition, LI had lower feedback SCRs associated to wins in real stimulation (t (15) = -2.169, p = .047), and in sham (t (15) = -2.334, p = .034), as well as lower anticipatory SCRs associated with losses (t (15) = -2.798, p = .014) and feedback SCRs (t (15) = -2.456, p = .027) in sham. Moreover, in decreased condition, LI had lower anticipatory SCRs and feedback SCRs (t (15) = -2.273, p = .038) associated with wins (t (15) = -2.410, p = .029), and lower anticipatory responses (t (15) = -2.517, p = .024) and feedback responses (t (15) = -2.271, p = .038) associated with losses in sham.

To investigate the interactions outcome x response, paired t-tests were conducted revealing that in real stimulation, the anticipatory responses were significantly lower compared with feedback responses in fixed condition wins (t (16) = -5.080, p = .001), and losses (t (16) = -2.354, p = .032), and in decreased condition wins (t (16) = -3.631, p = .002) and losses (t (16) = -4.072, p = .001). In sham condition, differences between anticipatory and feedback response were found in fixed condition wins (t (16) = -2.495, p = .002), and losses (t (16) = -2.495, p = .001), and decreased wins (t (16) = -6.488, p = .001) and losses (t (16) = -5.701, p = .001). There were no significant differences between wins and losses in any of the variables (all effects p > .05). Paired t-tests to investigate interactions with tDCS showed that there were no significant differences in SCRs between real stimulation and sham (all effects p > .05). These results are presented in Figure 5-9.

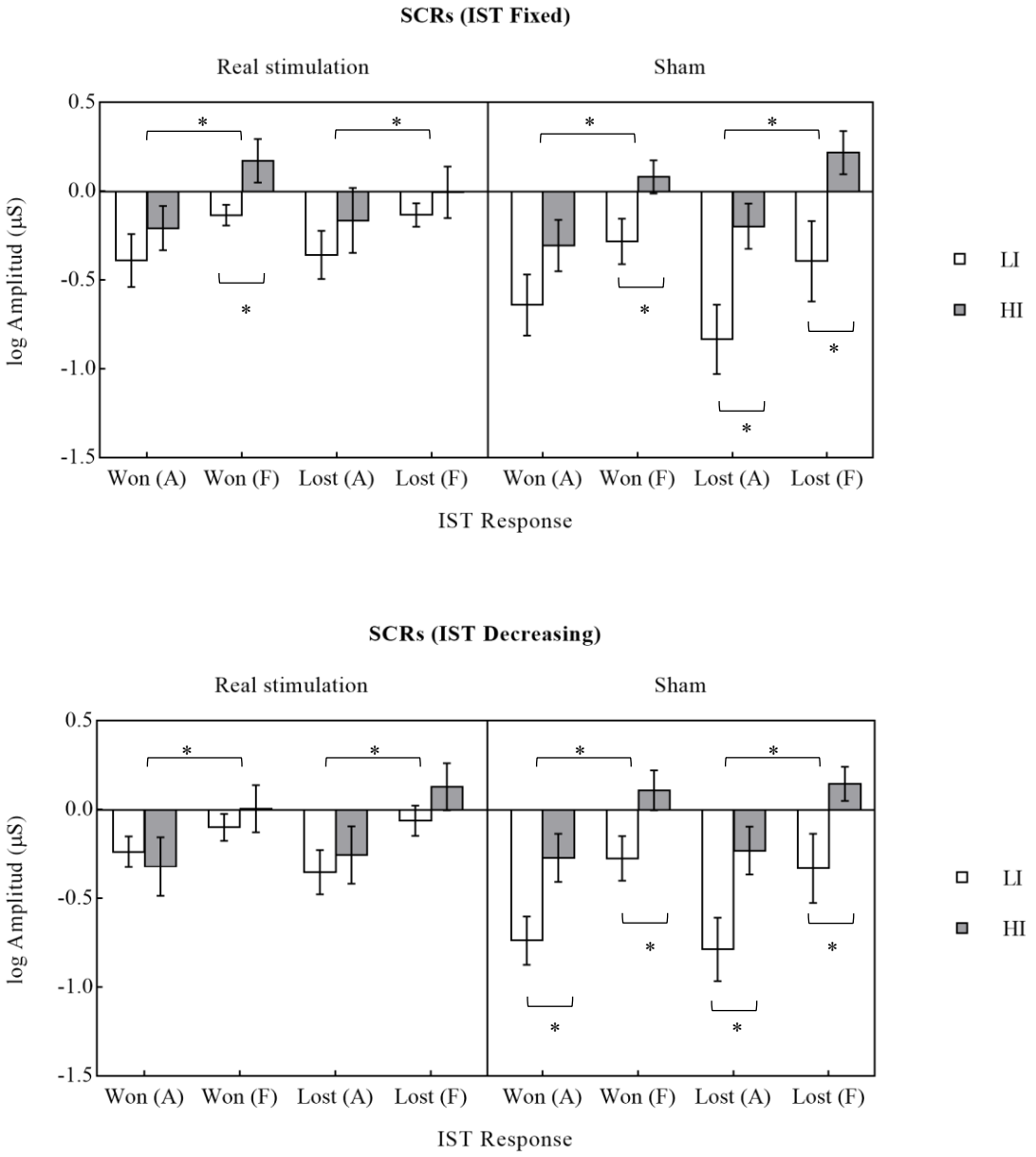

Figure 5-9. Skin conductance responses (SCRs) during information sampling task (IST) performance in Experiment IV. SCRs log amplitude (µS), including reward anticipatory (A) and feedback (F) responses, when low impulsive (LI) and high impulsive (HI) participants won or lost each trial in the IST, during tDCS real stimulation and sham, and fixed and decreased tasks conditions. Data represents mean and SEM (* $p < .05$).

Stop signal task (SST)

Results showed a main effect of outcome in SST (F (1, 15) = 8.529, p =.011, ηp^2 =.362), indicating that the SCRs were higher in losses than in wins. There was also a main effect of response (F (1, 15) = 62.043, p =.001, ηp^2 =.805), showing that SCRs were higher in the feedback responses than in the anticipatory responses. There were no significant main effects of tDCS (p > .05). Results revealed an interaction outcome x response (F (1, 15) = 4.776, p =.045, ηp^2 =.241), showing that anticipatory responses were similar between wins and losses, however feedback responses were higher in losses compared to wins.

To investigate the interactions outcome x response, paired t-tests revealed lower anticipatory responses compared with feedback responses in SST wins (t (16) = -5.140, p = .001) and in losses (t (16) = -2.495, p = .001) in real stimulation. In sham condition, differences between anticipatory and feedback response were also found in wins (t (16) = -4.375, p = .001), and losses (t (16) = -6.019, p = .001). In real stimulation condition, there were lower feedback responses associated with wins than with losses (t (16) = -4.118, p = .025), as well as in sham condition, in which feedback responses had lower SCRs in wins than in losses (t (16) = -2.441, p = .027). These results are represented in Figure 5-10.

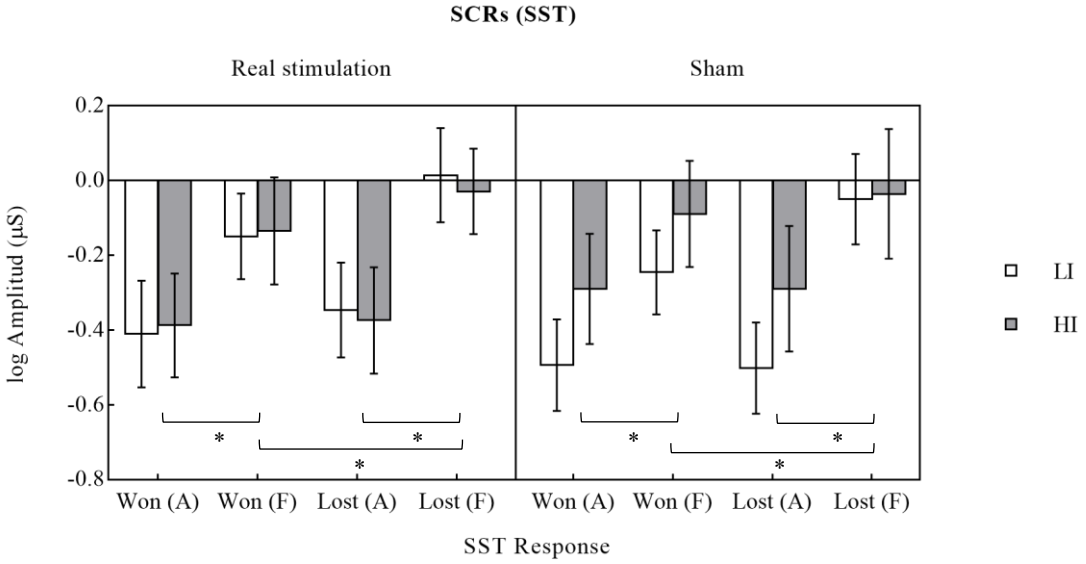

Figure 5-10. Skin conductance responses (SCRs) during stop signal task (SST) performance in Experiment IV. SCRs log amplitude (µS), including reward anticipatory (A) and feedback (F) responses, when low impulsive (LI) and high impulsive (HI) participants won or lost each trial in the SST during tDCS real stimulation and sham. Data represents mean and SEM (* $p < .05$).

5.4.4 Heart Rate Variability (HRV)

5.4.4.1 Low frequency (LF) and high frequency (HF) measures during the whole session

Results showed a main effect of period in total power (F (1, 15) = 2.586, p =.017, ηp^2 =.147), indicating that the periods from lowest to highest total power were: baseline, CGT descending, EEG1, CGT ascending, SST, EEG2, IST fixed and IST decreased. However, there were no significant main effects of tDCS, or significant interactions (all effects p > .05).

5.4.4.2 Low frequency (LF) and high frequency (HF) measures during pre-tDCS and post-tDCS rest periods

Results revealed no significant main effects of tDCS, period or any interactions (all effects p > .05).

5.4.4.3 Low frequency (LF) and high frequency (HF) measures during task performance

Results showed a main effect of period in absolute LF power (F (1, 15) = 2.823, p =.033, ηp^2 =.158) showing that the tasks from lowest to highest total power were: CGT descending, CGT ascending, SST, IST decreased and IST fixed. However there were no significant main effects of tDCS (p > .05). In LF relative power there was an interaction tDCS x period x group (F (1,15) = 3.617, p =.010, ηp^2 =.194), showing that in real stimulation, LF relative power was higher in HI compared with LI in all periods except in SST, in which both groups had similar LF power. The largest difference between HI and LI was found in IST decreased condition. In contrast, in sham, HI had higher LF power in all tasks compared to LI, with the smallest difference between groups being IST decreased condition and the largest being CGT ascending condition and SST.

There were no main effects of tDCS or period in HF absolute and relative power (all effects p > .05). However, in HF relative power there was an interaction tDCS x task x group (F (1,15) = 3.563, p =.011, ηp^2 =.192), showing that in real stimulation, relative HF power was higher in LI compared with HI in all tasks, except in SST, in which both groups had the same level. The largest difference between the groups was found in IST decreased condition. In sham, LI had higher HF power than LI in all tasks, with the smallest difference between the groups found in IST decreased condition, and the largest in CGT descending condition.

To investigate HRV interactions with groups, independent t-tests revealed that LI had significantly lower LF relative power than HI in real stimulation during IST decreased condition ($t(15) = -3.246$, $p = .005$) and in sham, during CGT descending condition ($t(15) = -3.432$, $p = .004$) and SST ($t(15) = -3.475$, $p = .003$). Moreover, LI had significantly higher HF relative power than HI in real stimulation during IST decreased condition ($t(15) = 3.156$, $p = .007$), and in sham, during CGT descending condition ($t(15) = 3.392$, $p = .004$) and SST ($t(15) = 3.451$, $p = .004$). These results are represented in Figure 5-11. To investigate tDCS interactions, paired t-tests comparing LF and HF relative power between tDCS conditions real stimulation and sham showed no significant differences.

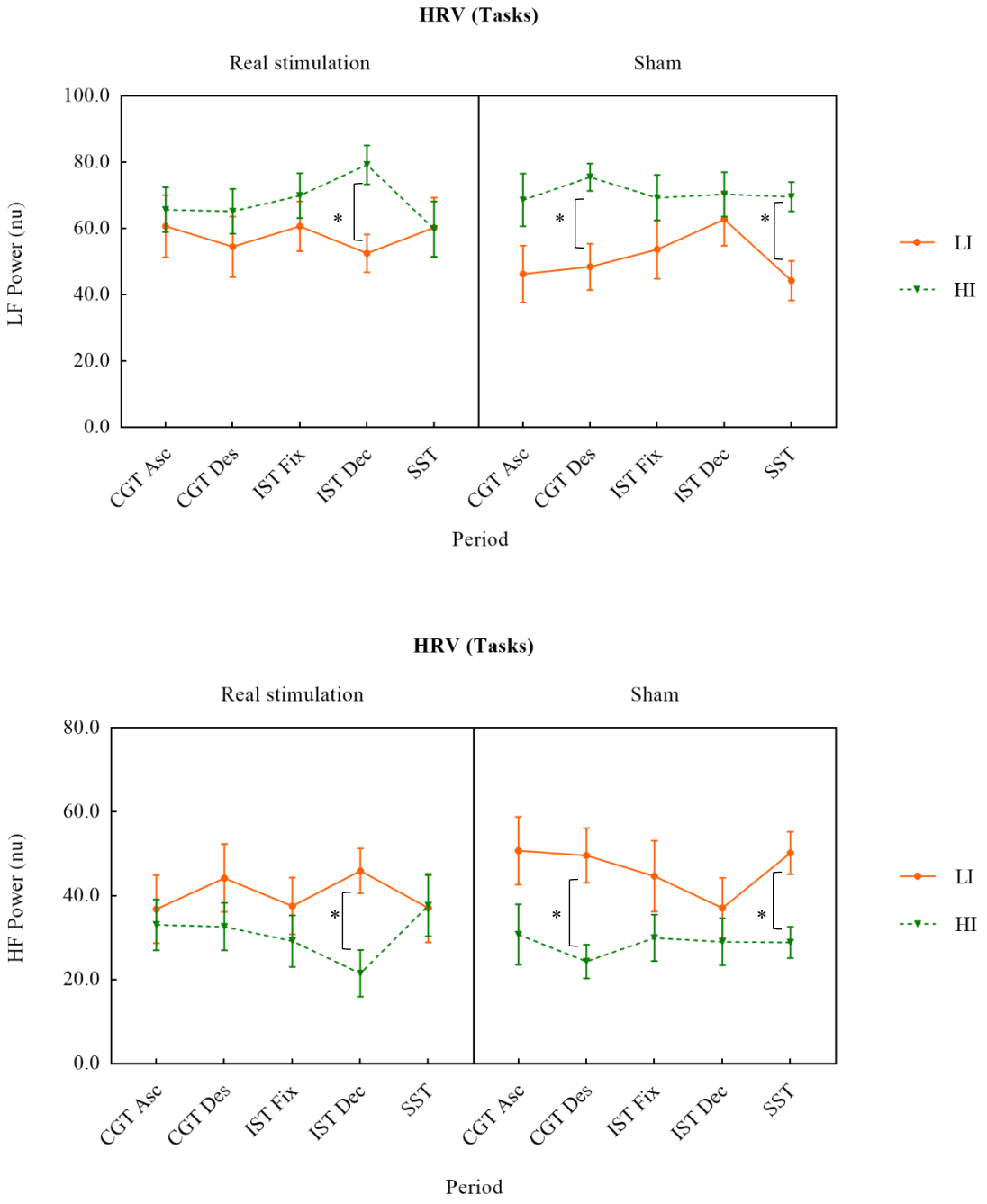

Figure 5-11. Heart rate variability (HRV) during task performance periods in Experiment IV. Low frequency (LF) and high frequency (HF) relative power (nu) in low impulsive (LI) and high impulsive (HI) participants, in tDCS real stimulation and sham conditions, during tasks performance periods including: Cambridge gambling task (CGT) ascending (asc) and descending (des) conditions; information sampling task (IST) fixed (fix) and decreased (dec) conditions; and stop signal task (SST). Data represents mean and SEM (* $p < .05$).

5.4.4.4 Heart rate during the tasks performance periods

HR during task performance results showed no differences between tasks in mean HR, or maximum HR (all effects $p > .05$). However, a main effect of period in minimum HR ($F (2.510, 37.649) = 3.421$, $p = .034$, $\eta p^2 = .186$) showed that minimum HR was higher in CGT descending condition with around 60 bpm, followed by CGT ascending and SST, IST decreased and lowest in IST fixed condition, with around 45 bpm.

5.4.4.5 Heart rate during Cambridge gambling task risk conditions

Results revealed no significant effects of HR during CGT risk conditions (all effects $p > .05$).

5.4.4.6 Heart rate during task reward outcomes (wins and losses)

Cambridge gambling task (CGT)

Results showed no significant effects in CGT during reward outcomes (all effects $p > .05$).

Information sampling task (IST)

Mean HR showed a significant main effect of outcome ($F (1, 15) = 5.931$, $p = .028$, $\eta p^2 = .283$), indicating that mean HR was higher in wins than in losses. However no significant main effects of tDCS or condition were found (all effects $p > .05$). There was an interaction outcome x group ($F (1, 15) = 4.920$, $p = .042$, $\eta p^2 = .247$), showing that LI had similar mean HR between wins and losses, however HI showed a large difference between wins (with higher mean HR) and losses. HI had higher mean HR in wins than LI, but lower mean HR in losses compared to LI. There was also an interaction tDCS x outcome ($F (1, 15) = 4.559$, $p = .050$, $\eta p^2 = .233$), showing that in both, real stimulation and sham, mean HR was higher in wins than in losses, but the difference between wins and losses was larger in real stimulation, being slightly higher for wins than sham, but much lower than sham in losses.

In maximum HR, there was a main effect of condition ($F (1, 15) = 5.509$, $p = .033$, $\eta p^2 = .269$), indicating that maximum HR was higher in IST decreased condition, and a main effect of outcome ($F (1, 15) = 7.757$, $p = .014$, $\eta p^2 = .341$), showing that maximum HR was higher in wins compared to

losses. There was no main effect of tDCS ($p > .05$). Results showed an interaction outcome x group ($F (1, 15) = 4.878$, $p = .043$, $\eta p^2 = .245$), showing that both groups had higher maximum HR in wins than in losses, but maximum HR was higher in HI compared to LI in wins but lower than LI in losses, and the difference between the groups was larger in wins than in losses.

Similarly, in minimum HR, there was a main effect of condition ($F (1, 15) = 4.958$, $p = .042$, $\eta p^2 = .248$), showing that minimum HR was lower in fixed condition compared to deceased condition. No main effects of tDCS were found ($p > .05$). There was an interaction condition x group ($F (1,15) = 4.867$, $p = .043$, $\eta p^2 = .245$), showing that in LI minimum HR was similar between both conditions, but in HI, minimum HR was lower in fixed condition, and higher in decreased condition. The difference between the groups was small in fixed condition, in which LI had higher minimum HR compared to HR, and larger in decreased condition, where HI had greater minimum HR than LI. There was an interaction outcome x groups ($F (1,15) = 4.652$, $p = .048$, $\eta p^2 = .237$), showing that in LI had lower minimum HR in wins compared with HI, The difference between groups was larger in wins than in losses. There was an interaction condition x outcome ($F (1, 15) = 4.761$, $p = .045$, $\eta p^2 = .241$), showing that the difference in minimum HR between tasks conditions fixed and decreased was larger in losses than in wins.

Stop signal task (SST)

In SST, there were no main effects of tDCS or outcome (all effects $p > .05$). However, there was an interaction outcome x groups ($F (1, 15) = 4.678$, $p = .047$, $\eta p^2 = .238$) in minimum HR, showing that LI had lower minimum HR than HI in wins and losses, and that the difference between the groups was larger in losses. LI had higher minimum HR in wins compared to losses, but HI had lower minimum HR in wins compared to losses.

To investigate the group interactions with mean HR, minimum HR and maximum HR, during task performance outcomes, independent samples t-tests revealed that in sham, LI had significantly lower mean HR ($t (15) = -2.627$, $p = .019$) and lower minimum HR ($t (15) = -2.523$, $p = .023$) in IST decreased wins, compared with HI. In SST, LI had lower minimum HR ($t (15) = -3.405$, $p = .004$) associated with wins, and losses ($t (15) = -3.214$, $p = .006$) compared with HI. To investigate tDCS condition interactions, paired t-tests revealed no significant differences between mean HR during IST task outcomes. These results are represented in Figure 5-12.

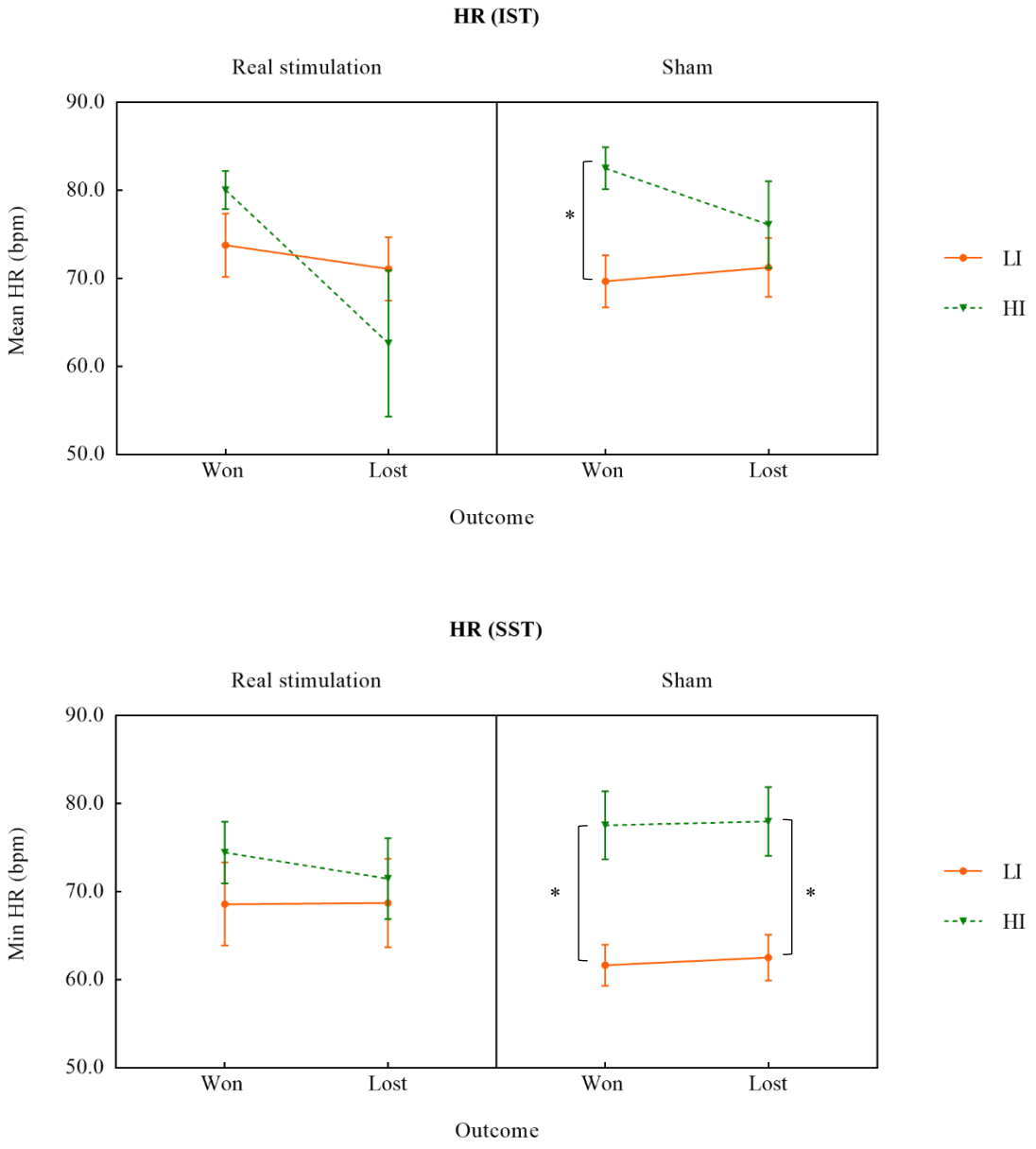

Figure 5-12. Heart rate (HR) during task performance outcomes in Experiment IV. Mean HR (bpm) in information sampling task (IST), and minimum HR (bpm) in stop signal task (SST), in low impulsive (LI) and high impulsive (HI) participants, in tDCS real stimulation and sham conditions, during tasks performance outcomes won and lost. Data represents mean and SEM (* $p < .05$).

5.4.5 Electroencephalogram (EEG)

5.4.5.1 Theta/beta ratio

Results showed a main effect of tDCS (F (1, 105) = 5.244, p =.037, ηp^2 =.259), indicating that theta/beta ratio was lower in real stimulation compared with sham. There was a main effect of electrode (F (7, 15) = 7.906, p =.001, ηp^2 =.345), indicating that the electrodes with higher to lower theta/beta ratio were Fpz followed by Cz and AF7 followed by AF4 and F4, then AF8 and lastly FC6. There were no significant main effects of tDCS or interactions (all effects p > .05).

5.4.5.2 Mean frequency power

Results showed a main effect of electrode, indicating that the electrodes with higher to lower mean frequency power were: FC6, F3, AF8, F4, AF4, Cz, AF7, and Fpz. There were no main effects of tDCS or tDCS time (all effects p > .05). There was an interaction tDCS condition x tDCS time, showing that in real stimulation, mean frequency power decreased from EEG1 (pre-tDCS) to EEG2 (post-tDCS), but in sham, mean frequency increased from EEG1 to EEG2. There was an interaction tDCS x group, showing that LI had higher mean frequency power in real stimulation compared with sham, however HI showed lower mean frequency power in real stimulation compared with sham. In addition, the difference between real stimulation and sham was smaller in HI. In addition, HI showed higher mean frequency power than LI, with the difference between the groups being smaller in real stimulation and larger in sham.

To investigate interactions with group, independent t-tests revealed that LI had lower mean frequency power than HI in sham EEG2, in AF8 (t (15) = -2.597, p = .020) and F3 (t (15) = -2.587, p = .021). To investigate interactions with tDCS time, paired sample t-tests showed that EEG1 was lower than EEG2 in sham, electrode position F3 (t (15) = -2.292, p = .036). To investigate interactions with tDCS condition, paired t-tests showed that mean frequency power was higher in real stimulation compared with sham in EEG1 in all electrodes except in Cz and F3: AF8 (t (16) = 2.434, p = .027), AF4 (t (16) = 2.885, p = .011), Fpz (t (16) = 2.403, p = .029), F4 (t (16) = 2.615, p = .019), FC6 (t (16) = 3.015, p = .008) and AF7 (t (16) = 2.220, p = .041). However, in EEG2 there were no significant differences between real stimulation and sham (all effects p > .05). These results are represented in Figure 5-13. In addition, EEG power spectrograms showing inter-individual and intra-individual variability across sessions and are presented in Appendix B, in Figure 0-1, Figure 0-2, Figure 0-3 and Figure 0-4.

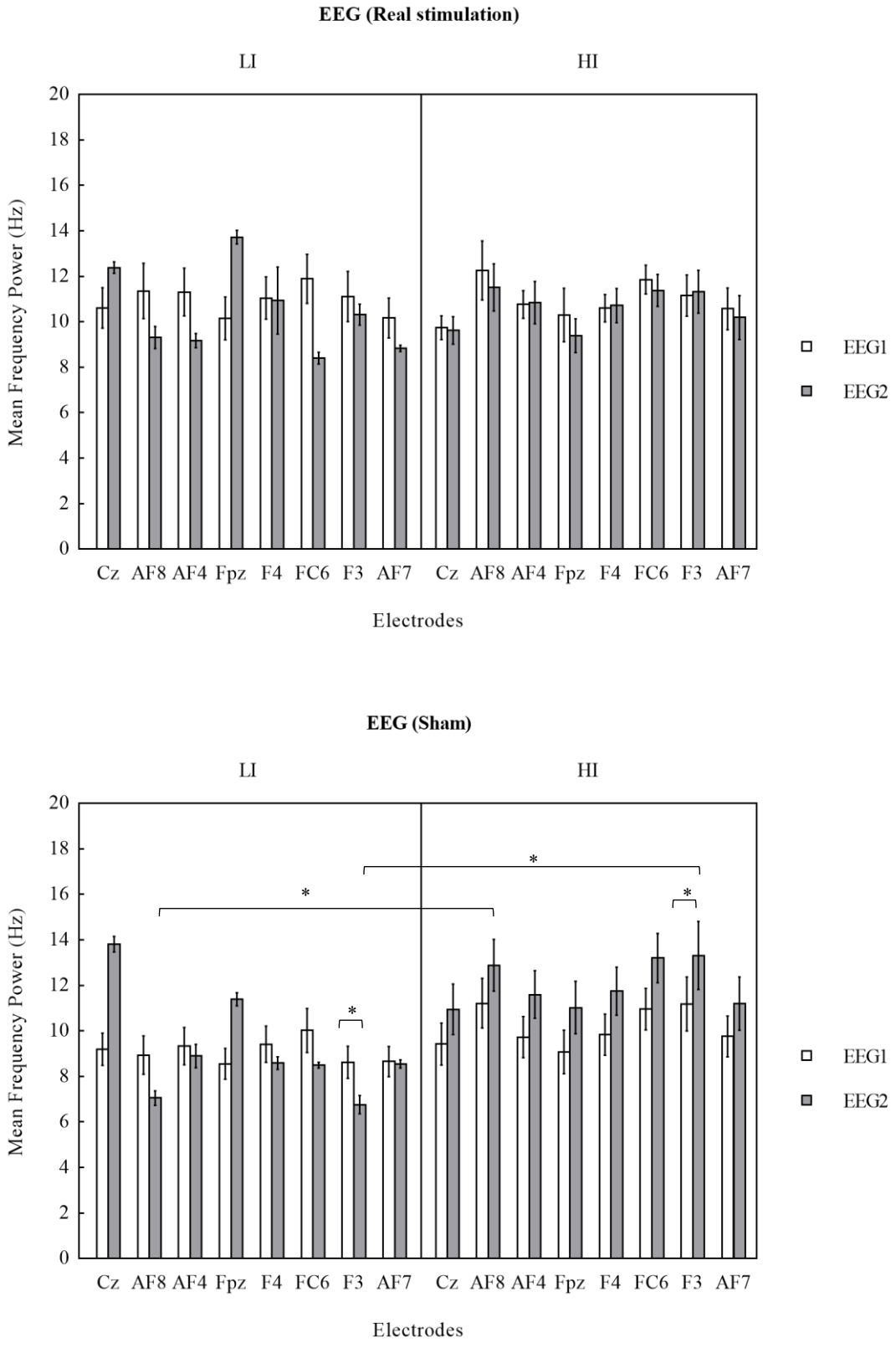

Figure 5-13. Electroencephalogram (EEG) mean frequency power in Experiment IV. Mean frequency power (Hz) of EEG resting state recordings pre-tDCS (EEG1) and post-tDCS (EEG2), across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7) for low impulsive (LI) and high impulsive (HI) participants in tDCS real stimulation and sham conditions. Data represents mean and SEM (* $p < .05$).

5.4.5.3 Delta absolute and relative power

Results showed no significant effects in delta absolute power in (all effects $p > .05$). In delta relative power, there was a main effect of tDCS ($F (1, 15) = 9.350$, $p = .008$, $\eta p^2 = .384$), showing that lower relative delta power was associated with real stimulation compared with sham. There was also a main effect of electrode ($F (7, 105) = 10.490$, $p = .001$, $\eta p^2 = .412$), showing that electrodes from highest to lowest delta relative power were AF7, Fpz, AF8, AF4, F3, FC6, F4 and Cz. There was an interaction time x group $F (1,15) = 5.229$, $p = .037$, $\eta p^2 = .258$, showing that in LI, delta relative power increased from EEG1 to EEG2, however in HI relative delta power decreased from EEG1 to EEG2. The groups showed a greater difference in EEG1, where HI had higher delta relative power than LI, and a smaller difference in EEG2, where HI had lower delta relative power than LI. There was also an interaction tDCS x tDCS time ($F (1,15) = 9.252$, $p = .008$, $\eta p^2 = .381$), showing that in real stimulation, delta relative power increased from EEG1 to EEG2, however in sham, delta relative power decreased from EEG1 to EEG2. In both, EEG1 and EEG2, real stimulation was associated with lower delta relative power compared to sham, finding the largest difference between real stimulation and sham in EEG1.

To investigate groups interactions, independent t-tests revealed that LI had higher relative delta power than HI in sham EEG2, in electrode position F3 ($t (15) = 2.334$, $p = .034$). To investigate tDCS time interactions, paired t-tests showed that EEG1 was lower than EEG2 in real stimulation, in electrode positions AF8 ($t (16) = -2.400$, $p = .029$), AF4 ($t (16) = -2.400$, $p = .015$), Fpz ($t (16) = -2.400$, $p = .002$) and FC6 ($t (16) = -2.400$, $p = .011$). However, EEG1 was higher than EEG2 in sham, in electrode positions Cz ($t (16) = 2.876$, $p = .011$), AF8 ($t (16) = 2.160$, $p = .046$), AF4 ($t (16) = 2.494$, $p = .024$), Fpz ($t (16) = 2.202$, $p = .043$), and F4 ($t (16) = 2.722$, $p = .015$). To investigate tDCS condition interactions, paired t-tests showed that delta relative power was lower in real stimulation compared with sham, in EEG1, in all electrode positions: Cz ($t (16) = -3.212$, $p = .005$), AF8 ($t (16) = -2.964$, $p = .009$), AF4 ($t (16) = -3.719$, $p = .002$), Fpz ($t (16) = -3.423$, $p = .003$), F4 ($t (16) = -3.518$, $p = .003$), FC6 ($t (16) = -3.794$, $p = .002$), F3 ($t (16) = -2.710$, $p = .015$) and AF7 ($t (16) = -2.494$, $p = .024$). However, in EEG2, there were no significant differences between real stimulation and sham conditions (all effects $p > .05$).

5.4.5.4 Theta absolute and relative power

Results showed a main effect of electrode ($F (7, 105) = 20.262$, $p = .001$, $\eta p^2 = .575$) in theta absolute power, showing that electrodes from highest to lowest theta absolute power were: Fpz, AF7, F3, Cz, F4, AF4, AF8 and FC6. There were no main effects of tDCS or tDCS time (all effects $p > .05$). There was an interaction tDCS x electrode x group ($F (7, 105) = 3.168$, $p = .037$, $\eta p^2 = .174$), showing that LI had higher theta absolute power than HI in real stimulation in all electrodes, and that the largest difference between the groups was found in F4. However, in sham LI had lower absolute theta power than HI in all electrodes. Independent t-tests to compare absolute theta power between groups revealed that there were no significant differences (all effects $p > .05$).

In theta relative power, results showed a main effect of tDCS ($F (1, 15) = 38.627$, $p = .001$, $\eta p^2 = .720$) indicating that real stimulation was associated with lower theta relative power compared with sham. There was a main effect of tDCS time ($F (1, 15) = 39.274$, $p = .001$, $\eta p^2 = .724$), showing that theta relative power decreased from EEG1 to EEG2. There was a main effect of electrode ($F (7, 105) = 44.268$, $p = .001$, $\eta p^2 = .747$), showing that the electrode with higher theta relative power was F4, and the rest of electrodes showed lower theta relative power. There was an interaction tDCS x tDCS time ($F (1,15) = 42.082$, $p = .001$, $\eta p^2 = .737$), showing that in real stimulation, similar theta relative power was found in both EEG1 and EEG2 conditions, however in sham, theta relative power decreased from EEG1 to EEG2. There was an interaction tDCS x electrode ($F (7, 105) = 44.268$, $p = .001$, $\eta p^2 = .747$), showing that the only electrode that differed between real stimulation and sham was F4, which showed higher theta relative power in sham compared with real stimulation. There was also an interaction time x electrode ($F (7, 105) = 44.088$, $p = .001$, $\eta p^2 = .746$), showing that the only electrode that differed between EEG1 and EEG2 was F4, showing higher theta relative power in EEG1. Lastly, there was a three way interaction tDCS x tDCS time x electrode ($F (7, 105) = 43.884$, $p = .001$, $\eta p^2 = .745$), showing that in real stimulation, all electrodes showed an increase in theta relative power from EEG1 to EEG2, however in sham, all electrodes showed no difference between EEG1 and EEG2 conditions, except for F4 that showed higher theta relative power in EEG1, whereas in EEG2 theta relative power was lower, as in the rest of electrodes.

To investigate group interactions, independent sample t-tests showed no significant differences in theta absolute power between LI and HI (all effects $p > .05$). To investigate tDCS time interactions, paired t-tests showed no significant differences in theta relative power between EEG1 and EEG2. To

investigate tDCS condition interactions, paired t-tests showed no significant differences between real stimulation and sham in EEG1 (all effects $p > .05$). However, in EEG2, theta relative power was higher in real stimulation compared with sham in electrode positions: AF8 ($t(16) = 2.180$, $p = .045$), Fpz ($16) = 2.608$, $p = .019$), FC6 ($16) = 2.397$, $p = .029$) and AF7 ($16) = 3.474$, $p = .003$).

5.4.5.5 Alpha absolute and relative power

Results showed a main effect of electrode ($F(7, 105) = 15.981$, $p = .001$, $\eta p^2 = .516$) in alpha absolute power, indicating that electrodes from highest to lowest alpha absolute power were: Cz, F3, AF7, Fpz, F4 and AF4, AF8 and the lowest was FC6. There were no main effects of tDCS or tDCS time, nor interactions (all effects $p > .05$).

In relative power, results showed a main effect of tDCS ($F(1, 15) = 5.066$, $p = .040$, $\eta p^2 = .252$), indicating that real stimulation was associated with higher alpha relative power compared to sham. There was also a main effect of electrode ($F(7, 105) = 19.926$, $p = .001$, $\eta p^2 = .571$), showing that electrodes from highest to lowest alpha relative power were: Cz, F4, AF4, F3, FC6, Fpz, AF7 and AF8. However there was no main effect of tDCS time ($p > .05$). There was also a four way interaction tDCS x tDCS time x electrode x group ($F(7, 105) = 3.801$ $p = .009$, $\eta p^2 = .202$), showing that in real stimulation, alpha relative power decreased from EEG1 to EEG2 in all electrodes, and in greater amount for LI compared with HI. However, in sham, alpha relative power increased from EEG1 to EEG2, and the increase was greater in HI than in LI. In both tDCS conditions LI had higher relative alpha power than HI.

To investigate group interactions, independent sample t-tests revealed that LI had significantly higher relative alpha power than HI in real stimulation EEG1 in electrodes positions Fpz ($t(15) = 2.261$, $p = .039$) and AF7 ($t(15) = 2.324$, $p = .035$), as well as in EEG2, in electrodes AF8 ($t(15) = 3.108$, $p = .007$), AF4 ($t(15) = 2.636$, $p = .019$), Fpz ($t(15) = 2.257$, $p = .039$) and AF7 ($t(15) = 2.630$, $p = .019$). In sham condition, LI had higher alpha relative power compared with HI in EEG1 in electrodes Cz ($t(15) = 3.170$, $p = .006$), AF8 ($t(15) = 2.406$, $p = .029$), AF4 ($t(15) = 2.638$, $p = .019$), Fpz ($t(15) = 2.576$, $p = .021$), F4 ($t(15) = 2.859$, $p = .012$), F3 ($t(15) = 2.653$, $p = .018$) and AF7 ($t(15) = 2.382$, $p = .031$), whereas no differences were found in EEG2 (all effects $p > .05$). To investigate tDCS time interactions, paired t-tests showed no significant differences between EEG1 and EEG2. To investigate tDCS condition interactions, paired t-tests showed that alpha relative power was

significantly higher in real stimulation compared with sham in EEG1, in electrode positions: Cz (16) = 2.384, p = .030), AF4 (16) = 2.168, p = .046), Fpz (16) = 2.179, p = .045), F4 (16) = 2.388, p = .030) and FC6 (16) = 2.502, p = .024). However, in EEG2 there were no significant differences between real stimulation and sham (all effects p > .05).

5.4.5.6 Beta absolute and relative power

There were no main effect of tDCS, tDCS time or electrode (all effects p > .05). However results revealed an interaction tDCS x group (F (1, 15) = 16.518, p =.005, ηp^2 =.412) in beta absolute power showing that LI had higher beta absolute power in real stimulation compared with sham. In contrast, HI had higher beta absolute power in sham compared with real stimulation. The largest difference between the groups was found in sham condition, where HI had higher beta absolute power than LI, but in real stimulation, the levels were higher in LI, but with a smaller difference between the groups. Beta relative power analysis revealed a main effect of electrode, indicating that electrodes from highest to lowest beta relative power were: FC6, F3, F4, AF8, AF4, Cz, AF7 and Fpz. There were no main effects of tDCS, tDCS time or interactions (all effects p > .05).

To investigate group interactions, independent samples t-tests showed no significant differences between LI and HI in beta absolute power. To investigate interactions with tDCS condition, paired samples t-tests showed no significant differences in beta absolute power between real stimulation and sham (all effects p > .05).

5.4.6 Correlations

Correlations between questionnaires and physiological measures during task performance with gambling severity and physiological measures in the baseline are summarised in Table 5-2[17].

[17] Further details are provided in the main text to include different conditions of each variable.

5.4.6.1 Correlations between gambling severity and self-report measures

Gambling severity measured with PGSI correlated positively with UPPS-NU (r = .647, n = 17, p = .005), and with VAS in both sessions, real stimulation (r = .796, n = 17, p = .001), and sham (r = .521, n = 17, p = .032).

5.4.6.2 Correlations between gambling severity and electrodermal activity measures

During CGT performance, gambling severity (PGSI) correlated positively in real stimulation with: SCL in CGT ascending condition (r = .585, n = 17, p = .014); with SCL in CGT ascending highest risk condition (r = .536, n = 17, p = .027); with SCRs in CGT wins (r = .617, n = 17, p = .008) and losses (r = .616, n = 17, p = .009) in ascending condition; with SCL in CGT descending condition (r = .483, n = 17, p = .049); with SCL in risk condition 8:2 (r = .503, n = 17, p = .040); with SCL in risk condition 7:3 (r = .492, n = 17, p = .045); and with SCRs during wins (r = .551, n = 17, p = .022) and losses (r = .508, n = 17, p = .038). In sham, PGSI correlated positively with: SCL during CGT ascending condition (r = .823, n = 17, p = .001); with SCL in CGT risk condition 9:1 (r = .755, n = 17, p = .001); with SCL in risk condition 8:2 (r = .733, n = 17, p = .001); with SCL in CGT risk condition 7:3 (r = .862, n = 17, p = .001); and with SCL in risk condition 6:4 (r = .631, n = 17, p = .007); with SCRs during wins (r = .799, n = 17, p = .001) and losses (r = .797, n = 17, p = .001); with SCL CGT descending condition (r = .771, n = 17, p = .001); with SCL in risk condition 9:1 (r = .712, n = 17, p = .001); with SCL in risk condition 8:2 (r = .703, n = 17, p = .002); with SCL in risk condition 7:3 (r = .796, n = 17, p = .001); and with SCL in risk condition 6:4 (r = .632, n = 17, p = .006); and also with SCRs during wins (r = .696, n = 17, p = .002) and losses (r = .738, n = 17, p = .001).

During IST performance, PGSI correlated positively with real stimulation SCRs during wins in fixed condition (r = .528, n = 17, p = .030). In sham, PGSI correlated positively with: SCL in IST fixed condition (r = .533, n = 17, p = .028); with SCRs during decreased condition in wins (r = .682, n = 17, p = .003) and losses (r = .531, n = 17, p = .028); with SCL in decreased condition (r = .581, n = 17, p = .015); and with SCRs during wins (r = .686, n = 17, p = .002) and losses (r = .504, n = 17, p = .039).

During SST performance, PGSI correlated positively with anticipatory SCRs in losses (r = .503, n = 17, p = .039) in real stimulation, and in sham with SCL during SST (r = .568, n = 17, p = .017); with feedback SCRs during wins (r = .491, n = 17, p = .046), and with anticipatory SCRs in losses (r = .517, n = 17, p = .034).

Furthermore, baseline EDA recordings were used to assess the relationship between skin conductance at rest and gambling-related behavioural and physiological measures during the experimental session. Pearson´s correlation analysis showed that baseline EDA in the session of real stimulation correlated positively with variables in the same session: ASRS total (r = .732, n = 17, p = .001), with EDA during EEG1 (r = .896, n = 17, p = .001), and EEG2 (r = .880, n = 17, p = .001), with maximum HR during the baseline (r = .675, n = 17, p = .003), with mean HR during CGT ascending condition wins (r = .852, n = 17, p = .001) and losses (r = .853, n = 17, p = .001), and with descending condition wins (r = .875, n = 17, p = .001), and losses (r = .881, n = 17, p = .001), as well as with mean HR during IST fixed condition wins (r = .801, n = 17, p = .001) but not losses, and with decreased condition wins (r = .801, n = 17, p = .001) and losses (r = .818, n = 17, p = .001), and also with mean HR during SST wins (r = .792, n = 17, p = .001) and losses (r = .705, n = 17, p = .002). Lastly, baseline EDA correlated negatively with EEG1 delta absolute power in electrode positions Cz (r = .535, n = 17, p = .027), AF4 (r = .595, n = 17, p = .012), Fpz (r = .543, n = 17, p = .024), F4 (r = .562, n = 17, p = .019), and F3 (r = .646, n = 17, p = .005), as well as with delta relative power in electrode positions AF8 (r = .604, n = 17, p = .010), Fpz (r = .646, n = 17, p = .005), F4 (r = .604, n = 17, p = .010), F3 (r = .654, n = 17, p = .004), and AF7 (r = .599, n = 17, p = .011). Baseline EDA correlated positively also with EEG2 delta absolute power in AF8 (r = .522, n = 17, p = .032) and with relative power in AF8 (r = .558, n = 17, p = .020) and F3 (r = .499, n = 17, p = .041). However, there were no significant correlations between baseline EDA in sham session and other variables except for EDA during EEG1a (r = .718, n = 17, p = .001), and EEG2a (r = .659, n = 17, p = .004).

In addition, baseline EDA in the session of real stimulation correlated positively with variables in the session of sham: EDA during EEG1 (r = .507, n = 17, p = .038), and EEG2 (r = .727, n = 17, p = .001), with mean HR during baseline (r = .486, n = 17, p = .048), with mean HR during CGT ascending condition wins (r = .712, n = 17, p = .001) and losses (r = .749, n = 17, p = .001), and descending condition wins (r = .737, n = 17, p = .001) and losses (r = .770, n = 17, p = .001), as well as with mean HR during IST fixed condition wins (r = .687, n = 17, p = .002), but not losses, and with decreased condition wins (r = .743, n = 17, p = .001), and losses (r = .653, n = 17, p = .004), and also with mean

HR during SST wins (r = .679, n = 17, p = .003) and losses (r = .698, n = 17, p = .002), and with mean HR during EEG2 (r = .604, n = 17, p = .010), but there were no significant correlations with EEG in sham.

5.4.6.3 Correlations between gambling severity and electrocardiogram measures

Gambling severity correlated negatively with maximum HR during CGT ascending condition (r = -.373, n = 17, p = .003), and positively with maximum HR during wins (r = . 651, n = 17, p = .005) and losses (r = .630, n = 17, p = .007), and with CGT descending condition (r = .664, n = 17, p = .004) and descending 9:1 (r = .638, n = 17, p = .006). In addition, gambling severity correlated negatively with minimum HR during EEG1; r = -.497, n = 17, p = .042), and in IST during fixed condition losses with mean HR (r = -.557, n = 17, p = .020), minimum HR (r = -.575, n = 17, p = .016) and maximum HR (r = -.541, n = 17, p = .025).

Furthermore, baseline ECG recordings were used to assess the relationship between HRV measures at rest and gambling-related behavioural and physiological measures during the experimental session. Pearson´s correlation analysis showed that baseline ECG in the session of real stimulation correlated with variables in the same session: UPPS-P total score correlated positively with minimum HR (r = .551, n = 17, p = .022) and LF/HF (r = .674, n = 17, p = .003). ASRS symptomatology correlated positively with LF power (r = .702, n = 17, p = .002) and LF/HF ratio (r = .638, n = 17, p = .006), and negatively with HF power (r = -.734, n = 17, p = .001). Delay aversion correlated positively with LF/HF ratio in both sessions, real stimulation (r = .552, n = 17, p = .022) and sham (r = .510, n = 17, p = .037). Maximum HR correlated negatively with SCRs during CGT ascending wins (r = -.554, n = 17, p = .021) and losses (r = -.507, n = 17, p = .038) and descending wins (r = -.537, n = 17, p = .026) but not in losses. Baseline HR variables correlated positively with HR during CGT wins (r = .853, n = 17, p = .001) and losses (r = .852, n = 17, p = .001), IST wins (r = .794, n = 17, p = .001) and losses (r = .591, n = 17, p = .012) and SST wins (r = .844, n = 17, p = .001) and losses (r = .844, n = 17, p = .001), and also with HR during EEG1 (r = .813, n = 17, p = .001) and EEG2 (r = .656, n = 17, p = .004). In addition, minimum HR correlated negatively with beta absolute power in Cz EEG1 (r = -.484, n = 17, p = .049), and EEG2 (r = -.495, n = 17, p = .043), and beta relative power in Cz EEG1 (r = -.611, n = 17, p = .009), and EEG2 (r = -.560, n = 17, p = .019), and delta absolute power in Fpz EEG1 (r = -.575, n = 17, p = .016). Minimum HR correlated positively with delta relative power in EEG1 in Cz (r = .491, n = 17, p = .045), and in FC6 (r = .507, n = 17, p = .038), and in EEG2 delta

relative power in AF4 (r = .554, n = 17, p = .021). LF power correlated negatively with EEG1 beta absolute power in AF8 (r = -.561, n = 17, p = .019), beta relative power in AF8 (r = -.618, n = 17, p = .008), in Fpz (r = -.557, n = 17, p = .020), and in beta relative power in EEG2 AF7 (r = -.500, n = 17, p = .041). LF power correlated positively with theta/beta ratio in AF8 (r = .527, n = 17, p = .030), Fpz (r = .501, n = 17, p = .041), AF7 (r = .546, n = 17, p = .023), and in EEG2 delta relative power in AF7 (r = .508, n = 17, p = .037). HF power correlated positively with beta absolute power in AF8 (r = .537, n = 17, p = .026), with beta relative power in AF8 (r = .586, n = 17, p = .014), and in Fpz (r = -.518, n = 17, p = .033), and negatively with theta/beta ratio in AF8 (r = -.511, n = 17, p = .036), Fpz (r = -.488, n = 17, p = .047), AF7 (r = -.525, n = 17, p = .030), and in with delta relative power in EEG2 AF7 (r = -.529, n = 17, p = .029), and beta relative power in AF7 (r = .499, n = 17, p = .041).

Lastly, baseline ECG variables in the real stimulation session correlated with baseline ECG variables in the sham session: variables that correlated positively between both sessions included total power (r = .652, n = 17, p = .005), maximum HR (r = .550, n = 17, p = .022) and minimum HR (r = .647, n = 17, p = .005). In addition, total power correlated negatively with LF/HF ratio (r = -.498, n = 17, p = .042), maximum HR correlated with LF power (r = .544, n = 17, p = .024) and mean HR (r = .520, n = 17, p = .033), and negatively with HF power (r = -.545, n = 17, p = .024).

5.4.6.4 Correlations between gambling severity and electroencephalogram measures

Gambling severity in real stimulation EEG1, correlated negatively with beta absolute power in electrode AF8 (r = -.525 n = 17, p = .030), and with alpha relative power in electrode positions: AF4 (r = -.500, n = 17, p = .041), Fpz (r = -.533, n = 17, p = .028), F4 (r = -.525, n = 17, p = .030), FC6 (r = -.549, n = 17, p = .023), F3 (r = -.492, n = 17, p = .045) and AF7 (r = -.486, n = 17, p = .048), as well as with theta/beta ratio in electrode positions AF4 (r = -.546, n = 17, p = .023), F4 (r = -.595, n = 17, p = .012), FC6 (r = -.565, n = 17, p = .018),), and theta/beta ratio in AF7 (r = -.528, n = 17, p = .029). In EEG2, gambling severity correlated negatively with absolute theta power in AF4 (r = -.512, n = 17, p = .036), and with relative theta power in Fpz (r = -.570, n = 17, p = .017) and F4 (r = -.512, n = 17, p = .035) and AF7 (r = -.499, n = 17, p = .041) as well as with theta/beta ratio in FC6 (r = -.492, n = 17, p = .045), In sham EEG1, gambling severity correlated positively with mean power frequency in F3 (r = .559, n = 17, p = .020) and alpha relative power in F3 (r = .560, n = 17, p = .020). In sham EEG2, gambling severity correlated positively with mean power frequency in F3 (r = .701, n = 17, p = .002), absolute beta power in F3 (r = .641, n = 17, p = .006) and relative beta power in F3

(r = .672, n = 17, p = .003), and negatively with theta absolute power in electrode positions Cz (r = -.483, n = 17, p = .050), AF4 (r = -.569, n = 17, p = .017), Fpz (r = -.500, n = 17, p = .041), F4 (r = -.506, n = 17, p = .038) F3 (r = -.522, n = 17, p = .032) and AF7 (r = -.518, n = 17, p = .033), and with delta relative power in F3 (r = -.494, n = 17, p = .044), theta relative power in F3 (r = -.491, n = 17, p = .045),and also with theta/beta ratio in FC6 (r = -.505, n = 17, p = .039), and F3 (r = .626, n = 17, p = .007).

Table 5-2. *Summary of correlations between questionnaires and physiological measures during task performance with gambling severity and physiological measures in the baseline, in Experiment IV.*

	PGSI		Baseline EDA		Baseline HR		Baseline HRV	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
UPPS-P NU	.647	.005 *	.178	.493	.110	.675	.425	.089
UPPS-P total	.372	.142	.199	.445	.551	.022 *	.674	.003 *
VAS	.796	.001 **	.092	.727	.183	.483	.013	.961
ASRS	.092	.724	.732	.001 **	.308	.229	.638	.006 *
AUDIT	-.260	.314	.271	.293	.382	.131	-.044	.866
SCRs in CGT wins	.617	.008 *	.037	.887	-.554	.021 *	.336	.187
SCRs in CGT losses	.616	.009 *	-.099	.705	-.507	.038 *	-.052	.844
SCRs in IST wins	.682	.003 *	-.075	.775	.424	.090	.384	.128
SCRs in IST losses	.531	.028 *	.039	.882	.302	.239	.244	.345
SCRs in SST wins	.491	.046 *	.311	.225	.143	.585	.336	.187
SCRs in SST losses	.517	.034 *	.236	.363	.273	.289	.058	.825
SCL in baseline	-.031	.905	-	-	.675	.003 *	.339	.183
SCL in EEG1	-.277	.380	.896	.001 **	.724	.001 **	.283	.271
SCL in EEG2	-.268	.298	.880	.001 **	731	.001 **	.106	.687
HR in baseline	.274	.288	.675	.003 *	-	-	-	-
HR in EEG1	-.497	.042 *	.829	.001 **	.813	.001 **	.279	.278
HR in EEG2	-.020	.941	.180	.490	.656	.004 *	.514	.035 *
HR in CGT wins	.651	.005 *	.852	.001 **	.853	.001 **	.303	.236
HR in CGT losses	.630	.007 *	.881	.001 **	.852	.001 **	.288	.262
HR in IST wins	-.069	.792	.801	.001 **	.794	.001 **	.251	.331
HR in IST losses	-.557	.020 *	.818	.001 **	.591	.012 *	.311	.224
HR in SST wins	.090	.732	.792	.001 **	.844	.001 **	.339	.183
HR in SST losses	.016	.950	.705	.002 *	.844	.001 **	.313	.222
EEG delta power	.120	.647	.562	.019 *	-.575	.016 *	-.529	.029 *
EEG theta power	-.512	.036 *	.083	.752	.222	.392	-.149	.569
EEG alpha power	-.533	.028 *	.130	.620	-.168	.519	-.262	.310
EEG beta power	-.525	.030 *	-.041	.875	-.484	.049 *	-.557	.020 *
EEG theta/beta ratio	-.546	.023 *	-.073	.780	-.241	.352	-.488	.047 *

Note: UPPS-P NU, negative urgency trait of urgency, premeditation, perseverance and sensation seeking scale; VAS, visual analogue scale; ASRS, attention deficit hyperactivity disorder self-report scale; CGT, Cambridge gambling task; IST, information sampling task; SST, stop signal task; SCL, skin conductance level; SCRs, skin conductance responses; EDA, electrodermal activity; HR, heart rate; EEG; electroencephalogram; PGSI, problem gambling severity index; HRV, heart rate variability (* $p \leq .005$; ** $p \leq .001$).

5.5 Discussion

In this experiment, the assessment of physiological characteristics of participants with different impulsivity levels during gambling-related task performance was used to investigate the effects of tDCS over the rDLPFC. The PFC modulates subcortical pathways that regulate the sympathetic and parasympathetic nervous systems. The sympathetic nervous system is linked to increased LF HRV, EDA and to cortical hyperactivity of slow waves (delta and theta), whereas the parasympathetic nervous system plays a role in inhibiting these physiological activations. In particular, it was investigated whether participants with higher impulsivity levels would show higher sympathetic activity compared with lower impulsive participants, and whether tDCS could help shifting the ANS towards parasympathetic dominance. Sympathetic dominance would indicate a state of stress and higher arousal, whereas parasympathetic dominance would be associated with relaxation.

Participants attended two tDCS sessions (real stimulation and sham) one week apart, in a counterbalanced order. Groups of participants were divided by UPPS-P NU scores into LI and HI groups. Assessment included self-report measures (PGSI, VAS), CANTAB cognitive tasks (CGT, IST and SST) as well as EDA, ECG and EEG resting state physiological measures. Alcohol dependence was assessed and controlled for with the AUDIT; ADHD symptoms with ASRS; and the blinding procedure with the events sham questionnaire. Overall, there were tDCS effects on cognitive task performance in all tasks, however post-hoc comparisons revealed that none of the interactions with tDCS conditions reached significance, nor did the interaction effects between LI and HI groups. Nevertheless, physiological data helped to demonstrate the effects of tDCS at rest and during gambling-task performance. EDA and EEG showed tDCS effects within session, comparing variables during pre-tDCS and post-tDCS periods, and in addition, all physiological measures (ECG, EEG, EDA) showed specific physiological characteristics associated with each participant group (LI and HI) at rest, and during gambling task performance.

5.5.1 Self-reported measures and CANTAB cognitive task performance

Results showed no differences in demographic and participant characteristics between LI and HI groups, including gambling severity measured with PGSI. The blinding procedure was assessed with the events sham questionnaire, showing that all participants were aware of the real stimulation condition and 65% of participants were aware of the sham condition, which means that beyond the effects of tDCS on physiological substrates, results might have been influenced psychologically

through the arousal created by the sensations during stimulation, or by participants expectations (Rabipour et al., 2018). No adverse events were reported.

Regarding CANTAB tasks performance, to study all phases of the experiment in regards to the stimulation it has to be taken into account that only IST, SST are performed during tDCS, and only EEG2 after the stimulation, therefore, comparisons of the other tasks between real stimulation and sham have to be carefully interpreted. Results are reported from each phase according to the session type, even though some parts are performed before the stimulation, to not discard the potential cumulative effect of the stimulation across sessions (see section 5.4.2 of this chapter). There is some controversy between studies that assume no carry-over effects with sessions conducted one week apart (Shahbabaie et al., 2014; Wu et al., 2020) to compare stimulation and sham conditions, however other studies suggest that crossover designs might not be free of carry-over effects (Spagnolo et al., 2020; Zucchella et al., 2020). In any case, tDCS session order effects were not significant for any of the physiological measures, so if there is an effect of tDCS in phases that have been performed before the stimulation, beyond the potential accumulative effect of tDCS if real stimulation has been conducted in the first session, it could also be interpreted as a session effect regardless of the stimulation type, instead of a tDCS effect.

The tasks IST and SST were performed during tDCS, however CGT was used as a priming task before tDCS, and therefore results indicating differences between real stimulation and sham conditions in this task, have been interpreted as a session effect, rather than a tDCS effect[18]. There were significant main effects of tDCS in all tasks, showing that the real stimulation session was associated with lower RT in CGT, higher total correct trials, but lower P (correct) in IST, and lower total correct stop trials in SST, compared with sham. In addition, there were also interaction effects of tDCS in all tasks, however pairwise comparisons revealed that none of the differences between real stimulation and sham conditions reached significance, nor differences found in the interaction effects between LI and HI groups.

[18] Although the CGT is performed before tDCS, and therefore potential effects of tDCS have been interpreted as a session effect. Nevertheless, it was decided to use the same terminology as with the other variables to refer to the factor analysed (tDCS condition, rather than session), for simplicity, and to consider as much information as possible that could, directly or indirectly be related to each session.

As mentioned in CGT, the tDCS condition factor has been interpreted as a session effect. Beyond tDCS effects, there was also a main effect of condition. The fact that QDM was lower in the ascending condition could be due to the descending condition being performed after the ascending condition, and participants having more practice in the task, however previous studies found no effects on condition order in the CGT (Lawrence et al., 2009). QDM was lower in higher risk conditions as it was expected, but there were no significant differences in task performance between groups, in contrast with previous experiments conducted in the project, and previous research showing that GD participants showed higher RT than non GD participants in the CGT, and that decision-making impairments were associated with increased urgency trait of impulsivity (Kräplin et al., 2014). However, differences in results might be due to discrepancies in the type of participant samples between studies.

The tDCS results obtained in the IST indicating an increase in total correct trials in real stimulation compared with sham was consistent with the hypothesis, however there was also a decrease of P (correct), and a lack of difference in number of boxes opened between tDCS conditions, contrary to the hypothesised. It is possible that the increase of number of total correct trials was not an effect of tDCS, given that the results on the other two variables did not support the effects on total correct trials: the number of boxes opened did not differ between tDCS conditions, and the fact that the P (correct) was lower in real stimulation (which relates to the level of uncertainty tolerated during decision-making), could be a result of the participant guessing the majority of the colour on the IST by chance, but not choosing the most advantageous option. Nevertheless, a recent systematic review suggested that while tDCS seems to modulate response inhibition, RT and cravings, results on impulsivity are not consistent, which would be in line with the lack of tDCS results found in reflection impulsivity measured with the IST in this experiment (Mayer et al., 2020).

In addition, real stimulation was associated with lower total correct stop trials than sham in the SST. This result indicates that participants were less able to inhibit their responses under real stimulation. This might be similar to the effect found in previous experiments of the project, showing higher RT associated with real stimulation compared with sham. It was hypothesised that tDCS would help improve inhibitory control, based on previous studies showing that anodal tDCS improved response inhibition in the SST (Jacobson et al., 2011), however the results found are consistent with other studies that showed no tDCS-related effects on impulse control with the SST (Ouellet et al., 2015).

5.5.2 Electrodermal activity

EDA was measured in different situations: SCL was measured during rest periods and task performance; SCL was measured at rest before and after the tDCS; local SCL was measured in different task-related risk conditions; and SCRs associated with reward outcomes (wins and losses) were measured during task performance. Results showed that SCL was lowest in rest phases, followed by IST and SST, and was highest during CGT. Changes in SCL reflect general changes in autonomic arousal, therefore it is reasonable that rest periods present the lowest SCL. Considering the cognitive tasks, the IST is the task that has the lowest work-load in terms of the inter-stimulus interval (ISI), which is around 30-45 seconds, compared with ISIs of around 10-15 seconds in the SST and CGT. And beyond ISI influence, SST requires more activation from the participant to react quickly and to try to inhibit a prominent response, and CGT is the task that resembles more a gambling activity, with the descending condition requiring the participant to select quickly the highest bet, or otherwise wait for the lowest bet to appear on the screen. Therefore, this could explain why the CGT descending condition was associated with the highest SCL in this experiment.

These results are consistent with the changes that would be expected in SCL, and importantly, help support the rational for using CGT to be performed before tDCS, as a priming task to influence the brain state, with the aim to facilitate the potential stimulation effects of tDCS on gambling-related cognitive functions. In addition, there was an interaction tDCS x period, which pairwise comparisons showed a significant difference between real stimulation and sham in the IST fixed condition, indicating that real stimulation was associated with higher SCL than sham. This result might be explained by the fact that the IST fixed condition was the first task and condition that participants performed during tDCS, so it could be that the initial sensations from real stimulation affected participants arousal, resulting in increased SCL compared with sham.

Analysis of SCL before and after tDCS (EEG1 and EEG2, respectively) showed no significant differences in SCL between EEG1 and EEG2. However, EEG recordings were subdivided in two halves to investigate whether tDCS effects would be more detectable during the first parts of the recordings after the stimulation. As predicted, dividing the SCL data recorded during the EEG section into two parts (see section 5.3.5.3), allowed to identify significant effects that were not detectable during the EEG period as a whole. Significant effects were found in the first part of the EEG (a). SCL was lower in EEG1a compared with EEG2a in real stimulation, but not in EEG1b, EEG2b, or any of the EEG subdivisions in sham. This demonstrates the effects of anodal tDCS on SCL, and therefore

on the sympathetic branch of the ANS. In this case, real stimulation was associated with increased sympathetic activity reflected by higher SCL, contrary to the hypothesis, nevertheless, other research also found that tDCS increased SCRs (Feeser et al., 2014). However, in the current study, the blinding procedure was not effective, and participants in the real stimulation condition were able identify the electrical current sensations. This could have had a direct influence on the increase of sympathetic activation in this condition, and therefore the changes of arousal might not be directly related to the effects of tDCS on cortical excitability.

In addition, SCL was higher in HI compared with LI in both real stimulation and sham. The differences between groups seemed to be more consistent in sham than in real stimulation. In real stimulation, there were significant differences between the groups only in pre-tDCS, in EEG1a, (but not in EEG2a) or only in post-tDCS, in EEG2 and EEG2b (but not in EEG1 or EEG1b). However, in sham, differences were found in both pre-tDCS and post-tDCS subdivisions (in EEG1 and EEG2, and in EEG1b and EEG2b). This might indicate that real stimulation affected in a different way both impulsive groups, in comparison with sham. Furthermore, results of local skin conductance associated with different risk conditions in the CGT, showed also that LI had lower SCL than HI, in the highest risk condition (6:4) in both ascending and descending conditions of the task. These results are not consistent with the hypothesis, and with previous research that showed decreased SCRs to reward in GD compared with healthy controls (Lole et al., 2014). In addition, results also indictaed that participants in high risk-taking groups showed decreased SCRs compared with lower risk-taking groups (Agren et al., 2019), and that risk-taking was associated negatively with SCRs in high impulsive participants (Hüpen, Habel, Schneider, Kable, & Wagels, 2019). Nonetheless, there are no enough studies investigating differences in SCRs and SCL between different types of gamblers, and therefore these results warrant further exploration.

Results from SCRs during reward outcomes revealed that SCRs were higher in wins than in losses in the CGT. This is consistent with the hypothesis, and with previous studies with healthy participants, showing that wins produced increases of SCL, but losses did not elicit physiological changes (Wilkes, Gonsalvez, & Blaszczynski, 2010). In the IST, there were no differences between wins and losses, consistent also with other research measuring SCRs during gambling task performance (Agren et al., 2019). In the SST, SCRs were higher in losses than in wins. In this case, wins refer to successful stops to inhibit a response, whereas losses refer to unsuccessful stops to inhibit a response, however for simplicity it was used the same term across tasks (wins and losses). SST performance involves

inhibition mechanisms different to the reward processing associated with the CGT or IST. Therefore, results are consistent with research that found increased brain activation in medial PFC with SCRs following unsuccessful stops in SST, but decreased activation following a stop success (Zhang et al., 2012).

In addition, results showed that SCRs were higher in feedback responses than in anticipatory responses in wins and losses in CGT, IST and SST, and higher anticipatory SCRs were associated with wins than with losses in CGT. On the contrary, previous research showed that the anticipation phase (when participants wait to receive the outcome) was associated with higher arousal response than the outcome phase, suggesting that anticipation is a major contributor during gambling (Agren et al., 2019). In this study, feedback responses produced increased arousal levels, however, it is possible that the manual method employed to log in the stimuli on the computer causing some delays, as specified in section 5.3.5 of this chapter, would have affected the measurements and results, making it more difficult to compare consistently with other studies.

Considering the groups, HI had higher feedback SCRs in wins than LI, and higher anticipatory and feedback SCRs associated with losses compared with LI in the CGT. Therefore, HI showed increased SCRs compared with LI, except in feedback SCRs losses, in which groups did not differ. This is in line with a study that showed no differences between GD participants and healthy participants in losses, but different SCRs in wins, with the difference that in that case, lower SCRs associated with wins was linked with GD participants (Lole et al., 2014). In IST, HI had higher anticipatory and feedback SCRs than LI in wins and losses. In none of the three tasks there were significant differences in SCRs associated with wins and losses between both sessions, which makes sense in the case of CGT, given that tDCS was delivered after CGT performance. However, this is contrary to the hypothesis in the case of IST and SST, as in these tasks it was expected to find lower SCL in real stimulation compared with sham, based on previous research (Wang et al., 2016).

5.5.3 Electrocardiogram

Results comparing at rest and task periods, showed that the lowest total power, which reflects overall ANS activity, was found in the baseline, and the highest in IST. No other significant results were found on HF or LF components of HRV. There were no significant differences between pre/post tDCS periods in HRV measures. During task performance, the lowest total power was found in CGT, and

the highest total power in IST. The fact that HRV total power was lowest during CGT (indicating lower activation of inhibitory processes), coincides with the higher SCL effect (see above) indicative of sympathetic predominance, and therefore supporting the function of the task as a priming mechanism to activate the brain network before tDCS.

There were no significant tDCS effects in HF or LF components between real stimulation and sham. This is not consistent with the hypothesis, and research showing that real stimulation tDCS increased HF compared with sham, including a task-related reduction of HF (Nikolin et al., 2017). In addition, considering participant groups, results showed that LI had lower LF and higher HF relative power than HI during CGT, IST and SST performance. This is consistent with the hypothesis, and with research showing that higher impulsive individuals had lower HRV, independently of the presence of GD (Maniaci, Goudriaan, Cannizzaro, & van Holst, 2018).

HR during task performance periods, results showed no differences between tasks in mean HR or maximum HR, however minimum HR was highest in CGT descending condition. This is in line with the results obtained in SCL, in which CGT descending condition had the highest SCL (indicative of sympathetic activation), supporting the choice of this task as a priming mechanism to activate the gambling-related brain network before tDCS. During the different risk conditions of the CGT, there were no significant effects of HR. This is not consistent with the hypothesis, however, it is possible that the actual risk conditions in the CGT did not provoke a physiological response, as would happen in other types of tasks, in which participants could choose between more risky and safer options in the same trial, like in the Iowa gambling task (IGT). Previous research showed that IGT performance correlated with LF HRV (Drucaroff et al., 2011), and that anticipatory HR was lower in disadvantageous relative to advantageous options in the IGT (Crone, Somsen, Beek, & Der, 2004).

There were no differences in HR during wins and losses in the CGT, contrary to the hypothesis. However considering that across tasks, minimum HR was highest during CGT, it is possible that there was a smaller range for variation of HR in this task. This could explain the lack of differences detected in HR during reward outcomes, which would be supported by the fact that total power HRV was lowest in CGT too. In IST, mean HR and maximum HR were higher in wins than in losses, and maximum HR and minimum HR were higher in decreased condition than in fixed condition. There were no significant effects of tDCS on HR, contrary to the hypothesis based on changes on HRV

measures and tDCS effects over ANS (Nikolin et al., 2017), but in line with previous research showing no differences of anodal or cathodal tDCS on HR (Vitor-Costa, Okuno, & Bortolotti, 2015). Regarding to the participant groups, HI had higher mean and minimum HR in wins than LI in IST, and HI had higher minimum HR in wins (successful stops) and losses (unsuccessful stops) than LI in SST. These results are supported by research showing that lower HR response to disadvantageous options compared to advantageous options was seen only in participants who were classified as good performers on the IGT, and lower HR was associated with losses compared with wins (Crone et al., 2004).

5.5.4 Electroencephalogram

Theta/beta ratio was lower in real stimulation compared with sham, with the highest power found in vmPFC, and the lowest in right PFC. This is in line with the hypothesis, and research showing that tDCS over the DLPFC increased beta frequency power (Song et al., 2014) and reduced theta activity over frontal areas (Powell, Boonstra, Martin, Loo, & Breakspear, 2014). Mean frequency power increased from EEG1 to EEG2 in left DLPFC, in the sham condition. In addition, mean frequency power was higher in real stimulation compared with sham in EEG1, but in EEG2 there were no differences in mean frequency power between real stimulation and sham. These results indicate that during tDCS real stimulation, mean frequency power remained stable, whereas in sham, mean frequency power increased from EEG1 to EEG2. Previous research showed a decrease in mean frequency power after tDCS real stimulation and after sham, using a tDCS protocol without cognitive tasks coupling (Boonstra et al., 2016). Considering that in this experiment, IST and SST were performed during tDCS, the possible increase of mean frequency power between EEG1 and EEG2 in sham, and the lack of significant decreases found in real stimulation, could be explained by task-related cognitive effects, which could have augmented mean frequency power (Gill et al., 2015; Newson & Thiagarajan, 2019).

Moreover, real stimulation was associated with lower delta relative power compared with sham, especially in the left PFC. This is in line with the hypothesis, and research that found reduced left frontal delta absolute power after tDCS real stimulation (Keeser et al., 2011). However, post-hoc analysis showed that in real stimulation, delta relative power increased in EEG2 in relation with EEG1, however, in sham, delta relative power decreased from EEG1 to EEG2, and that differences between real stimulation and sham were significant in EEG1, but not in EEG2. This might indicate that real stimulation could have increased delta relative power compared with sham, but participants

initial resting state might have been different between sessions (with lower delta relative power before the tDCS in the session of real stimulation, compared with the sham session). This result is contrary to the hypothesis and previous research that showed reduced delta in left PFC (Keeser et al., 2011), but it would be consistent with research that also showed increased delta power after anodal tDCS (Donaldson, Kirkovski, Yang, Bekkali, & Enticott, 2019).

Furthermore, theta absolute power was highest in vmPFC, being lower in real stimulation compared with sham. Theta absolute power decreased from EEG1 to EEG2, especially in right DLPFC. Post-hoc analysis showed that in pre-tDCS, there were no differences in theta relative power between real stimulation and sham, but in post-tDCS, theta relative power was higher in real stimulation compared with sham. These results might indicate that theta relative power decreased in both real stimulation and sham sessions, however the decrease in sham might have been stronger than in real stimulation, showing larger differences in EEG2. This is consistent with previous research with tDCS that showed a reduction of theta power after real stimulation (Jacobson, Ezra, Berger, & Lavidor, 2012).

Alpha absolute power was higher in central and left PFC. In addition, alpha relative power was higher in real stimulation compared with sham, especially in central and right PFC. In particular, real stimulation was associated with higher relative alpha power in EEG1, whereas in EEG2, there were no differences between real stimulation and sham. This might indicate that real stimulation decreased alpha relative power, whereas in sham alpha relative power could have remained constant or increased from EEG1 to EEG2, with participants having different initial resting states between both sessions (higher alpha relative power in the real stimulation session compared with the sham session).This result is contrary to previous research (Ulam et al., 2015) and the hypothesis that tDCS would increase alpha power. However, other research also found a reduction of alpha in frontal, parietal and temporal regions after tDCS real stimulation (de Melo, de Oliveira, dos Santos Andrade, Fernández-Calvo, & Torro, 2020; Maeoka, Matsuo, Hiyamizu, Morioka, & Ando, 2012).

Lastly, beta relative power was higher in right and left DLPFC, and lowest in vmPFC, and there were no significant differences between real stimulation and sham conditions. This distribution of power is consistent with research indicating that beta power is associated with cognitive effort (Schestatsky, Morales-Quezada, & Fregni, 2013) and that DLPFC is associated with executive functions (Koenigs & Grafman, 2009). However, it was expected to find an increase of beta power after real stimulation

compared with sham, contrary to results found, and previous research in healthy controls (Mangia et al., 2014; Song et al., 2014). Nevertheless, another tDCS study found also effects on delta, theta and alpha but not in beta, consistently with the current results (Ulam et al., 2015).

Regarding participant groups, LI had lower mean frequency power than HI in sham EEG2. In addition, LI showed higher delta relative power than HI in sham EEG2, which perhaps could be interpreted as a higher task performance effect on cognitive activation in LI compared with HI. LI had higher alpha relative power than HI in left and ventral PFC in EEG1, and in right PFC in EEG2, during real stimulation. In sham EEG1, LI had higher alpha power across the whole PFC, whereas no differences between the groups where found in EEG2. These results might indicate that real stimulation over rDLPFC decreased alpha relative power in higher extent in LI compared with HI. There were no significant differences between LI and HI in theta or beta frequencies.

In this experiment, it was hypothesised that tDCS would help decrease EEG slow waves (delta and theta) and theta/beta ratio, associated with low cortical arousal and lower inhibition control, while helping increase fast waves, associated with hyperexcitability of the nervous system (Kim et al., 2018; Lansbergen et al., 2007), and that HI participants would show increased slow wave and decreased fast wave bands compared with LI (Lee et al., 2017b). Results showed that theta/beta ratio activity was higher in vmPFC, and lower in DLPFC areas. Similarly, theta absolute power was higher in vmPFC, whereas beta relative power was higher in DLPFC and lower in vmPFC areas.

These results are consistent with research associating dorsal areas of the PFC with cognitive functions, such as goal-directed behaviour and attention, and ventral PFC with reward, emotions and motivational processing (Koenigs & Grafman, 2009; Miller & Cohen, 2001). In line with the hypothesis, results showed that tDCS real stimulation decreased theta/beta ratio, mean frequency power and theta relative power, and increased alpha relative power compared with sham. Participants initial resting state, before the tDCS procedure, was different between the sessions of real stimulation and sham, therefore, studying the effects of tDCS within each session has provided more accurate information about the actual effects of each condition, rather than comparing only results between sessions. In addition, LI showed lower mean frequency power, and higher delta and alpha relative power than HI, with no differences in beta band between groups.

Differences in EEG power between LI and HI were seen especially in EEG2 periods, suggesting that the stimulation combined with cognitive task performance affected both groups differently. These results add evidence to research investigating the effects of tDCS on gambling-related cognitive mechanisms in gamblers according to their impulsivity levels. However, research results are not homogeneous, with studies showing that high impulsive GD participants had decreased theta, alpha and beta absolute power in left, right and midline PFC (Lee et al., 2017b), and others showing that high impulsive individuals did not show high theta, theta/beta ratio or decreased beta power (Lansbergen et al., 2007). The effects of tDCS on EEG waves and reward processing mechanisms are still not clear. While these results are supported by previous research (as mentioned above), other studies found that tDCS over DLPFC showed an increase of delta, theta and alpha frequencies and a decrease of beta frequencies, resulting on a general slowing of the resting state (Boonstra et al., 2016). The heterogeneity of the results is in part influenced by the variety of outcome measures used, tDCS protocols, and participant characteristics. Further research might help to clarify the specific effects of tDCS on gambling-related behaviour.

5.5.5 Correlations

Gambling severity correlated positively with the impulsivity subscale of negative urgency (NU) and with gambling cravings. In addition, gambling severity correlated positively with EDA, particularly with SCL in CGT, and SCRs associated with wins and losses in CGT, IST, and with anticipatory SCRs in losses and feedback SCRs in wins in SST. Moreover, gambling severity correlated positively with maximum HR during wins and losses in CGT, and negatively with minimum HR during EEG1. Considering that EEG1 was recorded just after CGT performance, the correlations found between gambling severity and HR measures during and just after CGT performance support the results obtained in EDA, showing that the highest SCL across tasks was during CGT, and ECG results showing the highest minimum HR and the lowest HRV total power in CGT. Moreover, gambling severity correlated negatively with HR during IST fixed condition. This could be due to the work load of this task and the associated characteristics of the condition. Since this was the task with longer inter-stimulus interval (see section 5.5.2 of this chapter), and particularly, the fixed condition had not the same incentive of risk as compared with the decreased condition (see section 4.3.2, Chapter 4), it is possible that participants with higher GD severity would lose motivation to play during this task condition, and therefore display a slower HR. Lastly, gambling severity correlated negatively with beta absolute power in right PFC, and alpha relative power, theta absolute, and theta relative power in right, left and ventral PFC, as well as with theta/beta ratio in right and left PFC.

In addition, with the aim to find biological markers that could be used to identify individual variability factors between gamblers, baseline EDA was compared against the rest of the variables. Results revealed that baseline EDA correlated positively with ADHD symptomatology, with mean HR during wins and losses in CGT and SST, and with mean HR during IST wins (but not losses) in fixed condition, and mean HR in wins and losses in decreased condition. Considering that the chances of losing in IST fixed condition are lower compared with the chances of losing in the decreased condition (because participants can open all the boxes to identify the dominant colour without losing points in the fixed condition, but in decreased condition participants would lose points for every box opened), therefore, this might explain the difference in results regarding losses between task conditions in IST.

In addition, baseline EDA correlated negatively with delta absolute and relative power in EEG1, and positively with delta absolute and relative power in EEG2. However, these correlations were found only in one of the sessions (in real stimulation). Nevertheless, baseline EDA in real stimulation correlated positively with EEG periods in sham (but not with baseline EDA in sham), and mean HR during wins and losses in each task in sham, in the same way that it did in the real stimulation session. Therefore, this might indicate that similar mechanisms were present in both sessions during task performance involving EDA and HR during reward outcomes, even though baseline EDA recording in the sham session did not correlate with the variables during the same session. Given that gambling severity correlated with EDA during task performance, and that baseline EDA correlated with HR during reward based task performance in both sessions, EDA might be a useful biological marker that could inform about GD symptoms during situations involving reward.

Furthermore, correlation analysis to assess the relationship between baseline ECG measures and gambling-related behavioural and physiological measures during the experimental session, revealed that UPPS-P total score correlated positively with minimum HR and LF/HF ratio, and that ADHD symptomatology correlated positively with LF power and LF/HF ratio and negatively with HF power. Moreover, DA correlated with LF/HF ratio. These results highlight the link between impulsivity and HRV variables. This is consistent with research showing that impulsivity correlated positively with inter beat interval (Maniaci et al., 2018), and that increased HR and decreased HRV was associated with internet gaming disorder, which has been associated with high impulsivity (Park et al., 2019). In addition, maximum HR correlated negatively with EDA during wins and losses in CGT, whereas minimum HR correlated negatively with beta absolute and relative power, and positively with delta

relative power. LF power correlated negatively with theta/beta ratio and negatively with beta absolute and relative power in right, left and ventral PFC. Similarly, HF power correlated negatively with theta/beta ratio, and positively with beta absolute power in right, left and ventral PFC. Nevertheless, correlation analysis in small sample sizes are vulnerable to type I and type II statistical errors, therefore these results should be interpreted carefully (Knudson & Lindsey, 2014).

Results are overall consistent with the hypothesis, and with research that has found that participants with gambling problems showed increased brain activation associated with wins (Dymond et al., 2014), increased EDA associated with reward outcomes (Lole et al., 2014), with research suggesting that parasympathetic dominance is associated with improved cognitive performance (Nikolin et al., 2017), and with research that showed positive correlations of gambling severity with theta activity (Dymond et al., 2014), and negative correlations between alpha power in frontal and central regions and impulsivity (Lee et al., 2017b).

5.5.6 General conclusions and limitations

In summary, tDCS results on gambling-task performance were not consistent with the hypothesis. However, task performance was used in this experiment not only to assess the cognitive results associated with the tasks, but to inform about physiological states during gambling-related scenarios. Previous research suggested that the combination of behavioural and physiological variables could help reveal results that might not be detected using only behavioural variables (Gomis-Vicent et al., 2019), and that it can be difficult to relate personality traits to the underlying brain function (Grant et al., 2016). EDA results revealed that sympathetic activation was associated with task performance, particularly higher SCL in CGT (which was the task used as a priming mechanism to activate the brain network before tDCS). The effects of tDCS were measurable through SCL at rest, being more evident during the first part of the EEG recording, when comparing the effects within session (pre/post-tDCS periods).

In addition, different reward outcomes were associated with EDA during task performance, with higher EDA in HI compared with LI, and showing higher SCRs associated with wins in CGT and IST compared with losses, and higher SCRs associated with losses, compared with wins, in SST. ECG results did not reveal tDCS effects on HRV variables, however results showed that LI had higher HF and lower LF power, as well as lower HR associated with wins and losses compared with HI

participants. EEG results helped to demonstrate the effects of tDCS, showing that real stimulation was associated with lower theta/beta ratio, theta relative power and mean frequency power, but higher delta and alpha relative power compared with sham. In addition, participant groups showed different EEG wave activation with lower mean frequency power and higher delta relative power in LI compared with HI. Lastly, correlation analysis showed that baseline EDA might be a useful biological marker to inform about gambling-related behavioural and physiological states.

In this experiment, some of the limitations highlighted in the first experiments were addressed. A triple-blinded design (in which neither the participant, nor the researcher when conducting the experiment and the data analysis were aware of the stimulation condition) was used to ensure that data analysis was less likely to be biased. In addition, a computational model was used to create the most effective tDCS montage to target the brain area of interest more accurately. Finally, neurophysiological data, including EEG, EDA and ECG, were used to further quantify the effects of the intervention.

However, other limitations might have influenced the results of this experiment. Firstly, the blinding of the participants during real stimulation was not effective, and this could have influenced the results of the experiment. Future studies would benefit from the use of "ActiSham", an algorithm that creates and an active sham condition, that induces the same sensations than the tDCS real stimulation without affecting cortical excitability, therefore improving participants blinding (Neri et al., 2020). Secondly, participant groups were created by using a median-split of the impulsivity self-report measures which is less than optimal given that GD is a key focus of the PhD. Recruiting a larger sample of participants that allowed groups to be divided by gambling severity measures, would yield results more comparable with other studies about GD. Moreover, there was large number of significant correlations found in this experiment, however correlation analysis in small sample sizes are vulnerable to type I and type II statistical errors (Knudson & Lindsey, 2014), therefore replication of the results are warranted for a more reliable interpretation. Additionally, self-report questionnaires were used to control for ADHD and alcohol dependence, however controlling for other common GD comorbid psychiatric disorders, such as other addictive behaviours, anxiety or depression, might provide a better understanding of the individual characteristics of the sample. Finally, the manual method of logging the physiological data caused a delay that had to be accounted for in the analysis, and therefore the data obtained was not as accurate as it could be, compared to interfacing with a software prepared for experimental testing with the cognitive tasks.

In conclusion, this study broadened understanding of the mechanism of tDCS during gambling task-performance, informing about cognitive and physiological mechanisms associated with tDCS, and with particular characteristics of different groups of gamblers according to self-reported impulsivity levels. Results might help refine knowledge of the effects of tDCS at an individualised level, and contribute to the development of more specific protocols that target components of addiction and gambling disorder.

6 Chapter 6. General discussion

6.1 General overview

The overall aim of this research project was to investigate the potential use of transcranial direct current stimulation (tDCS) as a neuromodulation intervention to improve treatment approaches for gambling disorder (GD), and to inform current understanding of the neurophysiology of decision-making related of gambling behaviours. According to previous literature, results from non-invasive brain stimulation (NIBS) research are highly heterogeneous (Buss, Fried, & Pascual-Leone, 2019; Coles et al., 2018; Zucchella et al., 2020). This is in part due to the variability of methodologies and protocols used, together with participants individual differences that directly impact the responsiveness to NIBS.

There is a lack of consensus on which protocols might be more effective to potentially treat specific conditions. In particular, GD is not an homogeneous disorder (Quintero, 2017), and although impulsivity appears to be a shared characteristic across the majority of gamblers and individuals with GD (Grant et al., 2016), multiple interrelated factors, such as heritability, traumatic background, personality traits and psychiatric comorbidities, contribute to the complexity of finding adequate treatment interventions for each individual (Yau & Potenza, 2015). In order to develop more individualised treatment interventions, the understanding of specific cognitive and neurophysiological characteristics underlying GD needs to be refined, as the knowledge of how neuromodulation interventions act on brain circuits. Specifically, investigating autonomic responses during reward anticipation, decision-making and reward outcomes, might help to capture particular risk-factors associated with GD in more realistic scenarios.

6.2 Summary of results

In Experiment I, high definition (HD) tDCS montages with a 1 x 4 ring configuration were designed to target right dorsolateral prefrontal cortex (rDLPFC) and ventromedial prefrontal cortex (vmPFC), brain areas that have been previously associated with GD. Results showed tDCS effects on Cambridge gambling task (CGT) performance, but no difference between tDCS effects depending on brain target or participant groups split by impulsivity self-reported levels. In Experiment II, the HD montage to target rDLPFC was manipulated to explore whether altering tDCS focality would reveal differences in tDCS effects between both brain regions, and between participants with different impulsivity levels.

Therefore, the same HD tDCS montage designed for vmPFC used in Experiment I was compared against a new montage designed to target rDLPFC. This new montage was created to increase the amount of current traveling through the scalp to the target area, by expanding the distance between the anode and the return electrodes. Results in Experiment II replicated the findings found in the previous experiment, showing tDCS effects on CGT performance, but no difference between the effects depending on brain target or participant impulsive groups. In both experiments participant samples included non-gamblers and at risk gamblers that were grouped according to their impulsivity negative urgency (NU) scores. Results showed that tDCS was associated with higher quality of decision-making (QDM) and risk-taking (RT) behaviour, but did not affect delay aversion (DA).

In Experiment III, the cumulative effects of tDCS, in combination with cognitive behavioural therapy (CBT), were investigated across eight weekly sessions, through two separate case studies conducted at the National Problem Gambling Clinic (NPGC). The experimental design was originally planned as a randomised control trial (RCT), however a necessary change of approach due to recruitment complications, meant that data analysis had to be conducted according to a single participant design. Results indicated that in both patients diagnosed with GD (each allocated to one tDCS condition, real stimulation or sham), gambling severity and cravings were reduced at the end of the intervention. In addition, electrophysiological measures revealed correlations between gambling severity and electroencephalogram (EEG) power. Importantly, however, in the patient receiving tDCS real stimulation, there were further alterations in the experimental procedure due to changes of schedule in the individualised CBT treatment. These changes themselves could have increased the variability in EEG results across sessions, not allowing to establish reliable conclusions. Although tDCS seemed to produce a short term increase of mean frequency power in the majority of sessions, no clear direction of long term effects was found.

The electrophysiological findings in the patient allocated to sham tDCS appeared to have more consistent interpretations. In particular, concurrent SST performance could have explained both, the short and long term changes in EEG power in the period after receiving sham tDCS (that was coupled with SST), whereas cumulative effects of CBT could account for the reduction of mean frequency power, in the period before receiving sham tDCS with SST at the end of the intervention. In addition, in the patient allocated to sham, gambling severity correlated negatively with SST total correct trials and with mean frequency power in left DLPFC. Moreover, theta/beta ratio in left DLPFC correlated positively with cravings. The significant correlations found between these variables are particularly

relevant for neuromodulation research in GD, given that these electrophysiological measures could be used in future research as biomarkers to assess changes in cognitive states, and as targets for neuromodulation studies.

The results from Experiment III are from single cases, with unavoidable differences in testing protocols compared to the originally planned RCT, and so it is not possible to reliably infer the validity of any observed qualitative differences or similarities between the cases. As such, the limitations did not allow direct comparisons between tDCS real stimulation and sham conditions, or to distinguish between the effects of the tDCS protocol, involving cognitive task performance, and the effects of the CBT. In sum, with no qualitative differences in outcomes between the patients allocated to sham and real stimulation, it could be argued that there is no evidence here to support the use of tDCS to improve CBT treatment outcomes, contrary to the hypothesised. However, based on previous studies, cumulative effects of tDCS across multiple sessions might increase when delivered daily (Alonzo et al., 2012; Martinotti et al., 2018), compared with weekly administration (Boggio, 2007)[19]. Further research investigating the combination of tDCS and CBT using a more frequent schedule, might help to clarify the potential use of neuromodulation interventions to improve current treatment approaches for GD.

Experiment IV sought for a deeper understanding of the tDCS effects over the autonomous nervous system (ANS) during different reward phases, in participants with low and high risk gambling behaviour, grouped by their self-reported impulsivity levels. Results demonstrated the capability of tDCS to modulate the ANS, producing an increase of skin conductance level (SCL) after real stimulation compared with sham. Results also revealed tDCS effects on cortical excitability. In particular, tDCS produced an increase of delta power and a decrease of alpha power compared with sham, and a decrease of theta power in both real stimulation and sham, whereas no changes were found in beta power. These results are contrary to the hypothesis, however are supported by previous tDCS research (see section 5.5.4 in Chapter 5), and highlight the existent variability on tDCS results between studies. The results also revealed participant intra-individual variability, showing that pre-tDCS EEG measures were different between sessions. In addition, results suggested that cortical excitability might have changed due to task-related effects. In fact, task related effects on cortical

[19] At the National Problem Gambling Clinic (where the case studies were conducted), the CBT sessions were delivered to patients on a weekly basis. Therefore, it was decided to investigate a weekly neuromodulation protocol to explore the potential effects of coupling tDCS with CBT on treatment outcomes.

excitability might have had higher effects on low impulsive (LI) gamblers compared with high impulsive (HI) gamblers, indicated by an increased delta power in the sham post-tDCS period in LI.

In addition, tDCS seemed to produce a higher decrease in alpha power in LI compared with HI, whereas no differences were found in theta and beta power between the groups. Moreover, inter individual differences during gambling-related task performance were identified through heart rate variability (HRV), heart rate (HR) and skin conductance responses (SCRs). In particular, higher sympathetic activation was found in HI compared with LI, during reward outcome phases compared with anticipation phases, and during wins compared with losses. Lastly, gambling severity correlated positively with cravings, with EDA and HR during task-performance reward outcomes and negatively with EEG measures.

Correlations between baseline EDA and various variables measuring response to reward and cortical excitability might indicate the potential use of this measure as a biomarker to disentangle specific features of GD during different reward phases. To summarise, this experiment showed that autonomic arousal was different between groups of low and high impulsive gamblers, and between different reward phases during gambling-related task performance. The findings suggested that EDA might be a useful biomarker to use in future neuromodulation studies in GD. The results showed certain evidence of the capability of tDCS to modulate the ANS and cortical excitability. However, due to the lack of effectiveness of blinding during real stimulation, these results should be carefully interpreted, given that participants awareness of the stimulation sensations during tDCS could have caused the increase of sympathetic arousal, rather than this being directly affected by the neuromodulation of PFC activity.

6.3 Novel contributions

The majority of tDCS studies have investigated the effects of neuromodulation targeting one brain area, exploring separately decision-making mechanisms related to dorsal PFC areas (Fecteau et al., 2014; Feeser et al., 2014; Ouellet et al., 2015), and reward processing mechanisms related to ventral PFC areas (Abend et al., 2019; Bertossi et al., 2017; Manuel et al., 2019; Vergallito et al., 2018). The evidence about the functional connectivity between these two areas has been widely discussed (Hare et al., 2014; Kahnt, Heinzle, Park, & Haynes, 2011; Koenigs & Grafman, 2009). Furthermore, tDCS over DLPFC has been associated with vmPFC activation (Nakamura-Palacios, 2016). However, it

was not until very recently that research has started to compare directly the effects of tDCS on both brain circuits.

Neuromodulation of DLPFC has been compared with orbitofrontal cortex (Nejati, Salehinejad, & Nitsche, 2018), and ventrolateral PFC (Marques, Morello, & Boggio, 2018). More recently, tDCS effects over DLPFC and vmPFC have been directly compared in a study investigating attentional processing (Martínez-Pérez, Campoy, Palmero, & Fuentes, 2020), and in a study investigating smoking (Fischell, Ross, Deng, Salmeron, & Stein, 2020). The latter, found significant tDCS effects using functional magnetic resonance imaging, showing that anodal tDCS over left DLPFC and cathodal over the right vmPFC, modified the cognitive circuit associated with nicotine withdrawal. They found that tDCS had no significant effects on behavioural measures. However, they revealed that neuromodulation helped to reduce the default mode network (associated with ventral areas of the PFC) that is involved in self-referential thought and rumination, while increasing the salience network (associated with the anterior cingulate cortex (ACC)), which is involved in attention and inhibitory control. To the author´s knowledge no studies seem to have compared DLPFC and vmPFC as tDCS targets regarding gambling-related cognitive processing. Therefore, the current experiments contribute to the field of NIBS in the context of normative gambling behaviour and GD.

Results from Experiments I and II showed that both tDCS montages designed to target rDLPFC and vmPFC modulated decision-making and risk-taking behaviour, but did not show significant differences on tDCS results between brain targets. The significant tDCS effects found on cognitive-task performance, together with the lack of significant differences identified on tDCS effects between both brain areas, raised the need for further research investigating neuromodulation effects over this circuitry in combination with neuroimaging techniques. This might help to disentangle interrelated factors influencing gambling behaviour, such us decision-making, risk-taking, delay aversion, inhibition control and behavioural impulsivity, and help identify specific anatomical targets for future tDCS studies. Nevertheless, to interpret the current results, it has to be considered the lack of data about the tDCS focality and electrical current distribution in the brain in these experiments. Therefore, it is possible that shunting effects produced that the electrical current reached similarly the two brain areas with both tDCS montages, or that reciprocal connections between DLPFC and vmPFC produced similar alterations on top-down regulated cognitive processes (Brevet-Aeby et al., 2016).

A recent systematic review identified only four studies that have investigated the effects of tDCS in GD (Zucchella et al., 2020). None of these have investigated a combination of tDCS with cognitive behavioural therapy (CBT) as a potential intervention to improve current treatment approaches for GD. Currently, CBT shows the strongest evidence base of any therapeutic approaches (Cowlishaw et al., 2012; Menchon et al., 2018), and therefore, investigating the potential improvements of CBT outcomes through its combination with neuromodulation interventions seems a promising approach. Additionally, no studies using tDCS on GD have measured EEG neuro-correlates. In fact, only one study using tDCS in GD included physiological measures (Dickler et al., 2018).

Therefore, Experiment III, which investigated tDCS in combination with CBT using EEG measures in GD, represents a further contribution to novel research. Results showed that GD symptomatology decreased after the intervention with both tDCS real stimulation and sham conditions, in combination with CBT. Short and long term effects associated with the intervention with tDCS were recognised in cortical excitability, and individual biomarkers were identified, showing that EEG mean frequency power correlated negatively with gambling severity and inhibitory control, whereas theta/beta ratio correlated positively with cravings. However, due to the numerous limitations related to the changes of experimental design, the results represent preliminary pilot work from which valid extrapolations cannot easily be made. Nonetheless, there are methodological implications from the study (see section 6.4 of this chapter) which provide insights for future research attempting to conduct clinical trials involving neuromodulation in GD.

Lastly, tDCS capability to modulate autonomic control has not been broadly investigated (Clancy et al., 2014). Research investigating emotional regulation has studied tDCS effects using SCRs and SCL (Allaert, De Raedt, Sanchez-Lopez, Baeken, & Vanderhasselt, 2020; Feeser et al., 2014), showing a reduction of arousal associated with tDCS compared with sham. Internet gaming disorder has also been investigated using rDLPFC tDCS and SCRs, revealing improved arousal regulation after tDCS compared with sham (Wu et al., 2020). However, there are no tDCS studies using EDA in gamblers/gambling research. Similarly, some tDCS studies have investigated the effects of neuromodulation on cardiovascular measures (Beeli, Casutt, Baumgartner, & Jäncke, 2008; Montenegro et al., 2011; Nikolin et al., 2017), however no studies have investigated tDCS effects using HRV and HR measures in relation to gambling. Only one study using repetitive transcranial magnetic stimulation (rTMS) in GD measured also HR (Sauvaget et al., 2018). Lastly, several studies have investigated tDCS effects using EEG measures (Jacobson et al., 2012; Meiron et al., 2018;

Miller, Berger, & Sauseng, 2015; Schestatsky, Morales-Quezada, et al., 2013), however, again, no studies have done so in gamblers.

This research project has investigated tDCS effects on SCL, SCRs, HRV, HR and EEG in gamblers, and furthermore, all measures were obtained in the same experiment. Concurrent measurement of EDA and HR has been suggested to offer deeper understanding of individual variability factors that characterise specific behaviours, because both measures provide different information (Sohn et al., 2001; Studer et al., 2016). Generally, EDA is more sensitive to affective states and HR is more sensitive to the valence of the emotion and cognitive load. In addition, this research complements the information provided by these measures through the investigation of physiological responses during different gambling phases (anticipation of reward outcome and reward outcome receipt), and to wins and losses, during gambling-related task performance. The majority of research has investigated tonic SCL in gambling, however phasic SCRs provide more detailed information about the underlying mechanisms of gambling behaviour (Agren et al., 2019).

In the current study, SCL results demonstrated the capability of tDCS to modulate ANS, showing an increase of sympathetic activity. Furthermore, sympathetic activation was higher during reward feedback phases compared with anticipatory phases, and during wins compared with losses. In addition, HI gamblers showed increased sympathetic dominance compared with LI. This has potential implications for how HI gamblers engage in gambling activities, respond to gambling related stimuli and the management of treatment for such individuals. It potentially represents a problematic cycle where impulsivity and arousal feed one another in relation to certain stimuli. Therefore, Experiment IV, offered a novel approach to investigate neuromodulation and physiological responses during different gambling phases. Results are relevant for the understanding on tDCS effects on the ANS during reward processing, and contributed to identify differences in autonomic arousal between LI and HI gamblers. Collectively, the data from this experiment support the development of more individualised neuromodulation interventions for GD, and highlight the potential significance of physiological differences between high and low impulsive gamblers, which may have important implications for the assessment and management of GD.

6.4 General methodological implications and future directions

In reviewing the extensive literature on tDCS and other NIBS work, and its importance for the development of the studies in this thesis, the protocols, procedures and analysis, and in embedding findings within this literature base, a number of concerns have emerged which appear to challenge aspects of the research landscape in this field. This section highlights some of these key issues, alongside suggestions for future directions arising from the studies conducted.

There is a need for standardisation of neuromodulation procedures and outcome measures in tDCS research to improve the reproducibility of results. Studies need to adhere to rigorous reporting practices, in which stimulation protocols should be described in detail. This would include the type and location of electrodes according to standard references, direction, intensity and duration of the electrical current, reference electrode location, description of the sham or control condition, and when possible, details of current distribution when using computational modelling approaches (Miranda, Callejón-leblic, Salvador, & Ruffini, 2018). Particularly, the use of active sham conditions in tDCS studies, known as "ActiSham", improves participant blinding by inducing the same sensations as real stimulation but avoiding significant effects on cortical excitability (Neri et al., 2020).

In addition, details on the stimulation procedure should be described, including for example the timing of events that may be coupled with the stimulation (such as cognitive tasks performance), the type of blinding (that may be accompanied by blinding control questionnaires), the tDCS exclusion criteria employed, and other relevant characteristics such as room temperature (Mikkonen, Laakso, Tanaka, & Hirata, 2020; Nitsche et al., 2008). Similarly, outcome measures should be standardised, including questionnaire and cognitive tasks, reporting detailed information about specific characteristics of the variables measured. In addition, analysis methodologies should be described in detail, including data pre-processing protocols and exact definition of calculated variables (for example providing a specific range of frequency in each EEG band, rather than noting the nomenclature).

Ecological validity of the studies could improve by using incentives, such as real money in GD research, and considering different characteristics associated with different types of gambling products (such as roulette, sports betting etc.), which would allow the investigation of behaviours in more realistic scenarios (Newson & Thiagarajan, 2019; Sharpe, 2002). Lastly, pre-registration of studies, which involves reporting the study protocol, design, hypothesis and analysis plan before data

collection, should help to ensure that the research is conducted according to the design and analysis planned, and addresses the outlined research questions. This would require that researchers distinguish between confirmatory analysis through hypothesis testing and exploratory analysis.

Considering that tDCS effects are highly dependent on participant brain states (Hsu, Juan, & Tseng, 2016), controlling for influencing factors might help improve reproducibility of results. For example, controlling for substance intake (including energy drinks and caffeine) prior the experiment would help identify factors that might influence the results (Newson & Thiagarajan, 2019). This may be particularly relevant in research involving addiction and GD, given the high comorbidity with SUDs (Yip & Potenza, 2014). Specifically, using biological tests (such as blood tests), to control both acute use (intoxication or hangover at time of testing) and more chronic, undisclosed drug misuse (which could be captured through hair analysis to some extent, possibly other hepatic function measures), would be more rigorous than the use of self-report questionnaires. In addition, controlling for the time of the day during which the study is conducted, sleep quality, and participants physiological and emotional states through questionnaires and baseline recordings of physiological arousal, including hormonal influences such as cortisol, would inform about intra-individual variability across sessions, which in turn, might help interpret inter-individual variability factors (Krause & Kadosh, 2014; Spagnolo et al., 2020).

Moreover, neuromodulation research would benefit from the use of computational modelling approaches that evaluate the most effective tDCS montage to stimulate the brain area of interest, by considering electrical flow through anatomical and tissue characteristics (Ruffini et al., 2013). In addition, the application of control sites for the stimulation would allow further investigation of anatomical specificity (Bertossi et al., 2017). Functional specificity of tDCS can be improved through the combination of cognitive tasks or therapeutic interventions during the stimulation. Improving anatomical and functional specificity, and the combination of tDCS with neuroimaging techniques, would also facilitate the investigation of functional connectivity between different regions, helping to identify behaviours associated with the underlying network, and study whether it is possible to disentangle specific cognitive components that may, or not, be associated to particular brain regions (Bikson & Rahman, 2013; Nejati et al., 2018).

It is also clear that the number of stimulation sessions influences tDCS effects, however standard practices are not established yet, and there is no evidence that indicates the most effective number and frequency of sessions to modulate each clinical condition (Lefaucheur et al., 2017). Short and long terms effects of tDCS should also be investigated. In addition, in crossover designs, studies should control for condition order, and account for possible stimulation carry-over effects according to the time interval between sessions (Nitsche et al., 2008).

Participant samples should generally be larger and more representative, including all ages and gender groups, multiple sessions to control for both, intra and inter-individual variability, with a detailed description of demographic characteristics that may be relevant for the condition investigated (Newson & Thiagarajan, 2019). Studies should also investigate common comorbid disorders, and interactions of neuromodulation with other treatment interventions. Appropriate distribution of participants across groups regarding relevant characteristics, is also warranted. Consistency of stimulation protocols across sessions and across participants is crucial to maintain experimental variability to a minimum. Similarly, having the same time interval between sessions across all participants would allow to better understand long term effects of the intervention, avoiding to add more external factors influencing the results.

In research involving clinical samples, particular attention is given to ethical approval procedures, given the potential complexity and vulnerability associated with this population. The ethical application procedures usually requires extensive documentation that is reviewed through multiple phases and different clinical and research teams, which can take several months to complete, if not longer. In addition, once ethical approval is in place, a significant number of factors that may have direct implications in the development of the trials should be considered: notably, the number and availability of clinical sites. Low level availability of clinics (for treatment and research locations) might imply that treatment demand outweighs the site capability to treat patients, and could result on waiting lists, which might impact research recruitment procedures. The existence of adequate available facilities on the clinical site to conduct the experiment procedures should also be considered. There may be a need to invest clinical staff time to collaborate with the research trial, and to establish a continuous coordination between the research team and the clinical team during identification, screening, recruitment and testing of participants.

The data for the 2019/20 period presented in the Annual Statistics from the National Gambling Treatment Service report in Great Britain (GambleAware, 2020), which includes data from health care providers GamCare, Gordon Moody Association and Central and North West London NHS Foundation Trust (London National Problem Gambling Clinic), shows that a total of 60,413 appointments were recorded for clients treated in 2019/2020, which 80% were made for treatment purposes. From those, a small proportion of 9,008 individuals were treated within gambling services within that period. Crucially, those numbers include only people who contacted treatment services, however previous studies showed that only 7-12% of individuals with GD seek treatment (Slutske, 2006), which was in part explained by the limited availability of services offering treatment for GD (Petry et al., 2017). Treatment services continue to increase since previous reports indicating that 5909 individuals received treatment in 2015/2016. Nevertheless, current data, highlights the important shortfall in provision, and the urgent need to improve treatment delivery and strategies in GD.

Additional complications may arise in cases when different treatments are intended to be coupled. In these cases, it will be crucial to coordinate the patient testing and treatment session, considering the availability of the facilities and the clinicians for the whole duration of the research intervention. Also, there may be different treatment formats offered at the clinical site depending on the patient characteristics. This could interact with the research inclusion criteria, if patients allocated to the type of treatment of interest have specific features that exclude them from participation in the trial.

Therefore, in view of all these potential complications, it would be advisable to conduct a feasibility study prior a clinical trial, which would allow to identify potential problems to consider, and determine the possibilities to complete the clinical trial. As an alternative, home-based treatment through remote therapy interventions has already shown to have specific benefits, such as the increased accessibility and convenience, reduced costs, anonymity and privacy (Gainsbury & Blaszczynski, 2011). This type of treatment could potentially be applied in combination of remotely controlled and supervised tDCS, which represents a promising approach for clinical neuromodulation (Palm et al., 2018). However, further research is necessary to assess safety and technical monitoring and the feasibility of these interventions.

6.5 Limitations

This research has a number of limitations that compromise the potential implications of the results. Some of the existing limitations in the first experiments were addressed as the project progressed, however other limitations arose with new designs and methodologies used in later experiments. In Experiments I and II, a single blinding design was used, however this was substituted by a more rigorous triple blinding design in Experiments III and IV. Moreover, in Experiment I participant allocation to groups was not randomised due to changes of experimental design during recruitment, but randomisation of participant allocation to groups was applied in the rest of the experiments. In addition, in Experiments I and II, HD tDCS montages were created using a 1 x 4 ring configuration without current flow calculations (Villamar et al., 2013), but in Experiments III and IV a computational model was used to identify the most effective montage to target the brain area of interest (Ruffini et al., 2013).

Moreover, in Experiments I and II only behavioural measures were used to investigate the effects of tDCS, which might have left undetected effects that could have been identified with neurophysiological techniques. Therefore, whether the current reached the desired brain areas could only be speculated based on previous studies using similar protocols. However, in Experiment III, behavioural measures were accompanied by neurophysiological data, including EEG measures to investigate cortical excitability. In addition, Experiment IV, included EEG, electrocardiogram (ECG) and electrodermal activity (EDA) measures to quantify the physiological effects of tDCS on the ANS, which provided a more complete and rigorous approach for the study of neuromodulation effects. Experiments I, II and IV followed a two session crossover design to investigate the short term effects of tDCS, however possible carry over effects between sessions separated a week cannot be disregarded since there is not consensus on how long the short and long term effects of tDCS last (Nitsche et al., 2008).

Experiment III had numerous limitations due to changes of experimental design, which did not match the type of intervention conducted. Consequently, the results obtained did not allow to differentiate the intervention effects between real stimulation and sham conditions, or the specific effects of tDCS and CBT. Therefore, it was not possible to reach a conclusion about the effectiveness of the tDCS intervention. In Experiment IV, the methodology to log in the physiological data caused a delay that had to be accounted for in the analysis. This reduced the accuracy of the results, which could

compromise the generalisation to other studies that may have interfaced the tasks with experimental software, producing more reliable data. In addition, participants blinding the in real stimulation condition was not effective, which may have influenced results of the experiment. Future studies employing active sham protocols might be able to avoid this issue.

6.6 Final remarks

Elucidating the underlying cognitive and neurobiological mechanisms associated with dysfunctional decision-making, inhibitory control and emotion regulation, is essential for prevention and treatment of psychiatric disorders, including addiction (Goschke, 2014). The prospect of creating more effective individualised treatment interventions for GD has been reinforced by tDCS research. This is particularly relevant in view of the rising incidence of GD, and the preliminary state of its treatment (Paglieri et al., 2014).

This project represents a further contribution to the scientific literature pursuing a more profound understanding of tDCS neuromodulation capabilities. The findings embrace previous evidence suggesting that there is not a unique mechanism explaining how all neuromodulation interventions act (Bashir & Yoo, 2016). Refining neuromodulation protocols to modify dysfunctional behaviours is a rapidly evolving field, which is likely to be facilitated by the identification of cognitive and physiological underpinnings of GD. The current work sheds more light on the influence of impulsivity in gambling, and highlights the importance of studying the psychophysiological impact of tDCS, and the interface between such measures, impulsivity and gambling-related behaviour. And through it utilises a range of measures and interventions which could in the future be embedded in research and also treatment approaches for GD, addictions and other areas of psychiatric illness, as potentially cost effective methods to expand knowledge and improve clinical outcomes.

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Appendix A. Extended Results from Experiment III

Electroencephalogram (EEG) absolute and relative power results and discussion

Results

Participant allocated to tDCS sham condition

Delta relative power:

One way repeated measures ANOVA analysis of delta relative power revealed a significant main effect of session (F (5.553, 394.262) = 7.121, p= .001, ηp^2 = .091), showing that session four had the lowest delta relative power (≈ 50%), and sessions five, six and seven had the highest (≈ 60-80%). There was also a significant main effect of electrode (F (4.197, 297.987) = 62.794, p= .001, ηp^2 = .469) indicating that Fpz had the highest delta relative power (≈ 60%), and similar levels were found across the other electrodes (≈ 60%). In addition, there was an interaction session x tDCS time (F (5.818, 413.104) = 7.292, p= .001, ηp^2 = .093) showing that delta relative power was lower in EEG1 (≈ 55%) compared with EEG2 (≈ 80%) in session five, whereas delta relative power was higher in EEG1 compared with EEG2 in sessions four (≈ 50% and 45%), seven (≈ 70% and 60%), and eight (≈ 60% and 55%). Similar levels between both tDCS conditions were found in sessions one, two (≈ 55%) and six (≈ 75%). There was a significant interaction session x electrode (F (18.675, 1325.910) = 21.058, p= .001, ηp^2 = .229), showing that sessions six and five had the highest relative delta power in Fpz (≈ 65%), and the lowest delta relative power was in session four for F4 (≈ 55%).

There was also an interaction tDCS time x electrode (F (3.288, 282.730) = 11.057, p= .001, ηp^2 = .135), indicating that all electrodes showed similar levels of delta relative power between tDCS conditions (a slight difference of ≈ 5% of the power between EEG1 and EEG2), with lower delta relative power in EEG1 compared with EEG2 in electrodes Fpz and Cz, and higher delta relative power in EEG1 compared with EEG2 in electrodes F4, FC6, F3 and AF7. A three way interaction session x tDCS time x electrode (F (19.030, 1351.137) = 16.511, p= .001, ηp^2 = .189), showed that session five had the highest delta relative power in EEG2 (≈ 80%) while EEG1 was around 55%, and sessions six and seven had the highest delta relative power in EEG1 (≈ 70%). Higher delta relative power in EEG1 compared with EEG2 was found in session 7 (≈ 70% and 60%) and eight (≈ 60% and 55%). Delta relative power values across sessions and electrode positions are represented in Figure 0-1.

To assess the final outcome of the intervention, delta relative power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 delta relative power in session one was significantly lower than EEG1 delta relative power in session eight, only in electrode positions Fpz (t (117) = -3.606, p =.001) and AF4 (t (117) = -5.902, p =.001). EEG2 delta relative power in session one was significantly higher than EEG2 delta relative power in session eight, for all electrodes except for AF7: Cz (t (116) = 3.536, p =.001), AF8 (t (116) = 2.472, p =.015), AF4 (t (116) = 2.519, p =.013), Fpz (t (116) = 3.909, p =.001), F4 (t (116) = 5.016, p =.001), FC6 (t (116) = 5.533, p =.001) and F3 (t (116) = 3.737, p =.001). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 was significantly lower than EEG2 in session one for electrodes AF4 (t (120) = -3.343, p =.001), Fpz (t (120) = -4.095, p =.001) and F4 (t (120) = -2.161, p =.033) and higher in EEG1 compared with EEG2 in F3 (t (120) = 18.336, p =.001). In session eight EEG1 was higher than EEG2 in all electrodes except in Cz and F3: AF8 (t (116) = 2.044, p =.043), AF4 (t (116) = 3.898, p =.001), Fpz (t (116) = 2.530, p =.013), F4 (t (116) = 2.451, p =.016), FC6 (t (116) = 4.142, p =.001) and AF7 (t (116) = 2.584, p =.011).

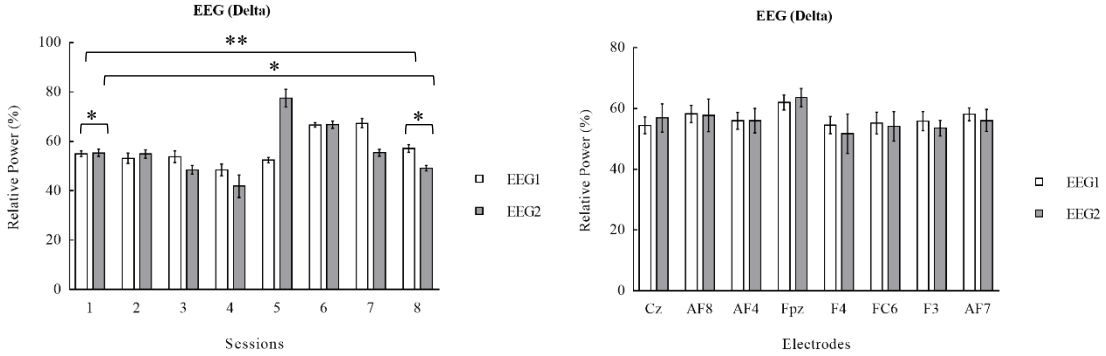


Figure 0-1. Electroencephalogram (EEG) delta relative power in sham condition. Delta relative power (%) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found only in electrodes Fpz and AF4. Significant differences between EEG2 in session one and EEG2 in session eight, were found in all electrodes except in AF7. Furthermore, significant differences between EEG1 and EEG2 in session one were found in electrodes AF4, Fpz, F4 and F3, however in session eight EEG1 and EEG2 were significantly different in all electrodes except Cz and F3.

Delta absolute power:

One way repeated measures ANOVA analysis of delta absolute power revealed a significant main effect of session (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that session five had the highest delta absolute power at around 50 μV^2, whereas the rest of the sessions had delta absolute power around 10 μV^2, except in session six, which was approximately 20 μV^2. There was also a significant main effect of tDCS time (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that delta absolute power was higher in EEG2 compared with EEG1, and a significant main effect of electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167) indicating that electrodes from the highest (≈ 20 μV^2) to the lowest (≈ 10 μV^2) delta absolute power were AF8, AF7, Fpz, F3, Cz, AF4, F4 and FC6. In addition, there was an interaction session x tDCS time (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that lower delta absolute power in EEG1 compared with EEG2 was found especially in session five (≈ 5 μV^2 and 50 μV^2, respectively) and six (≈ 10 μV^2 and 20 μV^2, respectively). However, in the rest of sessions both tDCS time conditions had similar delta absolute power.

There was a significant interaction session x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showing that session two had the highest delta absolute power in all electrodes. A significant interaction tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showed that in all electrodes, lower delta absolute power was associated with EEG1 compared with EEG2. Lastly, a three way interaction session x tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showed that in EEG1, session two had the highest delta absolute power in all electrodes, however in EEG2, session six had the highest in all electrodes except for AF8, in which session five had the highest delta absolute power. In addition these data indicated that in EEG1, all sessions had similar delta absolute power, with the exception of session two, but in EEG2, session six had the highest delta absolute power followed by sessions five, seven, three, one, and lastly, session eight that the lowest. Delta absolute power values across sessions and electrode positions are represented in Figure 0-2.

To assess the final outcome of the intervention, delta absolute power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 delta absolute power in session one was significantly lower than EEG1 delta absolute power in session eight, in all electrode positions except F3: Cz (t (117) = -2.373, p =.019), AF8 (t (117) = -5.137, p =.001), AF4 (t (117) = -3.917, p =.001), Fpz (t

(117) = -3.233, p =.002), F4 (t (117) = -2.690, p =.008), FC6 (t (117) = -2.351, p =.020) and AF7 (t (117) = -2.243, p =.027). EEG2 delta absolute power in session one was significantly higher than EEG2 delta absolute power in session eight, for all electrodes: Cz (t (116) = 5.933, p =.001), AF8 (t (116) = 7.515, p =.001), AF4 (t (116) = 8.329, p =.001), Fpz (t (116) = 5.902, p =.001), F4 (t (116) = 5.918, p =.001), FC6 (t (116) = 5.761, p =.001), F3 (t (116) = 5.941, p =.001) and AF7 (t (116) = 5.835, p =.001).

To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 delta absolute power was significantly lower than EEG2 delta absolute power in session one, for all electrodes except AF7: Cz (t (120) = -4.394, p =.001), AF8 (t (120) = -4.164, p =.001), AF4 (t (120) = -2.553, p =.012), Fpz (t (120) = -3.233, p =.002) and F4 (t (120) = -4.465, p =.001), FC6 (t (120) = -4.053, p =.001) and F3 (t (120) = -4.508, p =.001). In session eight, EEG1 delta absolute power was higher than EEG2 delta absolute power in all electrodes: Cz (t (116) = 2.044, p =.043), AF8 (t (116) = 2.044, p =.043), AF4 (t (116) = 3.898, p =.001), Fpz (t (116) = 2.530, p =.013), F4 (t (116) = 2.451, p =.016), FC6 (t (116) = 4.142, p =.001), F3(t (116) = 2.044, p =.043) and AF7 (t (116) = 2.584, p =.011).

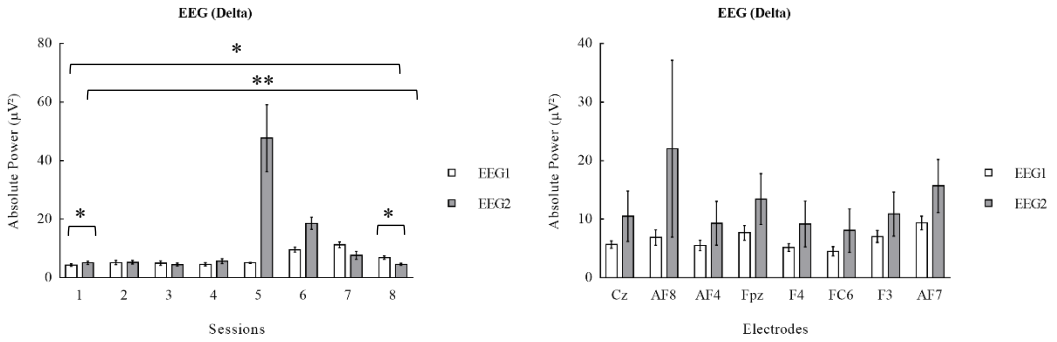


Figure 0-2. Electroencephalogram (EEG) delta absolute power in sham condition. Delta absolute power (μV^2) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes except in F3. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found in ell electrodes except AF7, and in session eight, EEG1 and EEG2 were significantly different in all electrodes.

Theta relative power:

One way repeated measures ANOVA analysis of theta relative power revealed a significant main effect of session (F (7, 497) = 7.694, p= .001, ηp^2 = .098), showing that session three had the highest theta relative power (≈ 15%) and session five had the lowest (≈ 8%). There was also a significant main effect of electrode (F (3.886, 275.939) = 138.430, p= .001, ηp^2 = .661) indicating that electrodes from the highest (≈ 15%) to the lowest (≈ 10%) theta relative power were AF4, Fpz, F4, FC6, Cz, F3, AF8 and AF7. In addition, there was an interaction session x tDCS time (F (7, 497) = 2.589, p= .012, ηp^2 = .035), showing that theta relative power was lower in EEG1 compared with EEG2 (≈ 10 % and 14 %, respectively) in sessions six and seven, however in session five EEG1 had higher theta relative power than EEG2 (≈ 12 % and 8 %, respectively) and in the rest of the sessions both tDCS time conditions seem to have similar theta relative power.

There was a significant interaction session x electrode (F (18.500, 1313.496) = 9.917, p= .001, ηp^2 = .123), showing that sessions seven and three had the highest theta relative power in all electrodes, and the lowest was in session five for the rest of electrodes, except for AF7, in which the lowest theta relative power was session five. There was also an interaction tDCS time x electrode (F (4.288, 304.427) = 4.311, p= .001, ηp^2 = .057), indicating that electrodes Fpz and AF4 showed the highest relative theta power, and the lowest in AF7 and AF8 in both with EEG1 and EEG2. A three way interaction session x tDCS time x electrode (F (18.959, 1345.982) = 5.087, p= .001, ηp^2 = .067), showed that session three had the highest relative theta power in both EEG1 and EEG2, whereas session five had the lowest, particularly in EEG2. All electrodes had similar theta relative power between tDCS time conditions. Relative theta power values across sessions and electrode positions are represented in Figure 0-3.

To assess the final outcome of the intervention, theta relative power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 theta relative power in session one was significantly higher than EEG1 theta relative power in session eight, only in electrode positions AF4 (t (117) = 2.896, p =.005) and Fpz (t (117) = 2.254, p =.026). EEG2 theta relative power in session one was significantly higher than EEG2 theta relative power in session eight, only in electrode position Fpz (t (116) = 2.571, p =.011). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 theta relative

power was significantly higher than EEG2 theta relative power in session one for electrodes AF4 (t (120) = 2.866, p =.005) and Fpz (t (120) = 3.442, p =.001) and lower in EEG1 compared with EEG2 in AF8 (t (120) = -18.004, p =.005). In session eight there were not significant differences between EEG1 and EEG2 theta relative power.

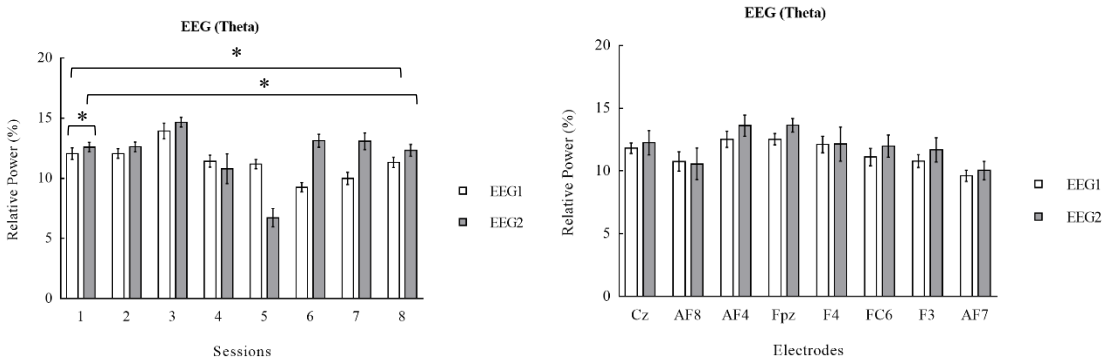


Figure 0-3. Electroencephalogram (EEG) theta relative power in sham condition. Theta relative power (%) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found only in electrodes AF4 and Fpz. Significant differences between EEG2 in session one and EEG2 in session eight were found only in Fpz. Furthermore, significant differences between EEG1 and EEG2 in session one were found only in electrodes AF8, AF4 and Fpz. However, in session eight, there were no significant differences between theta relative power between EEG1 and EEG2.

Theta absolute power:

One way repeated measures ANOVA analysis of theta absolute power revealed a significant main effect of session (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that session five and six had the highest theta absolute power (≈ 3.8 μV^2) whereas in the rest of the sessions it was around 1 μV^2. There was also a significant main effect of tDCS time (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that theta absolute power was lower in EEG1 compared with EEG2. A significant main effect of electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167) indicated that electrodes from the highest to the lowest absolute power were AF7, Fpz, F3, Cz, F4, AF4, AF8 and FC6 . In addition, there was an interaction session x tDCS time (F (1, 71) = 138.740, p= .001, ηp^2 = .661) showing that EEG1 had lower theta absolute power compared with EEG2 (≈ 1 μV^2 and 3.8 μV^2, respectively) in sessions five and six, however in the rest of sessions both tDCS time conditions seem to have similar theta absolute power. There was a significant interaction session x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showing that session two had the highest theta absolute power in all electrodes. There was also an interaction tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showing that in all electrodes lower theta absolute power was associated with EEG1 compared with EEG2. A three way interaction session x tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showed that in EEG1 session two had the highest absolute theta power in all electrodes, however in EEG2 session six had the highest theta absolute power in all electrodes except for AF8, in which session five showed the highest theta relative power. Theta absolute power values across sessions and electrode positions are represented in Figure 0-4.

To assess the final outcome of the intervention, theta absolute power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 theta absolute power in session one was significantly lower than EEG1 theta absolute power in session eight, in all electrode positions: Cz (t (117) = -4.232, p =.001), AF8 (t (117) = -4.836, p =.001), AF4 (t (117) = -4.292, p =.001), Fpz (t (117) = -3.044, p =.003), F4 (t (117) = -4.320, p =.001), FC6 (t (117) = -4.920, p =.001), F3 (t (117) = -3.834, p =.001) and AF7 (t (117) = -2.147, p =.034). EEG2 theta absolute power in session one was significantly higher than EEG2 theta absolute power in session eight, for all electrodes: Cz (t (116) = 4.134, p =.001), AF8 (t (116) = 9.318, p =.001), AF4 (t (116) = 10.362, p =.001), Fpz (t (116) = 7.657, p =.001), F4 (t (116) = 4.241, p =.001), FC6 (t (116) = 4.322, p =.001), F3 (t (116) = 3.842, p =.001) and AF7 (t (116) = 8.086, p =.001). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 theta

absolute power was significantly lower than EEG2 theta absolute power in session one, in all electrodes except for AF4 and AF7: Cz (t (120) = -2.168, p =.032), AF8 (t (120) = -2.820, p =.006), Fpz (t (120) = -2.337, p =.021) and F4 (t (120) = -2.097, p =.038), FC6 (t (120) = -2.027, p =.045) and F3 (t (120) = -2.058, p =.042). In session eight, EEG1 theta absolute power was higher than EEG2 theta absolute power in all electrodes: Cz (t (116) = 15.511, p =.001), AF8 (t (116) = 13.303, p =.001), AF4 (t (116) = 14.452, p =.001), Fpz (t (116) = 12.367, p =.001), F4 (t (116) = 15.758, p =.016), FC6 (t (116) = 15.600, p =.001), F3(t (116) = 15.071, p =.001) and AF7 (t (116) = 11.744, p =.001).

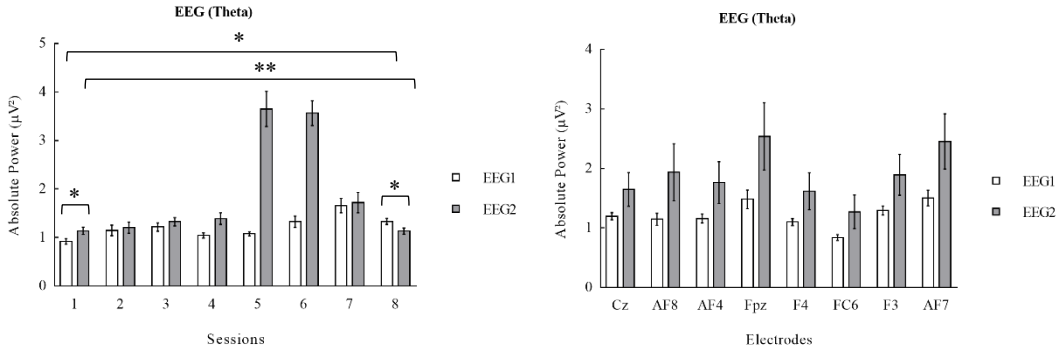


Figure 0-4. Electroencephalogram (EEG) theta absolute power in sham condition. Theta absolute power (μV^2) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes except in F3. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found in all electrodes except in AF7, and in session eight EEG1 and EEG2 were significantly different in all electrodes.

Alpha relative power:

One way repeated measures ANOVA analysis of alpha relative power revealed a significant main effect of session (F (5.742, 407.651) = 7.693, p= .001, ηp^2 = .098), showing that sessions three, four and eight had the highest alpha relative power (≈ 10%), and sessions five, six and seven had the lowest (≈ 5%). A significant main effect of tDCS time (F (1, 71) = 26.854, p= .001, ηp^2 = .274) showed that EEG1 had higher alpha relative power than EEG2, (but the differences were only around 2% of the power). There was also a significant main effect of electrode (F (3.897, 276.654) = 69.441, p= .001, ηp^2 = .494), indicating that AF7 had the highest alpha relative power (≈ 10%), and similar levels were found across the other electrodes (≈ 7%). In addition, there was an interaction session x tDCS time (F (5.716, 405.844) = 5.279, p= .001, ηp^2 = .069) showing that alpha relative power was higher in EEG1 compared with EEG2 in sessions four (≈ 10% and 7%), five (≈ 9% and 3%) and eight (≈ 10% and 8%), whereas alpha relative power was lower in EEG1 compared with EEG2 in session seven (≈ 5% and 7%). Similar levels between both tDCS conditions were found the rest of the sessions.

There was a significant interaction session x electrode (F (20.681, 1468.366) = 9.834, p= .001, ηp^2 = .122), showing that sessions two, four and eight had the highest alpha relative power across all electrodes, whereas the lowest alpha relative power was in session five for all electrodes, except for AF4 and AF8, in which session seven was the lowest. There was also an interaction tDCS time x electrode (F (4.320, 306.719) = 2.604, p= .032, ηp^2 = .035), indicating that all electrodes showed similar levels of alpha relative power between tDCS conditions (with a small difference of ≈ 2% of the power between EEG1 and EEG2). A three way interaction session x tDCS time x electrode (F (20.589, 1461.821) = 3.922, p= .001, ηp^2 = .052), showed that the largest difference between tDCS time conditions was found in session five, which had the lowest alpha relative power in EEG2 (≈ 3%) while EEG1 was around 8%. All electrodes had similar levels of alpha relative power in each session. Alpha relative power values across sessions and electrode positions are represented in Figure 0-5.

To assess the final outcome of the intervention, alpha relative power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 alpha relative power in session one was significantly lower than EEG1 alpha relative power in session eight, in electrode positions F4 (t (117) = -2.591, p =.011), FC6 (t (117) = -2.243, p =.027), F3 (t (117) = -2.729, p =.007) and higher EEG1 alpha relative

power in session one compared with EEG1 alpha relative power in session eight in AF7 (t (117) = 2.245, p =.027). EEG2 alpha relative power in session one was significantly lower than EEG2 alpha relative power in session eight in electrodes Fpz (t (116) = -2.822, p =.006), F4 (t (116) = -2.803, p =.006) and F3 (t (116) = -3.455, p =.001). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 alpha relative power was significantly higher than EEG2 alpha relative power in session one for electrodes AF4 (t (120) = 2.870, p =.005), Fpz (t (120) = 2.653, p =.009), and F3 (t (120) = 2.360, p =.019). Lower EEG1 alpha relative power compared with EEG2 alpha relative power was found in session one in FC6 (t (120) = -2.001, p =.048). In session eight EEG1 was higher than EEG2 only in FC6 (t (116) = 2.948, p =.004).

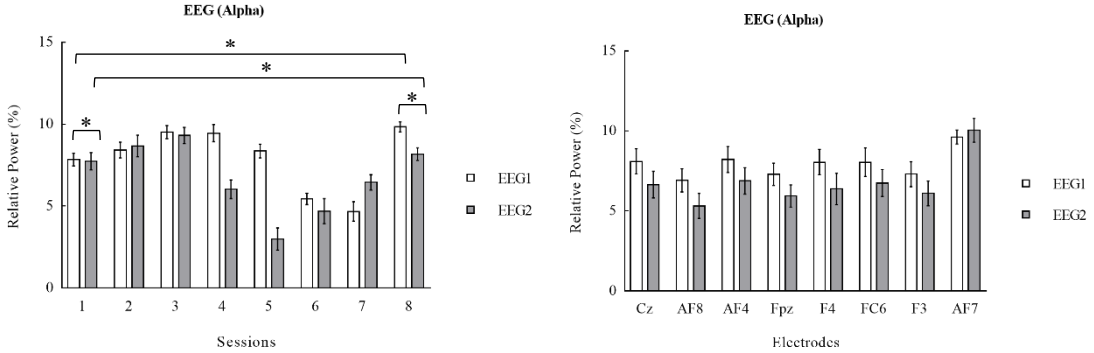


Figure 0-5. Electroencephalogram (EEG) alpha relative power in sham condition. Alpha relative power (%) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found only in electrodes F4, FC6, F3 and AF7. Significant differences between EEG2 in session one and EEG2 in session eight were found only in electrodes Fpz, F4 and F3. Furthermore, significant differences between EEG1 and EEG2 in session one were found in electrodes AF4, Fpz, FC6 and F3, whereas in session eight EEG1 and EEG2 were significantly different in FC6.

Alpha absolute power:

One way repeated measures ANOVA analysis of alpha absolute power revealed a significant main effect of session (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that sessions four and eight had the highest alpha absolute power at around 1.5 μV^2, whereas the lowest was alpha absolute power was found in session seven with approximately 1 μV^2. There was also a significant main effect of tDCS time (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that alpha absolute power was higher in EEG1 compared with EEG2. A significant main effect of electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167) indicated one electrode (AF8) showed the highest alpha absolute power at around 4.5 μV^2, and the rest of electrodes showed similar alpha absolute power (1 μV^2). In addition, there was a significant interaction session x tDCS time (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that higher alpha absolute power in EEG1 compared with EEG2 was found in all sessions except sessions five and six. There was a significant interaction session x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showing that session two had the highest alpha absolute power in all electrodes. A significant interaction tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showed that in all electrodes higher alpha absolute power was associated with EEG1 compared with EEG2. Lastly, a three way interaction session x tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showed that in EEG1, session two had the highest alpha absolute power in all electrodes, however in EEG2, session five had the highest alpha absolute power in all electrodes. Alpha absolute power values across sessions and electrode positions are represented in Figure 0-6.

To assess the final outcome of the intervention, alpha absolute power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 alpha absolute power in session one was significantly lower than EEG1 alpha absolute power in session eight, in all electrode positions: Cz (t (117) = -4.137, p =.001), AF8 (t (117) = -5.143, p =.001), AF4 (t (117) = -4.660, p =.001), Fpz (t (117) = -5.073, p =.001), F4 (t (117) = -4.598, p =.001), FC6 (t (117) = -4.141, p =.001), F3 (t (117) = -4.517, p =.001) and AF7 (t (117) = -5.110, p =.001). EEG2 alpha absolute power in session one was significantly higher than EEG2 alpha absolute power in session eight, for all electrodes: Cz (t (116) = 5.701, p =.001), AF8 (t (116) = 13.671, p =.001), AF4 (t (116) = 15.683, p =.001), Fpz (t (116) = 12.246, p =.001), F4 (t (116) = 6.759, p =.001), FC6 (t (116) = 7.646, p =.001), F3 (t (116) = 5.189, p =.001) and AF7 (t (116) = 13.101, p =.001). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that

EEG1 alpha absolute power was significantly lower than EEG2 alpha absolute power in session one, only in electrode AF8 (t (120) = -2.038, p =.044). In session eight, EEG1 alpha absolute power was significantly higher than EEG2 alpha absolute power in all electrodes: Cz (t (116) = 8.897, p =.001), AF8 (t (116) = 8.676, p =.001), AF4 (t (116) = 8.635, p =.001), Fpz (t (116) = 9.147, p =.001), F4 (t (116) = 8.463, p =.016), FC6 (t (116) = 8.492, p =.001), F3(t (116) = 9.687, p =.001) and AF7 (t (116) = 11.184, p =.001).

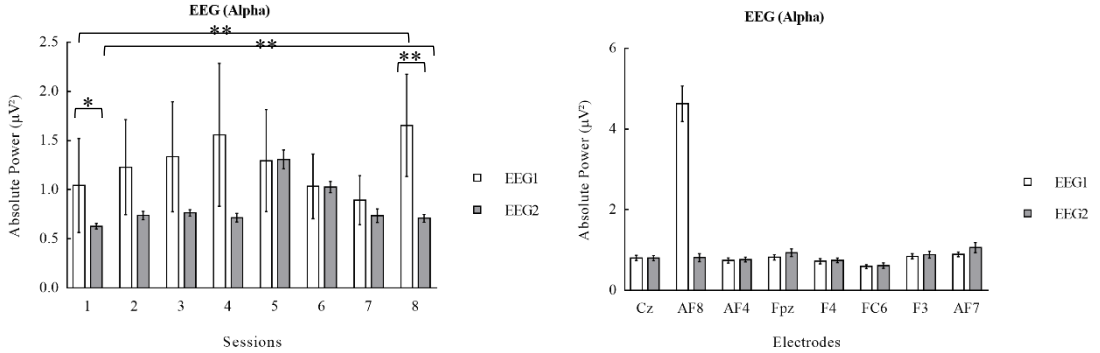


Figure 0-6. Electroencephalogram (EEG) alpha absolute power in sham condition. Alpha absolute power (μV^2) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrode positions. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found only in electrode AF8, however in session eight, EEG1 and EEG2 were significantly different in all electrodes.

Beta relative power:

One way repeated measures ANOVA analysis of beta relative power revealed a significant main effect of session (F (5.369, 381.201) = 14.479, p= .001, ηp^2 = .169), showing that session four had the highest beta relative power (≈ 40%), and session six the lowest (≈ 20%). A significant main effect of tDCS time (F (1, 71) = 1.229, p= .003, ηp^2 = .118) showed that EEG1 had lower beta relative power than in EEG2. There was also a significant main effect of electrode (F (3.582, 254.321) = 296.778, p= .001, ηp^2 = .807), indicating that F4 and AF7 had the highest beta relative power (≈ 30%), and the lowest was in Fpz (≈ 20%). In addition, there was an interaction session x tDCS time (F (5.831, 414.026) = 11.778, p= .001, ηp^2 = .142) showing that beta relative power was lower in EEG1 compared with EEG2. The difference between both tDCS conditions was around less than 5% difference of power in sessions one, two and six, and larger in sessions three (≈ 20% and 30%, respectively), four (≈ 28% and 40%, respectively), seven (≈ 18% and 28%, respectively), and eight (≈ 20% and 30%, respectively). However, in session five, EEG1 was higher than EEG2 (≈ 28% and 18%, respectively).

There was also a significant interaction session x electrode (F (17.199, 1221.126) = 40.381, p= .001, ηp^2 = .363), showing that session four had the highest beta relative power in all electrodes, except for F3, in which beta relative power was highest in session five, and Fpz in session two. A significant interaction tDCS time x electrode (F (3.478, 246.924) = 15.764, p= .001, ηp^2 = .182), indicated that the largest difference between tDCS time conditions was in AF7 (EEG1 was around 10% and EEG2 around 35%), and in the rest of electrodes the difference between tDCS time conditions was around 5% of the power. A three way interaction session x tDCS time x electrode (F (18.363, 1303.740) = 25.176, p= .001, ηp^2 = .262), showed that the largest difference between tDCS time conditions was found in session four, which had the highest beta relative power in EEG2 (≈ 40%), while EEG1 was around 28%. In EEG2, beta relative power increased from sessions one to four, decreased in session five, and increased again from sessions six to eight. Beta relative power was similar across electrodes, except in Fpz and AF7, which had the lowest levels. Beta relative power values across sessions and electrode positions are represented in Figure 0-7.

To assess the final outcome of the intervention, beta relative power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 beta relative power in session one was significantly higher

than EEG1 beta relative power in session eight, in electrode positions AF4 (t (117) = 6.355, p =.001), Fpz (t (117) = 4.184, p =.001) and FC6 (t (117) = 3.370, p =.001). EEG2 beta relative power in session one was significantly lower than EEG2 beta relative power in session eight in all electrodes except AF7: Cz (t (116) = -4.547, p =.001), AF8 (t (116) = -2.247, p =.027), AF4 (t (116) = -3.190, p =.002), Fpz (t (116) = -2.856, p =.005), F4 (t (116) = -5.492, p =.001), FC6 (t (116) = -5.994, p =.001) and F3 (t (116) = -3.561, p =.001). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 beta relative power was significantly higher than EEG2 beta relative power in session one, in electrodes AF4 (t (120) = 2.062, p =.041), Fpz (t (120) = 3.078, p =.003), and F3 (t (120) = 2.066, p =.041). In session eight EEG1 was lower than EEG2 in all electrodes except in F3: Cz (t (116) = -3.258, p =.001), AF8 (t (116) = -5.742, p =.001), AF4 (t (116) = -3.801, p =.001), Fpz (t (116) = -3.477, p =.001), F4 (t (116) = -4.355, p =.001), FC6 (t (116) = -8.031, p =.001) and AF7 (t (116) = -3.098, p =.002).

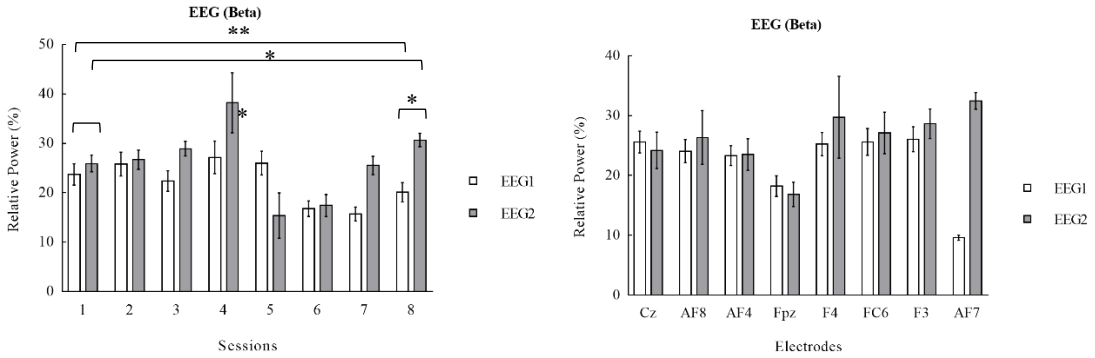


Figure 0-7. Electroencephalogram (EEG) beta relative power in sham condition. Delta relative power (%) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found only in electrodes AF4, Fpz and FC6. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes except in AF7. Furthermore, significant differences between EEG1 and EEG2 in session one were found in electrodes AF4, Fpz and F3, and in session eight EEG1 and EEG2 were significantly different in all electrodes except in F3.

Beta absolute power:

One way repeated measures ANOVA analysis of beta absolute power revealed a significant main effect of session (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that sessions four and five had the highest beta absolute power at around 8 μV^2, whereas the lowest was beta absolute power was found in sessions one, two and three with approximately 3 μV^2. There was also a significant main effect of tDCS time (F (1, 71) = 138.740, p= .001, ηp^2 = .661), showing that beta absolute power was lower in EEG1 compared with EEG2. A significant main effect of electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167) indicated the electrode AF8 showed the highest beta absolute power at around 15 μV^2, followed by AF7 and F3, with around 8 μV^2, and the rest of electrodes showed similar beta absolute power (around 3 μV^2). In addition, there was a significant interaction session x tDCS time (F (1, 71) = 138.740, p= .001, ηp2 = .661), showing that lower beta absolute power in EEG1 compared with EEG2 was found in all sessions, with the greatest differences (from 3 μV^2 to 8 μV^2) in sessions four and five.

There was a significant interaction session x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showing that session two had the highest beta absolute power in all electrodes. A significant interaction tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp2 = .167), showed that lower beta absolute power was associated with EEG1 compared with EEG2 with the greatest difference in AF8 (from 3 μV^2 to 15 μV^2) followed by AF7, F3 and F4, with differences of around 4 μV^2 of the power. Lastly, a three way interaction session x tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showed that EEG1 had similar levels of beta absolute power across sessions and across electrodes, however EEG2 beta absolute power increased specially in sessions four and five, and electrodes AF8 and AF7. Beta absolute power values across sessions and electrode positions are represented in Figure 0-8.

To assess the final outcome of the intervention, beta absolute power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 beta absolute power in session one was significantly lower than EEG1 beta absolute power in session eight, in all electrode positions except in FC6: Cz (t (117) = -4.490, p =.001), AF8 (t (117) = -4.108, p =.001), AF4 (t (117) = -4.858, p =.001), Fpz (t (117) = -5.150, p =.001), F4 (t (117) = -4.728, p =.001), F3 (t (117) = -4.634, p =.001) and AF7 (t (117) = -5.161, p =.001). EEG2 beta absolute power in session one was significantly higher than EEG2 beta

absolute power in session eight, in all electrodes: Cz (t (116) = 21.197, p =.001), AF8 (t (116) = 23.526, p =.001), AF4 (t (116) = 22.413, p =.001), Fpz (t (116) = 19.963, p =.001), F4 (t (116) = 21.324, p =.001), FC6 (t (116) = 21.184, p =.001), F3 (t (116) = 20.805, p =.001) and AF7 (t (116) = 18.161, p =.001).

To compare the effects of tDCS before and after the stimulation within each session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 beta absolute power was significantly lower than EEG2 beta absolute power in session one, only in electrode Cz (t (120) = -11.894, p =.001). In session eight, EEG1 beta absolute power was significantly higher than EEG2 beta absolute power in all electrodes: Cz (t (116) = 21.987, p =.001), AF8 (t (116) = 19.628, p =.001), AF4 (t (116) = 20.527, p =.001), Fpz (t (116) = 19.599, p =.001), F4 (t (116) = 21.392, p =.016), FC6 (t (116) = 19.208, p =.001), F3(t (116) = 22.524, p =.001) and AF7 (t (116) = 24.418, p =.001).

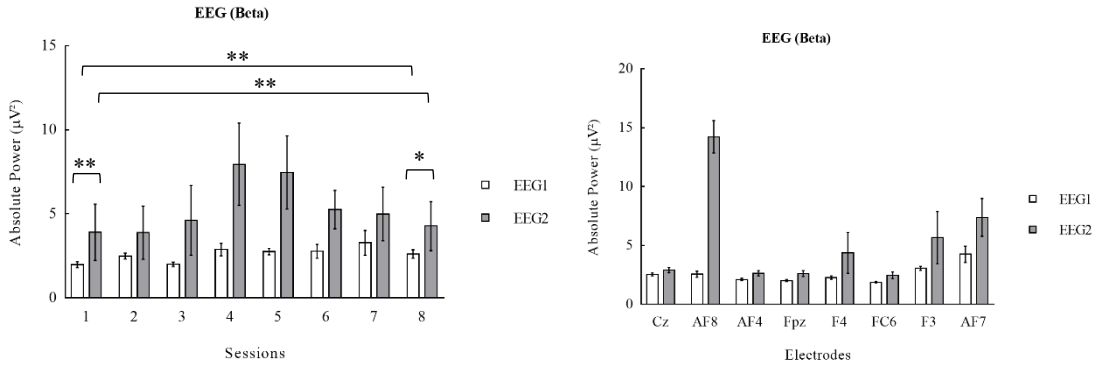
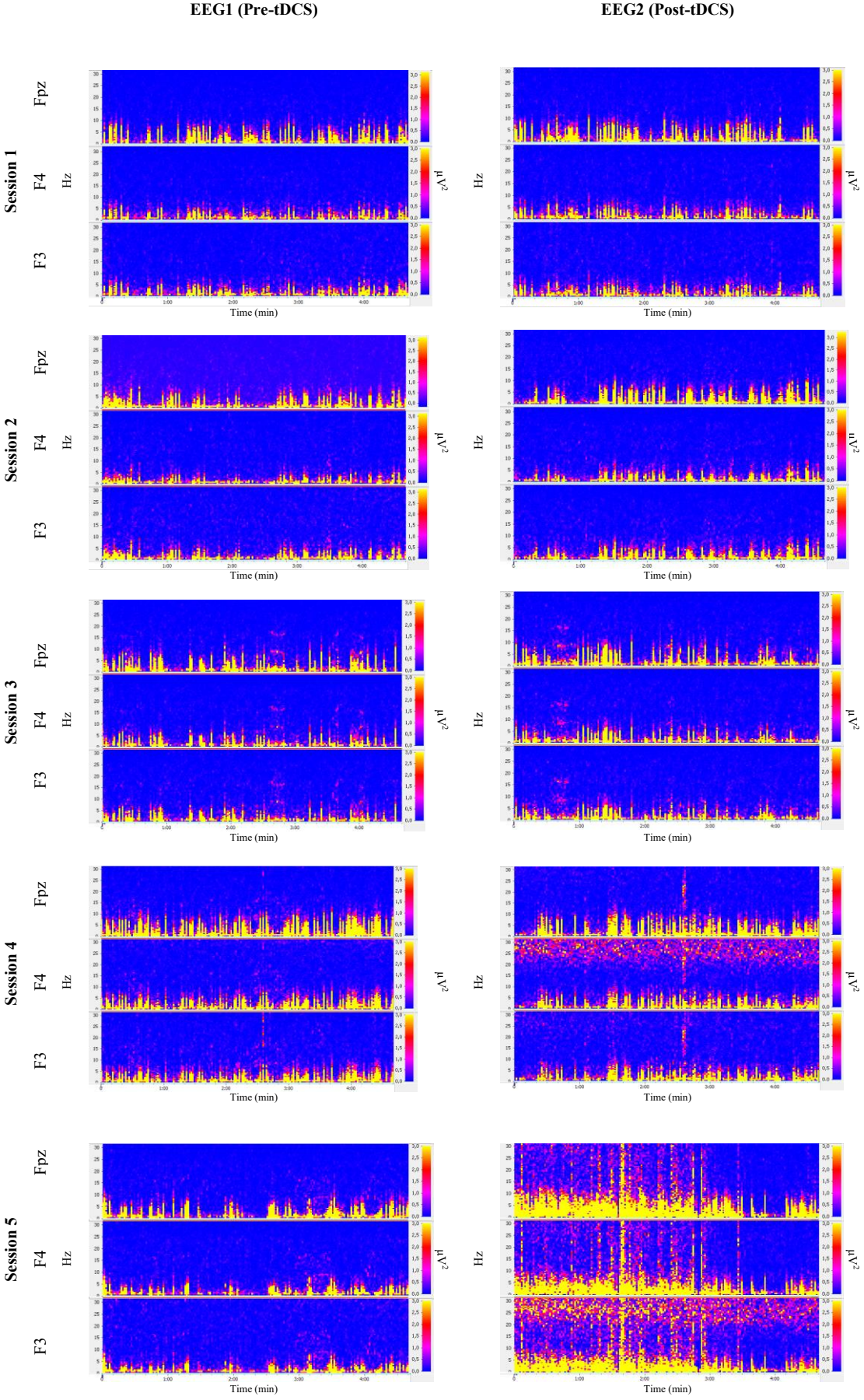


Figure 0-8. Electroencephalogram (EEG) beta absolute power in sham condition. Beta absolute power (μV^2) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes except in FC6. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found only in electrode Cz, however in session eight EEG1 and EEG2 were significantly different in all electrodes except F3.

Correlations:

To investigate the relationship between gambling severity and the rest of the variables, 2-tailed Pearson's correlation indicated that gambling severity measured with PG-YBOCS correlated positively with VAS for gambling cravings (r =.835, n = 8, p =.010). In addition, PG-YBOCS correlated negatively with SST total correct stop and go trials (r = -.737, n = 8, p =.037), total correct stop trials (r = -.715, n = 8, p =.046), total correct go trials (r = -.771, n = 8, p =.025) and with EEG2 beta relative power (r = -.942, n = 8, p =.001) in F3. Correlations with CGT could not be computed due to the lack of enough data points.

Having found all significant correlations between gambling severity and EEG variables, specifically in the electrode position F3, during the post-tDCS recordings (EEG2), these variables were further explored with the aim to find physiological markers that could inform about individuals gambling severity, gambling-related behaviours and symptomatology. Results showed that VAS gambling cravings correlated positively with theta/beta ratio in F3 (r = .739, n = 8, p =.036), and negatively with beta relative power in F3 (r = -.942, n = 8, p =.001). Moreover, negative correlations were found between SSRT and EEG2 absolute power, in all frequency bands in F3: delta (r = -.843, n = 8, p =.009), theta (r = -.742, n = 8, p =.035), alpha (r = -.860, n = 8, p =.006) and beta (r = -.844, n = 8, p =.008). In addition, IST total correct in win condition fixed, correlated negatively with EEG2 absolute power in F3, in frequency bands delta (r = -.780, n = 8, p =.022), theta (r = -.792, n = 8, p =.019) and alpha (r = -.774, n = 8, p =.024), and also with relative power in delta frequency (r = -.838, n = 8, p =.009), and positively in alpha frequency (r = .739, n = 8, p =.036). In addition, IST total correct correlated negatively with EEG2 in F3, in absolute beta power (r = -.837, n = 8, p =.010), and positively with relative theta power (r = .793, n = 8, p =.019.) Lastly, EEG2 absolute alpha power in F3, correlated negatively with IST mean number of boxes opened per trial (r = -.757, n = 8, p =.030) and mean number of boxes opened per trial in condition fixed (r = -.744, n = 8, p =.034). An EEG power spectrogram displaying electrode positions Fpz, F4 and F3 for ventral, right and left PFC respectively, is presented in Figure 0-9.


EEG1 (Pre-tDCS)
EEG2 (Post-tDCS)
Session 1
Session 2
Session 3
Session 4
Session 5
Fpz
F4
F3
Hz
μV²
Time (min)

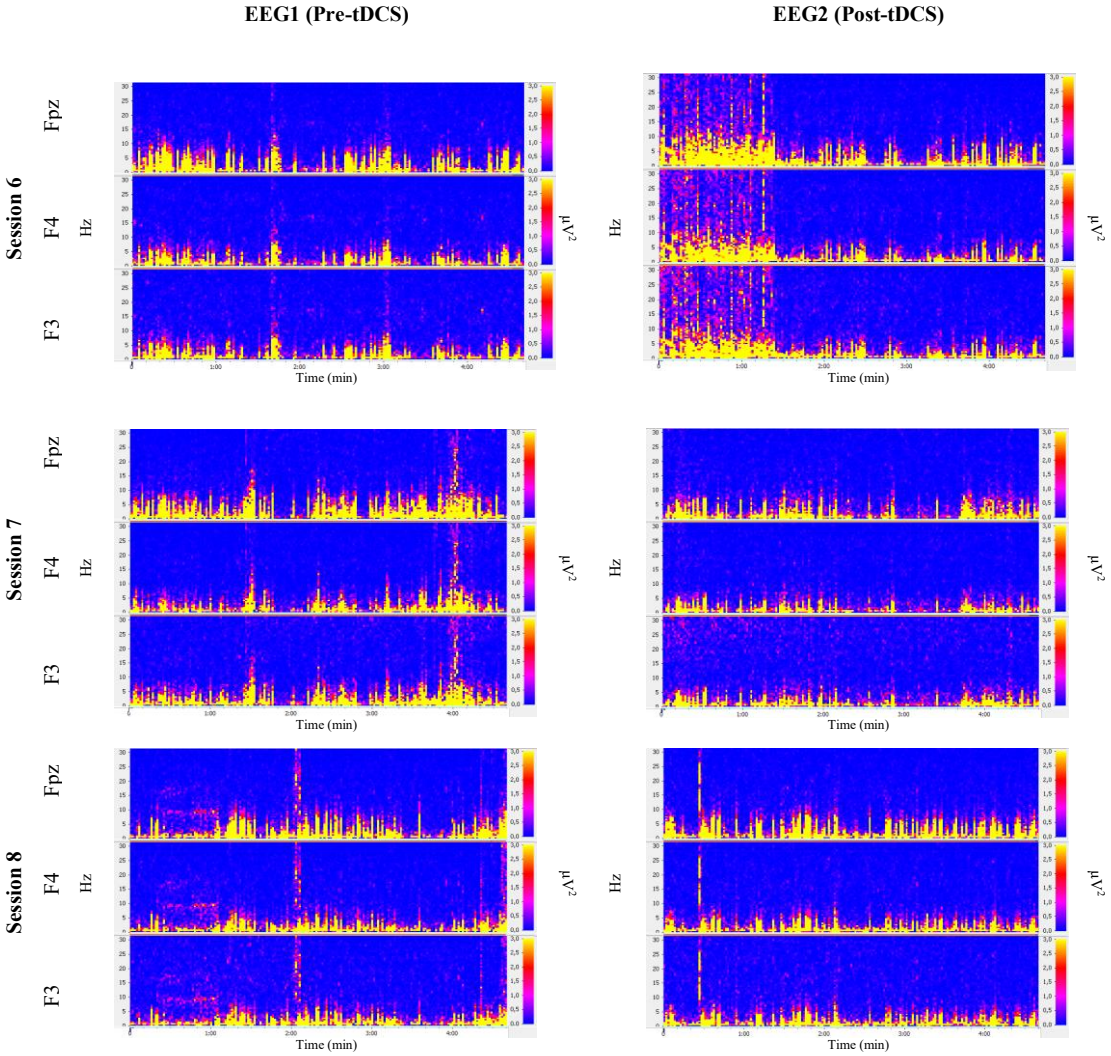


Figure 0-9. Electroencephalography (EEG) spectrogram during transcranial direct current stimulation (tDCS) in sham condition. EEG power (μV^2) in frequencies 0-30 Hz during five minutes EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions in sham condition. Electrodes positions displayed in the spectrogram with the international EEG 10/10 system are Fpz, F4 and F3 for ventral, right and left prefrontal cortex (PFC), respectively.

Participant allocated to tDCS real stimulation condition

Delta relative power:

One way repeated measures ANOVA analysis of delta relative power revealed a significant main effect of session (F (6.123, 600.006) = 83.760, p= .001, ηp^2 = .461), showing that session four had the lowest delta relative power (≈ 50%), and the rest of the sessions had around (≈ 75-90 %) delta relative power. A main effect of tDCS time (F (1, 98) = 188.496, p= .001, ηp^2 = .658), showed that EEG1 was higher than EEG2. There was also a significant main effect of electrode (F (3.733, 365.828) = 113.298, p= .001, ηp^2 = .536) indicating that similar levels of delta relative power were found across electrodes with FC6 having slightly lower delta relative power. In addition, there was an interaction session x tDCS time (F (6.185, 606.140) = 22.095, p= .001, ηp^2 = .184) showing that delta relative power was higher in EEG1 (≈ 90%) in all sessions but session four that had around 75% delta relative power, however EEG2 delta relative power changed from sessions one, two, three, four and six (≈ 85%, 75%, 65%, 55% and 60%, respectively) whereas it was higher (≈ 90%) in sessions five, six and eight. There was a significant interaction session x electrode (F (17.406, 1705.782) = 15.728, p= .001, ηp^2 = .138), showing that sessions six and eight had the highest relative delta power in all electrodes and the lowest delta relative power was in sessions four and three, especially in FC6.

There was also an interaction tDCS time x electrode (F (4.254, 416.915) = 44.280, p= .001, ηp^2 = .311), indicating that all electrodes showed similar levels of delta relative power between tDCS conditions with higher delta relative power in EEG1 compared with EEG2 with the highest difference being in FC6. A three way interaction session x tDCS time x electrode (F (16.960, 1662.096) = 10.209, p= .001, ηp^2 = .094), showed that session four had the lowest delta relative power in EEG2 (≈ 55%) while EEG1 was around 80%, and sessions three showed the largest difference between EEG1 and EEG2 delta relative power (≈ 90% and 65%, respectively), whereas in sessions five, six and eight the difference was the smallest. Delta relative power values across sessions and electrode positions are represented in Figure 0-10.

To assess the final outcome of the intervention, delta relative power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 delta relative power in session one was significantly lower than EEG1 delta relative power in session eight, in all electrodes except in AF7: Cz (t (100) = -3.765, p =.001), AF8 (t (100) = -3.181, p =.002), AF4 (t (100) = -4.843, p =.001), Fpz (t (100) = -2.999, p

=.003), F4 (t (100) = -3.623, p =.001), FC6 (t (100) = -3.664, p =.001) and F3 (t (100) = -4.527, p =.001). EEG2 delta relative power in session one was significantly higher than EEG2 delta relative power in session eight, in all electrodes: Cz (t (100) = 17.560, p =.001), AF8 (t (100) = 22.635, p =.001), AF4 (t (100) = 21.618, p =.001), Fpz (t (100) = 22.735, p =.001), F4 (t (100) = 19.683, p =.001), FC6 (t (100) = 15.401, p =.001), F3 (t (100) = 24.231, p =.001) and AF7 (t (100) = 28.615, p =.001). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 was significantly higher than EEG2 in session one in all electrodes: Cz (t (125) = 34.349, p =.001), AF8 (t (125) = 40.882, p =.001), AF4 (t (125) = 30.776, p =.001), Fpz (t (125) = 28.605, p =.001), F4 (t (125) = 36.382, p =.001), FC6 (t (125) = 31.116, p =.001), F3 (t (125) = 29.893, p =.001) and AF7 (t (125) = 34.327, p =.001). In session eight EEG1 was higher than EEG2 in all electrodes: Cz (t (100) = 53.398, p =.001), AF8 (t (100) = 55.544, p =.001), AF4 (t (100) = 57.657, p =.001), Fpz (t (100) = 35.690, p =.001), F4 (t (100) = 58.003, p =.001), FC6 (t (100) = 47.525, p =.001), F3 (t (100) = 48.540, p =.001) and AF7 (t (100) = 32.893, p =.001).

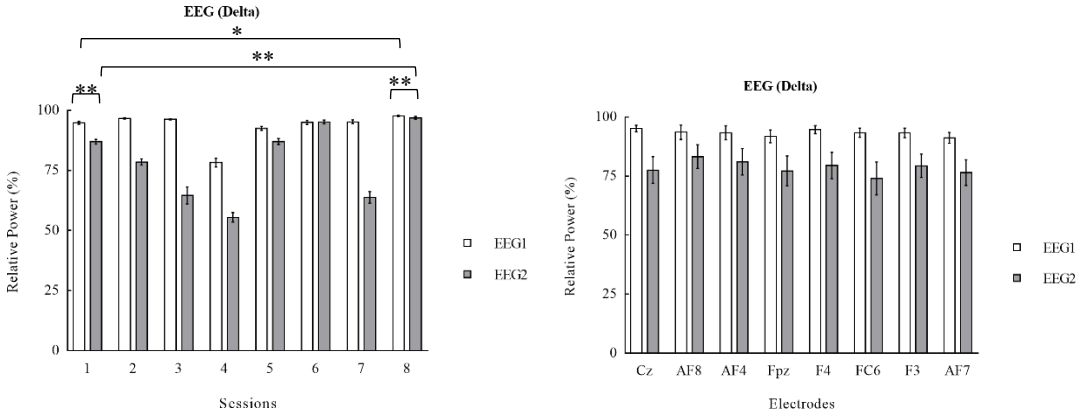


Figure 0-10. Electroencephalogram (EEG) delta relative power in real stimulation condition. Delta relative power (%) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes except in AF7. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one and in session eight were significantly different in all electrodes.

Delta absolute power:

One way repeated measures ANOVA analysis of delta absolute power revealed a significant main effect of session ($F (2.091, 204.958) = 21.748$, $p= .001$, $\eta p^2 = .182$), showing that session eight had the highest delta absolute power (over 1000 μV^2)[20], followed by session two with around 500 μV^2 delta absolute power and with minimum values in sessions three and four (around 45 μV^2). There was also a significant main effect of tDCS time ($F (1, 98) = 39.544$, $p= .001$, $\eta p^2 = .288$), showing that delta absolute power was higher in EEG1 compared with EEG2, and a significant main effect of electrode ($F (1.208, 118.415) = 75.130$, $p= .001$, $\eta p^2 = .434$) indicating that electrodes from the highest ($\approx$ 550 μV^2) to the lowest ($\approx$ 20 μV^2) delta absolute power were Cz, F4, AF8, FC6, Af4, F3, Fpz and AF7.

In addition, there was an interaction session x tDCS time ($F (2.097, 205.510) = 6.905$, $p= .001$, $\eta p^2 = .066$), showing that higher delta absolute power in EEG1 compared with EEG2 was found especially in sessions eight ($\approx$ 1000 μV^2 and 500 μV^2, respectively), two ($\approx$ 500 μV^2 and 40 μV^2, respectively), one ($\approx$ 400 μV^2 and 70 μV^2, respectively) and three ($\approx$ 400 μV^2 and 15 μV^2, respectively). In the rest of sessions a more similar delta absolute power was found between both tDCS time conditions. There was a significant interaction session x electrode ($F (2.647, 259.453) = 17.870$, $p= .001$, $\eta p^2 = .154$), showing that session eight had the highest delta absolute power in all electrodes followed by session two, in which electrodes Fpz and AF7 had the lowest delta absolute power. The session with lowest delta absolute power in all electrodes was session three.

A significant interaction tDCS time x electrode ($F (1.195, 117.145) = 35.422$, $p= .001$, $\eta p^2 = .265$), showed that in all electrodes higher delta absolute power was associated with EEG1 compared with EEG2, but this difference was smallest in electrodes Fpz and AF7. Lastly, a three way interaction session x tDCS time x electrode ($F (2.620, 256.807) = 8.320$, $p= .001$, $\eta p^2 = .078$), showed that in EEG1, session eight followed by session two had the highest delta absolute power in all electrodes reaching over 1000 μV^2, however in EEG2, delta absolute power values were lower (maximum around 500 in session eight followed by session six showing around 200 μV^2), but lower than 100 μV^2 in the rest of the sessions. Delta absolute power values across sessions and electrode positions are represented in Figure 0-11.

[20] Data processing was not able to remove all artefacts in this frequency band and absolute power values appear extremely high. Testing conditions that might have caused the artefacts will be described in the discussion.

To assess the final outcome of the intervention, delta absolute power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 delta absolute power in session one was significantly lower than EEG1 delta absolute power in session eight, in all electrode positions: Cz (t (100) = -3.696, p =.001), AF8 (t (100) = -3.485, p =.001), AF4 (t (100) = -4.202, p =.001), Fpz (t (100) = -2.566, p =.012), F4 (t (100) = -3.487, p =.001), FC6 (t (100) = -3.293, p =.001), F3 (t (100) = -3.951, p =.001) and AF7 (t (100) = -2.065, p =.041). EEG2 delta absolute power in session one was significantly lower than EEG2 delta absolute power in session eight, for all electrodes: Cz (t (110) = -5.370, p =.001), AF8 (t (110) = -5.222, p =.001), AF4 (t (110) = -5.296, p =.001), Fpz (t (110) = -4.899, p =.001), F4 (t (110) = -5.322, p =.001), FC6 (t (110) = -5.300, p =.001), F3 (t (110) = -5.157, p =.001) and AF7 (t (110) = -3.373, p =.001).

To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 delta absolute power was significantly higher than EEG2 delta absolute power in session one, in all electrodes except AF7: Cz (t (118) = 6.528, p =.001), AF8 (t (118) = 6.429, p =.001), AF4 (t (118) = 5.480, p =.001), Fpz (t (118) = 3.246, p =.001) and F4 (t (118) = 6.552, p =.001), FC6 (t (118) = 6.646, p =.001) and F3 (t (118) = 5.345, p =.001). In session eight, EEG1 delta absolute power was higher than EEG2 delta absolute power in all electrodes except in Fpz and AF7: Cz (t (100) = 2.986, p =.004), AF8 (t (100) = 2.759, p =.007), AF4 (t (100) = 2.726, p =.008), F4 (t (100) = 2.738, p =.007), FC6 (t (100) = 2.855, p =.005) and F3 (t (100) = 2.365, p =.020).

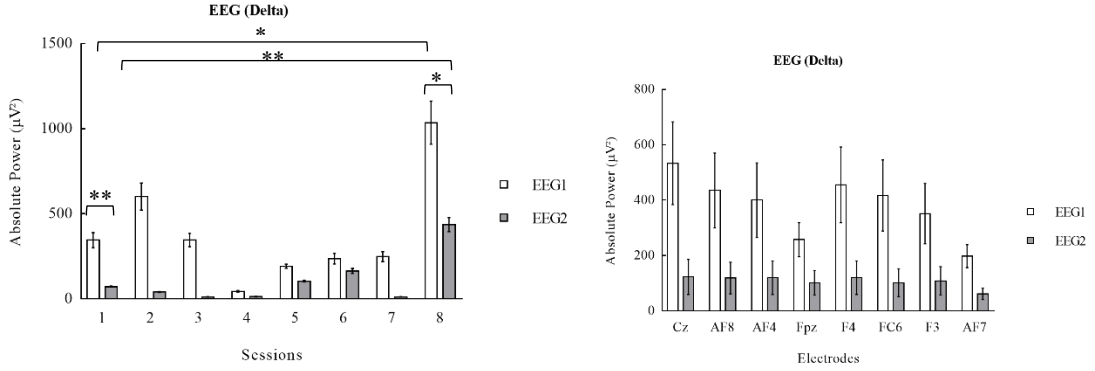


Figure 0-11. Electroencephalogram (EEG) delta absolute power in real stimulation condition. Delta absolute power (μV^2) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Notes: data was contaminated with multiple artefacts that were not completely removed after data processing, possibly due to sweating during the experimental sessions, which would explain that absolute power values appear extremely high low frequencies. Significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes locations. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found in ell electrodes except AF7, and in session eight, EEG1 and EEG2 were significantly different in all electrodes except in Fpz and AF7.

Theta relative power:

One way repeated measures ANOVA analysis of theta relative power revealed a significant main effect of session (F (6.117, 599.506) = 48.927, p= .001, ηp^2 = .333), showing that sessions three, seven and four had the highest theta relative power (≈ 7%) and session eight had the lowest level of theta relative power (≈ 1%). A main effect of tDCS time (F (1, 98) = 65.568, p= .001, ηp^2 = .401), showed that EEG1 had lower theta relative power than EEG2. There was also a significant main effect of electrode (F (3.785, 272.769) = 138.430, p= .001, ηp^2 = .736) indicating that electrodes from the highest (≈ 7%) to the lowest (≈ 1%) theta relative power were AF4, F3, Fpz, AF8, Cz, F4, AF7 and FC6. In addition, there was an interaction session x tDCS time (F (7, 686) = 28.298, p= .001, ηp^2 = .224), showing that theta relative power was lower in EEG1 compared with EEG2 in sessions one (≈ 2 % and 4%, respectively), two (≈ 1 % and 5 %, respectively), three (≈ 1 % and 7 %, respectively), four (≈ 5 % and 7 %, respectively) and seven (≈ 1 % and 7 %, respectively), whereas in the rest of the sessions both tDCS time conditions had more similar levels of theta relative power. There was a significant interaction session x electrode (F (18.943, 1859.359) = 10.932, p= .001, ηp^2 = .100), showing that sessions three had the highest theta relative power in all electrodes, followed by session seven, and the lowest theta relative power was in session eight.

There was also an interaction tDCS time x electrode (F (3.955, 387.632) = 28.164, p= .001, ηp^2 = .223), indicating that electrodes AF4, F3 and Fpz showed the highest relative theta power in EEG2, and the lowest was in EEG1, especially in electrodes Cz, F4 and FC6. A three way interaction session x tDCS time x electrode (F (19.459, 1906.991) = 8.452, p= .001, ηp^2 = .079), showed that in EEG1 session three had the highest delta relative power in all electrodes, and in EEG2 session seven and session two had the highest theta relative power in all electrodes, whereas in both tDCS conditions the lowest theta relative power was in session eight. Relative theta power values across sessions and electrode positions are represented in Figure 0-12.

To assess the final outcome of the intervention, theta relative power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 theta relative power in session one was significantly higher than EEG1 delta relative power in session eight, in all electrode positions except in AF7: Cz (t (100) = 3.090, p =.003), AF8 (t (100) = 3.081, p =.003), AF4 (t (100) = 4.872, p =.001), Fpz (t (100) = 3.427, p =.001) and F4 (t (100) = 3.426, p =.001), FC6 (t (100) = 2.532, p =.013) and F3 (t (100) =

3.193, p =.002). EEG2 theta relative power in session one was significantly higher than EEG2 theta relative power in session eight, in all electrode positions: Cz (t (110) = 6.304, p =.001), AF8 (t (110) = 5.548, p =.001), AF4 (t (110) = 6.219, p =.001), Fpz (t (110) = 5.617, p =.001) and F4 (t (110) = 5.924, p =.001), FC6 (t (110) = 5.050, p =.001), F3 (t (110) = 3.940, p =.001) and AF7 (t (110) = 3.041, p =.003). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 theta relative power was significantly lower than EEG2 theta relative power in session one in all electrodes: Cz (t (118) = -6.773, p =.001), AF8 (t (118) = -7.119, p =.001), AF4 (t (118) = -5.712, p =.001), Fpz (t (118) = -5.048, p =.001), F4 (t (118) = -6.659, p =.001), FC6 (t (118) = -6.088, p =.001), F3 (t (118) = -4.137, p =.001) and AF7 (t (118) = -5.134, p =.001). In session eight, EEG1 theta relative power was lower than EEG2 theta relative power only in AF7 (t (100) = -2.203, p =.030).

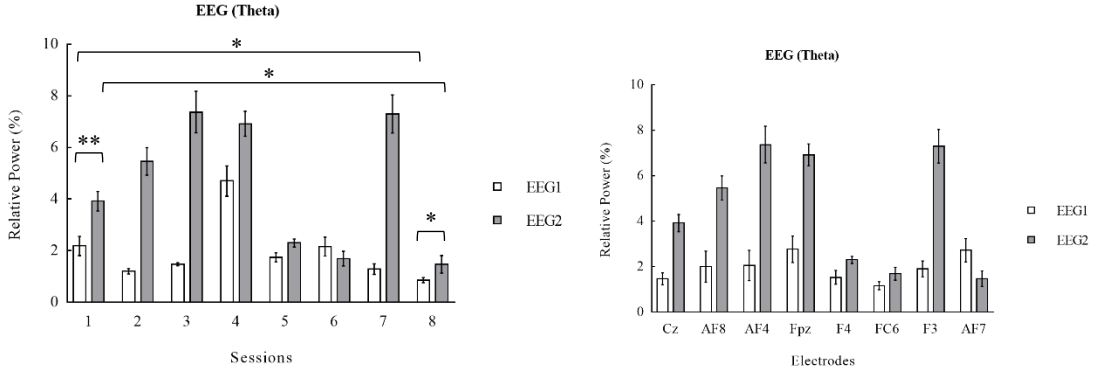


Figure 0-12. Electroencephalogram (EEG) theta relative power in real stimulation condition. Theta relative power (%) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes positions except in AF7. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrode positions. Furthermore, significant differences between EEG1 and EEG2 in session one were found in all electrodes, however in session eight only in electrode AF7.

Theta absolute power:

One way repeated measures ANOVA analysis of theta absolute power revealed a significant main effect of session (F (2.713, 265.880) = 15.134, p= .001, ηp^2 = .134), showing that sessions eight (≈ 8 μV^2), one and two (≈ 7 μV^2) had the highest theta absolute power, whereas the lowest theta absolute power was found in sessions three, four and seven (≈ 1 μV^2). There was also a significant main effect of tDCS time (F (1, 98) = 24.140, p= .001, ηp^2 = .198), showing that theta absolute power was higher in EEG1 compared with EEG2. A significant main effect of electrode (F (1.418, 139.008) = 14.242, p= .001, ηp^2 = .129) indicated that theta absolute power was similar across electrodes, with slightly higher values shown in Cz and Fpz, and the lowest in FC6. In addition, there was a significant interaction session x electrode (F (5.050, 494.882) = 3.738, p= .002, ηp^2 = .037), showing that session eight had the highest theta absolute power in all electrodes, followed by session two, and the lowest in session three. There was also an interaction tDCS time x electrode (F (1.479, 144.944) = 6.912, p= .004, ηp^2 = .066), showing that in all electrodes higher theta absolute power was associated with EEG1 compared with EEG2. A three way interaction session x tDCS time x electrode (F (4.921, 482.240) = 4.696, p= .001, ηp^2 = .046), showed that in EEG1 sessions eight and two had the highest delta relative power in all electrodes except in Fpz in which session one and session six where the highest, and in EEG2 session eight had the highest theta relative power in all electrodes, whereas in both tDCS conditions the lowest theta relative power was in session eight. Theta absolute power values across sessions and electrode positions are represented in Figure 0-13.

To assess the final outcome of the intervention, theta absolute power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 theta absolute power in session one was significantly lower than EEG1 theta absolute power in session eight, in electrode positions: Cz (t (100) = -2.093, p =.039), AF4 (t (100) = -1.475, p =.036) and F3 (t (100) = -2.120, p =.039). EEG2 theta absolute power in session one was significantly lower than EEG2 theta absolute power in session eight, in all electrodes: Cz (t (100) = -3.078, p =.003), AF8 (t (100) = -2.961, p =.004), AF4 (t (100) = -2.776, p =.006), Fpz (t (100) = -2.166, p =.032), F4 (t (100) = -3.523, p =.001), FC6 (t (100) = -3.627, p =.001), F3 (t (100) = -3.978, p =.001) and AF7 (t (100) = -2.765, p =.007). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 theta absolute power was significantly higher than EEG2 theta absolute power in session one, in all electrodes except for Fpz and AF7: Cz (t (118) = 6.521, p =.001), AF8 (t (118) = 4.013, p =.001), AF4 (t (118) = 2.879, p =.005) and F4 (t (118) = 6.278, p =.001), FC6 (t

(118) = 6.983, p =.001) and F3 (t (118) = 2.351, p =.020). In session eight, EEG1 theta absolute power was higher than EEG2 theta absolute power in electrodes Cz (t (100) = 3.039, p =.003), and FC6 (t (100) = 2.406, p =.018).

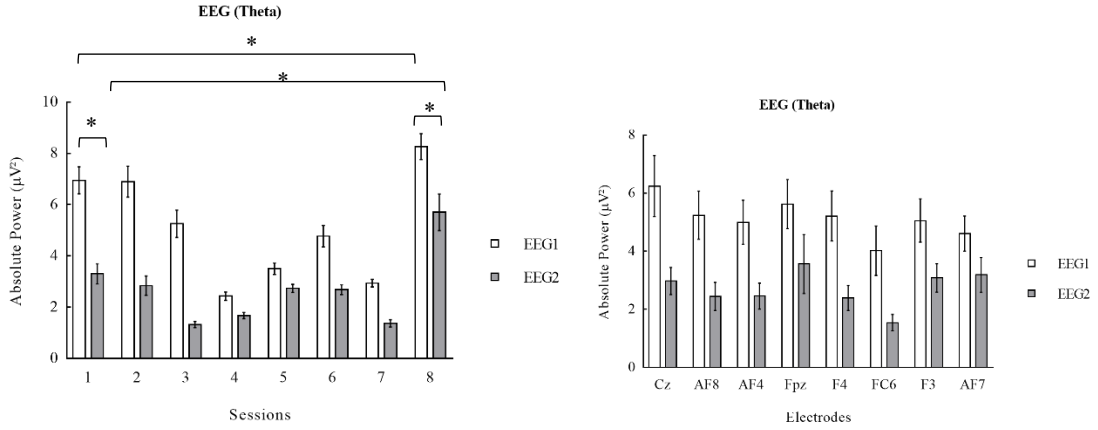


Figure 0-13. Electroencephalogram (EEG) theta absolute power in real stimulation condition. Theta absolute power (μV^2) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in electrode positions Cz, AF4 and F3. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found in all electrodes except in Fpz and AF7, and in session eight EEG1 and EEG2 were significantly different in electrodes Cz and FC6.

Alpha relative power:

One way repeated measures ANOVA analysis of alpha relative power revealed a significant main effect of session (F (4.860, 476.277) = 40.781, p= .001, ηp^2 = .294), showing that sessions two, three, four and seven had the highest alpha relative power (≈ 6%), and sessions six and eight had the lowest (≈ 1%). A significant main effect of tDCS time (F (1, 98) = 127.890, p= .001, ηp^2 = .566) showed that EEG1 had lower alpha relative power than in all sessions except in sessions six and eight. There was also a significant main effect of electrode (F (4.278, 419.223) = 228.116, p= .001, ηp^2 = .699), indicating that relative alpha power was similar across electrodes being slightly higher in Cz and lower in FC6. In addition, there was an interaction session x tDCS time (F (4.849, 475.207) = 14.217, p= .001, ηp^2 = .127) showing that alpha relative power was lower in EEG1 compared with EEG2 in sessions one (≈ 1% and 3%), two (≈ 1% and 6%), three (≈ 1% and 6%), four (≈ 4% and 6%), five (≈ 1% and 2%) and seven (≈ 1% and 6%), whereas similar levels between both tDCS conditions were found in sessions six and eight (≈ 1%).

There was a significant interaction session x electrode (F (18.732, 1835.708) = 10.182, p= .001, ηp^2 = .094), showing that sessions two, and three had the highest alpha relative power across all electrodes, whereas the lowest alpha relative power was in session eight for all electrodes. There was also an interaction tDCS time x electrode (F (3.874, 379.603) = 26.172, p= .001, ηp^2 = .211), indicating that all electrodes showed similar levels of alpha relative power between tDCS conditions (EEG1 having around 1-2% and EEG2 around 3-5%). A three way interaction session x tDCS time x electrode (F (17.442, 1709.364) = 7.419, p= .001, ηp^2 = .070), showed that in all electrodes in EEG1 the highest alpha relative power was in session three, whereas in EEG2 the highest was session two. In both tDCS conditions the lowest alpha relative power was shown in session eight and the lowest alpha relative power was in electrode FC6 in both conditions. Alpha relative power values across sessions and electrode positions are represented in Figure 0-14.

To assess the final outcome of the intervention, alpha relative power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 alpha relative power in session one was significantly higher than EEG1 alpha relative power in session eight, in all electrode positions: Cz (t (100) = 4.479, p =.001), AF8 (t (100) = 4.296, p =.001), AF4 (100) = 5.503, p =.001), Fpz (t (100) = 4.487, p =.001), F4 (t (100) = 4.625, p =.001), FC6 (t (100) = 3.518, p =.001) F3 (t (100) = 5.075, p =.001) and AF7

(t (100) = 3.457, p =.001). EEG2 alpha relative power in session one was significantly higher than EEG2 alpha relative power in session eight in all electrodes: Cz (t (110) = 3.124, p =.002), AF8 (t (110) = 4.085, p =.001), AF4 (110) = 5.198, p =.001), Fpz (t (110) = 5.114, p =.001), F4 (t (110) = 4.135, p =.001), FC6 (t (110) = 3.609, p =.001) F3 (t (110) = 4.336, p =.001) and AF7 (t (110) = 2.902, p =.004). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 alpha relative power was significantly lower than EEG2 alpha relative power in session one, in all electrodes: Cz (t (118) = -6.489, p =.001), AF8 (t (118) = -4.310, p =.001), AF4 (118) = -3.560, p =.027), Fpz (t (118) = -3.006, p =.003), F4 (t (118) = -5.489, p =.001), FC6 (t (118) = -5.681, p =.001), F3 (t (118) = -2.249, p =.026) and AF7 (t (118) = -2.492, p =.014). In session eight there were no significant differences in alpha relative power between EEG1 and EEG2.

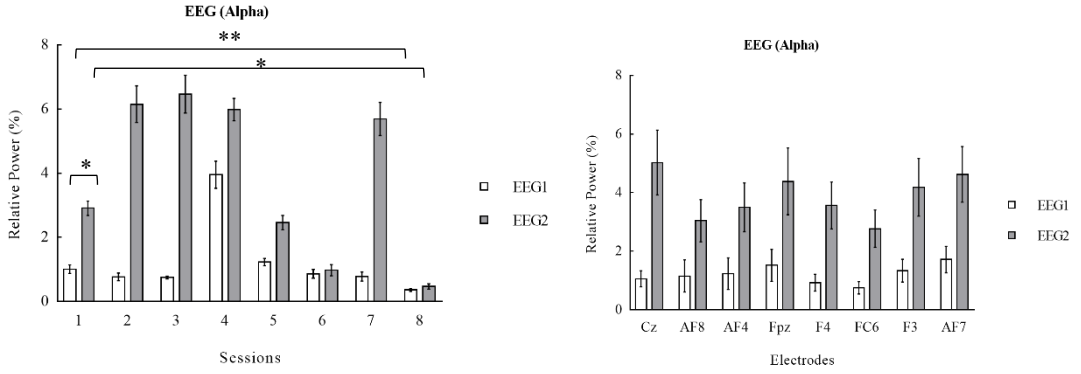


Figure 0-14. Electroencephalogram (EEG) alpha relative power in real stimulation condition. Alpha relative power (%) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrode positions. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found in all electrodes, however in session eight, there were no significant differences between alpha relative power between EEG1 and EEG2.

Alpha absolute power:

One way repeated measures ANOVA analysis of alpha absolute power revealed a significant main effect of session (F (4.731, 463.328) = 32.697, p= .001, ηp^2 = .250), showing that sessions three, eight and one had the highest alpha absolute power at around 3-4 μV^2, whereas the lowest was alpha absolute power was found in sessions three and seven with approximately 1 μV^2. There was also a significant main effect of tDCS time (F (1, 98) = 31.214, p= .001, ηp^2 = .242), showing that alpha absolute power was higher in EEG1 compared with EEG2. A significant main effect of electrode (F (3.165, 310.174) = 332.581, p= .001, ηp^2 = .772) indicated Cz showed the highest alpha absolute power at around 4 μV^2, the lowest was FC6 with around 1.5 μV^2, and the rest of electrodes showed similar alpha absolute power (1.5-2.5 μV^2). In addition, there was a significant interaction session x tDCS time (F (4.834, 473.737) = 3.396, p= .006, ηp^2 = .033), showing that higher alpha absolute power in EEG1 compared with EEG2 was found in all sessions except in session five. There was a significant interaction session x electrode (F (3.266, 320.105) = 16.325, p= .001, ηp^2 = .143), showing that session two had the highest alpha absolute power in all electrodes followed by session five in all electrodes except in Cz in which session six was the second highest, and the lowest alpha absolute power was found in session three, in all electrodes.

A significant interaction tDCS time x electrode (F (1.722, 122.288) = 14.242, p= .001, ηp^2 = .167), showed that in all electrodes higher alpha absolute power was associated with EEG1 compared with EEG2 with the smallest difference shown in AF7. Lastly, a three way interaction session x tDCS time x electrode (F (11.892, 1165.459) = 9.903, p= .001, ηp^2 = .092), showed that in both EEG1 and EEG2, session two had the highest alpha absolute power in all electrodes, and session three had the lowest, however in EEG2, session two still had the highest alpha absolute power but sessions three and seven were associated with the lowest alpha absolute power in all electrodes. Alpha absolute power values across sessions and electrode positions are represented in Figure 0-15.

To assess the final outcome of the intervention, alpha absolute power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 alpha absolute power in session one was significantly lower than EEG1 alpha relative power in session eight, in electrode positions AF8 (t (100) = -2.068, p =.011) and AF4 (t (100) = -2.539, p =.027). EEG2 alpha absolute power in session one was significantly higher than EEG2 alpha absolute power in session eight in electrodes Cz (t (110) = 3.523,

p =.001), F4 (t (110) = 2.601, p =.011), FC6 (110) = 2.163, p =.033), F3 (t (110) = -2.047, p =.043) and AF7 (110) = 3.371, p =.011). To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 alpha absolute power was significantly higher than EEG2 alpha absolute power in session one, in all electrodes except Fpz and AF7: Cz (t (118) = 3.474, p =.001), AF8 (t (118) = 3.755, p =.001), AF4 (118) = 3.305, p =.027), F4 (t (118) = 4.825, p =.001), FC6 (t (118) = 2.224, p =.001), and F3 (t (118) = -2.360, p =.028). In session eight EEG1 alpha absolute power was higher than EEG2 alpha absolute power in all electrodes except in Fpz: Cz (t (100) = 5.269, p =.001), AF8 (t (100) = 4.762, p =.001), AF4 (100) = 4.840, p =.001), F4 (t (100) = 4.864, p =.001), FC6 (t (100) = 5.630, p =.001), F3 (t (100) = 3.977, p =.001) and AF7 (t (100) = 2.324, p =.022).

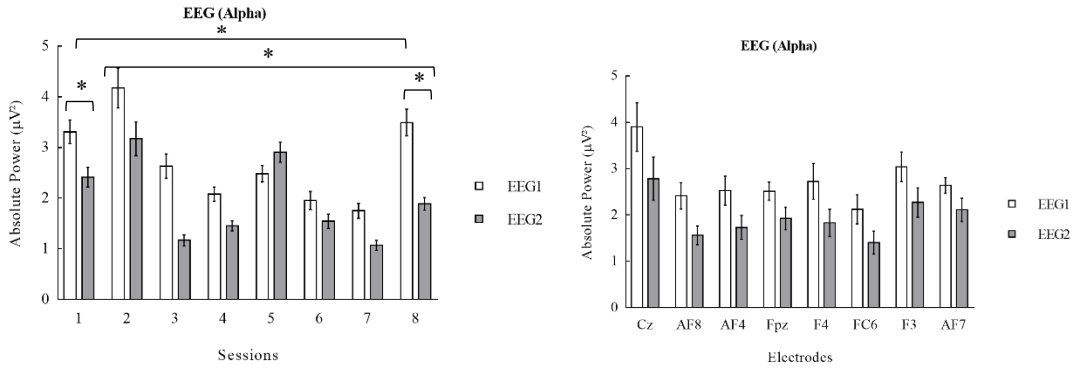


Figure 0-15. Electroencephalogram (EEG) alpha absolute power in real stimulation condition. Alpha absolute power (μV^2) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found only in electrodes AF8 and AF4. Significant differences between EEG2 in session one and EEG2 in session eight were found in electrodes Cz, F4, FC6. F3 and AF7. Furthermore, significant differences between EEG1 and EEG2 in session one were found in all electrodes except in Fpz and AF7, and in session eight, EEG1 and EEG2 were significantly different in all electrodes except in Fpz.

Beta relative power:

One way repeated measures ANOVA analysis of beta relative power revealed a significant main effect of session (F (5.910, 579.132) = 101.779, p= .001, ηp^2 = .509), showing that session four had the highest beta relative power (≈ 30%), and session eight had the lowest (≈ 3%). A significant main effect of tDCS time (F (1, 98) = 176.341, p= .001, ηp^2 = .643) showed that EEG1 had lower beta relative power than in EEG2. There was also a significant main effect of electrode (F (3.505, 343.510) = 446.450, p= .001, ηp^2 = .820), indicating that FC6 had the highest beta relative power (≈ 20%), and the lowest was in Cz (≈ 3%), but all electrodes had similar levels of beta relative power except the highest (FC6). In addition, there was an interaction session x tDCS time (F (5.891, 557.340) = 20.332, p= .001, ηp^2 = .172) showing that beta relative power was lower in EEG1 compared with EEG2. The difference between both tDCS conditions was around less than 5% difference of power in sessions one, five, six and eight, but larger in sessions three (≈ 3% and 20%, respectively), four (≈ 13% and 30%, respectively) and seven (≈ 5% and 25%, respectively).

There was also a significant interaction session x electrode (F (16.741, 1640.593) = 27.571, p= .001, ηp^2 = .220), showing that session four (especially in FC6) had the highest beta relative power in all electrodes, followed by session seven, and the lowest beta relative power in all electrodes was found in sessions six and eight. A significant interaction tDCS time x electrode (F (4.176, 409.285) = 103.483, p= .001, ηp^2 = .514), indicated that the largest difference between tDCS time conditions was in FC6 (EEG1 was around 6% and EEG2 around 22%), and in the rest of electrodes the difference between tDCS time conditions was around 10% of the power. A three way interaction session x tDCS time x electrode (F (17.017, 1667.620) = 15.529, p= .001, ηp^2 = .137), showed that in EEG1 the highest beta relative power was found in session three in all electrodes except in FC6 in which session five was the highest. In EEG2 the highest beta relative power was found in session four in all electrodes. Session eight had the lowest beta relative power in both tDCS conditions. Beta relative power was similar across electrodes except for FC6 that had the highest levels. Beta relative power values across sessions and electrode positions are represented in Figure 0-16.

To assess the final outcome of the intervention, beta relative power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 beta relative power in session one was significantly higher than EEG1 beta relative power in session eight, in all electrode positions except in Fpz and AF7: Cz

(t (100) = 2.977, p =.004), AF8 (t (100) = 2.221, p =.029), AF4 (100) = 3.604, p =.001), F4 (t (100) = 2.734, p =.007), FC6 (t (100) = 3.538, p =.001) and F3 (t (100) = 3.827, p =.001). EEG2 beta relative power in session one was significantly higher than EEG2 beta relative power in session eight in all electrodes: Cz (t (110) = 9.717, p =.001), AF8 (t (110) = 7.245, p =.001), AF4 (t (110) = 6.998, p =.001), Fpz (t (110) = 5.059, p =.001), F4 (t (110) = 8.646, p =.001), FC6 (t (110) = 11.212, p =.001), F3 (t (110) = 6.369, p =.001) and AF7 (t (110) = 2.031, p =.045).

To compare the effects of tDCS before and after the stimulation within session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 beta relative power was significantly lower than EEG2 beta relative power in session one, in all electrodes: Cz (t (118) = -8.713, p =.001), AF8 (t (118) = -5.592, p =.001), AF4 (t (118) = -4.192, p =.001), Fpz (t (118) = -3.354, p =.001), F4 (t (118) = -6.654, p =.001), FC6 (t (118) = -8.097, p =.001), F3 (t (118) = -2.614, p =.010) and AF7 (t (118) = -3.016, p =.003). In session eight there were no significant differences between beta relative power in EEG1 and EEG2.

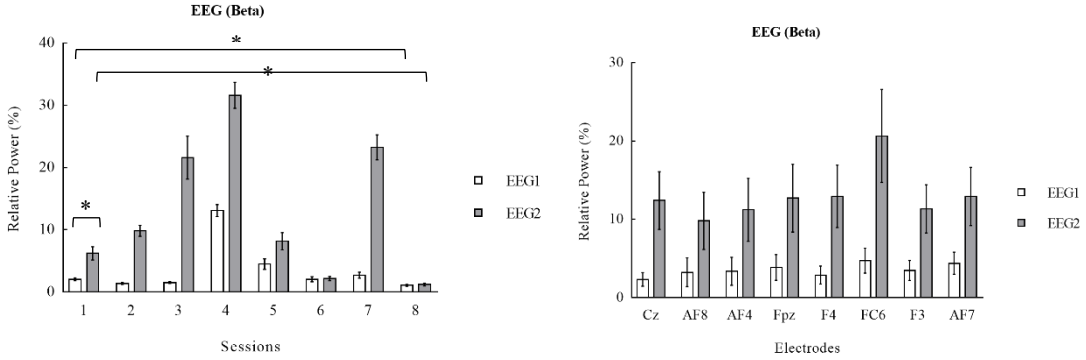


Figure 0-16. Electroencephalogram (EEG) beta relative power in real stimulation condition. Delta relative power (%) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes positions except in Fpz and AF7. Significant differences between EEG2 in session one and EEG2 in session eight were found in all electrodes. Furthermore, significant differences between EEG1 and EEG2 in session one were found in all electrodes, however in session eight, there were no significant differences in beta relative power between EEG1 and EEG2.

Beta absolute power:

One way repeated measures ANOVA analysis of beta absolute power revealed a significant main effect of session (F (2.760, 270.448) = 14.672, p= .001, ηp^2 = .130), showing that sessions five and eight had the highest beta absolute power at around 10 μV^2, whereas the lowest beta absolute power was found in sessions three and six, with approximately 4 μV^2. There was also a significant main effect of tDCS time (F (1, 98) = 10.883, p= .001, ηp^2 = .100), showing that beta absolute power was higher in EEG1 compared with EEG2. A significant main effect of electrode (F (2.023, 198.268) = 95.886, p= .001, ηp^2 = .495) indicated the electrode FC6 showed the highest beta absolute power at around 12 μV^2, and the rest of electrodes showed similar beta absolute power (4-7 μV^2). In addition, there was a significant interaction session x tDCS time (F (2.846, 278.870) = 4.619, p= .004, ηp^2 = .045), showing that higher beta absolute power in EEG1 compared with EEG2 was found in all sessions except in sessions four and five (in which tDCS conditions had similar beta absolute power), with the greatest difference between EEG1 and EEG2 found in session eight (10 μV^2 to 5 μV^2, respectively). There was a significant interaction session x electrode (F (2.245, 219.965) = 13.608, p= .001, ηp^2 = .122), showing that sessions two and four had the highest beta absolute power in all electrodes except in FC6 and F3 in which session five had the highest absolute beta power. Beta absolute power values across sessions and electrode positions are represented in Figure 0-17.

To assess the final outcome of the intervention, beta absolute power results were compared between sessions one and eight with paired t-tests (2-tailed) for each electrode position and each frequency band separately. Results showed that EEG1 beta absolute power in session one was significantly lower than EEG1 beta absolute power in session eight, in all electrode positions except in FC6: Cz (t (100) = -3.227, p =.002), AF8 (t (100) = -4.906, p =.001), AF4 (t (117) = -5.330, p =.001), Fpz (t (117) = -8.303, p =.001), F4 (t (100) = -3.256, p =.002), F3 (t (100) = -4.483, p =.001) and AF7 (t (100) = -3.758, p =.001). EEG2 beta absolute power in session one was significantly higher than EEG2 beta absolute power in session eight, in electrodes Cz (t (110) = 2.679, p =.009) and FC6 (t (110) = 3.162, p =.002), and absolute beta power was significantly lower in EEG1 than in EEG2 in Fpz (t (110) = -2.203, p =.030). To compare the effects of tDCS before and after the stimulation within each session, considering sessions one and eight, paired t-test (2-tailed) revealed that EEG1 beta absolute power was significantly higher than EEG2 beta absolute power in session one, in all electrodes, except in Fpz in which absolute beta power was lower in EEG1 compared with EGG2: Cz (t (118) = 4.243, p =.001), AF8 (t (118) = 6.398, p =.001), AF4 (t (118) = 5.147, p =.001), Fpz (t (118) = -7.086, p =.001), F4 (t (118) = 7.720, p =.016), FC6 (t (118) = 9.712, p =.001), F3(t (118) = 5.032, p =.001)

and AF7 (t (118) = 3.835, p =.001). In session eight, EEG1 beta absolute power was significantly higher than EEG2 beta absolute power in all electrodes: Cz (t (100) = 5.962, p =.001), AF8 (t (100) = 6.647, p =.001), AF4 (t (100) = 4.465, p =.001), Fpz (t (100) = 4.662, p =.001), F4 (t (100) = 4.275, p =.016), FC6 (t (100) = 4.731, p =.001), F3 (t (100) = 5.092, p =.001) and AF7 (t (100) = 3.923, p =.001).

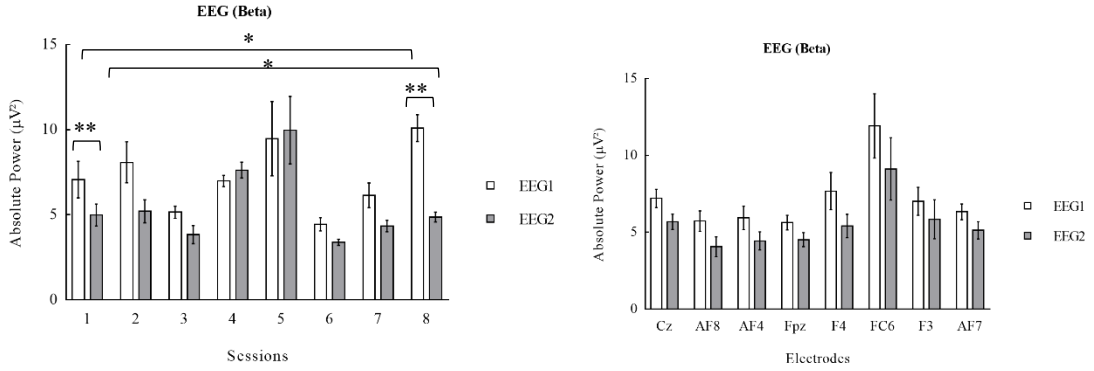
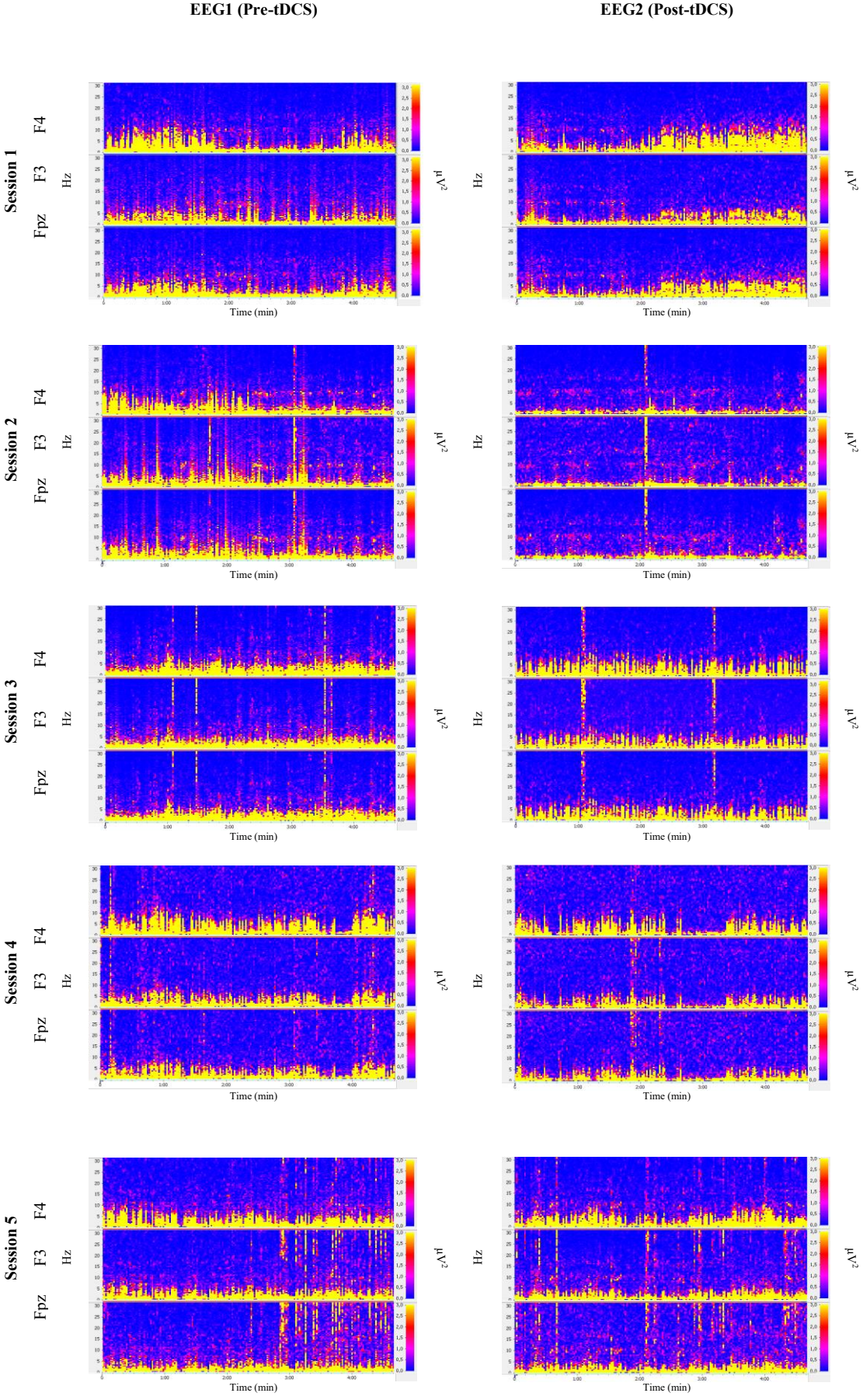


Figure 0-17. Electroencephalogram (EEG) beta absolute power in real stimulation condition. Beta absolute power (μV^2) of EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions, and across electrode positions with the international EEG 10/10 system (Cz, AF8, AF4, Fpz, F4, FC6, F3 and AF7). Data represents mean and SEM (* $p < .05$; ** $p \leq .01$). Note: significant differences between EEG1 in session one and EEG1 in session eight were found in all electrodes except in FC6. Significant differences between EEG2 in session one and EEG2 in session eight were found in electrodes Cz, Fpz and FC6. Furthermore, significant differences between EEG1 and EEG2 in session one were found in all electrodes, and in session eight EEG1 and EEG2 were significantly different in all electrodes.

Correlations

To investigate the relationship between gambling severity and the rest of the variables, 2-tailed Pearson's correlation indicated that gambling severity measured with PG-YBOCS correlated positively with EEG1 relative power across different electrode positions and frequency bands: PG-YBOCS correlated negatively with delta relative power in AF8 (r = -.743, n = 8, p =.035), AF4 (r =-.764, n = 8, p =.027), Fpz (r =-.751, n = 8, p =.032), F4 (r =-.709, n = 8, p =.049), F3 (r = -.735, n = 8, p =.038) and AF7 (r = -.811, n = 8, p =.015). PG-YBOCS correlated positively with theta relative power in Cz (r =.744, n = 8, p =.034), AF8 (r =.804, n = 8, p =.016), AF4 (r =.840, n = 8, p =.009), Fpz (r =.909, n = 8, p =.002), F4 (r =.812, n = 8, p =.014), FC6 (r =.774, n = 8, p =.024), F3 (r =.845, n = 8, p =.008) and AF7 (r =.921, n = 8, p =.001). PG-YBOCS correlated positively with alpha relative power in AF8 (r =.742, n = 8, p =.035), AF4 (r =.771, n = 8, p =.025), Fpz (r =.737, n = 8, p =.037), F4 (r =.725, n = 8, p =.042), F3 (r =.725, n = 8, p =.042) and AF7 (r =.725, n = 8, p =.042). PG-YBOCS correlated positively with beta relative power in AF8 (r =.717, n = 8, p =.045), AF4 (r =.729, n = 8, p =.040) and AF7 (r =.727, n = 8, p =.041). Correlations with CGT could not be computed due to the lack of enough data points.

Having found all significant correlations between gambling severity and EEG variables, in the EEG1 relative power (pre-tDCS) recordings, these variables were further explored with the aim to find physiological markers that could inform about gambling-related behaviours and symptomatology. There were no significant correlations between EEG1 relative power variables and any other self-report or behavioural variable. An EEG power spectrogram displaying electrode positions Fpz, F4 and F3 for ventral, right and left PFC respectively, is presented in figures Figure 0-18.


EEG1 (Pre-tDCS)
EEG2 (Post-tDCS)
Session 1
Session 2
Session 3
Session 4
Session 5
Fpz
F3
F4
Hz
μV²
Time (min)

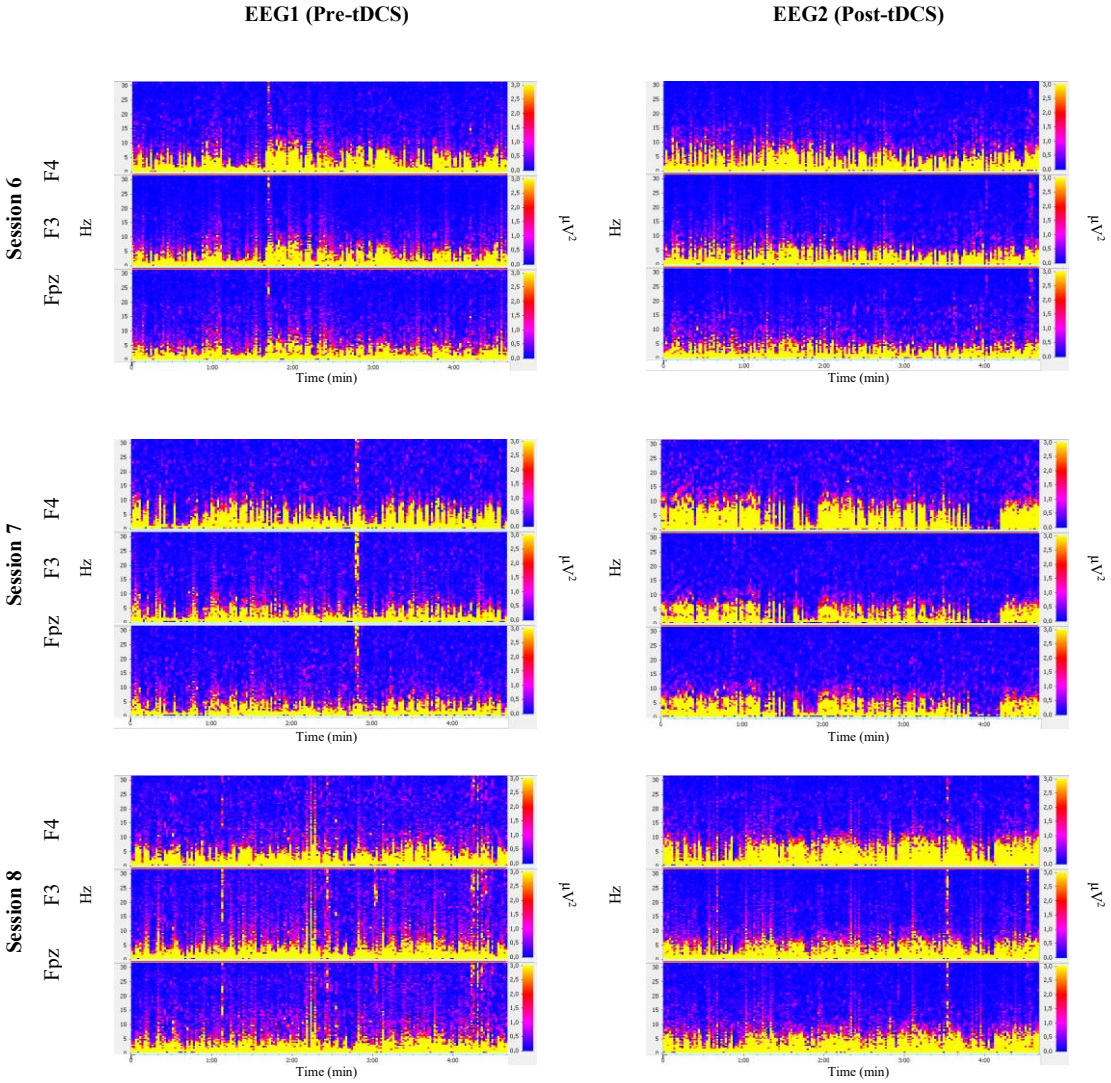


Figure 0-18. Electroencephalography (EEG) spectrogram during transcranial direct current stimulation (tDCS) in real stimulation condition. EEG power (μV^2) in frequencies 0-30 Hz during five minutes EEG resting state recordings before tDCS (EEG1) and after tDCS (EEG2), across eight sessions in real stimulation condition. Electrodes positions displayed in the spectrogram with the international EEG 10/10 system are Fpz, F4 and F3 for ventral, right and left prefrontal cortex (PFC), respectively.

Discussion

This experiment was originally designed as an RCT, with the aim to study the effects of tDCS across sessions in combination with the CBT treatment offered at the NPGC, to investigate the potential use of this particular intervention to improve current treatment approaches for GD. The trial planned followed a crossover design with two groups, real stimulation and sham, with parallel assignment. The data was collected according to the RCT design, however, the analysis plan had to be adapted to fit a single-participant design, due to the recruitment complications described in the clinical settings at the NPGC section 4.1.3 in Chapter 4. The data collected consisted of gambling symptomatology self-report measures, cognitive behavioural tasks and EEG resting state, from two participants who attended eight sessions of tDCS combined with CBT. The participants were assigned to two different tDCS groups (one participant received real stimulation, and the other participant received sham). Data was analysed according to two different methodologies usually employed to analyse single-participant designs: percentage change of scores across sessions was calculated as in case report studies, and statistical analysis was performed, based on single-case study analysis methods. However, given that data was collected following the RCT design, both, the case report and the single-case study analysis approaches employed, had to be adapted to fit the existing data, as described in the analysis plan section 4.3.6 in Chapter 4. Results revealed significant effects of the intervention combining the tDCS procedure with CBT on gambling severity, cravings, cognitive task performance, and EEG power frequency, in both participants.

Participant allocated to tDCS sham condition

In the participant allocated to sham condition, results showed significant differences across sessions in gambling severity measured with PG-YBOCS, gambling cravings measured with VAS, and inhibitory control measured with SSRT (which indicates de ability to stop a prominent response). To assess the final outcome of the intervention, session eight was compared against session one, revealing that there were statistically significant changes, which were also quantified using percentage change of scores: a decrease of 44% in PG-YBOCS, a decrease of 38% in G-SAS, a decrease of 64% in DA, an increase of 10% of SST total correct trials and a decrease of 16% of SSRT. VAS statistics could only be calculated across sessions due to a lack of enough data points, which showed significant differences across sessions, and in addition, percentage change between the last and the first session revealed a reduction of 86% in gambling cravings. Across sessions, it was identified that session five had the lowest scores in self-report measures PG-YBOCS and VAS. Similarly, session five in the cognitive behavioural tasks variables show the lowest scores in: IST mean number of boxes opened

and total correct, and SST total correct stop and go, total correct stop and SSRT[21]. The final outcome of the intervention showed that the sham tDCS procedure combined with CBT was associated with a significant improvement of GD severity and symptomatology, including cravings, as well as behavioural impulsivity, and the ability to inhibit prominent responses. Another GD case study using a longitudinal intervention with real stimulation tDCS over bilateral DLPFC reported similar improvements, showing a reduction of 62.5% of PG-YBOCS, a reduction of 100% of VAS for gambling cravings, a reduction of 69.2% in G-SAS and a reduction of 23.6% of self-reported impulsivity (Martinotti et al., 2018). The effectiveness of tDCS to improve addiction symptomatology is still unclear (Trojak et al., 2017). Literature reviews identified a medium size effect in favour of active tDCS compared with sham to reduce cravings (Jansen et al., 2013), however, beyond cravings modulation, research also found no differences between active and sham groups in cigarette consumption, suggesting that real tDCS might not be more effective than sham to reduce addictive behaviours (Mondino et al., 2018). The effectiveness of tDCS to treat addictive behaviours needs to be further investigated (Coles et al., 2018).

EEG frequency power analysis results showed significant differences in relative and absolute power of the four frequency bands (delta, theta, alpha and beta), across sessions, between the first and the last session, and within session one and session eight. In particular, to measure of the cumulative effects of tDCS, the EEG outcomes were compared at the beginning and at the end of the eight session intervention, considering separately the EEG recordings before and after the tDCS. Results revealed an increase of EEG1 delta, theta, alpha and beta absolute power, an increase of EEG2 mean frequency power, delta and beta relative power, and an increase of alpha relative power in both EEG1 and EEG2 in session eight compared with session one. However, there was also a decrease in session eight compared with session one in EEG1 mean frequency power, delta relative power and beta relative power, as well as a decrease of EEG2 delta, theta, alpha and beta absolute power, and an increase of theta relative power in both EEG1 and EEG2. To quantify the short term effects of tDCS, results within each session showed that there were EEG power changes after the participant received sham tDCS while completing the cognitive task SST: in session one, there was an increase delta, theta and beta absolute power, and an increase of delta and beta relative power. There was also a decrease of

[21] Statistical analysis comparing each of the eight sessions against the rest was not performed (paired comparisons were conducted only between the first and the last session), and therefore, the results discussed about any other specific session, refer simply to the variables values observed. The statistical analysis was more focused on assessing the final outcome of the intervention, and to compare each session against the others (involving between and within sessions analysis), would extend the work load and amount of data reported excessively, considering the limitations associated to the adapted analysis methodologies used, the time available for the project and the limited space to report the results. Nevertheless, this will be considered to further explore the data in the future.

theta and alpha relative power and a decrease of alpha absolute power. In session eight, there was an increase of delta, theta and beta relative power, as well as a decrease of delta, theta, alpha and beta absolute power and alpha relative power.

These results showed that there was an increase of absolute power across all frequency bands (delta, theta, alpha and beta) in session eight compared with session one. Particularly, the increase was in the pre-tDCS EEG recording (EEG1), which occurred just after the participant completed the IST (which measures reflection impulsivity, and was used as a priming task to activate the brain networks associated with gambling behaviours), and just before the participant received sham tDCS. Furthermore, there was a decrease of absolute power across all frequency bands (delta, theta, alpha and beta) in session eight compared with session one, in the post-tDCS EEG recording (EEG2), just after the participant received sham tDCS and completed the SST, which measures inhibition control. Across all EEG variables, changes of power frequency comparing session one against session eight were more consistent between electrode locations in EEG2 compared with EEG1, showing that significant differences were shown in a higher number of electrodes in EEG2.

Within each session, significant differences were shown in a higher number of electrodes in session eight, compared with session one. In addition, the increases of EEG power within each session were more consistent between session one and session eight, however, the decreases of EEG power involved more frequency variables in session eight compared with session one, which could perhaps be interpreted as a result of the accumulative effects of the intervention. However, the more widespread decrease of EEG power within session eight compared with session one, could also be explained by the fact that EEG1 power increased between session one and session eight, and therefore the decrease seen from EEG1 to EEG2 within session eight, might have been driven more by the increase of EEG1 between sessions, than by a greater decrease from EEG1.

Assuming that sham tDCS had no effects over physiological substrates, the changes of EEG absolute power observed could be explained, in part, by an effect of the cognitive task performance before each EEG recording, on the brain circuitry underlying the associated cognitive functions to each task. This would mean that IST performance across sessions could have facilitated the increase of EEG absolute power in all frequency bands, whereas SST performance might have facilitated the decrease of EEG absolute power in all frequency bands. However, participants received CBT sessions after the

tDCS procedure, and with the current data is not possible to differentiate the specific effects of CBT from the effects of the experimental procedure, which includes the cognitive tasks and sham tDCS. Therefore, the changes in EEG power seen across sessions could have been facilitated by the tDCS experimental protocol, which included cognitive task performance, the CBT alone, or the combination of the tDCS protocol with CBT.

Moreover, the absence of physiological effects on the brain associated with sham tDCS is still under debate (Neri et al., 2020), so an alternative explanation of the results could be that the electrical current delivered during the ramp up and ramp down of the sham condition, contributed to produce the changes observed in the EEG data, showing the mentioned increase of absolute power in all frequency bands from session one to session eight before the sham tDCS, but the decrease of absolute power in all frequency bands from session one to session eight after the sham tDCS. Furthermore, this possibility, could have an added psychological influence, due to the fact that the participant was confident that received real stimulation condition. These interpretations would be supported by previous research that sham tDCS modulated EEG mean frequency power (Boonstra et al., 2016), and studies investigating the brain state dependent effects of tDCS, including influences produced by task performance (Gill et al., 2015), and participants motivations and expectations (Brangioni et al., 2018; Rabipour et al., 2018).

A closer examination of the EEG data, revealed that there were also other common findings between EEG frequency bands results, when comparing outcome values across sessions[22]: in delta frequency, session five showed the highest relative power in EEG2, and the largest difference between EEG1 and EEG2 absolute power; in theta frequency, session five showed the lowest EEG2 relative power and the largest difference between EEG1 and EEG2, whereas sessions five and six showed the highest EEG2 absolute power; in alpha frequency, session five showed the lowest relative power in all electrodes and the largest difference between EEG1 and EEG2, as well as the highest EEG2 absolute power in all electrodes; and in beta frequency, relative power values in EEG2 increased from sessions one to four, decreased to the lowest value in session five, and increased again from sessions six to

[22] Statistical analysis comparing each of the eight sessions against the rest was not performed (paired comparisons were conducted only between the first and the last session), and therefore, the results discussed about any other specific session, refer simply to the variables values observed. The statistical analysis was more focused on assessing the final outcome of the intervention, and to compare each session against the others (involving between and within sessions analysis), would extend the work load and amount of data reported excessively, considering the limitations associated to the adapted analysis methodologies used, the time available for the project and the limited space to report the results. Nevertheless, this will be considered to further explore the data in the future.

eight, whereas the greatest difference in absolute power between EEG1 and EEG2 was shown in sessions four and five. Results showing that session five outcomes differentiated from the outcomes of the rest of the sessions, are consistent between EEG variables, self-reported measures and behavioural task performance, as reported above. In line with this findings, correlation analysis showed that gambling severity correlated positively with cravings and EEG2 theta/beta ratio in F3 (electrode position associated with left DLPFC). In addition, correlation analysis showed that gambling severity correlated negatively with SST variables total correct stop and go trials, total correct stop trials and total correct go trials, as well as with EEG2 beta relative power in left DLPFC (lDPFC). An additional assessment of the neurophysiological variables that correlated with gambling severity was conducted, and correlation analysis between EEG2 variables in F3 and gambling related behavioural and self-report measures revealed that: theta/beta ratio correlated positively with cravings and with IST total correct, whereas beta relative power correlated negatively with cravings. Also, beta absolute power correlated negatively with IST total correct and total correct in win condition fixed. In addition, absolute power in all frequency bands (delta, theta, alpha and beta) correlated negatively with SSRT. In addition, relative alpha power correlated positively with IST total correct in win condition fixed, whereas relative delta power correlated negatively with IST total correct in win condition fixed. Lastly, absolute alpha power correlated negatively with IST mean number of boxes opened and mean number of boxes opened in win condition fixed.

Previous research showed that gambling severity correlated positively with beta absolute power in the left PFC (Kim et al., 2018), however in this experiment gambling severity correlated negatively with beta relative power in the left DLPFC. Negative correlations between self-reported impulsivity and alpha absolute power were shown in previous GD research (Lee et al., 2017b), whereas this experiment revealed negative correlations between behavioural impulsivity measured with IST and absolute alpha power. Also, decreased theta/beta ratio has been associated with several psychiatric disorders including addition (Newson & Thiagarajan, 2019), and with decreased inhibition control measured with SSRT (Lansbergen et al., 2007). Research also found a negative correlation between theta/beta ratio and reward and punishment-related reversal learning (Schutte et al., 2017), and considering that reversal learning has been associated with gambling problems (Jara-rizzo et al., 2020), those findings would be consistent with the positive correlation between gambling severity and theta/beta ratio found in this experiment.

Participant allocated to tDCS real stimulation condition

In the participant allocated to real stimulation condition, results showed significant differences across sessions in gambling severity measured with PG-YBOCS, reflection impulsivity measured with IST, in particular results showed differences in IST variables number of boxes opened per trial, probability of a correct decision, and in addition there were significant differences in in the SST variable SSRT. VAS statistics could only be calculated across sessions due to a lack of enough data points, which showed significant differences across sessions, and in addition, percentage change between the last and the first session revealed a reduction of 71% in gambling cravings. To assess the final outcome of the intervention, session eight was compared against session one, revealing that there were statistically significant changes only in PG-YBOCS, which showed a reduction of 66%. In addition, at the end of the intervention the participant thought that received tDCS real stimulation. These results are consistent with the findings from the case study mentioned previously showing a reduction of 62.5% of PG-YBOCS, and a reduction of 100% of VAS for gambling cravings after a longitudinal intervention with real stimulation tDCS over DLPFC in GD (Martinotti et al., 2018).

EEG frequency power analysis results showed significant differences in relative and absolute power of the four frequency bands (delta, theta, alpha and beta), across sessions, between the first and the last session, and within session one and session eight. In particular, comparing the EEG outcomes at the beginning and at the end of the eight session intervention, and considering separately the EEG recordings before and after the tDCS, results revealed long term effect of tDCS, showing an increase of EEG1 delta and theta relative power, delta, theta, alpha and beta absolute power, and a decrease of EEG1 alpha and beta relative power, in session eight compared with session one. There was also an increase of EEG2 delta and theta absolute power, and a decrease of EEG2 delta, theta, alpha and beta relative power, and alpha and beta absolute power, in session eight compared with session one. To assess short term effects of tDCS, within each session, there were EEG power changes after the participant received real stimulation tDCS while completing the cognitive task SST: in session one, there was an increase of theta, alpha and beta relative power, and a decrease of delta relative power, and delta, theta, alpha and beta absolute power. In session eight, there was an increase of theta relative power (only in electrode position AF7), and a decrease of delta relative power, and delta, theta, alpha and beta absolute power, however there were no changes in in alpha and beta relative power. Previous research combining tDCS and EEG, showed an increase of theta, alpha and beta power (Mangia et al., 2014), as well as an increase of delta, theta and alpha power, a reduction of beta power (Boonstra et al., 2016), and a reduction of delta power after real stimulation tDCS (Keeser et al., 2011), which is consistent with the results found in this experiment. Moreover, significant correlations between

gambling severity and EEG relative power were found, particularly in the pre-tDCS recording (EEG1): gambling severity correlated positively with delta, theta, alpha and beta relative power, which is consistent with previous research that showed that gambling severity correlated positively with beta absolute power (Kim et al., 2018).

The results showed that there was an increase of absolute power across all frequency bands (delta, theta, alpha and beta) as well as an increase of delta and theta relative power and a decrease of alpha and beta relative power in session eight compared with session one. Particularly, the increase of absolute power across all frequency bands was in the pre-tDCS EEG recording (EEG1), which occurred just after the participant completed the IST (which measures reflection impulsivity, and was used as a priming task to activate the brain networks associated with gambling behaviours), and just before the participant received real stimulation tDCS. Furthermore, in the post-tDCS EEG recording (EEG2), just after the participant received sham tDCS and completed the SST (which measures inhibition control), there was an increase of delta and theta absolute power, as well as a decrease of relative power across all frequency bands (delta, theta, alpha and beta) and alpha and beta absolute power, in session eight compared with session one. Across all EEG variables, changes of EEG power comparing session one against session eight were more consistent between electrode locations in EEG2 compared with EEG1, showing that significant differences were shown in a higher number of electrodes in EEG2. Within each session, significant differences were shown in a higher number of electrodes in session one, compared with session eight. In addition, in session one, the increases and decreases of EEG power happened in similar number of variables, however, in session eight, the decreases of EEG power were found across a higher number of variables than the increases of EEG power.

To embrace these results, it is essential to consider the particular experimental conditions that did not follow the planned procedure: the participant had a break of one weak between session two and session three, in which the CBT treatment was delivered remotely without the combined tDCS protocol. In session six, the participant received two sessions of CBT after the tDCS procedure, and in sessions seven and eight only the tDCS procedure was administered, without CBT following the experimental session. In addition, the participant was tested during hot days in the summer season, in a room without controlled temperature, after traveling for four hours to attend the treatment at the clinic. There were considerable amounts of sweat on the scalp that probably affected the EEG data, which would explain the high amount of artefacts that could not be completely removed after data

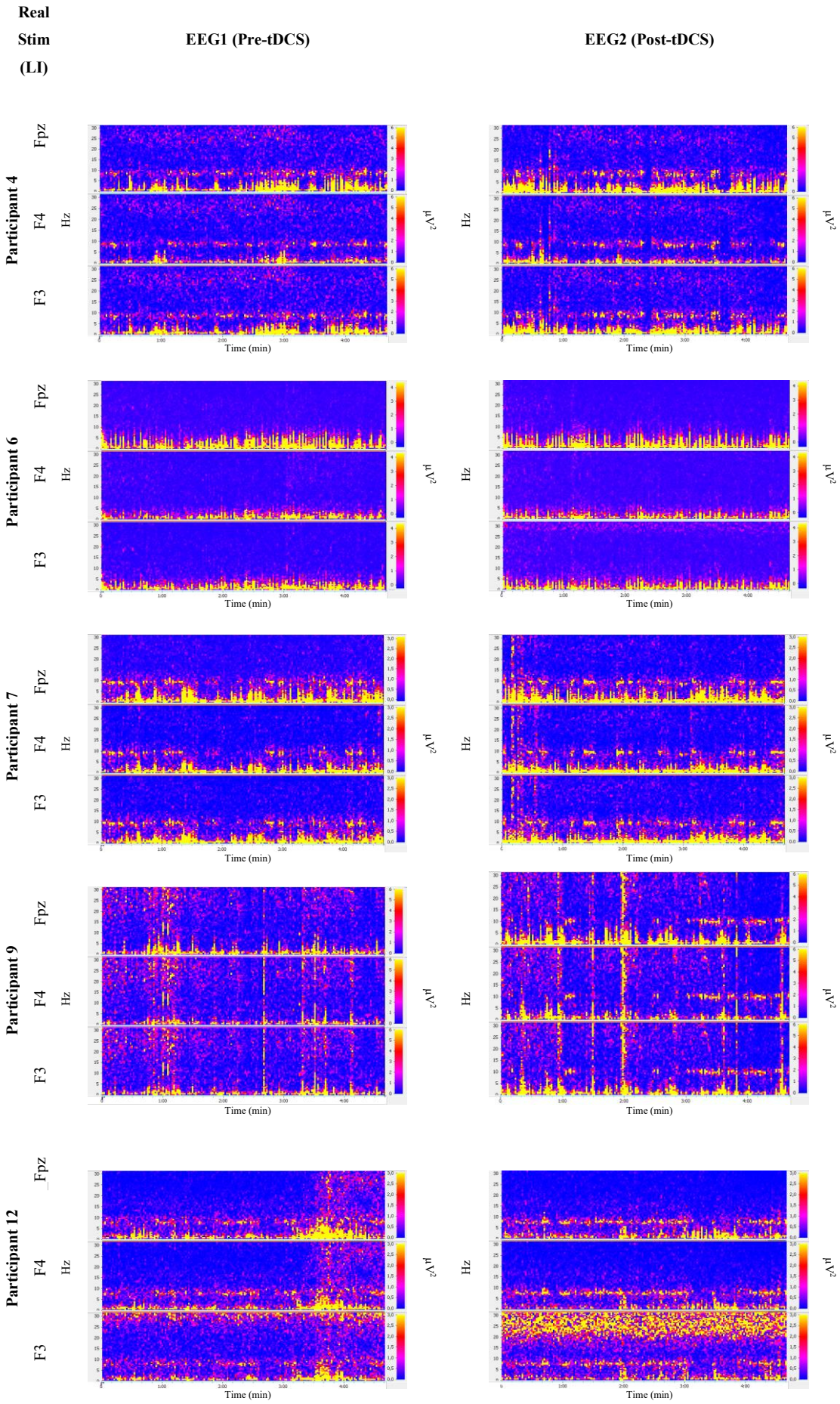
processing, and the extremely high absolute power values, especially in low EEG frequencies (Thompson et al., 2008). Under these conditions, unfortunately it is not possible to draw reliable conclusions about the effectiveness of the intervention, however data from each session might provide some information about the mechanisms of tDCS.

General discussion and limitations

In this experiment, some of the limitations highlighted in previous experiments were addressed: a triple-blinded design (in which neither the participant, nor the researcher conducting the experiment and the data analysis were aware of the stimulation condition) was used to ensure that data analysis was less likely to be biased; a longitudinal design was planned to investigate the potential accumulative effects of tDCS; a computational model was used to create the most effective tDCS montage to target the brain area of interest more accurately; and neurophysiological data (EEG) was used to quantify the effects of the intervention. Results revealed significant effects of the intervention with tDCS and cognitive behavioural therapy (CBT) on gambling severity, cognitive task performance, and changes on electroencephalogram (EEG) power frequency in both participant cases. In one case (in sham tDCS condition), gambling severity correlated with behavioural measures, and in both cases, gambling severity correlated with EEG variables. Nevertheless, even though previous limitations were tackled, additional limitations arose in this experiment, given that the original design planned as an RCT had to be adapted to analyse data following single-participant designs due to recruitment complications.

Therefore, the results obtained cannot be used to draw conclusions about the effectiveness of the intervention combining tDCS and CBT, and the particular adaptations prevent contrasting the findings consistently against previous studies results. Especially, in the participant allocated to real stimulation condition (with whom the testing procedure did not follow the planned protocol, and multiple artefacts contaminated the EEG data), results obtained are not reliable to interpret the intervention outcomes congruently. However, in the participant allocated to sham condition (with whom experimental testing was carried out as planned), results revealed associations between self-report measures, behavioural outcomes and neurophysiological data that support further investigation of these variables to study potential biological markers that could be used to assess the effectiveness of treatment interventions.

Appendix B. EEG power spectrograms from Experiment IV


Real Stim (LI)
EEG1 (Pre-tDCS)
EEG2 (Post-tDCS)
Participant 4
Participant 6
Participant 7
Participant 9
Participant 12
Fpz
F4
F3
Hz
μV²
Time (min)

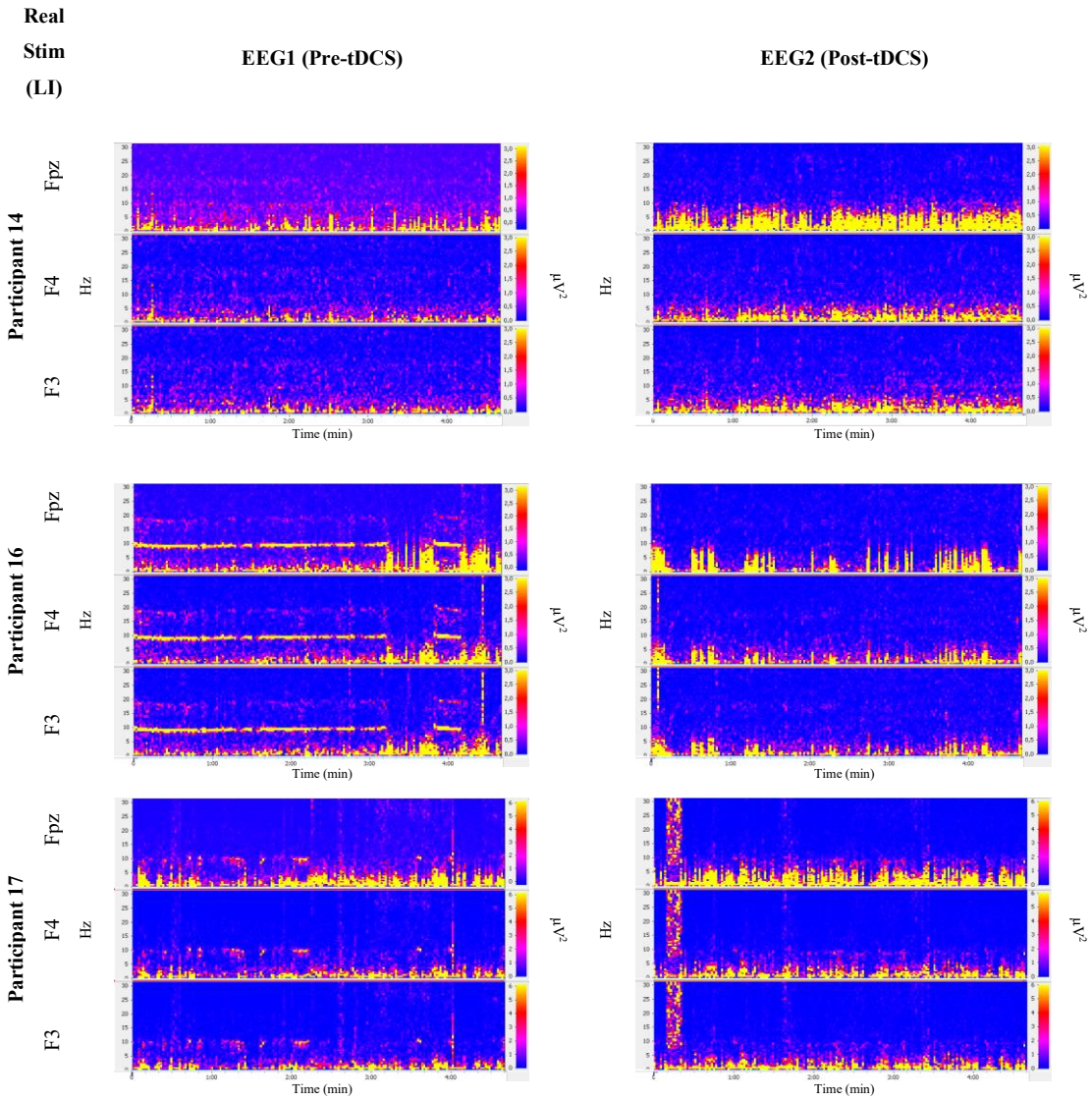
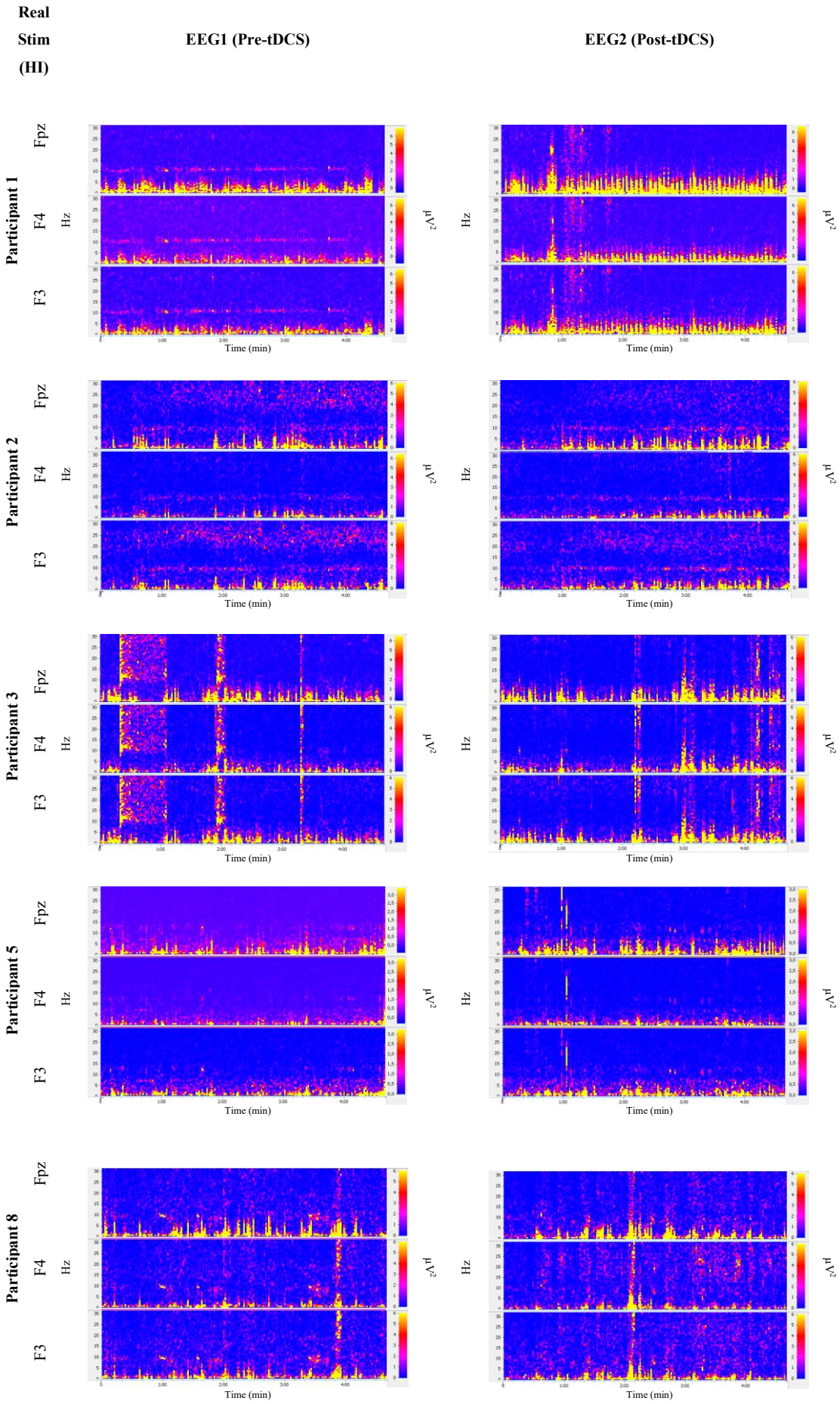


Figure 0-1. Electroencephalography (EEG) spectrogram across low impulsive (LI) participants during transcranial direct current stimulation (tDCS) in real stimulation condition. EEG power (μV^2) in frequencies 0-30 Hz during five minutes EEG resting state recordings pre-tDCS (EEG1) and post-tDCS (EEG2), across LI participants in tDCS real stimulation condition. Electrodes positions displayed in the spectrogram with the international EEG 10/10 system are Fpz, F4 and F3 for ventral, right and left prefrontal cortex (PFC), respectively.


Real Stim (HI)
EEG1 (Pre-tDCS)
EEG2 (Post-tDCS)
Participant 1
Participant 2
Participant 3
Participant 5
Participant 8
Fpz
F4
F3
Hz
μV²
Time (min)

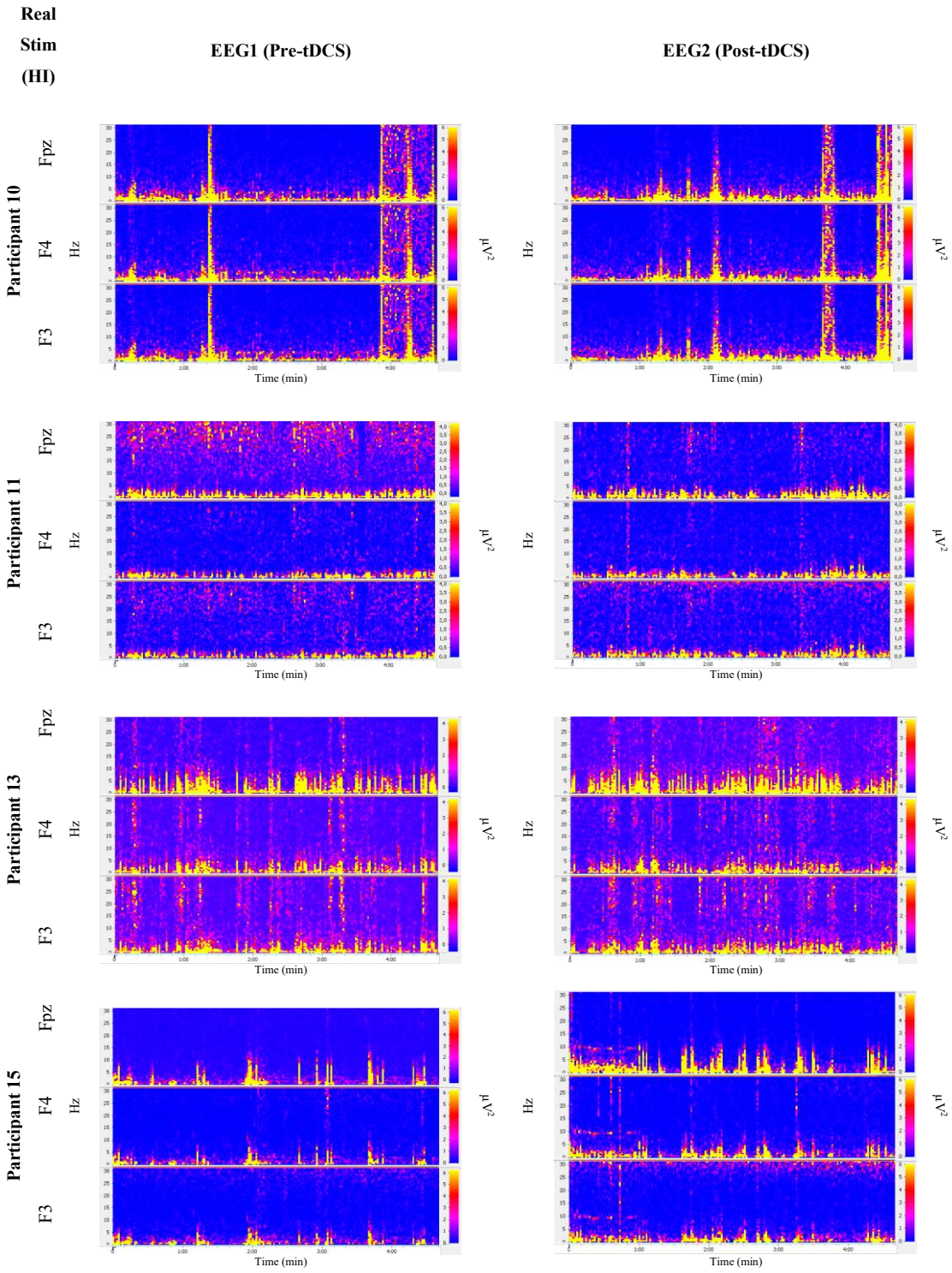
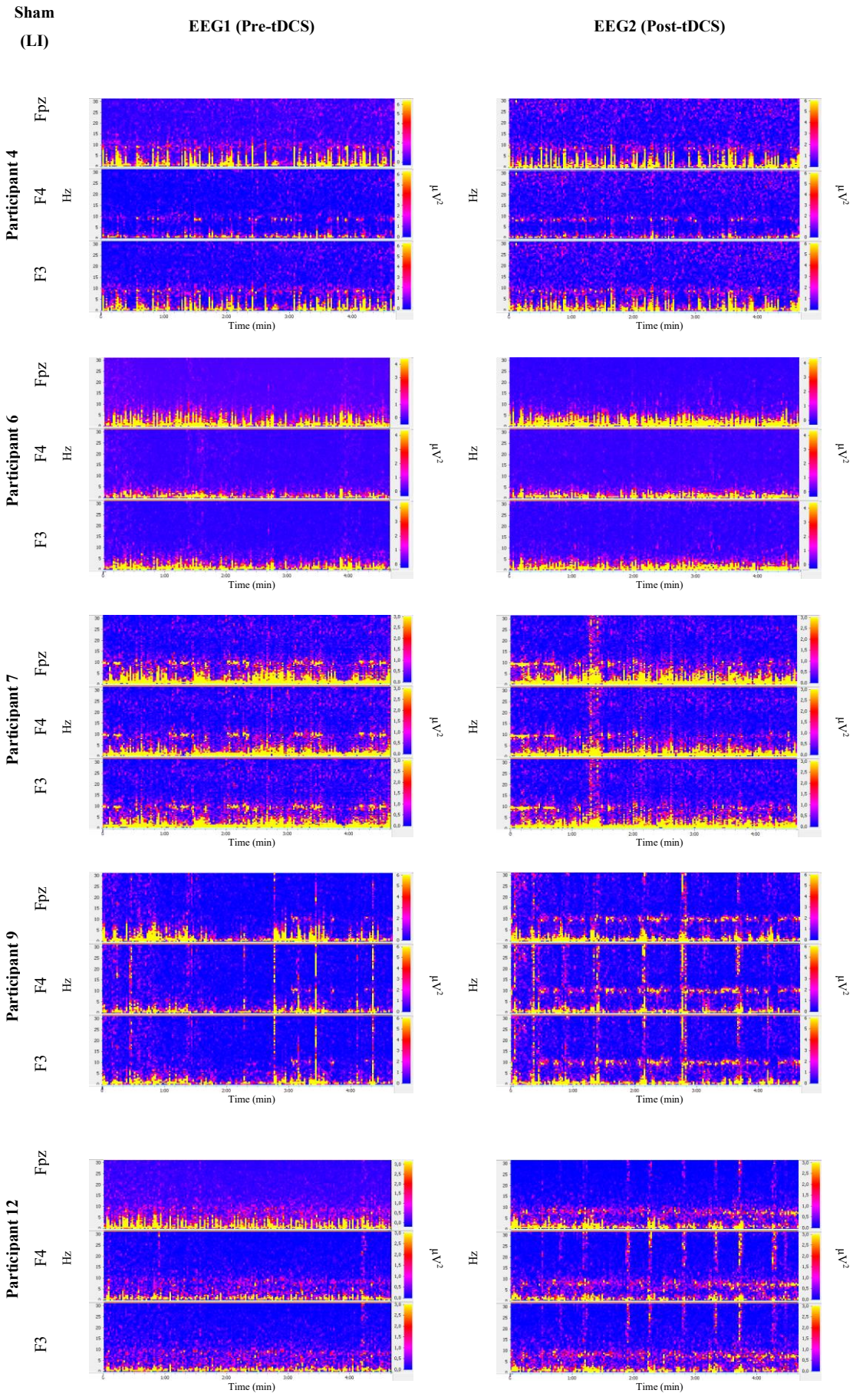


Figure 0-2. Electroencephalography (EEG) spectrogram across high impulsive (HI) participants during transcranial direct current stimulation (tDCS) in real stimulation condition. EEG power (μV^2) in frequencies 0-30 Hz during five minutes EEG resting state recordings pre-tDCS (EEG1) and post-tDCS (EEG2), across HI participants in tDCS real stimulation condition. Electrodes positions displayed in the spectrogram with the international EEG 10/10 system are Fpz, F4 and F3 for ventral, right and left prefrontal cortex (PFC), respectively.


Sham
(LI)
EEG1 (Pre-tDCS)
EEG2 (Post-tDCS)
Participant 4
Participant 6
Participant 7
Participant 9
Participant 12
Fpz
F4
F3
Hz
μV²
Time (min)

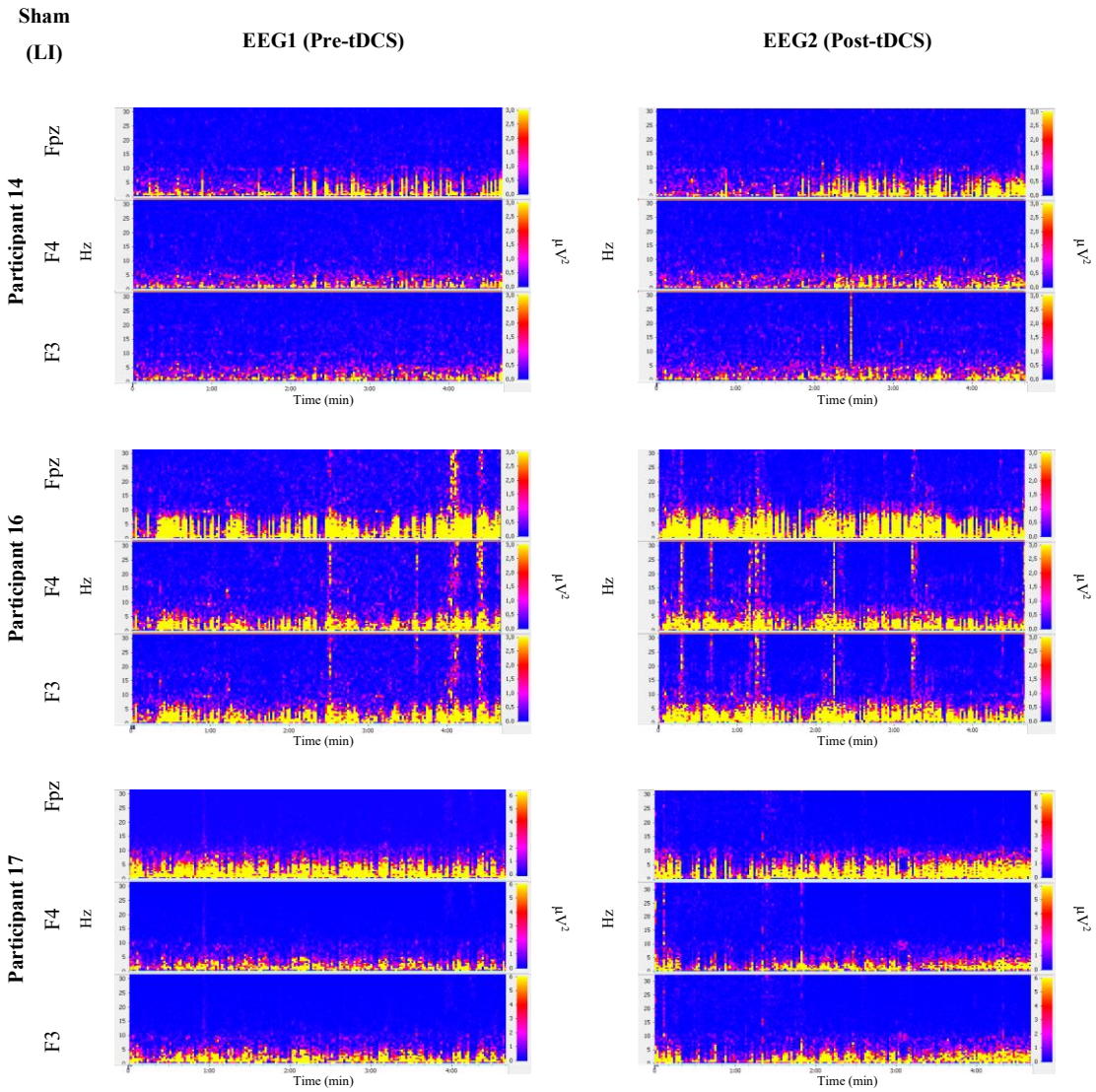
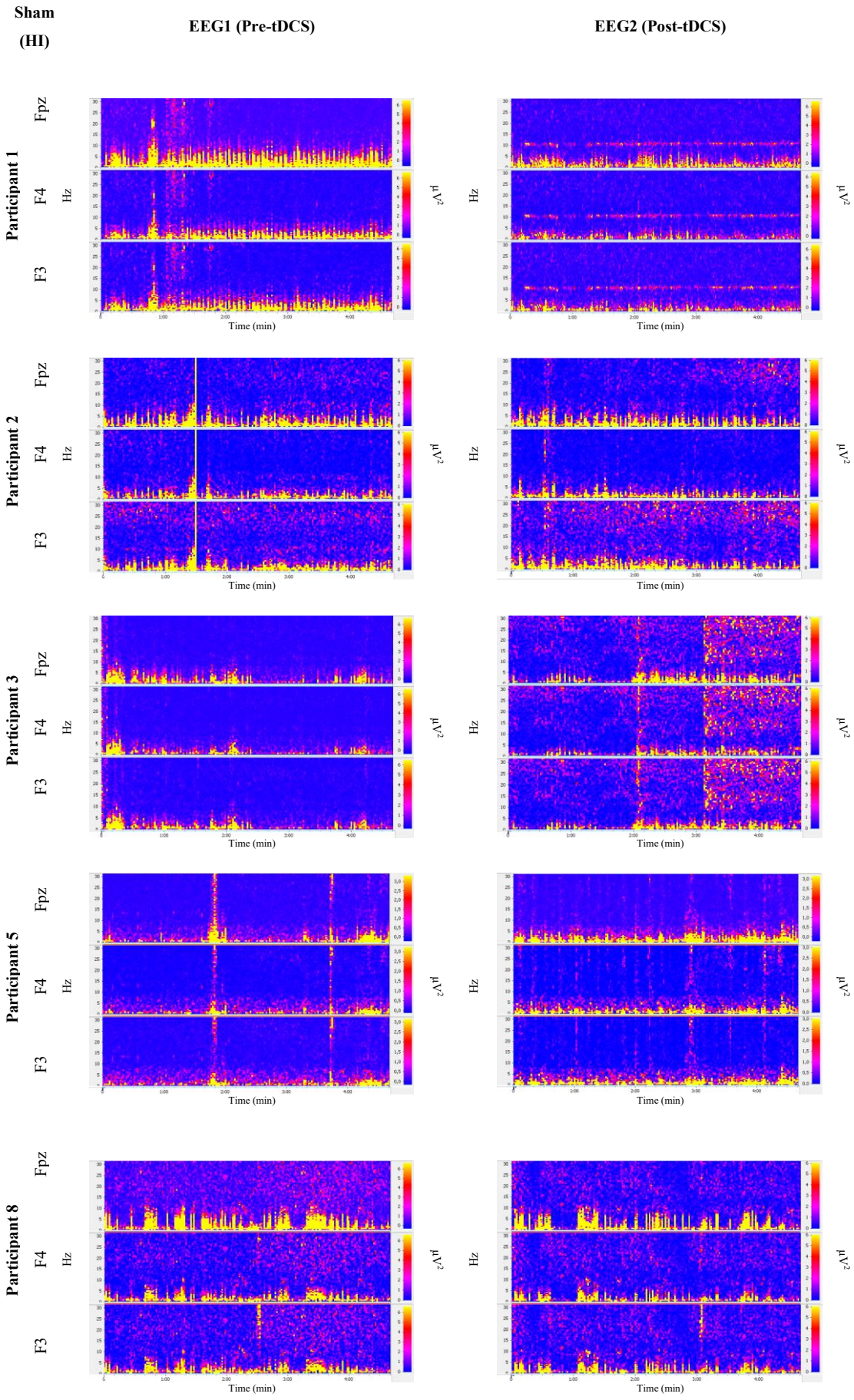


Figure 0-3. Electroencephalography (EEG) spectrogram across low impulsive (LI) participants during transcranial direct current stimulation (tDCS) in sham condition. EEG power (μV^2) in frequencies 0-30 Hz during five minutes EEG resting state recordings pre-tDCS (EEG1) and post-tDCS (EEG2), across LI participants in tDCS sham condition. Electrodes positions displayed in the spectrogram with the international EEG 10/10 system are Fpz, F4 and F3 for ventral, right and left prefrontal cortex (PFC), respectively.


Sham
(HI)
EEG1 (Pre-tDCS)
EEG2 (Post-tDCS)
Participant 1
Participant 2
Participant 3
Participant 5
Participant 8
Fpz
F4
F3
Hz
μV²
Time (min)

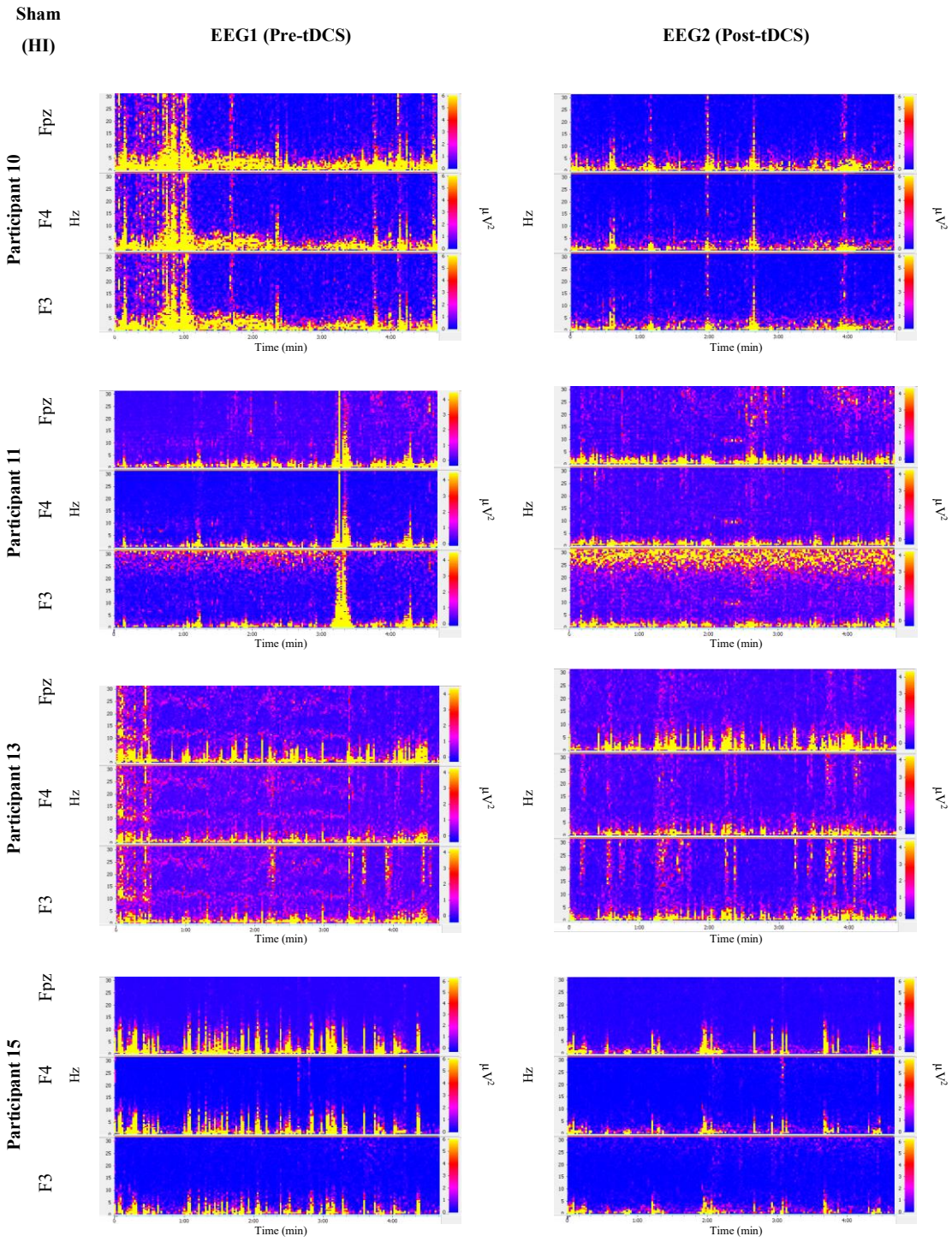


Figure 0-4. Electroencephalography (EEG) spectrogram across high impulsive (HI) participants during transcranial direct current stimulation (tDCS) in sham condition. EEG power (μV^2) in frequencies 0-30 Hz during five minutes EEG resting state recordings pre-tDCS (EEG1) and post-tDCS (EEG2), across HI participants in tDCS sham condition. Electrodes positions displayed in the spectrogram with the international EEG 10/10 system are Fpz, F4 and F3 for ventral, right and left prefrontal cortex (PFC), respectively.

Appendix C. Publications and poster presentations

Article publication

Gomis-Vicent, E., Thoma, V., Turner, J. J., Hill, K. P., & Pascual-Leone, A. (2019). Non-Invasive Brain Stimulation in Behavioral Addictions: Insights from Direct Comparisons with Substance Use Disorders. *The American journal on addictions*, 28(6), 431-454.

Abstract publication

Vicent, E. G., Thoma, V., Turner, J., Rivolta, D., & Bowden-Jones, H. (2019). Transcranial direct current stimulation (tDCS) of dorsolateral versus ventromedial prefrontal cortex: impact on gambling task performance. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, 12(2), 577-578.

Most relevant poster presentations

Gomis-Vicent, E., Turner, J. J., Rivolta, D., Thoma, V. (2017, December). The role of neuromodulation for cognitive processing and behavioural inhibition in gambling disorder. Poster presented at the GambleAware Harm Minimisation Conference, London, United Kingdom.

Gomis-Vicent, E., Thoma, V., Turner, J. J., (2018, November). The effects of transcranial direct current stimulation in impulsivity and gambling disorder. Poster presented at the Society for the Study of Addiction Annual Conference, Newcastle, United Kingdom.

Gomis-Vicent, E., Thoma, V., Turner, J. J., Rivolta, D., & Bowden-Jones, H. (2019, February). Transcranial direct current stimulation (tDCS) of dorsolateral versus ventromedial prefrontal cortex: impact on gambling task performance. Poster presented at the 3rd International Brain Stimulation Conference, Vancouver, Canada.

Gomis-Vicent, E., Thoma, V., Turner, J. J., & Bowden-Jones, H. (2019, May). Transcranial direct current stimulation (tDCS) for gambling disorder: developing a clinical trial protocol at the National Problem Gambling Clinic (NPGC). Poster presented at the NHS Central and North West London Research Conference, London, United Kingdom.

Appendix D. Ethical approval

Dear Miss Gomis Vicent,

Application ID: ETH2021-0004

Original application ID: ETH1920-0081

Amendment to change project title to: Cognitive and physiological assessment of prefrontal cortex neuromodulation in low and high risk gambling

Former project title:

The role of neuromodulation, cognitive processing and behavioural inhibition in problem gambling

Lead researcher: Miss Elena Gomis Vicent

Your application to University Research Ethics Sub-Committee (URES) was considered on 13th August 2020.

The decision is: **Approved**

In view of the Covid-19 pandemic, URES has taken the decision that all face-to-face projects that include participant interactions should cease to use this method of data collection. For example, in person participant interviews or focus groups. The University supports Microsoft Teams for remote work. New research projects must not recruit participants using face-to-face interactions and all data collection should occur remotely. These regulations should be followed until further notice by URES.

The Committee's response is based on the protocol described in the application form and supporting documentation.

Your project has received ethical approval for 2 years from the approval date.

If you have any questions regarding this application please contact your supervisor or the secretary for the University Research Ethics Sub-Committee.

Approval has been given for the submitted application only and the research must be conducted accordingly.

Should you wish to make any changes in connection with this research project you must complete 'An application for approval of an amendment to an existing application'.

Approval is given on the understanding that the UEL Code of Practice for Research and the Code of Practice for Research Ethics is adhered to.

Any adverse events or reactions that occur in connection with this research project should be reported using the University's form for Reporting an Adverse/Serious Adverse Event/Reaction.

The University will periodically audit a random sample of approved applications for ethical approval, to ensure that the research projects are conducted in compliance with the consent given by the Research Ethics Committee and to the highest standards of rigour and integrity.

Please note, it is your responsibility to retain this letter for your records.

With the Committee's best wishes for the success of the project.

Yours sincerely,

Catherine Hitchens

Research Integrity & Ethics Manager

Dear Elena

Application ID: ETH1920-0081

Original application ID: 161731

Project title: The role of neuromodulation, cognitive processing and behavioural inhibition in problem gambling

Lead researcher: Miss Elena Gomis Vicent

Your application to Research, Research Degrees and Ethics Sub-Committee meeting was considered on the 21st of November 2019.

The decision is: **Approved**

The Committee's response is based on the protocol described in the application form and supporting

documentation. Your project has received ethical approval for 2 years from the approval date.

If you have any questions regarding this application please contact your supervisor or the secretary for the Research, Research Degrees and Ethics Sub-Committee meeting.

Approval has been given for the submitted application only and the research must be conducted accordingly.

Should you wish to make any changes in connection with this research project you must complete 'An application for approval of an amendment to an existing application'.

Approval is given on the understanding that the UEL Code of Practice for Research and the Code of Practice for Research Ethics is adhered to.

Any adverse events or reactions that occur in connection with this research project should be reported using the University's form for Reporting an Adverse/Serious Adverse Event/Reaction.

The University will periodically audit a random sample of approved applications for ethical approval, to ensure that the research projects are conducted in compliance with the consent given by the Research Ethics Committee and to the highest standards of rigour and integrity.

Please note, it is your responsibility to retain this letter for your records.

With the Committee's best wishes for the success of the project

Yours sincerely,

Fernanda Silva
Research, Research Degrees and Ethics Sub-Committee

13th December 2018

Dear Elena,

Project Title	**The role of neuromodulation for cognitive processing and behavioural inhibition in gambling disorder**
Researcher	**Elena Gomis-Vicent**
Principal Investigator	**Elena Gomis-Vicent**

I am writing to confirm that the application for the aforementioned NHS research study reference **241677** has received UREC ethical approval and is sponsored by the University of East London.

The lapse date for ethical approval for this study is **13th December 2022**. If you require UREC approval beyond this date you must submit satisfactory evidence from the NHS confirming that your study has current NHS R&D ethical approval and provide a reason why UREC approval should be extended.

Please note as a condition of your sponsorship by the University of East London your research must be conducted in accordance with NHS regulations and any requirements specified as part of your NHS R&D ethical approval.

Please confirm that you will conduct your study in accordance with the consent given by the Trust Research Ethics Committee by emailing researchethics@uel.ac.uk.

Please ensure you retain this approval letter, as in the future you may be asked to provide proof of ethical approval.

With the Committee's best wishes for the success of this project.

Yours sincerely,

Fernanda Silva
Administrative Officer for Research Governance
For and on behalf of
University Research Ethics Committee (UREC)
Email: **researchethics@uel.ac.uk**

24th October 2018

Dear Elena,

Project Title:	**The role of neuromodulation for cognitive processing and behavioural inhibition in problem gambling**
Researchers:	**Elena Gomis Vicent, Dr Davide Rivolta, Dr John Turner, Daniel Edgcumbe, Janine Brierley and Nicholas Wethered**
Principal Investigator:	**Dr Volker Thoma**
Amendment reference number:	**AMD 1819 09**
UREC reference no of original approved application:	**UREC 1718 23**

I am writing to confirm that the application for an amendment to the aforementioned research study has now received ethical approval on behalf of University Research Ethics Committee (UREC).

Should you wish to make any further changes in connection with your research project, this must be reported immediately to UREC. A Notification of Amendment form should be submitted for approval, accompanied by any additional or amended documents: http://www.uel.ac.uk/wwwmedia/schools/graduate/documents/Notification-of-Amendment-to-Approved-Ethics-App-150115.doc

Approved Research Site

I am pleased to confirm that the approval of the proposed research applies to the following research site:

Research Site	Principal Investigator / Local Collaborator
National Problem Gambling Clinic - London	Dr Volker Thoma

Summary of Amendments

Two UEL MSc students will collaborate with data collection at the NHS site, the National Problem Gambling Clinic. The volunteer assistants will help the PhD student, Elena Gomis Vicent with participant testing until data collection finishes, in June 2019. All the data will be anonymised in the study, as stated in the approved ethical application, and the volunteer assistants will not have access to the participant health records. The amendment has received approval from the NHS to include the student's details in the application.

Assistant 1
Title: Miss
Forename: Janine
Surname: Brierley
Email:
Post: Volunteer Research Assistant
Qualifications: BSc Biological Sciences (UCL); MSc Psychology (UEL)
Approximately how much time you will allocate to this research (in Whole Time Equivalents, WTE): 0.40
Has this person has been accepted by the NHS organisation? YES

Assistant 2
Title: Mr
Forename: Nicholas
Surname: Wethered
Work email:
Post: Volunteer Research Assistant
Qualifications:
BA Philosophy and Theology, University of Oxford (2011)
MA Legal & Political Theory, University College London (2014)
Graduate Diploma in Law, BPP University (2015)
Legal Practice Course, BPP University (2016)
Admitted as a Solicitor in England and Wales (September 2018)
Approximately how much time you will allocate to this research (in Whole Time Equivalents, WTE): 0.27
Has this person has been accepted by the NHS organisation? YES

Ethical approval for the original study was granted on 10th January 2018.

Approval is given on the understanding that the UEL Code of Good Practice in Research is adhered to.

With the Committee's best wishes for the success of this project.

Please ensure you retain this letter, as in the future you may be asked to provide evidence of ethical approval for the changes made to your study.

Yours sincerely,

Fernanda Silva
Administrative Officer for Research Governance
University Research Ethics Committee (UREC)
Email: **researchethics@uel.ac.uk**

2nd October 2018

Dear Elena,

Project Title:	**The role of neuromodulation for cognitive processing and behavioural inhibition in problem gambling**
Researchers:	**Elena G Vicent, Dr Volker Thoma, Dr Davide Rivolta, Dr John Turner and Daniel Edgcumbe**
Principal Investigator:	**Dr Volker Thoma**
Amendment reference number:	**AMD 1819 06**
UREC reference no of original approved application:	**UREC 1718 23**

I am writing to confirm that the application for an amendment to the aforementioned research study has now received ethical approval on behalf of University Research Ethics Committee (UREC).

Should you wish to make any further changes in connection with your research project, this must be reported immediately to UREC. A Notification of Amendment form should be submitted for approval, accompanied by any additional or amended documents: http://www.uel.ac.uk/wwwmedia/schools/graduate/documents/Notification-of-Amendment-to-Approved-Ethics-App-150115.doc

Approved Research Site

I am pleased to confirm that the approval of the proposed research applies to the following research site:

Research Site	Principal Investigator / Local Collaborator
National Problem Gambling Clinic - London	Dr Volker Thoma

Summary of Amendments
1. Nomenclature (substitute Gambling Disorder for Problem Gambling) 2. Design: • Reducing the number of treatment arms to two (instead of 4). • Increasing the number of sessions that each participant will attend (8 instead of 5). 3. Consent form, participant information sheet and other supporting documents format had to be changed to meet the specific requirements of a successful NHS application, and are enclosed in this document. In addition, two further changes were suggested after Ms Gomis' summer training at Harvard medical school in July and August 2018: 4. Materials: • Reduction of the number of cognitive tasks (3 instead of 5). • Change from the 3 original cognitive questionnaires for 3 new ones (all validated). 5. tDCS Protocol: Change of the intensity of tDCS from 1.5mA to 2mA.

Ethical approval for the original study was granted on 10th January 2018.

Approval is given on the understanding that the UEL Code of Good Practice in Research is adhered to.

With the Committee's best wishes for the success of this project.

Please ensure you retain this letter, as in the future you may be asked to provide evidence of ethical approval for the changes made to your study.

Yours sincerely,

Fernanda Silva
Administrative Officer for Research Governance
University Research Ethics Committee (UREC)
Email: **researchethics@uel.ac.uk**

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Ms Elena Gomis-Vicent
Water Lane
Department of Psychological Sciences
London
E15 4LZ

Email: hra.approval@nhs.net
Research-permissions@w ales.nhs.uk

02 October 2018

Dear Ms Gomis-Vicent

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**The role of neuromodulation for cognitive processing and behavioural inhibition in gambling disorder**
IRAS project ID:	**241677**
Protocol number:	**UREC 1718 23**
REC reference:	**18/LO/1454**
Sponsor	**University ofEast London**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment .

Following the arranging of capacity and capability, participating NHS organisations should **formally confirm** their capacity and capability to undertake the study. How this will be confirmed is detailed in the "*summary of assessment*" section towards the end of this letter.

You should provide, if you have not already done so, detailed instructions to each organisation as to how you will notify them that research activities may commence at site following their confirmation of capacity and capability (e.g. provision by you of a 'green light' email, formal notification following a site initiation visit, activities may commence immediately following confirmation by participating organisation, etc.).

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study . Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?
The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?
You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Catherine Fieulleteau
Tel: 02082236683
Email: researchethics@uel.ac.uk

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **241677**. Please quote this on all correspondence.

Yours Sincerely

Beverley Mashegede
Assessor

Email: hra.approval@nhs.net

Copy to: Catherine Fieulleteau, Sponsor Contact

Mabel Saili, Central and North West London NHS Foundation Trust , Lead NHS R&D Contact

List of Documents

The final document set assessed and approved by HRA and HCRW Approval is listed below.

Document	*Version*	*Date*
Contract/Study Agreement template [Study_Agreement_Funder_Sponsor_IRAS241677]		26 July 2016
Covering letter on headed paper [Letter from Academic Supervisors]		16 September 2018
Covering letter on headed paper [Response to Provisional]		27 September 2018
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Employer's Liability]		
HRA Schedule of Events	1	22 August 2018
HRA Statement of Activities	1	28 August 2018
Instructions for use of medical device [Device_Manual_IRAS241677v1]	1	05 July 2018
IRAS Application Form [IRAS_Form_27092018 (updated q's in cover letter)]		27 September 2018
IRAS Application Form XML file [IRAS_Form_27072018]		27 July 2018
IRAS Checklist XML [Checklist_27092018]		27 September 2018
Letter from funder [Funder_Letter_IRAS241677]		17 June 2016
Letters of invitation to participant [Participant_Invitation_Letter_IRAS241677v1.1]	1.1	10 September 2018
Other [Response to Validation]		20 August 2018
Participant consent form [Participant_Consent_form_IRAS241677v1.1]	1.1	10 September 2018
Participant information sheet (PIS) [Participant_Information_Sheet_IRAS241677v1.1]	1.1	10 September 2018
Referee's report or other scientific critique report [Annual_Monitorin_Review_18_IRAS241677]		28 March 2018
Referee's report or other scientific critique report [Transfer_PhD_IRAS241677]		26 March 2018
Referee's report or other scientific critique report [Peer_Review_IRAS241677]		25 April 2018
Research protocol or project proposal [Protocol_IRAS241677v1.1]	1.1	10 September 2018
Summary CV for Chief Investigator (CI) [CV_CI_EGomisVicent_IRAS241677v1]	1	05 July 2018
Summary CV for student [CV_CI_EGomisVicent_IRAS241677v1]	1	05 July 2018
Summary CV for supervisor (student research) [CV_Supervisor_VThoma_IRAS241677v1]	1	05 July 2018
Summary of any applicable exclusions to sponsor insurance (non-NHS sponsors only) [UEL_Insurance_IRAS241677v1]	1	29 May 2018
Summary of any applicable exclusions to sponsor insurance (non-NHS sponsors only) [UEL_Insurance_IRAS241677v1.1]	1.1	18 July 2018
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Flow_Chart_IRAS241677v1]	1	05 July 2018
Validated questionnaire [Cognitive_Questionnaire_G-SAS_IRAS241677v1]	1	05 July 2018
Validated questionnaire [Cognitive_Questionnaire_PG-YBOCS_IRAS241677v1]	1	05 July 2018
Validated questionnaire	1	05 July 2018

[Cognitive_Questionnaire_VAS_IRAS241677v1]		
Validated questionnaire [Screening_Questionnaire_NDQ_IRAS241677v1]	1	29 May 2018
Validated questionnaire [Screening_Questionnaire_SADQ_IRAS241677v1]	1	29 May 2018
Validated questionnaire [Screening_Questionnaire_SADQ_IRAS241677v1]	1	29 May 2018

Summary of assessment

The following information provides assurance to you, the sponsor and the NHS in England and Wales that the study, as assessed for HRA and HCRW Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England and Wales to assist in assessing, arranging and confirming capacity and capability.

Assessment criteria

Section	Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	The sponsor intends to use the Statement of Activities as the form of agreement with the participating organisation.
4.2	Insurance/indemnity arrangements assessed	Yes	Valid insurance certificate submitted.
4.3	Financial arrangements assessed	Yes	Funded by Responsible Gambling Trust. No funds will be provided to the participating organisation to support this study.
5.1	Compliance with the Data Protection Act and data	Yes	No comments

Section	Assessment Criteria	Compliant with Standards	Comments
	security issues assessed		
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	Provisional Opinion issued 10 September 2018. Further Information Favourable Opinion issued 02 October 2018.
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England and Wales

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.
This is a non-commercial student (PhD) study and there is one site type. The Chief Investigator or sponsor should share relevant study documents with participating NHS organisations in England and Wales in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. Where applicable, the local LCRN contact should also be copied into this correspondence. If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England and Wales which are not provided in IRAS, the HRA or HCRW websites, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net or HCRW at Research-permissions@wales.nhs.uk. We will work with these organisations to achieve a consistent approach to information provision.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and Wales, and the minimum expectations for education, training and experience that PIs should meet (where applicable).
A Local Collaborator is expected at the participating organisation. GCP training is not a generic training expectation, in line with the HRA/HCRW/MHRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken
Where arrangements are not already in place, network staff (or similar) undertaking any research activities that may impact on the quality of care of the participant (listed in A18 and A19), would be expected to obtain an honorary research contract from one NHS organisation (if university employed), followed by Letters of Access for subsequent organisations. This would be on the basis of a Research Passport (if university employed) or an NHS to NHS confirmation of pre-engagement checks letter (if NHS employed). These should confirm enhanced DBS checks, including appropriate barred list checks, and occupational health clearance.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England and Wales to aid study set-up.
The applicant has indicated that they intend to apply for inclusion on the NIHR CRN Portfolio.

10th January 2018

Dear Elena,

Project Title:	**The role of neuromodulation for cognitive processing and behavioural inhibition in problem gambling**
Principal Investigator:	**Dr Volker Thoma**
Researcher(s):	**Elena G Vicent, Dr Volker Thoma, Dr Davide Rivolta, Dr John Turner and Daniel Edgcumbe**
Reference Number:	**UREC 1718 23**

I am writing to confirm the outcome of your application to the University Research Ethics Committee (UREC), which was considered by UREC on **Wednesday 15 November 2017.**

The decision made by members of the Committee is **Approved**. The Committee's response is based on the protocol described in the application form and supporting documentation. Your study has received ethical approval from the date of this letter.

Should you wish to make any changes in connection with your research project, this must be reported immediately to UREC. A Notification of Amendment form should be submitted for approval, accompanied by any additional or amended documents: http://www.uel.ac.uk/wwwmedia/schools/graduate/documents/Notification-of-Amendment-to-Approved-Ethics-App-150115.doc

Any adverse events that occur in connection with this research project must be reported immediately to UREC.

Approved Research Site

I am pleased to confirm that the approval of the proposed research applies to the following research site.

Research Site	Principal Investigator / Local Collaborator
National Problem Gambling Clinic - London	Dr Volker Thoma

Approved Documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
UREC application form	2.0	4 January 2018
Participant Information sheet	2.0	4 January 2018
Consent form	1.0	31 October 2017
Debrief sheet	2.0	4 January 2018
Research advertisement	2.0	4 January 2018
Cognitive Reflection Task (CRT)	1.0	31 October 2017
The Gambling Related Cognition Scale (GRCS)	1.0	31 October 2017
Severity of Alcohol Dependence Questionnaire (SADQ-C)[1]	1.0	31 October 2017
Notes on the use of the SADQ	1.0	31 October 2017
South Oaks Gambling Screen (SOGS)	1.0	31 October 2017
South Oaks Gambling Screen – Score Sheet (SOGS)	1.0	31 October 2017
The Actively Open Minded Thinking Scale	1.0	31 October 2017
The Need for Cognition Scale	1.0	31 October 2017
Severity of Dependence Scale (SCS)	1.0	31 October 2017
Response Card	1.0	31 October 2017
Participant number – UPPS-P	1.0	31 October 2017

Approval is given on the understanding that the UEL Code of Practice in Research is adhered to.

The University will periodically audit a random sample of applications for ethical approval, to ensure that the research study is conducted in compliance with the consent given by the ethics Committee and to the highest standards of rigour and integrity.

Please note, it is your responsibility to retain this letter for your records.

With the Committee's best wishes for the success of this project.

Yours sincerely,

Fernanda Silva
Administrative Officer for Research Governance
University Research Ethics Committee (UREC)
Email: **researchethics@uel.ac.uk**

3rd March 2017

Dear Elena,

Project Title:	**The role of neuromodulation, cognitive processing and behavioral inhibition in problem gambling**
Principal Investigator:	**Dr Volker Thoma**
Researcher:	**Elena Gomis Vicent**
Reference Number:	**UREC 1617 31**

I am writing to confirm the outcome of your application to the University Research Ethics Committee (UREC), which was considered by UREC on **Wednesday 18 January 2017.**

The decision made by members of the Committee is **Approved**. The Committee's response is based on the protocol described in the application form and supporting documentation. Your study has received ethical approval from the date of this letter.

Should you wish to make any changes in connection with your research project, this must be reported immediately to UREC. A Notification of Amendment form should be submitted for approval, accompanied by any additional or amended documents: http://www.uel.ac.uk/wwwmedia/schools/graduate/documents/Notification-of-Amendment-to-Approved-Ethics-App-150115.doc

Any adverse events that occur in connection with this research project must be reported immediately to UREC.

Approved Research Site

I am pleased to confirm that the approval of the proposed research applies to the following research site.

Research Site	Principal Investigator / Local Collaborator
Behavioural, tCS testing will take place in the Cognitive Lab, Room AE.G. 12 in the School of Psychology	Dr Volker Thoma

Approved Documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
UREC application form	3.0	14 February 2017
Annexe 1 – Participant Information sheet	2.0	13 February 2017
Annexe 2 - Consent form	2.0	13 February 2017
Debrief after tDCS (tCS) session	2.0	13 February 2017
Standard procedure for Transcranial current stimulation (tCS) Recording and participant safety	1.0	26 December 2016
References	1.0	26 December 2016
Recruitment Advertisement	2.0	13 February 2017
Cognitive Reflection Task (CRT)	1.0	13 February 2017
Kirby Monetary-Choice Questionnaire	2.0	13 February 2017
Severity of alcohol dependence questionnaire (SADQ-C)	1.0	26 December 2016
Notes on the use of the SADQ	1.0	26 December 2016
South Oaks Gambling Screen (SOGS)	2.0	13 February 2017
South Oaks Gambling Screen – Score sheet (SOGS)	1.0	26 December 2016
The Actively Open Minded Thinking Scale	1.0	26 December 2016
The Gambling Related Cognition Scale (GRCS)	1.0	26 December 2016
Scoring	1.0	26 December 2016
The Need for Cognition Scale	1.0	26 December 2016
Severity of Dependence scale (SDS)	1.0	26 December 2016
Response Card SDS	1.0	26 December 2016
UPPS-P	1.0	26 December 2016
Scoring Instructions	1.0	26 December 2016

Approval is given on the understanding that the UEL Code of Practice in Research is adhered to.

The University will periodically audit a random sample of applications for ethical approval, to ensure that the research study is conducted in compliance with the consent given by the ethics Committee and to the highest standards of rigour and integrity.

Please note, it is your responsibility to retain this letter for your records.

With the Committee's best wishes for the success of this project.

Yours sincerely,

Fernanda Silva
Administrative Officer for Research Governance
University Research Ethics Committee (UREC)
Email: **researchethics@uel.ac.uk**