NEUROMODULATION IN BASIC, TRANSLATIONAL AND CLINICAL RESEARCH IN PSYCHIATRY

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NEUROMODULATION IN BASIC, TRANSLATIONAL AND CLINICAL RESEARCH IN PSYCHIATRY

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EDITORIAL
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Editorial: Neuromodulation in Basic, Translational and Clinical Research in Psychiatry

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Keywords: neuromodulation, electroconvulsive therapy (ECT), repetitive transcranialmagnetic stimulation (rTMS), transcranial direct current stimulation (tDCS), neurofeedback, psychiatry, rehabilitation, resting state network (RSN)

Editorial on the Research Topic

Neuromodulation in Basic, Translational and Clinical Research in Psychiatry

There has been emerging evidence of non-pharmacologic therapeutics for psychiatric illnesses to modulate brain activity. These methodologies are often referred as "neuromodulation," which is a broad term that could technically be considered to cover any medical, surgical, or physiologic therapy designed to alter the function of the nervous system in some manner. In the clinical neurosciences, however, neuromodulation is understood to refer specifically to therapies that involve targeted delivery of electrical current or magnetic field, which includes electroconvulsive therapy (ECT), one of the oldest treatments in psychiatry, and vagus nerve stimulation (VNS), approved by the Food and Drug Administration (FDA) in 2005 for severe depression, and repetitive transcranial magnetic stimulation (rTMS), approved by the FDA in 2008 for the treatment of major depression. Recently, studies using transcranial direct current stimulation (tDCS), electric trigeminal nerve stimulation (eTNS), deep brain stimulation (DBS), and neurofeedback have been also reported in a growing trend. To develop more effective treatments for psychiatric diseases, translational approaches bridging basic and clinical evidence deserve considerations.

In this e-book, we tried to provide a forum for researchers interested in basic, translational, and clinical research of neuromodulation for psychiatric illnesses and aim to facilitate an integrative view of neuromodulation. It was our unexpected pleasure to have 16 papers in this topic which reported new exciting findings and cutting edge methodologies. The included papers were divided into four groups as follows: (1) the clinical application of tDCS, ECT, and rTMS for psychiatric diseases, (2) the brain function enhancement by tDCS, (3) new application of neurofeedback, and (4) translational research in neuromodulation.

The clinical application of neuromodulation for psychiatric diseases, mainly mood disorders and cognitive impairments, was described in the papers of the first group. To examine the effects of ECT on neuronal oscillatory pattern and phase synchronization, and the relationship between clinical response or cognitive change and electroencephalogram (EEG) measurements, Takayima et al. analyzed resting 19-lead EEG data recorded from 13 depressed patients before and after a course of ECT by exact low resolution electromagnetic tomography (eLORETA). They found ECT modulation on resting-state EEG oscillatory patterns and phase synchronization in central

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nodes of the default mode network (DMN) and suggested that changes in beta synchronization in the left hemisphere might explain the ECT-related cognitive side effects. Nishida et al. evaluated the immediate impact on anxiety of tDCS to the left dorsolateral prefrontal cortex (DLPFC) or dorsomedial prefrontal cortex (DMPFC) in 14 patients with major depressive disorder (MDD) and 19 healthy controls (HCs) and its association with pre-stimulus brain activity by using eLORETA. They suggested that the association between pre-tDCS brain activity and the anxiety reduction effect of tDCS depends on psychopathology (depressed or non-depressed) as well as the site of stimulation (DMPFC or left DLPFC) and insomnia. To compare rTMS-induced cortical plasticity changes in patients with MDD and in healthy volunteers, Vignaud et al. used motor evoked potentials (MEPs) evoked by single-pulse TMS before and after a single and continuous intermittent theta-burst stimulation (TBS). They observed impaired TBS-induced neuroplasticity in patients with MDD compared to that in controls and suggested impaired long-term potentiation (LTP)-like mechanisms in MDD. Kazemi et al. reported the effects of bilateral rTMS of DLPFC on the activity of resting state network (RSN) as well as relevant cognitive function in patients with bipolar depression that responded to treatment. They suggested that bilateral rTMS of DLPFC changed the activity of RSN and consequently improves verbal memory and executive functions in patients with bipolar depression. Inagawa et al. assessed the safety and efficacy of tDCS during cognitive training on cognitive functioning in patients with mild or major neurocognitive disorders by adopting two-arm, parallel, randomized, and sham-controlled trial and reported tDCS is safe and tolerable but causes no statistically significant cognitive effects in patients with mild or major neurocognitive disorders. Cruz Gonzalez et al. conducted the systematic review on the literature about the efficacy of tDCS in improving cognitive outcomes in mild cognitive impairment (MCI) and dementia, including 12 studies with 195 patients with dementia and four studies with 53 patients with MCI. They concluded that tDCS improves memory in dementia in the short term and have a mild positive effect on memory and language in MCI. These studies showed clinical validity and usefulness of neuromodurational methodology with the effectiveness on brain activity and pathophysiology related to certain brain areas and connectivity related to various types of cognitive process and psychiatric symptoms.

The second group of papers investigated the possibility of the enhancement of brain function of normal subjects by applying tDCS. To test whether anodal offline tDCS over the left prefrontal cortex (PFC) enhances working memory (WM) capacity by modulating the oscillatory activity in the left dorsolateral PFC (DLPFC) using magnetoencephalography (MEG), Ikeda et al. investigated the cortical oscillatory changes induced by anodal tDCS during a WM task. They elucidated no-correlation between stable WM capacity and increased gamma-band oscillation induced by tDCS. Gold and Ciorciari applied tDCS to "flow states," considered a positive, subjective experience during an optimal balance between skills and task demands. Although they found the increased flow experience by real stimulation for both trained and untrained Tetris players compared to sham stimulation, improved performance effects were only seen with untrained groups. They concluded that tDCS may encourage flow experiences in complex real-life motor tasks that occur during sports, games, and everyday life. Steinberg et al. reviewed the literature about acute behavioral, neurophysiological, and neurochemical effects and the mechanistic pathways of tDCS and aerobic exercise (AE) and discusses potential interactions and synergies between tDCS and AE that might be provoked when directly combining both techniques. They suggested that a direct combination of tDCS and AE provides multiple beneficial opportunities for synergistic effects both within non-clinical settings in health and for treating several psychiatric and neurologic conditions. These papers proved quite wide and promising utility of tDCS on the brain function enhancement and augmentation which would provide huge markets for normal subjects.

The third group contains the new application of neurofeedback from Karch et al. assessing the combination of real-time fMRI (rtfMRI) and neurofeedback (NF) to predict the outcome of NF training plus group psychotherapy at the beginning of the treatment for patients with tobacco use disorder. They reported that they could estimate a successful withdrawal in patients with tobacco use disorder by analyzing the first rtfMRI NF session: a pronounced reduction of frontal responses during NF training in patients might be the functional correlate of better therapeutic success. They suggested that the results of the first NF sessions could be useful as predictor whether a patient will be able to achieve success after the behavioral group therapy and NF training in quitting smoking or not. Chiba et al. conducted a systematic review to compare Decoded Neurofeedback (DecNef) effect with those of conventional EEG/fMRI-based neurofeedback on post-traumatic stress disorder (PTSD) amelioration. They suggested that DecNef could be a promising therapy that bypasses the unpleasantness of conscious exposure associated with conventional therapies for fear related disorders, including PTSD. The other types of neurofeedback studies, especially electrophysiological procedures, which have some advantages like cheaper running costs, smaller apparatus, and non-invasiveness without any exposure to radiation or strong magnetic fields, would be encouraged and provoked by these MRI neurofeedback studies.

The fourth group of articles tried to expand the new frontiers for translational research of neuromodulation, combining electroacupuncture and neurogenesis in PTSD rats (Zhou et al.), TMS and brain-derived neurotrophic factor (BDNF) in fibromyalgia and depression (Cardinal et al.), acoustic startle response (ASR), and locomotor dynamics in autism spectrum disorder (ASD) (Ebishima et al.; Ogino et al.), P300 and heart rate in emotional processing (Matsuo et al.). The future translational approach bridging between clinical application on neuropsychiatric diseases and basic pathophysiological research about the mechanism of these neuromodurational methodologies will be expected.

In summary, this e-book presented novel methodologies and various applications of neuromodulation in psychiatry. As a result, these papers established the feasibility and plausibility of neuromodulation in psychiatry with new evidence and threw impacts on new directions for expanding new possibility of neuromodulation for basic and clinical application.

AUTHOR CONTRIBUTIONS

RI and KN discussed about this Research Topic and wrote the manuscript. NY, KJ, and ST gave significant advice and helped the editing the manuscript.

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ORIGINAL RESEARCH
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Bilateral Transcranial Magnetic Stimulation on DLPFC Changes Resting State Networks and Cognitive Function in Patients With Bipolar Depression

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Introduction: Bipolar patients have abnormalities in cognitive functions and emotional processing. Two resting state networks (RSNs), the default mode network (DMN) and the sensorimotor network (SMN), play a decisive role in these two functions. Dorsolateral prefrontal cortex (DLPFC) is one of the main areas in the central executive network (CEN), which is linked to the activities of each of the two networks. Studies have found DLPFC abnormalities in both hemispheres of patients with bipolar depression. We hypothesized that the bilateral repetitive transcranial magnetic stimulation (rTMS) of DLPFC would produce changes in the activity of both the SMN and DMN as well as relevant cognitive function in patients with bipolar depression that responded to treatment.

Methods: 20 patients with bipolar depression underwent 10 sessions of 1 Hz rTMS on right DLPFC with subsequent 10 Hz rTMS on left DLPFC. Changes in electroencephalography resting networks between pre and post rTMS were evaluated utilizing low-resolution electromagnetic tomography (eLORETA). Depression symptom was assessed using the Beck Depression Inventory (BDI-II) and cognitive function was assessed by Verbal Fluency Test (VFT), Rey Auditory Verbal Learning Test (RAVLT), Stroop Test, and Wisconsin Card Sorting Test (WCST).

Results: Responders to rTMS showed significantly lower DMN activity at baseline and a significant decrease in SMN connectivity after treatment. Non-responders did not significantly differ from the control group at the baseline and they showed higher activity in the SMN, visual network, and visual perception network compared to control group following treatment. Bilateral rTMS resulted in significant changes in the executive functions, verbal memory, and depression symptoms. No significant changes were observed in selective attention and verbal fluency.

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Conclusion: Bilateral stimulation of DLPFC, as the main node of CEN, results in changes in the activity of the SMN and consequently improves verbal memory and executive functions in patients with bipolar depression.

Keywords: sensorimotor network, default mode network, bipolar depression, bilateral transcranial magnetic stimulation, low-resolution electromagnetic tomography, resting state networks, cognitive functions

INTRODUCTION

Emotional processing (Wessa and Linke, 2009) and cognitive functions (Torrent et al., 2006) are two areas known to be impaired in individuals with bipolar disorder. Those with bipolar disorder may be unable to use cognitive functions to regulate and maintain emotional states. This can lead to a dysfunction in their emotional processing and emotion regulation (Keener and Phillips, 2007; Phillips and Vieta, 2007). Studies on resting state neural networks in bipolar disorder have identified abnormalities in four networks, including the default mode network (DMN; Fan et al., 2012), central executive network (CEN; Baker et al., 2014), salience network (SN; Lopez-Larson et al., 2017), and sensorimotor network (SMN; Martino et al., 2016). The DMN and SMN have a major role in emotional and cognitive processing. Several studies have focused on DMN abnormalities in individuals with bipolar disorder (Calhoun et al., 2008; Allin et al., 2010; Öngür et al., 2010). It has been reported that DMN deactivation plays an important role in cognitive functions (Daselaar et al., 2004; Shulman et al., 2007); decreased DMN activity is correlated with successful functioning in various cognitive domains (Anticevic et al., 2012). One trial identified greater activity in the areas of middle temporal gyrus, middle frontal gyrus, and caudate in individuals with bipolar disorder compared to healthy controls (Fan et al., 2012). Similarly, reduced DMN deactivation has been observed in patients with bipolar depression during cognitive tasks (Fernández-Corcuera et al., 2013).

Research has also suggested the CEN and DMN act conversely (26), with the SN mediating activity between the two (Goulden et al., 2014). Both CEN and SN negatively regulate DMN function (Sridharan et al., 2008; Murphy et al., 2009). A recent study has shown that inhibition of a major node in CEN by 1 Hz repetitive transcranial magnetic stimulation (rTMS) leads to disinhibition of DMN. Conversely, stimulation by single pulse TMS leads to a negative connectivity of DMN with CEN and SN (Chen et al., 2013). SMN has an important role in emotional functions [e.g., emotion discrimination (Banissy et al., 2010) and emotion recognition (Wood et al., 2016; Davis et al., 2017)] and cognitive functions [e.g., working memory (D'Esposito and Postle, 2015) and social cognition (Pineda, 2008)]. Recently, it has been argued that abnormality in interhemispheric activities in SMN is the basis of emotion processing dysfunction in patients with bipolar disorder (Ishida et al., 2017). In fact, in a number of mental health disorders, the interaction between SMN and DMN has been shown to be dysfunctional (Chenji et al., 2016).

Dorsolateral prefrontal cortex (DLPFC) is a major node in the CEN, associated with both cognitive (Townsend et al., 2010) and emotional (Hassel et al., 2008) abnormalities in bipolar disorder. Abnormality of DLPFC function has been identified in both hemispheres in patients with bipolar depression (Brooks et al., 2009). Decreased DLPFC metabolism has been reported in some studies, (Baxter et al., 1989; Martinot et al., 1990) while metabolic increase is reported in others (Ketter et al., 2001). rTMS to the DLPFC is expected to have an impact on both the SMN and the DMN networks, particularly to the pre-SMA (Wang et al., 2005; Nachev et al., 2008) and mPFC (Chai et al., 2011), respectively. As discussed, both of these interconnected networks are involved in bipolar disorder, with reduced SMN activity and increased DMN activity evident in bipolar depression (Martino et al., 2016).

A recently published meta-analysis suggested rTMS is a safe and relatively effective therapy to treat bipolar depression (McGirr et al., 2016). There is a very low risk for treatment-emergent affective switches, while no increased risk of future manic episodes from a course of active rTMS treatment has been observed (McGirr et al., 2016). Given the involvement of the DMN, SMN, and DLPFC in bipolar depression (Fernández-Corcuera et al., 2013) and that DLPFC stimulation can affect the DMN and SMN, the current study proposed that rTMS provided to the DLPFC would alter DMN and SMN function, with subsequent improvement in cognitive function and emotional processing relevant to bipolar depression. Given the reported involvement of the DLPFC in both hemispheres in patients with bipolar depression (Brooks et al., 2009), sequential bilateral rTMS was selected as the intervention for this study.

Electroencephalography (EEG) was selected as a tool to obtain new insights on neurophysiological features in patients with bipolar depression, especially in regards to the role of the DMN and SMN in mediating clinical response. Changes in EEG resting state networks (RSNs) between pre and post rTMS were explored utilizing EEG functional network analysis, evaluated by exact low-resolution electromagnetic tomography (eLORETA; Pascual-Marqui et al., 2011). eLORETA is a three-dimensional, discrete, linear, and weighted minimal norm inverse solution method. It is uniquely endowed with the property of exact localization to a test point source at any location, albeit with low spatial resolution. Because of the principles of linearity and superposition, the method produces a low-resolution estimate of any distribution of electric neuronal activity. In a detailed and exhaustive comparison with other competing linear inverse solution methods, it was shown that eLORETA has improved localization properties in the presence of noise and in multiple source situations (Pascual-Marqui et al., 2011). In a previous study utilizing resting state EEG data of 80 healthy subjects, five resting state independent networks were identified with the eLORETA system (Aoki et al., 2015).

Hypotheses of the current study were therefore (1) patients with bipolar depression will show abnormal DMN connectivity compared to controls; (2) DLPFC rTMS will produce changes in regions of the DMN and SMN which contribute to improvement

of cognitive functions and clinical symptoms in responders to rTMS; and (3) DLPFC rTMS will produce changes in DMN and SMN connectivity in patients with bipolar depression who respond to treatment.

MATERIALS AND METHODS

This was an open-label study in which 20 patients with bipolar disorder received 10 sessions of sequential bilateral rTMS, one session a day, 6 days a week. Patients were evaluated at baseline (pre-treatment) and at the end of the treatment course (post-treatment). Resting EEG data from these 20 patients was compared to data from 80 healthy controls collected in a previous study (38).

Participants

The clinical sample consisted of twenty patients (8 men and 12 women; M ± SD = 28.65, age range of 16–47) referred to the Atieh Clinical Neuroscience Center (Tehran, Iran) from April to September 2015. All patients had a diagnosis of bipolar disorder, and were experiencing a current depressive episode as verified by a psychiatrist based on DSM-IV-TR criteria. The inclusion criteria were (1) age range of 16–70 years, (2) diagnosis of bipolar depression confirmed by a psychiatrist and based on DSM-IV-TR, (3) current treatment under supervision of a psychiatrist, (4) a score higher than 14 (mild depression) on the Beck Depression Inventory (BDI-II; Beck et al., 1996), and (5) unchanged medication regime during the treatment process. The study exclusion criteria were (1) a history of rTMS treatment for any disorder, (2) presence of intracranial implants (such as shunts, irritations, electrodes) or any other metal object inside or near the head (e.g. mouth) which could not be removed, (3) cardiac pacemaker, (4) acute heart disease, (5) a history of epilepsy or seizure in the individual or first degree relatives, (6) a history of head trauma, and (7) pregnant or breastfeeding women. Medication was unchanged from a month before starting the treatment until the end of the course of rTMS. If the treating psychiatrist identified any need to change a patient's medication, the patient was excluded from the study.

Resting state EEG data, collected from 80 healthy control participants in a previous study (27), were utilized for comparison purposes. The control sample consisted of 57 males and 23 females (mean age = 44, standard deviation = 20) without any history of neurological or psychiatric disorder. Control participants 60 years of age or older were screened for global cognitive deficits [i.e., mini-mental state examination (MMSE) and clinical dementia rating (CDR)]. A CDR score of zero was obtained for all screened participants and a median MMSE score of 30 (interquartile range; 29–30). A 120-s window of recorded EEG was selected and artifact rejected after strict visual inspections of the certified electroencephalographers.

Table 1 shows demographic and clinical information for clinical and control participants. Informed consent was obtained prior to commencement of this trial from all participants who received TMS. The study was approved by the University of Tehran ethics committee.

rTMS

Repetitive transcranial magnetic stimulation was administered with a Magstim Rapid 2 machine (Magstim Company Ltd., Whitland, United Kingdom) and a 70-mm figure-of-eight coil (air film coil). rTMS treatment was applied on F3 and F4 EEG regions based on the international 10-20 system. Right DLPFC stimulation was applied at 1 Hz for a 10-s train of stimulation, 2-s inter-train interval, and a total of 150 pulse trains. This resulted in 1500 pulses per session for a total of 15,000 pulses to the right DLPFC over 10 sessions. Within each session, right side stimulation was immediately followed by left DLPFC stimulation at a frequency of 10 Hz, 5 s of stimulation, 10-second inter-train interval, and 75 pulse trains. This resulted in 3750 pulses per session and a total of 37,500 pulses over 10 sessions to left DLPFC.

Resting motor threshold (RMT) was determined prior to treatment. RMT is defined as the minimum intensity required to stimulate the motor cortex and lead to a contraction in the abductor policies brevis (APB) muscle. Stimulation at threshold should cause APB muscle contraction in at least five out of 10 attempts. Treatment stimulation intensity was set at 120% of the RMT on the right side and 100% of the RMT on the left side.

EEG Recording

Electroencephalography data were recorded by a 19-channel amplifier (Mitsar, Russia) using an ElectroCap (ElectroCap, Inc, OH). Electrodes were located on a cap based on a 10-20 system. A1+A2 electrode was used as the reference. Electrode impedance was kept below 5 kΩ and the sampling rate was 250 Hz. EEG was recorded for 5 min while patients were resting in an acoustics room with closed eyes. EEG data were filtered using a band-pass filter (0.3–40 Hz). Electroocular artifacts were removed by setting the amplitude threshold to ±70 μV. Independent component analysis (ICA) was also performed to remove muscle artifacts. After artifact removal an interval of 60 s of the data for each subject were used for further analyses.

eLORETA Network Analysis

The eLORETA brain model and electrode coordinate system are based on the Montreal Neurological Institute average MRI brain map (MNI 152; Mazziotta et al., 2001). The solution space is limited to the cortical gray matter, comprising 6239 voxels of 5-mm^3 space resolution. The validity of eLORETA tomography as a reliable and effective tool for exploring brain activities has been confirmed by several studies using intracranial EEG (Zumsteg et al., 2006), PET (Dierks et al., 2000), structural MRI (Worrell et al., 2000), and fMRI (Vitacco et al., 2002; Mulert et al., 2004). eLORETA images in the current study were evaluated in the following five frequency bands: delta (2–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), and gamma (30–60 Hz).

The 60 s of artifact-free EEG from all participants' recordings were fragmented into 2-s fragments offline. The processed 2-s artifact-free EEG fragments were analyzed with eLORETA software, exploring functional EEG activity based on the five RSNs reported in the previous study (Aoki et al., 2015; see **Figure 1**). These networks included (1) independent component

TABLE 1 | Demographic and clinical characteristics of study participants and paired t-test results in test variables of Wisconsin Card Sorting Test, Rey Auditory Verbal Learning Test, Verbal Fluency Test, Stroop Test, and Beck Depression Inventory.

	***N* rTMS group**		***N* control group**					
Sex								
Male	8 (40%)		57 (71/25%)					
Female	12 (60%)		23 (28/75%)					
Medication								
Tricyclics	11 (24/4%)							
Selective serotonin reuptake inhibitor	4 (9%)							
Atypical antipsychotics	14 (31/1%)							
Mood stabilizers	16 (35/5%)							
	Mean	**SD**	**Mean**	**SD**	***t***	***df***	***P***	**Eta squared**
Executive function								
Preservative errors (WCST)	4.95	2.76	2.25	2.40	4.64	19	0.0001	0.51
Total errors (WCST)	14.45	4.80	11.80	4.84	2.38	19	0.03	0.23
Total efforts (WCST)	57.35	4.20	53.55	5.50	2.52	19	0.02	0.25
Completed number of categories (WCST)	4.70	1.71	5.10	1.48	−1.32	19	0.20	0.08
Phonetic fluency	34.65	15.45	37.40	14.73	−1.28	19	0.21	0.07
Semantic fluency	53.95	9.70	57.05	12.37	−1.72	19	0.10	0.14
Memory and verbal learning								
Total recall	12.45	1.82	13.95	1.57	−4.56	19	0.0001	0.52
Immediate recall	10.55	2.80	13.45	1.60	−5.66	19	0.0001	0.62
Delayed recall	10.88	3.02	13.30	2.57	−3.84	19	0.001	0.43
Recognition	13.90	1.21	14.45	0.88	−1.99	19	0.06	0.17
Attention and focus								
Stroop interference	0.77	2.10	0.50	1.85	0.63	19	0.54	0.002
Depression symptoms								
Beck depression inventory	30.15	10.05	15.25	8.37	4.77	19	0.0001	0.54

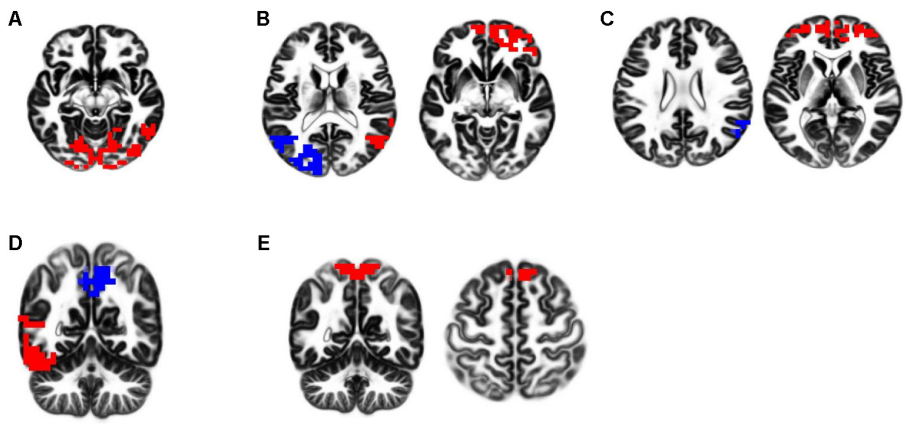

FIGURE 1 | eLORETA-neurophysiological independent components (ICs). Five neurophysiological network activities were identified in the previous study. In respective figures, red and blue voxels indicate increasing and decreasing in power, respectively, with increasing ICs activities. The definitions of these networks are described as follows. **(A)** IC-4; the visual network in alpha frequency band. **(B)** IC-5; dual-process of visual perception network, characterized by a negative correlation between the right ventral visual pathway (VVP) in alpha and beta frequency bands and left posterior dorsal visual pathway (DVP) in alpha frequency band. **(C)** IC-6; self-referential processing network (DMN), characterized by a negative correlation between the medial prefrontal cortex (mPFC) in beta frequency band and right temporoparietal junction (TPJ) in alpha frequency band. **(D)** IC-9; dual-process of memory perception network, functionally related to a negative correlation between the left VVP and the precuneus in alpha frequency band. **(E)** IC-10; sensorimotor network (SMN) in beta and gamma frequency bands.

4 (IC-4): the visual network in alpha frequency band; (2) IC-5: dual-process visual perception network, characterized by a negative correlation between the right ventral visual pathway (VVP) in alpha and beta frequency bands and left posterior dorsal visual pathway (DVP) in alpha frequency band; (3) IC-6: self-referential processing network (DMN), characterized

by a negative correlation between the medial prefrontal cortex (mPFC) in beta frequency band and right temporoparietal junction (TPJ) in alpha frequency band; (4) IC-9: dual-process of memory perception network, functionally related to a negative correlation between the left VVP and the precuneus in alpha frequency band; and (5) IC-10: SMN in beta and gamma frequency bands.

Detailed explanations of the methodology utilized in this RSN analysis can be found in the original Aoki et al. (2015) study described above. However, a brief description of eLORETA-ICA, by which these five RSNs were obtained is provided here. ICA is a mathematical and statistical technique which disintegrates mixed signals into statistically independent components. Electrical activities of desired vertices (cortical solutions) are computed by eLORETA using scalp EEG recordings. Cortical solutions for subjects over frequency bands are arranged in data matrices with the format: subject × frequency band × cortical solutions. Data matrices are consequently processed by a group ICA application embedded in eLORETA (Pascual-Marqui and Biscay-Lirio, 2011), yielding a set of independent components. The independence of these components was maximized based on fourth-order cumulant as its difference criterion (Cardoso, 1989; Cichocki and Amari, 2002). Subsequently, independent components with red and blue colored brain maps (red and blue indicate increasing and decreasing cortical activities respectively, as independent component activities are increased) were derived in total power order. Therefore, differences in functional network construction in two sets of EEG data can be evaluated by comparing their corresponding independent components' coefficients.

Thus, in this study, we used these five networks to investigate the difference of functional network constituents between patients with bipolar depression and healthy controls (Aoki et al., 2015). To explore difference in network coefficients we calculated z-scores. All coefficients were adjusted by age in linear regression based on Aoki et al. (2015). The significance level was set at $p = 0.05$ following Bonferroni correction.

Cognitive and Clinical Assessment

The primary outcome measure was cognitive function: executive functioning, selective attention, and verbal memory was assessed before and after treatment by the (1) Verbal Fluency Test (VFT) (Lezak, 2004), (2) Rey Auditory Verbal Learning Test (RAVLT) (Jafari et al., 2010), (3) Stroop Test (Stroop, 1935), and (4) Wisconsin Card Sorting Test (WCST; Nelson, 1976). The secondary outcome measure was response rate (50% or greater reduction in mean BDI-II scores from baseline to end of treatment). Paired *t*-tests were used to evaluate change in cognitive functions and depressive symptoms.

RESULTS

All participants with bipolar disorder completed a course of rTMS treatment. No side effects of rTMS were observed. Two patients were not able to participate in post-treatment EEG recording because of issues undertaking the EEG testing.

Effect of rTMS on Cognitive Functions and Depressive Symptoms

Effects of rTMS on Cognitive Functions

T-tests (**Table 1**) showed a decrease in preservation errors [$t_{(19)} = 4.64$, $P < 0.0001$], total errors on the WCST [$t_{(19)} = 2.38$, $P = 0.03$], and total efforts [$t_{(19)} = 2.52$, $P = 0.02$] following a course of rTMS. However, there was no significant increase in the completed number of WCST categories, or any change in verbal fluency, or change on the STROOP test (all $p > 0.05$). Verbal memory was enhanced post-rTMS, evident in total recall ($P = 0.0001$), immediate recall ($P = 0.0001$), and delayed recall ($p = 0.0001$). No significant effect on recognition memory was seen ($p > 0.05$).

Effects of rTMS on Depressive Symptoms

A decrease in depression symptoms was evident from baseline to treatment end [$t_{(19)} = 4.77$, $P < 0.0001$). Eleven of 20 patients (55%) met response criteria (≥50% reduction in BDI-II score), while three of 20 patients met remission criteria (final BDI-II score < 8).

eLORETA Network Analysis

Baseline Comparison of Patients vs Healthy Controls

At baseline, the only difference between the patients ($n = 18$) and the control group was in the DMN (mPFC and TPJ). The patients with BPAD at baseline exhibited significantly less activity in IC-6 coefficient (self-referential processing network, characterized by a negative correlation between the mPFC in beta frequency band and right TPJ in alpha frequency band) compared to the controls ($p = 0.007$).

Responders and Non-responders vs Controls Before Treatment

Similar to the overall sample, the only difference between responders ($n = 10$) and the control group prior to treatment was in DMN (mPFC and TPJ; $p = 0.0009$). Non-responders ($n = 8$) did not differ from the control group across any networks. Compared with healthy controls prior to treatment, responders exhibited a significant lack of activity in IC-6 coefficient (self-referential processing network, characterized by a negative correlation between the mPFC in beta frequency band and right TPJ in alpha frequency band). Non-responders ($n = 8$) showed no significant differences from controls prior to treatment.

Responders Post-treatment

After treatment, the only difference between responders and the control group was in the SMN. Post-treatment, responders exhibited significantly less activity in the IC-10 coefficient (SMN in beta and gamma frequency bands) compared with the model of healthy controls ($p = 0.012$).

Non-responders Post-treatment

Non-responders exhibited significantly higher activity in IC-4 coefficient (the visual network in alpha frequency band; $p = 0.015$) and IC-10 coefficient (SMN in beta and gamma frequency bands; $p = 0.0009$) compared with the healthy controls post-treatment. In addition, they demonstrated significantly higher activity in

IC-9 coefficient (dual-process of memory perception network, functionally related to a negative correlation between the left VVP and the precuneus in alpha frequency band) after rTMS ($p = 0.00002$).

Comparison of Patients vs Controls Post-treatment

After treatment, there was a significant difference observed between participants with bipolar disorder and the control group in SMN and the memory perception network. Patients with BPAD after intervention exhibited significantly higher activity in IC-9 (dual-process of memory perception network, functionally related to a negative correlation between the left VVP and the precuneus in alpha frequency band; $p = 0.003$) and significantly less activity in IC-10 (SMN in beta and gamma frequency bands; $p = 0.00002$).

DISCUSSION

Our study demonstrated noticeable changes in RSNs activity in patients with bipolar depression following bilateral rTMS to DLPFC. Compared to the control group, responders to rTMS treatment showed significantly lower DMN activity at baseline assessment and non-responders did not significantly differ from the control group at baseline. However, responders showed a significant decrease in SMN connectivity and higher activity in the SMN, visual network, and visual perception network compared to controls was observed in non-responders following treatment. Bilateral rTMS resulted in significant changes in executive functions, verbal memory, and depression symptoms in patients with bipolar depression.

DMN and SMN Changes at Baseline and Following Bilateral Stimulation Among Responders

In the present study, a reduction of gamma activity in pre-SMA and beta activity in the postcentral area occurred among the responders to rTMS. The results were in line with those in a previous study on patients with bipolar depression, in which gamma frequency activity in postcentral areas was significantly decreased among the responders to unilateral rTMS stimulation (Kazemi et al., 2016). In another study, an imbalance was observed in the DMN/SMN activity of bipolar patients, compared to other resting networks such as DMN/SN and DMN/CEN. Further, a high ratio of DMN/SMN activity was reported in the depression phase while the opposite happened in the manic phase. The relationship between these two networks was considered as a diagnostic marker for this disorder (Martino et al., 2016). The activity of the pre-SMA and precentral areas of SMN is probably related to motor and sleep functions in bipolar patients. Sleep disorders and psychomotor problems are regarded as two predictors of response to rTMS treatment (Brakemeier et al., 2007). Furthermore, the symptom of psychomotor retardation and agitation among depressed patients is a predictor of response to drug therapy (Yoshimura et al., 2004; Mallinckrodt et al., 2007; Herrera-Guzman et al., 2008), ECT (Van Diermen et al., 2015), and rTMS (Brakemeier et al., 2007). Regarding the patients with major depressive disorder (MDD), psychomotor retardation is related to the changes in the integrity of pre-SMA and SMA-proper white matter (pathway), as well as the changes in structural connectivity in rACC-pre-SMA and DLPFC-pre-SMA (Bracht et al., 2012). Some abnormalities were observed in the motor cortex of patients with bipolar disorder when they were doing motor tasks. In addition, an increase in rCBF in the right SMA was reported among these patients (Berns et al., 2002; Caligiuri et al., 2004). The medications used to manage the symptoms of bipolar disorders suppress the activities across motor cortical regions with greater effects in primary motor cortex areas (Caligiuri et al., 2004). Suppressing motor cortex activity among the patients treated by mood-stabilizing medications is regarded as a positive predictor for treatment in bipolar disorder (Caligiuri et al., 2004).

The precentral area is considered as another part of SMN, in which the activities are negatively related to DMN activities (Tomasi and Volkow, 2011). In some studies, sleep disturbance has been considered as a predictor for the response to rTMS treatment (Brakemeier et al., 2007). However, insomnia, as the most common problem, can affect the patients with bipolar depression (Winokur et al., 1969; Casper et al., 1985). Patients with primary insomnia experience some defects in the size of brain gray matter in precentral and postcentral areas (Joo et al., 2013). The activity in the beta frequency band is related to both types of insomnia. Further, an increase in beta activity was observed among the patients with insomnia and healthy people at the onset of sleep and during the NREM phase in sensorimotor areas (Wang et al., 2005). A reduction in the beta activity indicates a decrease in the activities in the postcentral area, and the changes in this area can be related to sleep problems among patients.

The Changes in Resting State Networks Among Non-responders to Bilateral rTMS

Regarding the non-responders to rTMS, some changes were observed in IC-4, IC-9, and IC-10 after the treatment. These patients experienced a decrease in alpha frequency activity in the areas related to occipital visual network, compared with the healthy controls. In addition, an increase occurred in the IC-9 activities, i.e., increased alpha frequency activities in precuneus and VVP.

A small number of analyses have focused on investigating the electrophysiological correlates of not responding to rTMS treatment (Arns et al., 2012, 2014). These studies have largely focused on EEG power rather than connectivity analysis. For example, non-responders had slower alpha peak at baseline(Arns et al., 2012). Recently, in another study, non-responders to rTMS treatment had low connectivity within the dopaminergic pathway, which is correlated to anhedonia (Downar et al., 2014). However, in the present study, anhedonia was not directly measured. Thus, future studies can focus on anhedonia as a predictor of not responding to treatment in bipolar patients.

Difference in DMN Functions Between Bipolar Patients and Healthy Controls at Baseline

Less beta and alpha activity at the baseline was observed in mPFC and right TPJ, respectively, among all patients, compared to those in the healthy group. Few studies have evaluated resting EEG abnormalities in bipolar patients. Regarding the results of previous research, the present study supports the role of alpha and beta frequency bands in bipolar disorder (Ozerdem et al. 2008; 2013). Recently, source localization analysis was studied to compare depressive and manic phases in bipolar patients. Based on the results, bipolar patients in the manic phase had lower theta in brodmann areas 13, 38, and 47, compared to bipolar patients in the depressive phase. In addition, higher Beta-2 and Beta-3 were reported in brodmann area 6 and cingulate cortex among the patients. In line with the results in the present study, this study emphasized the role of frontal and temporal lobes in both phases of bipolar disorder (Painold et al., 2014). The results of previous studies indicated that some problems are available in the DMN function (Brady et al., 2017) and the affective network (Luking et al., 2011; Pannekoek et al., 2014) among the patients with mood disorder. According to a recent meta-analysis, there is a hyper-connectivity among depressed patients, compared to healthy people in the socio-affective network (Schilbach et al., 2014). The findings indicate that the abnormalities of these two areas of DMN can be related to the etiology of bipolar depression, which can be regarded as a probable neuromarker.

Improvements in Cognitive Functions

Considering the literature, the present study pioneered to explore the effectiveness of bilateral stimulation treatment on improving cognitive functions among patients with bipolar depression. However, the findings of this study are consistent with those addressing bilateral stimulation on unipolar patients, which have indicated that rTMS could produce improvements in cognitive functions (Loo et al., 2003; McDonald et al., 2006; Fitzgerald et al., 2012). Further, some significant changes took place in verbal memory, which are consistent with the results of the present study.

Furthermore, the impairment of verbal memory is considered as the only cognitive problem which continues during mania phase, depression, and euthymic mood in patients with BPAD (Basso et al., 2002). An impairment in verbal memory can be considered as a unique feature of bipolar depression, as well as the endophenotype of this disorder, due to its persistence in depressive phase (Malhi et al., 2007). Generally, successful treatment methods have similar effects on the neuropsychological profiles among these patients in treating bipolar depression. Pharmacological treatment (lamotrigine) (Pavuluri et al., 2010) and other therapies (McIntyre et al., 2012) improve the executive functions or verbal memory. Electrophysiological studies demonstrated that cognitive deficits in bipolar patients are related to frontal-temporal dysfunctions (Andersson et al., 2008). In a normal verbal memory, apart from optimal performance in the temporal lobe, the cooperation of frontal lobe, especially ventro mPFC, is required (Gilboa et al., 2009).

The present study demonstrated significant improvement in executive functions after rTMS treatment, in addition to the improvement in verbal memory. Usually, normal function in WCST requires a proper functioning in the prefrontal cortex. The area which is mostly associated with the preservation errors is the DLPFC (Berman et al., 1995). Further, neuroimaging studies have also focused on another area of the frontal cortex called "ventrolateral prefrontal cortex," which is related to the performance of this test (Konishi et al., 1998). Some studies emphasized that this area of the brain experiences some abnormalities such as a reduction in the volume of gray matter among bipolar patients (Ellison-Wright and Bullmore, 2010). In addition, the stimulation of this area instead of DLPFC has been recently suggested for increasing the response to treatment in bipolar patients (Downar and Daskalakis, 2013).

Limitations of the Study

One serious limitation of our study was simultaneous use of medicine and rTMS, which made the interpretation of results complex and difficult. But to overcome this problem, patients' medications remained unchanged from 1 month before treatment to the end of treatment. Furthermore, eLORETA network template was extracted from medicine-free normal patients. Thus, the potential medicine effects on neurophysiological activities could not be completely discounted. To the best of the authors' knowledge, no previous study has demonstrated medication effects on eLORETA ICA analysis. Nevertheless, cautious interpretation of our results is required. Another potential limitation of this study was the reliance on the BDI-II to evaluate treatment outcomes. However, in previous research where expert-based assessment tools were used alongside self-reports, no significant difference was observed between these methodologies in their evaluation of treatment response and recovery rates (Pridmore et al., 2000). In a previous study, we used 80 healthy subjects with a wide age range (44.2 ± 20.0 years) and revealed that there were common five EEG-RSNs across a wide age range and their activities showed no age dependences (Aoki et al., 2015). This result indicates that age related changes of EEG-RSN activities are better described by cognitive functions rather than age itself which was suggested by fMRI studies (Balsters et al., 2013; Staffaroni et al., 2018). Therefore, in this study, although there was a significant age difference between these 80 healthy subjects and bipolar patients, we could compare EEG-RSN activities between 80 healthy subjects and bipolar patients.

CONCLUSION

Targeting the main nodes of SMN and DMN with rTMS appears to be useful in the treatment of depression. The current study suggests targeting the mPFC and TPJ areas of DMN, areas related to the socio-affective network, can be effective in treating bipolar depression.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Declaration of Helsinki, University of Tehran ethics committee. The protocol was approved by the University of Tehran ethics committee.

AUTHOR CONTRIBUTIONS

RK and RR designed the study. RK, RR, MR, and SK carried out the study. GB, MH, YA, RI, and MI analyzed the EEG data. RK, MH, RI, and PF interpreted the results and wrote the manuscript. All authors read and approved the final manuscript.

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SYSTEMATIC REVIEW
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Can Transcranial Direct-Current Stimulation Alone or Combined With Cognitive Training Be Used as a Clinical Intervention to Improve Cognitive Functioning in Persons With Mild Cognitive Impairment and Dementia? A Systematic Review and Meta-Analysis

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Background: Transcranial direct-current stimulation (tDCS) facilitates cognitive improvement in healthy and pathological populations. It has been increasingly used in cases of mild cognitive impairment (MCI) and dementia. Our research question is: Can tDCS serve as a clinical intervention for improving the cognitive functions of persons with MCI (PwMCI) and dementia (PwD)?

Objective: This systematic review evaluated the evidence to determine the efficacy of tDCS in improving cognitive outcomes in PwD and PwMCI.

Methods: A systematic review was conducted of studies published up to November 2017 involving tDCS in cases of MCI and dementia. Studies were ranked according to the level of evidence (Oxford Center for Evidence-Based Medicine) and assessed for methodological quality (Risk of Bias Tool in the Cochrane Handbook for Systematic Reviews of Interventions). Data was extracted on all protocol variables to establish a reference framework for clinical interventions. Different modalities, tDCS alone or combined with cognitive training, compared with sham tDCS were examined in both short and long-term effects. Four randomized control trials (RCTs) with memory outcomes were pooled using the fixed-effect model for the meta-analysis.

Results: Twelve studies with 195 PwD and four with 53 PwMCI met the inclusion criteria. Eleven articles were ranked as Level 1b. The results on the meta-analysis on pooled effects of memory indicated a statistically significant medium effect size of 0.39 ($p = 0.04$) for immediate effects. This improvement was not maintained in the long term 0.15 ($p = 0.44$).

Conclusion: tDCS improves memory in PwD in the short term, it also seems to have a mild positive effect on memory and language in PwMCI. However, there is no conclusive advantage in coupling tDCS with cognitive training. More rigorous evidence is needed to establish whether tDCS can serve as an evidence-based intervention for both populations.

Keywords: tDCS (transcranial direct-current stimulation), neuromodulation, MCI (mild cognitive impairment), dementia, cognitive rehabilitation, cognitive training, systematic review, meta-analysis

INTRODUCTION

Transcranial direct-current stimulation (tDCS) is a type of non-invasive brain stimulation (NIBS). tDCS delivers weak direct currents to the brain that can alter spontaneous firing rates on neural activity, which subsequently translates into behavioral changes (Nitsche et al., 2008). It is a process that has been described as "portable, painless, inexpensive and safe" (Kadosh et al., 2012). During the administration of tDCS, depolarization or hyperpolarization of the neuronal membrane of target neurons may be induced, even though the small electric fields of tDCS are considered to be below the intensity required to evoke action potentials (Nitsche et al., 2003; Miniussi et al., 2013; Tatti et al., 2016). In other words, tDCS causes a shift in the membrane potential threshold which is likely to change the probability that an incoming action potential will result in post-synaptic firing during and after its administration (Prehn and Flöel, 2015). Such changes in neuronal excitability modulates the cognitive processes and tDCS can induce physiological processes. Due to the proposed resemblance of the effects of tDCS and cognitive processes on cerebral physiology, researchers have been using NIBS to alter cognition (Kuo and Nitsche, 2012; Prehn and Flöel, 2015).

Mild Cognitive Impairment (MCI) is defined as the stage between normal and dementia-type pathological aging. MCI is a syndrome of cognitive decline in non-demented persons that does not affect the capacity to be independent in activities of daily living (ADLs; Portet et al., 2006). In contrast, people who suffer from dementia present a more severe cognitive decline and do not preserve independence in functional abilities and ADLs (Langa and Levine, 2014). Epidemiological investigations suggest a range of prevalence for MCI of 7–24% among adults aged over 65, and the manifestation of MCI is consistently shown to have a high risk of progression to dementia (Langa and Levine, 2014; Petersen et al., 2014). To date, there is no pharmaceutical treatment shown to be effective in improving cognitive functioning in MCI and dementia (Langa and Levine, 2014), although cognitive training interventions show promise for improving targeted cognitive functions in elderly persons without cognitive impairments (Ball et al., 2002). Cognitive Rehabilitation (CR) is defined as "the therapeutic process of increasing or improving an individual's capacity to process and use incoming information so as to allow increased functioning in everyday life." This includes methods to train and restore cognitive functioning as well as compensatory techniques (Sohlberg and Mateer, 1989, p. 871).

CR is therefore essential and research has indicated that NIBS can positively affect the cognitive performance of populations affected by cognitive disorders (Miniussi et al., 2008). Differences in tDCS experimental protocols regarding the parameters employed such as the montage, the current, the intensity or the size of the electrodes can affect the electric field strength. All of these variables contribute to increase the heterogeneity of the electric field's properties among studies thus producing different outcomes (Woods et al., 2016). Furthermore, targeting a neural network with tDCS while it is engaged by a cognitive stimulation activity, during or after the administration of tDCS, may yield better therapeutic effects than stimulating the same cortical region lacking cognitive stimuli (Cruz Gonzalez et al., 2018). tDCS may increase the strength of transmission across synaptic circuits in pathways that are stimulated by cognitive practice. Thus, coupling both techniques could create a synergistic positive effect on behavior (Miniussi et al., 2013; Birba et al., 2017; Cruz Gonzalez et al., 2018). The effectiveness of tDCS in CR targeting people with MCI or dementia must therefore be established. It is fundamentally important to learn about all the different configurations and protocols in which tDCS has been employed to assess its utility.

We systematically reviewed the literature regarding effects of tDCS on persons with MCI and dementia to address the following questions: (1) Does tDCS alone improve cognitive functioning in persons with MCI and dementia? (2) Does tDCS coupled with cognitive training, or as a priming to other cognitive interventions yield greater benefits in cognitive functioning than the administration of tDCS alone? (3) Are the effects of tDCS on the cognitive functions able to maintain across time?

In this study, we reviewed and evaluated the effects of tDCS on cognitive functions in people with MCI or dementia from all the available clinical trials. A systematic review of the available information up to the present will enable researchers to better understand the potential of tDCS to offer solutions for cognitive deterioration, with the aim of outlining more robust interventions in the future for people with MCI and dementia. Other reviews involving the use of different NIBS on healthy aging (Prehn and Flöel, 2015), dementia (Freitas et al., 2011; Hsu et al., 2015), MCI (Birba et al., 2017) have been carried out since 2011, but we provide an update and meta-analysis of recent trials to focus exclusively on the use of tDCS in MCI and dementia populations.

METHODS

Eligibility Criteria

We performed a systematic review and meta-analysis following the PRISMA guidelines (Liberati et al., 2009). Studies were selected based on the following criteria:

- Participants: Participants included in the study were older adults with MCI and persons with a diagnosis of dementia. The criteria for MCI includes (a) subjective memory complaint; (b) objective cognitive decline; (c) preserved ADLs, and (d) not demented (Petersen et al., 1999). The diagnosis of dementia followed the criteria of the NINCDS-ADRDA (McKhann et al., 1984) and the DSM-IV (American Psychiatric Association, 2000). Participants with any other neurological disease that was not dementia, such as only the Parkinson's type, were excluded.
- Interventions: tDCS alone (anodal, cathodal, or sham), or a combination of tDCS (online or offline) with an additional cognitive task (CT).
- Comparisons: The comparison group could be a placebo with sham tDCS, sham tDCS in combination with a CT, or a control group performing a cognitive intervention. In order to establish evidence on tDCS protocols for people with MCI or dementia, studies without sham tDCS were included.
- Outcome measurements: The outcomes were measurements of cognitive functions and neuroimaging techniques.
- Study design: All clinical trials published in English from January 2007 to November 2017 were included.

Search Strategy

Studies were identified by a systematic literature search in the following databases: PubMed, Web of Science, Science Direct, MEDLINE, and PsycINFO. A search was performed combining all the chosen keywords across the above databases. The keywords and the search strategy are presented in **Table 1**. A hand search was also performed to identify relevant studies.

TABLE 1 | Sample search strategy and databases.

Search strategy	Database	Articles yielded
Aged OR aging OR old adult OR old people OR old person OR aged OR aging/aging OR elder OR geriatric	PubMed	2282878
	Web of science	20020579
	Science direct	160098
	Medline	2215444
	PsycINFO	990595
Mild cognitive impairment OR MCI OR subtle cognitive impairment OR mild dementia OR prodromal dementia	PubMed	39043
	Web of science	32402
	Science direct	26522
	Medline	18949
	PsycINFO	13300
Dementia OR Alzheimer's disease OR AD OR vascular dementia OR VD OR dementia with Lewy bodies OR DLB OR mixed dementia OR frontotemporal dementia	PubMed	680614
	Web of science	230907
	Science direct	8365
	Medline	218682
	PsycINFO	67559
1 AND 2 OR 3	PubMed	688964
	Web of science	234611
	Science direct	1936
	Medline	221967
	PsycINFO	69699
Cognition OR executive function OR attention OR memory or working memory OR cognitive training OR cognitive intervention OR cognitive stimulation OR cognitive rehabilitation OR cognitive remediation OR brain training OR mental training OR memory training OR mnemonic training OR executive function training OR attention training or working memory training	PubMed	688598
	Web of science	934342
	Science direct	24133
	Medline	462185
	PsycINFO	815917
Transcranial direct-current stimulation OR tDCS OR direct-current stimulation OR TES OR DC stimulation OR electrical stimulation OR transcranial stimulation OR non-invasive brain stimulation OR NIBS OR neuromodulation	PubMed	65155
	Web of science	60269
	Science direct	11106
	Medline	44985
	PsycINFO	36695
4 AND 5 AND 6	PubMed	1135
	Web of science	601
	Science direct	43
	Medline	460
	PsycINFO	333
Randomized control trials OR clinical trial OR crossover studies OR case control studies OR case series OR case report OR placebos OR sham OR control	PubMed	3021385
	Web of science	3889523
	Science direct	231043
	Medline	2521985
	PsycINFO	744877
7 AND 8	PubMed	434
	Web of science	317
	Science direct	31
	Medline	235
	PsycINFO	181

Selection Criteria

After removing duplicates, the abstracts of the articles retrieved were screened to make a final decision for further review. Two investigators realized the search and the selection of studies to be included. Any disagreements were resolved by a third reviewer.

Data Extraction

The data extracted from the selected studies were conducted by two investigators using a standardized data extraction sheet which included study design, study population, number of participants, mean participant age, gender ratio, general cognitive level, number of intervention sessions, experimental/sham tDCS parameters, combination of tDCS with other interventions, outcome measures, neuroimaging techniques, assessment sequence, follow-up, effect(s) of the intervention, and intervention safety reports.

Methodological Quality

The studies selected for review were categorized and leveled according to their design based on the hierarchy level of evidence [Oxford Center for Evidence-based Medicine—Levels of Evidence (March 2009)—CEBM[1]]. All randomized control

[1]https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009 (Accessed March 29, 2018).

TABLE 2 | Methodology's heterogeneity assessment of RCT'S.

Study	Stimulated region	Intensity (mA)	Sessions	Duration (min)
André et al., 2016	LDLPFC	2	4	20
Cotelli et al., 2014	LDLPFC	2	10	25
Khedr et al., 2014	LDLPFC	2	10	25
Suemoto et al., 2014	LDLPFC	2	6	20
Bystad et al., 2016a	Temporal cortex (T3)	2	6	30

LDPFC, Left dorsolateral prefrontal cortex.

trials (RCTs) were then rated by the first two authors using the Risk of Bias Tool in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2008).

Data Analysis

Only RCTs, excluding crossover designs, were considered for meta-analysis. In some cases, authors were contacted to obtain data from their studies. After the review of the clinical methodology's heterogeneity of each study (**Table 2**), the selected papers were further assessed for statistical heterogeneity, using the I-squared and Chi-squared statistics of the outcome measures.

Data of pooled memory outcomes comparing: (1) Short-term effects of tDCS treatments vs. sham tDCS that targeted the dorsolateral prefrontal cortex (DLPFC) were calculated based on the differences between post-intervention evaluations relative to the baseline to assess the immediate effects of tDCS; (2) Long-term effects of tDCS treatments vs. sham tDCS that targeted the DLPFC; were assessed according to the differences between follow-up evaluations relative to the baseline.

All outcomes were analyzed as continuous variables with the mean change, the largest standard deviation, and the sample size in each group. The standardized mean difference and 95% confidence intervals were calculated for all meta-analyses using the fixed-effect model. The effect size was considered to be small between 0.2–0.49, moderate (0.5–0.79), and a value of 0.8 or above was considered to be large (Cohen, 1992). If I^2 was below 40%, it was considered to not represent statistical heterogeneity. Otherwise, the random-effect model was used instead. Significance was set at $p = 0.05$ and both meta-analyses were conducted using Review Manager Software 5.3.

RESULTS

Study Selection

The search strategy identified 1,198 published articles from the selected databases: PubMed ($n = 434$), Web of Science ($n = 317$), Science Direct ($n = 31$), Medline ($n = 235$), and PsycINFO ($n = 181$) (**Table 1**). Sixteen articles met the eligibility criteria (**Figure 1**).

Study Characteristics

Eleven studies (Ferrucci et al., 2008; Boggio et al., 2009, 2012; Cotelli et al., 2014; Khedr et al., 2014; Suemoto et al., 2014; Penolazzi et al., 2015; André et al., 2016; Bystad et al., 2016a,b, 2017; Costa et al., 2017) involved the application of tDCS on persons with dementia (PwD). These articles included three randomized crossover studies (Ferrucci et al., 2008; Boggio et al., 2009, 2012), five RCTs (Cotelli et al., 2014; Khedr et al., 2014; Suemoto et al., 2014; André et al., 2016; Bystad et al., 2016a), two single-subject pretest-post-test case studies (Bystad et al., 2016b, 2017), and two single-subject crossover-design studies (Penolazzi et al., 2015; Costa et al., 2017). Four articles (Meinzer et al., 2015; Yun et al., 2016; Ladenbauer et al., 2017; Murugaraja et al., 2017) exposed persons with MCI (PwMCI) to the application of tDCS. These four studies each used a different design: a randomized crossover (Meinzer et al., 2015), an RCT (Yun et al., 2016), a group pretest-post-test (Murugaraja et al., 2017), and a balanced crossover (Ladenbauer et al., 2017).

These studies included a total of 195 participants with dementia and 53 participants with MCI. Eleven studies applied tDCS "alone" (Ferrucci et al., 2008; Boggio et al., 2012; Khedr et al., 2014; Suemoto et al., 2014; André et al., 2016; Bystad et al., 2016a,b, 2017; Yun et al., 2016; Ladenbauer et al., 2017; Murugaraja et al., 2017) and five paired tDCS with CT (Boggio et al., 2009; Cotelli et al., 2014; Meinzer et al., 2015; Penolazzi et al., 2015; Costa et al., 2017). The details of the studies' characteristics and protocols are set out in **Table 3**.

tDCS Parameters

Two studies randomly assigned participants to anodal, cathodal, and sham groups (Ferrucci et al., 2008; Khedr et al., 2014). The majority of the studies involved anodal and sham groups (Boggio et al., 2009, 2012; Cotelli et al., 2014; Suemoto et al., 2014; Meinzer et al., 2015; Penolazzi et al., 2015; André et al., 2016; Bystad et al., 2016a; Yun et al., 2016; Costa et al., 2017; Ladenbauer et al., 2017; Murugaraja et al., 2017). In contrast, three studies focused on anodal stimulation lacking sham tDCS (Bystad et al., 2016b, 2017; Murugaraja et al., 2017). Regarding the dose, we found a high level of heterogeneity among experiments. Only four studies were single-session (Ferrucci et al., 2008; Boggio et al., 2009; Meinzer et al., 2015; Ladenbauer et al., 2017) whereas the number of sessions for the rest of studies ranged from 4 to 10 (Cotelli et al., 2014; Khedr et al., 2014; Suemoto et al., 2014; Penolazzi et al., 2015; André et al., 2016; Bystad et al., 2016a; Yun et al., 2016). Bystad carried out two case studies adopting unusual approaches, the first study with a daily dose of tDCS for a duration of 8 months (Bystad et al., 2017) and the second study using tDCS twice daily consecutively for 6 days (Bystad et al., 2016b). With respect to the electric fields, more homogeneous parameters were chosen among studies. The majority of the studies applied 2 mA of intensity and the targeted region for the active electrode was the DLFPC and the right supraorbital region for the cathode (**Figure 2**).

Six studies reported mild adverse reactions such as itchy and tingling sensations, redness in the area of electrode application, burning scalp, headache, dizziness, and pricking (Ferrucci et al., 2008; Khedr et al., 2014; Suemoto et al., 2014; Bystad et al., 2017; Ladenbauer et al., 2017; Murugaraja et al., 2017).

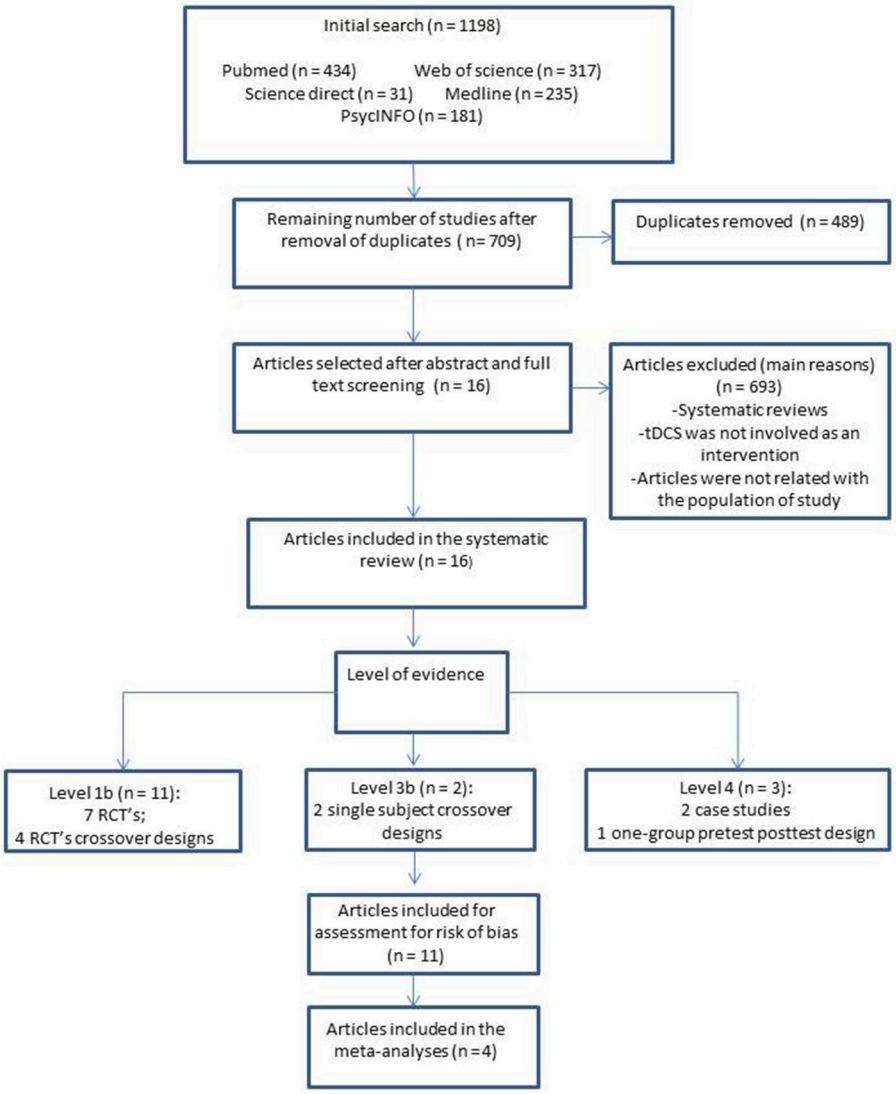

FIGURE 1 | Flow chart for study selection and level of evidence. RCT's, Randomized control trials.

Effectiveness of tDCS "Alone"

Seven studies on the dementia population reported positive effects of anodal (Ferrucci et al., 2008; Boggio et al., 2012; Khedr et al., 2014; André et al., 2016; Bystad et al., 2016b, 2017) and cathodal tDCS (Khedr et al., 2014) on cognition. All these cognitive improvements were associated with memory and global cognition. All outcomes but two (Boggio et al., 2012; Bystad et al., 2017) were statistically significant. However, two of these studies failed to report positive effects in the attention domain (Ferrucci et al., 2008; Boggio et al., 2012). Two others did not report any positive effects of anodal tDCS on cognition (Suemoto et al., 2014; Bystad et al., 2016a).

Four studies (Boggio et al., 2012; Cotelli et al., 2014; Khedr et al., 2014; Bystad et al., 2016b) assessed the long-term effects of tDCS. Three of these reported significant changes: one showed that the improvement caused by anodal tDCS persisted 4 weeks after the end of stimulation (Boggio et al., 2012), another indicated that either anodal or cathodal tDCS improved mean MMSE score at 1- and 2-month follow-up (Khedr et al., 2014), and the third study revealed that 2 months after the end of the intervention, anodal tDCS was clinically significant (Bystad et al., 2016b).

Only two studies performed neuroimaging tests. In the first, an ERP experiment confirmed significant effects reducing P300 latency after both anodal and cathodal tDCS (Khedr et al., 2014). The second used EEG, although it did not prove changes from baseline (Bystad et al., 2016b).

Three studies evaluated the efficacy of anodal tDCS on PwMCI. Overall, anodal tDCS achieved significant improvement in memory (Yun et al., 2016; Murugaraja et al., 2017). Furthermore, two of these studies investigated the neural effects

TABLE 3 | Study characteristics.

Study and design	Participants	tDCS montage	Sham tDCS montage	Number of sessions	Combination with other intervention	Outcomes	Assessment sequence	Effect of intervention
AD/MD								
Ferrucci et al., 2008 Randomized crossover design	*N* = 10 (3 groups) AD participants MMSE = 22.7 ± 1.8 Age= 75.2 ± 7.3 70% Females	1.5 mA 15 min (1) Anode (P3-T5) Cathode (right deltoids) (2) Cathode (P6-T4) Anode (right deltoids)	10 s Same tDCS montage	1	No	Assessments: WRT (modified from ADAS-cog), VAT (exogenous cue version of the Posner paradigm) Imaging: No	At baseline, 30 min after tDCS 1 week wash out period FUP: No	Accuracy in WRT increased significantly after anodal tDCS but decreased after cathodal tDCS Safety: Itching sensation
Boggio et al., 2009 Randomized crossover design	*N* = 10 (3 groups) AD participants MMSE = 17 ± 4.9 Age = 79.1 ± 8.8 60% Females	2 mA 30 min 35 cm^2 (1) Anode (LDPFC) Cathode (Fp2) (2) Anode (T7) Cathode (FP2)	30 s Same tDCS montage	1	VRT with faces (IBV software); Stroop test; DST (starting 10 min after the onset of the stimulation)	Assessments: VRT, Stroop test, DST Imaging: No	10 min after tDCS onset 2 days wash out period FUP: No	A significant effect of both tDCS experimental conditions on VRT, as compared with sham tDCS Safety: No adverse effects
Boggio et al., 2012 Randomized crossover design	*N* = 15 (2 groups) AD participants MMSE = 20 ± 3 Age = 79.05 ± 8.2 46.6% Females	2 mA 30 min Anode (T3 and T4) 35 cm^2 Cathode (right deltoids) 64 cm^2	30 s Same tDCS montage	5 (in a row)	No	Assessments: MMSE, Adas-Cog, VRT (IBV software), VAT (using endogenous cue version of the Posner task) Imaging: No	At baseline, right after the last tDCS session, 1 week and 1 month FUP Average wash out period 71.1 days	VRT improved significantly after anodal tDCS than after sham tDCS VRT performance kept improving in tDCS group at 1 month FUP Safety: No adverse effects
Cotelli et al., 2014 RCT	*N* = 36 (3 groups) AD participants MMSE; expC = 20.1 ± 2.4 expM= 22.1 ± 2.3 sham= 20.8 ± 2.1 Age; expC = 76.6 ± 4.6; expM = 78.2 ± 5.2 sham = 74.7 ± 6.1 Female proportion; expC = 83.3% expM = 83.3% sham = 75%	2 mA 25 min Anode (LDLPFC) 25 cm^2 Cathode (right deltoids) 50 cm^2	40 s (20 s at first, 20s at the end) Same tDCS montage	10 (5 per week for 2 weeks)	Memory training (based on the performance of the, FNAT, at the baseline) or motor training Both interventions started at the same time as the onset of tDCS	Assessments: FNAT, MMSE, ADL, IADL, Tinetti scale, NPIT, Picture naming task, BADA, RMBT, Rey auditory verbal learning test, Complex figure-copy, TMT Imaging: No	At baseline, post-intervention, 3 and 6 months FUP	tDCS plus memory training and sham tDCS plus memory training showed significantly improved performance on FNAT compared with the tDCS plus motor training group after the intervention and at 12 weeks FUP Safety: No adverse effects

(Continued)

TABLE 3 | Continued

Study and design	Participants	tDCS montage	Sham tDCS montage	Number of sessions	Combination with other intervention	Outcomes	Assessment sequence	Effect of intervention
Suemoto et al., 2014 RCT	*N* = 40 (2 groups) AD participants MMSE; Exp = 15.0 ± 3.1 sham = 15.4 ± 2.6 Age; Exp = 79.4 ± 7.1 sham = 81.6 ± 8.0 Female proportion; exp = 37.5% sham = 32.5%	2 mA 20 min 35 cm^2 (1) Anode (LDLPFC) 25 cm2 Cathode (Fp2)	20 s Same tDCS montage	6 (during 2 weeks)	No	Assessments: Apathy Scale; ADAS-Cog (word list learning, word recognition and digit cancellation) Imaging: No	At baseline, post-intervention, 1 week FUP	No significant effects Safety: Tingling, sking redenss, scalp burning
Khedr et al., 2014 RCT	*N* = 34; NexpA = 11 NexpC = 12 Nsham = 11 AD participants MMSE; expA = 18.4 ± 3.9 expC = 18.8 ± 2.9 sham = 16.9 ± 2.9 Age; expA = 68.5 ± 7.2 expC = 70.7 ± 5.4 sham = 67.3 ± 5.9 Female proportion; expA = 45.4% expC = 33.3% sham = 54.5%	2 mA 25 min Anode (LDPFC) 24 cm^2 Cathode (Fp2) 100 cm^2 (2) Cathode (LDPFC) 24 cm2 Anode (Fp2) 100 cm2	40 s (20 s at first, 20s at the end) Same tDCS montage	10 (in a row)	No	Assessments: MMSE, WAIS-III Imaging: ERP, resting motor threshold, cortical silent periods	At baseline, post-intervention, 1 and 2 months FUP	WAIS IQ performance significantly improved after cathodal tDCS, MMSE improved and reduced P300 latency occurred after both anodal and cathodal tDCS Safety: Itching, headache and dizziness
Bystad et al., 2016a RCT	*N* = 25; Nexp = 12 Nsham = 13 AD participants MMSE; exp = 20.5 ± 8.0; sham = 22.1 ± 13.0 Age; exp = 70.25 ± 21.0; sham = 75.0 ± 30.0 Female proportion; exp = 42% sham = 47%	2mA 30 min 35 cm2 Anode (T3) Cathode (Fp2)	60 s (30s at first, 30s at the end) Same tDCS montage	6 (in 10 days)	No	Assessments: CVLT-II, MMSE, clock-drawing test; TMT, WAIS (Abbreviated version) Imaging: No	At baseline, post-intervention FUP: No	No significant effects Safety: No adverse effects
Bystad et al., 2016b Case study	*N* = 1 AD case MMSE = 23.2 Age = 59 0% Females	2mA 30 min 35 cm^2 Anode (T3) Cathode (Fp2)	No sham	12 (during a 6-day period, twice a day)	No	Assessments: CVLT-II, MMSE Imaging: EEG	At baseline, 2 days after the last session, 2 months FUP EEG at baseline and 2 months FUP	Significantly improvement on MMSE. CVLT-II delayed recall test was clinically significant No changes in EEG Safety: No adverse effects

(Continued)

TABLE 3 | Continued

Study and design	Participants	tDCS montage	Sham tDCS montage	Number of sessions	Combination with other intervention	Outcomes	Assessment sequence	Effect of intervention
Bystad et al., 2017 Case study	*N* = 1 AD case MMSE = 20 Age = 60 0% Females	2mA 30 min 35 cm2 Anode (T3) Cathode (Fp2)	No sham	Daily (for 8 months)	No	Assessments: RBANS Imaging: NO	Baseline, at 5 months, at 8 months FUP: No	The patient's cognitive functions were stabilized except for visuospatial functioning. At 8 months, immediate recall and delayed recall improved Safety: Tingling and itchy sensation
Penolazzi et al., 2015 Single subject crossover design	*N* = 1 (2 groups) AD case MMSE = 23.2 Age = 60 0% Females	2 mA 20 min Anode (LDPFC) 35 cm2 Cathode (Fp2) 100 cm2	10 s Same tDCS montage	10 (in 2 weeks)	WRT; VWMT; PFT; CPT (All these activities were administered right after the tDCS administration for 45 min)	Assessments: WRT, VWMT, PFT, CPT, DST, TMT, overlapping figures, clock drawing Imaging: No	At baseline, post-intervention,2 weeks FUP 2 month wash out period	A significant accuracy improvement in WMT for tDCS + CT Safety: No adverse effects
Costa et al., 2017 Single subject crossover design	*N* = 1 (2 groups) AD case MMSE = 14.27 Age = 67 100% Females	2 mA 30 min 35 cm^2 Anode (Broca's area) Cathode (Fp2)	30 s Same tDCS montage	5	Linguistic exercises; as writing-to-dictation, reading aloud, and repetition of words and pseudowords. (exercise were administered 7 min after the onset of tDCS)	Assessments: Naming, auditory, comprehension of nouns and verbs tasks Imaging: No	At baseline, immediately after end of intervention, 2 weeks FUP 2 week wash out period	Significant improvement of comprehension of verbs Safety: No adverse effects
André et al., 2016 RCT	*N* = 21; Nexp =19 Nsham = 9 VD/MD participants MMSE; exp = 24.5 ± 1.8 sham = 22.4 ± 2.6 Age; exp = 80.3 ± 5.8 sham = 75.8 ± 7.4 Females	2 mA 20 min 35 cm^2 Anode (LDPFC) Cathode (Fp2)	8 s Same tDCS montage	4 (in a row)	No	Assessments: ADAS, picture-naming task, 2-back task, Go/no-go task Imaging: No	Baseline, after intervention, 2 weeks FUP	2-back task and the go/no-go test improved. Picture naming task increased the number of memorized words after intervention Safety: No adverse effects
MCI								
Meinzer et al., 2015 Randomized crossover design	*N* = 18 (2 groups) MCI participants MMSE = 27.17 ± 1.34 Age = 67.44 ± 7.27 Females 38.8%	1 mA 20 min Anode (IFG) Cathode (Fp2)	30 s Same tDCS montage	1	Overt semantic word-retrieval task	Assessments: Overt semantic word-retrieval task Imaging: fMRI	Anodal tDCS vs. sham tDCS with concurrent fMRI recording during a word-retrieval task and resting state One week wash out period FUP: No	Significant improvement of the semantic word-retrieval task to the level of healthy controls Reduced task-related prefrontal hyperactivity during resting-state fMRI Safety: No adverse effects

(Continued)

TABLE 3 | Continued

Study and design	Participants	tDCS montage	Sham tDCS montage	Number of sessions	Combination with other intervention	Outcomes	Assessment sequence	Effect of intervention
Yun et al., 2016 RCT	*N* = 16 MCI participants MMSE; exp = 26.75 ± 1.58; sham = 25.12 ± 2.74 Age; exp = 74.75± 7.47 sham = 73.12 ± 4.25 Female distribution; exp = 37.5% sham = 25%	2 mA 20 min 25 cm^2 Anode (LDPFC) Cathode (RDPFC)	20 s Same tDCS montage	9 (3 times per week for 3 weeks)	No	Assessments: Modified MMQ Imaging: PET	Baseline, post-intervention FUP: No	Subjective memory satisfaction and memory strategies significantly improved. Increased regional cerebral metabolism Safety: No adverse effects
Murugaraja et al., 2017 One group pretest-post-test	*N* = 11 MCI participants MMSE = 28 Age = 59.6 ± 4.3 Females 54.5%	2 mA 30 min 35 cm^2 Anode (IFG) Cathode (Fp2)	No sham	5 (in a row)	No	Assessments: PMIT Imaging: No	Baseline and 1 h after end of the intervention 1 month FUP	Immediate and delayed recall performance improved, persisting at 1 month FUP Safety: Pricking, burning sensation
Ladenbauer et al., 2017 Randomized crossover design	*N* = 8 (2 groups) MCI participants MMSE = 28.3 ± 1.4 Mean age = 71 ± 9 Females 43.7 %	so-tDCS frequency of 0.75 Hz (0–262.5 uA) 5 min blocks(3–5 blocks in total) 8 mm Anodes (F3 and F4) Cathodes (mastoids)	Same tDCS montage tDCS device remained off	1	No	Assessments: Visuospatial memory task, verbal memory task, sequential finger tapping task Imaging: EEG	Cognitive test at baseline and after tDCS and EEG during tDCS. 2 weeks wash-out period. FUP: No	Visual declarative memory improved so-tDCS significantly increased overall SO and spindle power Safety: Tingling sensation

AD, Alzheimer's disease; ADAS-cog, Alzheimer's disease assessment scale-Cog; BADA, battery for analysis of aphasic deficits; CVLT-II, California verbal learning Test; CPT, continuous performance task; DST, digit span test; expC, cognitive experimental group; exp, experimental group; expA, anodal experimental group; expC, cathodal experimental group; expM, motor experimental group; FNAT, face-name memory association task; FUP, Follow-up; I/ADL, instrumental/activities of daily living; IFG, inferior frontal gyrus; L/RDFPC, left/right dorsolateral prefrontal cortex; MD, Mixed dementia; MCI, mild cognitive impairment; MMQ, metamemory questionnaire for older adults; MMSE, Mini-mental state examination; N, sample size; PFT, phonemic fluency task; PMIT, picture memory impairment test; RBANS, assessment of neuropsychological status; RMBT, Rivermead behavioral memory test; so-tDCS, slow oscillatory tDCS; TMT, Trail making test; VAT, visual attention task; VD, Vascular dementia; VRT, visual recognition task; VWMT, verbal working memory task; RCT, randomized control trial; WAIS-III, Weschler abbreviated scale of intelligence; WRT, word recognition task. Values are means ± SD or as otherwise.

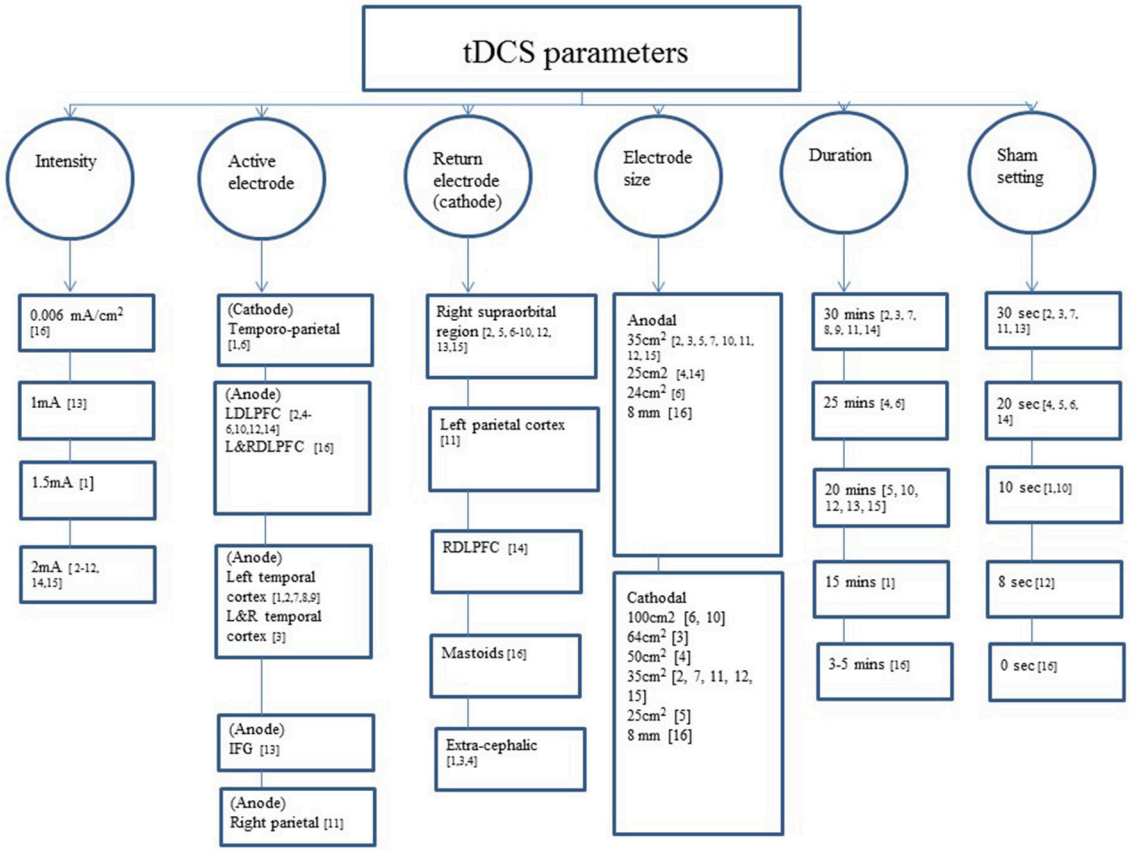

FIGURE 2 | tDCS parameters used across the studies included. IFG: 1, (Ferrucci et al., 2008); 2, (Boggio et al., 2009); 3, (Boggio et al., 2012); 4, (Cotelli et al., 2014); 5, (Suemoto et al., 2014); 6, (Khedr et al., 2014); 7, (Bystad et al., 2016a); 8, (Bystad et al., 2016b); 9, (Bystad et al., 2017); 10, (Penolazzi et al., 2015); 11, (Costa et al., 2017); 12, (André et al., 2016); 13, (Meinzer et al., 2015); 14, (Yun et al., 2016); 15, (Murugaraja et al., 2017); 16, (Ladenbauer et al., 2017); IFG, inferior frontal gyrus; L/DLPFCT, left/right dorsolateral prefrontal cortex; L&R, left and right.

of anodal tDCS. Yun et al. (2016) utilized PET to demonstrate a significantly increased metabolism in cortical regions. In the same way, the work of Ladenbauer et al. (2017) made clear, through the use of concurrent EEG, that slow oscillatory tDCS significantly increased overall slow oscillations (SO) and spindle power (Ladenbauer et al., 2017).

Effectiveness of tDCS Combined With CT

Details and methods about the CT operated among studies are shown in **Table 3**. All the studies involving PwD showed significant benefits after receipt of anodal tDCS paired with a CT. Boggio et al. (2009)applied tDCS while participants completed cognitive assessments, enhancing memory in a visual recognition memory task, but there were no effects on attention. The work of Cotelli et al. (2014) combining memory training with tDCS and sham tDCS resulted in improved memory performance illustrated in a face-name association memory task, as compared to a group which received tDCS paired with motor training; this improvement persisted significantly after 12 weeks. However, it failed to produce significant effects on standardized cognitive tests. In one single-subject crossover study, the cognitive training associated with memory components was started right after the end of tDCS administration and the findings revealed a significant accuracy improvement in a verbal working memory task. In contrast, there is no indication of amelioration in other cognitive assessments (Penolazzi et al., 2015). Alternatively, one case study that focused on stimulating the production and comprehension of language through a combination of anodal tDCS and linguistic training found a significant effect in an auditory comprehension task (Costa et al., 2017).

The work of Meinzer et al. (2015) targeting PwMCI revealed that during exposure to anodal tDCS, participants performed significantly better in a semantic word-retrieval task than those who received sham tDCS, achieving the level of healthy elderly subjects. Furthermore, the application of anodal tDCS led to reduced task-related prefrontal hyperactivity shown by resting-state fMRI.

Details of the CT

Study Quality

The level of evidence of all the trials is displayed in **Figure 1**. Details can be found in **Table 4**. Most of the studies reported a

TABLE 4 | Methodological quality (Cochrane Risk of Bias Tool).

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Ferrucci et al., 2008	Unclear	High	High	Low	Low	Low	Low
Boggio et al., 2009	Unclear	Low	High	High	Low	Low	Low
Boggio et al., 2012	Unclear	High	Low	High	Low	Low	Low
Cotelli et al., 2014	Unclear	Unclear	High	Low	High	Low	Low
Suemoto et al., 2014	Low	Low	High	Low	Low	Low	Low
Khedr et al., 2014	Low	Low	Low	Low	Low	Low	Low
Bystad et al., 2016a	Low	High	Low	Low	Low	Low	Low
André et al., 2016	Unclear	High	High	High	Low	Low	Low
Meinzer et al., 2015	Unclear	High	High	Low	Low	Low	Low
Yun et al., 2016	Low	High	Low	Low	Low	Low	Low
Ladenbauer et al., 2017	Unclear	High	High	High	Low	Low	Low

risk of bias describing the method used to conceal the allocation sequence (Ferrucci et al., 2008; Boggio et al., 2012; Meinzer et al., 2015; André et al., 2016; Bystad et al., 2016a; Yun et al., 2016; Ladenbauer et al., 2017). The most common methodological limitation of these studies was the issue of the blinding of the personnel due to the nature of most tDCS devices.

Meta-Analysis

Four studies (Cotelli et al., 2014; Khedr et al., 2014; Suemoto et al., 2014; André et al., 2016) involving 119 PwD in total were included in the meta-analysis. One RCT study was excluded because the region of stimulation was the temporal region (Bystad et al., 2016a). The results revealed a statistically significant mean effect size of 0.39 [95% CI, 0.02, 0.74] ($p = 0.04$) that favored real tDCS over sham stimulation for immediate effects. There was no evidence of heterogeneity across studies ($Q = 4.73$, $I^2 = 37\%$, $p = 0.19$). An overall small non-significant effect of 0.15 [95% CI, −0.023, 0.52] ($p = 0.44$) was noted in long-term effects of tDCS in comparison with sham tDCS. Heterogeneity was not found ($Q = 2.18$, $I^2 = 0\%$, $p = 0.53$; **Figure 3**).

DISCUSSION

All the 11 articles (RCTs) whose evidence was ranked as level 1b presented a commendable methodological quality with a general presence of low risk of bias. From the MMSE admission scores in the AD studies that ranged from 15 to 24.5 and MCI studies from 26.75 to 28.3, we noticed that the effects of tDCS benefits on cognition were significantly better for patients with mild to moderate cognitive decline.

When comparing the effectiveness of tDCS, in single and multisession interventions, positive changes occurred in both behavioral and neural systems. In this systematic review, we aimed to reveal robust interventions by identifying similar elements across studies. One main concern when designing interventions in NIBS is the treatment duration in multisession trials. There is similarity in terms of the number of sessions across the selected studies: four to ten sessions, staggered over 1–2 weeks. These short interventions can provide valuable data that allow tDCS to be proposed as a potential option in CR. However, the benefit is rather short-term with a medium effect size of 0.39. This also contrasts with other long intervention frameworks for clinical use in which more time is needed to evaluate whether the changes have a real benefit in reversible conditions such as MCI (Portet et al., 2006) or have an impact in long-term neurodegenerative processes such as dementia. For example, an alternative was proposed by Bystad et al. (2017) that adopted an 8-month protocol of daily tDCS use in a person with AD to stabilize cognitive decline. The long-term outcome probably requires prolonged periods of intervention.

Although six studies reported side effects (Ferrucci et al., 2008; Khedr et al., 2014; Suemoto et al., 2014; Bystad et al., 2017; Ladenbauer et al., 2017; Murugaraja et al., 2017), all participants tolerated the therapies well and the sensations experienced were mild. This suggests that the parameters employed are sufficiently safe (up to 30 min, 2 mA). Another concern is that the range of the parameters for intensity and duration stimulation and the size of the electrodes were highly diverse, making it difficult to draw conclusions in order to select a specific protocol for future research.

Another view is that when selecting a region of interest for stimulation, most of the studies targeted the temporal regions (Ferrucci et al., 2008; Boggio et al., 2012; Bystad et al., 2016a,b, 2017), for the role this area plays in certain memory processes (Brown et al., 1987; Kaye et al., 1997) as well as language (Nguyen et al., 2018). Another common region of interest is the DLPFC because of its importance in high-order cognitive mechanisms (Tremblay et al., 2014). Language-oriented work has targeted the inferior frontal gyrus and DLPFC as well, successfully achieving better performance in semantic word retrieval (Meinzer et al., 2015) and comprehension of language (Costa et al., 2017). In the same way, studies that applied tDCS combined with CT operated a CT related with a cognitive domain associated with the brain area targeted by tDCS. Although this approach is reasonable and consistent, the studies failed to assess if other cognitive domains associated with other brain regions were affected. Due to the

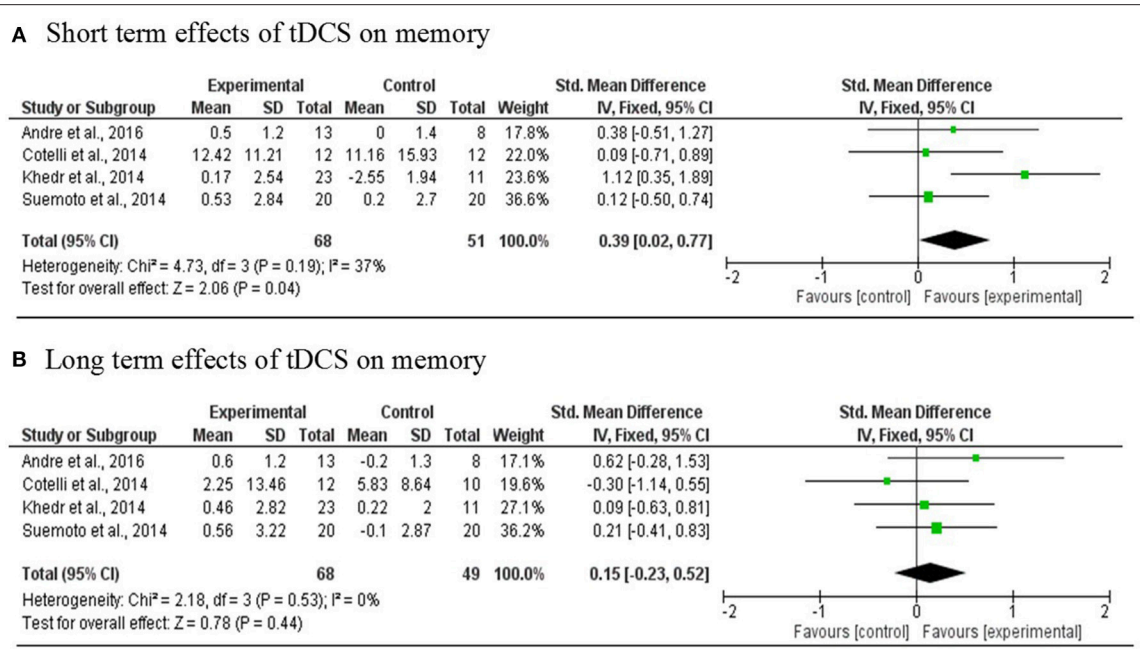

FIGURE 3 | Meta-analyses forest plot. **(A)** Short term effects of tDCS on memory. Data derived from a fixed effect model. Each line represents an individual effect size of each study. The diamond at the bottom shows the standardized effect size (0.39). Relative weight for each trial is illustrated by the sized of the corresponding square. **(B)** Long term effects of tDCS on memory. Data derived from a fixed effect model. Each line represents an individual effect size of each study. The diamond at the bottom shows the standardized effect size (0.15). Relative weight for each trial is illustrated by the sized of the corresponding square.

lack of focality of tDCS and the variability of the current flow direction, there is a possibility that other neural networks, not directly targeted by tDCS, could have been affected (Woods et al., 2016).

Three studies used an extracephalic cathodal montage (Ferrucci et al., 2008; Boggio et al., 2012; Cotelli et al., 2014) but the majority of the studies selected a cephalic montage by placing the cathode on the supraorbital region (Fp2) (Boggio et al., 2009; Khedr et al., 2014; Suemoto et al., 2014; Meinzer et al., 2015; Penolazzi et al., 2015; André et al., 2016; Bystad et al., 2016a,b, 2017; Costa et al., 2017; Murugaraja et al., 2017).

Overall, these studies have selected predominantly global cognition and memory domain as experimental evaluators. Despite the fact that these constructs are similar in nature, there is great variability in terms of assessment and CT chosen. All the studies but two (Suemoto et al., 2014; Bystad et al., 2016a) report positive effects of the application of tDCS. Against this trend, among the other articles, we must emphasize that only six studies translated these improvements into standardized cognitive assessments (Ferrucci et al., 2008; Khedr et al., 2014; André et al., 2016; Bystad et al., 2016b; Yun et al., 2016; Ladenbauer et al., 2017) while other studies reporting improvements in non-standardized CT to prove the effects of tDCS. Yet it must be acknowledged that certain cognitive functions are mediated by networks of various brain sites and might be difficult to be influenced by targeting only a subset of their brain regions (Reinhart et al., 2017), besides the short length of the intervention might have contributed to these changes being insufficient to translate into standardized test results.

It is hypothesized that targeting a neural circuit with tDCS paired with a CT may produce stronger therapeutic effects than stimulating the same brain area without cognitive stimuli (Birba et al., 2017; Cruz Gonzalez et al., 2018). The evidence on whether using tDCS alone or in combination with other CT yields identical results and seems to be inconclusive in both PwD or PwMCI. Recently, a single-subject design study using cognitive stimulation practice across sessions in combination with simultaneous anodal tDCS showed significantly stronger effects on planning ability, processing speed, and attention of cognitive stimulation practice than both sham tDCS and the application of cognitive stimulation practice alone in PwMCI (Cruz Gonzalez et al., 2018). This finding prompts the plausible speculation that tDCS, combined with cognitive training, might have synergic effects. A recent review of CR or cognitive training interventions with control conditions for PwD shows that RCTs on the effect of cognitive training on PwD are limited and there is no indication of any significant benefits from cognitive training (Bahar-Fuchs et al., 2013). Following this line of thought, future studies would carry more weight if they considered combining both interventions in comparison with control groups receiving tDCS or cognitive training alone, and would report not just benefits in the trained CT but also generalization to the trained cognitive domains and daily functioning.

Only five studies reported the use of brain imaging as an outcome demonstrating the neuromodulatory effects of tDCS (Khedr et al., 2014; Meinzer et al., 2015; Bystad et al., 2016b; Yun et al., 2016; Ladenbauer et al., 2017). In the absence of imaging techniques, we can only speculate on the results of behavioral

tests without examining the underlying neural mechanism of tDCS in MCI or dementia.

This is the first meta-analysis to explore the short- and long-term effects of tDCS in the memory domain, targeting the DLPFC in PwD. We have found evidence that tDCS has a significant immediate effect but that it is not significantly sustained with the passage of time. We suggest that future research address the need to evaluate the long-lasting effects of tDCS on the cognitive domain, implementing both behavioral and imaging follow-up evaluations.

This study has several limitations. For instance, although the pooled outcomes for meta-analysis were all memory-based, the selected studies used different tests. In addition, only four studies could be included, this might contribute to making the meta-analyses somewhat underpowered, thus the findings should be interpreted with cautions. Another striking example is the AD stage, which varied among the studies. Moreover, we have not included the most recent work published since November 2017 (Cruz Gonzalez et al., 2018), because of the time eligibility criteria. This systematic review included all tDCS trials carried out in dementia and MCI populations, and subsequently reported a few papers that did not use a comparison group (sham tDCS), which weakens the conclusions somewhat.

CONCLUSION

Our meta-analysis suggests that there is modest evidence supporting tDCS on the DLPFC ameliorates memory in PwD, however, the benefits are not long-term. Our review shows that tDCS alone seems to have a positive effect on cognition particularly for memory and language in PwD, with mild to moderate cognitive decline, and MCI. Whether tDCS might produce better outcomes on PwMCI and PwD in coupling with another CT than when administered alone remains unclear.

Although all these findings are promising, the administration of tDCS might not yet be a valid option for clinical intervention for dementia or MCI. Some of the results come from non-RCT studies, and the heterogeneity of the clinical trials does not allow one to define a clear protocol with optimal parameters. Furthermore, the interventions were too short to determine the real effects on cognitive functions and none of the studies assessed the impact of treatments on everyday cognition in daily functioning, which is an essential domain to be considered due to the functional consequences of dementia. We recommend that future studies include prolonged periods of intervention, neuroimaging techniques, and consider more robust, standardized methodology of tDCS in order to establish whether tDCS can serve as an evidence-based clinical intervention for PwMCI and PwD.

AUTHOR CONTRIBUTIONS

PC and KF designed the study, collected the data, conducted the statistical analysis, and wrote the manuscript. RC supervised the statistical analysis. K-HT and LL provided advice writing the manuscript. TB supervised the design and provided guidance.

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frontiers in Human Neuroscience

BRIEF RESEARCH REPORT
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Negatively Skewed Locomotor Activity Is Related to Autistic Traits and Behavioral Problems in Typically Developing Children and Those With Autism Spectrum Disorders

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An important objective for researchers and clinicians is to gain a better understanding of the factors that underlie autism spectrum disorders (ASDs). It is possible that investigating objective and quantitative behavioral phenotypes and their relationship to clinical characteristics, such as autistic traits and other emotional/behavioral problems, might facilitate this process. Given this, in the current study we examined the link between locomotor dynamics and clinical characteristics, including autistic traits and emotional/behavioral problems, in children with ASD (n = 14) and typically developing (TD) children (n = 13). A watch-type actigraph was used to continuously measure locomotor activity which was assessed in terms of mean activity levels and the skewness of activity. Parents assessed quantitative autistic traits using the Japanese version of the Social Responsiveness Scale (SRS) and emotional and behavioral problems using the Japanese version of the Strengths and Difficulties Questionnaire (SDQ). Results showed that among all children, all-day activity was more negatively skewed, suggesting sporadic large all-day "troughs" in activity and was significantly correlated with the SRS social awareness subscale score (ρ = −0.446, p = 0.038). In addition, the more negatively skewed daytime locomotor activity was associated with the SDQ Hyperactivity Inattention subscale score (ρ = −0.493, p = 0.020). The results of this study indicate that investigating locomotor dynamics may provide one way to increase understanding of the neurophysiological mechanisms underlying the clinical characteristics of ASD.

Keywords: autism spectrum disorders, autistic traits, social awareness, hyperactivity/inattention, locomotor activity, quantitative behavioral phenotypes

INTRODUCTION

The potential importance of translational research for autism spectrum disorder (ASD) is increasing given that its biological pathology and fully effective treatments have not yet been determined. Specifically, the acquisition of more knowledge about objective and quantitative neurobiological and behavioral indices may facilitate the development of basic and clinical research as well as lead to possible ASD phenotypes being identified.

In this context, locomotor dynamics may constitute a useful objective and quantifiable measure for translational research, as investigation of this behavioral index is less invasive and can be continued for an extended period of time in both laboratory (animal) and clinical research for the study of psychiatric and developmental disorders (Nakamura et al., 2008). Focusing on higher-order statistics such as skewness might be especially useful when characterizing behavioral alterations in psychiatric and developmental disorders. For example, we recently reported that in children with ASD, locomotor activity was significantly more negatively skewed—defined as a left-skewed distribution (or a long left tail relative to the right tail) with extreme values lower than their mean—for all-day activity, and also tended to be more negatively skewed for daytime activity, and, that this pattern of locomotor activity was related to acoustic hyper-reactivity (Takahashi et al., 2018). Further extending the use of actigraphy to help determine the clinical relevance of locomotor activity in ASD may help reveal important mechanisms in the underlying neurophysiology of this condition, while using higher-order statistics might further contribute to the realization of this purpose.

The objective of this study therefore was to investigate the relationship between locomotor activity and different clinical characteristics to determine the clinical relevance of locomotor activity in ASD and typically developing (TD) children. More specifically, as the investigation of locomotor activity is more common in attention-deficit hyperactivity disorder (ADHD; Cheung et al., 2015, 2016; De Crescenzo et al., 2016) compared to ASD, and ASD is known to have several comorbid psychiatric and developmental problems including attention deficit and hyperactivity (Lai et al., 2014), in this study we investigated the relationship between locomotor activity and emotional/behavioral problems as well as autistic traits. We hypothesize that locomotor activity indexes measured in daily life, such as negative skewness—which are related to ASD—will also be related to these clinical characteristics of ASD.

MATERIALS AND METHODS

Participants

Data were used from 27 Japanese children aged from 7 to 16 years old. Fourteen had ASD (13 boys) while 13 were TD children (10 boys). All participants were included in our previous study (Takahashi et al., 2018). Diagnoses were made by child psychiatrists after medical records were reviewed and a clinical interview had been performed based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (American Psychiatric Association, 2000). Diagnoses were confirmed by using the Autism Diagnostic Observation Schedule (Lord et al., 2000) and Autism Diagnostic Interview-Revised (Lord et al., 1995). There were no significant differences between the groups in terms of sex, age (age in months; ASD 125.6 ± 30.9; TD 138.5 ± 38.2; $U = 76$, $p = 0.467$). The Wechsler Intelligence Scale for Children-Third Revision (Wechsler, 1991) showed that, the estimated IQ was above 70 for every child included in the study, and did not differ between groups (IQ: ASD 105.7 ± 23.3; TD 104.7 ± 18.3; $U = 31$, $p = 0.958$). No children were being medicated with psychotropic substances and none of them smoked. Besides having autism, no children had any central nervous system abnormalities. For the TD group having a previous or current psychiatric diagnosis or learning disability served as exclusion criteria.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Helsinki Declaration with institutional review-board approval being granted by the Research Ethics Committee of the National Center of Neurology and Psychiatry (#A2013-112) and the research ethics committee of the Graduate School of Education, the University of Tokyo (#13-119). After the details of the study were explained to them, all participants and their parents provided written informed consent before being included in the study.

Assessment of Autistic Traits and Emotional/Behavioral Problems

Parents used the Japanese version (Kamio et al., 2013) of the Social Responsiveness Scale (SRS; Constantino and Gruber, 2005) to assess quantitative autistic traits. This scale consists of five treatment subscales: social awareness, social cognition, social communication, social motivation, and autistic mannerisms. Higher SRS scores indicate increased social impairment.

To assess emotional and behavioral problems, we used the Japanese version (Moriwaki and Kamio, 2014) of the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997). This measure also has five subscales: four difficulty subscales (Emotional Symptoms, Conduct Problems, Hyperactivity Inattention, and Peer Problems) and one strengths subscale (Prosocial Behavior). The scores of the four difficulty subscales can be summed to create a total difficulties score. As with the overall SDQ difficulties score, higher scores on the individual difficulty subscales reflect greater difficulties. In contrast, higher scores on the prosocial behavior subscale indicate increased prosociality.

Assessment of Locomotor Dynamics

In order to assess locomotor dynamics participants were informed that they should wear the MicroMini Motionlogger actigraph (Ambulatory Monitors Inc., Ardsley, NY, USA; Teicher, 1995) on the wrist of their non-dominant hand. Specifically, that they should wear it on their wrist for > 7 days during the spring, summer, or winter school vacations (TD:

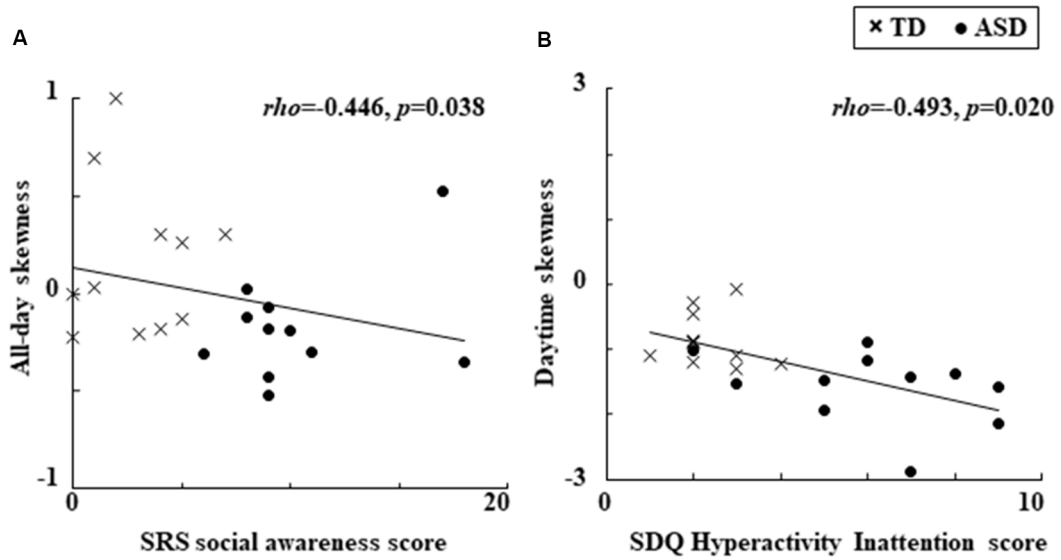

FIGURE 1 | Significant relationships observed between locomotor dynamics and clinical characteristics. **(A)** Relationship between all-day skewness and the SRS social awareness score. **(B)** Relationship between daytime skewness and the SDQ Hyperactivity Inattention score. ASDs, Autism Spectrum Disorders; SDQ, Strengths and Difficulties Questionnaire; SRS, Social Responsiveness Scale; TD, Typical Development.

7.7 ± 1.9 days, ASD: 7.8 ± 1.8 days, U = 80, p = 0.574). Full details of the assessment of locomotor dynamics are provided in our previous study (Takahashi et al., 2018). It was expected that children would wear this device continuously throughout the study period. However, they were instructed to remove it when either bathing, or during rigorous exercise, or when performing any activity that might result in the device being damaged. They were also informed that they should just lead their lives normally while wearing the actigraph device. After the recording period had finished, three boys with ASD and two boys with TD had to be excluded from the actigraph behavioral data analysis for not wearing the actigraph for a long enough period of time during the daytime hours.

As described previously (Takahashi et al., 2018), sleep–wake cycles were scored using Action W-2 software, and we now further examine the mean and skewness, the first- and third-order statistical moments, of the locomotor distributions, for all-day and daytime activity. Sleep parameters were not examined, since none of them differed significantly between ASD and TD children.

Statistical Analysis

To examine categorical differences in the participants' scores we used Chi-square tests (and Fisher's exact tests when necessary). Due to the non-normal distributions of most of the locomotor activity and clinical characteristic variables nonparametric analyses were performed. The Mann-Whitney

TABLE 1 | Clinical characteristics of the participants.

	Typical development		Autism spectrum disorders			
	Mean	*SD*	*Mean*	*SD*	*U*	*P*
Social Responsiveness Scale						
Total Score	20.5	10.0	70.1	34.4	8	<0.001
Social Awareness	3.2	2.3	9.9	3.5	2	<0.001
Social Cognition	4.0	3.2	14.6	5.4	7.5	<0.001
Social Communication	6.6	3.9	22.2	13.7	16.5	<0.001
Social Motivation	4.3	3.6	8.7	6.3	49	0.043
Autistic Mannerisms	2.5	1.5	14.7	9.1	12	<0.001
Strengths and Difficulties Questionnaire						
Total Difficulties Score	6.3	3.7	15.8	6.6	17.5	<0.001
Emotional Symptoms	1.2	1.7	2.9	2.3	52.5	0.610
Conduct Problems	1.6	1.4	2.9	2.0	57	0.105
Hyperactivity Inattention	2.3	0.9	5.9	2.3	19	<0.001
Peer Problems	1.2	1.2	4.1	2.8	32.5	0.003
Prosocial Behavior	7.7	1.4	4.9	2.9	33	0.004

SD, standard deviation; Mann-Whitney U test. Number of participants (typical development: autism spectrum disorders) = 13:14.

U test was used to compare mean parameter values. Spearman's rank order correlation coefficients were used to examine the relationships between variables. The level of statistical significance was set at $p < 0.05$. As multiple tests were performed a Bonferroni adjustment was subsequently applied to determine statistical significance. SPSS Ver. 22 (IBM Japan, Tokyo, Japan) was used to perform all statistical analyses.

RESULTS

Differences in Clinical Characteristics Between Children With Autism Spectrum Disorders and Controls

All SRS scores were significantly higher in the ASD group than in the controls (**Table 1**). Compared with the TD group, the ASD group had a significantly lower SDQ Prosocial Behavior score and significantly higher scores for Total Difficulties and the SDQ Hyperactivity Inattention and Peer Problems subscales (**Table 1**).

Relationship of Locomotor Dynamics to Clinical Characteristics

We found significant relationships between the locomotor dynamics and clinical characteristics which are presented in **Figure 1**. Specifically, for all children combined there was a significant negative relationship between all-day skewness and the SRS social awareness score (**Figure 1A**). In addition, there was also a significant negative relationship between daytime skewness and the SDQ Hyperactivity Inattention score (**Figure 1B**). No other significant relationships were observed for the clinical characteristics of locomotor dynamics, while the significant relationships described above became non-significant when we divided the children into groups (ASD and TD).

DISCUSSION

The results of this study showed that more negatively skewed all-day locomotor activity evaluated with an actigraph was related to the SRS social awareness score when the ASD and TD children were combined in one group. In addition, more negatively skewed locomotor activity during the daytime was related to the SDQ Hyperactivity Inattention score. To the best of our knowledge, this is the first time an association has been reported between locomotor dynamics and clinical characteristics, including autistic traits and emotional/behavioral problems, in children with ASD and TD. Our results suggest that besides using standard descriptive statistics when examining behavioral characteristics in ASD children, it may also be beneficial to utilize third-order statistical moments such as skewness.

For all children combined, the autistic trait of social awareness was related to significantly more negative skewness in all-day locomotor activity (defined as a left-skewed distribution with extreme values lower than their mean, which suggests behavior marked by an increase in large sporadic "troughs" below mean activity levels), with this behavioral characteristic having been previously found in ASD compared to TD children (Takahashi et al., 2018). Although the biological background of social awareness is uncertain, it might be associated with von Economo's neurons, which are also known to be involved in motor awareness (Cauda et al., 2014). Thus, the use of higher order statistics, such as skewness to examine locomotor activity in animals and humans might help clarify the biological background of autistic traits, including social awareness.

The behavioral problem of hyperactivity/inattention was related to significantly more negative skewness of daytime locomotor activity, which tended to be more negative in children with ASD compared to control children (Takahashi et al., 2018). These results support the idea that ADHD may be highly comorbid in ASD (Lai et al., 2014), and suggest daytime locomotor activity and its skewness might serve as a potential behavioral phenotype that is connected with the comorbid clinical features of hyperactivity/inattention in ASD children. Several comorbid problems are frequently reported in ASD (Lai et al., 2014), including hyperactivity, inattention as well as motor abnormalities, such as motor delay, deficits in coordination and movement planning. Atypical movement in ASD may be regulated by emotion (Trevarthen and Delafield-Butt, 2013; Vernazza-Martin et al., 2013). Future studies investigating these comorbid features in relation to locomotor activity and emotional regulation in daily life might help elucidate the neurophysiological mechanisms that may underlie these features in ASD.

The small number of children in the ASD and TD groups is a significant study limitation. While it was still possible to detect significant associations between some aspects of locomotor dynamics and clinical characteristics for all children combined, significant relationships were not observed when the children were divided into groups. Further, gender differences exist in many aspects of ASD (Lai et al., 2014), however, participants in this study were mainly boys, while the age span was rather large. In addition, although we did not find significant differences in sleep parameters in our previous study (Takahashi et al., 2018), sleep problems are frequently observed in ASD children (Lai et al., 2014) and might possibly be seasonal (Hayashi, 2001), which could have impacted on our analysis of both nighttime and daytime locomotor activity. Given this, research that uses a larger number of children of both sexes with a narrower age range that also controls for season is now warranted to more clearly determine the relationship between locomotor dynamics and different clinical characteristics.

CONCLUSION

Negatively skewed all-day locomotor activity (as seen in activity that was characterized by large sporadic all-day "troughs,") might be a potentially useful quantitative behavioral index related to ASD, especially autistic social awareness. For all children,

more negatively skewed daytime locomotor activity was also associated with comorbid hyperactivity/inattention behavioral problems. The results of this study thus build on and extend previous research on locomotor dynamics and further develop understanding of the potential neurophysiological mechanisms that may underlie clinical characteristics in ASD.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

HT, ToN, JK, HK, KY, TA, YY, and YK conceived and designed the experiments. HT, ToN, YY, and YK supervised the project. KO, HT and YK confirmed diagnoses. KO, HT, ToN, JK, and TaN performed the experiments. KO, HT, ToN, JK, YY, and YK analyzed the data. KO, HT, ToN, JK, KE, TS, AS, YY, and YK wrote the manuscript. All authors read and approved the final manuscript.

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REVIEW
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A Review of Acute Aerobic Exercise and Transcranial Direct Current Stimulation Effects on Cognitive Functions and Their Potential Synergies

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Today, several pharmaceutic and non-pharmaceutic approaches exist to treat psychiatric and neurological diseases. Because of the lack of treatment procedures that are medication free and without severe side effects, transcranial direct current stimulation (tDCS) and aerobic exercise (AE) have been tested to explore the potential for initiating and modulating neuroplasticity in the human brain. Both tDCS and AE could support cognition and behavior in the clinical and non-clinical context to improve the recovery process within neurological or psychiatric conditions or to increase performance. As these techniques still lack meaningful effects, although they provide multiple beneficial opportunities within disease and health applications, there is emerging interest to find improved tDCS and AE protocols. Since multimodal approaches could provoke synergetic effects, a few recent studies have begun to combine tDCS and AE within different settings such as in cognitive training in health or for treatment purposes within clinical settings, all of which show superior effects compared to single technique applications. The beneficial outcomes of both techniques depend on several parameters and the understanding of neural mechanisms that are not yet fully understood. Recent studies have begun to directly combine tDCS and AE within one session, although their interactions on the behavioral, neurophysiological and neurochemical levels are entirely unclear. Therefore, this review: (a) provides an overview of acute behavioral, neurophysiological, and neurochemical effects that both techniques provoke within only one single application in isolation; (b) gives an overview regarding the mechanistic pathways; and (c) discusses potential interactions and synergies between tDCS and AE that might be provoked when directly combining both techniques. From this literature review focusing primarily on the cognitive domain in term of specific executive functions (EFs; inhibition, updating, and switching), it is concluded that a direct combination of tDCS and AE provides multiple beneficial opportunities for synergistic effects.

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A combination could be useful within non-clinical settings in health and for treating several psychiatric and neurologic conditions. However, there is a lack of research and there are several possibly interacting moderating parameters that must be considered and more importantly must be systematically investigated in the future.

Keywords: non-invasive brain stimulation, neuro-rehabilitation, cognitive training, transcranial electric stimulation, executive functions, physical activity, cognitive enhancement, tDCS

INTRODUCTION

One of the most striking characteristics of the human brain is its ability to respond to changing internal and external states by reorganizing and restructuring neural circuitry on different timescales ranging from milliseconds to many years. This ability, called neuroplasticity, is extremely important throughout a person's entire life, whether it is in skill or knowledge acquisition or within recovery processes after brain injury (Kays et al., 2012). Synaptic strengthening and building new synaptic connections is a key element within neuroplasticity and must be studied to determine the best means to recover or initiate optimal neuroplastic processes in case of disruptive events through disease or injuries that provoke maladaptive brain functions and abnormal neuroplasticity (Nitsche et al., 2012). Traditional and conventional treatments of maladaptive brain functions and neuroplasticity include medications; however, these are often accompanied by severe side-effects (De Hert et al., 2011). Therefore, there is an increasing need for non-pharmacological, cost-effective, harmless, and easily applicable, yet still effective treatments that can be used to replace drugs or as supplements to increase overall treatment success. Two techniques are of relevance to fulfill such needs: transcranial current stimulation (tCS), such as transcranial direct current stimulation (tDCS), and aerobic exercise (AE).

The tDCS technique provides a non-invasive and safe way to modulate neuronal activity and provoke neuroplastic changes, which has been predominantly shown in the human motor cortex, such as the primary motor cortex (M1) (Huang et al., 2017). tDCS applies a weak and constant current via surface electrodes attached to the scalp and placed over a target brain area. While most tDCS studies stimulated motor regions, several works have been applied to investigate the effects of tDCS on non-motor regions, such as the prefrontal cortex (PFC). In particular, prefrontal tDCS is a promising technique of initiating neural plasticity within neurological and psychiatric conditions that are associated with maladaptive neuroplasticity (for a review see Flöel, 2014). Different forms of regular AE have also been associated with functional and structural brain adaptations enabling the individual to better adapt to new demands (Hötting and Röder, 2013). Moreover, the research of acute effects of AE have documented a series of effects on the cognitive domain in health and disease (McMorris, 2016b). These techniques provide promising and multiple beneficial opportunities in neurologic and psychiatric diseases (Fregni et al., 2006a; Knöchel et al., 2012; Kuo et al., 2014; Forbes et al., 2015) and also in health (Choe et al., 2016; Ward et al., 2017). More specifically, there is increasing evidence from clinical trials that both tDCS and AE can be beneficial in stroke (Duncan et al., 2003; Fregni et al., 2005; Tang et al., 2009; Brunoni et al., 2012; Marquez et al., 2015), fibromyalgia (Castillo-Saavedra et al., 2016; Fink and Lewis, 2017), Alzheimer's disease (Ferrucci et al., 2008; Intlekofer and Cotman, 2013; Farina et al., 2014; Hsu et al., 2015; Inagawa et al., 2018), Parkinson's disease (Fregni et al., 2006c; Schenkman et al., 2012), major depressive disorder (Fregni et al., 2006b; Kuo et al., 2014; Schuch et al., 2016; Yokoi et al., 2018), and schizophrenia treatments (Gorczynski and Faulkner, 2010; Kuo et al., 2014; Smith et al., 2015; Yokoi et al., 2018).

However, despite such promising results, clinical effectiveness is not yet fully supported as both techniques often lack meaningful effect sizes (Kekic et al., 2016). Therefore, recent research has explored optimal exercise protocols such as the ideal dosage and modalities. Also, optimal tDCS stimulation protocols, e.g., electric intensity, electrode configuration, and duration, were studied (Paulus, 2011; Woods et al., 2016). Moreover, improved techniques such as optimized focality (Datta et al., 2009), multi-electrode (Dmochowski et al., 2011; Pixa et al., 2017a,b), brain priming (Christova et al., 2015; Hurley and Machado, 2017), or network stimulation (Fischer et al., 2017) were used to improve tDCS effects.

Other researchers have proposed that multimodal approaches (i.e., more than one intervention technique) may initiate synergistic or additive effects and increase effectiveness (Ward et al., 2017; Cespón et al., 2018). Multimodal approaches can only be effective if supposed mechanistic pathways of both to-be-combined techniques justify an additive outcome with respect to the targeted system. Recent works took advantage of the exclusive and converging mechanistic pathways of tDCS and AE, including their possibilities to initiate cortical plasticity processes. Those possibilities presented theoretical considerations (Moreau et al., 2015) and promising empirical findings in the combined use of tDCS and AE in therapeutic, non-clinical, and sports settings (Okano et al., 2015; Angius et al., 2017; Edwards et al., 2017; Ward et al., 2017). In a randomized control trial (RCT), tDCS and AE resulted in greater improvements in multiple cognitive domains in a 4-month cognitive training intervention in healthy persons more than each technique alone, indicating promising opportunities for complex occupational settings (Ward et al., 2017). Moreover, combined tDCS/AE applications in a RCT reduced pain perception in fibromyalgia (Mendonca et al., 2016) and reduced appetite sensation in a single session experiment (Montenegro et al., 2012). Other directly sport-related studies were also able to modulate perceived exertion in a submaximal cycling exercise task (Okano et al., 2015). In addition, improved endurance performance was found (Angius et al., 2017, 2018; Edwards et al., 2017). Based on this potential of tDCS to improve

sport performance, potential applications in sport-related skills are currently discussed (Colzato et al., 2017).

When reviewing the new and multimodal empirical studies, the rationale for a combination of tDCS and AE and their potentially targeted mechanisms, which are responsible for desired additive/synergistic effects, often remained vague and on a superficial level. This lack of knowledge may be because: (a) the exact mechanistic pathways of tDCS and AE in exerting their impact on the brain are still not fully understood; (b) there is a large number of moderating parameters and variabilities known for each technique alone; and (c) there is a complete lack of knowledge about how those moderating parameters interact specifically and how both techniques interact generally when applied together. This work describes the mechanistic pathways of tDCS and AE and asserts that understanding the possible interactions of these pathways is critical when designing combination interventions aimed at improving cognition in healthy individuals or patients suffering neuropsychiatric or neurological diseases.

Based on chronic exercise effects (i.e., the effects of exercise training for several weeks or months), Moreau et al. (2015) provide a description and rationales of mechanistic pathways (2015) for tDCS/AE, and Hendrikse et al. (2017) also discussed the effects of the combination of AE with repetitive transcranial magnetic stimulation (rTMS). Acute effects and their possible interactions provoked by tDCS and AE methods on behavioral, neurophysiological and neurochemical levels even within a single application and short time succession have not been systematically addressed when combining tDCS and AE (Moreau et al., 2015; Hendrikse et al., 2017). This article reviews the reports of acute effects (specifically, only one application) in the tDCS and AE field, and the effects that should be carefully considered when combining both methods for future research or clinical use. Due to the large variety of effects on several brain functions and behaviors, the primary focus is on the acute effects of AE and tDCS on cognitive abilities and particularly on executive functions (EFs).

Our literature search in central databases (i.e., MEDLINE, Web of Science, PsycINFO and Google Scholar) for this narrative review primarily included studies that investigated the effects of AE (i.e., no resistance exercise) or tDCS (i.e., no other brain stimulation method) on tests that measure EFs or cognition in a single session intervention. Based on this search we also checked the reference list of selected articles. The intention of such a broad search strategy was to find as many studies as possible, simultaneously reducing the risk of missing any studies that combined tDCS and AE in one single session intervention on the cognitive system. We used the logical operators "OR" and "AND" between exercise-related terms (i.e., exercise and physical activity) and brain stimulation terms (tDCS and transcranial direct current stimulation) and the cognition search modifier cogniti* (i.e., cognition, cognitive). We did not restrict the time interval of the search but concentrated our study description on tasks that are thought to measure EFs (see section "Executive Functions and Their Neuroanatomical Basis"). We also excluded studies that investigated effects in more than one session (e.g., chronic exercise effects on cognitive functions or tDCS repetition across days) and in other domains (e.g., the motor or emotional domain) as this would require additional reviews. Based on this review and the performed comparison of tDCS and AE effects on EFs, possible interacting pathways and mechanisms are discussed while additionally consulting insights from studies focusing on other functions (e.g., motor domain). As there are several forms of exercise paradigms, we use the term AE when cardiovascular exercise had been performed within highly automated movements such as in cycling, walking or running. AE includes physiological changes on the metabolic, respiratory and cardiovascular system of the body, while anaerobic exercise includes higher intensity activities which can be maintained only during a short time frame. Other exercise paradigms are resistance exercise (i.e., strength training) which affects also metabolic systems but also intra- and inter-muscular coordination, or coordinative exercise which often involve less physiological demand on the human body (Voelcker-Rehage and Niemann, 2013; Voelcker-Rehage et al., 2017). This review primarily focuses those effects that are elicited by AE protocols, while indicating whether another paradigm has been applied in a specific study.

Executive Functions and Their Neuroanatomical Basis

Early researchers in the field of EFs described a "central executive," (Baddeley and Hitch, 1974) or a "supervisory attentional control system" (Norman and Shallice, 1986). Both models propose a specific kind of a superordinate control instance that acts as the central mechanism which coordinates and processes higher cognitive functions. Despite controversy about exact definitions and cognitive models, there is a consensus about the complexity of EFs and significance to human adaptive behavior (Jurado and Rosselli, 2007). Today, it is widely accepted that EFs are an umbrella term for a set of lower-level cognitive processes that serve higher cognitive processes such as self-regulation, coping with novel situations, complex planning, and decision making (Miyake et al., 2000; Friedman et al., 2008; Miyake and Friedman, 2012; Niendam et al., 2012; Diamond, 2013). One key question that remained unresolved was whether there is one single executive functioning (unity) or whether there are distinct functions (diversity) (Jurado and Rosselli, 2007). Current influential assumptions in cognitive psychology and neuroscience provide evidence for both perspectives and indicate that EFs are unitary and non-unitary in nature (e.g., Duncan et al., 1996; Godefroy et al., 1999). Based on confirmatory factor analysis, Miyake et al. (2000) stated that the three following unique, but not completely separable, EFs form the core aspects of cognitive control: updating relevant information in the working memory (i.e., updating ability), switching between different tasks and rule sets (i.e., shifting ability), and inhibiting responses to dominant, pre-potent stimuli (i.e., inhibition ability; Miyake et al., 2000).

More generally, EFs are mainly processed in a superordinate, widespread frontal–cingulate–parietal–subcortical cognitive control network of the brain (Niendam et al., 2012). Thus, activations of different brain areas are integrated to guide

behavior, attention regulation, thought, goal setting, and other higher-level cognitive abilities (Alvarez and Emory, 2006; Jurado and Rosselli, 2007). Despite the fundamental necessity of the integrity of the entire brain in forming controlled behavior, the PFC is of exceptional relevance as this brain area and its sub regions orchestrate behavior by integrating information coming from other cortical and subcortical areas (Stuss and Alexander, 2000; Stuss et al., 2002). Such involvement of the PFC in EFs has been identified in neuropsychological studies on human patients with lesions in PFC structures by neuroimaging studies and also in various animal studies (Stuss and Alexander, 2000; Stuss et al., 2002). Brain lesions in the PFC provoke decreased performance in various tasks requiring EF abilities, including flexibility, working memory, and inhibition (Niendam et al., 2012), indicating that the intact function of this area is fundamental for optimal performance (Stuss and Alexander, 2000).

Similar brain activation patterns are observable across EF tasks, but there is also a unique and specific activation pattern within single EFs (Niendam et al., 2012). Experimental evidence exists that EFs are processed by various interconnected brain regions, ranging from frontal and motor areas to subcortical structures (Niendam et al., 2012). More specifically, the dorsolateral PFC (DLPFC), medial frontal cortex, anterior cingulate cortex (ACC), frontal and posterior parietal cortex, motor areas, and cerebellum are all involved in EF processing (Fuster, 2002; Badre and D'Esposito, 2007; Bellebaum and Daum, 2007; D'Esposito, 2007). Further analyses and meta-analysis of brain areas activated between EFs (i.e., domain-specific areas) indicate different activity patterns in the anterior PFC, anterior and midcingulate regions, and even in unique subcortical regions such as the basal ganglia, cerebellum and thalamic pathways (Kassubek et al., 2004; Lewis et al., 2004; Monchi et al., 2006; Niendam et al., 2012). Thus, those differences in neural activation patterns across cognitive processing together with the coactivation of common structures during cognitive task support the unity-diversity perspective that was proposed based on behavioral data (Miyake et al., 2000; Miyake and Friedman, 2012; Snyder et al., 2015).

Executive Function Tests

Many test procedures exist that have been developed to test various EFs. Some of the more complex tests [e.g., the Tower of London (TOL), Wisconsin Card Sorting Test] tap into multiple EFs or have been used in one study to test one specific EF and in another to test a different EF. For example, the well-known Stroop test has been used in AE studies to measure inhibition ability in one study (Peruyero et al., 2017), but it is often used or termed as being a measure of cognitive flexibility (Masley et al., 2009). This non-specificity, lack of definition and terminology across studies has been criticized in some influential models; thus, it is often difficult to clearly state which test measures a specific ability (Miyake et al., 2000; Miyake and Friedman, 2012; Snyder et al., 2015). Nevertheless, according to recent works, the updating ability represents a specific kind of working memory that has often been tested by the N-back, Keep track, Sternberg and other, more complex neuropsychological tests (Snyder et al., 2015). This function is concerned with storing a specific amount of information in working memory while this information has to be continuously updated with new incoming information; in other words, irrelevant "old" information must be removed from working memory and new information must be stored and handled mentally. Typical performance parameters include reaction time and accuracy. The shifting function is responsible for applying a new task rule that must be fulfilled. Specifically, the shifting function controls the flexible switch from one concept to the other based on task demands. Tests to measure the switching ability are, for example, the Global-local, Trail-making or the Number-letter tasks. Typical performance parameters include the switching costs, which can be calculated by the differences in reaction times between stimuli (within a test) where no switch was necessary and with those stimuli preceding a rule switch (i.e., the score represents the time for rule shifting). The inhibition function is thought to control the correct identification of relevant task stimuli while ignoring task-irrelevant, yet pre-potent stimuli. In other words, the inhibition function ensures that responses to task-irrelevant stimuli must be inhibited. Some of the most common tests are the Wisconsin Card Sorting, Stroop, Flanker, Simon, Stop-signal, or Go/no-go tests.

AEROBIC EXERCISE EFFECTS ON EXECUTIVE FUNCTIONS

Early and systematic investigations on the acute exercise-cognition interaction observed that several cognitive abilities can be modulated (positively and negatively) by a short period of physical whole-body exercise (McMorris, 2016c). Typical study designs include a baseline measure of the cognitive function of interest with a subsequent AE intervention and a retesting of the cognitive function. Retesting was performed either during or after the AE intervention with the focus of online or offline effects, respectively. Usually, a control condition was included where either the same (cross-over) or another participant group (between-design) performed the same tests in the same sequence in rest or in another activity. Thereby, exercise was traditionally defined as being a stressor; thus, its interaction with cognition would follow an inverted-U profile derived from the Yerkes-Dodson arousal-performance theory (see McMorris and Hale, 2012 for an overview). This theory posits the existence of an optimal relationship between the arousal level and cognitive performance (Yerkes and Dodson, 1908). As long as arousal is on a "too low" or "too high" level and not at the peak of the inverted-U, it means that optimal cognitive performance cannot be achieved without any change in the arousal level. This model was transferred to the AE field, suggesting that physical exercise modulates the arousal level based on the intensity (see further below on mechanistic pathways of how AE modulates arousal).

One limitation of this access is that exercise intensity definitions have varied across research on the exercise-cognition relationship, leading to inconsistent definitions and consequently heterogeneous findings. However, several exercise intensity definitions exist when describing relative or absolute

physiological or subjective parameters (Norton et al., 2010; American College of Sports Medicine, 2018). Relative indices include parameters such as the individual heart rate ranges (i.e., % of maximal heart rate), oxygen uptake (i.e., % of maximum oxygen uptake = VO_2max), and ratings of perceived exertion scales (e.g., range between 6 = very low and 20 = absolute limit). Absolute intensity metrics include parameters such as the metabolic equivalent (MET). One current categorization (American College of Sports Medicine, 2018) of exercise intensity, such as the % of maximal heart rate (%HRmax), includes five distinct exercise intensities: inactive below 50%, low between 50% and 64%, moderate between 65% and 74%, high (or vigorous) above 75% and maximal at 100%. Based on those or comparable categorizations, several studies have hypothesized that as exercise intensity is low (low arousal), the performance in various cognitive tasks is low (including EF tasks using the Stroop, Flanker or more complex neuropsychological tests such as the TOL test and including memory, attention, and choice-reaction time tasks); in addition as exercise intensity rises to moderate levels, performance (and arousal) increases. Subsequently, the cognitive performance decreases again with rising exercise intensity levels (McMorris, 2016c). However, less empirical evidence supports the inverted-U hypothesis, and various reviews and meta-analyses are inconsistent and even provide opposite conclusions (Brisswalter et al., 2002; Tomporowski, 2003; Lambourne and Tomporowski, 2010; McMorris et al., 2011; Chang et al., 2012; McMorris and Hale, 2012, 2015). Specifically, cognitive performance was not consistently associated with intensity levels following an inverted-U profile (e.g., Chang et al., 2012).

Heterogeneous findings are due to many multifaceted and interacting parameters that can moderate the exercise-cognition interaction. It has been extensively shown that exercise intensity can influence the performance of EFs (Kamijo et al., 2007) and cognition in general (Chang et al., 2012). Labelle et al. (2013), as an example, found that the accuracy scores in a Stroop test declined during a high intensity AE cycling task compared to moderate intensity. It has also been repeatedly shown that the timing of cognitive test administration can affect the outcome of the cognitive test. According to a meta-analysis across different cognitive domains by Chang et al. (2012), no effects on cognitive functions occurred within the first 10 min of exercise, negative effects emerged between 11 min and 20 min of exercise, and positive effects appeared after 20 min (Chang et al., 2012). Such a conclusion has been confirmed for the EF inhibition ability (reaction time of incompatible stimuli in a Stroop test), as 20 min of moderate exercise intensity improved inhibition, while 10 and 45 min had no effects on inhibition (Chang et al., 2015b). The Chang et al. (2012) meta-analysis also showed positive effects on cognitive functions regardless whether cognitive tests were administered during exercise, immediately following exercise, or after a time delay. Another meta-analysis observed a small negative effect of cognitive performance during AE (Lambourne and Tomporowski, 2010). This discrepancy potentially occurred because the latter study focused only on healthy participants and crossover designs (see Chang et al., 2012).

Also, exercise modality has an effect on EFs since Pontifex et al. (2009) showed that reaction times in a Sternberg task (working memory) were faster (compared to rest condition) after only 30 min of AE and not after a bout of resistance exercise of 30 min. Interestingly, even a change in environmental factors (e.g., changed gravity or confinement) affected cognitive functions differently compared to AE conditions (Schneider et al., 2013; Vogt et al., 2014). Other studies have shown that the exercise-cognition relationship can be modulated by cognitive task difficulty, the cognitive domain (i.e., whether EFs or other cognitive tasks relating to attention or pure memory task are measured), and age (Kamijo et al., 2009; Weng et al., 2015; Voelcker-Rehage et al., 2017). Despite the age and cognitive domain, a further crucial factor seems to be the individual fitness status (Labelle et al., 2013). During exercise (i.e., online), individual fitness level is associated with enhanced cognition for highly fit subjects, but is negligible in moderately fit and decreased for unfit participants; while only unfit and highly fit, but not moderately fit participants benefited after exercise (i.e., offline) (Chang et al., 2012). A recent systematic review focusing on high-intensity exercise in trained people found that acute effects are dependent on the cognitive domain. In 10 reviewed studies simple tasks were not affected while the effects were stronger in parameters indicating speed of processing compared to accuracy parameters in complex tasks (Browne et al., 2017).

Several meta-analyses and reviews, however, suggest that EFs, within the broad domain of cognition, benefit from AE regardless of exercise paradigm, modality, intensity and time of testing (Chang and Etnier, 2009; Lambourne and Tomporowski, 2010; Chang et al., 2012, 2015a,b). In addition, it is argued that moderate exercise intensity is most beneficial; however, these positive effects in cognitive tasks have been mostly found in terms of speed of processing (across cognitive domains) and not accuracy (e.g., the number of errors made in a specific test), while the observed effect sizes were highest for EFs compared to tasks of alertness/attention and recall (McMorris and Hale, 2012).

Moreover, moderate exercise sessions affect speed of processing positively and accuracy slightly negatively, especially in working memory tasks (e.g., in N-back tests) (McMorris et al., 2011; McMorris and Hale, 2012). However, in this meta-analysis, the inclusion criteria of working memory tasks included all EF tasks (e.g., Stroop and flanker tasks), thus not agreeing with the discussed definition (e.g., a Stroop test does not assess working memory performance). In contrast, recent studies, such as Tempest et al. (2017), show that performing high-intensity exercise improved inhibition performance (reaction time in a flanker test) but decreased updating performance (aggravated d' value of a 2-back test; d' value represents task performance accounting for accuracy and reaction time) suggesting that EFs are not uniformly affected by exercise, despite having the same test protocol (Weng et al., 2015; Tempest et al., 2017). These different effects indicate that exercise selectively affects neural networks and possibly prefrontal sub-regions that support the different EF abilities. However, whether this is due to time or intensity dependent properties of exercise and thus due to

differences in methodological study designs remains an open question. Furthermore, caution is needed as most evidence for the acute exercise effects on EFs have been derived based on EF tests mainly testing inhibition (Ludyga et al., 2016), and there are also several studies suggesting no effects or even detrimental effects (Basso and Suzuki, 2017).

tDCS EFFECTS ON EXECUTIVE FUNCTIONS

Due to the significant involvement of the PFC in cognitive processing and in EFs, a growing amount of studies have analyzed tDCS-induced modulations of PFC activity and its possibility to enhance cognitive functions including EFs (see Strobach and Antonenko, 2017) in the short-term (see Tremblay et al., 2014) and in the long term (Park et al., 2014; Metzuyanim-Gorlick and Mashal, 2016). The typical study design for analyzing acute tDCS effects on EF ability or other cognitive domains includes a pretest of the cognitive function of interest, followed by an intervention, where the cognitive test is performed during (i.e., online effects) or after (i.e., offline effects) tDCS. Usually, the same test sequence was performed either in a cross-over or between-subject design, where a sham stimulation (i.e., placebo) was provided and/or real tDCS at a brain region suspected not to be involved in cognitive processing (e.g., M1). If applicable, the experiment was performed with a single-blind (participant was not aware whether stimulation is real or sham) or at best cases, with a double-blind protocol (neither participant nor the investigator were aware whether the stimulation is real or sham).

Meta-analyses and reviews on acute tDCS effects on cognition performed so far provide mixed results about their capacity to modulate cognitive performance. While some studies report small to moderate beneficial effects (Dedoncker et al., 2016; Hill et al., 2016; Mancuso et al., 2016), others report no effects using tDCS within one single session in healthy young humans (Horvath et al., 2015) or when individuals perform working memory tasks (Medina and Cason, 2017). However, due to various experimental designs regarding electrode configurations, intensities, durations, electrode size and state-dependency, no general assumption on efficacy appears to be valid until more clarity is gathered (Jacobson et al., 2012; Tremblay et al., 2014). It is widely accepted within the motor domain that anodal stimulation of motor regions facilitates neural networks and that cathodal stimulation inhibits neural networks engaged in several motor tasks (for a review see Buch et al., 2017). This dichotomy is not yet well established for the stimulation of non-motor regions such as prefrontal areas. Consequently, some authors argue that tDCS-effects observed in the motor-domain cannot simply be transferred to the cognitive domain (Miniussi et al., 2008; Jacobson et al., 2012). Nonetheless, combined neurophysiological and behavioral studies present evidence for altered neural excitability (Nitsche and Paulus, 2000; Nitsche et al., 2005) and comparable polarity-specific effects in the PFC (review in Wörsching et al., 2016). However, one major caveat of tDCS is the high inter-subject variability suggesting that tDCS effects are dependent of individual factors, such as the instantaneous state of the brain (Antal et al., 2008; Dutta, 2015; Li et al., 2015) or genetic variations (Plewnia et al., 2013).

In terms of executive function research, few studies (review in Strobach and Antonenko, 2017) have investigated shifting ability using 10–30 min of tDCS, while only two have shown specific task-shifting effects (Leite et al., 2011, 2013). Leite et al. (2011), using two different shifting tasks, observed that stimulating the DLPFC either with an anodal or cathodal electrode configuration modulated the response speed of the shifting ability. The active electrode was placed over F3 (F3 refers to the electrode position according to the 10-20 international system for electrodes positioning) and the return electrode over the contralateral supraorbital area. Anodal stimulation improved the task performance, while cathodal stimulation decreased the task performance. Using cross-hemispheric tDCS stimulation (i.e., either left PFC anodal and right PFC cathodal electrode positions or vice versa) on two task-switching tests in a subsequent study, Leite et al. (2013) concluded that such effects were critically dependent on the laterality and the task. This was deduced because performance improvements in accuracy and speed of processing were reversed based on whether the right or the left PFC was stimulated, and which of the two task-shifting tests were used (Leite et al., 2013).

More evidence exists for the ability to update information in the working memory. Several studies have shown that this ability can be positively modulated by tDCS applied to the left DLPFC with anodal tDCS lasting 10–30 min (Fregni et al., 2005; Ohn et al., 2008; Andrews et al., 2011; Teo et al., 2011; Zaehle et al., 2011b; Hoy et al., 2013; Meiron and Lavidor, 2013). Those studies indicate that especially the left DLPFC is critically for working memory tasks. More specifically, Ohn et al. (2008) found significantly increased accuracy scores for a verbal 3-back task during and even 30 min after stimulation. Also, in the Fregni et al. (2005) study, positive effects on working memory performance were observed after only 10 min of tDCS. Comparable positive effects in clinical trials for one stimulation session were also found in patients with Parkinson's disease (Boggio et al., 2006; Fregni et al., 2006c). Notably, in the Boggio et al.'s (2006) study only 2 mA but not 1 mA, yielded any performance improvements, indicating intensity-dependent effects. Intensity-dependent effects on working memory capacity (n-back task) were also observed in healthy participants in the Teo et al. (2011) study. Thus, tDCS current intensity is a critical factor that should be considered in health and disease tDCS interventions. Another interesting finding reported by Gill et al. (2015) showed that higher demands on the cognitive systems during tDCS had a significant effect on post-stimulation performance, indicating that the task and the timing of stimulation is an additional critical factor to consider. Not only left DLPFC, but also right DLPFC could be a potential target area for improving working memory performance as anodal tDCS over right DLPFC improved performance within a spatial working memory task, and particularly in the more complex task components (Wu et al., 2014).

There is also some evidence that tDCS can modulate the inhibition ability when the right PFC hemisphere is stimulated by anodal tDCS lasting 10–20 min as indicated by improved response inhibition (i.e., reaction times) in Go/No-Go and Stop-Task paradigms (Jacobson et al., 2012; Kwon et al., 2013; Hogeveen et al., 2016). In contrast, when using the Stroop task, increased performance has been identified by right DLPFC anodal tDCS and by left DLPFC anodal tDCS (Jeon and Han, 2012). Both anodal tDCS-initiated (right and left hemisphere) improvements were observable as long as 2 weeks after the stimulation session. Another study also using the Stroop task and a bilateral left anodal/ right cathodal tDCS electrode configuration, observed a positive effect in the response speed (Loftus et al., 2015). Other studies by Zmigrod et al. (2016), using the Eriksen flanker and Simon test (inhibition function), showed that cathodal tDCS over the right DLPFC influenced performance of the flanker test, but not of the Simon test (Zmigrod et al., 2016). An additional study also showed that only cathodal tDCS over the right DLPFC, but not anodal tDCS, influences the control of impulsiveness (Beeli et al., 2008). While the latter two studies showed selective tDCS effects (i.e., only cathodal effects), Hsu et al. (2011) reported that both anodal and cathodal tDCS over the superior medial frontal cortex either improved or deteriorated inhibitory control ability compared to sham stimulation. There are also some studies that explored prefrontal tDCS effects on more complex EFs measures such as the TOL, a test that taps into multiple EFs abilities. (Welsh et al., 1999; Miyake et al., 2000; Snyder et al., 2015). Dockery et al. (2009) found that 15 min of both anodal and cathodal tDCS over the left DLPFC improved planning performance measured by the TOL. Interestingly they observed that these effects were phase dependent, i.e., cathodal tDCS improved performance in an early learning phase while anodal tDCS was effective only in a later phase, suggesting training-phase-specific effects (Dockery et al., 2009). Comparable TOL improvements (initial thinking time) were found in a prefrontal bilateral tDCS protocol with anodal left DLPFC (cathode placed on the right DLPFC) improved TOL performance but cathodal left DLPFC tDCS had no effects (Heinze et al., 2014). Although so far only few studies exist, taken the reviewed single studies and reviews together there is some evidence that tDCS can modulate all three EFs even within one single application. However, due to different stimulation protocols (intensity, duration and timing), other non-significant effects and one contrasting meta-analysis (Horvath et al., 2015; Strobach et al., 2016), much more research is necessary to be certain how EFs are modulated by tDCS.

NEUROPHYSIOLOGICAL AND NEUROCHEMICAL EFFECTS OF ACUTE EXERCISE

Early animal studies on the exercise-cognition interaction proposed that increased arousal due to aerobic whole-body exercise increases brain concentration of neurotransmitters such as noradrenaline. This, in turn, activates the reticular formation, a heterogeneous and not well-defined structure of the nervous system that contains various nuclei associated with neurotransmitter releases. Due to this function (for a historical overview see McMorris, 2016b) in the noradrenergic, serotonergic and the dopaminergic pathways, the reticular formation is integral to the arousal activation system (review in Meeusen and De Meirleir, 1995). Currently, it is thought that only one session of exercise induces the synthesis and release of diverse neurochemical substances such as noradrenaline, adrenaline, dopamine, brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), lactate, vascular endothelial growth factors (VEGF) and hypothalamic–pituitary–adrenal axis (HPA) hormones (e.g., cortisol) and, as shown in animal studies, they can potentially pass either directly or indirectly through the brain-blood-barrier to modify the arousal level and neuroplastic mechanisms (McMorris and Hale, 2015; McMorris et al., 2016; Basso and Suzuki, 2017). This modification, which is emphasized and detailed in the catecholamine hypothesis first proposed by Cooper (1973) and further developed by works of McMorris (Cooper, 1973; McMorris et al., 2008; McMorris, 2016a), leads to either the optimal or suboptimal preparation of a person for action and aids in neurogenesis and neuroplasticity.

The catecholamine hypothesis (McMorris et al., 2008) provides a rational explanation for the discussed meta-analytical findings of moderate positive effects sizes in cognitive performance (McMorris and Hale, 2012). Based on the findings of neurochemicals associated with acute bouts of exercise, it is thought that elevated concentrations of the neurotransmitter's dopamine and norepinephrine observed during and following moderate-intensity exercise, should theoretically facilitate cognition. However, observations of catecholamine releases (and thus reticular system) in response to low-intensity AE indicate that processing speed in various cognitive tasks is reduced due to less activation in the relevant brain areas. In contrast, high-intensity exercise induces more massive catecholamine releases during and following the exercise session and introduces neural noise, ultimately leading to decreased cognitive performance. However, such an inverted-U relationship has never been unequivocally observed, and recent considerations on this hypothesis are only conceivable by adding data from animal studies (McMorris et al., 2008; McMorris, 2016a).

More specifically, moderate increase of catecholamines due to moderate exercise intensity mostly performed between 20 min and 30 min were optimal for working memory improvements. In contrast, longer lasting moderate exercise provoked interaction between HPA hormones and catecholamine, leading to the inhibition of cognitive performance (McMorris et al., 2016). Furthermore, high-intensity AE sessions induced activation of β-adrenoreceptors in the hippocampus and lead to increased memory performance and elevated BDNF concentrations (Neeper et al., 1995; Piepmeier and Etnier, 2015; Szuhany et al., 2015), agreeing with the observation that a short period of high-intensity exercise increased BDNF more than a long-lasting moderate exercise intensity session (Winter et al., 2007). Single

studies are confirmed by meta-analytic evidence with moderate effect sizes showing BDNF releases after only one-session exercise (Szuhany et al., 2015). In addition, suggesting against an exercise-intensity dependence of BDNF release (review in Piepmeier and Etnier, 2015), low-intensity exercise elevated BDNF in the same amount as in a high-intensity condition. In contrast, Ferris et al. (2007) confirmed the exercise intensity dependence of BDNF release after 30-min cycle exercise, but could not find any correlation of BDNF changes with EFs, such as inhibition ability measured by a Stroop test (Ferris et al., 2007). Moreover, no intensity effect was observed since the Stroop task performance improved pretest- to posttest at the same extent, regardless of exercise intensity. In a recent comprehensive review of acute exercise effects on neurophysiological parameters, it was reported that there is consistent observation that high exercise intensity around the anaerobic threshold induces approximately 15% increases in BDNF which last up to 20 min after exercise. However, one limitation of studies on intensity-dependent BDNF effects in humans is the risk that peripherally measured BDNF may not sufficiently reflect cortical BDNF level (Basso and Suzuki, 2017).

There are also many electroencephalography (EEG) studies investigating tasks that evoke EEG responses during, immediately after, or with a time delay after AE. They are mostly quantified using event-related potentials (ERPs) P300, contingent negative variation (CNV), error-related negativity (ERN) and error positivity (EP). P300 is a prominent marker that can be seen as a positive deflection in the EEG amplitude about 300 ms after a stimulus and P300 is an index of attentional resources devoted to task completion (Polich, 2007). Thereby, difficult and complex tasks reduce P300 and increase latency. Kamijo et al. (2004a), as an example, observed decreased P300 amplitude after a high-intensity cycling exercise in a Go/no-go task and increased P300 amplitude after moderate intensity exercise (Kamijo et al., 2004a). Based on this pattern, they suggested that reduced attentional resources were available after high-intensity exercise and increased resources after moderate exercise. In another study by Kamijo et al. using the CNV as a marker of attention and arousal, they found further evidence for intensity dependent effects of exercise on central neurocognitive markers (Kamijo et al., 2004b).

By using additional markers such as the ERN and EP (markers observable in evaluation of conflict during instances of erroneous), those ERPs clearly demonstrate that the exercise-cognition relationship is reflected in task-evoked brain activity (Yanagisawa et al., 2010; Chang, 2016), with further evidence for intensity-dependent effects (Olson et al., 2016). In contrast, early stimulus-locked components of the EEG such as N1 and P2 (reflecting early processing steps that are not directly related to EFs) are not affected by exercise, while P300 and CNV reflect exercise-associated behavioral changes. A review by Chang (2016) concluded that a moderate exercise protocol lasting between 18 min and 30 min exerts beneficial effects on cognition and neurocognitive markers (Hillman et al., 2003, 2009; Scudder et al., 2012; Drollette et al., 2014).

Moreover, oscillatory EEG (i.e., the synchronized activity patterns of neurons in a functional neural network) activity recorded during and after exercise indicated increases in the alpha band (8–12 Hz oscillation indicating a marker of arousal); however, this was not different from the increase measured for other frequency bands (Crabbe and Dishman, 2004). Subsequent research observed frontal alpha asymmetry (FAA) changes. FAA is a marker of different activity in the left and right PFC hemispheres, and this study indicated higher relative left prefrontal activity elicited by moderate and strenuous exercise intensity. This pattern was measurable up to 38 min post-exercise and associated with post-exercise mood modification (Woo et al., 2009; Hall et al., 2010; Hicks et al., 2017). Gutmann et al. (2015), for example, showed that the resting state individual alpha peak frequency (iAPF), which is a marker of arousal and attention and is associated with the speed of information processing, was increased after an exhaustive exercise compared to steady state moderate exercise intensity. This finding suggests there is a mechanism leading to an optimal brain state for cognitive performance (Gutmann et al., 2015). Furthermore, resting-state functional magnetic resonance imaging (fMRI) analysis proposes that AE increases the integration of attention and executive control networks indicative of functional network connections that are particularly sensitive to moderate-intensity AE (Weng et al., 2017). A review on acute exercise effects including animal and human studies summarized that a single bout of exercise increases hippocampal theta activity and other frequency bands across the entire cortex, positively modulates the P300 and indicates that cognitive enhancement is accompanied by increase in cerebral blood flow (Basso and Suzuki, 2017).

However, no clear association exists between brain oxygenation, cerebral blood flow, and cognitive performance during exercise, although alterations of these parameters have repeatedly been reported (review in Ando, 2016). This conclusion contrasts with the influential reticular-activating hypofrontality model (Dietrich, 2003; Dietrich and Audiffren, 2011). This complex theory principally suggests that the human brain has a limited information-processing capacity that eventually leads to decreased cognitive performance with rising exercise intensity. According to this model, moderate exercise intensity leads, through activation of the reticular system, to increased arousal, and in turn, to increased cognitive performance, but this effect only applies in tasks that are well-learned. As soon as the intensity is high, the model proposes that motor areas of the brain must be higher activated due to resource allocations for motor task completion. If exercise intensity level is to be maintained, this can only occur at the expense of the PFC areas, which are downregulated. In turn, this PFC deactivation results in poorer cognitive performance especially in tasks that rely on PFC structures such as EFs. However, this well-defined model, while it has some striking empirical support (for an overview see Dietrich and Audiffren, 2011), only accounts for any online (effects observed during AE) exercise-cognition interactions (i.e., limited explanatory power for any offline effects), and there are other empirical findings, which disagree with this model (e.g., Tempest et al., 2017).

NEUROPHYSIOLOGICAL AND NEUROCHEMICAL EFFECTS OF tDCS

In tDCS, the transcranially induced, weak, direct current flows from the anode to the cathode, passing the neural tissue, and is thought to exert transient alterations in the neural processes of the stimulated brain region. Although the exact physiological mechanisms of tDCS are still being studied, it is presumed that the primary mechanism of action derives from a polarity-specific subthreshold polarization of the resting membrane potential of neurons (Nitsche and Paulus, 2000, 2001). Recently, it was indicated by intracranial recordings in primates and epilepsy patients that 1–2 mA of anodal tDCS slightly elevated the resting membrane potential by approximately 0.2–0.5 mV (Opitz et al., 2016). In turn, such a transiently increased membrane excitability is suggested to boost the likelihood of action potentials (APs), resulting in a higher spontaneous firing rate of neurons. In contrast, cathodal tDCS exert the opposing effect of a transient hyperpolarization, thereby more likely diminishing cortical excitability and the spontaneous firing rate of neurons (Creutzfeldt et al., 1962; Bindman et al., 1964; Nitsche and Paulus, 2000; Romero Lauro et al., 2014).

Consequently, anodal tDCS is typically associated with increased cortical excitability, while cathodal tDCS is associated with decreased cortical excitability. The polarity-specific effects of tDCS on resting membrane potentials originate from tDCS-induced changes in the conductivity of voltage-dependent ion channels. Anodal tDCS is assumed to increase cortical excitability due to modulations of N-Methyl-D-Aspartate-receptor-mediated (NMDA) Ca^{2+}-channels, causing alterations in Ca^{2+} influx (Nitsche et al., 2003a). However, polarity-specific alterations of cortical excitability are predominantly demonstrated in motor regions of the brain (e.g., M1), such as by higher amplitudes of TMS-induced motor-evoked potentials (MEPs) (Nitsche and Paulus, 2000, 2001). Excitability changes were also observed in non-motor regions by means of sensory-evoked potentials (SEPs) in the somatosensory cortex (Dieckhöfer et al., 2006), auditory-evoked potentials (AEPs) in the auditory cortex (Zaehle et al., 2011a), and visually-evoked potentials (VEPs) in the visual cortex (Antal et al., 2004).

Short durations of tDCS application can elicit effects that last a few seconds, whereas longer-lasting tDCS application can prolong the after effects on membrane excitability and cortical activity. Exemplarily, it was demonstrated that 13 min of anodal and 9 min of cathodal tDCS can initiate long-lasting after-effects for 30 to 120 min (Nitsche and Paulus, 2001; Nitsche et al., 2003b; Kidgell et al., 2013) and can last up to 24 h when tDCS was repeatedly applied throughout 1 day (Bastani and Jaberzadeh, 2014). Longer-lasting after-effects of tDCS are rendered to provoke neuroplastic processes, as indicated by tDCS-related modulations of several neuroplasticity markers, such as glutamate (Nitsche et al., 2003a), gamma-aminobutyric acid (GABA) (Nitsche et al., 2004), dopamine (Nitsche et al., 2006; Monte-Silva et al., 2009), serotonin (Nitsche et al., 2009), acetylcholine (Kuo et al., 2007) and BDNF (Fritsch et al., 2010). In the corresponding primary mechanism of action, anodal tDCS causes a reduced concentration of GABA with a concurrent increase in glutamate concentration, while cathodal tDCS brings about the opposing effect (Stagg et al., 2009; Stagg and Nitsche, 2011). Furthermore, the reduced GABA concentration and the concurring reduced GABA-gated intracortical inhibition (ICI) provoked by anodal tDCS leads to a facilitative effect on glutamate-driven neuroplasticity (Stagg et al., 2009; Kim et al., 2014). Therefore, tDCS-related neuroplasticity appears mainly as a glutamatergic process comprising NMDAs, and is based on their numbers upon glutamatergic synapses causing increased synaptic strength and responsiveness to glutamate (Gillick and Zirpel, 2012), and NMDA-mediated influx of Ca^{2+} (Liebetanz et al., 2002; Nitsche et al., 2003a). Fluctuations of Ca^{2+} are further associated with both long-term potentiation (LTP) and long-term depression (LTD) (Bennett, 2000), synaptic processes, which are contingent upon BDNF (Fritsch et al., 2010).

In addition, fMRI, EEG, and functional near-infrared spectroscopy (fNIRS) approaches reported local functional synchronization due to tDCS (Polanía et al., 2011, 2012; Kunze et al., 2016) and altered brain activity in nearby and functionally connected brain areas (Baudewig et al., 2001). fMRI studies observed extended and large-scale network changes following one session of cathodal stimulation (Ardolino et al., 2005; Lang et al., 2005). fNIRS approaches reported hemodynamic changes after prefrontal anodal tDCS (Merzagora et al., 2010) and EEG recordings found that the oscillatory activity of the brain adapts its frequency in response to tDCS (Keeser et al., 2011; Zaehle et al., 2011b; Jacobson et al., 2012). In particular, anodal tDCS applied over the left DLPFC increased the cortical perfusion in the stimulated area, while a strong decrease was observed after the stimulation (Stagg et al., 2013). Moreover, prefrontal tDCS reduces low-frequency EEG oscillations and increases event-related activity (for an overview see Wörsching et al., 2016). Also, surface brain area activity changes and alterations in neural processing in deeper prefrontal structures have been described (Keeser et al., 2011). Studies on resting-network activities consistently report tDCS induced influences on regional activity on functional connectivity across brain regions interhemispheric and intrahemispheric (Turi et al., 2012; Hartwigsen and Siebner, 2013; Hartwigsen et al., 2015; Bergmann et al., 2016; Kunze et al., 2016).

Further neurophysiological measures during EF execution (inhibition tasks, a combined Go/no-go and Stop signal task) using a combined tDCS-EEG approach observed that an anodal bilateral tDCS (right hemispheric anodal/left hemispheric cathodal) of the right inferior frontal cortex (IRF) modulated the P300 ERP component, while no clear effects were observed on a behavioral level (Cunillera et al., 2016). This study was one of the first to report EEG data during the stimulation as the EEG is usually compromised by the tDCS electrical field. Although P300 was affected by tDCS, the authors concluded that bilateral tDCS of the right IRF is not the best option to modulate response inhibition. Another study targeted the medial frontal cortex with cathodal tDCS and induced EP modifications of the EEG (Bellaïche et al., 2013). Since the EP reflects error monitoring, the authors concluded that targeting those brain areas involved in error monitoring with a neuromodulatory

technique such as tDCS would be valuable for therapy. This conclusion was based on the error monitoring ability in patients suffering neuropsychiatric conditions being frequently impaired. Keeser et al. (2011) observed modulations of the P300 ERP component of the EEG, together with behavioral improvements in an updating ability task (N-back) after anodal tDCS (cathode placed over the left supraorbital cortex) of the left DLPFC (Keeser et al., 2011). Further source analysis using EEG based standardized low resolution tomography (sLORETA) revealed enhanced para-hippocampal gyrus activity, suggesting that tDCS alters the regional surface neural activity and reaches deeper PFC structures.

Based on the reviewed studies targeting the PFC, there is converging evidence that prefrontal tDCS impacts resting state activity, task-evoked activity, brain oscillations, brain perfusion and oxygenation, bioenergetics, functional connectivity, event-related spectral perturbations (ERSPs) and ERPs. Consequently, tDCS of the PFC initiates comparable synaptic-plasticity and associated neuroplastic markers known from motor cortex studies (see Wörsching et al., 2016). However, direct measures of excitability changes using TMS, which induces a direct measure of cortical excitability (i.e., MEPs), are not available for prefrontal structures.

INTERACTIONS AND SYNERGIES BETWEEN ACUTE tDCS AND ACUTE PERIODS OF AEROBIC EXERCISE

Converging evidence exist indicating that both tDCS and AE involve promising mechanistic pathways that initiate changes in cortical excitability and neuroplasticity that can improve cognitive functions even in the short-term. Therefore, it is conceivable that a combination of both techniques could act in a synergistic fashion to improve cognitive performance beyond the level known for each technique alone. The idea of combining multiple techniques is far from being new, as several recent studies and reports have shown complementary effects when tDCS and exercise are combined in an interventional approach (Okano et al., 2015; Angius et al., 2017; Edwards et al., 2017; Ward et al., 2017). There are also several other multimodal interventions using AE without tDCS, aimed to improve cognitive functions. For example, a direct combination of meditation with AE mitigated symptoms of major depressive disorder, provoked enhanced neurocognitive markers (P300) and indicated increased synchronous brain activity during a cognitive control task (Alderman et al., 2016). Thus, similar to meditation, tDCS may synergistically modulate neural networks in combination, as this technique has been shown to mitigate depressive symptoms, increasing cognitive functions and modifying neural activity (Fregni et al., 2006b; Schuch et al., 2016). Other more direct interventions with the purpose of improving cognitive functions have combined cognitive training with AE training, either in a direct combination (i.e., cognitive training during exercising) or in an indirect fashion (i.e., sequentially). In a meta-analysis of studies with older aged participants, mixed results compared to single interventions appeared; however, this result could have been due to the heterogeneity of training protocols (Zhu et al., 2016). In particular, studies applying a sequential approach have either revealed positive effects on EFs (Rahe et al., 2015; Lai et al., 2017) or no superior effects on EFs compared to single-modality training (Shatil, 2013). Importantly, the latter study applied AE training and cognitive training on different days, while the other two studies combined cognitive and AE training in one session by having cognitive training followed directly by AE. Thus, one bout of AE and its acute effects could initiate an ideal environment for increases in a subsequent training of EF or other cognitive functions and might thus serve as a brain primer for subsequent tDCS intervention.

In line with the presented argumentation that multimodal interventions provide a possibility for initiating neuroplastic processes and superior performance gains compared to single-modality interventions based on the literature reviewed thus far, AE might be capable of positively interacting with the tDCS induction of synaptic plasticity. It could well be, although not tested so far, that combining these techniques may enhance synaptic processing and network activity that are associated with EFs. For example, following the catecholamine hypothesis, a session of moderate AE intensity elicits an ideal amount of catecholamine release for cognitive enhancement. This enhancing pathway is potentially mediated through catecholamine, because pharmacological studies have shown that central EF tasks require the activation of the noradrenergic and dopaminergic pathways (Luciana et al., 1998; Berridge et al., 2006; Chamberlain et al., 2006). The enhancement of EFs by anodal tDCS during stimulation (i.e., online), however, is thought to be based primarily on changes in resting membrane potential (Stagg and Nitsche, 2011). Those divergent mechanisms, capable of initiating cognitive enhancement on its own, might be one possibility when combined in one session for synergistic effects on EF performance. While tDCS may modulate the frontal neural networks in a more specific and focused manner when stimulating the PFC structures such as the DLPFC [e.g., even more focally using smaller electrodes, i.e., high-definition tDCS systems (Datta et al., 2009; DaSilva et al., 2011; Villamar et al., 2013)], the AE may activate broader networks through reticular arousal activations pathways and neural oscillatory modifications (Hall et al., 2007; Woo et al., 2009; Gutmann et al., 2015; McMorris and Hale, 2015; McMorris, 2016a,b; Basso and Suzuki, 2017; Hicks et al., 2017). The involvement of the two converging pathways may then, as an example, enhance EF inhibition. In particular, brain areas involved in inhibition, such as the ACC (van Veen et al., 2001; Mansouri et al., 2009; Kühn et al., 2016; Weng et al., 2017), are not directly accessible by tDCS, but they can be reached by arousal activation through physical exercise (Critchley, 2004). This, in turn, might be supportive in a synergistic fashion for the executive function ability inhibition and possibly other EFs.

Alternatively, AE performed directly prior to an anodal tDCS session of the PFC, in which EFs are trained or tested may act complimentary for the induction of neuroplasticity based

on BDNF expression. The increase in BDNF might create an optimal environment for longer lasting tDCS induced LTP initiation. As BDNF factors are necessary for successful and efficient LTP induction (Cotman and Berchtold, 2002), these pathways (AE initiated BDNF and tDCS initiated LTP) might converge into optimal learning processes and synaptic plasticity. In particular, animal studies at which rodents performed exercise sessions have indicated that BDNF levels in the hippocampus are related to enhanced learning and memory processes (Vaynman et al., 2004), and, as reviewed, BDNF expression in humans due to exercise has been repeatedly observed (Winter et al., 2007; Szuhany et al., 2015). Cortical concentrations of BDNF are reduced concomitant with disturbed LTP mechanisms in several diseases, such as Alzheimer's disease, Parkinson's disease, depression, anorexia, and many other diseases (Mariga et al., 2017). Thus, AE in combination with tDCS might act for disease prevention or to enhance cognition and cognitive training regimens and restore maladaptive neural functions in such diseases. However, in specific cases, and based on the targeted system, the AE intensity plays an additional critical role since high-intensity exercise has several positive effects such as a superior elevation of BDNF compared to moderate intensity. Exercise-induced BDNF increase in general (Piepmeier and Etnier, 2015; Szuhany et al., 2015) is higher due to heavy exercise compared to a longer-lasting moderate exercise session (Winter et al., 2007). Thus, the exercise intensity may have a critical role for initiating/restoring optimal neuroplasticity, which is mediated by BDNF, such as in major depression (Brunoni et al., 2008), a psychiatric state which can be influenced by tDCS and AE (Fregni et al., 2006b; Kuo et al., 2014; Schuch et al., 2016).

Due to heterogeneous findings on cognitive abilities during exercise, it is only possible to speculate about how this cognitive-exercise interaction could be additionally modulated with parallel anodal or cathodal tDCS. Although the tDCS-induced electrical field might be affected during whole body movements through movement artifacts and head transpiration, behavioral modifications due to stimulation during exercise have already been observed repeatedly (Angius et al., 2017). Several recent works provide first evidence that single bouts of tDCS can improve exercise performance (for an overview, see Angius et al., 2017; Edwards et al., 2017). In most of these studies (9 out of 12), anodal tDCS was applied to the left or right M1 and the cathode was mostly placed at an extra-cephalic position (e.g., shoulder) or a contralateral site, such as the contralateral forehead. In two studies, the anode was placed over the temporal area (T3) and in another study in a central position (Cz). However, with the exception of two studies, anodal tDCS was applied prior to the exercise protocol and lasted for 10–20 min. In sum, the studies indicated mixed results but point toward a tendency to improve exercise performance following tDCS application. Primarily, it is thought that tDCS reduces and delays supra-spinal fatigue accompanied by a reduced subjective perceived exertion (Williams et al., 2013; Okano et al., 2015; Angius et al., 2016). Therefore, one additive effect of tDCS and AE might emerge due to the fact that people can endure higher exercise intensities, especially in aged or clinical populations, when tDCS is applied to the motor cortex for example prior to an AE session. As outlined, heavy AE seems to be more beneficial in terms of BDNF releases compared to moderate AE (Basso and Suzuki, 2017) which, in turn, may have a stronger effect on cognitive performance.

However, to the best of our knowledge, no study has investigated a possible interaction between exercise, tDCS, and cognition. Therefore, only indirect conclusions for the cognitive domain can be derived from combined tDCS-AE studies within the motor- and sports-related domain. To the best of our knowledge, so far, there is only one published RCT trial that implemented in a multimodal study design a group that received tDCS and AE treatments (Ward et al., 2017). In this comprehensive 4-month study, cognitive training success (computer-based EF and working memory training) was compared between five different groups, whereas one of those groups received tDCS over DLPFC (HD-tDCS: two small anodes were placed on left and right PFC and two return electrodes at occipital areas) during cognitive training and a physical exercise training. The rationale is that previous studies have shown that cognitive training success accompanied by tDCS or by AE training outperformed cognitive training in isolation (Ward et al., 2017). When they are combined in one group, converging mechanisms, as reviewed in this work (LTP, neural excitability, network modifications, and synaptic plasticity) and addressed by Ward et al. (2017), should ultimately be expressed in enhanced cognitive learning success compared to unimodal interventions (e.g., only cognitive training). Indeed, they found that the combined tDCS-exercise group outperformed other unimodal and multimodal groups in cognitive performance, suggesting synergetic effects on the cognitive system. Unfortunately, there is no specific information about the time delay between the exercise session and the tDCS-cognitive training session. Moreover, exercise sessions also included resistance training and non-standardized AE training protocols, making it difficult to clearly conclude whether the higher cognitive training effects were due to the combinatory effects evoked by each single technique alone, or whether there were any direct and acute interactions between exercise and subsequent tDCS effects (e.g., exercise that serves as a brain primer; more details regarding this aspect are in the next paragraphs).

One key parameter for the interaction between tDCS and AE seems to be the timing of the AE and tDCS sessions, i.e., whether tDCS is applied prior, during, or after AE. Based on tDCS studies stimulating the motor areas, the timing of tDCS relative to a given task seems to be a crucial factor with a strong impact on study outcomes (Stagg and Nitsche, 2011). In experiments where tDCS is applied during task performance (online tDCS), the specific neural network involved to perform the task is mainly stimulated and the tDCS-induced neuroplastic changes primarily occur within the task-related neuronal circuits (Huang et al., 2017). However, studies indicated behavioral improvements following online tDCS and after tDCS administration in the absence of task performance (offline tDCS). As reviewed, the timing of AE with regards to cognitive

test administration is important, but the timing interacts with exercise intensity especially during AE (i.e., online). In addition, both the online and offline cognitive testing within AE seem to be an option to enhance behavioral output, as AE effects may serve as an ideal primer to initiate an optimal environment to improve brain functions in both cases. No explicit knowledge is available for the best timing of tDCS and AE. However, if an intervention wants to exert the online-effects or any after-effects of one technique directly on another technique, then the timing appears to be crucial, considering the observation of tDCS after-effects of no longer than 120 min (Nitsche and Paulus, 2001; Nitsche et al., 2003b; Kidgell et al., 2013).

Further hints for the optimal timing come from priming studies (Parkin et al., 2015), since a specific kind of priming or "pre-conditioning" (a task or intervention applied before a second "conditioning" task or intervention) is thought to initiate "metaplastic" processes by using a short priming (or pre-conditioning) period of tDCS stimulation before the actual stimulation (TMS or tDCS) is applied. There is emerging evidence from motor, cognitive, and vision studies that metaplasticity provokes more robust results and even boosts effects of conventional protocols (Hurley and Machado, 2017). The term metaplasticity refers to the Bienenstock et al. (1982) model (BCM) of synaptic modification. The BCM implies that a preceding inhibition of cortical activity decreases the threshold of a subsequent excitation and vice versa for a preceding elevation of cortical excitation (i.e., increased threshold for excitation) (Bienenstock et al., 1982). Consequently, the plasticity of the state of a neuron depends on earlier states induced by separate prior events (Abraham, 2008; Hulme et al., 2013). It is thought that such an experience-dependent neural plasticity mechanism requires the existence of a state-dependent fast-reacting system that can dynamically adapt in response to preceding synaptic activity to retain network stability and permit enduring LTP or LTD-like mechanisms (Abraham, 2008; Feldman, 2009).

Thus, the modification or state of a neural network prior to an intervention can have an impact on a subsequent intervention where the same network is targeted (within the timeframe of after-effects). This mechanism was tested by Carvalho et al. (2015) using two tDCS sessions with a time delay and different polarities in a working memory task (N-back) (Carvalho et al., 2015). Notably, anodal tDCS over the DLPFC as a pre-conditioning period decreased working memory performance during subsequent anodal tDCS of DLPFC (conditioning stimulation). This decrease occurred when a time delay was placed between the two stimulations; thus, metaplastic processes may have changed the direction of polarity. In contrast, using cathodal tDCS as a primer 10 min before a second cathodal tDCS conditioning stimulation enhanced working memory performance, suggesting a metaplastic mediated compensation with an upregulation process due to the prior inhibition period induced by cathodal tDCS. Thus, the effects reversed, agreeing with the BCM model for retaining network stability and a phenomenon called the "rebound effect" (Creutzfeldt et al., 1962). Consequently, due to the aforementioned AE acute effects, a short bout of high-intensity AE, as an example, could be an additional tool and taken as a primer (pre-conditioner) for subsequent anodal or cathodal tDCS. Considering that one bout of AE increases excitability of the brain and increases the aforementioned BDNF levels, AE might synergistically interact with LTP-like mechanisms induced by metaplasticity. Therefore, either AE or tDCS could be considered a versatile tool to initiate a specific desirable state of the brain. Thereby, AE—with its possibility to modify the excitability of the brain could be used as a brain primer, and tDCS could be used as a therapeutic tool to synergistically target specific brain areas more specifically. Alternatively, AE could be applied after a tDCS phase to facilitate consolidation in the hours after tDCS.

A further aspect to consider may be that meta-analyses provide evidence that moderate exercise intensity is most beneficial for cognitive functions, but affects only the speed of processing and not accuracy (McMorris et al., 2011; McMorris and Hale, 2012). Increases in catecholamine with working memory performance improvements were observable at between 20 min and 30 min of moderate exercise intensity, while longer lasting moderate AE resulted in cognitive inhibition (McMorris et al., 2016). For tDCS, studies show that offline stimulation improves accuracy in working memory tasks and helps neuropsychiatric patients during online stimulation to a greater extent than tDCS is able to improve processing speed (Dedoncker et al., 2016; Hill et al., 2016). Thus, there might be a synergistic pathway for a combined short and moderate AE session and subsequent tDCS to improve both the accuracy and speed of working memory performance.

Another potential pathway for its combined effect is the inhibitory system, as recent evidence shows that increased tonus of the inhibitory system is essential for increasing cognitive performance due to its important regulatory activity of multiple competing systems. Interestingly, the lack of cognitive demand leads to a lack of inhibition, likely due to a compensatory mechanism (Capano et al., 2015). Exercise seems to have a dual effect with an initial increase in excitatory circuits activity followed by an increase in inhibitory circuits, and has been used in children to increase inhibitory control, especially in cases of attention deficit hyperactivity disorder (ADHD) (Chang et al., 2014). Anodal tDCS seems to lead to a similar effect. Although anodal tDCS does lead initially to an increase in spontaneous neuronal firing, it is followed by an increase in intra-cortical inhibition (Nitsche et al., 2005; Vignaud et al., 2018). Thus, combining both therapies may enhance these effects; however, in this case, the timing of both therapies needs to be planned, as the AE session may not be during the compensation phase and increase in the inhibitory tonus. There is currently a lack of data to test this hypothesis, and simultaneous application of both therapies seems the best option (Mendonca et al., 2016).

Future Implications

The outcome of a direct combination of tDCS and AE (i.e., tDCS occurs either during or in a sequential fashion with AE) on

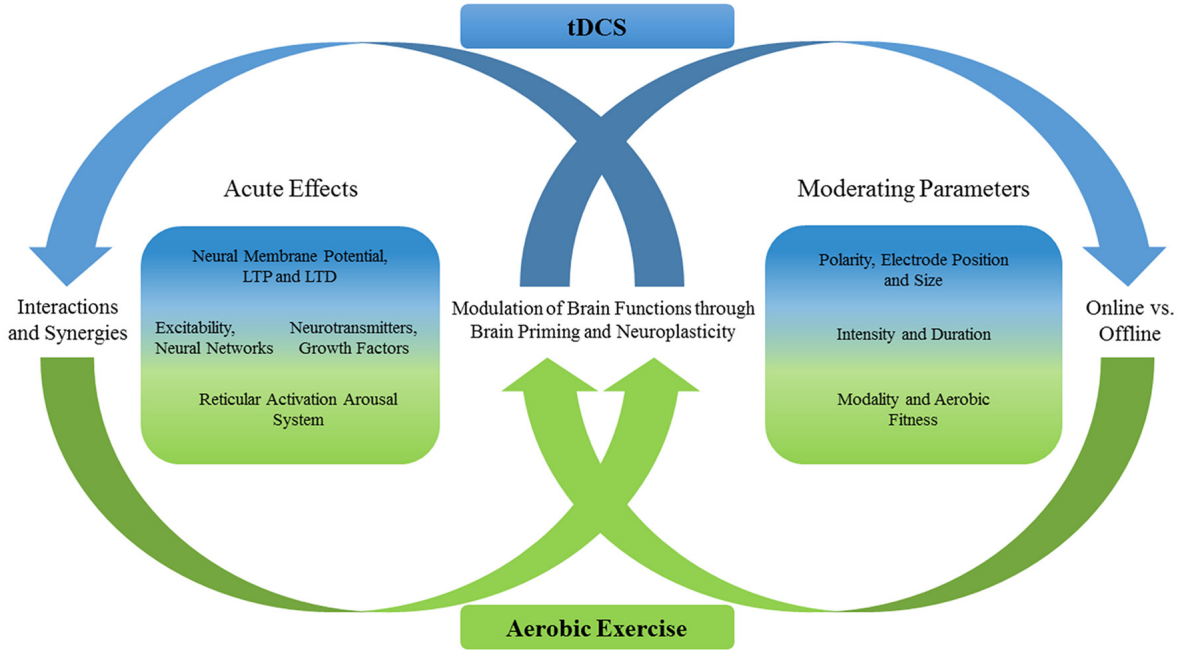

FIGURE 1 | Acute effects of transcranial direct current stimulation (tDCS) and aerobic exercise (AE) and possible interactions. Shown is a schematic overview/summary of the key concepts/effects reviewed in this article. The figure shows some acute effects and interacting parameters that could play a role when both techniques are applied in combination for supporting cognitive performance. The green color represents the AE effects and the blue color those of tDCS. The more a specific effect (or moderating parameter) is displayed in the middle of the two-colored boxes the more the effects are comparable between both techniques. This drawing does not focus on providing a comprehensive overview of all acute effects that have been observed for tDCS and AE (e.g., those for the motor, perceptual or affective domain). Instead, this figure includes those that might be supportive of the cognitive domain and those that should be critically evaluated in future multimodal approaches. LTP means long-term potentiation and LTD long-term depression.

a molecular and system level has not yet been investigated. However, the outcome of both techniques is modulated by many parameters, and all of which may be of relevance when both techniques are directly combined (see **Figure 1** for an overview). Following our working hypothesis that moderating parameters need to be considered, the timing of cognitive test administration (or the training of EF) and the AE intensity are important factors to be investigated in the future with regards to whether tDCS and AE are sequentially or directly combined.

One practical argumentation of combining both techniques come from an applied perspective as both techniques have remarkable similarities besides converging mechanisms and modulatory capacity of brain functions. For both techniques, 20 min of application has been found to be especially supportive in initializing high effects with enhancement of EFs and other cognitive abilities. As an example, tDCS of the DLPFC or moderate-intensity AE of about 20 min have shown to enhance the EF inhibition, in both online and offline situations (Kamijo et al., 2007; Davranche et al., 2009; Loftus et al., 2015; Strobach and Antonenko, 2017). Moreover, the techniques can be performed in a direct combination (i.e., stimulation during exercising) when mobile wireless tDCS hardware is used. Critical aspects such as movement artifacts (compared to EEG or fNIRS) do not have a major role, and tDCS and AE are relatively easy to apply, cost-effective and without known severe side effects. Thus, they can be easily applied in almost all settings in real life, in health, in sport, peak performance, and in the treatment of disease (except in patients with contraindications) without any significant restrictions in body movement.

The various effects of both techniques on cognition and brain functions, their potential synergistic interactions, and their promising possibilities from an applied perspective suggest that further systematical investigations into the value of their combined use in health and disease is worthwhile. Because of the possibility of both tDCS and AE modulating brain functions and EFs, it would be desirable to investigate whether AE can prime the brain to foster an ideal brain state for optimized brain stimulation using tDCS. Thus, if a method wants to prime the brain or aims to initiate meta-plasticity processes by combined tDCS/AE, exercise intensity and duration (e.g., long moderate or short high intensity), timing (i.e., the delay between exercise and tDCS session), sequence (i.e., exercise before tDCS or after tDCS), and polarity (i.e., cathodal or anodal) of tDCS are critical factors to be considered. Regardless of metaplasticity, depending on the study objective or clinical objectives, whether downregulation of a given brain area (such as in depressive patients) or upregulation (such as in cognitive training for healthy people) is intended, should be carefully considered.

Especially with respect to the reticular activation hypofrontality theory (see section "Neurophysiological and

Neurochemical Effects of Acute Exercise"), it might also be worth systematically modulating prefrontal cortical activity with tDCS or transcranial alternating stimulation (tACS) to actively upregulate or downregulate PFC activity during exercise. Using tDCS or tACS (through application of alternating current to brain areas, this technique aims to modulate brain oscillations and thus the functions that are associated with specific frequency bands) as a versatile tool and possibility for making causal inferences may help to confirm or reject the idea of rising hypofrontality with increasing exercise intensity (Dietrich and Audiffren, 2011). However, if there is a downregulation of the PFC by high AE intensity according to this hypofrontality model, a combined cathodal tDCS might synergistically support the down regulative capacity of AE on PFC structures. This could be experimentally investigated and actively used to optimize AE treatment protocols that have been shown to help depressive patients (Melo et al., 2016), possibly by the pathways defined in the hypofrontality model (Dietrich and Audiffren, 2011). In turn, anodal tDCS administered to the motor cortex during high AE intensity may release brain capacities which are available for cognitive functioning. The increased motor cortical activity required for intensive motor performance, might be supported by increasing motor cortical activity with anodal tDCS applied over the motor cortex. Hence, one may speculate that the higher cortical activity induced exogenously by anodal tDCS provokes lower needs for endogenous brain activity for motor performance, which in turn, release brain capacities (e.g., information-processing) and enhance cognitive performance during high AE.

Moreover, it might worth considering that modulations in brain oscillatory activity such as in the individual alpha band (e.g., FAA) can be provoked by exercise and tDCS and tACS (Zaehle et al., 2010, 2011b; Herrmann et al., 2013; Soekadar et al., 2016; Herrmann and Strüber, 2017; Kasten and Herrmann, 2017). Because several neurological and psychiatric conditions are associated with dysfunctional brain synchronization and maladjusted oscillatory communication (Stam et al., 2003; Schnitzler and Gross, 2005), a combined application of AE and tACS/tDCS could help research to modulate brain oscillations and study the associated behavior systematically.

Despite any potential and promising possibilities of a combined tDCS-AE use, however, there are several important aspects to consider. Although there is a multitude of studies indicating beneficial tDCS effects on cognitive function and possible synergistic pathways, there are also several studies suggesting no or even detrimental effects. Moreover, there are hints that the current state of the brain and dynamically changing brain physiology can have a significant impact on any tDCS-related effects, a factor which must be carefully considered and which might be especially susceptible by exercise and its moderating factors. As an example, one could assume that the effects elicited by a combined tDCS-exercise protocol on cognitive functioning may be contingent upon the individual fitness level, thus making it important to account for inter-subject variability. Moreover, the present review did not consider the different effects of tDCS and AE being the result of factors such as age (e.g., very young and old people), gender or specific neurological and psychiatric states, all of which might be differently affected by the two techniques and specifically by the moderating parameters reviewed here. Lastly, even if there are any potential synergistic combined tDCS-AE effects from one application, it must also be examined whether this has an impact on any longer lasting (i.e., repeated application of a combined AE-tDCS) interventional approaches, where additional factors must also be considered.

CONCLUSIONS

AE and tDCS can have remarkably similar effects on EFs and other cognitive domains and support rapid initiating of neuroplasticity in the human brain within a short timescale. This similarity offers multiple beneficial opportunities within clinical research, such as treatment of psychiatric diseases or neuro-rehabilitation and also in non-clinical settings, such as sport. Compared to other multimodal approaches, such as combined AE-TMS studies or treatments combined with pharmaceutics, tDCS-AE has the advantages of its easy-to-apply and time and cost-effective applications without any yet known severe side effects. The acute effects of both techniques on the neurophysiological, neurochemical, and behavioral level, such as the enhancement of cognitive skills, modifications of neural activity, and catecholamine, have striking similarities and provide synergistic mechanistic pathways that might improve brain functions and neuroplasticity in health and disease when both techniques are applied in direct combination. While AE might provoke more large-scale changes across the entire brain, serving as a brain primer to provoke a given desired brain state, tDCS may then: (a) be used to more focally and specifically modulate brain activity and behavior; and (b) lead to more robust and higher effects possibly due to lower intersubjective variability and potential metaplastic effects.

Due to various interacting and dynamically changing parameters, caution must be given to those moderating parameters that could significantly impact the interaction between both techniques, possibly leading to the abolishment of any effect (see Singh et al., 2016). As outlined, moderating parameters of AE and tDCS, such as exercise and electric intensity, tDCS polarity, exercise and tDCS duration, and timing of tDCS, provoke modifications on several levels of the human organism, even with only one application. Both AE and tDCS are capable of modulating cognitive functions, brain activity, and excitation in terms of brain oscillations, hemodynamic activity, NDMA mediating the release of neurochemicals, BDNF, and growth factors. This modification can initiate enhanced cognition and behavior or support treatments in neurologic and psychiatric conditions. However, such promising positive effects probably appear only under specific conditions that need to be carefully controlled and evaluated.

The acute interactions of one session of exercise and tDCS on cognition have not been empirically addressed so far and should be systematically and experimentally investigated

in the future. Special emphasis should determine whether there is a dose-response relationship in terms of exercise and tDCS intensity and duration, stimulation area and polarity, and whether the time course between exercise and stimulation could interact on several levels of the healthy and the impaired human brain. If synergistic or additive effects from acute combined effects can be experimentally observed in the cognitive or in the motor domain, future interventions may benefit from synergies between both techniques.

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All authors provided substantial contributions to the work. FS drafted the manuscript. FF and NP revised it critically.

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Relationship of the Acoustic Startle Response and Its Modulation to Adaptive and Maladaptive Behaviors in Typically Developing Children and Those With Autism Spectrum Disorders: A Pilot Study

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Background: Autism spectrum disorder (ASD) is associated with persistent impairments in adaptive functioning across multiple domains of daily life. Thus, investigation of the biological background of both adaptive and maladaptive behaviors may shed light on developing effective interventions for improving social adaptation in ASD. In this study, we examined the relationship between adaptive/maladaptive behaviors and the acoustic startle response (ASR) and its modulation, which are promising neurophysiological markers for ASD translational research.

Method: We investigated the ASR and its modulation in 11 children with ASD and 18 with typical development (TD), analyzing the relationship between startle measures and adaptive/maladaptive behaviors assessed with the Vineland Adaptive Behavior Scales (VABS) Second Edition.

Results: Peak-ASR latency was negatively correlated with the VABS total score and socialization domain score of adaptive behaviors, while the ASR magnitude for relatively weak stimuli of 75–85 dB was positively correlated with VABS maladaptive behavior scores. Prepulse inhibition (PPI) at the prepulse intensity of 70–75 dB was also correlated with VABS maladaptive behavior. However, these relationships did not remain significant after adjustment for multiple comparisons.

Conclusions: Our results indicate that the prolonged peak-ASR latency of ASD children might be associated with impairment in the developmental level of adaptive behavior, and that the greater ASR magnitude to relatively weak acoustic stimuli and smaller PPI of ASD children might increase the risk of maladaptive behavior. Future studies that have larger sample sizes will be important for further elucidating the neurophysiological factors that underpin adaptive as well as maladaptive behaviors in ASD.

Keywords: acoustic startle reflex, adaptive behavior, hypersensitivity, autism spectrum disorder, neurophysiology, sensorimotor gating

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INTRODUCTION

Autism spectrum disorder (ASD) is associated with persistent impairments in adaptive functioning across multiple domains including social, communicative, occupational, or other important areas of daily life (American Psychiatric Association, 2000). Development of adaptive behaviors and prevention of maladaptive behavior are primary and fundamental intervention aims in ASD.

Sensory abnormalities have often been reported as symptoms of ASD (Lai et al., 2014), and also reported to be related to adaptive/maladaptive behaviors (Lane et al., 2010; Schauder and Bennetto, 2016). Thus, investigating the biological background of sensory abnormalities and its relationship to adaptive as well as maladaptive ASD behaviors might uncover the neurobiological cascade of adaptive/maladaptive behaviors, which may shed light on developing effective interventions for improving social adaptation in ASD, however, there is a dearth of research on this issue.

Recently, we reported that the acoustic startle reflex (ASR) and its modulation, such as sensorimotor gating evaluated as prepulse inhibition (PPI), might serve as a promising and quantitative neurophysiological endophenotype of sensory processing and act as a diagnostic marker of ASD as well as comorbid psychiatric conditions (Takahashi et al., 2014, 2016). A prolonged peak-ASR latency (Takahashi et al., 2014, 2016) and a greater ASR magnitude (Takahashi et al., 2014, 2016) in response to weak stimuli of 65–85 dB was found in children with ASD compared to those with typical development (TD), and these indices were related to autistic traits and emotional/behavioral difficulties in ASD children (Takahashi et al., 2016).

Building on this earlier research, in this study, we investigated the influence of the ASR and its modulation including PPI on adaptive/maladaptive behaviors, assessed with the Japanese version (Kuroda et al., 2014) of the Vineland Adaptive Behavior Scales (VABS), in children with ASD and those with TD, in order to examine the neurophysiological background of these behaviors in ASD. We hypothesized that adaptive and maladaptive behaviors might be related to different aspects of the ASR and its modulation. We investigated several ASR intensities, as a greater ASR to relatively weak stimuli has been related to several clinical features in children with ASD (Takahashi et al., 2016).

MATERIALS AND METHODS

Participants

Eleven Japanese children with ASD (age 8–16 years old; eight boys) and 18 typically developing (TD) Japanese children (age 8–16 years old; 12 boys) participated in the study. Experienced child psychiatrists assigned diagnoses after reviewing medical records and performing clinical interviews based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (American Psychiatric Association, 2000). Diagnoses were confirmed using the Autism Diagnostic Interview-Revised (Lord et al., 1995) and the Autism Diagnostic Observation Schedule (Lord et al., 2000). Intelligence quotient (IQ) was assessed with the Wechsler Intelligence Scale for Children-Third Revision (WISC-III: Wechsler, 1991). Neither sex (χ^2 = 0.117, df = 1, p = 0.732), age (age in years; ASD 11.6 ± 2.1; TD 11.4 ± 2.1; U = 91, p = 0.719), or estimated IQ (ASD 85.6 ± 40.1; TD 95.6 ± 32.5; U = 44.5, p = 0.317) differed significantly between the two groups. Additionally, results from the WISC-III (Wechsler, 1991) also showed that the estimated IQ for every child in the study was above 70. No child smoked and none were currently being medicated with psychotropic substances. Children were excluded from the study if they had any degree of hearing loss or abnormalities of the central nervous system apart from autism. Additionally, members of the TD group were excluded if they had previous or current psychiatric diagnoses or a learning disability.

Ethics Approval and Consent to Participate

The Research Ethics Committee of the National Center of Neurology and Psychiatry, Japan granted institutional review-board approval for the study (#A2013-112) and it was undertaken in accordance with the principles laid out in the Helsinki Declaration 1964 and its subsequent amendments. The study procedures were fully explained to all participants and their parents who then provided written informed consent before being included in the study.

Startle Response Measurement

A commercial computerized human startle-response monitoring system (Startle Eyeblink Reflex Analysis System Map1155SYS, Nihonsanteku Co., Osaka, Japan) was used to deliver acoustic startle stimuli and to record and score the corresponding electromyographic activity. The specific methods for stimulus presentation and eyeblink acquisition have been described in detail previously (Takahashi et al., 2017, 2018). The following startle measures were examined: (1) ASR65, ASR75, ASR85, ASR95, and ASR105: average ASR eyeblink magnitude in response to pulse intensities of 65, 75, 85, 95, and 105 dB SPL, respectively; (2) the peak-ASR latency, defined as the average peak-ASR latency across trials with an ASR larger than 60 μV; (3) habituation of the ASR during the session, defined as the percentage of ASR amplitude reduction at 105 dB SPL and (4) PPI65, PPI70, PPI75: PPI at prepulse intensities of 65, 70, and 75 dB SPL, respectively. The PPI at each prepulse intensity was defined as the percentage of amplitude reduction between pulse alone and pulse with prepulse trials. Trials were discarded if the voltage of their peak electromyographic activity was above 60 μV within a latency window of 0–20 ms following the startle-eliciting stimulus onset. Startle measures were not calculated for conditions in which more than half of the trials had been discarded.

Assessment of Adaptive and Maladaptive Behaviors

The children's adaptive and maladaptive behaviors were assessed with the Japanese version (Kuroda et al., 2014) of the VABS Second Edition (Sparrow et al., 2005), which was administered to the mothers of the participants by a child psychiatrist. The VABS

Second Edition is composed of two parts, an "adaptive behavior evaluation" part, which measures the level of adaptive behavior [the skills needed by individuals to function and be self-sufficient within their everyday environments (Sparrow et al., 2005)], and a "maladaptive behavior evaluation" part, which measures behavior that is problematic with respect to individual social life. Among the four different domains of adaptive behavior evaluation, communication (conceptual), socialization (social), and daily living (practical) adaptive skills were evaluated, whereas the motor adaptive skills domain was not used in this study as this score is designed to be evaluated only in participants who are aged 6 years or under, or over 50 years. Each of these domains comprises subdomains (receptive, expressive, and written skills in communication; personal, domestic, and community skills of daily living; interpersonal relationships, play and leisure time, and coping skills of socialization; and gross and fine motor skills) with item sets assessing specific content areas (e.g., adaptive skills). Maladaptive behavior evaluation comprises three domains (internalizing problems, externalizing problems, and others). We obtained total standard scores (M = 100, SD = 15) as well as scores for each of the adaptive behavior domains, and a v-scale score (M = 15, SD = 3) for maladaptive behavior. The higher the adaptive behavior standard score, the higher the adaptive behavior level, the higher the maladaptive behavior v-scale score, the higher the risk of maladaptation in life.

Statistical Analysis

Chi-square tests were used to examine categorical proportions. As most of the variables relating to the ASR and VABS scores were not normally distributed, nonparametric analyses were performed. The Mann-Whitney U test was used to compare the median scores of parameter values. Spearman's rank order correlation coefficients were used to examine the relationships between variables. SPSS Ver. 22 (IBM Japan, Tokyo, Japan) was used to perform all statistical analyses with the level of statistical significance set at $p < 0.05$. A Bonferroni correction was subsequently used to adjust significance levels for multiple comparisons.

RESULTS

Differences in Adaptive/Maladaptive Behaviors and Startle Measures Between Children With Autism Spectrum Disorders and Controls

The VABS scores and ASR measures for both groups are presented in **Table 1**. All VABS scores except the maladaptive behavior score, were significantly lower in the ASD group than in the controls. Children with ASD had a significantly prolonged peak-ASR latency. Additionally, their ASR75 was significantly greater. A trend towards greater ASR65 and ASR85 was also observed in ASD children. There were no statistically significant differences observed between the groups for other ASR measures, including ASR modulation of habituation and PPI at all prepulse intensities. However, after correction for multiple comparisons, only group differences in the VABS total and socialization domain scores remained significant.

Relationship of Adaptive/Maladaptive Behaviors to Startle Measures

Figure 1 shows the scatter plot of significant relationships between the VABS scores and ASR measures for all subjects.

TABLE 1 | Adaptive/maladaptive behavior scores and startle measures.

	Typical development (N = 18)		Autism spectrum disorders (N = 11)				
	Median	Inter-quartile range	Median	Inter-quartile range	U	p-value	Effect size (r)
Vineland Adaptive Behavior Scales Adaptive behavior							
Total	104	92.5–128.0	91	69.0–94.0	17.0	<0.001	0.69
Communication	103	77.0–112.5	75	66.0–88.0	41.0	0.009	0.49
Socialization	99	91.5–119.5	83	79.0–92.0	8.0	<0.001	0.76
Daily living	110	104.0–132.0	98	72.0–117.0	41.0	0.009	0.49
Maladaptive behavior	16	13.5–17.5	18	13.0–20.0	75.5	0.287	0.20
Peak startle latency (ms)	72.4	67.0–79.5	85.0	71.9–90.1	36.0	0.005	0.53
Acoustic startle magnitude (μV)							
65 dB	30.4	20.1–35.9	37.6	35.6–46.3	61.5	0.092	0.31
75 dB	28.5	18.9–40.2	46.0	40.1–56.4	52.0	0.035	0.39
85 dB	37.7	32.4–44.3	57.1	50.0–81.5	57.0	0.059	0.35
95 dB	47.5	28.9–65.1	47.3	44.0–56.3	96.0	0.893	0.03
105 dB	75.9	61.0–82.0	65.7	51.0–149.6	84.0	0.500	0.13
Habituation (%)†	28.9	13.0–36.3	22.8	2.7–31.0	52.0	1.000	0.00
Prepulse inhibition (%)†							
65 dB	20.4	4.3–37.7	14.2	5.2–23.1	66.0	0.618	0.10
70 dB	30.2	13.3–42.4	39.8	5.5–48.4	87.5	0.778	0.05
75 dB	42.3	29.7–48.8	49.2	16.8–59.6	89.5	0.851	0.04

Mann-Whitney U test. The level of statistical significance was set at $p < 0.003$ after a Bonferroni correction. †Number of participants (typical development, TD: autism spectrum disorders, ASDs) for habituation = 13: 8; Prepulse inhibition (65-dB prepulse) = 15: 10; (70-dB prepulse) = 17: 11; (75-dB prepulse) = 17: 11.

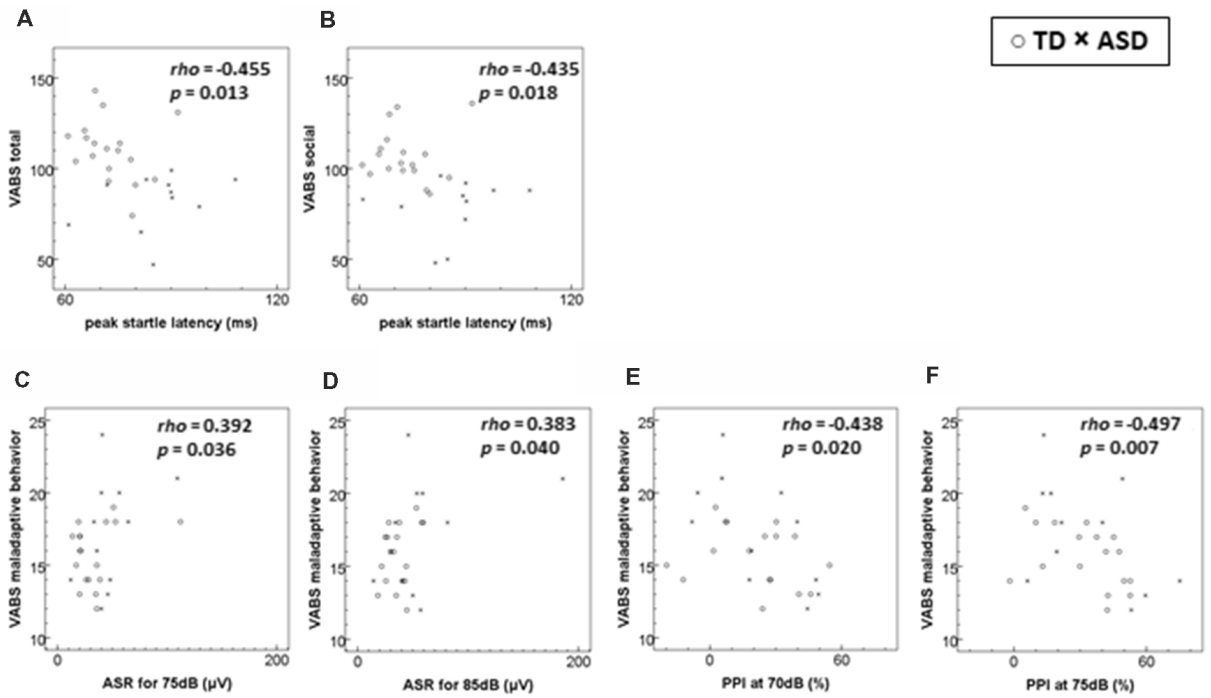

FIGURE 1 | Scatterplots of significant relationships of adaptive/maladaptive behavior scores to startle measures. **(A)** VABS total score to peak startle latency. **(B)** VABS socialization (social) domain score to peak startle latency. **(C)** VABS maladaptive behavior score to ASR for 75 dB stimuli. **(D)** VABS maladaptive behavior score to ASR for 85 dB stimuli. **(E)** VABS maladaptive behavior score to PPI at 70 dB prepulse. **(F)** VABS maladaptive behavior score to PPI at 75 dB prepulse. ASD, autism spectrum disorder; ASR, acoustic startle response magnitude; PPI, prepulse inhibition; TD, typical development; VABS, Vineland Adaptive Behavior Scales. The rho and the *p*-values are derived from Spearman's rank order correlations. The level of statistical significance was set at $p < 0.001$ after a Bonferroni correction. Number of participants (TD:ASD): **(A–D)** 18:11; **(E,F)** 17:11.

The peak-ASR latency was negatively correlated with the VABS total score and socialization domain score, while the ASR magnitudes for 75–85 dB stimulation were positively correlated with the VABS maladaptive behavior score. PPI at the prepulse intensity of 70–75 dB was also correlated negatively with the VABS maladaptive behavior score. However, these relationships did not remain significant after adjustment for multiple comparisons.

In the ASD group, we found a significant correlation between the VABS maladaptive behavior score and PPI70 ($\rho = -0.651$, $p = 0.030$). This relationship did not remain significant after adjustment for multiple comparisons. No other significant relationships between the ASR measures and VABS scores were observed in either group.

DISCUSSION

In this pilot study, we found possible relationships between adaptive and maladaptive behaviors and different aspects of the ASR in children with ASD and TD. Impaired adaptive behavior evaluated by the VABS total and socialization domain scores was negatively related to peak-ASR latency, while the VABS maladaptive behavior score was related not only to a greater startle magnitude to weak acoustic stimuli of 75 and 85 dB, but also to PPI.

Possible relationships were observed between impaired adaptive behaviors, especially for the socialization domain, and prolonged peak-ASR latency. Regarding the VABS adaptive behavior domains, previous research has highlighted that the socialization domain is consistently impaired in people with ASD irrespective of their cognitive level (Volkmar et al., 1987; Carter et al., 1998; Fenton et al., 2003; Klin et al., 2007; Perry et al., 2009). Thus, prolonged peak-ASR latency might serve as a possible marker of the impaired adaptive behavior which is seen in ASD.

We also found a possible relationship of maladaptive behavior to a greater startle response to relatively weak acoustic stimuli as well as PPI. The PPI is considered to be a stable neurophysiological marker, which continues to develop to full maturation until around 8 years of age (Takahashi et al., 2011). PPI impairment has been noted in several psychiatric diseases, such as schizophrenia, obsessive-compulsive disorder and posttraumatic stress disorder (Takahashi et al., 2011), however, consistent results have not been obtained with respect to the PPI of ASD in previous studies (Takahashi and Kamio, 2018), and we did not find a PPI difference between ASD and TD children. Thus, maladaptive behavior might be explained not only by greater hyper-reactivity to relatively weak stimuli, which was found in ASD, but also by impairment of sensorimotor gating, which is not specific to ASD.

This suggests that interventions for maladaptive behavior might be better started as early as possible before full PPI development has occurred.

The small sample size was a major limitation of this study. As significant ASR 65 group differences that were reported in previous studies (Takahashi et al., 2014, 2016) were not observed in the current study, and most of the associations between the ASR measures and adaptive/maladaptive behaviors became non-significant when the children were divided into groups, the small sample size might have affected the results. Further, although gender differences exist in ASD (Lai et al., 2014), this study consisted mainly of boys, while the age span of the children was also rather large. In addition, adaptive behaviors were evaluated with standardized scores while maladaptive behaviors were assessed with v-scale scores, and these scoring differences might also have affected the results. Future studies that have larger sample sizes of both sexes where the age range is narrower and that use other assessment tools standardized for maladaptive behavior will be important for further elucidating the neurophysiological factors that underpin adaptive as well as maladaptive behaviors in ASD.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

HT, KE and YK conceived and designed the experiments and analyzed the data. HT and YK supervised the project and confirmed the diagnoses. KE, HT and TN performed the experiments. KE, HT, AS, TN, TS and YK wrote the manuscript. All authors read and approved the final manuscript.

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Electroconvulsive Therapy Modulates Resting-State EEG Oscillatory Pattern and Phase Synchronization in Nodes of the Default Mode Network in Patients With Depressive Disorder

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Introduction: Electroconvulsive therapy (ECT) has antidepressant effects, but it also has possible cognitive side effects. The effects of ECT on neuronal oscillatory pattern and phase synchronization, and the relationship between clinical response or cognitive change and electroencephalogram (EEG) measurements remain elusive.

Methods: Individuals with unipolar depressive disorder receiving bilateral ECT were recruited. Five minutes of resting, eyes-closed, 19-lead EEG recordings were obtained before and after a course of ECT. Non-overlapping 60 artifact-free epocs of 2-s duration were used for the analyses. We used exact low resolution electromagnetic tomography (eLORETA) to compute the whole-brain three-dimensional intracortical distribution of current source density (CSD) and phase synchronization among 28 regions-of-interest (ROIs). Paired *t*-tests were used to identify cortical voxels and connectivities showing changes after ECT. Montgomery Asberg Depression Rating Scale (MADRS) and Mini-Mental State Examination (MMSE) were used to evaluate the severity of depression and the global cognitive function. Correlation analyses were conducted to identify the relationship between changes in the EEG measurements and changes in MADRS or MMSE.

Results: Thirteen depressed patients (five females, mean age: 58.4 years old) were included. ECT increased theta CSD in the anterior cingulate cortex (ACC), and decreased beta CSD in the frontal pole (FP), and gamma CSD in the inferior parietal lobule (IPL). ECT increased theta phase synchronization between the posterior cingulate cortex (PCC) and the anterior frontal cortex, and decreased beta phase synchronization between the PCC and temporal regions. A decline in beta synchronization in the left hemisphere was associated with cognitive changes after ECT.

Abbreviations: CSD, current source density; DMN, default mode network; ECT, electroconvulsive therapy; EEG, electroencephalography; LORETA, low resolution electromagnetic tomography.

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Conclusion: ECT modulated resting-state EEG oscillatory patterns and phase synchronization in central nodes of the default mode network (DMN). Changes in beta synchronization in the left hemisphere might explain the ECT-related cognitive side effects.

Keywords: electroconvulsive therapy, electroencephalography, depressive disorder, low resolution electromagnetic tomography, current source density, phase synchronization

INTRODUCTION

Electroconvulsive therapy (ECT) is the most effective antidepressant treatment (Kellner et al., 2012), but it also has potential cognitive side effects (Semkovska and McLoughlin, 2010). A better understanding of the biological mechanisms behind ECT-related antidepressant effects and cognitive side effects may have implications for developing new antidepressant treatments that have comparable efficacy to ECT without the cognitive side effects.

The electroencephalogram (EEG) is one of the principal methods for extracting information from the human brain noninvasively (Fingelkurts and Fingelkurts, 2015). Studies investigating the effects of ECT on electrophysiological measurements date back to the 1930s. Although the results of early studies using qualitative ratings were not consistent, quantitative analyses of EEG data have reported ECT-induced slow-wave increases in the fronto-temporal regions (Sackeim et al., 1996). Recent studies found that ECT-induced theta changes in the subgenual anterior cingulate cortex (ACC) were associated with improvement in psychotic symptoms (McCormick et al., 2009), and ECT modulated multi-scale entropy in depressed patients (Farzan et al., 2017). However, the number of studies examining the electrophysiological effects of ECT is still small compared to other modalities, such as magnetic resonance imaging (MRI; Abbott et al., 2014). Moreover, the relationship between changes in clinical response and/or cognitive function and changes in EEG measurements remains elusive.

Depression is now conceptualized as a system-level disorder (Mayberg et al., 2005), and it has been reported that depression showed increased resting-state EEG functional connectivity among multiple brain regions (Fingelkurts et al., 2007; Leuchter et al., 2012). The effects of antidepressant medications and transcranial magnetic stimulation (TMS) on brain electrophysiological measures have been examined by using a newly developed measurement of EEG functional connectivity, namely lagged non-linear connectivity or lagged phase synchronization (Pascual-Marqui et al., 2011; Olbrich et al., 2014; Iseger et al., 2017; Kito et al., 2017). Because phase synchronization is considered to be a fundamental neural mechanism relating to neural plasticity and cognitive processes (Fell and Axmacher, 2011), this measurement seems to be ideal for investigating the underlying mechanisms of ECT.

The aim of this study was to investigate the effects of ECT on cortical oscillatory activity and EEG phase synchronization throughout the brain. We also investigated whether changes in these EEG measurements were associated with clinical response as well as cognitive change.

MATERIALS AND METHODS

Trial Setting

We performed a longitudinal study to compare changes in neuronal oscillatory pattern and phase synchronization before [time point (TP1): time between admission and the first ECT] vs. after ECT (TP2: within 1 week of the completion of the ECT series). This study was conducted at Keio University Hospital from June 2013 through December 2015. Ethical approval was obtained from the Ethics Committee of Keio University School of Medicine, and the study was conducted in accordance with the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all the participants.

Participants

Individuals meeting the following inclusion criteria were recruited from Keio University Hospital: (1) International Classification of Disease 10th edition (ICD-10) diagnosis of depressive disorder (F32, F33; World Health organization, 1994); (2) inpatients at the psychiatric ward; (3) clinical indications for ECT including treatment resistance and a need for a rapid and definitive response; and (4) age $\geq$20 years. Exclusion criteria were the following: (1) a lifetime history of neurological or degenerative disorder; (2) unstable or severe medical illness; (3) ECT treatment within the last 3 months; (4) lifetime history of drug or alcohol misuse;and (5) difficulty in communication. These participants were originally collected for a previous study (Hirano et al., 2017).

Clinical Assessments

The following clinical assessments were performed by trained psychiatrists who were blinded to the EEG data at TP1 and TP2. Montgomery Asberg Depression Rating Scale (MADRS; Montgomery and Asberg, 1979) was used to evaluate the severity of depression, and Mini-Mental State Examination (MMSE; Folstein et al., 1975) was used for the assessment of global cognitive function. We also collected participants' demographic and clinical information including age, sex, past medical history, medications prescribed, and ECT data (e.g., the number of ECT sessions). Clinical response was defined as a decrease in MADRS score of at least 50% from baseline (Rush et al., 2003), and remission was defined as a total MADRS score of 10 or less (Zimmerman et al., 2004).

ECT Treatment

ECT was performed with bitemporal electrode placement using a brief-pulse square-wave device (Thymatron system IV device; Somatics, Inc., Lake Bluff, IL, USA). The intensity of the first ECT session was determined based on the half age method. Treatments were performed three times a week, and treatments were continued until a plateau was reached and no more improvement was seen in the last two sessions. EEG seizure manifestations were monitored to ensure adequate seizure. When the EEG seizure duration was less than 25 s, the patients were restimulated at a higher intensity after a 1 min interval. Thiopental (3.5 mg/kg) was used for general anesthesia, and succinylcholine (1 mg/kg) was used to induce muscle relaxation (A Task Force Report of the American Psychiatric Association, 2001).

EEG Recording

The participants underwent EEG before (TP1) and after (TP2) a series of ECT. The first recording was performed between admission and the first ECT, and the second recording was done within 1 week after the last ECT. EEG data was obtained and digitalized on Nihon Kohden EEG machines (Neurofax EEG-1200) by trained technicians at Keio University Hospital. Five minutes of resting EEG was recorded under eyes-closed conditions from 19 scalp locations according to the international 10/20 system (Fp1/2, F3/4, C3/4, P3/4, O1/2, F7/8, T3/4, T5/6, Fz, Cz, Pz) referenced to linked ear lobes (A1 and A2). Impedances were kept below 5 kΩ. Data were collected digitally with a sampling rate of 500 Hz. Simultaneous video recordings were used to check each segment for movements and to exclude these segments.

EEG Preprocessing

EEG raw data was first analyzed using the EEGLAB (Delorme and Makeig, 2004). The data were downsampled to 250 Hz to reduce computing time, filtered at 1.0 Hz (high-pass) and 50 Hz (notch-filter), and segmented in 2-s epocs. Then the EEG signal was decomposed into independent components (ICs) by Infomax IC analysis (Bell and Sejnowski, 1995), using the EEGLAB *runica* command. Each IC was visually examined and ICs corresponding to artifactual sources were removed. The cleaned EEG signal was reconstructed by retro-projecting only the ICs containing a cerebral signal. The reconstructed signals were referenced to Cz and the first 60 epocs were entered into the following analyses.

EEG-Source Localization Analysis

We used exact low resolution electromagnetic tomography (eLORETA) to compute the three-dimensional (3D) intracortical distribution of electric neuronal activity for the following six bands: delta (1.0–3.5 Hz), theta (4.0–7.5 Hz), alpha (8.0–12.0 Hz), beta 1 (12.5–20.0 Hz), beta 2 (20.5–30.0 Hz), gamma (30.5–45.0 Hz). The eLORETA method is a discrete, 3D-distributed, linear, weighted minimum norm inverse solution. Compared with previous versions, eLORETA has no localization bias in the presence of structured noise in simulated data (Pascual-Marqui, 2007a). Numerous studies using functional MRI (fMRI; Vitacco et al., 2002; Mulert et al., 2004), structural MRI (Worrell et al., 2000), positron emission tomography (PET; Pizzagalli et al., 2003; Zumsteg et al., 2005), and intracranial EEG (Zumsteg et al., 2006a,b) have validated LORETA to study brain activity. Studies using a relatively small number of electrodes (i.e., 19 electrodes) have applied LORETA source localization successfully (McCormick et al., 2009; Thatcher et al., 2014).

Several previous studies have reported abnormal current source density (CSD; Pizzagalli et al., 2002, 2004) and EEG functional connectivity (Olbrich et al., 2014) in depressed patients, as well as changes in EEG functional connectivity with antidepressant treatments, including antidepressant medications (Olbrich et al., 2014; Iseger et al., 2017), and TMS (Kito et al., 2017). The eLORETA solution space (6,239 voxels; spatial resolution; 5 mm) is restricted to the cortical gray matter. The Montreal Neurologic Institute average MRI brain (MNI152; Mazziotta et al., 2001) is used as a realistic head model for which the lead field was computed (Fuchs et al., 2002). At each voxel, LORETA values represent the power of the computed intracortical current density distribution for each frequency band. To eliminate variability for the total power changes of each subject, we used subject-wise data normalization implemented in LORETA before statistical analyses.

EEG Functional Connectivity Analysis

We selected 28 regions-of-interest (ROIs) covering the whole-brain based on Brodmann Areas (BAs) provided in the eLORETA software, as others did in a previous study (Di Lorenzo et al., 2015; **Supplementary Table S1**). We selected a single voxel in the center of each ROI as the representative voxel. We used lagged phase synchronization (Kito et al., 2017) as a measure of EEG functional connectivity between all pairs of ROIs. Lagged phase synchronization quantifies the non-linear relationship between two ROIs after the instantaneous zero-lag contribution has been excluded. This correction is important because zero-lag synchronization is usually due to non-physiological artifacts, such as volume conduction and low spatial resolution that usually affect other connectivity indices (Nolte et al., 2004; Stam et al., 2007). Details on the lagged phase synchronization algorithm can be found in several reports (Pascual-Marqui, 2007b; Kito et al., 2017).

Statistical Analysis

We conducted paired *t*-tests to compare differences in CSD and lagged phase synchronization between TP1 and TP2. We used statistical nonparametric mapping (SnPM; Nichols and Holmes, 2002). This method determined the critical probability threshold values for the actually observed *t*-values with correction for multiple comparisons across all voxels and all frequencies. A total of 5,000 permutations were conducted to calculate the critical threshold t_{crit} for $p = 0.05$ with correction for multiple comparisons among all voxels and frequencies. The omnibus null hypothesis was rejected if at least one *t*-value (i.e., voxel tmax) was above the t_{crit}. The use of SnPM in eLORETA has been validated in several studies (Pascual-Marqui et al., 1999; Canuet et al., 2012). To investigate

associations between EEG changes and clinical changes, we extracted individual eLORETA values and connectivity values from identified brain regions and connectivities using the above-mentioned paired *t*-tests, and we conducted correlation analyses as exploratory analyses. Clinical changes included MADRS reduction volume (post MADRS score − pre MADRS score) and MMSE reduction volume (post MMSE score − pre MMSE score). Statistical analyses were performed using SPSS ver 24.0 (IBM Inc., Armonk, NY, USA). Statistical significance was defined by a *p*-value of <0.05 (two-tailed). Multiple testing corrections were not conducted for the correlation analyses, as these analyses were exploratory.

RESULTS

Demographic and Clinical Characteristics

Demographics and clinical characteristics of the participants are summarized in **Table 1**. Thirteen individuals [five females, mean age: 58.4 (Standard Deviation: 13.6) years old] with unipolar depressive disorder completed this study. After ECT, the total MADRS score was significantly reduced from TP1 to TP2 [TP1: 30.3 (8.6), TP2: 6.5 (6.3), $df = 12$, $t = 9.04$, $p < 0.001$], whereas the total MMSE score did not change [TP1: 27.0 (3.0), TP2: 26.3 (2.9), $df = 11$, $t = 0.76$, $p = 0.46$]. Remission and response rate were 69.2% (9/13) and 92.3% (12/13), respectively. The number of ECT was mean 9.9 (SD: 1.8).

Longitudinal Effects of ECT on Whole Brain CSD

Whole-brain analyses using eLORETA showed the following changes in oscillatory cortical activity patterns after ECT ($t_{crit} = 1.52$, $p < 0.05$): increased theta ($t = 1.70$) in the ACC and the medial prefrontal cortex (MPFC); decreased beta 2 ($t = -1.75$) in the frontal pole (FP), and decreased gamma ($t = -1.74$) in the right inferior parietal lobule (IPL; **Figure 1**).

TABLE 1 | Clinical characteristics of the participants.

Number of patients	13
Age, years	58.4 (13.6)
Female, *n* (%)	5 (38.5%)
Psychotic features, *n* (%)	5 (38.5%)
Age at onset, years	48.2 (7.7)
Number of depressive episodes	2.5 (1.4)
Duration of current episode, months	10.2 (12.4)
Number of prior antidepressants	4.0 (1.8)
Number of ECT treatments	9.9 (1.8)
Time between the pre-EEG and the first ECT, days	9.1 (7.8)
Time between the last ECT and post-EEG, days	3.1 (1.7)
MADRS total score	
pre-ECT (TP1)	30.3 (8.6)
post-ECT (TP2)	6.5 (6.3)
Clinical Remitters, *n* (%)	9 (69.2%)
Clinical Responders, *n* (%)	12 (92.3%)
MMSE total score	
pre-ECT (TP1)	27.0 (3.0)
post-ECT (TP2)	26.3 (2.9)

Data are number or mean (standard deviation) unless stated otherwise. Abbreviation: TP1, pre-ECT series (baseline); TP2, post-ECT series (endpoint); ECT, Electroconvulsive therapy; EEG, Electroencephalogram; MADRS, Montgomery Asberg Depression Rating Scale; MMSE, Mini-Mental State Examination.

Longitudinal Effects of ECT on Lagged Phase Synchronization

Analyses of changes in lagged phase synchronization between TP1 and TP2 ($t_{crit} = 5.37$, $p < 0.05$) revealed that there was a significant increase in theta phase synchronization between the right anterior PFC (APFC) and the right posterior cingulate cortex (PCC; $t = 5.48$). There were significant decreases in the beta 1 phase synchronization between the right insula (INS) and the right superior parietal lobule (SPL; $t = -5.85$), between the left PCC and the left INS ($t = -6.60$), and between the left PCC and the left lateral temporal lobe (LTL; $t = -5.55$; **Figure 2**).

Correlation Between Changes in EEG Measurements and Clinical Changes

There were no correlations between changes in CSD in three identified regions (theta CSD in the ACC/MPFC, beta2 CSD in the FP, and gamma CSD in the right IPL) and changes in MADRS or MMSE (**Supplementary Table S2**). However, connectivity changes between the left PCC and the left INS ($r = -0.68$, $df = 10$, $p = 0.015$) as well as connectivity changes between the left PCC and the LTL ($r = -0.64$, $df = 10$, $p = 0.024$) had negative associations with changes in MMSE.

DISCUSSION

The current study revealed that ECT increased theta activity in the ACC/MPFC, decreased beta activity in the FP, and decreased gamma activity in the IPL. ECT increased theta phase synchronization between the PCC and the APFC, and decreased beta phase synchronization between the PCC and the temporal regions. We could not find any associations between clinical response and any EEG measurements, but we found a relationship between decreased beta phase synchronization and cognitive change after ECT. This is the first study to show the correlation between ECT-related cognitive change and beta phase synchronization.

Longitudinal Effect of ECT on Neural Oscillations

We found that ECT increased theta oscillations in the ACC/MPFC and decreased high frequency oscillations in the FP and the right IPL. Since the 1930s, many studies have reported ECT-induced slow wave oscillations in the frontal lobe (Krystal et al., 2000; Farzan et al., 2014), and a recent EEG study reported that ECT decreased high frequency oscillations, especially in patients who responded to ECT (Farzan et al., 2017). Our results are in line with these previous findings, which may support the validity of our findings.

According to fMRI and EEG studies, frontal medial theta activity was negatively correlated with blood oxygen level dependent (BOLD) signals in the default mode network (DMN) regions, namely medial frontal, inferior frontal, precuneus/PCC, inferior parietal, middle temporal cortices, and the cerebellum

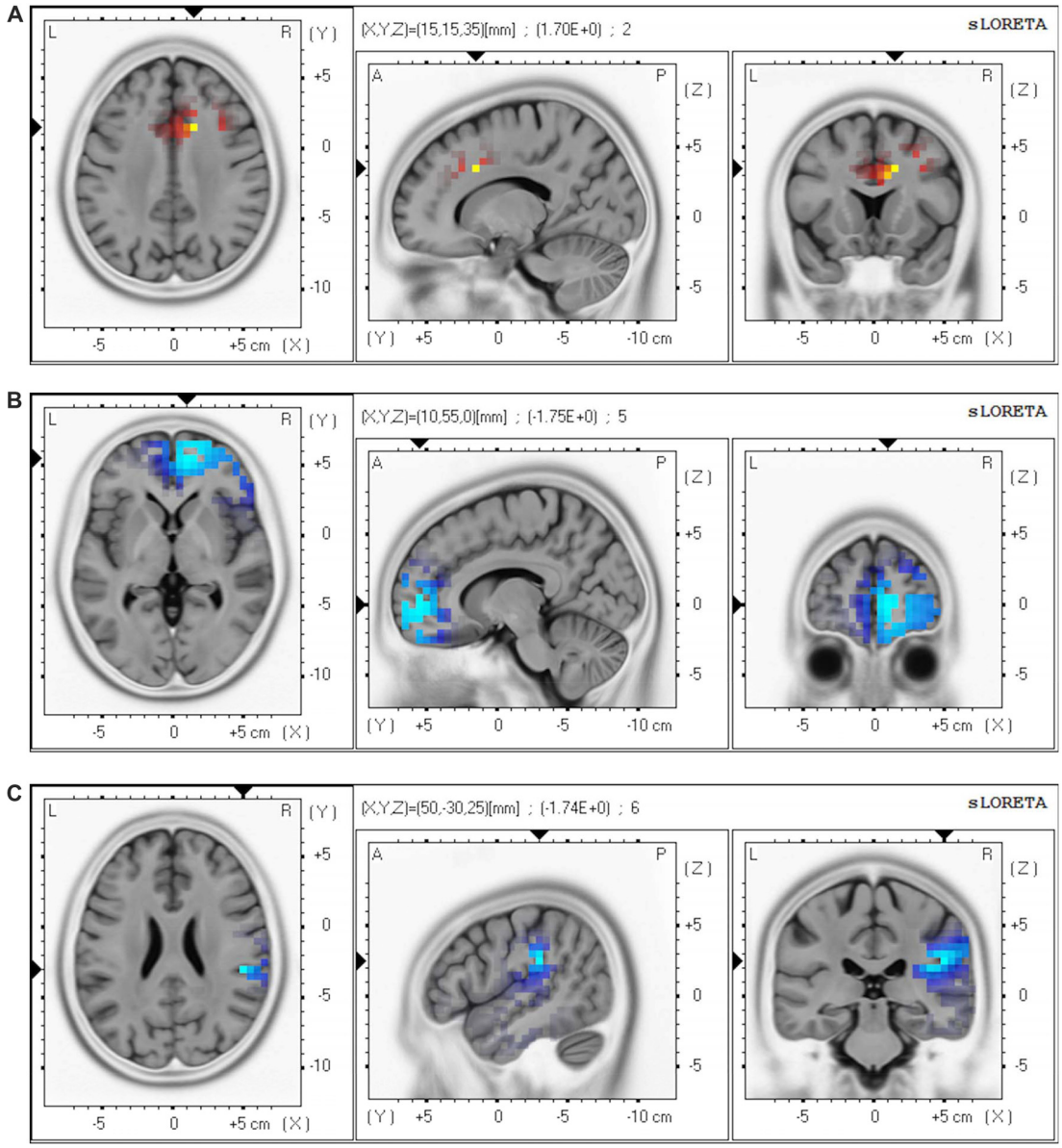

FIGURE 1 | Brain regions showing change in oscillatory patterns after a course of electroconvulsive therapy (ECT). Our analyses revealed the following changes after ECT: **(A)** increased theta (4.0–7.5 Hz) in the anterior cingulate cortex (ACC) and the medial prefrontal cortex (MPFC); **(B)** decreased beta 2 (20.5–30.0 Hz) in the frontal pole (FP); and **(C)** decreased gamma (30.5–45.0 Hz) in the right inferior parietal lobule (IPL). Red regions correspond to significantly increased CSD after ECT, and blue regions correspond to significantly decreased CSD after ECT. Abbreviation: CSD, current source density.

(Scheeringa et al., 2008). In addition, high-frequency bands, including beta (Laufs et al., 2003) and gamma (Mantini et al., 2007), were positively correlated with DMN BOLD signals. Considering these previous findings, the current results (increased theta in the ACC/MPFC, decreased beta in the MPFC, and decreased gamma in the IPL) may indicate that ECT decreased resting-state electrical activity in nodes of the DMN. A recent meta-analysis of PET studies investigating the effect of treatments for depression (i.e., antidepressant medications and ECT) on brain metabolism revealed that ECT decreased activity in central nodes of the DMN (Chau et al., 2017). Given that electroencephalographic oscillations are a relatively more direct measure of neuronal activity than other modalities (e.g., PET, MRI), the current study may provide additional evidence for the results from previous PET studies.

We could not find any correlations between oscillatory changes in nodes of the DMN regions and ECT and MADRS reduction. One potential interpretation is that an ECT-induced reduction in DMN activity may be just a by-product of electrical stimulation or seizure, and not related to clinical response.

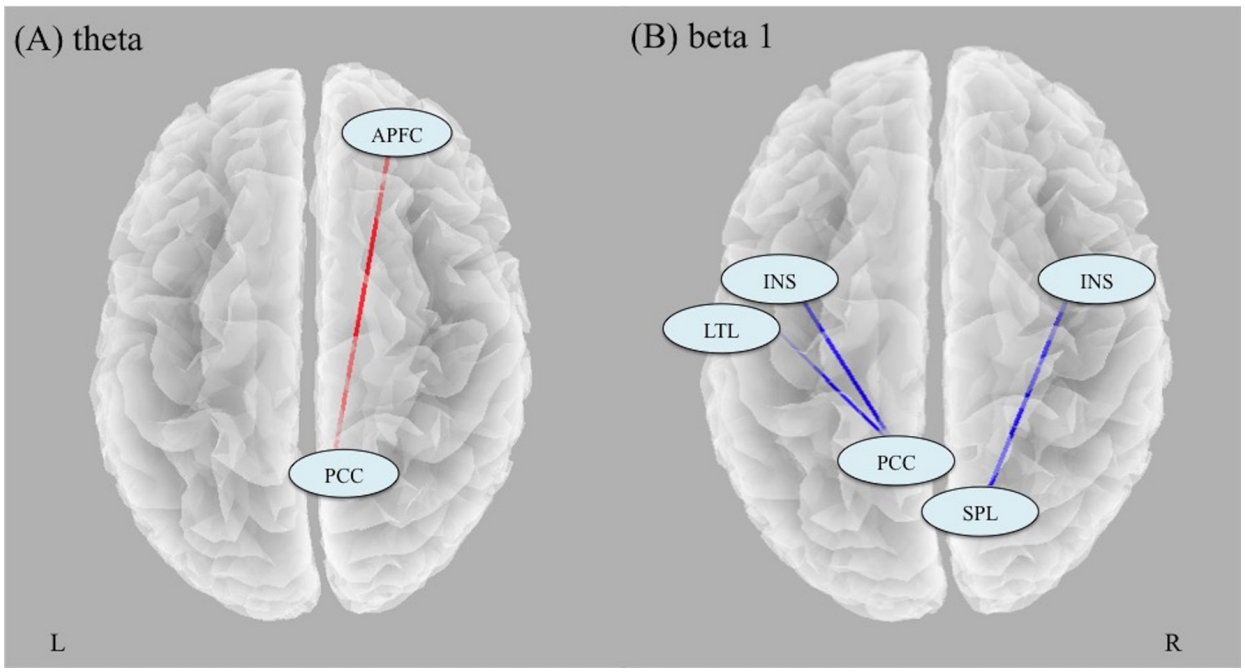

FIGURE 2 | Results of analyses of changes in lagged phase synchronization with ECT. **(A)** There was a significant increase in theta phase synchronization between the right APFC and the right PCC. **(B)** There were significant decreases in the beta 1 phase synchronization between the right INS and the right SPL, between the left PCC and the left INS, and between the left PCC and the left LTL. Abbreviation: APFC, anterior prefrontal cortex; PCC, posterior cingulate cortex; INS, insula; SPL, superior parietal lobule; LTL, lateral temporal lobe.

Another potential explanation is that a change in DMN activity due to ECT may be related to a change in specific psychiatric symptoms, and not related to a change in the entirety of depressive symptoms (i.e., total HAM-D scores). Since DMN activity is considered to be associated with rumination and autobiographical memory (Zhu et al., 2012), a future study should focus on the relationship between ECT-induced changes in DMN activity and specific symptoms (e.g., rumination) or autobiographical memory, which is known as ECT-related side effects (Semkovska and McLoughlin, 2013).

Longitudinal Effect of ECT on Lagged Phase Synchronization

ECT-induced EEG slowing suggests that synchronization occurs in the synaptic activity of large neuronal populations, with a reduction in firing rate (Sackeim et al., 1996). Our observed results of ECT-induced increased phase synchronization in theta frequency between the PCC and the APFC (BA9, 10) support this notion. These two regions (PCC and BA9/10) are located in the posterior and anterior central nodes of the DMN, suggesting that ECT may increase phase synchronization within the DMN. Since there were no correlations between changes in theta phase synchronization and those in MADRS and MMSE, the implication of our findings still remains unclear. Therefore, to elucidate the clinical relevance of changes in theta phase synchronization due to ECT, a large sample study that focuses on specific symptoms related to the DMN is needed.

Additionally, the current study revealed that ECT decreased beta phase synchronization between the PCC and the temporal regions. This is in line with a previous EEG study using graph theoretical analysis, which reported that a single session of seizure therapy decreased the phase synchronization in the beta frequency band (Deng et al., 2015). Furthermore, we found a significant correlation between changes in beta synchronization and changes in MMSE scores, which may suggest that depressed patients who present a larger decrease in beta synchronization after ECT show more cognitive decline after ECT. A prior study has also reported that lower beta band synchronization is associated with lower MMSE scores (Stam et al., 2003). Furthermore, the PCC has an important role in autobiographical memory (Leech and Sharp, 2014), which is one of the cognitive functions largely affected by ECT (Semkovska and McLoughlin, 2013). In addition, our finding was restricted to the left hemisphere. The short-term cognitive side effects of ECT change depending on the electrode placement (i.e., bilateral vs. unilateral). Right unilateral electrode placement has been shown to have less cognitive side effects than bilateral electrode placement (Kolshus et al., 2017), and left unilateral electrode placement tended to have more verbal memory impairment than right unilateral electrode placement (Kellner et al., 2017). The interpretation of these results is understandable based on the theory that the left hemisphere is dominant for language and verbal processing for most people. Left-lateralized results in the current study are consistent with this evidence. We used only bilateral electrode placement in the study because

our participants were severely depressed patients who needed rapid improvement, but future studies should compare the effects of ECT on neurophysiological and neuropsychological measurements between different electrode placements to test our hypothesis. Taken together, our findings of a decrease in beta synchronization between the left PCC and the left temporal regions may reflect the underlying electrophysiology of ECT-induced cognitive impairments.

LIMITATIONS

The current study should be interpreted with the following limitations. First, the number of participants was limited. A larger study is needed to confirm our preliminary results. Second, all patients continued their psychopharmacological medications, which may affect the EEG oscillatory pattern and phase synchronization. However, a previous study reported that antidepressant medications increased beta band phase synchronization as calculated by LORETA (Olbrich et al., 2014). The effects of ECT on EEG phase synchronization (i.e., ECT decreased beta band phase synchronization) may be stronger than the effects of antidepressant medications. Third, we did not conduct multiple testing corrections for correlation analyses, as the analyses were exploratory. The observed relationship between beta synchronization and cognitive change needs to be replicated. In addition, MMSE includes multiple cognitive domains, so future studies should focus on specific cognitive domains that relate to ECT. Fourth, our sample includes only depressive disorder to avoid heterogeneity, but this also limits the generalizability of our results.

CONCLUSION

ECT reduced resting-state EEG oscillatory activity in central nodes of the DMN regions and increased phase synchronization within the DMN. An ECT-induced reduction in beta phase synchronization was associated with the cognitive side effects experienced by patients after a series of ECT.

DATA AVAILABILITY

The datasets for this study will not be made publicly available because it might be possible if we get consents to provide data from all participants.

AUTHOR CONTRIBUTIONS

AT and JH designed the study, recruited the participants, conducted the clinical assessments, and analyzed the data. AT conducted a literature search and wrote the first draft. JH, BY, ST, TK, and MM wrote the final manuscript. All authors contributed to and have approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnhum.2019.00001/full#supplementary-material

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frontiers in Human Neuroscience

CLINICAL TRIAL
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Impaired Modulation of Corticospinal Excitability in Drug-Free Patients With Major Depressive Disorder: A Theta-Burst Stimulation Study

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Impaired neural plasticity may be an important mechanism in the pathophysiology of major depressive disorder (MDD). Coupled with electromyography (EMG), repetitive transcranial magnetic stimulation (rTMS) is a useful tool to evaluate corticospinal excitability and cortical neuroplasticity in living humans. The goal of this study was to compare rTMS-induced cortical plasticity changes in patients with MDD and in healthy volunteers. In this single-blind controlled study, 11 drug-free patients with MDD and 11 matched healthy controls were analyzed. Cortical excitability, measured by the amplitude of motor evoked potentials (MEPs) evoked by single-pulse TMS, was assessed before and repeatedly after (for 30 min) participants received a single session of intermittent theta-burst stimulation (iTBS) and continuous TBS (cTBS). rTMS was applied over the left motor cortex using a neuronavigation system. Intensity was set at 80% of the active motor threshold (AMT). A large interindividual variability was observed after both iTBS and cTBS in the two groups. At the group level, we observed impaired iTBS-induced neuroplasticity in patients with MDD compared to that in controls. No differences were observed between the groups regarding cTBS-induced neuroplasticity. Our results suggest impaired long-term potentiation (LTP)-like mechanisms in MDD.

Clinical Trial Registration: www.Clinicaltrials.gov, identifier #NCT02438163.

Keywords: major depressive disorder, transcranial magnetic stimulation, theta-burst stimulation, cortical excitability, neural plasticity

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INTRODUCTION

Unipolar major depressive disorder (MDD) is a very frequently occurring disorder associated with high impairment of global functioning and significant societal economic burden (Kessler et al., 2003; Whiteford et al., 2013). Despite current efforts, the pathophysiology of MDD is not completely elucidated. Among the several theories that have been proposed, the "neural plasticity abnormalities" theory in particular, which may bridge the prevailing theories, has gained attention (Wainwright and Galea, 2013). Neural plasticity encompasses an array of key brain mechanisms (birth, survival, migration, and integration of neurons, synaptogenesis and apoptosis). Several studies have reported impaired neural plasticity at different levels in patients

with MDD. For instance, postmortem studies have revealed a reduction in the number of synapses and a decreased expression of synaptic function-related genes in the prefrontal cortex (PFC) of patients with MDD (Kang et al., 2012). Patients with MDD also display a reduction in brain volume compared with healthy volunteers, especially in the hippocampus (Campbell et al., 2004) and in the PFC (Drevets, 2000). Taken together, these studies suggest a close relationship between abnormal neural plasticity and MDD, but more studies are needed to establish the key role of these mechanisms in MDD pathophysiology (for a review see Cantone et al., 2017).

Transcranial magnetic stimulation (TMS) is a noninvasive brain stimulation method that can be used to evaluate some indexes of neural plasticity in living humans. Some authors have suggested that the modulation of motor corticospinal excitability measured by single pulse TMS following a repetitive TMS (rTMS) session may reflect neural plasticity (Cantone et al., 2017). Among all the currently available rTMS protocols, the theta burst stimulation (TBS) is a brief rTMS protocol enabling an assessment of cerebral plasticity, especially at a synaptic level (Huang et al., 2005). Depending on the stimulation parameters, TBS can induce either an inhibition of corticospinal excitability (following continuous TBS, cTBS), or an enhancement (following intermittent TBS, iTBS). In these studies, the induced modulation of corticospinal excitability is assessed by the size of motor evoked potentials (MEPs) measured before and after the rTMS session. For instance, by measuring the modulatory effects of a single TBS session in individuals with Asperger's syndrome, Oberman et al. (2012) observed a significant alteration in the modulation of corticospinal excitability in patients compared to that in healthy volunteers in response to both iTBS and cTBS suggesting aberrant mechanisms of plasticity in patients. These results suggest that TBS may reveal abnormal neuroplasticity in patients with psychiatric neurodevelopmental conditions. However, to the best of our knowledge, the modulatory effect of TBS on neural plasticity has never been investigated in patients with MDD. We hypothesized that patients with MDD would display decreased TBS-induced modulation of corticospinal excitability compared with healthy volunteers.

MATERIALS AND METHODS

The study was approved by a local ethics committee (CPP Sud-Est 6), ANSM 2013-A00971-44 and registered in www.Clinicaltrials.gov (NCT02438163). All patients provided written, informed consent. The trial was conducted in the Hospital Le Vinatier, University Department for treatment-resistant depression, University of Lyon, France. All patients were consecutively recruited from March 2014 to October 2017.

Participants

Fourteen patients with unipolar MDD according to DSM 5 and 14 matched healthy controls were enrolled in the study. Three patients with MDD were not included in the final analyzed sample. One patient with MDD was excluded because of an unexpected cerebral lesion discovered during the magnetic resonance imaging (MRI); one patient withdrew her consent; one was excluded because we were not able to obtain a 1 mV baseline MEP. It should be note that one patient only took part in the cTBS session because of strong nausea on the morning of the iTBS session, and setting up another day of investigation with the patient was not possible due to the introduction of antidepressant medication. In the healthy control group, three participants were not included in the final analyzed sample: two participants withdrew their consent, and one was excluded because she presented with an antecedent of a major depressive episode. Therefore, the final analyzed sample consisted of 11 healthy participants, 10 patients with MDD who received cTBS and 11 patients with MDD who received iTBS.

Only right-handed patients (according to the Edinburgh Handedness Inventory) from both genders [eight females, three males, age range 28–61, mean = 44.6 (standard deviation = 10.8) years old] with a Montgomery–Åsberg Depression Rating Scale (MADRS) score between 20 and 35 and free from any psychotropic drugs (including antipsychotic, antidepressant, and antiepileptic drugs) were included. For patients with MDD under psychotropic drugs, the wash out period was at least of five half-life time of the concerned drugs. Exclusion criteria consisted of (i) melancholic features; (ii) presence of a neurological or psychiatric comorbidity, except for anxiety disorder; (iii) pregnancy; and (iv) contraindications for TMS.

The group of healthy controls was composed of 11 right-handed individuals [seven females, four males, age range 26–59, 42.3 (9.4) years old]. The inclusion criteria consisted of the following: (i) no current psychiatric, neurologic or infectious disease with a potential effect on the brain; and (ii) free from any psychotropic drug. The exclusion criteria consisted of (i) pregnancy; and (ii) contraindications for TMS. Further characteristics of the participants are given in **Table 1**.

TABLE 1 | Demographic and clinical characteristics of the participants.

	Patients with MDD	Healthy controls	*p*
n	11	11	
Gender (female/male)	8/3	7/4	1
Age	44.6 (10.7)	42.3 (9.4)	0.59
Number of prior episodes	1.6 (1.4)	0 (0)	<0.001
MADRS	29.8 (4.7)	0 (0)	<0.001
Duration of illness (months)	19.1 (22.6)	0 (0)	<0.001
STAI trait	55.5 (10.2)	34.8 (6.6)	<0.001
STAI state before iTBS	52.4 (11.1)	27.4 (7.0)	<0.001
1 mV MEP before iTBS	59.8 (13.9)	57.5 (8.2)	0.65
AMT before iTBS	33.9 (9.1)	35.5 (7.3)	0.67
STAI state before cTBS*	54.5 (13.4)	28.5 (7.6)	<0.001
1 mV MEP before cTBS*	58.8 (12.0)	58.3 (9.5)	0.91
AMT before cTBS*	34.8 (7.6)	34.5 (6.9)	0.93

*The results are given as the mean ± standard deviation. MADRS, Montgomery–Åsberg Depression Rating Scale; STAI, State-Trait Anxiety Inventory scale; AMT, activity motor threshold; 1 mV MEP, TMS intensity to obtain an MEP with a mean amplitude of 1 mV at baseline. *Only 10 patients with MDD received continuous theta-burst stimulation (cTBS).*

Transcranial Magnetic Stimulation to Assess TBS-Induced Neural Plasticity

Participants were seated in a comfortable chair with both arms supported passively. Electromyographic (EMG) recordings from the right first-dorsal interosseus muscle (FDI) were taken using Ag/AgCl surface electrodes (Disposable Surface Electrodes SEAg-C-0.7/100/22X30; Friendship Medical, Xi An, China). Raw signals were amplified and digitized using a commercially available amplifier (Keypoint Portable System). All recordings were manually analyzed offline.

TMS was applied over the left primary motor cortex (M1) using a posterior-anterior current direction through a standard figure-of-eight coil (Cool Coil Magnetic Stimulator B65, Mag2Health) connected to a MagPro-X100 stimulator. The coil was manually and tangentially placed with the handle pointing backwards at an angle of 45° to the midline. The stimulation site leading to large and stable MEPs was defined as the optimal coil position over the left M1. To ensure that the coil reliably remained over the same stimulation target throughout the entire experimental session (baseline, TBS protocol, and repeated MEP recordings), the coil was guided with an MRI-coupled neuronavigation system [SYNEIKA ONE (SYN1) version 1.5.1].

To record MEPs at baseline and repeatedly after TBS, the TMS intensity was set to evoke MEPs of approximately 1 mV (S1mV) amplitude at baseline. We measured the peak to peak amplitude of 15 MEPs at baseline and 10 MEPs (Groppa et al., 2012) at different time points: 5, 10, 20, and 30 min after the end of the TBS session.

Theta-Burst Stimulation Procedures

Participants were randomly assigned to receive two sessions of TBS delivered on two separate days. The experimental sessions were performed with a wash period between 2 and 7 days. Sessions took place at the same time of day (morning or afternoon) to prevent diurnal influences on neurophysiologic measures (Stagg and Nitsche, 2011; Kuo and Nitsche, 2012). All participants but one received one session of cTBS and one session of iTBS (Huang et al., 2005). The cTBS paradigm consisted of three pulses at 50 Hz every 200 ms for 40 s (for a total of 600 pulses). In the iTBS paradigm, participants received a 2-s train of cTBS repeated every 10 s for a total of 190 s (600 pulses). In both experiments, the intensity of stimulation was set at an intensity of 80% of the active motor threshold (AMT). AMT was assessed in the setting phase described above and was defined as the lowest intensity to obtain at least five MEPs of 200 μV over 10 stimulations in the FDI contracted at 20% of maximal strength (Huang et al., 2005). This strength was measured using a dynamometer (Hand Dynamometer Vernier HD-BTA, driven by the software Logger Pro 3); a continuous audio-visual EMG feedback was available to evaluate participants' relaxation or their level of muscle contraction. The experimental design is illustrated in **Figure 1**.

Clinical Assessments

The severity of depressive symptoms was assessed using the MADRS. State and trait anxiety levels were assessed using the State-Trait Anxiety Inventory questionnaire (STAI; Spielberger, 1989); the trait form of the questionnaire (STAI Y-B) was addressed during the inclusion visit and the state form (STAI Y-A) before each TBS session.

Data Analysis

The sociodemographic and clinical characteristics as well as the baseline MEP measures of participants were compared between groups using independent two-tailed sample *t*-tests and Fischer's exact tests for gender.

The relative MEP values calculated as the mean of 10 MEPs peak amplitudes post TBS/the mean of 15 MEPs peak amplitudes at baseline in each subjects were used as primary outcomes. A repeated measures ANOVA (RM-ANOVA) was undertaken with relative MEP value at the different time points as the dependent variable, group (healthy controls vs. patients with MDD) as the between-subject factor, and time as the within-subject factor. Two RM-ANOVAs were conducted to analyze the effect of iTBS on the one hand and the effect of cTBS on the other hand.

When appropriate (significant interactions in the RM-ANOVAs), *post hoc* comparisons were performed to more specifically determine the changes in MEP amplitude. TBS-induced modulation of MEP size across the five time points (baseline, 5, 10, 20 and 30 min) in both condition (iTBS and cTBS) were also investigated as the maximum peak amplitude at the individual level. Number of responders and non-responders after TBS according to Hamada et al. (2013) classification were also calculated and compared across groups using Fischer exact test. Responders and non-responders were defined according to the grand average of TBS responses below and above 1 for cTBS and iTBS, respectively (Hamada et al., 2013). SPSS 21 was used for all analyses, and the level of significance was set at $p < 0.05$.

RESULTS

Sociodemographic and Clinical Characteristics

There were no significant differences in age, gender, or AMT measures between groups at baseline. State and trait anxiety were significantly higher in patients with MDD than in healthy controls (**Table 1**).

TBS Induced Changes in Neural Plasticity

At baseline, there was no significant difference in the mean 1 mV MEP between the groups. Before the iTBS session, the mean amplitude of 1 mV MEP in the MDD group was 969.7 (SD 243) vs. 1055.7 (129) μV in the control group ($p = 0.317$). Before the cTBS session, the mean amplitude of 1 mV MEP in the MDD group was 1206.3 (385) vs. 975.4 (131) μV in the control group ($p = 0.074$).

The individual data illustrating the modulation of MEP amplitudes induced by iTBS and cTBS are displayed in **Figure 2**. A large interindividual variability was observed in the two

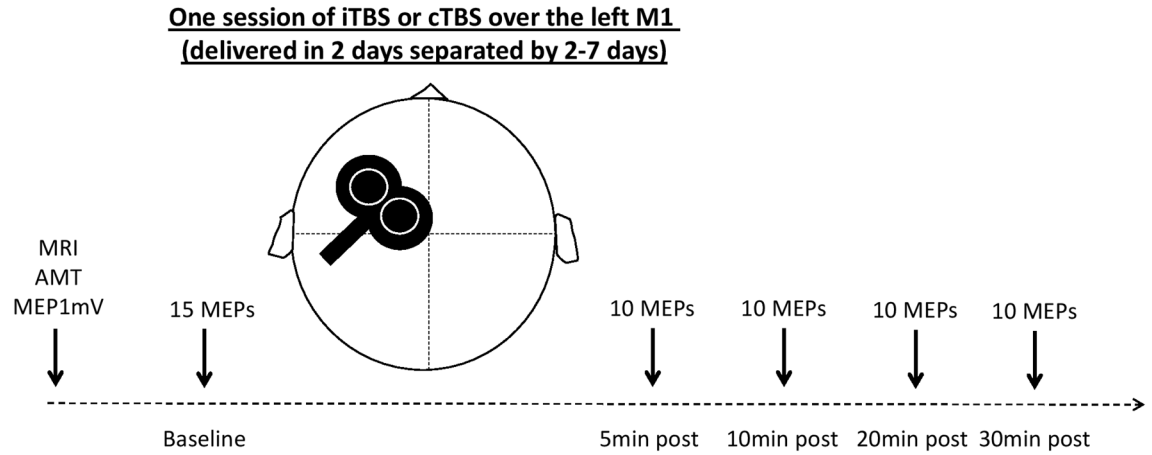

FIGURE 1 | Experimental design. MRI, magnetic resonance imaging (T1-weighted image acquired to be used with the neuronavigation system); AMT, activity motor threshold; 1 mV MEP, transcranial magnetic stimulation (TMS) intensity to obtain an MEP with a mean amplitude of 1 mV; MEP, motor evoked potential; iTBS, intermittent theta-burst stimulation; cTBS, continuous theta-burst stimulation.

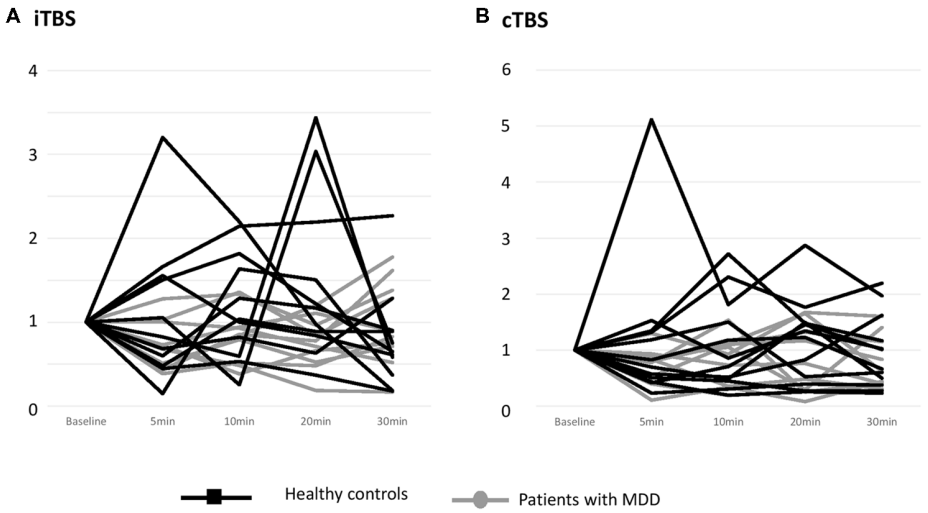

FIGURE 2 | Effects of one session of TBS on MEP amplitude at an individual level. **(A)** Effects of iTBS. **(B)** Effects of cTBS. Healthy controls are outlined with dark lines; patients with major depressive disorder (MDD) are outlined with gray lines. The results are given as the mean ± SEM.

groups. The mean effects of iTBS and cTBS on both groups are displayed in **Figure 3**.

iTBS-Induced Changes in Neural Plasticity

The RM-ANOVA revealed a significant group × time interaction when participants were exposed to iTBS ($F_{(4,21)} = 2.504$, $p = 0.049$).

The *post hoc* comparisons revealed that after iTBS, the difference between depressed subjects and healthy controls was significant at 20 min post iTBS ($p = 0.038$). The difference was not significant at the other time points (5 min: $p = 0.193$; 10 min: $p = 0.130$; 30 min: $p = 0.406$).

Measured by the peak, the MEP size was significantly elevated by iTBS in the control group ($p = 0.009$), whereas no modulation of MEP size was induced in patients with MDD ($p = 0.339$).

Three patients with MDD out of the 10 were classified as responders whereas six healthy controls out of 11 were responders. The difference did not reach significance $p = 0.39$ (**Figure 4**).

cTBS-Induced Changes in Neural Plasticity

The RM-ANOVA revealed no significant group × time interaction when participants were exposed to cTBS ($F_{(4,22)} = 0.986$, $p = 0.42$).

No significant effect of cTBS on MEP measured by the peak MEP size was observed in both groups.

Eight patients with MDD out of the 11 were classified as responders whereas seven healthy controls out of 11 were responders. The difference did not reach significance $p = 1.00$ (**Figure 4**).

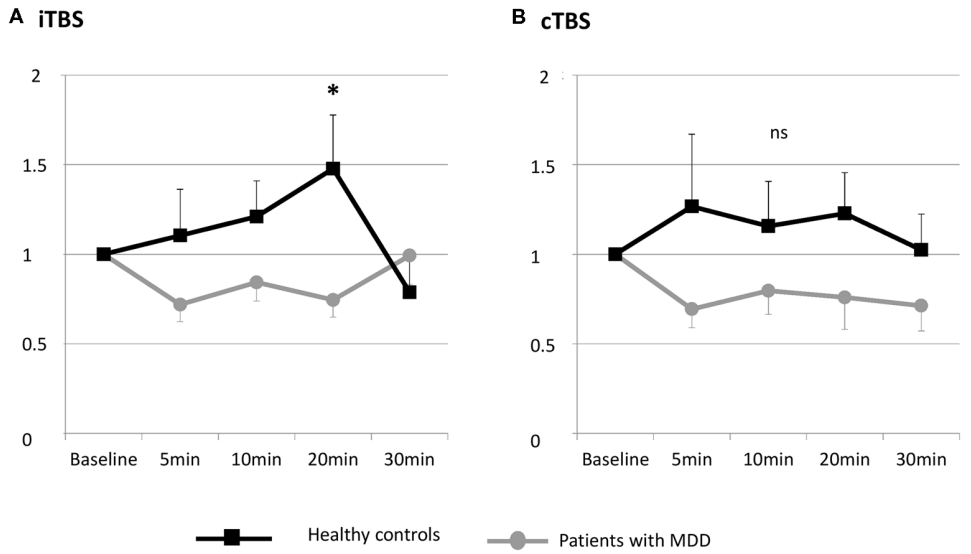

FIGURE 3 | Changes in MEP amplitude after one session of TBS in patients with MDD and healthy controls at the group level. **(A)** Effects of iTBS. **(B)** Effects of cTBS. ns, not significant.

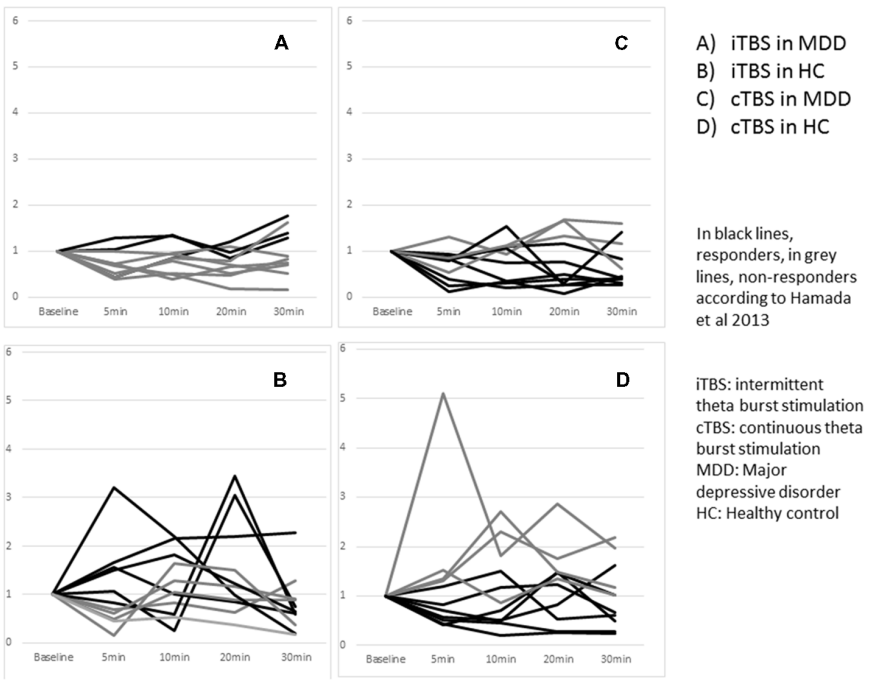

FIGURE 4 | Effects of one session of TBS on MEP amplitude according to responder and non responder status across groups at an individual level.

Safety

No serious adverse events were observed during the study. Three patients with MDD and four healthy controls reported mild headache during iTBS exposure. Two patients with MDD and one healthy control reported mild headache during cTBS exposure. This symptom disappeared after administration of a mild analgesic (paracetamol).

DISCUSSION

The aim of this study was to evaluate neural plasticity integrity in patients with MDD compared with that in healthy controls. Using TBS, we reported that iTBS-induced changes in neural plasticity were altered in patients with MDD. Whereas MEP size post iTBS was significantly increased in healthy controls,

no effects of iTBS on MEP size were observed in patients with MDD. No effects of cTBS were observed in either group. Importantly, in addition to those effects, we observed a large interindividual variability in the effects of both iTBS and cTBS on MEP amplitude regardless of group.

The effects of rTMS protocols have been proposed to relate to activity-dependent changes in synaptic neurotransmission, reflecting neural plasticity (Ziemann et al., 2008). Among the currently available rTMS protocols, the TBS protocol has been proposed to measure neural plasticity, especially at the synaptic level (Huang et al., 2005). Depending on the stimulation parameters, TBS is assumed to induce either an inhibition of corticospinal excitability (following cTBS) or an enhancement (following iTBS). These effects outlast the stimulation period for approximately 40 min in healthy subjects (Oberman et al., 2012). The transient suppression of corticospinal excitability following cTBS and its transient enhancement following iTBS appear to be mediated by cortical processes (Di Lazzaro et al., 2011) and are assumed to reflect indexes of long-term depression (LTD) and long-term potentiation (LTP)-like mechanisms, respectively (Huang et al., 2005; Huerta and Volpe, 2009). Moreover, cTBS has been shown to involve GABAergic neurotransmission, whereas iTBS involves the glutamatergic NMDA receptor pathway (Huang et al., 2007; Stagg et al., 2009). In light of these studies, our results suggest that the LTP-like mechanisms mediated by the glutamatergic NMDA receptor pathway are impaired in patients with MDD. No significant difference was observed between patients with MDD and healthy controls regarding LTD-like mechanisms.

Effect of iTBS on Cerebral Plasticity

The integrity of LTP-like mechanisms involving GABA and glutamatergic neurotransmission has already been investigated in patients with MDD using TMS. For instance, modulation of the duration of the interstimulus interval when applying paired-pulse TMS allows for the investigation of the inhibitory and facilitatory mechanisms mediated by GABAergic neurotransmission (short-interval intracortical inhibition–SICI, Ziemann et al., 1996) and glutamatergic neurotransmission (intracortical facilitation–ICF). Although discrepancies between studies investigating those phenomena exist, in a meta-analysis, Radhu et al. (2013) found that SICI was decreased in patients with MDD compared with that in controls. These results are in line with ours reporting alterations of neural plasticity in the motor cortex in patients with MDD. These observations are also consistent with animal studies reporting that the iTBS-induced LTP mechanisms could be modulated by the administration of GABA antagonists (Kotak et al., 2017).

Altered Cerebral Plasticity Following iTBS in Other Psychiatric Conditions

Our results in healthy controls are in line with the classically described effects of iTBS on MEP amplitude (Huang et al., 2005). Our results are also consistent with a previous study evaluating iTBS-induced neural plasticity in patients with psychiatric conditions. For instance, in a controlled study, Suppa et al. (2014) reported that healthy subjects displayed an increase in MEP amplitude after iTBS, whereas MEP amplitude remained unchanged in patients with Gilles de la Tourette syndrome. In the same study, the same group of authors also assessed the effect of iTBS on MEP amplitude in patients with obsessive compulsive disorder (OCD) and reported that iTBS induced an equal increase in MEP amplitude in both groups (Suppa et al., 2014). Finally, Oberman et al. (2012) reported that the iTBS-induced effects on MEP amplitude were significantly greater and longer lasting in patients with autism spectrum disorder than in healthy controls. Altogether, these results illustrate the usefulness of iTBS in revealing impaired neural plasticity in patients with psychiatric conditions, allowing us to distinguish patients with decreased iTBS-induced neural plasticity (MDD, Gilles de la Tourette syndrome), increased iTBS-induced neural plasticity (autism spectrum disorder) or similar iTBS-induced neural plasticity (OCD) compared to that in healthy controls.

Effect of cTBS on Cerebral Plasticity

We observed that cTBS did not modulate MEP amplitude in patients with MDD. These results were in line with several studies revealing no effects of cTBS on cerebral plasticity in patients with other psychiatric conditions. For instance, no effect of cTBS on MEP amplitude was reported in patients with schizophrenia (Hasan et al., 2015), in patients with obstructive sleep apnea (Opie et al., 2013), and in patients with Gilles de la Tourette syndrome (Suppa et al., 2014). A possible explanation is that cTBS may be less efficient at inducing cerebral plasticity in patients with psychiatric disease than iTBS.

In the current study, cTBS also had no effect on MEP amplitude in healthy controls. Although these results were unexpected, they are in line with several studies showing that the effects following different TBS paradigms are subject to high interindividual variability (McAllister et al., 2009; Todd et al., 2009; Goldsworthy et al., 2012; Hamada et al., 2013). For instance, in their study investigating the effect of cTBS in patients with schizophrenia, Hasan et al. (2015) did not report any significant changes on MEP amplitude following TBS in the control group. The current results were however not in line with findings from Oberman et al. (2012) showing longer cTBS response in patients with autism spectrum disorder than in controls. The lack of a significant effect of TBS in the current study suggests that high interindividual variability can mask a significant TBS effect at the group level. However, the size of our sample did not allow us to cluster participants into TBS responders and nonresponders to explore this question.

Strengths and Limitations

In the current study, only 10 MEPs were recorded to assess TBS-induced neuroplasticity. This could have hampered the reliability of the reported results and contribute to the observed high interindividual heterogeneity. Indeed a recent study indicated that 21 MEPs are required for reliable estimation of the MEP amplitude (Chang et al., 2016).

The lack of detailed neurocognitive assessment (allowing to detect a mild cognitive impairment which is a common

finding in MDD), the lack of a preliminary evaluation of the integrity of the cortico-spinal conductivity and the lack of a more accurate T2-MRI scan instead of only the T1-weight MRI (allowing to detect brain lesions in both white and gray matter for differential diagnosis) did not allow us to exclude that such comorbidities in our sample may have influenced current results.

Further studies should investigate the close relationship between depressed mood and cognitive dysfunction since this aspect has crucial implications in determining changes of cortical excitability to TMS (Guerra et al., 2015), that can induce neuroplastic phenomena at the level of M1 (Pennisi et al., 2015) and enhance the risk of clinical deterioration in depressed subjects, in depressed subjects, especially in subjects with vascular depression (VD; Pennisi et al., 2016).

Another limit is that we only assessed the TBS-induced plasticity on the dominant M1 and not on both sides. Indeed, given that several studies found an interhemispheric difference of motor threshold it would have been interesting to evaluate cortical excitability from both hemispheres, in order to obtain bilateral data to compare before and after cTBS/iTBS.

From a more cognitive perspective, it would have been of interest to assess neural plasticity induced in the dorsolateral PFC (DLPFC), a brain region critically involved in the pathophysiology of MDD (Concerto et al., 2015). In line with this, combining EEG and TMS, Noda et al. (2018) reported an impaired neuroplasticity in the DLPFC of patients with MDD compared to healthy subjects.

Lastly, an important limitation of the current study was the relatively small sample size of included patients, which might hide significant differences between healthy subjects and patients with MDD at the group level. Nevertheless, our sample size is within the range of other TBS studies in patients with neuropsychiatric conditions (Eggers et al., 2010; Huang et al., 2011; Hasan et al., 2015). A second limitation was in the single-blind procedure of the study and the lack of a sham TBS group. Indeed, in order to not leave patients without treatment for a too long period of time, we decided to perform only two measurements separated by 2–7 days: 1 day with iTBS, 1 day with cTBS. Added a sham arm or added an arm investigating DLPFC plasticity would have increase the time where patients did not received medication.

Despite these limitations, the main strength of our study is that the included patients with MDD were drug free. Indeed, medication and especially psychopharmacological drugs are known to highly influence the cortical excitability parameters assessed by TMS (for a review see Paulus et al., 2008); therefore, this bias did not influence the current results.

CONCLUSION AND PERSPECTIVES

In summary, iTBS-induced cerebral plasticity was altered in patients with MDD, whereas no effect of cTBS-induced cerebral plasticity was observed. These results suggested abnormal LTP-like plasticity mediated by glutamatergic neurotransmission in patients with MDD. These abnormalities should be considered an endophenotype biological marker of MDD. However, because of the small sample of the current study, results should be taken with caution and further studies are needed to explore this topic more thoroughly. Moreover, although MDD and the so called VD share some clinical similarities, VD may rely on distinct pathophysiological mechanisms (Concerto et al., 2013) that could be highlighted by distinct neuroplasticity alterations. In the perspective of a differential diagnosis, it would be of interest to replicate our experimental protocol in the sample of VD patients. Lastly, as TBS-induced neuroplasticity results in a large interindividual variability, other TMS paradigm such as quadripulse stimulation (QPS) that have showed less inter-subject variability in healthy controls (Nakamura et al., 2016) could be useful to evaluate alteration in patients with psychiatric condition.

DATA AVAILABILITY

The datasets for this manuscript are not publicly available because Data are available on reasonable request. Requests to access the datasets should be directed to philippe.vignaud@ch-le-vinatier.fr.

AUTHOR CONTRIBUTIONS

PV, EP and JB contributed to the conception and design of the study. PV and CD organized the database and acquired data. PV and JB performed the statistical analysis. PV wrote the first draft of the manuscript. All authors contributed to the manuscript revision, read and approved the submitted version.

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Real-Time fMRI Neurofeedback in Patients With Tobacco Use Disorder During Smoking Cessation: Functional Differences and Implications of the First Training Session in Regard to Future Abstinence or Relapse

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One of the most prominent symptoms in addiction disorders is the strong desire to consume a particular substance or to show a certain behavior (craving). The strong association between craving and the probability of relapse emphasizes the importance of craving in the therapeutic process. Former studies have demonstrated that neuromodulation using real-time fMRI (rtfMRI) neurofeedback (NF) can be used as a treatment modality in patients with tobacco use disorder. The aim of the present project was to determine whether it is possible to predict the outcome of NF training plus group psychotherapy at the beginning of the treatment. For that purpose, neuronal responses during the first rtfMRI NF session of patients who remained abstinent for at least 3 months were compared to those of patients with relapse. All patients were included in a certified smoke-free course and took part in three NF sessions. During the rtfMRI NF sessions tobacco-associated and neutral pictures were presented. Subjects were instructed to reduce their neuronal responses during the presentation of smoking cues in an individualized region of interest for craving [anterior cingulate cortex (ACC), insula or dorsolateral prefrontal cortex]. Patients were stratified to different groups [abstinence (N = 10) vs. relapse (N = 12)] according to their individual smoking status 3 months after the rtfMRI NF training. A direct comparison of BOLD responses during the first NF-session of patients who had remained abstinent over 3 months after the NF training and patients who had relapsed after 3 months showed that patients of the relapse group demonstrated enhanced BOLD responses, especially in the ACC, the supplementary motor area as well as dorsolateral prefrontal areas, compared to abstinent patients. These results suggest that there is a probability of estimating a successful withdrawal in patients with tobacco use disorder by analyzing the first rtfMRI

NF session: a pronounced reduction of frontal responses during NF training in patients might be the functional correlate of better therapeutic success. The results of the first NF sessions could be useful as predictor whether a patient will be able to achieve success after the behavioral group therapy and NF training in quitting smoking or not.

Keywords: real-time fMRI, neurofeedback, craving, tobacco use disorder, therapy success

INTRODUCTION

Smoking tobacco can lead to diverse symptoms and illnesses including cancer, respiratory and cardiovascular diseases and is one of the most significant causes of death in Europe (Ezzati and Lopez, 2003; Sasco et al., 2004; Thun et al., 2013). Worldwide more than 5 million people per year die as a result of tobacco use[1] (World Health Organization [WHO], 2018). In addition, tobacco users who die prematurely deprive their families of income, raise the cost of health care and slow down the economic development[1] (World Health Organization [WHO], 2018).

Important aspects of tobacco use disorder are a reduced control of tobacco intake, the inability to stop or reduce substance use, tolerance development, withdrawal symptoms and a strong desire to consume the particular substance (craving behavior). Even though more than 70% of smokers want to quit, only 5% are successful in doing so (Hatsukami et al., 2008). In addition, the relapse rate in patients with tobacco addiction is relatively high. According to different meta-analyses there is not enough evidence that behavioral therapies alone can prevent long-term relapse (Agboola et al., 2010; Hajek et al., 2013). Even with combined medication and cognitive behavioral therapies, the most common outcome 1 year after an attempt to quit is a relapse (Piasecki, 2006). A review about the effectiveness of different medication therapies combined with behavioral support in the United Kingdom showed that abstinence rates were comparable after 3 months with mean values of pooled point prevalence between 35 and 55% (Coleman et al., 2010). The common German cognitive behavioral therapy program called "Rauchfrei Programm" (translation: smoke-free program) showed an abstinence rate of 40% after 6 months and 31% after 1 year (Gradl et al., 2009; Wenig et al., 2013). Therefore, the need for new and improved treatments helping smokers to stop smoking seems obvious. Craving can be elicited, e.g., by the Presentation of Nicotine-Relevant Information (Saladin et al., 2012). The regional areas of brain activation associated with craving in nicotine-dependent smokers are scientifically well studied. Functional neuroimaging studies have examined increased craving-related responses, e.g., in the anterior cingulate cortex (ACC) (McClernon et al., 2005; Wilson et al., 2005; Brody et al., 2007; Goudriaan et al., 2010; Hartwell et al., 2011), the medial prefrontal cortex (mPFC) (Hartwell et al., 2011) and the precuneus/cuneus (Smolka et al., 2006; Hartwell et al., 2011) during the presentation of substance-related information, while these areas are linked to attentional processes (Augustus Diggs et al., 2013) and motivation (Augustus Diggs et al., 2013). Also, the insula has shown to play a major role in addictive behavior (Naqvi and Bechara, 2010). The role of the insula is not yet clear: it may be related to conscious interoception, emotional experience and decision-making. Naqvi and colleagues presented evidence that the insula represents the interoceptive effects of drug taking, making this information available to conscious awareness, memory and executive functions (Naqvi and Bechara, 2010; Naqvi et al., 2014). In addition, the orbitofrontal cortex (OFC) (Hartwell et al., 2011) which is thought to be related to cognitive reappraisal (Goldstein and Volkow, 2002) and regions involved in decision making and goal-directed behavior such as the dorsolateral prefrontal cortex (DLPFC) (Goldstein and Volkow, 2002) seem to be important.

Results of the meta-analysis focusing on neurobiological aspects of smoking cue reactivity in smokers indicate that smoking cues reliably evoke larger neuronal responses than neutral cues in the extended visual system, the precuneus, the posterior cingulate gyrus, the ACC, the dorsal prefrontal cortex (dPFC) and the mPFC, the insula, and the dorsal striatum (Engelmann et al., 2012). The areas that were found to be responsive to smoking cues agree in most parts with theories of the neurobiology of cue reactivity (Engelmann et al., 2012). Surprisingly, there was a reliable cue reactivity effect in the precuneus which is not typically considered a brain region important to addiction (Engelmann et al., 2012). Furthermore, the meta-analysis did not show any significant effect in the nucleus accumbens (Engelmann et al., 2012). Altogether, the authors of the meta-analysis emphasize that the extended visual system should receive more attention in future studies of smoking cue reactivity (Engelmann et al., 2012).

A good overview of cue-related activities and their functions has been presented by Miller (2013). They report that neuronal responses which are related to cues have been shown in brain regions that are associated with attention, reward and goal-directed behavior (Miller, 2013). Responses in the secondary and tertiary visual cortex, the precuneus as well as the gyrus fusiformis have been observed (Miller, 2013). Activations in these regions show an increased allocation of attention on the visual smoking cues (Miller, 2013). Activations of limbic and paralimbic structures including the hippocampus, the thalamus, the amygdala, the insula and the cingulate cortex reflect the contribution of emotional processes (Miller, 2013). Moreover, increased cue-related responses have been shown in the posterior cingulate cortex and particularly in the ACC (Miller, 2013). Activations in the ACC have been interpreted as reflecting, e.g., conflict monitoring and reward learning, as well as the emotional relevance of stimuli (Miller, 2013). Motivational processes of

[1] http://www.who.int/mediacentre/factsheets/fs339/en/

smoking-related cues have been linked to BOLD responses in the ventral tegmentum and the ventral striatum (Miller, 2013). In addition, BOLD responses in the PFC including the orbitofrontal cortex (OFC), the inferior frontal gyrus (IFG), the medial frontal gyrus (MFG) and the superior frontal gyrus (SFG) have been related to emotion and reward-related processes (OFC and IFG) and the mobilization of cognitive control and executive processes (MFG and SFG) (Miller, 2013).

Some researchers have investigated a possible difference between 'craving' and 'resisting craving' regarding the underlying neuronal brain regions. For example the ACC could be especially detected for 'craving' whereas 'resisting craving' was more assigned to the dorsomedial PFC (Hanlon et al., 2013). The cigarette cue resist condition elicited enhanced brain responses in the left dorsal ACC, the posterior cingulate cortex (PCC), and the precuneus compared with the cigarette cue crave condition (Brody et al., 2007). In addition, a study which focused on craving and resisting craving found a considerable overlap between the areas activated during craving and attempts to resist craving, supporting the idea that these two aspects do not have to be investigated separately because craving is almost always associated with some degree of resisting the urge to smoke, and vice versa (Hartwell et al., 2011).

Functional magnetic resonance imaging also enables the detection of functional connectivity which has been shown to be altered in several neurodegenerative and neuropsychiatric diseases, including addiction disorders. Concerning nicotine addiction, smokers show a loss of functional connectivity in brain areas of the executive control network and an increase of connectivity in brain areas of the default mode network modulated by the insula, or the salience network which contains the insula (Fedota and Stein, 2015; Vergara et al., 2017). Furthermore, the thalamostriatal connectivity seems to be increased in smokers. Recent prediction studies showed that the risk of relapse increases when addicted patients have decreased functional connectivity in corticolimbic and corticostriatal networks (Moeller and Paulus, 2018). In tobacco dependent people insula-related functional connectivity seems to be positively correlated with the success in smoking cessation. In this context connectivity between the insula and primary sensorimotor cortical areas or control-related brain regions, such as the dACC and the DLPFC, seems to play an important role in the potential to stop smoking and to stay abstinent (Janes et al., 2010; Addicott et al., 2015; Zelle et al., 2017).

Several studies focused on the relationship between craving and relapse rates. A systematic review showed mixed results with respect to the relationship between craving and relapse rate (Wray et al., 2013). By contrast, a recent study demonstrated that greater neural activation during pre-treatment exposure to smoking cues in the right ventral striatum, the left amygdala, and the anterior cingulate was associated with longer periods of abstinence following cessation (Owens et al., 2017). The authors concluded that these results suggest that pre-treatment reactivity to smoking cues in areas associated with cue reactivity may be associated with successfully maintaining abstinence during treatment (Owens et al., 2017). Another study demonstrated that subjects that were not successful in their attempt to quit smoking revealed heightened fMRI reactivity to smoking-related images in brain regions implicated in emotion, interoceptive awareness, and motor planning and execution (Janes et al., 2010). Additionally, these subjects had decreased functional connectivity between a network comprising the insula and brain regions involved in cognitive control (Janes et al., 2010). Overall there is some evidence for a relevant association between craving and/or craving-related neurobiological responses and the risk of relapse. This emphasizes its importance for future studies and within the therapeutic process. Hence, reducing craving and the physiological response to smoking cues could well have positive effects on smoking cessation outcomes.

Neurofeedback (NF) delivered via real-time functional magnetic resonance imaging (rtfMRI) enables the immediate visualization of brain activations or functional connectivity between brain areas, and offers the possibility to modulate voluntarily neuronal activity in circumscribed brain areas. It can also be seen as a training method whereby a person is confronted with a mental or emotional task while simultaneously receiving information about changes in neural activity in brain areas. This information can be used for self-regulation, control and modulation of the neural activity in a target region which is important for the ongoing task. The modulation of neuronal responses is expected to lead to changing behavior (Stoeckel et al., 2014). It is assumed that predominantly implicit learning processes, including operant conditioning, modulate distinct behavioral patterns (Caria et al., 2007; deCharms, 2007).

While EEG-based NF is restricted to the modulation of neuronal activity in cortical areas as well as relatively broad brain regions, fMRI-based NF can be used to modulate the activity in subcortical areas and small cortical brain regions, as well as functional connectivity between areas (Koush et al., 2017). Several studies refer to brain regions which are related to emotional and/or cognitive processes (Posse et al., 2003; Caria et al., 2007; Johnston et al., 2010; Dyck et al., 2011; Hamilton et al., 2011; Lee et al., 2011; Zotev et al., 2011). Other studies demonstrate the modulation leading to specific behavioral effects (Rota et al., 2009; Caria et al., 2010).

There is already some evidence about positive effects of EEG, respectively, fMRI NF training on patients with attention-deficit/hyperactivity disorder (ADHD) (Arnold et al., 2013; Zilverstand et al., 2017), as well as depression (Choi et al., 2011; Linden et al., 2012). RtfMRI studies in schizophrenic patients have shown that patients were able to influence their insular activity (Ruiz et al., 2013a,b). Functional variations were accompanied by an improvement to recognize negative facial expressions (Ruiz et al., 2013a). In depressive patients training of brain regions which are associated with emotion regulation was related to an improvement of depressive symptomatology (Linden et al., 2012).

There are only a few studies focusing on NF processes in persons with addiction-related symptoms (Hartwell et al., 2013). However, there are a lot of indices that neuromodulation can be a unique opportunity to directly apply neuroscientific knowledge to the treatment of addiction (Luigjes et al., 2013). Kirsch et al. (2016) examined the modulation of reward-related striatal brain responses in non-addicted heavy social drinkers:

subjects were instructed to downregulate the responses in their ventral striatum. RtfMRI led to a significant downregulation of striatal activations in the real group, whereby the sham conditions did not reveal comparable effects (Kirsch et al., 2016). A study of our own working group aimed at modulating craving-associated neuronal responses in patients with alcohol addiction using individualized feedback (Karch et al., 2015). The results showed a significant reduction of neuronal responses in patients at the end of the training compared to the beginning, especially in the ACC, the insula, the inferior temporal gyrus and the medial frontal gyrus. In addition, patients reported slightly reduced craving after the NF training, compared to before. The results suggest that it is feasible for patients with alcohol dependency to reduce their neuronal activity using rtfMRI NF (Karch et al., 2015).

Regarding nicotine dependence, Li et al. (2013) demonstrated that smokers with rtfMRI NF were able to reduce voluntarily neuronal responses in the ACC during the presentation of smoking-relevant information. These modulations were associated with a temporarily decreased craving for nicotine (Li et al., 2013). Hartwell et al. (2016) showed that individualized real-time fMRI NF can be an appropriate method to attenuate craving in nicotine-dependent smokers (Hartwell et al., 2016). The efficacy of multiple NF training sessions as well as the need to consider the nicotine-dependence severity was further supported by the fact that individuals with lower nicotine-dependence severity were more successful in reducing the activation in the ACC over time (Canterberry et al., 2013). Recent studies mention that the additional inclusion of functional connectivity information in fMRI-based NF could improve its efficacy in the reduction of cigarette craving (Kim et al., 2015). Overall, rtfMRI NF has been increasingly discussed as a potential therapeutic method (Augustus Diggs et al., 2013; Bruhl, 2015; Sitaram et al., 2017; Sokunbi, 2017).

Especially because of the comparatively great effort for patients, the high technical requirements and the high costs of rtfMRI NF training, it seems to be relevant to find indicators for a therapeutic indication. Former studies regarding the prediction of smoking relapse suggest that smokers high in anger trait may have greater mood difficulties during abstinence and may be more vulnerable to early relapse than smokers with low anger trait (al'Absi et al., 2007). Another study found differences in cue reactivity of smokers before participating in a cessation clinical trial predicting outcomes with 79% accuracy in combination with results of an Emotional Stroop task (Janes et al., 2010). In a resting state fMRI study, a logistic regression based on functional connectivity predicted relapse of smokers before medication therapy with 80.7% accuracy (Shen et al., 2017). Classifying abstinent smokers according to their individual relapse risk profile may be helpful in order to find the best therapeutic strategy, for example to switch to medication therapy or to modulate the existing strategy, or to even intensify the neurofeedback training. In this context, the use of neuroimaging data for prediction models seems to be promising for addiction disorders in general as fMRI data show altered brain reactivity to drug-related and non-drug-related cues and certain changes in functional connectivity and gray and white matter volumes (Moeller and Paulus, 2018). Concerning fMRI neurofeedback, there is no data as yet about the prediction of abstinent smokers regarding the risk of relapse.

The aim of the present project was to determine whether it is possible to predict functional differences of patients who remained abstinent and patients who relapsed after receiving rtfMRI NF training plus group psychotherapy. We focused especially on the question whether there are any brain activity differences between groups which appear already at the beginning of NF training. For that purpose, patients were stratified in two separate groups according to their individual treatment success 3 months after the NF training sessions. Neuronal responses during the first rtfMRI NF session of patients who then remained abstinent for at least 3 months were compared to those of patients with relapse. To our knowledge, none of the previous studies has combined NF training with behavioral group therapeutic strategies in patients with tobacco use disorder.

MATERIALS AND METHODS

Subjects

The study comprised the investigation of 54 patients with tobacco use disorder (♀ = 22, ♂ = 32). All patients were recruited through an advertisement in a regional daily newspaper. Key inclusion criteria were age between 18 and 65 years, no prior head injury or lifetime diagnosis of a neurological and/or psychiatric disorder, and the ICD-10 diagnosis of nicotine dependence (*F* 17.2). The exclusion criteria were, e.g., claustrophobia, pregnancy, any implanted metal or a cardiac pacemaker. All participants reported having a solid mental and physical constitution at the time of testing. The study received approval from the local research ethics committee of the Medical Faculty of LMU Munich and is in accordance with the Declaration of Helsinki and subsequent revisions. The participation in the rtfMRI sessions was compensated with 50€ per session. The participation in the group therapeutic program was free of charge.

After proving their study qualification by a short standardized questionnaire in a telephone interview, patients participated in a specialized therapeutic program for nicotine-dependence ("Das Rauchfrei Programm," IFT – Gesundheitsförderung Gesellschaft mbH, München, 2012) at the Department of Psychiatry and Psychotherapy, LMU Munich. RtfMRI NF training was provided three times as an add-on to the group therapy sessions. Standardized questionnaires were used in order to assess sociodemographic data, information about smoking and craving as well as psychopathological information.

18 patients had to be excluded from the study because of missing measurement appointments or technical problems (four patients), permanent makeup (one patient), dropping out of the group therapeutic program (six patients), structural anatomic brain abnormalities (two patients), medication because of clinical diagnosed depression (one patient) and deviant social behavior (one patient). Three more patients were not available in the follow-up telephone interview; for this reason, it was not possible to assign these patients to one of the groups (abstinence vs. relapse).

Taking into account the exclusions from the study, the results of 36 nicotine-dependent smokers (♀ = 11, ♂ = 25) aged between 19 and 65 years (M = 43.83, SD = 12.37) with a number of 3 to 51 pack-years (M = 26.29, SD = 14.43) were analyzed.

All nicotine-dependent smokers were randomized in a real NF training group (N = 22) and a sham NF group (N = 14). During the real condition, neuronal responses in a ROI that is located in an individual, craving-related area within the insula, the ACC or the DLPFC were presented parallel to the tobacco-associated pictures. During the sham condition, the participants received feedback about neuronal responses in brain areas that are not related to craving (e.g., parietal cortex).

In order to determine the significance of the first NF session for the therapeutic outcome after the complete NF training, we focus in this manuscript on the results of the real group. The results of the sham group will be presented elsewhere in detail (Karch et al., unpublished).

For that purpose in the present study the results of smokers of the real group who remained abstinent 3 months after the rtfMRI NF training and group therapy (abstinent group; N = 10) with those of smokers of the real group who relapsed within the first 3 months after the interventions (relapse group; N = 12) were compared.

Psychometric Questionnaires

Different psychometric tests were used as a screening for neurologic and/or psychiatric diseases. The symptomatology of the participants was determined using the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al., 1991) and Questionnaire on Smoking Urges – German (QSU-G) (Tiffany and Drobes, 1991). Verbal intelligence was assessed using the verbal intelligence test (WST) (Schmidt and Metzler, 1992). In addition, we used several questionnaires in order to determine affective symptoms including the Barratt Impulsiveness Scale (BIS-11) (Patton et al., 1995), the Aggression Questionnaire (AQ) (Buss and Perry, 1992), Beck Depressions Inventar (BDI) (Beck and Steer, 1987), State-Trait-Anger Expression Inventory (STAXI) (Schwenkmezger et al., 1992), State-Trait-Anxiety Inventory (STAI) (Laux et al., 1981), NEO-Five Factor Inventory (NEO-FFI) (Costa and McCrae, 1992).

Paradigm

FMRI measurements took place at the Department of Radiology, Ludwig Maximilian University of Munich. The three NF-training sessions were conducted after day 4, day 5, and day 6 or 7 of the smoking-free program (see **Figure 1**). Before and after each fMRI session participants' degree of craving was examined with the German version of the "Questionnaire on Smoking Urges" (QSU-G). CO-levels were measured using the UBLOW CO breath tester (Neomed Medizintechnik GmbH).

The visual stimulation utilized consisted of 20 neutral and 20 tobacco-related pictures. The neutral pictures originated from the International Affective Picture System (IAPS[2]) or were taken in the course of the study. Tobacco-relevant pictures contained specific triggers for tobacco consumption, e.g., persons smoking, cigarettes or cigarette packets. The tobacco-related pictures were taken from databases or in the course of the study.

Three paradigms were used during the fMRI measurements: (1) cue exposure, (2) resting state and (3) rtfMRI NF paradigm.

(1) *Cue exposure*: The cue exposure paradigm was used as functional localiser. Neutral and nicotine-related pictures were presented block-wise to the participants using the software program PsychoPy (v1.78.00, Peirce et al., 2019). A single run consisted of 9 blocks of 40 s each; during 5 blocks neutral pictures were presented, during 4 blocks nicotine-related pictures were presented. Each picture was shown for 4 s. Patients were instructed to look at the pictures. Neuronal response contrasts during tobacco-related cues and neutral pictures were then identified and compared using the multiplanar activation maps calculated in the TBV online analysis: the activation cluster with the most extensive BOLD response to addiction-related information in the ACC, DLPFC, and insula was defined as region of interest for each person and day individually (threshold t = 3). The ACC, the DLPFC and the insula were identified on the first acquired EPI image of the online analysis using conventional neuroanatomical MRI landmarks (Ulmer, 2010) and the multiplanar reconstructions offered by TBV, and later validated in the offline analysis after transfer to Talairach space.

(2) *Resting state:* Resting state-sequences were acquired on each day before and after the NF-task: the results of these sequences will be presented in elsewhere (Keeser et al., unpublished).

(3) *rtfMRI NF-paradigm:* The NF-training consisted of three sessions of NF training with three NF runs each. Apart from the NF-task during addiction-related cues, the paradigm of a single NF run was identical to the paradigm of a cue exposure run. During the presentation of tobacco-associated stimuli, participants were instructed to decrease their individual neuronal responses in the target ROI. ROI-based BOLD responses were calculated and visualized using the Turbo-BrainVoyager[3]. The BOLD responses in the target ROI were visualized using a 'graphical thermometer,' which based on the top one-third of voxels with the highest t-values for BOLD responses for the comparison of addiction-related and neutral stimuli. During the neutral condition, participants were requested to look at the pictures without any further instruction.

Between NF runs, participants of both groups were asked about their perceived success during the rtfMRI training run and received feedback from a staff member. All participants were encouraged to apply various strategies to identify the best individual method. The participants were not instructed to use a specific strategy for modulation. However, it was recommended that they could try methods that have demonstrated to be successful coping with craving in the past.

Group Therapy

All patients took part in a certified and manualized "smoke-free program" (Kroeger and Gradl, 2007) over 6 weeks, a program based on cognitive-behavioral and motivational concepts. It includes an induction session, 6 group sessions at

[2]http://csea.phhp.ufl.edu

[3]http://www.brainvoyager.com/TurboBrainVoyager.html

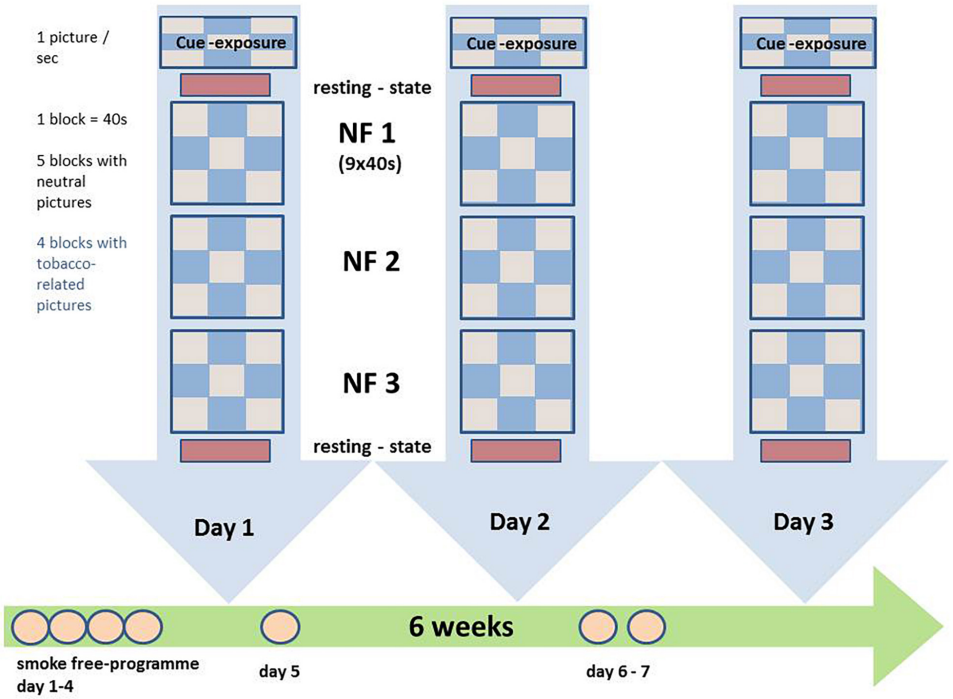

FIGURE 1 | Experimental procedures: the patients participated in three rtfMRI NF sessions within 5 weeks; during the NF training presentation of neutral and tobacco-related pictures in blocks of 40 s with 10 pictures of the respective category; participants were instructed to reduce brain activity during the presentation of tobacco-associated information; during the presentation of neutral information, participants were instructed to simply gaze at the pictures. Before and after each NF training session, resting-state activity was acquired. NF, neurofeedback.

90 min and, 2 individual telephone counseling appointments. The treatment can be divided in 3 phases: creation of motivation, preparation of smoking cessation and stabilization. The program focuses on the positive benefits of a nicotine-free life and uses different methods for behavioral change, e.g., psychoeducation, motivational communication, prevention, understanding, and treatment of relapse, etc. At the induction session, patients get an idea of a smoking-free life and information about smoking. The topics of the six group session is "the ambivalence of smoking," "errors in logic and alternatives," "preparations of smoking cessation," "experiences with the smoking stop," "identity as a smoking-free person," and "planning the future"[4].

MRI Data Acquisition and fMRI Data Analysis

A 3 Tesla Philips MR System Ingenia scanner with echo planar capability (Release 4.1 Level 3 2013-04-05, Philips Medical Systems Nederland B.V.) and a 32-channel phased array head coil was used for imaging. Subjects had to wear ear plugs and headphones for noise protection. We also used cushions in the coil to minimize head movement. A T1-weighted high-resolution 3D data set was acquired for each subject for anatomical referencing. Functional MR data were acquired using an EPI sequence in the identical position as the anatomical images [Field of View: 230 mm × 230 mm × 132 mm; spatial resolution: 3 mm × 3 mm × 4 mm; slice thickness: 4 mm; gap: 0.15 mm; repetition time: 2000 ms; echo time (TE): 35 ms; 25 axial slices].

The results of the resting state sequence will be presented elsewhere (Keeser et al., unpublished).

rtfMRI Pre- and Post Data Processing

We used the TurboBrainVoyager (Version 3.0, Brain Innovation, Maastricht, 2011) for the initial processing and real-time analysis as well as the feedback for the participants. For further analysis, raw-data in a DICOM-format were converted into a NIfTI-format using MRIConvert (Version 2.0.7 build 369, University of Oregon, Lewis Center for Neuroimaging, 2013). All subsequent data-analyses of the fMRI sequence were carried out with the BrainVoyager software package (Brain Innovation, Maastricht, Netherlands). In order to reduce relaxation time effects the first 5 images were excluded from any further analysis. The preprocessing of the fMRI data included high-pass filtering (cut-off: three cycles in a time course) to remove low-frequency signal drifts inherent in echo planar imaging. Additionally, a slice scan time correction (cubic), spatial smoothing (Gaussian filter with FWHM 8.0 mm), and a 3D motion correction (trilinear interpolation) were applied. Functional images were transferred to a standard Talairach brain. Significant BOLD activity was determined by a cross-correlation of the pixel intensity of MR images with an expected hemodynamic response function. Voxelwise *t*-tests

[4]https://www.rauchfrei-programm.de/images/Rauchfrei_Jahresbericht_2018.pdf

were used to identify those brain areas where the signal change differed significantly between tobacco-related responses and neutral stimuli. We used the Bonferroni correction at a threshold of $p < 0.05$ to counteract the problem of multiple testing. For each participant the conditions tobacco-relevant pictures and neutral were calculated as regressors.

Statistical Analysis

Statistical analysis of the questionnaire ratings of patients of abstinent versus relapse group was calculated with SPSS version 23 with a level of significance $p < 0.05$. Because of the small sample size, we first calculated the non-parametric Mann–Whitney-*U* test for independent samples or the Wilcoxon test for dependent samples. In a second step, the two-tailed *t*-test for independent or dependent samples was calculated. If the results of both tests did not differ, the *t*-test were mentioned instead of the non-parametic test. A general linear model with a repeated measure design was calculated in order to compare variations before and after the NF session.

RESULTS

Relapse Rates

Ten patients of the real group remained abstinent during the first 3 months after the therapeutic program, 12 patients relapsed. The abstinence rate was 45.5%. Regarding the sham group, 9 patients remained abstinent, 5 patients relapsed. The relapse rate did not differ significantly between groups ($p = 0.270$; Chi-Quadrat test).

Comparison of Psychometric Data Between Abstinent and Relapse Group on the Day of the First rtfMRI NF Session

The comparison of patients of the abstinent group compared to the relapse group did not show any significant differences regarding verbal intelligence, CO score and personality on day 1. In addition, there were hardly any significant differences regarding psychopathology. A significant difference between groups was only demonstrated in the Anger-In subscale of STAXI ($p = 0.001$). Additionally, differences between groups regarding pack-years or consumption of cigarettes per day did not differ significantly between abstinent and relapse (see **Table 1**).

Changes of Craving: Influence of Groups (Abstinent; Relapse)

The comparison of QSU-overall score revealed no significant difference between pre–post measurements [measurement before/after rtfMRI NF training session; $F(1,20) = 0.063$; $p = 0.805$]. In addition, the interaction

TABLE 1 | Comparison of the psychometric data of abstinent group vs. relapse group.

Questionnaire	Abstinent		Relapse		*p*-value
	M	*SD*	*M*	*SD*	
Pack-years	30.20	12.60	19.96	14.61	0.097
Consumption of cigarettes per day	22.00	5.87	17.67	6.21	0.111
C0 score	1.60	1.075	4.67	6.733	0.171
WST	106.30	7.45	112.08	15.22	0.287
Neo-FFI-Neuroticism	18.50	5.87	17.55	6.74	0.734
Neo-FFI-Extraversion	23.60	6.77	27.73	5.18	0.131
Neo-FFI-Openness to experiences	25.10	7.28	27.45	5.56	0.412
Neo-FFI-Compatibility	26.90	5.43	30.36	4.06	0.112
Neo-FFI-Conscentiousness	31.86	4.09	33.71	4.52	0.803
Fagerström	4.70	2.91	5.25	1.49	0.122
BDI	6.90	6.85	4.92	6.96	0.510
QSU-overall	61.70	24.08	71.75	14.37	0.239
STAI-State	36.39	10.30	39.00	6.99	0.497
STAI-Trait	38.11	10.41	35.00	10.55	0.518
STAXI-State	11.44	2.70	11.25	1.77	0.844
STAXI-Trait-Anger	19.22	3.23	18.17	7.31	0.692
STAXI-Anger-Control	23.89	2.93	21.67	5.98	0.319
STAXI-Anger-Out	13.44	2.96	12.92	4.14	0.749
STAXI-Anger-In	19.44	4.28	13.17	3.01	0.001
BIS-11 attention-to-details	25.44	3.59	23.00	4.44	0.272
BIS-11 motoric-impulsiveness	23.89	4.15	22.17	4.15	0.382
BIS-11 coping	23.11	2.68	22.50	4.30	0.659
AQ	65.80	17.63	69.45	11.32	0.622

M, mean, SD, standard deviation.

effect was not significant [pre–post treatment * group: $F(1,20) = 0.196$; $p = 0.662$]. The between groups (abstinent vs. relapse) difference [$F(1,20) = 0.893$; $p = 0.356$] was not significant.

The comparison of QSU-Factor 1 (strong desire and intention to smoke) did not show any significant differences regarding the pre–post measurements [$F(1,20) = 0.083$; $p = 0.777$], the interaction effect [pre–post treatment * group: $F(1,20) = 0.018$; $p = 0.894$] and the between groups difference [$F(1,20) = 0.723$; $p = 0.405$].

The comparison of QSU-Factor 2 (anticipation of relief from negative effect with an urge desire to smoke) revealed non-significant differences between the pre–post measurements [$F(1,20) = 0.063$; $p = 0.805$]. In addition, the interaction effect [pre–post treatment * group: $F(1,20) = 0.133$; $p = 0.720$] and the between groups effect [$F(1,20) = 0.280$; $p = 0.603$] were not significant.

Outcome-Based Comparison of Neuronal Responses During the Cue Exposure Task

During the cue exposure task of the 1st day, smokers that remained abstinent (see **Figure 2** and **Table 2**) and smokers that relapsed within the 3 months intervals after the NF training (see **Figure 2** and **Table 3**) demonstrated tobacco cue-related responses (tobacco-related pictures minus neutral pictures) especially in brain regions that are associated with the processing of visual information (e.g., visual association cortex).

The comparison of neuronal responses of smokers who relapsed and smokers who remained abstinent revealed only small differences within the fusiform gyrus (see **Figure 2** and **Table 4**).

Comparison of the Target ROIs for NF Training Between Groups

The following brain regions were used as target ROIs for the NF training: *abstinent group*: DLPFC left: 2 patients; DLPFC right: 1 patient; insula left: 6 patients; insula right: 1 patient; *relapse group*: DLPFC left: 2 patients; DLPFC right: 2 patients, insula left: 2 patients, insula right: 3 patients; ACC left: 1 patient; ACC right: 2 patients. Overall, we did not find any clear association between treatment success and brain region.

Functional Variations During Neurofeedback

Neuronal Responses of the Abstinent Group During the First NF Session

The comparison of BOLD responses during the presentation of smoking-related cues and neutral pictures during the first NF session in smokers who remained abstinent 3 months after the NF training demonstrated increased responses while smoking cues were presented, especially in the superior/medial frontal gyrus, the ACC, the inferior parietal lobule, the culmen, the fusiform gyrus, the superior/inferior parietal lobule, and the insular cortex. Neutral pictures led to increased BOLD responses in the superior/middle frontal gyrus, the ACC and the cerebellum (see **Figure 3** and **Table 5**).

Neuronal Responses of the Relapse Group During the First NF Session

In the relapse group, NF training during the presentation of tobacco-related cues led to increased responses especially in the ACC, the superior/medial/middle frontal gyrus, the insula, the thalamus, the precentral gyrus, the fusiform gyrus, the inferior parietal lobule/supramarginal gyrus, the inferior/middle temporal gyrus, the cuneus/precuneus, the culmen and the fusiform gyrus compared to the presentation of neutral stimuli (see **Figure 3** and **Table 6**).

Outcome-Based Comparison of Neuronal Responses During the First NF Session

The comparison of neuronal responses (tobacco-related pictures minus neutral pictures) during the first rtfMRI session of smokers who relapsed and smokers who remained abstinent showed increased responses in the relapse group, especially in frontal brain regions including the medial/middle/superior frontal gyrus, the ACC, the caudate nucleus and the superior temporal gyrus. By contrast, the responses in the inferior occipital gyrus and the fusiform gyrus were decreased in the relapse group (see **Figure 3** and **Table 7**).

DISCUSSION

The aim of the project was to assess neurobiological response differences between tobacco-dependent patients who benefitted from a combined individualized rtfMRI NF training and group therapeutic program and tobacco-dependent patients who relapsed within the first 3 months after these therapeutic interventions. We focused especially on functional differences between patients of both groups during the first NF training after a general stop of smoking in order to detect early functional features which may be helpful for a fast therapeutic decision-making regarding the application of NF training as add-on-therapy.

For the NF training an individualized target region within the DLPFC, the ACC or the insula was determined for each participant during a localiser run while craving-related tobacco-cues were presented. The selection of the brain region was based on the information from several prior studies showing that these areas are of special importance for cue-elicited craving (McClernon et al., 2005; Wilson et al., 2005; Brody et al., 2007; Goudriaan et al., 2010; Naqvi and Bechara, 2010; Hartwell et al., 2011; Naqvi et al., 2014). The functional localizer for the ROI selection was defined separately for each training session. Reason for this strategy was the consideration that the relevance of functional responses in each brain region can alter during the therapeutic process. This could probably lead to variations in the personal significance of brain regions between sessions. All participants were asked to downregulate craving-related BOLD responses using NF training.

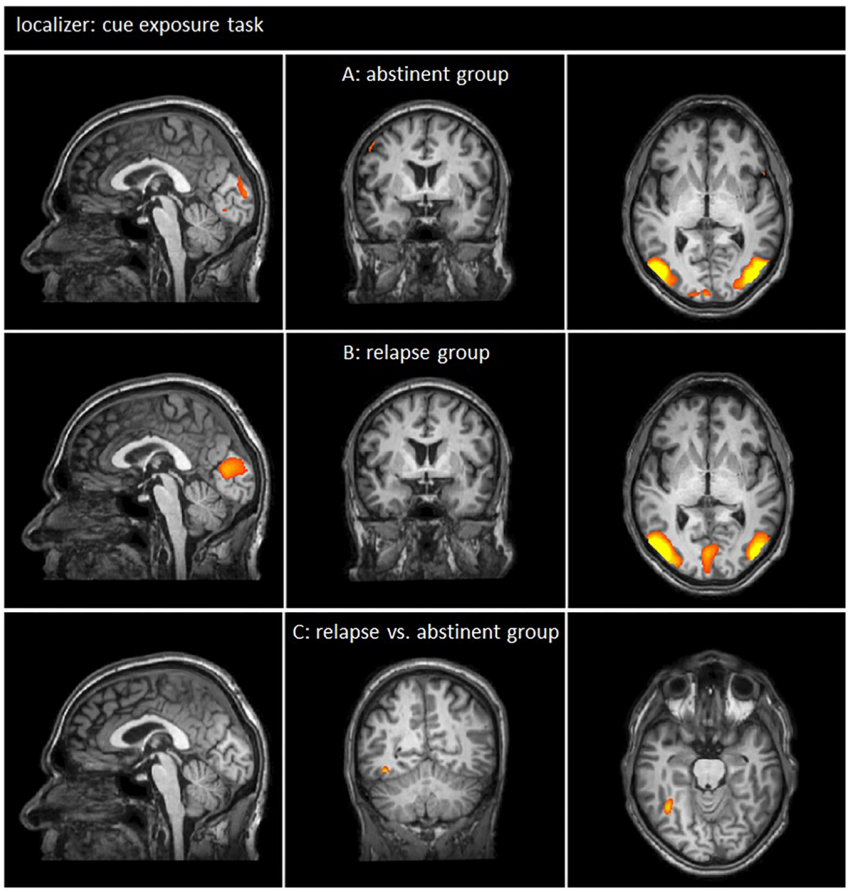

FIGURE 2 | Neuronal responses during cue exposure task [tobacco-related pictures > neutral pictures; *p*(Bonf) < 0.05, *T*-score: 4.830–8]. **(A,B)** Smokers of both groups demonstrated neuronal responses to tobacco related pictures especially in the brain regions associated with visual information processing ($x = 0$; $y = 0$; $z = 0$). **(C)** The comparison of brain responses of the relapse group and the abstinent group showed only very small functional differences within the fusiform gyrus were detected ($x = -1$; $y = -53$; $z = -18$).

TABLE 2 | Neuronal responses in abstinent group during the cue exposure task of the first fMRI session [tobacco-related pictures minus neutral pictures; clusters of >30 voxels, *p*(Bonf) < 0.05, *T*-score: 4.830–8].

			Abstinent group					
			Center of gravity			Size	*t*-score	
Brain region	**Side**	**BA**	***x***	***y***	***z***		**Ø**	**Max**
Tobacco-related pictures > neutral pictures								
Superior Parietal Lobule/Inferior Parietal Lobule	R	7	34	−54	47	6187	6.41	9.67
Superior Parietal Lobule/Inferior Parietal Lobule	L	7	−28	−58	47	2810	5.77	7.77
Middle Frontal Gyrus	R	6	50	5	37	1395	5.36	6.56
Inferior Occipital Gyrus/Lingual Gyrus	L	18/19	−37	−69	−4	15232	8.94	19.25
Inferior Occipital Gyrus/Lingual Gyrus	R	18/19	33	−70	−4	18026	8.77	15.19

BA, Brodman area; side, hemisphere; L, left; R, right; max, maximal t-score; Ø, average t-score; size, cluster size; voxels, number of activated voxels; x, Talairach coordinate x-axis; y, Talairach coordinate y-axis; z, Talairach coordinate z-axis.

Clinical Outcome

Relapse Rate

The abstinence rate was 45.5% after 3 months. The 'Rauchfrei Programm' is the most common cognitive behavioral group program for quitting smoking in Germany (Gradl et al., 2009; Rasch et al., 2010). Former studies indicated that immediately after the program, 60.9% of the participants stopped smoking. After 6 months, the abstinence rate was 40.2%, 31.8% after 1 year (Gradl et al., 2009; Wenig et al., 2013). In our study, the combination of this program with rtfMRI-Neurofeedback did not

TABLE 3 | Neuronal responses in relapse group during the cue exposure task of the first fMRI session [tobacco-related pictures minus neutral pictures; clusters of >30 voxels, *p*(Bonf) < 0.05, *T*-score: 4.830–8].

			Relapse group					
			Center of gravity			Size	*t*-score	
Brain region	**Side**	**BA**	***x***	***y***	***z***		**Ø**	**Max**
Tobacco-related pictures > neutral pictures								
Superior Parietal Lobule/Precuneus	R	7	29	−54	50	1698	5.59	7.08
Fusiform Gyrus	R	19/37	38	−65	−8	17700	8.90	19.63
Lingual gyrus/Cuneus	R	17/18	2	−80	5	4460	5.50	6.95
Fusiform Gyrus/Middle Occipital Gyrus	L	19/37	−47	−64	−9	11615	8.47	14.52
Tobacco-related pictures < neutral pictures								
Fusiform Gyrus/Parahippocampal Gyrus	L	36/37	−24	−42	−14	1582	−6.23	−8.79

BA, Brodman area; side, hemisphere; L, left; R, right; max, maximal t-score; Ø, average t-score; size, cluster size; voxels, number of activated voxels; x, Talairach coordinate x-axis; y, Talairach coordinate y-axis; z, Talairach coordinate z-axis.

TABLE 4 | Neuronal responses in relapse group minus abstinent group during the cue exposure task of the first fMRI session [tobacco-related pictures minus neutral pictures; clusters of >30 voxels, *p*(Bonf) < 0.05, *T*-score: 4.830–8].

Relapse group versus abstinent group (localizer of the first fMRI session)								
			Center of gravity			Size	*t*-score	
Brain region	**Side**	**BA**	***x***	***y***	***z***		**Ø**	**Max**
Relapse > abstinent (localizer)								
Fusiform gyrus	R	37	35	−50	−19	498	5.76	7.44

BA, Brodman area; side, hemisphere; L, left; R, right; max, maximal t-score; Ø, average t-score; size, cluster size; voxels, number of activated voxels; x, Talairach coordinate x-axis; y, Talairach coordinate y-axis; z, Talairach coordinate z-axis.

significantly change the entire abstinence rate. Unfortunately, the relapse rate of the real and sham group did not differ significantly.

Craving

The assessment of variations in the clinical data did not show any significant difference regarding craving on the first day. Findings about the association between craving and relapse rates are mixed: a systematic review revealed that (a) there were only a few cases of significant associations between craving collected as part of cue-reactivity studies and treatment outcome, (b) craving after quitting smoking was a stronger predictor of treatment outcome than craving before quitting smoking, and (c) several moderators are likely to influence the relationship between craving and cessation outcome (Wray et al., 2013). The authors conclude that craving is not a necessary condition of relapse. In addition, inconsistent relationships between craving and treatment outcome call the value of craving as a target of treatment into question and emphasize limitations in the prognostic utility of craving (Wray et al., 2013). However, other studies showed that the activation in the right ventral striatum predicted the duration of abstinence beyond the level of nicotine dependence (Owens et al., 2017). Additionally heightened neuronal reactivity in brain regions related to the regulation of emotions, interoception and motor planning/execution to smoking-related cues as well as decreased functional connectivity between insula and cognitive brain areas were presented in subjects that relapsed (Janes et al., 2010). The authors concluded that their data suggest that relapse-vulnerable smokers can be identified before quit attempts, which could enable personalized treatment, improve tobacco-dependence treatment outcomes, and reduce smoking-related morbidity and mortality (Janes et al., 2010).

Despite these inconsistent results regarding the association between craving and relapse, cue-induced craving to smoke has been considered one of the driving forces in continued smoking (Tiffany, 1990). Psychopharmacological interventions have demonstrated only a small impact on cue-induced craving (Tiffany et al., 2000; Ferguson et al., 2009).

Functional Imaging Data

BOLD Responses During the First NF Training Session of the Relapse Group Compared to the Abstinent Group

The comparison of BOLD responses during the NF first training session between patients who relapsed and patients who remained abstinent revealed increased responses in the relapse group, especially in the PFC cortex (SFG/medial PFG/middle PFG) and the ACC (see **Figure 3** and **Table 7**). BOLD responses in the PFC, especially the SFG and MFG, have been related to cognitive processes including executive control (Miller, 2013). The ACC can be attributed to cognitive as well as emotional processes (Miller, 2013): The ACC does have connections to both the 'emotional' limbic system and the 'cognitive' prefrontal cortex. Thus, the ACC seems to play an important role in the integration of neuronal circuitry to affect regulation (Stevens et al., 2011), including the ability to control and manage uncomfortable emotions. Avoidance of painful emotions is often the motivational force in negative behaviors such as substance abuse. These actions are taken as part of maladaptive approaches to control, avoid, or regulate painful emotions (Stevens et al., 2011). During NF training, a regulation of craving-associated emotions is necessary. Increased responses in the respective brain areas during the NF session in the relapse group could indicate that the downregulation of craving-related responses in

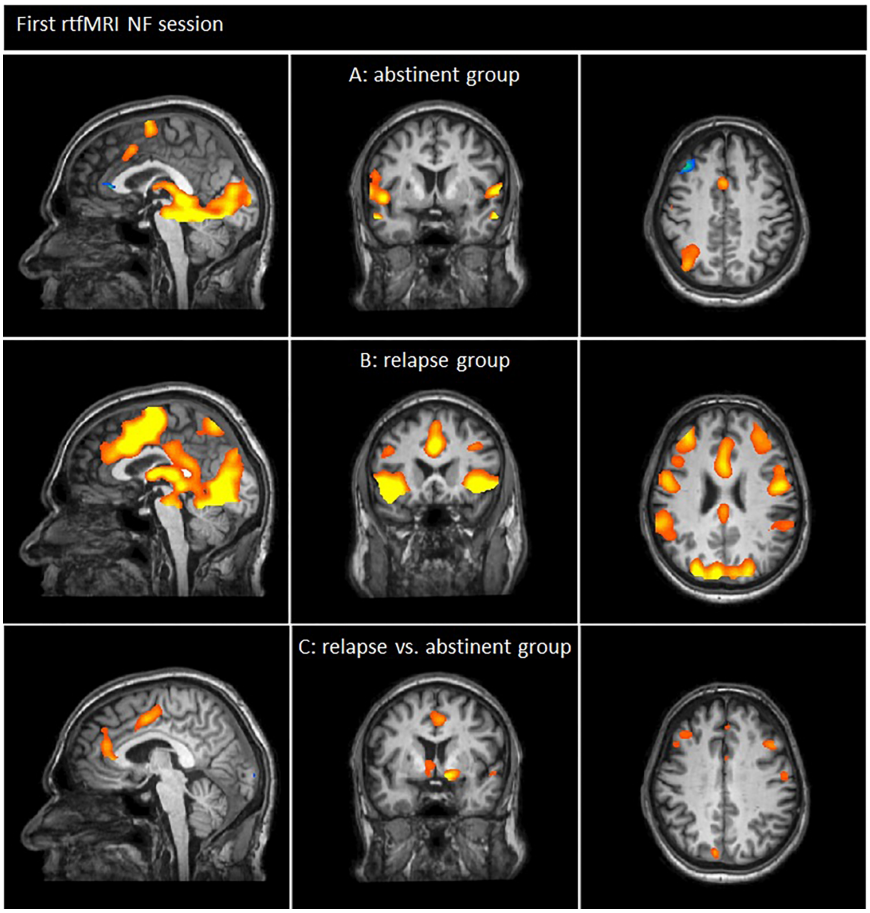

FIGURE 3 | Neuronal responses of the first neurofeedback-session [tobacco-related pictures > neutral pictures; p(Bonf) < 0.05, T-score: 4.830–8]. **(A)** Smokers of the abstinent group demonstrated enhanced neuronal responses during the presentation of smoking related cues especially in frontal brain regions (e.g., superior/medial frontal gyrus, ACC), the pariatal cortex and the insula (x = 0; y = 4; z = 42). **(B)** Smokers of the relapse group demonstrated enhanced neuronal responses during the presentation of smoking related cues compared to neutral pictures especially in frontal brain regions (e.g., superior/medial/middle frontal gyrus, ACC), the insula, pariatal areas as well as temporal regions and the cuneus/precuneues (x = 0; y = 18; z = 27). **(C)** Neuronal responses of smokers who relapsed compared to smokers who remained abstinent: patients of the relapse group demonstrated enhanced BOLD responses especially in the medial/middle and superior frontal gyrus, the ACC, the caudate nucleus and the superior temporal gyrus compared to patients that remained abstinent. By contrast, the responses in the inferior occipital gyrus and the fusiform gyrus were decreased in the relapse group (x = 4; y = 3; z = 34).

brain areas which are especially associated with cognitive and emotional processes is less successful in these patients than in patients who remained abstinent after therapeutic interventions.

Furthermore, increased BOLD responses in the relapse group compared to the abstinent group were shown in the caudate nucleus/claustrum. The caudate nucleus is one of the structures that make up the dorsal striatum which is a component of the basal ganglia (Yager et al., 2015). Apart from various motor functions, the caudate is also one of the brain structures which compose the reward system and functions as part of the cortico-basal ganglia-thalamic loop (Yager et al., 2015). This area has proved to play an important role in the context of dependence disorders, including tobacco. A review of Balfour (2004) indicated that the stimulation of dopamine (DA) projections to the nucleus accumbens play a complementary role in the development of nicotine dependence (Balfour, 2004). The hypothesis in the review proposes that increased extra-synaptic DA in the accumbens confers hedonic properties on behavior, such as smoking which deliver nicotine, and thereby increase the probability that the response is learned. The authors of the review argue that sensitisation of the DA projections to the accumbal core, and the behaviors which depend on this process, play a pivotal role in the maintenance of a tobacco smoking habit (Balfour, 2004). Against this background increased responses in the relapse group during the down-regulation NF task might indicate that these smokers were less able to modulate their neurobiological responses in the reward system which is influenced by dopaminergic innervation. The salience of tobacco-related cues may resist increased in this group compared to smokers who benefitted from the training.

By contrast, the responses in the inferior occipital gyrus and the fusiform gyrus were decreased in the relapse group compared to the abstinent group. These areas have been related to the secondary and tertiary visual cortex and can, e.g., be

TABLE 5 | Neuronal responses in abstinent group during the first rtfMRI NF session [tobacco-related pictures minus neutral pictures; clusters of >30 voxels, *p*(Bonf) < 0.05, *T*-score: 4.830–8].

Abstinent group								
			Center of gravity			Size	*t*-score	
Brain region	**Side**	**BA**	***x***	***y***	***z***		**Ø**	**Max**
Tobacco-related pictures > neutral pictures								
Superior Frontal Gyrus/Medial Frontal Gyrus	R	6	4	−5	61	3206	6.52	11.34
Medial Frontal Gyrus/Anterior Cingulate Gyrus	R/L	6/32	1	13	40	837	5.33	7.14
Precuneus/Superior Parietal Gyrus/Inferior Parietal Lobule	R	7/40	32	−51	47	4110	5.58	7.00
Superior Parietal Lobule/Inferior Parietal Lobule/Postcentral Gyrus	L	5/7/40	−30	−45	55	2886	2.82	8.65
Fusiform Gyrus/Lingual Gyrus	R	18/19	35	−65	−4	34888	10	25.75
Culmen	R/L		0	−53	−5	51795	7.02	14.29
Fusiform Gyrus	L	37/19	−40	−61	−6	25750	11.02	26.89
Sub-lobar/Insula/Superior Temporal Gyrus/Precentral Gyrus	L	13/22/44	−49	10	3	3564	6.34	10.79
Superior Frontal Gyrus/Middle Frontal Gyrus	R	10	30	49	17	999	5.84	8.36
Tobacco-related pictures < neutral pictures								
Middle Frontal Gyrus/Superior Frontal Gyrus	R	6/8	28	18	49	1892	−5.94	−8.14
Middle Frontal Gyrus/Superior Frontal Gyrus	L	6/8	−27	16	51	1217	−5.62	−6.86
Anterior Cingulate Gyrus/Medial Frontal Gyrus	L/R	32/24/9/10	−2	40	9	3982	−6.03	−9.24
Cerebellum	L		−24	−42	−16	1089	−6.74	−10.4

BA, Brodman area; side, hemisphere; L, left; R, right; max, maximal t-score; Ø, average t-score; size, cluster size; voxels, number of activated voxels; x, Talairach coordinate x-axis; y, Talairach coordinate y-axis; z, Talairach coordinate z-axis.

TABLE 6 | Neuronal responses in relapse group during the first rtfMRI NF session [tobacco-related pictures minus neutral pictures; clusters of >30 voxels, *p*(Bonf) < 0.05, *T*-score: 4.830–8].

Relapse group								
			Center of gravity			Size	*t*-score	
Brain region	**Side**	**BA**	***x***	***y***	***z***		**Ø**	**Max**
Tobacco-related pictures > neutral pictures								
Cingulate Gyrus/Medial Frontal Gyrus/Superior Frontal Gyrus	L	24/32/6	−1	4	46	29215	7.31	13.7
Precentral Gyrus/Middle Frontal Gyrus	R	6	36	−7	44	11388	5.95	8.11
Middle Frontal Gyrus/Superior Frontal Gyrus	R	6/9	34	33	28	5090	5.83	7.98
Insula/Precentral Gyrus/Inferior Frontal Gyrus	R	13/44/47/22	41	8	1	26082	6.9	14.11
Middle Frontal Gyrus/Superior Frontal Gyrus	L	9/10	−34	35	26	5703	5.64	7.04
Insula/Precentral Gyrus	L	13	−43	5	16	26699	6.86	15.88
Thalamus	L/R		0	−18	2	40474	6.01	10.12
Inferior Parietal Lobule/Supramarginal Gyrus	L	40	−35	−49	39	8650	5.71	8.67
Middle Occipital Gyrus/Fusiform Gyrus	L	19/37	−42	−64	−7	12976	9.16	18.17
Inferior Parietal Lobule/Supramarginal Gyrus	R	40	43	−43	36	12146	6.48	13.29
Cuneus/Precuneus	R	7/31/18	12	−70	30	22047	6.70	12.53
Inferior Temporal Gyrus/Middle Temporal Gyrus	R	37	39	−63	−3	21868	10.78	25.15
Culmen	R		5	−64	−9	20198	8.57	17.44
Cuneus/Precuneus	L	18/31	−14	−74	20	6345	5.88	8.25
Culmen/Declive/Lingual Gyrus	R	18	5	−65	−7	19420	8.45	17.44
Lingual Gyrus/Fusiform Gyrus	R	18	38	−65	−3	23581	10.50	25.15
Tobacco-related pictures < neutral pictures								
Fusiform Gyrus/Parahippocampal Gyrus	L	37/20	−26	−44	−14	1530	−6.60	−9.99
Lingual Gyrus/Inferior Occipital Gyrus/Fusiform Gyrus	L	17/18	−14	−89	−6	1878	−6.73	−10.5

BA, Brodman area; side, hemisphere; L, left; R, right; max, maximal t-score; Ø, average t-score; size, cluster size; voxels, number of activated voxels; x, Talairach coordinate x-axis; y, Talairach coordinate y-axis; z, Talairach coordinate z-axis.

TABLE 7 | Neuronal responses in relapse group minus abstinent group during the first rtfMRI NF session [tobacco-related pictures minus neutral pictures; clusters of >30 voxels, *p*(Bonf) < 0.05, *T*-score: 4.830–8].

Relapse group versus abstinent group								
			Center of gravity			Size	*t*-score	
Brain region	**Side**	**BA**	***x***	***y***	***z***		**Ø**	**Max**
Relapse > abstinent								
Cingulate Gyrus/Medial Frontal Gyrus	L	24/6	−2	−2	46	2715	5.63	7.17
Cingulate Gyrus/Medial Frontal Gyrus	R	24/6	7	−70	40	2060	5.96	9.49
Middle Frontal Gyrus	R	8/9	33	27	39	1609	5.88	8.39
	R	8/6	51	8	39	637	5.28	6.13
	R	9	41	21	34	545	5.37	6.28
Anterior Cingulate/Medial Frontal Gyrus	R	32/9/10	8	39	13	3390	5.59	7.36
Middle Frontal Gyrus/Superior Frontal Gyrus	L	10	−31	51	19	940	6.30	9.67
Extra-Nuclear/Lentiform Nucleus/Caudate	R		16	14	0	3852	5.55	7.94
Lentiform Nucleus/Extra-Nuclear/Claustrum	L		−22	12	−2	2261	5.90	10.35
Superior Temporal Gyrus	L	22	−49	13	−4	1117	5.69	8.94
Relapse < abstinent								
Lingual Gyrus/Fusiform Gyrus/Inferior Occipital Gyrus/Declive	L	18/19	−29	−74	−8	13679	6.39	11.56
Lingual Gyrus/Inferior Occipital Gyrus/Fusiform Gyrus	R	18/19	24	−85	−10	2263	6.44	8.92

BA, Brodman area; side, hemisphere; L, left; R, right; max, maximal t-score; Ø, average t-score; size, cluster size; voxels, number of activated voxels; x, Talairach coordinate x-axis; y, Talairach coordinate y-axis; z, Talairach coordinate z-axis.

influenced by attention. Bradley et al. (2003) for example have shown that both extent and strength of functional activity of the occipital cortex were linked to the judged affective arousal of the different picture contents. The author suggested that more extensive visual system activation reflects 'motivated attention' where motivational engagement directs attention and facilitates perceptual processing of important stimuli (Bradley et al., 2003). The increased down-regulation of craving-related responses in the relapse group in these areas could indicate that patients of this group chose a different strategy compared to smokers of the abstinent group. Apparently, these patients modulated more strongly visual perception processes which are influenced by motivation, personal significance of the visual information and attention rather than emotional processes or other cognitive processes (including cognitive control, executive functions), related to craving. These modulations seem to be indirect effects since the target ROIs for the modulation were located within the prefrontal cortex/insula.

Craving-related responses between groups before the NF training (during the localizer run) differed only marginally in a small region within the right fusiform gyrus (see **Figure 2** and **Table 4**). For that reason, we assume that differences between the relapse group and the abstinent group during rtfMRI NF cannot be attributed to craving-related responses before training.

NF Related Responses During the First NF Session in the Abstinent Group and the Relapse Group

In both groups BOLD responses during the NF trials (presentation of tobacco cues and NF information) compared to the neutral condition during the first NF training sessions were enhanced, especially in brain areas related to cognitive and/or emotional craving processes as well as attention/motivation (see **Figure 3** and **Tables 5**, **6**) including frontal/fronto-central areas, the insula, parts of the secondary visual processing system and brain regions which are important for reward processing.

In both groups, real NF had been provided and participants had been instructed to downregulate their brain responses in individually defined ROIs within the frontal cortex. Nevertheless, in both groups brain responses in most parts of the brain were increased during NF compared to the neutral condition. These results may indicate that craving-related responses stayed visible despite NF modulation. Less prominent differences between the cue-related stimulation plus NF and the neutral condition could be expected after several NF sessions (Ruiz et al., 2013b).

Participants who remained abstinent after the training also revealed decreased responses in several frontal areas, including the middle FG, the SFG and the ACC/medial FG. These patients seemed to be able to reduce their individual responses more strongly than patients of the relapse group.

Comparison of Psychometric Data of the Relapse Group With the Abstinent Group

An array of questionnaires and ratings were used in order to compare psychopathological aspects and aspects of the personality of patients who remained abstinent with those who relapsed. We did not find any differences regarding physical dependence (Fagerström test), pack-years, number of cigarettes per day, intelligence (WST), personality (Neo-FFI), craving (QSU-G), anxiety (STAI), impulsivity (BIS), and aggression (AQ). Concerning anger expression (STAXI), the anger-in subscale demonstrated an increased score on the day of the first rtfMRI NF session for patients who remained abstinent during 3 months after the NF training compared to patients who relapsed. This may indicate a varying approach in both groups regarding the

expression of negative emotions. However, the difference seems to be small: The results of all other subscales were comparable between groups. Altogether, the differences between groups were marginal and we did not detect any reliable variables influencing the success of the therapeutic approach.

Individualized ROI Definition

The selection of a personalized ROI enhances the probability for valid feedback: the selection of the target ROI was based on each individual's neuronal responses during the localizer run during the presentation of craving-related information. The individualized ROIs which were identified for feedback encompassed areas of the DLPFC, the insula or the ACC. These regions were selected based on information from former studies using exposure to smoking-related cues compared with neutral cues (Janes et al., 2016, 2017). In these studies the ACC has been reported to be involved in executive functioning such as decision making, choosing between alternatives and evaluating possible outcomes to optimize results. In addition, the ACC is an important area for emotional processing (Bush et al., 2000). Furthermore, previous rtfMRI NF studies have demonstrated that the activity within the ACC can be influenced by the participants (Weiskopf et al., 2003; Hamilton et al., 2011; Hartwell et al., 2016).

The challenge of not smoking following exposure to smoking-related cues presents both a cognitive and an emotional task for nicotine-dependent smokers while individual variations in the involvement of the ACC and the PFC is expected (Hartwell et al., 2016).

The selection of an individualized task-driven NF minimized the risk of providing NF from a non-activated area, e.g., owing to possible alterations as a result of previous NF sessions (Hartwell et al., 2016). It seems sensible for future studies to include the examination of an optimal target region for NF in patients with tobacco dependence.

Disadvantages of an individualized task-based ROI definition may be that the anatomical specificity is reduced and the possibility to compare the results between patients is limited.

Comparison With the Results of Other NF Studies With Patients With Tobacco Use Disorder

In some aspects the design of the present study was comparable to the design of the study of Hartwell et al. (2016). In both studies an individualized NF approach for craving-associated BOLD responses was used, based on an initial run during which smoking-related cues were employed to provoke craving; participants completed three neuroimaging visits with three NF runs each. The results of the study of Hartwell et al. (2016) reveal decreased subjective craving and cue-induced brain activation.

The results of the present study show small differences in terms of craving between groups: the difference reached trend level. In addition, BOLD responses were influenced by the day of measurement and the group. One main difference between these studies is the inclusion criteria: unlike our study Hartwell et al. (2016) did not include treatment seeking smokers.

Influence of Intensity of Craving on BOLD Responses

The study of Wilson and Sayette (2015) demonstrated that brain responses measured during mild states of desire fundamentally differ from those measured during states of overpowering desire (i.e., craving) to use drugs (Wilson and Sayette, 2015). A meta-analysis revealed that fMRI cue exposure studies using nicotine-deprived smokers produced different patterns of brain activation to those using non-deprived smokers (Wilson and Sayette, 2015). The authors conclude that the intensity of the urges does matter, and more explicit attention to urge intensity in future research should have the potential to yield valuable information about the nature of craving (Wilson and Sayette, 2015). In our study, the intensity of craving was comparable between groups: we did not find any differences concerning the craving intensity between the abstinent and relapse group.

Limitations

Several limitations should be noted in the interpretation and application of the results.

Our interpretation is based on the results of the real group and does not consider the results of the sham group that will be reported elsewhere in detail (Karch, Paolini et al., unpublished). Therefore, even if our results are suggesting neurofeedback specific effects, we cannot completely exclude that some of our findings are independent of the targeted NF approach. Various control groups, for example with alternative – not neurofeedback based – strategies are needed, perhaps in future studies with bigger sample sizes to address this question.

Concerning the paradigm, we did not include any cue-exposure scanning without feedback for training evaluation directly after the rtfMRI training. In addition, we did not record neurobiological data during the post-training survey 3 months after the NF sessions. For that purpose it is not possible to determine whether and to which extent neurobiological effects are enduring. During the rtfMRI training, tobacco-associated pictures as well as neutral pictures were presented repeatedly to the participants. This might have led to some kind of habituation and a diminished response at later repetitions. However, we expected that habituation effects would occur in the relapse group as well as in the abstinent group.

Future research in treatment-seeking smokers prepared to initiate a cessation attempt is needed and should include further fMRI sequences after the NF training in order to assess the persistence of neuronal effects.

The optimal number of NF training sessions is not yet clear. In the present study, all patients participated in three rtfMRI NF sessions in order to enhance the power compared to single session training. However, further studies are needed focusing on the systematic examination of this topic.

CONCLUSION

Patients with tobacco use disorder who remained abstinent for at least 3 months after a behavioral group therapy combined with

a rtfMRI NF training demonstrated decreased neural responses during the first cue-associated NF training session compared to patients who relapsed, especially in the ACC, the SMA as well as dorsolateral prefrontal areas. It seems that a pronounced neural reduction in frontal brain regions related to cognitive-emotional processes during craving in the first NF training may be used as an early predictor of a better therapeutic success for quitting smoking in patients with tobacco use disorder. As our NF target areas, i.e., the ACC, the insula and the DLPFC, were mainly included in these brain areas of decreased neural responses, the success in smoking cessation may be related to the success in conducting effective rtfMRI NF. This approach needs further research, exploration and development, especially in order to assess the persistence of neuronal and therapeutic effects.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

SK, MP, ArR, BE-W, OP, DK, and TR conceived and designed the experiments. The experiments were performed by MP, SG, HJ, AnR, OY, BR, and DK. Data analysing was done by SK, MP, SG, HJ, AnR, MM, CF, BR, AC, ArR, and DK. SK, MP, MM, CF, BE-W, OP, DK, and TR contributed in the writing of the manuscript.

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Anodal Transcranial Direct Current Stimulation Induces High Gamma-Band Activity in the Left Dorsolateral Prefrontal Cortex During a Working Memory Task: A Double-Blind, Randomized, Crossover Study

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Ikeda T, Takahashi T, Hiraishi H, Saito DN and Kikuchi M (2019) Anodal Transcranial Direct Current Stimulation Induces High Gamma-Band Activity in the Left Dorsolateral Prefrontal Cortex During a Working Memory Task: A Double-Blind, Randomized, Crossover Study. Front. Hum. Neurosci. 13:136. doi: 10.3389/fnhum.2019.00136

Transcranial direct current stimulation (tDCS) has been shown to have mixed effects on working memory (WM) capacity in healthy individuals. Different stimulation paradigms may account for these discrepancies, with certain features being favored. To determine the effect in the context of anodal tDCS, we investigated whether anodal tDCS induced cortical oscillatory changes during a WM task. Specifically, we tested whether anodal offline tDCS over the left prefrontal cortex (PFC) enhances WM capacity by modulating the oscillatory activity in the left dorsolateral PFC (DLPFC) using magnetoencephalography (MEG). This study employed a double-blind, randomized, crossover design, in which 24 healthy right-handed participants conducted MEG recordings during a 3-back task after administration of 2 mA tDCS or sham stimulation as a placebo. Our results showed that the effect of tDCS did not appear in the behavioral indices—WM accuracy (d') or reaction time (RT). From the results of the time-frequency analysis, significant event-related synchronization (ERS) in the high-gamma band (82–84 Hz) of the left DLPFC was found under the tDCS condition; however, ERS was not correlated with WM capacity. Furthermore, we calculated the modulation index (MI), which indicates the strength of phase-amplitude coupling (PAC). tDCS significantly decreased MI of the left DLPFC, representing the theta-gamma PAC during the n-back task using color names as verbal stimuli. Our results suggest that although tDCS increased the gamma-band oscillation indicating greater neural activity in the left DLPFC, it did not lead to an improvement of WM capacity; this may be due to the inability of gamma-band oscillation to couple with the task-induced theta wave. WM capacity might not increase unless theta-gamma PAC is not enhanced by tDCS.

Keywords: tDCS, working memory, DLPFC, MEG, phase-amplitude coupling, n-back task, color

INTRODUCTION

Working memory (WM) permits the maintenance of perceived information over a short period of time. WM has specialized buffers, a phonological loop and visuo-spatial sketchpad, and the central executive, which represent executive function (Baddeley and Hitch, 1974; Baddeley, 2012). Executive function has been a focus of recent research as it serves as an attention controller that allocates and coordinates attentional resources for a variety of cognitive tasks (Osaka et al., 2007). Executive function is needed to solve complex ("frontal lobe") tasks and is thought to comprise three subcomponents—shifting, updating, and inhibition (Miyake et al., 2000). Shifting describes the flexibility of switching between tasks or mental sets, updating is the ability to monitor and rapidly add to or delete WM contents, and inhibition is the ability to deliberately override dominant or prepotent responses (Miyake and Friedman, 2012). For example, the *n*-back task, which is frequently used to measure WM capacity, relies more heavily on concurrent updating ability than it does shifting (Kane et al., 2007; Snyder et al., 2015). Neuroimaging studies suggest that executive functions are located in the prefrontal cortex (PFC), cingulate cortex, and parietal cortex (Baddeley, 2003; Niendam et al., 2012). In particular, activation of the left dorsolateral PFC (DLPFC) has been observed in tasks that require executive function (Smith and Jonides, 1999). In electrophysiology, the relationship between WM and brain rhythms has been studied (Klimesch, 1999). Electroencephalography (EEG) and magnetoencephalography (MEG) studies have frequently reported event-related oscillatory changes, which are considered to represent the increase or decrease in synchronous activity of neuronal populations. When frequency-specific changes of the ongoing oscillatory power occur, the increase or decrease of power is called event-related synchronization (ERS) or desynchronization (ERD), respectively (Pfurtscheller and Lopes da Silva, 1999). Some studies have reported prominent theta power increases over frontal regions during various WM tasks (Ishii et al., 1999; Jensen and Tesche, 2002; Hsieh and Ranganath, 2014). Task-dependent theta band oscillations recorded over the frontal cortex have been shown to increase with memory demand (Jensen and Tesche, 2002). Furthermore, higher frequencies have also been shown to contribute to WM function. Inhibitory gamma-aminobutyric acid (GABA) neurons in the DLPFC mediate the synchronization of pyramidal neurons at the gamma frequency; accordingly, patients with schizophrenia, where synthesis of GABA is decreased, frequently present with WM deficits (Lewis et al., 2005). An integrated study using EEG and magnetic resonance spectroscopy confirmed that *in vivo* GABA measures, gamma-band oscillations, and WM capacity were tightly correlated (Chen et al., 2014).

Recently, advancements have been made in studies aimed at improving WM capacity through non-invasive stimulus methods (Steinberg et al., 2018). Transcranial direct current stimulation (tDCS) is a widely used technique for non-invasive brain stimulation, which is a subset of transcranial electrical stimulation (tES) methodology (Nitsche and Paulus, 2011). During its initial study, the effect of tDCS on motor function was investigated. tDCS over the motor cortex depends on its current polarity, with research suggesting that anodal tDCS increases excitability of the motor cortex, whereas cathodal tDCS decreases excitability (Nitsche and Paulus, 2000). The mechanism of excitability change caused by tDCS has been studied electrically and pharmacologically. One animal study found that anodal currents to the cortical surface depolarized pyramidal neurons, whereas cathodal currents hyperpolarized them (Purpura and McMurtry, 1965). In a human study, cortical excitability continued even after cessation of current stimulation; however, this aftereffect was blocked by an NMDA receptor antagonist (Nitsche et al., 2003). In addition, tDCS extending over a few minutes led to LTP-like plasticity, which could spread to other cortical and subcortical regions (Polania et al., 2012). Taken together, it is thought that direct current has a modulation effect on cortical plasticity (Stagg and Nitsche, 2011). Oscillatory changes caused by tDCS was also reported in some articles. Anodal tDCS applied to the occipital region has been found to elicit gamma band ERS in the visual cortex (Hanley et al., 2016; Wilson et al., 2017). Since tDCS has been shown to modulate brain activity, enhancement of cognitive function has also been studied. Among cognitive functions, of particular interest has been the acute influence of tDCS on executive functions (Strobach and Antonenko, 2017). Many studies have stimulated the left PFC, which is the core brain region involved in cognitive function (Santarnecchi et al., 2015). F3, the left prefrontal site in the international 10–20 system, is located approximately above the left DLPFC and is the primary candidate for placing an anode during tDCS. Anodal tDCS over F3 has been shown to improve WM capacity, compared to sham, cathodal tDCS, and anodal tDCS over the motor cortex (Fregni et al., 2005). The effect of polarity of direct current stimulation on cognitive function is difficult to study. From a meta-analysis study, the anodal-excitation effect is commonly found in cognitive studies, but cathodal-inhibition effects are unclear (Jacobson et al., 2012).

However, while positive effects of tDCS on WM capacity have been reported, negative results have also been found. For example, tDCS over the left DLPFC had no effect on n-back accuracy, reaction time (RT; Mylius et al., 2012; Hoy et al., 2013; Hill et al., 2018), or Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV) scores (Sellers et al., 2015). There are several possible reasons for these differences in results including stimulation site, polarity, current, cathode location, length of stimulation, and online vs. offline stimulation (Medina and Cason, 2017). One review reported that offline anodal tDCS applied to healthy participants improved WM accuracy and RT, whereas online did not (Hill et al., 2016). Thus, the impact of tDCS on WM capacity is still unclear and its neural basis should be better defined, ideally using the commonly used n-back task. Gamma oscillations are the key to interpreting the effect of anodal tDCS, WM capacity, and the left DLPFC.

Thus, we selected a stimulation method with a high possibility of improving WM capacity and investigated tDCS-induced neural activity changes. tDCS should be effective with anodal stimulation and an offline paradigm. Here we report the effects of tDCS on behavioral and neurophysiological state. We

hypothesized that anodal offline tDCS over F3 will enhance WM capacity by modulating the oscillatory activity in the left DLPFC using MEG. If tDCS effectively stimulates the left DLPFC, oscillatory changes should occur during a task which elicits strong activation in that region. WM capacity was measured by the 3-back task. The n-back task is a continuous performance test used to estimate WM capacity (Rosvold et al., 1956; Haatveit et al., 2010). The task requires participants to monitor whether the current stimulus is the same as the one presented *n* trials before—where *n* is a predefined number, usually 1, 2, or 3. As we assessed the effects of tDCS on WM performance, floor and ceiling effects should be avoided. For healthy young adults, the 2-back task can be performed easily (Ikeda and Osaka, 2007) and, without special training, the 4-back task is difficult (Buschkuehl et al., 2014); accordingly, the 3-back task was considered suitable to study the effects of tDCS on WM performance. In a previous fMRI study (Ikeda and Osaka, 2007) performed with right-handed participants, the 2-back task using verbal stimuli (Word condition) increased activity in the left PFC, which is an important region for verbal WM (Smith et al., 1998). In addition, presentation of visual color stimuli that belong to the same color category (Within condition) activates the right PFC, whereas using highly codable color stimuli (Cross condition) has intermediate properties among the other two conditions. These results indicate that the items to remember in the n-back task could bias the balance between the left and right hemispheres of activation areas according to participant's dominant language hemisphere. If tDCS activates verbal WM and updating ability together, WM capacity and/or neural oscillations would be enhanced in the Word condition.

MATERIALS AND METHODS

Participants

Twenty-four healthy adult male students (mean = 21.3 years old, SD = 1.26) were recruited from Kanazawa University and participated in this experiment. All participants were right-handed, which was assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). They had normal or corrected-to-normal vision. The Farnsworth Dichotomous Test for color blindness (Panel D-15) was used to assess color vision. One participant had a suspected case of Deuteranopia, however, he passed the color discrimination test described later. Participants were native Japanese speakers with normal hearing and had no medical or family histories of neurological or psychiatric disorders. Full IQ scores (mean = 108.4, SD = 5.83) were estimated using the Japanese version of the National Adult Reading Test (Matsuoka et al., 2006). Participants agreed to participate in this study with full knowledge of the experimental nature of the research. Each participant provided written informed consent prior to participation. The Ethics Committee of Kanazawa University approved this study, which conformed to the tenets of the Declaration of Helsinki.

Experimental Design

The study employed a randomized double-blind, controlled placebo, crossover design that included washout period of at least 1 month (mean = 57.4 days, SD = 25.9). Initially, all participants were randomly assigned to either the tDCS-Sham or Sham-tDCS group. At the beginning of each testing day, participants performed a practice session of the 1-, 2-, and 3-back task. Next, participants were administered tDCS or sham stimulation with 20-min rest between two 13-min stimulation. After the stimulation, participants were prepared for MEG recordings and received a 10-min explanation of the procedure. Following a 15-min auditory task (Miyagishi et al., 2018), we measured the MEG signal to investigate the neural effects of tDCS on the 3-back task (**Figure 1A**). After all the experiments were finished, participants conducted a color naming task and a color discrimination task to check that all color stimuli in this experiment were recognizable and discriminable.

tDCS

A direct current was induced through two saline-soaked surface sponge electrodes (5 × 7 cm) and delivered using a battery-driven, constant current stimulator (DC-STIMULATOR Plus, neuroConn GmbH, Germany). The anode electrode was placed over F3, and the cathode electrode was placed over F4 (see the international EEG 10–20 system) during stimulation (**Figure 1B**). Participants received the stimulus twice before MEG recording, and the duration of a stimulation was 13 min at a current strength of 2 mA to maximize the aftereffects of stimulation (Monte-Silva et al., 2013). During the sham stimulation, electrodes were also attached to the participant, but the current was only delivered during the first 10 s, which prevented the participants from noticing the absence of electrical stimulation.

n-Back Task

A block in each n-back task contained 15 trials to respond. In the 3-back condition, a block contained 18 trials as the first three trials were only for encoding (**Figure 2**). Each stimulus was presented for 1,000 ms followed by a 1,500 ms interstimulus interval (ISI). Participants had to respond with their right index or middle finger depending on whether the stimulus was the same or different from the one presented in three trials previously, using a response pad (LUMINA LU400-PAIR, Cedrus Corporation, San Pedro, CA, USA). The percentage of both "same" trials and "different" trials was 50% within each condition. WM accuracy was measured using d' which is calculated from hit rate and false-alarm rate (MacMillan and Creelman, 2004) and RT was defined as the time from a stimulus presentation to button press.

All participants had practice sessions using capital letters (from A to H) that were not presented in the MEG recording session to confirm that they understood how to perform the n-back task. At first, participants completed a 1-back and 2-back condition until they achieved an accuracy greater than 85%. Following the successful completion of these tasks, a fixed-length practice session of the 3-back task and six blocks were conducted. These practice sessions were conducted before tDCS or sham stimulation in the both days.

In the MEG recordings, we employed verbal (color word) or visual (color rectangle) stimuli as items to remember during an

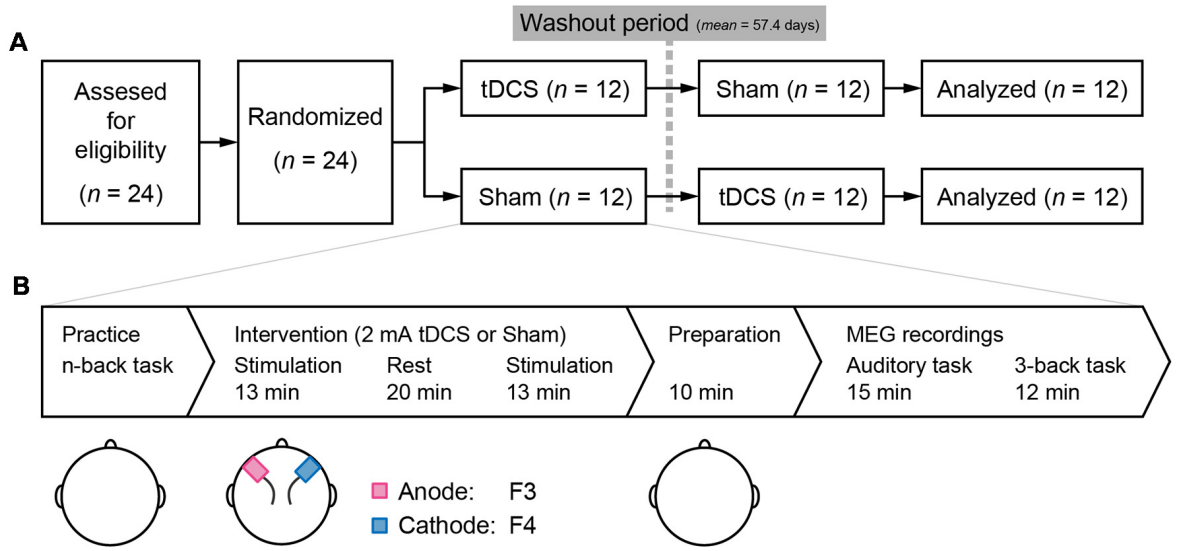

FIGURE 1 | (A) Study design: a double-blind, randomized, crossover design was employed. Twenty-four participants were recruited and randomly assigned to receive either transcranial direct current stimulation (tDCS) or Sham stimulation during the first session. After a washout period of at least 1 month, the second session was conducted. **(B)** Task flow of the experiments in each session: practice of the n-back task was conducted in the order of 1-, 2-, and 3-back conditions. tDCS or sham stimulus as a placebo was administrated. Two sponge electrodes, anode and cathode were on the F3 and F4 according to the international 10–20 system, respectively. Electrodes were removed and preparation for magnetoencephalography (MEG) recordings in a shielded room was initiated. The first MEG task was an auditory task reported in Miyagishi et al. (2018). The 3-back task was started approximately 25 min after the end of stimulation.

n-back task. In the Word condition, Japanese words describing the color name were in white (Meiryo font, 36 point). The color coordinates of stimuli are listed in **Supplementary Table S1**. Visual stimuli were presented on the screen in front of a participant using a liquid crystal projector (IPSiO PJWX6170N, Ricoh Company Ltd., Tokyo, Japan). All stimuli were controlled through Presentation (Version 13.1, Neurobehavioral Systems, Berkeley, CA, USA) running on Windows XP. The luminance and chromaticity of color stimuli were measured by a luminance and color meter (CS-200, Konica Minolta, Japan). The size of color stimulus was 5.6° × 5.6°, and the neutral gray background field was 24.1° × 21° (width × height). An optical sensor connected to the MEG system was attached outside of the background field, which generated a trigger signal synchronizing with the start time of visual stimulus presentation.

MEG Recordings

Magnetic fields were measured using a 160-channel whole-head-type system (MEGvision PQA160C; Ricoh Company, Ltd., Kanazawa, Japan). Sensors were configured as first-order coaxial gradiometers with a baseline of 50 mm; the diameter of each coil of the gradiometers was 15.5 mm. Magnetic fields were sampled at 2,000 Hz per channel with a 500 Hz low-pass filter. Using a Signa Excite HD 1.5T system (GE Yokogawa Medical Systems Ltd., Milwaukee, WI, USA), we obtained a T1-weighted structural image with spherical lipid markers placed at the five MEG fiducial points to enable us to superpose the MEG coordinate system on the MRI data. A T1-weighted image consisted of 166 sequential 1.2 mm-thick slices with a resolution of 512 × 512 points within a field of view of 261 × 261 mm. The cortex surface was reconstructed using Freesurfer software (version 5.3[1]).

Data Analysis

Behavioral data processing and analysis were performed using R software (version 3.5.1[2]). Each dependent variable, d' for accuracy and RT for speed, was analyzed using a two-way repeated measures analysis of variance (ANOVA), with Intervention (tDCS, Sham) and Condition (Word, Cross, Within) as the within-subject factors.

MEG data processing and analytical procedures were performed using Brainstorm software (Tadel et al., 2011) ran on MATLAB® (version R2016b, The MathWorks, Natick, MA, USA). Four noisy channels were eliminated from the analysis. Eye-movement and cardiac artifacts were removed using the signal-space projection (SSP) method. Segments that included head movement or muscle artifacts detected in a visual inspection or in the automatic processing procedure in Brainstorm, were discarded. Next, data were filtered using band-pass (0.5–100 Hz) and notch (60 Hz) filters. The epoch was defined as −1,000 to 3,000 ms relative to the visual stimulus onset (0 ms), followed by selecting correctly encoded trials.

We estimated the signal source using the anatomical cortical surface data of each subject tessellated with 15,000 vertices. The lead field was then computed using the overlapping spheres algorithm. The inverse solution was calculated for each session through the linearly constrained minimum variance vector

[1]http://surfer.nmr.mgh.harvard.edu/
[2]http://cran.r-project.org/

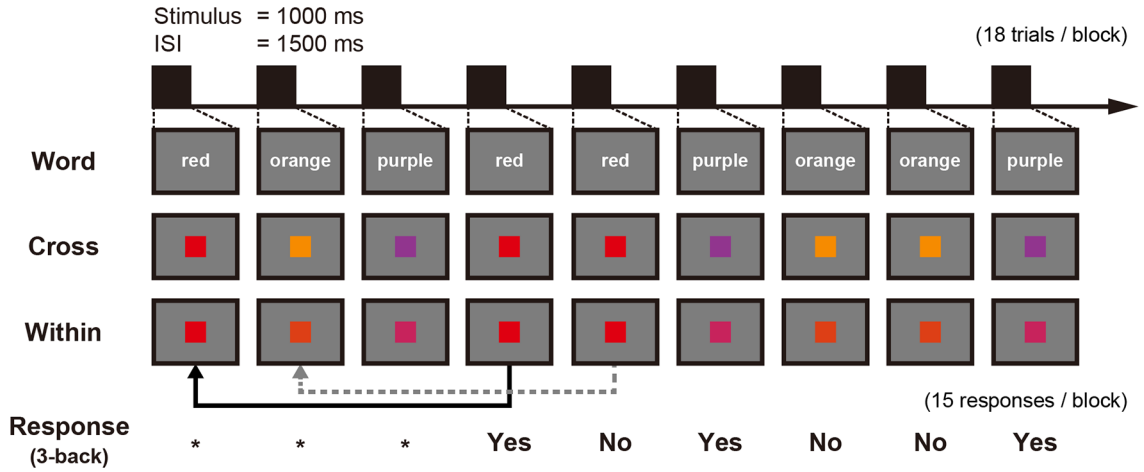

FIGURE 2 | Schematic figure of an experimental block showing three conditions and the corresponding 3-back responses: stimuli for the Word condition are represented here in English instead of Japanese Kana used within the tDCS-MEG study. *Means that no response is needed.

beamformer. A noise and data covariance matrix were calculated based on the MEG recordings obtained during the −100 to 0 ms, and 0–2,350 ms time windows of every epoch within a session.

Two regions of interest (ROIs: L/R DLPFC = Rostral Middle Frontal) were determined based on the Desikan-Killiany atlas (Desikan et al., 2006) implemented in Freesurfer. Signals were taken from the first mode of the principle component analysis decomposition of the signals within each ROI. A time-frequency analysis was conducted using a multi-taper convolution method with the Hanning window (0.3 s). The Event-related spectral perturbation (ERSP) represents the event-related percent changes in signal magnitude relative to a prestimulus baseline period (from −400 to −100 ms). To compare the neural activation under the tDCS and sham conditions, we conducted paired-sample permutation *t*-tests on the data, which contained the three following dimensions: ROI (left/right), time (–500 to 2,500 ms), and frequency (1–100 Hz). The statistical threshold was set at $p < 0.05$, two-tailed, with a false discovery rate (FDR) correction. The additional analysis on the gamma-band power, which was significantly affected by tDCS, was conducted using a two-way repeated measures ANOVA, with Intervention (tDCS, Sham) and Condition (Word, Cross, Within) as the within-subject factors. Furthermore, a correlation analysis was performed to explore the correlation between gamma-band oscillations and WM capacity (d').

RESULTS

Figure 3 summarizes the behavioral data of the 3-back task during MEG recordings. To assess the ceiling or floor effect on WM capacity, we calculated skewness of d' (range: −0.67 to 0.47). No highly skewed distribution was found, and thus the ceiling or floor effect was not observed. From the results of the ANOVA performed on d' data, the main effect of intervention was not significant ($F_{(1,23)} = 1.140$, $p = 0.297$, $\eta_p^2 = 0.047$),

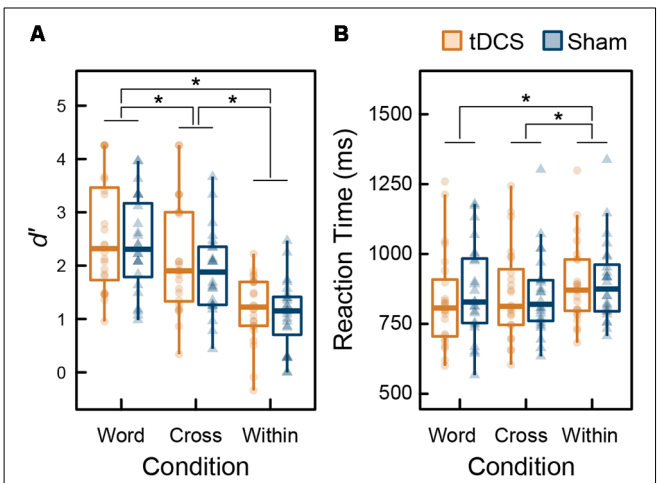

FIGURE 3 | Box plots with individual participant data of **(A)** d' and **(B)** reaction time (RT): stars denote significant difference at $p < 0.05$.

the main effect of condition was significant ($F_{(2,46)} = 58.038$, $p < 0.001$, $\eta_p^2 = 0.716$), and their interaction was not significant ($F_{(2,46)} = 0.244$, $p = 0.785$, $\eta_p^2 = 0.011$). From the results of the ANOVA for RT, similarly, the main effect of intervention was not significant ($F_{(1,23)} = 0.352$, $p = 0.559$, $\eta_p^2 = 0.015$), the main effect of condition was significant ($F_{(2,46)} = 12.140$, $p < 0.001$, $\eta_p^2 = 0.346$), and their interaction was not significant ($F_{(2,46)} = 1.324$, $p = 0.276$, $\eta_p^2 = 0.054$). All behavioral data were affected by condition factor only. The results following multiple comparisons using Holm's sequentially rejective Bonferroni method identified that d' under the Word condition was significantly higher than the Cross ($t_{(23)} = 4.118$, $p < 0.001$, $d = 0.492$) and Within condition ($t_{(23)} = 8.775$, $p < 0.001$, $d = 1.643$). Further, d' under the Cross condition was higher than that for the Within condition ($t_{(23)} = 8.053$, $p < 0.001$,

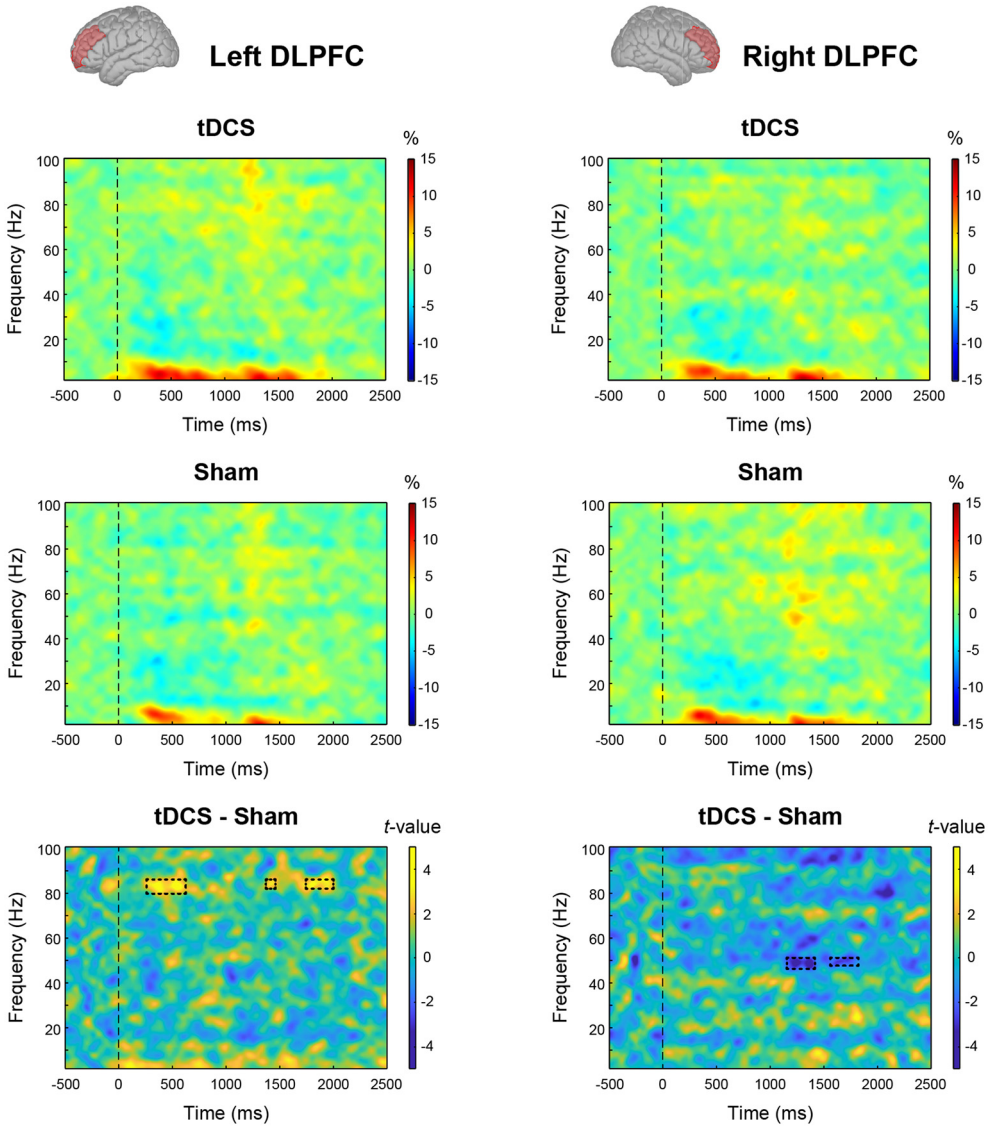

FIGURE 4 | Effect of intervention on oscillatory cortical activity: event-related spectral perturbation (ERSP) plots from the results of time-frequency analysis are given for the tDCS condition and Sham condition in the left and right dorsolateral prefrontal cortex (DLPFC). The bottom panels show the results of the permutation *t*-test (tDCS—Sham). The rectangle regions surrounded by a dotted line indicate significant event-related synchronization (ERS) or desynchronization (ERD) with false discovery rate (FDR) correction ($p < 0.05$).

$d = 1.113$). RTs under Word ($t_{(23)} = 3.623$, $p = 0.003$, $d = 0.291$) and Cross conditions ($t_{(23)} = 4.979$, $p < 0.001$, $d = 0.291$) were significantly faster than the Within condition; however, there was no significant difference between the Word and Cross condition regarding RT ($t_{(23)} = 0.570$, $p = 0.574$, $d = 0.032$).

We tested the main effect of intervention on MEG data. From the results of the permutation *t*-test on time-frequency data, tDCS increased high-gamma band power (82–84 Hz) in the left DLPFC from 270 to 600 ms and 1,750–2,000 ms after stimulus onset. In the right DLPFC, tDCS significantly reduced gamma band power in 47–49 Hz band from 1,180 to 1,400 ms and at 49 Hz from 1,610 to 1,720 ms (**Figure 4**). To explore this result in more depth, we analyzed the data where tDCS had a significant effect on high-gamma band ERS or gamma band ERD using two-way ANOVA. In the left DLPFC at 82–84 Hz, there were significant main effects of intervention ($F_{(1,23)} = 19.461$, $p < 0.001$, $\eta_p^2 = 0.458$) and condition ($F_{(2,46)} = 5.541$, $p = 0.007$, $\eta_p^2 = 0.194$) on high-gamma band ERS. Their interaction was not significant ($F_{(2,46)} = 1.579$, $p = 0.217$, $\eta_p^2 = 0.064$). The results following multiple comparisons showed that percent signal change under the Word condition was significantly higher than that under the Cross ($t_{(23)} = 2.655$, $p = 0.028$, $d = 0.501$) and Within ($t_{(23)} = 3.229$, $p = 0.011$, $d = 0.218$) conditions (**Figure 5A**). In the right DLPFC at 47–49 Hz, there was a significant main effect of intervention on gamma band ERD ($F_{(1,23)} = 15.048$, $p < 0.001$, $\eta_p^2 = 0.396$), and no significant main

effect of condition ($F_{(2,46)} = 0.367$, $p = 0.645$, $\eta_p^2 = 0.016$) and no interaction ($F_{(2,46)} = 0.582$, $p = 0.563$, $\eta_p^2 = 0.025$; **Figure 5B**).

There was a significant correlation between d' and percent signal change in the high-gamma band oscillation in the left DLPFC after the sham stimulation ($t_{(70)} = 2.101$, $r = 0.244$, $p = 0.039$). There were no other significant correlations (**Figures 5C,D**). Furthermore, in each ROI and in each intervention, percent signal change data were divided into the three groups corresponding to Word, Cross, and Within conditions; we then conducted correlation analyses in each group (2 ROIs × 2 interventions × 3 conditions). No significant correlations were found within these groups ($p > 0.05$).

Further analyses were performed to explore the phase-amplitude coupling (PAC) between high-gamma band and theta bands. We also analyzed the modulation index (MI) showing the strength of theta (4–7 Hz) phase and high-gamma (82–84 Hz) amplitude coupling in the left DLPFC within the time of interest, in which tDCS significantly increased high-gamma band power (270–600 ms). In this time window, task-related gamma-band oscillations were present in this region. An increase of MI indicates a phase-dependent increase in amplitude (Canolty et al., 2006; Tort et al., 2010). From the ANOVA results for the MI, the main effect of intervention ($F_{(1,23)} < 0.001$, $p = 0.987$, $\eta_p^2 < 0.001$) and condition ($F_{(2,46)} = 0.212$, $p = 0.810$, $\eta_p^2 = 0.009$) were not significant; however, their interaction was significant ($F_{(2,46)} = 5.574$, $p = 0.007$, $\eta_p^2 = 0.195$). The simple main effect of intervention in the Word condition was significant ($F_{(1,23)} = 8.819$, $p = 0.007$, $\eta_p^2 = 0.277$), but those in the Cross ($F_{(1,23)} = 0.492$, $p = 0.490$, $\eta_p^2 = 0.021$) and the Within condition ($F_{(2,46)} = 1.956$, $p = 0.175$, $\eta_p^2 = 0.078$) were not significant. Regarding tDCS intervention, the condition factor was significant ($F_{(2,46)} = 3.640$, $p = 0.034$, $\eta_p^2 = 0.137$). At that level, MI in the Word condition was significantly lower than the Within condition ($t_{(23)} = 3.335$, $p = 0.009$, $d = 0.715$) following a *post hoc t*-test using the Holm's sequentially rejective Bonferroni method. In summary, the significant reduction effect of tDCS on the MI was found in the Word condition (**Figure 6A**). There was no significant correlation between d' and PAC (tDCS: $t_{(70)} = -1.492$, $r = -0.176$, $p = 0.140$; Sham: $t_{(70)} = -0.010$, $r = -0.001$, $p = 0.992$; **Figure 6B**). Furthermore, no significant correlations were found with the groups ($p > 0.05$).

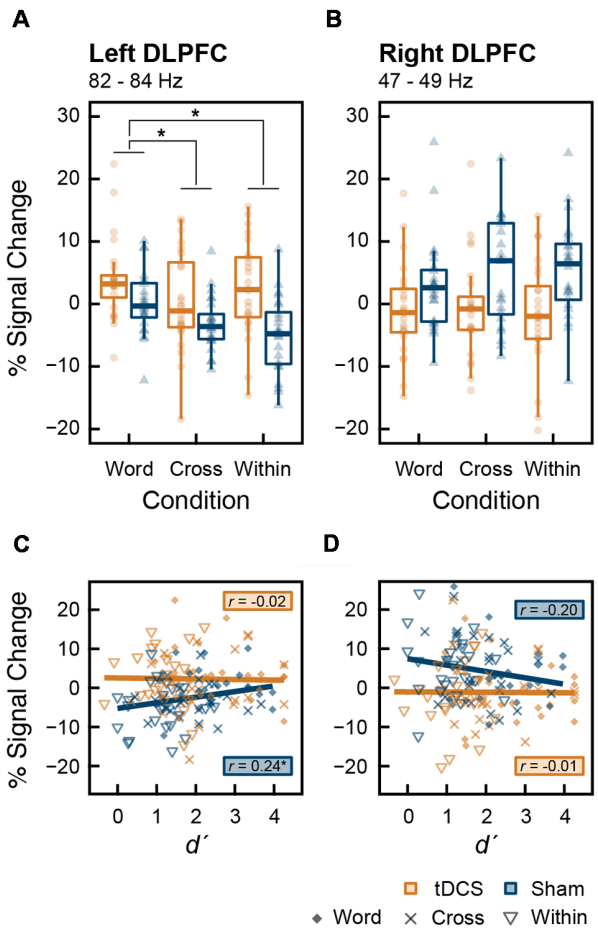

FIGURE 5 | Box plots with individual participant data of percent signal change in **(A)** the left DLPFC and **(B)** the right DLPFC. Data from the left DLPFC were extracted from 270 to 600 ms at 82–84 Hz, and data from the right DLPFC were extracted from 1,180 to 1,400 ms at 47–49 Hz, during which tDCS had significant effects. Stars denote the significance at $p < 0.05$; however, the stars indicating the significant main effect of intervention are omitted. Scatter plots **(C,D)** show the correlation between d', indicating working memory (WM) capacity, and percent signal change that appeared above in the **(A)** left and **(B)** right DLPFC, respectively. The results of correlation analysis (Pearson's correlation coefficient, r) at each intervention are shown in **(C,D)**.

DISCUSSION

We found that offline anodal tDCS over F3 did not improve WM performance in accuracy and speed, partially rejecting our hypothesis (**Figure 3**). Despite the lack of behavioral changes, tDCS significantly induced high-gamma band ERS (82–84 Hz) in the left DLPFC and gamma band ERD (47–49 Hz) in

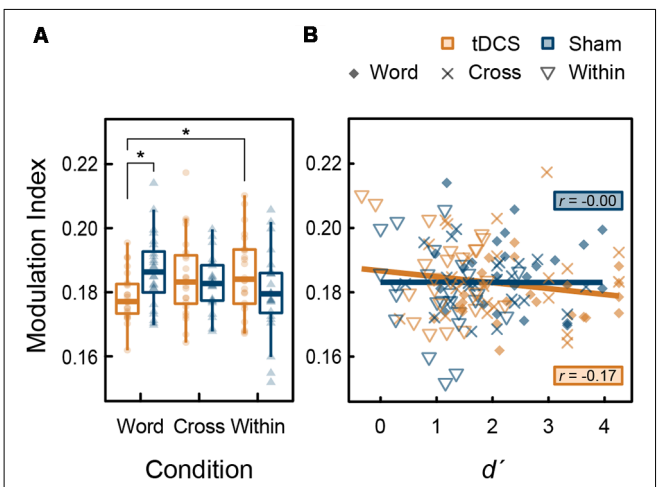

FIGURE 6 | (A) Box plots with individual participant modulation index (MI) data showing the strength of phase-amplitude coupling (PAC) in the left DLPFC: stars denote significant difference at $p < 0.05$. **(B)** Scatter plots showing the correlation between d', and MI.

the right DLPFC (**Figure 4**). At first, we found that tDCS significantly enhanced high-gamma band power regardless of the condition, because the interaction (intervention × condition) was not significant. However, the main effect of condition was significant, and the Word condition had a higher power than the two other conditions. This implies that high-gamma band power in the left DLPFC could be responsible for activation of verbal WM rather than a domain-general updating ability. Given this, it may be possible that WM capacity does not increase, even if tDCS activated verbal WM, in the Cross and Within conditions, in which colored rectangles were visually remembered. Furthermore, we found a significant positive correlation between the high-gamma band power and WM capacity (d') after the sham stimulus. However, when the data were divided into groups corresponding to the three conditions, group-wise correlations were not significant. Accordingly, the relationship between high-gamma power and WM capacity was spurious, which could be explained by the nature of the task condition. In other words, high-gamma oscillation in the left DLPFC might not affect WM capacity, and it could be altered by the items to be remembered. Our findings also raise the possibility that there are optimal frequencies for updating verbal WM as a mental rehearsal system. During 3-back task, tDCS induced oscillations of a higher frequency than the frequency band (30–45 Hz) known to be effective for the 2-back task accuracy (Hoy et al., 2015a). High-gamma ERS over 50 Hz in the left DLPFC has also been observed in language-related tasks, such as a verb generation task (Hashimoto et al., 2017) and an object naming task (Babajani-Feremi et al., 2014). Another possibility is that the relationship between gamma band power and WM capacity has an "inverted-U" shape, much like that of dopamine and WM (Takahashi et al., 2008). Healthy adults might have an appropriate level of gamma band activity, and tDCS could have a smaller impact on WM capacity than it might in patients with cognitive impairment, whose gamma band power is decreased.

We also observed significant gamma band ERD in the right DLPFC after cathodal tDCS over F4, whereas the effect of condition and the interaction was not significant (**Figure 5B**). In addition, gamma band power in the right DLPFC was not correlated with WM capacity (**Figure 5D**). The right DLPFC has been suggested to be responsible for executive function inhibitory control during a Stroop task (Vanderhasselt et al., 2009). The ERD observed in our study seems not to be important for updating ability, verbal WM, or items to remember, because no significant result was found.

From our results, it is still unclear why the tDCS-induced gamma oscillation did not affect WM capacity. There is a possibility that increasing high-gamma band oscillations which do not interact with the lower-band rhythm may not align with improving WM capacity (Turi et al., 2018). From a local field potential study, when the memory system holds multiple items, the population of neurons in the PFC of a rhesus monkey shows phase-dependent activity (Siegel et al., 2009). In human studies, high-gamma (80–150 Hz) amplitude couples to the theta (4–8 Hz) and alpha (8–12 Hz) trough recorded by electrocorticogram; in particular, during several verbal tasks, theta-gamma coupling was prominent in the left DLPFC (Voytek et al., 2010). The MI (Canolty et al., 2006), indicating theta-gamma PAC measured by EEG, has been shown to be greater in healthy adults than patients with mild cognitive impairment or Alzheimer's dementia during the 2-back task (Goodman et al., 2018). These studies suggest that the complex waves where gamma-band amplitude is coupled to theta-band phase could convey sequential information necessary to perform n-back tasks (Roux and Uhlhaas, 2014).

We found the significant interaction of tDCS and task condition in theta-gamma PAC during the verbal 3-back task (**Figure 6A**). Indeed, anodal tDCS induced greater high-gamma band power in the left DLPFC (**Figure 5A**); however, theta-gamma PAC was not affected, or rather reduced, during the task which recruits the left DLPFC. Considering with high-gamma band oscillation mentioned above (**Figure 5A**), it is possible that, in the Word condition, the decrease in PAC canceled out the enhancement of the high-gamma band power induced by tDCS, which might have activated the verbal WM. While gamma band ERS in the left DLPFC is known to be positively correlated with WM capacity (Hoy et al., 2015a, 2016), the timing of emergence of gamma-band oscillation may also play an important role. One transcranial alteration current stimulation (tACS) study reported that gamma band tACS did not improve WM capacity in patients with schizophrenia (Hoy et al., 2015b). Future studies should aim to uncover the most effective timing of gamma band oscillations for WM capacity in more detail. Moreover, we found no significant correlation between PAC and WM capacity (**Figure 6B**). Similar to the high-gamma ERS induced by tDCS in this experiment, the frequency of PAC might be also important for WM capacity. In conclusion, our findings provide neurophysiological evidence that the effect of tDCS on WM capacity is not always robust.

Our study has some limitations from the inherent nature of the n-back task. For estimating WM capacity, the n-back task is useful; however, memory functions, such as encoding, maintenance and retrieval, are not clearly distinguishable in time. During the time of interest (270–600 ms), a new item is encoded into WM storage and is compared with the stored item simultaneously. In addition, the pre-stimulus baseline period in a trial may also be the end section of the previous trial as trials were presented continuously. Therefore, baseline correction processes may affect the values in the latter time period of a trial. If we reveal the effect of tDCS on the memory process in detail, memory tasks that have a pre-trial baseline period and distinguish between encoding, maintenance, and recognition, such as a reading span task (Daneman and Carpenter, 1980; Osaka and Osaka, 1992) should be used. Furthermore, a WM task that can overcome the immediate learning effect, introducing a pre-post design for each day would increase the statistical power.

ETHICS STATEMENT

The Ethics Committee of Kanazawa University approved this study, which conformed to the tenets of the Declaration of Helsinki (UMIN Clinical Trials Registry: UMIN000021058).

AUTHOR CONTRIBUTIONS

TI, TT, and HH contributed to the conception and design of the study. TI, TT, HH, and DS collected the data. TI performed the statistical analysis and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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frontiers in Human Neuroscience

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Differential Neuroplastic Changes in Fibromyalgia and Depression Indexed by Up-Regulation of Motor Cortex Inhibition and Disinhibition of the Descending Pain System: An Exploratory Study

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Background: Major depressive disorder (MDD) and fibromyalgia (FM) present overlapped symptoms. Although the connection between these two disorders has not been elucidated yet, the disruption of neuroplastic processes that mediate the equilibrium in the inhibitory systems stands out as a possible mechanism. Thus, the purpose of this cross-sectional exploratory study was: (i) to compare the motor cortex inhibition indexed by transcranial magnetic stimulation (TMS) measures [short intracortical inhibition (SICI) and intracortical facilitation (ICF)], as well as the function of descending pain modulatory systems (DPMS) among FM, MDD, and healthy subjects (HS); (ii) to compare SICI, ICF, and the role of DPMS evaluated by the change on Numerical Pain Scale (NPS) during the conditioned pain modulation test (CPM-test) between FM and MDD considering the BDNF-adjusted index; (iii) to assess the relationship between the role of DPMS and the BDNF-adjusted index, despite clinical diagnosis.

Patients and Methods: A cohort of 63 women, aged 18 to 75 years [FM (n = 18), MDD (n = 19), and HC (n = 29)].

Results: The MANCOVA analysis revealed that the mean of SICI was 53.40% larger in FM compared to MDD [1.03 (0.50) vs. 0.55 (0.43)] and 66.99% larger compared to HC [1.03 (0.50) vs. 0.34 (0.19)], respectively. The inhibitory potency of the DPMS assessed by the change on the NPS during CPM-test was 112.29 % lower in the FM compared to MDD [0.22 (1.37) vs. −0.87 (1.49)]. The mean of BDNF from FM compared to MDD

was 35.70% higher [49.82 (16.31) vs. 14.12 (8.86)]. In FM, the Spearman's coefficient between the change in the NPS during CPM-test with the SICI was Rho = −0.49, [confidence interval (CI) 95%; −0.78 to −0.03]. The BDNF-adjusted index was positively correlated with the disinhibition of the DPMS.

Conclusion: These findings support the hypothesis that in FM a deteriorated function of cortical inhibition, indexed by a higher SICI parameter, a lower function of the DPMS, together with a higher level of BDNF indicate that FM has different pathological substrates from depression. They suggest that an up-regulation phenomenon of intracortical inhibitory networks associated with a disruption of the DPMS function occurs in FM.

Keywords: fibromyalgia, depression, primary motor cortex, pain, CPM, BDNF

INTRODUCTION

Major depressive disorder (MDD) and fibromyalgia (FM) present overlapped symptoms. Although the connection between these two disorders has not been elucidated yet, the disruption of neuroplastic processes that mediate the equilibrium in the inhibitory systems stands out as a possible mechanism. These processes comprise a central pathologic mechanism of the sensitization syndrome (CSS) (Maletic et al., 2007; Woolf, 2012). The CSS embodies the long-term consequence of an abnormal stress-response system (Lyon et al., 2011) that culminates in the amplification of sensory inputs. It covers the decline of top-down inhibitory activity (dysregulation of dopamine, serotonin, norepinephrine, epinephrine, and endogenous opioids) (Wallace and Gotto, 2008) and the enhancement of bottom-up excitatory activity.

Both MDD and FM present a robust association with an imbalance of glutamatergic (Glu) and GABAergic transmission. Motor cortex disinhibition indexed by transcranial magnetic stimulation (TMS) measurements became a robust common feature of MDD (Fidalgo et al., 2014; Lewis et al., 2016) and FM (Caumo et al., 2016). In chronic pain, changes in the short intracortical inhibition (SICI) (a surrogate marker of GABAergic activity) are mixed. Some studies in neuropathic pain (Nijs et al., 2014), chronic myofascial, FM, and migraine found a reduction in the SICI (Chadaide et al., 2007; Dall'Agnol et al., 2014). And, a similar result has been found in depression (Antal et al., 2010; Conforto et al., 2012; Cantone et al., 2017). Regarding to intracortical facilitation (ICF) (a proxy of glutamatergic activity), an increased activity of excitatory intracortical interneurons (Dall'Agnol et al., 2014; Vidor et al., 2014; Botelho et al., 2016; Caumo et al., 2016; Dussán-Sarria et al., 2018) was found in chronic pain, while it is decreased in MDD (Cantone et al., 2017). Another biomarker associated with both MDD and FM is the brain-derived neurotrophic factor (BDNF) (Zhou et al., 2017). A reduction of the serum BDNF has been observed in MDD (Zhou et al., 2017), while an increment has been found in FM (Zanette et al., 2014; Deitos et al., 2015; Caumo et al., 2016).

The BDNF has a central role in the clinical picture of dysfunctional neuronal circuits. It strengthens glutamatergic synapses, while it weakens GABAergic synapses (Zhang et al., 2013). In chronic musculoskeletal pain, the serum BDNF was inversely correlated with the SICI and positively correlated with a decreased inhibitory role of the descending pain modulatory system (DPMS). Thereby, it is reasonable to consider the serum BDNF and the motor cortex excitability measured by TMS as probing neural plasticity indexes to improve the comprehension of the neural substrates shared by FM and MDD, as well as their interplay with the inhibitory function of DPMS. The DPMS function is assessed by the conditioned pain modulation (CPM) paradigm. CPM engages activation of a cortically regulated spinal-bulb-spinal loop by the diffuse noxious inhibitory control (DNIC) mechanism, where "pain-inhibits pain" phenomenon (Bars et al., 1979; Yarnitsky, 2010). The neurobiological mechanism involved in the CPM-test includes several neurobiological systems, such as serotoninergic, opioidergic, and noradrenergic (Lindstedt et al., 2011; Baba et al., 2012; Treister et al., 2013). These neurobiological systems are also involved with psychological characteristics of chronic pain, i.e., anxiety (Karg et al., 2011; Horjales-Araujo et al., 2013), depression (Karg et al., 2011), and pain catastrophizing (Horjales-Araujo et al., 2013). Thus, the DPMS is also influenced by psychological characteristics, which explain at least part of the interpersonal variability in pain perception (Racine et al., 2012). According to earlier studies, a higher score on the CS Inventory for chronic pain was positively associated with greater dysfunction of DPMS and correlated positively with serum BDNF (Caumo et al., 2017). While another study with in chronic myofascial pain found a positive association of DPMS with increase in ICF, serum BDNF levels, and disability due to pain (Botelho et al., 2016). At the same way in chronic pain (e.g., FM and chronic myofascial pain) compared to osteoarthritis the SICI was associated with greater dysfunction in DPMS (Caumo et al., 2016). We hypothesize that a deteriorated function of cortical inhibition, the dysfunction of the inhibitory DPMS and serum BDNF can differentiate FM from MDD and HS.

Considering that homeostatic plasticity is the ability of neurons to maintain their levels of excitability within a narrow range, thereby, the disruption of this equilibrium is likely to have a central role in the physiopathology of FM and MDD. Thus, this exploratory study tested the hypothesis that FM patients would present higher disinhibition of the motor cortex compared to

MDD and HS. Another hypothesis was that the dysfunction of the DPMS is related to the disinhibition of the motor cortex evaluated by the SICI in FM. Thus, this study was meant to meet the following objectives: (i) to compare the motor cortex inhibition indexed by the TMS measures SICI and ICF as well as the DPMS to evaluate the neuroplastic changes in FM, MDD, and HS; (ii) to compare the inhibitory function at the cortical level indexed by the SICI and ICF as well as the descending pain inhibitory system between clinical diagnoses (FM and MDD) considering the BDNF adjusted index as a marker of neuroplasticity; (iii) to examine the relationship between the role of DPMS with the BDNF adjusted index despite clinical diagnosis.

MATERIALS AND METHODS

Study Design, Settings, and Subjects

We conducted an exploratory cross-sectional study following the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement. The Ethics Committee Board of the Hospital de Clínicas de Porto Alegre (HCPA) (Institutional Review Board IRB 0000921) approved the protocol. All individuals provided oral and written consent before their engagement in the study.

Participants

The study's subject recruitment and data collection were conducted from August 2017 to July 2018. The sample included literate, right-handed females, aged from 18 to 75 years. The inclusion and exclusion criteria pertaining each one of the three groups (FM, MDD, and HC) are presented in **Figure 1**.

Major depressive disorder subjects were recruited from the Basic Health Unit (BHU). Diagnosis of MDD was performed according to the diagnostic criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (American Psychiatric Association , 2013).

Fibromyalgia patients were recruited by direct contact from the institutional chronic pain clinic, by referrals from other clinic units, and from the BHU at HCPA. They were reached by phone and answered a screening questionnaire. Those who met the inclusion criteria were invited to medical evaluation for history collection and a detailed description of their symptoms. FM diagnosis was established according to the criteria of the American College of Rheumatology (2010–2016) by experienced pain physicians. Those patients who reported at least a pain score on the Numerical Pain Scale (NPS) greater than 5, on most days of the last 3 months, were included.

Volunteers were recruited from the community by advertisement postings in universities and in public places in Porto Alegre city. They answered a structured questionnaire that assessed the following variables: current acute or chronic pain conditions, use of analgesics in the past week, rheumatologic disease, clinically significant or unstable medical psychiatric disorder, history of alcohol abuse in the past 6 months, neuropsychiatric comorbidity, and use of psychotropic substance or drugs. They were excluded when presenting scores higher than 13 on the Beck Depression Inventory (BDI) (Beck et al., 1996; Gomes-Oliveira et al., 2012).

Instruments and Assessment

Outcomes

The primary outcome was the motor cortex inhibitory function indexed by SICI and ICF, and the DPMS assessed by the change on NPS during CPM-test, ranging from 0 to 10 during CPM-test. A secondary outcome was the heat pain threshold (HPT).

Dependent Variables

TMS measures

Left primary motor cortex (M1) measures were assessed through TMS MagProX100 stimulator (MagVenture Company, Lucernemarken, Denmark) with figure-eight coil (MagVenture Company). Ag-AgCl electrodes were placed over the first dorsal interosseous (FDI) belly muscle and in its corresponding tendon on the distal phalanx of the index finger. Then, we recorded the responses to stimuli from the FDI muscle of the right hand by surface electromyography (EMG). Before to start the measures, patients were placed in a comfortable chair and informed about the TMS procedure, including possible sensations that might experience.

To identify motor "hot spot," the coil was placed over the left M1 at 45°angle to the sagittal line tangential to the scalp. To ensure the proper placement of the coil during cortical excitability assessments, researchers marked the site with a soft-tipped pen. To reduce variability, the same researcher performed all TMS assessments. The measures of TMS, such as amplitudes of the single and paired-pulse, and the latency and the measures of the cortical silent period (CSP) were recorded on an Excel spreadsheet.

Motor threshold (MT) defined as the lowest stimulus used to induce 50% of the evoked potentials of resting FDI. Initially, it was set the minimum amplitude of 50 mV peak-to-peak, in at least 5 of 10 (at least 50%) of successive trials. Subsequently, single-pulse TMS protocol with an intensity of 130% of MT was applied to record ten motor evoked potential (MEP). It is a measure that reflects the excitability of the membrane potential of pyramidal neurons in M1 (Nielsen and Norgaard, 2002).

Cortical silent period has been associated with inhibitory network influenced by GABAB-receptors (Werhahn et al., 1999). Likewise, ten CSPs (measured during muscle activity measured on a dynamometer set to approximately 20% of the maximal force) were recorded using an intensity of 130% of the RMT.

Short intracortical inhibition mainly reflects GABA(A) receptor-mediated inhibitory function (Ilić et al., 2002; Cash et al., 2017), while ICF denotes excitatory transmission mostly through the glutamatergic N-methyl-D-aspartate receptor (Ziemann et al., 1998).

We used a paired-pulse TMS protocol to measure SICI and ICF with an interstimulus interval (ISI) to evaluate the SICI equal to 2 and 12 ms for ICF, respectively.

We set individual conditioning stimulus (first) at 80% of the MT to measure the ICF and SICI, while for the test stimulus (second) we set at 130% (Kujirai et al., 1993a). A total of 30 randomized paired-pulse trials were conducted

Major Depressive Disorder (MDD), Fibromyalgia (FM) and Healthy Controls (HC) groups (n=76)

Common inclusion criteria	*literate females, aged from 18 to 75y*		
Common exclusion criteria	*Alcohol, drug and/or psychotropic substance abuse six months prior to the study. Formal contraindication for TMS, such as: pregnancy, individuals with metal implants in the head, shunts, implanted simulators, or defibrillators, history of seizures or active seizure disorder.*		
	***MDD group* (n=18)**	***FM group* (n=17)**	***HC groups* (n=41)**
Inclusion criteria	Diagnosis of MDD, according to Structured Clinical Interview for DSM-V, performed by two independent psychiatrists.	Diagnosis of FM, according to American College of Rheumatology, performed by two independent physicians. Pain levels assessed by visual analogue scale (VAS) ≥ 50mm during most days of the last three months.	Absence of any diagnosis of acute or chronic disease or medication use
Exclusion criteria	Acute or chronic pain Recent use of analgesics or corticosteroids	Other chronic clinical disorder Alcohol, drug and/or psychotropic substance abuse six months prior to the study.	Recent use of analgesics, corticosteroids or medications with recognized effect under central nervous system.
	Clinical sample		***Non - clinical sample***
Recruitment	*Outpatient from primary health - care units or psychiatric outpatient clinic and tertiary hospital (MDD and FM groups, respectively)*		*Newspaper advertisement*

FIGURE 1 | The flow of the study with the inclusion and exclusion criteria of three groups: patients with depression or fibromyalgia and healthy subjects.

(ten for each measure: SICI, ICF, and control stimuli). The units for these parameters were: MEP in mV; SICI and ICF in their ratio to the MEP; and the CSP in milliseconds (ms) (Pascual-leone et al., 1994).

Conditioned pain modulation test (CPM-test)

Conditioned pain modulation test (CPM-test) to evaluate the DPMS a nociceptive tonic stimulus was used, such as immersion of the non-dominant hand in water at a temperature of zero up to 1°C for 1 min. To control if water temperature was maintained in the range between zero to 1°C, a thermostat was used to control temperature variation. The QST procedure was introduced after 30 s of cold-water immersion. The CPM TEST was determined by the difference between the pain score on NPS during the QST at the same time they maintained their dominant hand in cold water immersion (QST+CPM) than the temperature of the subjects felt 6/10 pain on the NPS scale [during the initial period (T0)].

Heat pain threshold

It was assessed through quantitative sensory testing (QST), which uses the method of limits with a computer Peltier-based device thermode (30630 mm) (Schestatsky et al., 2011). Firstly, the thermode was attached to the skin on the ventral position of the mid-forearm. Baseline temperature was set at 32°C and increased at a rate of 1°C/s to a maximum of 52°C. Each participant was instructed to push the button immediately at the moment the stimulation became painful. This trial was composed of three assessments with an ISI of 40 s (Schestatsky et al., 2011), and then an average temperature of the three assessments was calculated. The position of the thermode was slightly altered between evaluations to avoid sensitization of nociceptors.

Independent Variables

Assessments of Demographic and Clinical Characteristics

Standardized questionnaire

A standardized query was used to assess demographic data and medical comorbidities. Patients were requested to provide information about their age, sex, level of education, marital status, and lifestyle habits. They also provided information about their health status, including clinical and psychiatric diagnosis.

Psychiatric diagnosis

Psychiatric diagnosis was based on the Structured Clinical Interview for DSM-V (SCID) applied by a trained psychiatrist. This instrument consists of a semi-structured diagnostic interview created from DSM – V. The answers identify the presence or absence of the symptoms, scored according to the judgment of the evaluator. It is composed of 10 modules, which

can be used in a combined or independent way (2012). In the study, the "A" module was used to diagnose mood episodes (MDDs). The translation and adaptation of this clinical interview into Portuguese language present, in general, good reliability for mood disorders (Del-Ben et al., 2001).

Psychological state and sleep quality

All instruments used were validated for the Brazilian population and the assessments were conducted by two trained evaluators. The following tools were applied: Beck Depression Inventory-II (Beck et al., 1996; Gomes-Oliveira et al., 2012), Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989; Bertolazi et al., 2011), Fibromyalgia Impact Questionnaire (FIQ) (Burckhardt et al., 1991; Marques et al., 2006), State-Trait Anxiety Inventory (STAI) (Kaipper et al., 2010), Brazilian Portuguese Pain-Catastrophizing scale (BP- PCS) (Sehn et al., 2012) and Visual Analog Pain Scale (0 no pain and ten worst pain). Analgesic use was defined by an average of analgesics used per week during the previous month. For data analysis, analgesic use was included as a dichotomous variable (more than 4 days per week or lower than 4 days per week).

BDNF dosage

Blood samples were collected and identified in a standardized manner. The blood samples were obtained in plastic tubes and centrifuged for 10 min at 4,500 rpm at 4°C. Serum was stored at −80°C for further BDNF assay. Serum-mediator concentrations were determined using BDNF (Chemicon CYT306, lower detection limit 7.8 pg/mL; EMD Millipore, Billerica, MA, United States) enzyme-linked immunosorbent assay kits, according to the manufacturer's instructions.

Efforts to Address Potential Sources of Bias

In order to reduce assessment bias, two researchers with the vast clinical expertise in treating outpatients in pain clinic were responsible for making the diagnostics according to pre-specified criteria. A trained psychiatrist with more than 10 years of experience in psychiatric care applied the psychiatric diagnosis based on the SCID-VR. Two evaluators with specific training were responsible for all assessments and for applying the standardized protocol to assess the QST and the CPM-test. To reduce the variability, the same researcher performed all TMS assessments.

Study Size

For type I and II errors of 0.05 and 0.20, respectively, and anticipating partial η^2 of 0.25 for multiple regression analysis, which allows for three predictors (diagnosis, age, and BDNF), a sample size of 60 patients was estimated. It was calculated using the *post hoc statistical power calculator for hierarchical multiple regression* at https://www.danielsoper.com/statcalc/calculator.aspx?id=17. Finally, considering the likely attrition rate and other unexpected factors, the required sample size was determined to be 63 patients.

Statistical Analysis

To assess if the data presented a normal distribution the Shapiro was used – Wilk test. Descriptive statistics were used to summarize the main characteristics of the sample. ANOVA was performed to compare the three groups in the univariate analysis. A MANCOVA was used to test the differences between groups (FM, MDD, and healthy controls) on the multiple outcome controlled for age (Huberty and Morris, 1989). The dependent variables included in the MANCOVA were the cortical excitability [SICI and ICF and the change on the NPS (0–10) during the CPM-test] and HPT (secondary outcomes).

To construct an adjusted surrogate index of factors related to neuroplasticity we created a BDNF adjusted index (dependent variable). For this purpose, we used a multivariate regression model with a stepwise method controlled by multicollinearity. We included in the model the following variables, which can affect the biological process of BDNF secretion: age, analgesic use, classes of antidepressants: [selective serotonin reuptake inhibitors (SSRIs), serotonin–norepinephrine reuptake inhibitors (SNRIs), tricyclic] and anticonvulsants uses (Yes/No).

Another MANCOVA model was used to assess the relationship between the SICI, ICF and the change on NPS during CPM-test (dependent variables) with the BDNF – adjusted index as a covariate, according to diagnosis group. To analyze the correlation between the SICI, change on NPS during CPM-test and BDNF adjusted index the Spearman's correlation analysis was used. All analyses were adjusted by multiple comparisons using the Bonferroni's Multiple Comparison Test. To analyze the data, we used the software SPSS version 22.0 (SPSS, Chicago, IL, United States).

RESULTS

Socio-Demographic, Clinical, and Psychological Characteristics of the Sample

The demographic, the clinical and the psychiatric characteristics are presented in **Table 1**. The analysis showed that compared to controls, both MDD and FM groups are older and have lower educational levels. In comparison to healthy controls, both FM and MDD presented higher levels of trait anxiety and depressive symptoms.

The cortical excitability parameters measured by TMS, psychophysical measures and serum BDNF according to diagnosis group are presented in **Table 2**. We observed that FM group, compared to MDD, showed lower ICF, higher SICI, and higher serum BDNF. However, in this univariate analysis, we did not find a difference in the efficiency of DPMS among groups.

Assessment of Cortical Excitability (SICI, ICF), and HPT According to Groups

A MANCOVA with the cortical excitability (SICI and ICF), the function of DPMS assessed by the change on NPS during CPM-test and HPT parameters as dependent variables and independent age revealed a significant difference between groups (Hotelling's Trace = 0.99, $F = 10.42$, and $P < 0.0001$). FM group compared to healthy controls showed lower HPT, higher SICI, and lower ICF. While the MDD group compared to healthy controls presented

TABLE 1 | Demographic characteristics.

	Fibromyalgia	Major depressive disorder	Healthy control
	(*n* = 17)	(*n* = 18)	(*n* = 28)
Demographic			
Age (years)*	50.5 (±8.7)[a]	45.2 (±15.9)[a]	43.8 (±13.0)[b]
BMI[2]* (Kg/m^2)	31.31 (±7.3)[a]	25.89 (±5.2)[b]	22.78 (±2.9)[b]
Years of education [median (Q25–75)]°	11.0 (6.5–12.5)[a]	11.5 (10.0–16.2)[a]	17.0 (15.7–18.5)[b]
Employed (yes/no)	10/7	14/4	41/0
Smoking (yes/no)	4/13	1/17	1/40
Alcohol use (yes/no)	4/13	7/11	12/29
Clinical and psychiatric			
Use of psychotropic medications (yes/no)	11/6	18/0	
Selective serotonin reuptake inhibitors (SSRIs) (yes/no)	11/6	16/02	
Tricyclic antidepressant (yes/no)	10/7	10/8	
Dual antidepressant (yes/no)	2/15	2/16	
Pregabalin (yes/no)	6/11	0/18	
Antipsychotic (yes/no)	0/17	3/15	
Clinical chronic disease (yes/no)*	11/6[a]	11/7[a]	1/40[b]
Hypertension (yes/no)	10/7	3/15	1/40
Type 2 Diabetes mellitus (yes/no)	1/16	3/15	
Asthma (yes/no)	3/14	1/17	
Psychiatric disorder according to the SCID-V (yes/no)*	12/5	18/0	
Major depressive episode	12/5	18/0	
Generalized anxiety disorder	3/14	1/17	
Beck Depression Inventory – BDI – II*	25.4 (±12.8)[a]	22.3 (±14.4)[a]	3.4 (±4.5)[b]
Pain Catastrophizing Scale – PCS*	33.9 (±12.0)[a]	15.7 (±13.6)[b]	6.1 (±8.0)[c]
State-Trait Anxiety Inventory – STAI*			
STAI – State*	27.3 (±5.3)[a]	28.4 (±3.6)[a]	28.4 (±3.6)[a]
STAI – Trait*	29.35 (±8.1)[a]	27.4 (±4.5)[a]	22.1 (±5.3)[b]
Pittsburgh Sleep Quality Index – PSQI*	12.6 (±4.8)[a]	7.1 (±2.3)[b]	3.74 (±2.0)[c]
Fibromyalgia impact questionnaire (FIQ)	70.4 (±14.5)	–	–
Pain measures			
Analgesic doses used per week median (Q25–75)°	16 (6.5 – 24.5)	–	–
Pain on the VAS (0 – 100 mm) median (Q25–75)°	6.7 (5.8 – 8.2)	–	–
Quantitative sensory testing (QST)			
QST: heat pain threshold*	38.0 (±3.5)[a]	39.8 (±3.7)[a,b]	42.1 (±3.2)[b]
Pressure pain threshold (kg/cm^2)*	2.4 (±1.1)[a]	4.1 (±1.3)[a]	4.1 (±1.3)[b]

*Data area presented as mean and standard deviation (SD) or frequency (n = 63). [2]Body Mass Index. *Comparisons using ANOVA. Post hoc differences from each other are indicated via superscript numbers. °Comparison by Kruskal–Wallis Test, values represented as median and P25 – P75 comparisons using ANOVA. Post hoc differences from each other are indicated via superscript numbers.*

TABLE 2 | Cortical excitability measures assessed by the TMS, psychophysical pain measures, and BDNF.

	Fibromyalgia (*n* = 17)	Major depressive disorder (*n* = 18)	Healthy control (*n* = 28)	*F*	*P*
Motor evocate potential – MEP	1.28 (±0.25)	1.56 (±0.52)	1.45 (±0.43)	1.750	0.183
Intracortical facilitation – ICF	0.33 (±0.23)[a]	1.39 (±1.02)[b]	1.14 (±0.27)[b]	16.268	<0.001
Short intracortical inhibition – SICI	1.03 (±0.50)[a]	0.55 (±0.43)[b]	0.34 (±0.19)[b]	18.701	<0.001
Cortical silent period – CSP	67.21 (±19.51)[a]	48.58 (±12.21)[b]	70.90 (±25.38)[a]	8.168	0.001
BDNF ng/ml	49.82 (±16.31)[a]	14.12 (±8.86)[b]	18.04 (±10.19)[b]	50.246	<0.001
Heat pain threshold (C)	38.03 (±3.45)[a]	39.83 (±3.70)[a,b]	42.11 (±3.23)[b]	7.903	0.001
Change on NPS during CPM TEST	0.22 (±1.37)[a]	−0.87 (±1.49)[a]	−2.54 (±2.46)[b]	11.208	<0.001

Data are presented as mean and standard deviation (SD) (n = 63). Different superscripts (a,b) indicate significant difference among treatment groups after post hoc analysis adjusted by Bonferroni (P < 0.05). Analysis of variance (ANOVA) to compare mean (SD).

larger SICI. However, MDD did not show a difference in the ICF. The age did not correlate with the SICI, ICF, and HPT. The results of this adjusted multivariate model are presented in **Table 3**.

Relationship Between Cortical Excitability and Descendent Pain Modulatory System With the BDNF According to MDD and Fibromyalgia

Factors such as age, antidepressant, anticonvulsant, antipsychotic and analgesics can influence either BDNF secretion, the cortical excitability or the function of descending pain modulating system. They are involved in the neuroplasticity processes. Thus, we construct a BDNF adjusted index as a surrogate marker of the neuroplasticity. For this purpose, the multiple regression analysis was used. The variables antidepressant selective serotonin reuptake inhibitors (SSRIs), anticonvulsants and analgesic use were retained in the model. Age and tricyclic antidepressant were excluded from the model. The mean (SD) of serum BDNF according to SSRIs users and no-users was 27.77 (5.63) vs. 43.91 (25.63), respectively. The R^2 = 0.38, [β coefficient = −14.50, confidence interval (CI) 95% = −26.43 to −2.56, t = 2.48]. Whereas, the mean (SD) of serum BDNF according to anticonvulsant use or not was 60.87 (15.54) vs. 25.37 (18.18), respectively. The R^2 = 0.54, (β coefficient = 22.71, CI 95% = 8.19 to 37.22). The mean (SD) when they used analgesics more than 4 days per week or lower than 4 days per week was 48.03 (17.51) vs. 20.41 (17.79), respectively. The R^2 = 0.54, (β coefficient = 20.94, CI 95% = 9.84 to 32.04).

A MANCOVA model was used to assess the relationship of dependent variables (SICI, ICF, and CPM-test) according to FM and MDD groups adjusted by the BDNF adjusted index. This analysis revealed a significant difference between diagnostic groups (Hotelling's Trace = 0.70, F = 7.06, and

TABLE 3 | Multivariate linear regression model of the cortical excitability and heat pain threshold measures among FM, MDD, and HC groups (n = 63).

Dependent variables	Type III sum of squares	*df*	Mean square	*F*	*P*	Partial eta squared
(A) Main effects						
Corrected model						
Heat pain threshold (°C degree)	33.462[a]	3	11.154	7.269	0.000	0.326
Change on NPS during CPM-test	53.593[b]	3	17.864	5.129	0.004	0.255
Short intracortical inhibition [(SICI), ratio: SICI/test stimulus]	4.255[c]	3	1.418	7.923	0.000	0.346
Intracortical facilitation [(ICF), ratio: ICF/ test stimulus]	11.506[d]	3	3.835	8.972	0.000	0.374

	B	*SEM*	*t*	*P*	*CI 95%*
(B) Beta coefficients					
Dependent variable: heat pain threshold (°C degree)					
Intercept	3.387	0.699	4.844	0.000	(1.97 to 4.79)
Fibromyalgia	−1.816	0.457	−3.973	0.000	(−2.74 to −0.89)
Major depressive disorder	−0.067	0.442	−0.153	0.879	(−0.95 to 0.82)
Healthy controls	0[reference]				
Age	0.017	0.014	1.232	0.224	(−0.01 to 0.05)
Dependent variable: Change on NPS during CPM-test					
Intercept	−3.104	1.053	−2.947	0.005	(−5.22 to −0.98)
Fibromyalgia	2.394	0.689	3.476	0.001	(1.07 to 3.78)
Major depressive disorder	1.335	0.666	2.005	0.051	(−0.06 to 2.67)
Healthy controls	0[reference]				
Age	0.022	0.021	1.055	0.297	(−0.02 to 0.07)
Short intracortical inhibition [(SICI), ratio: SICI/test stimulus]					
Intercept	0.245	0.239	1.026	0.310	(−0.24 to 0.73)
Fibromyalgia	0.698	0.156	4.469	0.000	(0.38 to 1.02)
Major depressive disorder	0.215	0.151	1.428	0.160	(−0.09 to 0.52)
Healthy controls	0[reference]				
Age	0.002	0.005	0.428	0.671	(−0.008 to 0.01)
Dependent variable: intracortical facilitation [(ICF), ratio: ICF/ test stimulus]					
Intercept	1.618	0.369	4.385	0.000	(0.87 to 2.36)
Fibromyalgia	−0.731	0.241	−3.030	0.004	(−1.22 to −0.25)
Major depressive disorder	0.273	0.233	1.169	0.249	(−0.19 to 0.74)
Healthy controls	0[reference]				
Age	−0.011	0.007	−1.496	0.142	(−0.02 to 0.004)

[a] R^2 = 0.326 (Adjusted R^2 = 0.282). [b] R^2 = 0.255 (Adjusted R^2 = 0.205). [c] R^2 = 0.346 (Adjusted R^2 = 0.302). [d] R^2 = 0.374 (Adjusted R^2 = 0.333).

P = 0.001). The BDNF adjusted index did not correlate with the SICI, ICF, nor with the change on NPS during CPM-test. The power of this analysis was 96%. The results of this adjusted multivariate model are presented in **Table 4**. The analysis revealed that the FM group compared to MDD showed a greater dysfunction of the descending pain inhibitory system compared to MDD. However, FM showed higher SICI compared to MDD, in the sense that there is a disengagement between the inhibitory motor cortex function and the descending pain inhibitory system. Whereas, we did not find a difference between groups in the ICF.

Figures 2A,B present the relationships between the SICI and the CPM (primary outcomes) according to FM and MDD. The means were compared using MANCOVA, and *post hoc* adjusted for multiple comparisons using Bonferroni correction (the model is presented in **Table 4**).

Secondary Analysis: Relationship Between SICI, Change on NPS During CPM-Test and BDNF Adjusted Index

The Scatter plots of the raw change on NPS during CPM-test and SICI according to diagnosis group FM and MDD is presented in **Figures 3A,B**, respectively. The change on NPS during CPM-test and SICI in the FM showed a conversely non-parametric correlation. Such non-parametric correlation means that in patients with FM a greater SICI is related to lower scores in the CPM-test or vice – versa. It is important to highlight that lower scores in the CPM-test indicates better function of the DPMS as assessed by the change on the NPS during CPM-test. The correlation coefficient between the scores in the NPS (0 – 10), during CPM TEST and the SICI in the FM was Spearman's Rho = −0.49 and its CI 95% was (−0.78 to −0.03); P = 0.04. The correlation coefficient between the NPS, during CPM-test and the SICI in the MDD was Spearman's Rho = 0.17, and its CI 95% was (−0.32 to 0.59); P = 0.5.

The BDNF adjusted index and change on NPS during CPM-test, despite diagnosis group, showed a positive non-parametric correlation. Such non-parametric correlation means that a greater score in the BDNF adjusted index was correlated with higher dysfunction of the DPMS or vice-versa. The Spearman's Rho = 0.35, and its CI 95% was (0.02 to 0.61); P = 0.03.

DISCUSSION

These results extent evidence that FM displays a deteriorated function of cortical inhibition, indexed by higher SICI parameter compared to MDD and HC. This finding contrasts to our initial hypothesis that it would be decreased. On the other hand, it confirms the assumption that there is greater disinhibition of the DPMS in FM compared to MDD and that it is conversely correlated with the SICI in FM but not in MDD. Also, they showed a positive relationship between the change in the NPS during CPM-test with a measure of neuroplasticity composed by the BDNF adjusted index, despite the clinical diagnostic.

These results demonstrate the relevance of using the motor cortex measures to understand the imbalanced inhibitory or

TABLE 4 | Relationship between intracortical inhibition (SICI and ICF) and descendent pain modulating as assessed by the change on NPS during CPM-test with the BDNF according to diagnosis group (FM and MDD) (n = 35).

	Type III sum of squares	*df*	*F*	Mean square error	*P*	Partial eta squared
Corrected model						
Intracortical facilitation (ICF)	10.755[a]	2	5.377	9.751	0.000	0.38
Change on NPS during CPM TEST	13.812[c]	2	6.906	3.907	0.030	0.20
Short intracortical inhibition (SICI)	2.110[b]	2	1.055	4.786	0.015	0.23
	B	**SEM**	***t***	***P***	***CI 95%***	
Dependent variable: intracortical facilitation [(ICF), ratio: ICF/ test stimulus]						
Intercepted	1.662	0.278	5.978	0.000	(1.09 to 2.23)	
Fibromyalgia	−0.641	0.419	−1.531	0.136	(−1.49 to 0.21)	
Major depressive disorder	0[reference]					
BDNF – adjusted – index	−0.015	0.012	−1.260	0.217	(−0.04 to 0.009)	
Dependent variable: change on NPS during CPM-test						
Intercepted	−0.385	0.498	−0.773	0.445	(−1.39 to 0.63)	
Fibromyalgia	1.760	0.749	2.349	0.025	(0.23 to 3.28)	
Major depressive disorder	0[reference]	.	.	.	.	
BDNF – adjusted – index	−0.021	0.022	−0.970	0.339	(−0.07 to 0.02)	
Short intracortical inhibition [(SICI), ratio: SICI/test stimulus]						
Intercepted	0.659	0.176	3.752	0.001	(0.30 to 1.02)	
Fibromyalgia	0.640	0.265	2.418	0.021	(0.10 to 1.18)	
Major depressive disorder	0[reference]	.	.	.	.	
BDNF – adjusted – index	−0.006	0.008	−0.77	0.443	(−0.021 to 0.01)	

[a] R^2 = 0.379 (Adjusted R^2 = 0.340). [b] R^2 = 0.230 (Adjusted R^2 = 0.182). [c] R^2 = 0.196 (Adjusted R^2 = 0.146).

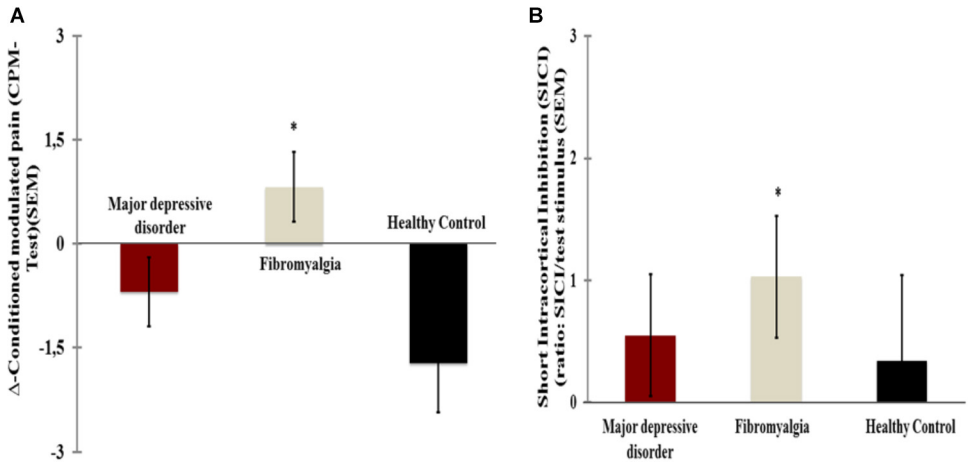

FIGURE 2 | Comparisons between [fibromyalgia (n = 17) and major depressive disorder (n = 18)]. **(A)** Short intracortical inhibition [(SICI) ratio: SICI/test stimulus]. **(B)** Change on Numerical Pain Scale (NPS) during CPM TEST. Error bars indicate standard error of the mean (S.E.M.). *Positioned above the bars indicate differences between groups (fibromyalgia and major depressive disorder) assessed by MANCOVA with *post hoc* Bonferroni's multiple comparison test.

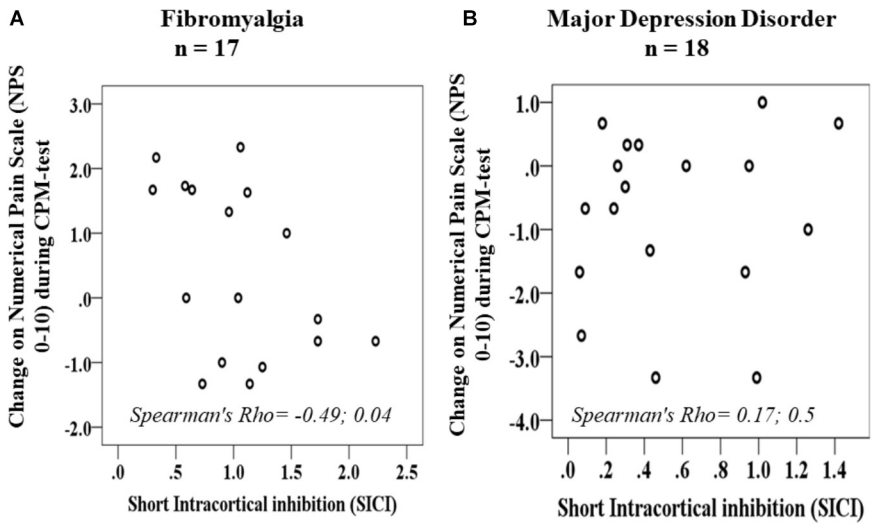

FIGURE 3 | Scatter plots of change on NPS during CPM-test and SICI according to fibromyalgia **(A)** and major depressive disorders **(B)**.

excitatory intracortical neurochemical circuitry to comprehend the underpinning pathophysiology process of FM and MDD. The most relevant result was to show that the increase of SICI is conversely correlated with the change in the NPS during CPM-test only in FM, in the sense that more substantial intracortical inhibition is associated to a higher potency of the descending pain inhibitory system. Here, it is important to realize that negative values in the CPM-test indicate a higher effect of heterotopic stimulus inhibits the test stimulus (i.e., "pain-inhibits pain"), in other words, a better function of the DPMS. Indeed, the change in the SICI may indicate that a disruption of mechanism mediated by inhibitory gamma-aminobutyric acid (GABAergic) occurs interneurons within the primary motor cortex (Di Lazzaro et al., 2006) in FM, at the same time, it indicates an up-regulation phenomenon of intracortical inhibitory networks mediated by GABAA receptors. As previously demonstrated by the tiagabine use (a GABA-reuptake inhibitor) that decreased the SICI (Werhahn et al., 1999) or reduced excitability of intracortical facilitatory systems (van Elswijk et al., 2007). In the same way, earlier studies found that changes in pain pathways that facilitate convergent stimuli are associated with nerve injury, which can induce selective apoptosis of inhibitory GABAergic interneurons (Moore et al., 2002). These processes decreased the inhibitory receptors expression on primary afferent terminals and postsynaptic neurons, and it culminates with a higher perception of repetitive nociceptive stimuli (Staud et al., 2007). Both phenomena comprise a primary mechanism of the pathophysiology of neuropathic pain syndromes, but it also has been pointed out as a possible mechanism in FM, while the CPM-test is a marker of dysfunction of DPMS in chronic pain.

Thus, this increased cortical inhibition could be a compensatory response to contra-regulate the changes induced by the persistent hyperexcitability induced by chronic pain. This hypothesis finds support in a physiological protective reaction, when a prolonged effort at adaptation can result in the dysregulation of other systems, such as autonomic, metabolic, and inflammatory systems. Also, this assumption is substantiated by results of the previous study in chronic pain syndromes (trigeminal neuralgia, poststroke pain syndrome, back pain, and FM), which demonstrated a decreased intracortical inhibition after anodal stimulation, concurrently with the improvement in pain scores (Antal et al., 2010). Likewise, the rTMS induced a long-lasting reduction in the SICI by a possible mechanism mediated by activation of NMDA receptors associated to downregulate hyper excitability associated with the mal-adaptive neuroplasticity (Kobayashi and Pascual-Leone, 2003).

In contrast, observational studies found a decreased SICI in chronic pain (Mhalla et al., 2010). Although the reasons to explain these differences are not clear, it is necessary to consider that the FM is a syndrome with complex pathophysiology involving a neurochemical imbalance in the excitatory and inhibitory mechanisms mediated by multiple systems (i.e., GABAergic, glutamatergic, noradrenergic, serotonergic, etc.). Likewise, it is possible that these incongruences between the results of studies may be explained by the sample characteristics related to the severity of disorders, the medication used, disability, comorbidities, etc.

This difference in the SICI between FM and depression suggest that these two disorders may have considerable overlaps in neuroplasticity processes, but the TMS patterns together with the distinct standard of dysfunction in the DPMS as well in the BDNF serum indicate that these two disorders have substantial differences in their pathophysiological mechanisms. This way, these results give support to understand differences in the cardinal symptoms of each one of these two disorders (i.e., fatigue, migratory pain, pain catastrophizing, etc.), which are prototypical symptoms of FM. Besides, it can help to personalize the therapeutic approach. Despite the absence of a FDA-approved neuromodulation protocol targeted to patients with comorbidity MDD and FM, the effectiveness of neuromodulatory techniques (i.e., tDCS and TMS) has been supported by consistent evidence to treat both FM and depression (Kauffmann et al., 2004; Antal et al., 2010; Brunoni et al., 2011; Marie, 2014; Fagerlund et al., 2015; Castillo-Saavedra et al., 2016; Cheng et al., 2018; Karina do Monte-Silva et al., 2019). Considering that these two disorders are frequently overlapped, it poses a considerable challenge to decide if it would be better to stimulate the M1 or the dorsal lateral prefrontal cortex for the treatment of pain accompanied by depression.

However, the interpretation of the SICI measure should consider that it is a low-threshold inhibition test elicited during paired-pulse TMS, which does not influence the descending corticospinal volleys (Di Lazzaro et al., 1999) neither alters spinal reflexes (Kujirai et al., 1993b). Thus, the SICI might be a tool to identify the cortical inhibition. However, the values of SICI should not be interpreted in isolation, since it is influenced by several factors such as the precision of measurement, the mechanism of pain (i.e., inflammatory vs. neuropathic pain), the severity of pain and the psychotropic medications, etc., Accordingly, the M1 may be an entry port to assess the complex pain-related neural network, also to understand the M1 role to inhibit or interrupt pain signals and as a measure to evaluate the cortical process on the neuroplasticity of chronic pain. This hypothesis finds support in a previous study, which showed that in FM a strong M1–ventral lateral thalamus connectivity at baseline predicted a more significant reduction in pain across tDCS treatment (Cummiford et al., 2016). A similar effect was found when the invasive chronic motor cortex stimulation decreased the thalamic hyperactivity in patients with thalamic pain (Tsubokawa et al., 1993). Aligned with this assumption, we found extensive literature showing that the transcranial stimulation (i.e., tDCS and TMS) might improve the disrupted neurochemical processes in chronic pain (Cheng et al., 2010).

Both FM and MDD are disorders associated with chronic stress that share several symptoms and sometimes co-exist in the same patient. In this study greater serum levels of BDNF in FM compared to MDD and healthy controls was observed. The current finding is in agreement with the previous studies that found higher serum BDNF in FM (Deitos et al., 2015), whereas in MDD there is a vast literature showing lower serum BDNF (Karege et al., 2002). Thus, these results suggest that this neurotrophic factor could be a correlate marker of distinct mechanisms that underpin the pathophysiology of FM and MDD. The BDNF is secreted by the microglia and it participates in the adaptative and protective neuroplasticity processes. However, in chronic pain, this mechanism is likely to be overactivated and raise a counterproductive response, in the sense that the microglia-to-neuron communication might attenuate the pain inhibitory action of GABA and the glycine receptor-mediated inhibition (Ferrini and De Koninck, 2013). This hypothesis is supported by compelling evidence that BDNF is a ubiquitous pain mediator at many levels of the nervous system. Given this, it would be hard to conclude that the generation of BDNF is a compensatory mechanism specific to chronic pain conditions (i.e., FM, chronic inflammatory, and neuropathic pain). Although in the current study we have not observed a significant relationship between the BDNF adjusted index and the inhibitory function of motor cortex indexed by the SICI, this may be explained by an error type II, since other studies found a significant correlation between the SICI and BDNF. Indeed, the adjusted index of BDNF was used as a measure to summarize several factors associated with the BDNF secretion (i.e., antidepressant, anticonvulsants, age, etc.). Thereby, we need parsimony in the interpretation of this inter-relationship, since this study is an exploratory and approximately 65% of FM presented psychiatric diagnosis and used psychotropic medications (tricyclic antidepressant, pregabalin, etc.). Thereby, it is possible that intermediates confounding factors did not have entirely controlled (Cole and Hernán, 2002) or a non-significant *p*-value after adjustment reflects the absence of a relevant effect these relationships in this sample (Baguley, 2004).

Also, we identified a more substantial dysfunction in DPMS in FM compared to MDD, and the BDNF adjusted index was positively correlated with the disinhibition of DPMS. This result

is aligned with an earlier study that found similar results related to the increase of serum BDNF and the disruption of the inhibitory function of DPMS in chronic musculoskeletal pain (Botelho et al., 2016; Caumo et al., 2016). Likewise, it has been demonstrated that the increased synthesis of BDNF in the nociceptive pathways is responsible for increasing neuronal excitability by causing disinhibition in dorsal horn neurons in the spinal cord (Ferrini and De Koninck, 2013). In the brain, the BDNF has been shown to activate descending nociceptive facilitation in the nucleus raphe magnus (Zhang et al., 2013). Also, at the periaqueductal gray neurons, the BDNF has a central role for orchestrating descending antinociception (Lewis et al., 2012; Nijs et al., 2014). Thus, the disinhibition of the motor cortex indexed by SICI together with the dysfunction of the descending antinociceptive mechanisms is an essential feature of FM, which we did not observe in depression. However, it is difficult to determine whether the deterioration of cortical inhibition, changes in BDNF and the dysfunction of DPMS may be an underlying pathophysiological mechanism of the disease or a disease severity state-dependent phenomenon.

Although our results are likely to help to advance in the comprehension of changes in measures related to neuroplasticity in the two disorders, our results are correlational and do not allow a causality relationship. This study has some limitations: Firstly, TMS consists of an indirect neurophysiological measure intended to assess the activity of a neurotransmitter system. Second, psychiatric disorders remain a potential confounding factor, and they cannot have been adequately controlled, even if anxiety, depression, catastrophizing pain behavior, and psychiatric diagnosis were assessed. More than 70% (12/17) of FM group suffered from any mental illnesses. However, this finding is expected, as the emotional burden is a recurrent finding in chronic pain syndromes. Third, we must address the effect of psychotropic medicines under cortical excitability because the regular prescription of these medicines deliberates the proper treatment of both disorders. Nevertheless, it is critical to mention that different changes in cortical excitability produced using psychotropic medications might produce distinctive outcomes in acute and long-term use. Fourth, we performed this study only in females, and it is essential to consider a sex effect in pain perception and modulation. Likewise, our results must be carefully interpreted, given the design of this study. Further, research designed to address differences and similarities between FM and MDD are required to claim if the neuroplastic and neurophysiological measures constitute differential biomarkers of their pathophysiological mechanisms.

CONCLUSION

In conclusion, these findings support the hypothesis that in FM a deteriorated function of cortical inhibition, indexed by a higher SICI parameter, and a lower function of the DPMS, together with higher levels of BDNF indicate that FM has different pathological substrates from depression. They suggest that an up-regulation phenomenon of intracortical inhibitory networks associated with a disruption of the DPMS function occurs in FM.

ETHICS STATEMENT

This study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. The protocol was approved by the Ethics Committee Board of the Clínicas Hospital de Porto Alegre (Institutional Review Board IRB 0000921). All individuals provided oral and written consent before their engagement in the study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Electroacupuncture Pretreatment Ameliorates PTSD-Like Behaviors in Rats by Enhancing Hippocampal Neurogenesis via the Keap1/Nrf2 Antioxidant Signaling Pathway

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Electroacupuncture (EA) pretreatment is a clinically useful therapy for several brain disorders. However, whether and via which exact molecular mechanisms it ameliorates post-traumatic stress disorder (PTSD) remains unclear. In the present study, rats received EA stimulation for seven consecutive days before exposure to enhanced single prolonged stress (ESPS). Anxiety-like and fear learning behaviors; hippocampal neurogenesis; the expression of nuclear factor erythroid 2-related factor 2 (Nrf2), Kelch-like ECH-associated protein 1 (keap1), and heme oxygenase 1 (HO-1); and the activity of AMP-activated kinase (AMPK) were evaluated at 14 days after ESPS. EA pretreatment improved hippocampal neurogenesis and ameliorated anxiety-like behaviors in ESPS-treated rats. EA pretreatment also increased the expression of Nrf2 and HO-1 and the activity of AMPK. Furthermore, Nrf2 knockdown by a short hairpin RNA affected anxiety-like behaviors and expression of neuroprotective markers (BDNF, DCX) in a manner similar to ESPS alone and dampened the neuroprotective effects of EA pretreatment. In contrast, Keap1 knockdown increased the expression of HO-1, improved hippocampal neurogenesis, and alleviated PTSD-like behaviors. Altogether, our results suggest that EA pretreatment ameliorates ESPS-induced anxiety-like behaviors and prevents hippocampal neurogenesis disruption in a rat model of PTSD possibly through regulation of the keap1/Nrf2 antioxidant defense pathway.

Keywords: electroacupuncture, pretreatment, post-traumatic stress disorder, hippocampus, keap1/Nrf2

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a fear-based biopsychosocial disorder that is caused by exposure to severely traumatic events, such as sexual violence, war, and life-threatening accidents (Careaga et al., 2016). PTSD severely affects patients' quality of life and social stability. Epidemiological studies have revealed that more than half of the world's population experiences stressful events and that the lifetime and average prevalence of PTSD are 6.8% and 8%, respectively (McLaughlin et al., 2015; Liu et al., 2017). Currently, PTSD is mainly treated with psychological

intervention and drugs. However, none of these approaches have proved to constitute a satisfying method to improve the clinical symptoms of these patients (Sripada et al., 2016). Thus, the development of novel treatment strategies is an urgent need in PTSD.

It is well known that the hippocampus plays a crucial role in memory processes and fear conditioning responses—highly relevant phenomena to the pathogenesis of PTSD (Corcoran and Maren, 2001; Kheirbek et al., 2013; Girardeau et al., 2017). Physical or psychosocial stressors may induce morphological changes in the hippocampus, including reduced neurogenesis and loss of pyramidal neurons (Cohen et al., 2014). Patients with PTSD have reduced hippocampal volumes (Filipovic et al., 2011) and animal models of PTSD show suppressed hippocampal cell proliferation, inhibited neurogenesis, and increased neuronal apoptosis (Peng et al., 2013). It has been suggested that brain oxidative damage may be the cause of hippocampal structure and function impairments (Miller and Sadeh, 2014). In the hippocampus of PTSD-like rats, total reactive oxygen species (ROS), peroxynitrite and superoxide levels are elevated (Wilson et al., 2013). Moreover, cranially irradiated superoxide dismutase (SOD)-deficient mice exhibit decreased neurogenesis in the hippocampus due to long-term ROS accumulation (Yuan et al., 2015). Interestingly, preclinical studies have also shown that antioxidants can improve hippocampal neurogenesis and attenuate anxiety-like behaviors in animal models (Bouayed et al., 2009; Moustafa, 2013). One may then hypothesize that inhibiting oxidative damage may improve hippocampal function and be beneficial for treating PTSD.

Nuclear factor erythroid 2-related factor 2 (Nrf2) and its antioxidant signaling pathway are key regulators of neuroprotection against oxidative stress (Mitsuishi et al., 2012; Ahuja et al., 2016). The activation of Nrf2 confers protective effects to many central nervous system diseases (Gan and Johnson, 2014), and Nrf2 silencing in the brain increases anxiety-like behaviors in rats (Khalifeh et al., 2015). Under physiological conditions, Nrf2 binds to Kelch-like ECH-associated protein 1 (keap1) in the cytoplasm. However, upon exposure to ROS, Nrf2 disassociates from keap1 and then translocates to the nucleus, where it activates the transcription of several antioxidant enzymes genes, including *SOD* and heme oxygenase 1 (*HO-1*) (Kubben et al., 2016; Cai et al., 2017), which protect hippocampal neurons against oxidative stress (Lee et al., 2015). A recent study has shown that Nrf2 activation in lipopolysaccharide-treated mice or cells is accompanied by an increase in the phosphorylation of AMP-activated kinase (AMPK) and inhibition of AMPK blocked aucubin-induced expression of Nrf2 and its downstream effector HO-1 (Qiu et al., 2018). Taken together, inhibition of oxidative damage via activation of the keap1/Nrf2 antioxidant defense pathway may improve hippocampal function and be beneficial for treating PTSD.

Electroacupuncture (EA) combines the advantages of acupuncture and electrophysiological stimulation. Its beneficial effects, including during the pretreatment phase, have been demonstrated for several neuropsychiatric disorders. Although the precise mechanisms remain to be fully elucidated, enhanced neurogenesis and synaptic plasticity, and prevention of oxidative damage and inflammation have been described following EA pretreatment in previous studies (Feng et al., 2010; Chen et al., 2012, 2016). It has been suggested that the keap1/Nrf2 pathway may be involved in the protective effect of EA (Yu et al., 2014, 2015). Activation of both AMPK and HO-1 is required for EA to exert its therapeutic effects (Yu et al., 2014, 2015). Thus, we hypothesize that EA pretreatment is beneficial for the prevention of PTSD and that the keap1/Nrf2 pathway might play a role in this process. In this study, we sought to determine whether EA pretreatment could ameliorate stress-associated behaviors in a rat model of PTSD. We also aimed to examine whether changes in the activity of keap1/Nrf2 and its downstream antioxidative proteins in the hippocampus could be involved in the EA pretreatment effects.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (280–320 g) were obtained from the Animal Center of Fourth Military Medical University (FMMU). Rats were group-housed (four per cage) and maintained at 20–25°C on a 12-h light/dark daily cycle with free access to food and water. The experiment procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Use and Protection Committee of the FMMU.

Experimental Design

Experiment I

To determine the effect of EA pretreatment on PTSD, after 7 days of acclimatization, 32 rats were randomly divided into four groups (eight rats per group): control, EA, enhanced single prolonged stress (ESPS), and EA + ESPS. Rats in the control group were given false stimulation (EA treatment without electricity) for seven consecutive days (30 min every day) and then housed in their home cage for 2 weeks. Rats in the EA and EA + ESPS groups were stimulated with EA at a frequency of 2/15 Hz and an intensity of 1 mA for seven consecutive days (30 min every day). Rats in the ESPS group were given false stimulation (EA treatment without electricity) for seven consecutive days and then subjected to ESPS. The researchers performing the behavioral testing were blinded to the animals' group allocation. Then, hippocampal neurogenesis, the expression of BDNF, DCX, Nrf2, and HO-1, as well as the activity of AMPK were evaluated.

Experiment II

To investigate the role of the Nrf2 antioxidant signaling pathway in the hippocampus in the neuroprotective effect of EA pretreatment, 64 rats were randomly divided into eight groups (eight rats per group): Scramble, Scramble + ESPS, Scramble + EA + ESPS, shNrf2, shNrf2 + ESPS, shNrf2 + EA, shNrf2 + EA + ESPS, and shkeap1 + ESPS. Rats in the Scramble, Scramble + ESPS, and Scramble + EA + ESPS groups were injected with scramble short hairpin RNA (shRNA) (a

lentivirus carrying scramble shRNA), while rats in the shNrf2, shNrf2 + ESPS, shNrf2 + EA, shNrf2 + EA + ESPS, and shkeap1 + ESPS groups were injected with Nrf2-shRNA or keap1-shRNA lentivirus in the hippocampus. Two weeks after the lentivirus injection, rats in the Scramble + EA + ESPS, shNrf2 + EA and shNrf2 + EA + ESPS groups received EA stimulation (2/15 Hz, 1 mA) for seven consecutive days (30 min every day), while rats in the Scramble, Scramble + ESPS, shNrf2, shNrf2 + ESPS and shkeap1 + ESPS groups received false stimulation as described in Experiment I, after which rats in the Scramble + ESPS, Scramble + EA + ESPS, shNrf2 + ESPS, shNrf2 + EA + ESPS, and shkeap1 + ESPS groups were subjected to ESPS. The behavioral tests were performed 2 weeks after ESPS. Then, hippocampal neurogenesis and gene expression were determined as in Experiment I.

EA Treatment

Electroacupuncture treatment was performed as described previously (Feng et al., 2010; Wang et al., 2014). Briefly, rats were maintained on a platform (10 cm × 10 cm × 50 cm) without anesthesia and the acupoint "Bai hui" (GV20), which is located at the intersection of the sagittal midline and the line linking the rat ears, was stimulated for 30 min daily (frequency: 2/15 Hz, waveform: dilatational wave, intensity: 1 mA) by using the G6805–2 EA instrument (No. 227033; Qingdao Xinsheng Ltd.). False stimulation was performed at the same acupoint without electricity.

Enhanced Single Prolonged Stress (ESPS)

Enhanced single prolonged stress was performed in accordance with our previous study (Wang H. N. et al., 2015). Rats were restrained for 2 h and then immediately exposed to forced swimming in water (diameter: 24 cm, height: 50 cm, water temperature: 24°C) for 20 min and then exposed to diethyl ether until they lost consciousness after recuperation for 15 min. Finally, rats were exposed to a single electric foot shock (1 mA for 4 s) after 30 min of recovering in a rectangular box with stainless steel rods floors and aluminum and acrylic walls.

Behavioral Tests

All the behavioral tests began 14 days after ESPS exposure. Rats were acclimatized to the separate experimental room for at least 30 min prior to each test, and all experiments were conducted under low light conditions in order to minimize anxiety effects. The area was cleaned with 75% ethanol between tests. Besides, the open field test was conducted prior to the elevated plus maze test on the same day, while the fear conditioning test was performed 24 h after the elevated plus maze test.

Open Field Test (OFT)

According to previous studies (Sullivan et al., 2003; Missault et al., 2019), the OFT was used to assess anxiety-related behavior and locomotor activity in an open field arena (47 cm × 47 cm × 50 cm). Rats were gently placed in one of the arena's corners and recorded from the soundproof box, which was illuminated by a red fluorescent light (30 W). After each trial, the apparatus was cleansed with 75% ethanol. The time spent in the center of the arena that could be used for the quantification of rodent anxiety and exploratory drive was recorded for 10 min and analyzed by using an automatic system (Top Scan, Clever Sys Inc., United States). Rats with high levels of innate anxiety typically avoid the center arena and spend more time in close proximity to the walls. The total distance moved in the open field was also measured to analyze general locomotor activity according to previous work (Yang et al., 2016).

Elevated Plus Maze Test (EPMT)

The EPMT has been well validated for detecting anxiety-like behavior. The Plexiglas apparatus (Dig Behav, Ji Liang Co., Ltd., Shanghai, China) consisted of two opposite open arms (50 cm × 10 cm) and two enclosed arms (50 cm × 10 cm, surrounded by a 40 cm-high black wall) elevated 50 cm above the floor. Rats were placed in the center area of the maze for each individual trial lasting 5 min. The number of entries and the time spent in the open arms were recorded and measured by an automatic analyzing system (Top Scan, Clever Sys Inc., United States) and used as indices of anxiety (Kim et al., 2016). The area was cleansed with 75% ethanol between tests.

Fear Conditioning Test

The experiments were performed in the shock chamber (Context A: a rectangular box with stainless steel rod floors and aluminum and acrylic walls) and a neutral test context (Context B: a rectangular box with white acrylic floor and acrylic frame roof) as described previously (Nie et al., 2014; Liu et al., 2016). For shock application, rats were placed into the shock chamber (Context A) for 16 s and then exposed to a tone (3 min, 80 dB, 9 kHz); then, rats received a foot shock (4 s, 1.0 mA) and remained in the shock chamber for 60 s, after which they were returned to their home cages. The contextual fear conditioning test was performed 4 h after shock application. Rats were placed in the same chamber where they were trained (Context A) but without a tone or foot shock application for 3 min, and then they were immediately returned to their home cages. The auditory-cued fear test was performed 24 h later; rats were placed in the chamber (Context B) for 3 min, and received a neutral tone (3 min, 80 dB, 9 kHz). Then, rats remained in the test chamber (Context B) for another 60 s. The freezing behavior was recorded and analyzed by using a computerized automatic analyzing system (Freezing Scan, Clever Sys Inc., Reston, VA, United States).

RNA Isolation and Quantitative Real-Time PCR (qRT-PCR) Analysis

Rat brains were rapidly dissected on ice after sacrificed and the hippocampus were isolated and placed on dry ice immediately. Then, total RNA was isolated by using the RNAiso Plus kit according to the manufacturer's protocol (Takara Bio, Inc., Otsu, Japan). The quality and quantity of RNA were analyzed by spectrophotometry using the Multiskan Sky Microplate Spectrophotometer (Thermo Fisher Scientific, Inc.). The optical density at 260/280 nm of RNA in all samples ranged from 1.8 to

2.0 and the concentration ranged from 400 to 1,000 ng/μl. For real-time PCR analysis, 1,000 ng of RNA from each sample was reverse transcribed (37°C for 15 min, 85°C for 5 s, and 4°C for 10 min) by using the Prime-Script RT Reagent Kit (Takara Bio, Inc., Otsu, Japan). The cDNA was quantified by using real-time PCR with SYBR Premix Ex Taq^{TM} II (RR820Q, Takara) according to the manufacturer's protocol on a Bio-Rad IQ5 Real-Time PCR Detection System. The primers used for real-time PCR were designed and synthesized by Takara Biotechnology Co., Ltd. (Dalian, China) according to the target mRNA sequence (*GAPDH*: NM_017008.4; *Nrf2*: NM_031789.2; and *keap1*: NM_057152.2). The primer sequences were as follows: *GAPDH*, forward and reverse: 5′-CCAATGTGTCCGTCGTGGATCT-3′ and 5′-GTTGAAGTCGCAGGAGACAACC-3′, respectively; *Nrf2*: forward and reverse, 5′-TTGGCAGAGACATTCCCA TTTGTA-3′ and 5′-GAGCTATCGAGTGACTGAGCCTGA-3′, respectively; and *keap1*: forward and reverse, 5′-CATCGGCATCGCCAACTTC-3′ and 5′-GCTGGCAGT GTGACAGGTTGA-3′, respectively. Each reaction consisted of 2 μl of cDNA product from the diluted reverse transcription reaction (5×), 0.5 μM of primers (forward and reverse), and 12.0 μl of SYBR Green real-time PCR master mix. The reactions were incubated in a 96-well plate and the two-step qRT-PCR program used was as follows: 1 cycle of 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, 60°C for 30 s, and 1 cycle of 95°C for 15 s, and then maintained at 4°C. Subsequently, the relative changes in gene expression of Nrf2 and keap1 were normalized to the level of GAPDH mRNA of each sample (Jo et al., 2014; Chen et al., 2019) and analyzed by $2^{-\Delta\Delta Cq}$ method and shown relative to expression in control samples (Livak and Schmittgen, 2001).

Lentivirus

A recombinant lentivirus coding for green fluorescent protein (GFP) carrying Nrf2-shRNA, keap1-shRNA or non-silencing RNA were purchased from Shanghai Genechem Co., Ltd. (Shanghai, China). Based on rat Nrf2 and keap1 mRNA sequences (accession number: NM_031789; NM_057152), three shRNA targeting different regions of Nrf2 mRNA (shNrf2-a, shNrf2-b, shNrf2-c), keap1 mRNA (shkeap1-a, shkeap1-b, shkeap1-c) and a scrambled non-silencing control shRNA (scramble) were generated. The targeting sequences were as follows: shNrf2-a, 5′-CTGGATGAAGAGACCGGAGAA-3'; shNrf2-b, 5′-GAGAAAGAATTGCCTGTAATT-3′; shNrf2-c, 5′-CCAAAGAGCAGTTCAATGATT-3′; shkeap1-a, 5′-GGA CAAACCGCCTTAATTCTT-3′; shkeap1-b, 5′-CAGCAGAACT GUACCTGTTTT-3′; shkeap1-1c, 5′-GGGCGTGGCTGTCCT CAATTT-3′; and scramble, 5′-TTCTCCGAACGTGTCACGT-3′.

Primary Culture of Astrocytes and Transfection

As described previously (Zhou et al., 2017), astrocytes were harvested from the brains of newborn rats. Briefly, the hippocampus of newborn rats was isolated and single-cell suspensions were obtained. Then, the cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and plated in 75-cm^2 flasks coated with poly-L-lysine and incubated at 37°C with 5% CO_2 for 10 days. After shaking at a speed of 240 rpm, astrocyte-enriched cultures were obtained. To screen and validate the efficiency of the produced lentivirus in targeting the Nrf2 and keap1 mRNAs, astrocytes were infected with the lentiviral particles according to the previous study (Li et al., 2012). Three days after transfection, the transfected astrocytes were harvested for real-time PCR and immunohistochemistry analysis.

To verify the identity of the astrocytes and the lentivirus infection, astrocytes were fixed in 4% paraformaldehyde at 4°C for 0.5 h. After washes in phosphate-buffered solution (PBS), the astrocytes were incubated with mouse anti-glial fibrillary acidic protein (GFAP, ab7260, 1:1,000, Abcam) diluted in buffer (1% w/v bovine serum albumin and 0.3% Triton in PBS), overnight at 4°C. The cells were washed and incubated with fluorescent secondary antibodies (Alexa Fluor 594 donkey anti-rabbit IgG, R37119, 1:1,000, Invitrogen) for 2 h and then incubated with DAPI for 20 min to stain the cellular nuclei. The preparations were analyzed under a laser-scanning confocal microscope (FV-1000, Olympus, Tokyo, Japan) and the silencing efficiency of shNrf2 and shkeap1 was assessed by real-time PCR. Ultimately, the shNrf2-a and shkeap1-c constructs were selected for the following *in vivo* experiments.

Stereotaxic Surgery and Microinjections

As described previously (Uzakov et al., 2015), the concentrated titer-matched lentiviral suspension (5 μl, 2.5 μl for each side) was injected into the dentate gyrus (DG) (AP −3.0 mm; L ±1.8 mm; H 3.6 mm from dura) by an automatic nanoinjector at a rate of 0.25 μl/min. Then the syringe needle was left in position for 5 min after delivery to prevent reflux.

Immunohistochemistry and Bromodeoxyuridine (BrdU) Detection

As described previously (Peng et al., 2018), rats were injected with 100 mg/kg BrdU (B5002, Sigma-Aldrich) for three consecutive days intraperitoneally. Twenty-four hours after the last BrdU injection, rats were anesthetized (chloral hydrate solution, i.p. 40 mg/kg) and then perfused with 4% paraformaldehyde in PBS. Brains were removed and transferred to 30% sucrose in PBS for 1 week to dehydrate and then sectioned (16-μm brain coronal sections) with a cryostat and mounted on gelatinized slides.

To assess cell proliferation, the brain sections were incubated in hydrochloric acid (2 N) at 37°C for 30 min and washed in 0.1 M sodium borate (pH 8.5) and PBS. Then, the sections were incubated with the primary antibody: anti-NeuN (ab177487, 1:500, Abcam) and anti-BrdU (B8434, 1:500, Sigma-Aldrich) at 4°C overnight. Next, they were incubated with secondary antibodies: Alexa Fluor 594 donkey anti-mouse (R37115, 1:1,000, Invitrogen) and Alexa Fluor 488 donkey anti-rabbit IgG (A-21206, 1:1,000, Invitrogen) or Alexa Fluor 405 goat anti-rabbit IgG (A-31556, 1:1,000, Invitrogen). The sections were observed under a fluorescence microscope and the BrdU-labeled cells were quantified.

For the immunofluorescence detection of DCX and cell-specific analysis of Nrf2, brain sections were incubated

with primary antibody DCX (D9943, 1:1000, Sigma-Aldrich) and NeuN (ab177487, 1:500, Abcam), Nrf2 (AB413, 1:100, Sigma-Aldrich) and NeuN (MAB377B, 1:200, Sigma-Aldrich) or Nrf2 and GFAP (ab10062, 1:500, Abcam) overnight at 4°C after blocking with 5% (w/v) bovine serum albumin for 1 h. Subsequently, sections were incubated with Alexa Fluor 405 goat anti-rabbit IgG (A-31556, 1:1,000, Invitrogen) and Alexa Fluor 488 donkey anti-rabbit IgG (A-21206, 1:1,000, Invitrogen) or Alexa Fluor 594 donkey anti-rabbit IgG (R37115, 1:1,000, Invitrogen) for 2 h with or without DAPI at room temperature. The images were captured by the Olympus FV1200 confocal laser-scanning microscope (Olympus, Japan) and processed for further quantification. The percentage of double labeling of NeuN/Nrf2 and GFAP/Nrf2 was quantified by using Image-pro Plus 6.0 analysis software.

According to previous unbiased stereology protocol (Hill et al., 2018), every sixth section throughout the entire rostral caudal extent of the hippocampus was used to determine the number of BrdU-labeled cells or DCX^+ in the DG. The number of BrdU+ or DCX+ cells was counted under a fluorescence microscope (Olympus, Japan) in the area of the subgranular zone (SGZ). The total number of positive cells in the SGZ of the hippocampal DG was estimated by multiplying the number of cells counted in every sixth section by six. For each marker, four animals were analyzed. All counts were performed by an experimenter blinded to the purpose of the study.

Western Blot Analysis

Rat brains were rapidly dissected on ice after sacrificed and the hippocampus were isolated and washed with ice PBS. Then tissues were cut into pieces and weighed and lysed in a buffer composed of 62.5 mM Tris-HCl, 2% w/v sodium dodecyl sulfate, 10% glycerol, 50 mM dithiothreitol, and 0.1% w/v bromophenol blue. The protein concentrations of the supernatant were determined by the BCA Protein Assay Kit (Invitrogen). Then, samples were separated by 10% polyacrylamide gel (40 μg of total protein per lane) and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat dried milk and incubated with anti-Nrf2 (ab137550, 1:1000, Abcam), HO-1 (ab13248, 1:2000, Abcam), DCX (D9943, 1:1000, Sigma-Aldrich), AMPKα (2603, 1:1000, Cell Signaling), p-AMPKα (2535, 1:1000, cell signaling), BDNF (ab205067, 1:1000, Abcam), keap1 (ab139729, 1:1000, Abcam), and β-actin antibodies (ab8227, 1:5000, Abcam) overnight at 4°C. The membranes were then washed and incubated with secondary antibodies for 1 h at room temperature. Immunoreactive bands were detected using the Super Signal West Pico Chemiluminescent Substrate (34077; Thermo Fisher Scientific, Inc.) and visualized on X-ray films. Quantifications were performed by using densitometric analysis implemented in the Bio-Rad QuantityOne1-D Analysis Software.

Statistical Analyses

Data are presented as mean ± standard deviation and statistical analyses were performed by using SPSS 19.0 software (SPSS Inc., Chicago, IL, United States). Experimental data were subjected to Levene's test and the Kolmogorov–Smirnov test for equality of variances and normal distribution, and then subjected to two- or one-way analysis of variance (ANOVA) with Tukey's *post hoc* test was performed to compare means of different groups and $P < 0.05$ was defined as the threshold for statistically significance.

RESULTS

EA Pretreatment Ameliorates Anxiety-Like and Fear Learning Behaviors in ESPS-Treated Rats

First, we determined the effect of EA pretreatment on PTSD (**Figure 1A**). Two-way ANOVA revealed that ESPS and EA treatment did not induce any motor impairment in rats because there were no differences in the total distance traveled in the OFT in both stress (control vs. ESPS, $F = 0.486$, $P = 0.489$) and EA treatment (control EA vs. EA, $F = 0.275$, $P = 0.603$) factors (**Figures 1B,D**). There were significant differences in the time spent in the center in the OFT for the stress factor ($F = 13.606$, $P < 0.01$, **Figure 1E**), as well as in the number of entries in the open arms ($F = 8.532$, $P < 0.01$) and the time spent in the open arms ($F = 10.653$, $P < 0.01$) in the EPMT (**Figures 1C,F,G**). However, there were no significant differences for the EA treatment factor neither in the time spent in the center in the OFT ($F = 1.009$, $P = 0.321$) nor as in the number of entries in the open arms ($F = 1.298$, $P = 0.261$) and the time spent in the open arms ($F = 1.058$, $P = 0.310$) in the EPMT. In addition, there were significant differences for the stress factor in the freezing times both during the contextual fear ($F = 5.816$, $P < 0.05$, **Figure 1H**) and the cued fear conditioning ($F = 10.686$, $P < 0.05$, **Figure 1I**) tests. Furthermore, there were also significant differences for the EA treatment factor in the freezing times during the contextual fear test ($F = 4.474$, $P < 0.05$). *Post hoc* comparisons further showed that ESPS markedly reduced the time spent in the center in the OFT as well as the number of entries into the open arms and the time spent in the open arms in the EPMT (ESPS vs. control, $P < 0.05$). Further, EA pretreatment increased values of these parameters (EA + ESPS vs. ESPS, $P < 0.05$). Additionally, rats in the ESPS group showed a significant increase of freezing time in both contextual fear and cued fear conditioning tests when compared to the control group ($P < 0.05$). EA pretreatment significantly decreased freezing times and enhanced fear learning in ESPS-treated rats (EA + ESPS vs. ESPS, $P < 0.05$, **Figures 1H,I**). These results suggest that EA pretreatment could ameliorate anxiety-like behaviors and fear learning in ESPS-treated rats.

EA Pretreatment Increased Neurogenesis and BDNF Expression in the Hippocampus of ESPS-Treated Rats

As shown in **Figure 2**, there were significant differences for the stress factor in the number of BrdU-positive ($BrdU^+$) ($F = 15.455$, $P < 0.01$, **Figures 2A,B**) and DCX-positive (DCX^+) ($F = 78.030$, $P < 0.01$, **Figure 2C**) cells in the DG of the hippocampus. There were also significant differences for the EA treatment factor in the number of $BrdU^+$ cells ($F = 5.181$,

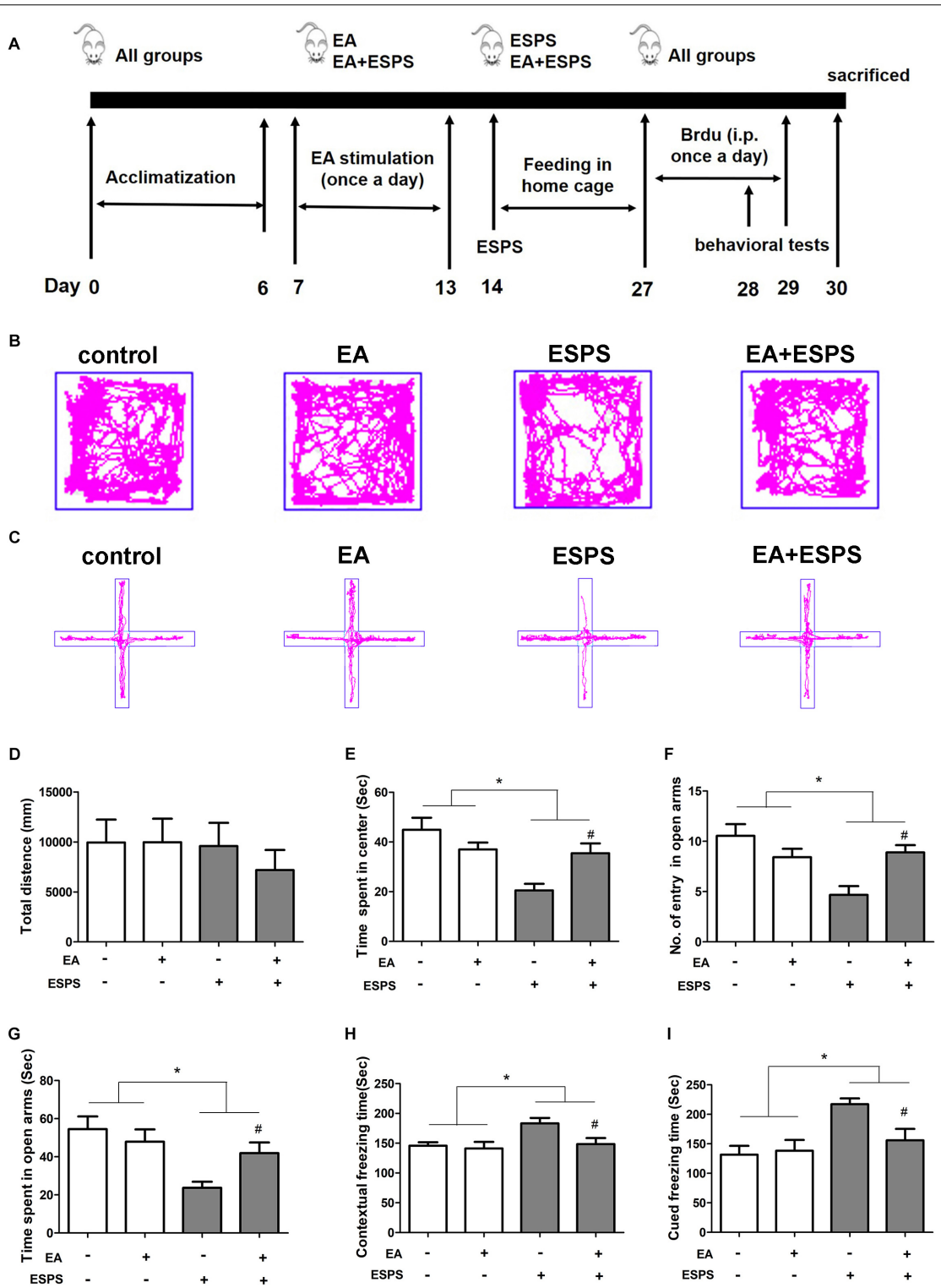

FIGURE 1 | Electroacupuncture pretreatment ameliorates anxiety-like and fear learning behavior in ESPS-treated rats. **(A)** Timeline of the EA pretreatment, ESPS exposure, and behavioral testing in Experiment I. All animals were subjected to 1 week of adaptation, and then EA (EA at 1 mA in intensity and 2/15 Hz in frequency or EA without current) was administered once a day from days 7 through 13. ESPS treatment was performed on day 14. BrdU was administered once a day from days 27 through 29 by intraperitoneal injection and behavioral alterations were measured from days 28 through 30 before rats were sacrificed. **(B)** Real-time movement traces in the open field. **(C)** Elevated plus maze movement traces. **(D)** Quantification of the total distance traveled in the open field box. **(E)** The time spent in center of the open filed box. **(F)** Numbers of entries in the open arms of the elevated plus maze test. **(G)** The time spent in the open arms of the elevated plus maze test. **(H)** Freezing time of the contextual fear response. **(I)** Freezing time of the cued fear response. $^{*}P < 0.05$, $^{\#}P < 0.05$ vs. ESPS.

$P < 0.05$, **Figure 2B**) but not DCX$^+$ cells ($F = 1.256$, $P = 0.274$, **Figure 2C**) in the DG. Meanwhile, there were significant differences of BDNF and DCX expression (**Figures 2D,E**) for both the stress ($F_{BDNF} = 44.189$, $P < 0.01$; $F_{DCX} = 39.830$, $P < 0.01$) and EA treatment ($F_{BDNF} = 10.566$, $P < 0.01$; $F_{DCX} = 7.902$, $P < 0.05$) factors. *Post hoc* comparisons further showed that ESPS decreased the number of BrdU$^+$ and DCX$^+$ cells in the hippocampus (ESPS vs. control, $P < 0.01$), and EA pretreatment prevented this damage induced by ESPS (EA + ESPS vs. ESPS, $P < 0.05$). In addition, ESPS stimulation markedly decreased the expression of DCX and BDNF (ESPS vs. control, $P < 0.01$), while EA pretreatment effectively

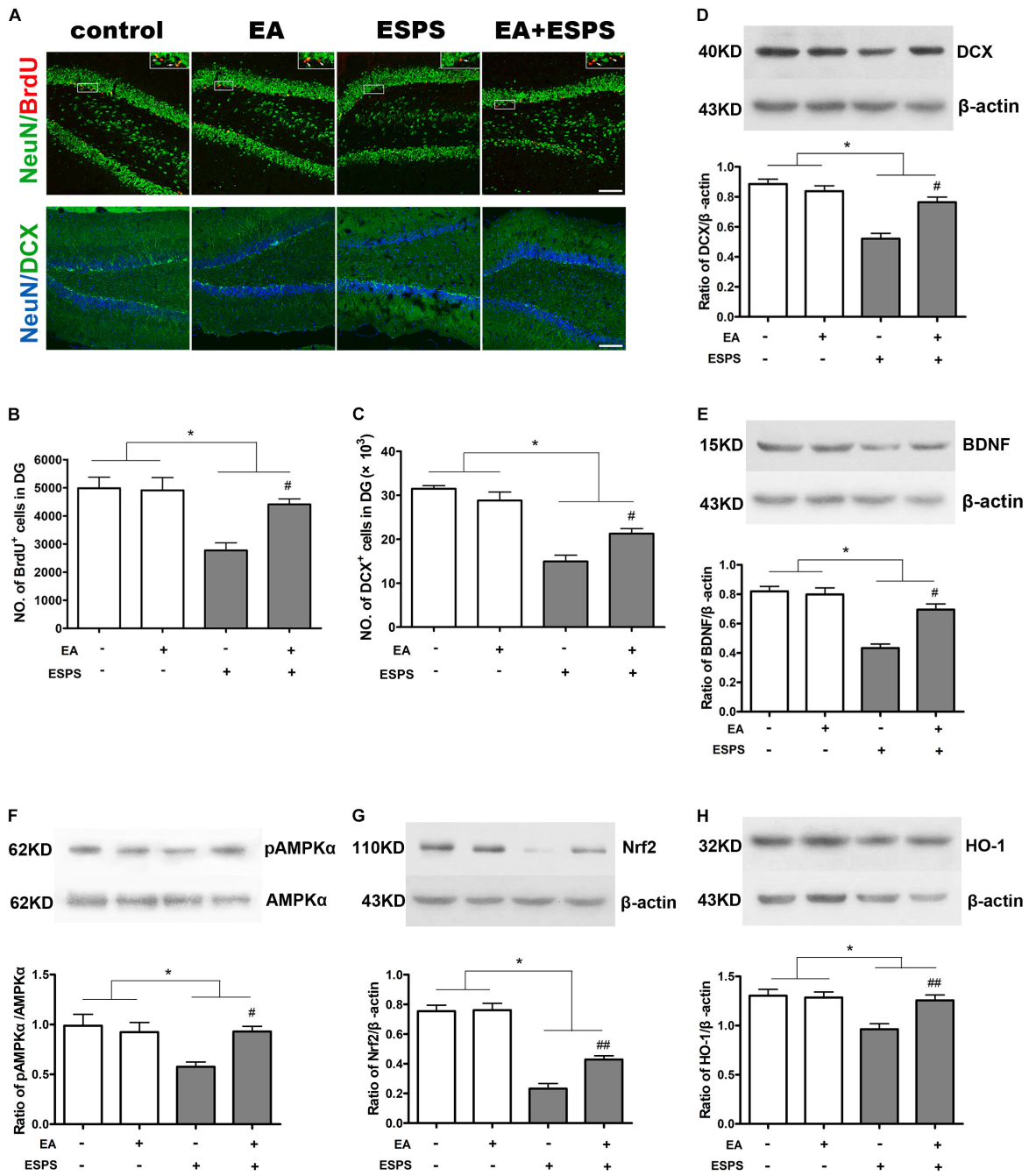

FIGURE 2 | Electroacupuncture pretreatment improved hippocampal neurogenesis and the expression of BDNF, DCX, and Nrf2 and their related genes in ESPS-treated rats. **(A)** Microphotographs and **(B,C)** histograms of BrdU-positive proliferating cells and of the DCX-positive immature neurons in the dentate gyrus. **(D–H)** Representative immunoblots and densitometry analysis of **(D)** DCX, **(E)** BDNF, **(F)** p-AMPK, **(G)** Nrf2, and **(H)** HO-1 expression in the total hippocampus of control, EA, ESPS, and EA + ESPS groups. $^*P < 0.05$, $^{\#}P < 0.05$ vs. ESPS, $^{\#\#}P < 0.01$ vs. ESPS. Bar: 100 μm.

reversed these changes (EA + ESPS vs. ESPS, $P < 0.05$). These results suggest that EA pretreatment is effective in preventing impairments of the hippocampal neurogenesis in ESPS-treated rats.

EA Pretreatment Influences the Expression of Nrf2 and HO-1 and the Activity of AMPK in the Hippocampus of ESPS-Treated Rats

There were significant differences for the stress factor in the expression of Nrf2 ($F = 13.443$, $P < 0.01$, **Figure 2G**) and HO-1 ($F = 10.367$, $P < 0.01$, **Figure 2H**) and the activity of AMPK ($F = 5.883$, $P < 0.05$, **Figure 2F**). There were also significant differences for the EA treatment factor in the expression of Nrf2 ($F = 7.487$, $P < 0.05$) and HO-1 ($F = 5.766$, $P < 0.05$). However, there was no significant difference for the EA treatment factor in the activity of AMPK ($F = 3.054$, $P = 0.094$). Meanwhile, there were significant differences for the stress factor in the percentage of the $NeuN^+Nrf2^+$ ($F = 41.861$, $P < 0.05$, **Figures 3A,B**) and $GFAP^+Nrf2^+$ ($F = 18.449$, $P < 0.05$, **Figures 3A,C**) cells in DG. *Post hoc* comparisons further showed that ESPS decreased the activity of AMPK and the expression of Nrf2 and HO-1 (ESPS vs. control, $P < 0.05$), as well as the double labeling of NeuN and Nrf2 or GFAP and Nrf2, which were prevented by EA pretreatment (EA + ESPS vs. ESPS, $P < 0.05$). These results indicate that the neuroprotective effect of EA pretreatment in ESPS-treated rats could be mediated by the activation of AMPK/Nrf2 antioxidant pathway.

Nrf2 Knockdown in the Adult Hippocampus Blocks the Protective Effects of EA Pretreatment on ESPS-Treated Rats

In order to establish the contribution of Nrf2 on the effects of EA pretreatment (**Figure 4A**), we knocked down Nrf2 or Keap1 in the DG by bilateral injections of LV-GFP shRNA (shNrf2 or scramble) (**Supplementary Figure S1**). No significant differences in the total distance traveled in the open-field arena were observed ($F_{7,56} = 0.210$, $P = 0.982$, **Figures 4B,D**). However, there were significant differences in the time spent in the central area of the OFT ($F_{7,56} = 4.937$, $P < 0.01$, **Figure 4E**), the entry numbers ($F_{7,56} = 4.938$, $P < 0.01$, **Figures 4C,F**) and the time spent ($F_{7,56} = 4.041$, $P < 0.01$, **Figure 4G**) in the open arms of the EPMT, as well as the freezing time in the fear conditioning test (contextual freezing time: $F_{7,56} = 2.831$, $P < 0.05$, **Figure 4H**; cued freezing time $F_{7,56} = 4.195$, $P < 0.01$, **Figure 4I**). EA pretreatment successfully reversed all the changes associated to ESPS exposure (Scramble + EA + ESPS vs. Scramble + ESPS, $P < 0.05$). However, the protective effect of EA pretreatment was dampened by Nrf2 knockdown (shNrf2 + EA + ESPS vs. Scramble + EA + ESPS, $P < 0.05$). Moreover, downregulation of keap1 itself also ameliorated the deficits observed in behavior and in the hippocampal neurogenesis of ESPS-treated rats (shkeap1 + ESPS vs. Scramble + ESPS, $P < 0.05$). These results indicate that the keap1/Nrf2 antioxidant pathway may play a role in the anti-anxiety effects of EA pretreatment.

The Neuroprotective Effect of EA Pretreatment Is Inhibited by Downregulation of Nrf2

shNrf2 treatment effectively downregulated Nrf2 ($F_{7,24} = 7.425$, $P < 0.01$, **Figure 5D**) and HO-1 expression ($F_{7,24} = 6.559$, $P < 0.01$, **Figure 5E**), decreased AMPK ($F_{7,24} = 12.33$, $P < 0.01$, **Figure 5C**) activity, and dampened the effects of the EA pretreatment on the expression of BDNF ($F_{7,24} = 16.85$, $P < 0.01$, **Figure 5A**) and DCX ($F_{7,24} = 7.484$, $P < 0.01$, **Figure 5B**) in PTSD rats. In addition, shkeap1 significantly decreased keap1 protein level (**Figure 5F**) but increased BDNF and DCX expression in ESPS-treated rats, when compared to the Scramble + ESPS group ($P < 0.05$).

We also examined adult DG neurogenesis after lentiviral treatment. Significant differences in the number of $BrdU^+$ ($F_{7,24} = 8.057$, $P < 0.01$, **Figures 5G,H**) and DCX^+ ($F_{7,24} = 6.339$, $P < 0.01$, **Figures 5G,I**) cells were observed. EA pretreatment increased the number of $BrdU^+$ and DCX^+ cells (Scramble + EA + ESPS vs. Scramble + ESPS, $P < 0.01$). This effect was inhibited by Nrf2 downregulation (shNrf2 + EA + ESPS vs. Scramble + EA + ESPS, $P < 0.05$). Moreover, there were more $BrdU^+$ and DCX^+ cells in the shkeap1 + ESPS group than in the Scramble + ESPS group ($P < 0.01$).

DISCUSSION

In the present study, we provide first evidence that EA pretreatment can ameliorate the behavioral deficits and the impairments of hippocampus neurogenesis observed in ESPS-treated rats. EA pretreatment increased the expression of Nrf2, HO-1, and BDNF as well as the phosphorylation level of AMPK in the hippocampus of ESPS-treated rats. However, knockdown of Nrf2 in the hippocampus before the EA pretreatment dampened the therapeutic effects of the EA pretreatment, while keap1 knockdown in the hippocampus displayed similar neuro-protective effects to those observed for the EA pretreatment. We suggest that EA pretreatment may represent an effective preventive strategy for PTSD and its beneficial effects may involve the keap1/Nrf2 antioxidant pathway.

Acupuncture is a traditional Chinese medicine technique typically included in the field of complementary and alternative medicine (Langevin et al., 2011). It is widely used for managing chronic pain (Zhao et al., 2017; Hershman et al., 2018) and more recently has been suggested as a treatment for PTSD (Grant et al., 2018). The general sympatho-inhibitory effects of acupuncture depend on needle location and acupuncture type. Although research has not been as exhaustive as in manual acupuncture, EA has also been used in the treatment of several disorders as an improvement of traditional acupuncture. EA (particularly at low frequency) has been shown to produce more widespread effects in the brain than manual acupuncture, as

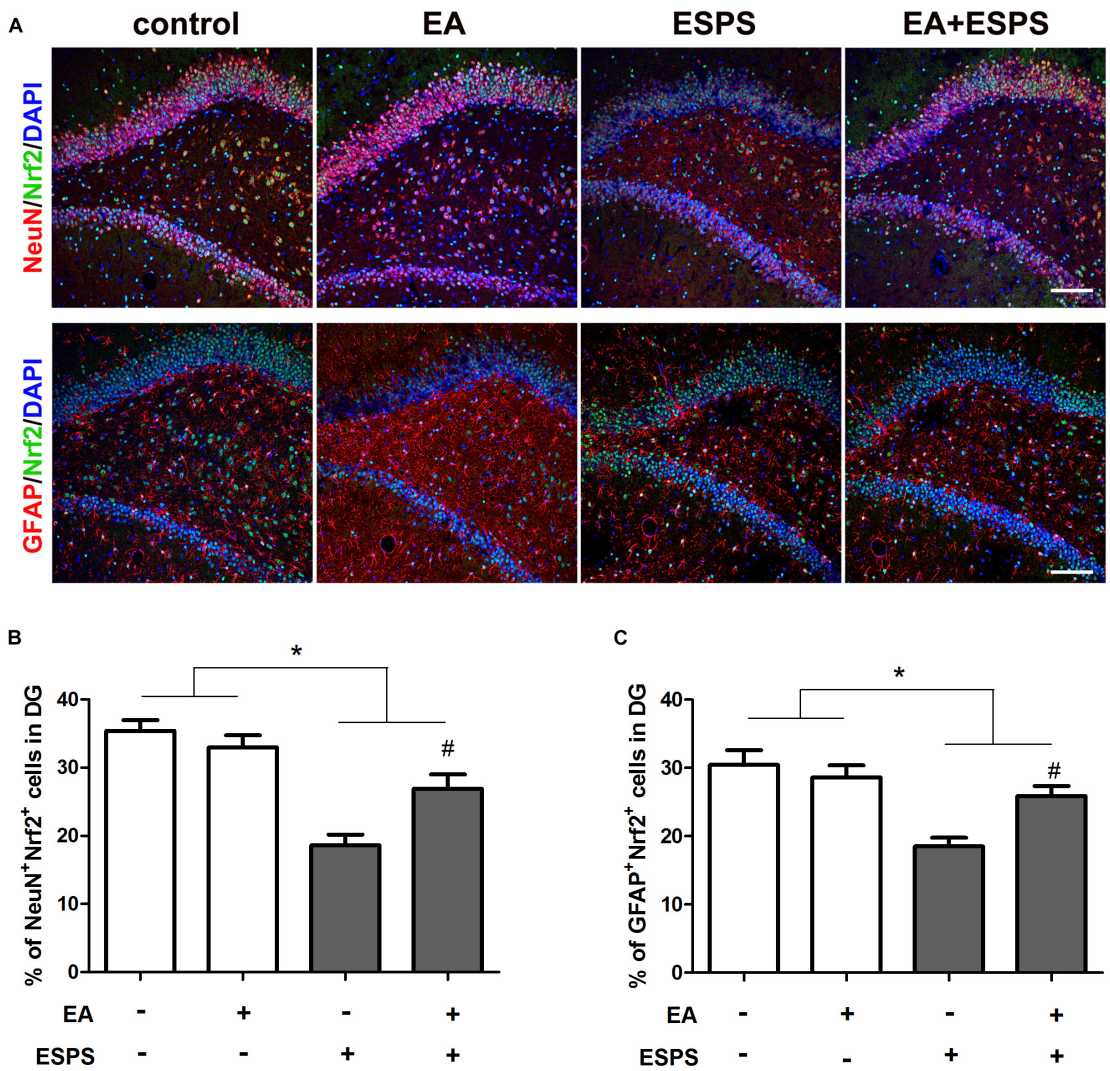

FIGURE 3 | Electroacupuncture pretreatment improved hippocampal Nrf2 expression in both neurons and astrocytes in DG of ESPS-treated rats. **(A)** Microphotographs and **(B,C)** histograms of the percentage of NeuN/Nrf2 and GFAP/Nrf2 double labeling cells in the DG. $^{*}P < 0.05$, $^{\#}P < 0.05$ vs. ESPS. Bar: 100 μmm.

assessed by functional magnetic resonance imaging (Napadow et al., 2005). Studies have found that EA influences the activity of the autonomic nervous system, as well as of prefrontal and limbic brain structures, including the amygdala, hippocampus, and the hypothalamus. EA has also been described to influence hypothalamic–pituitary–adrenal axis (HPA) function and plasma cortisol levels (Song et al., 2012; Mucuk et al., 2013; Le et al., 2016), which are involved in the pathophysiology of PTSD. In general, most of the preclinical and clinical studies on EA have focused on its role as a therapeutic agent. However, an ideal therapeutic scenario would involve prevention of symptoms even before its appearance. It is found that EA pretreatment (2/15 Hz) applied at the GV20 ("Bai hui") conferred neuroprotection against cerebral ischemia (Wang et al., 2012; Zhao et al., 2015), and EA pretreatment with the same frequencies applied at Fengfu and Fengchi (GB20) provided neuroprotective effects during craniocerebral tumor resection (Lu et al., 2010). Meanwhile, 2/15 Hz EA pretreatment also reduced glutamate toxicity and exerted antiapoptotic effects on experimental stroke rats (Zhou et al., 2013; Zhu et al., 2013). More importantly, Wang et al. (2009) showed that EA pretreatment applied at the GV20 with 2/15 Hz conferred neuroprotection against cerebral ischemia by stimulating the production of 2-AG and AEA in the brain and activating CB1R. Consistent with the above implications, the present study indicates that EA pretreatment applied at the GV20 with 2/15 Hz for seven continuous days before rats are exposed to ESPS ameliorate PTSD-like behavior, including the increases in anxiety and the alterations in fear learning typically observed in these animals. Altogether, our data support the notion that EA pretreatment may be an effective therapy for the prevention of PTSD.

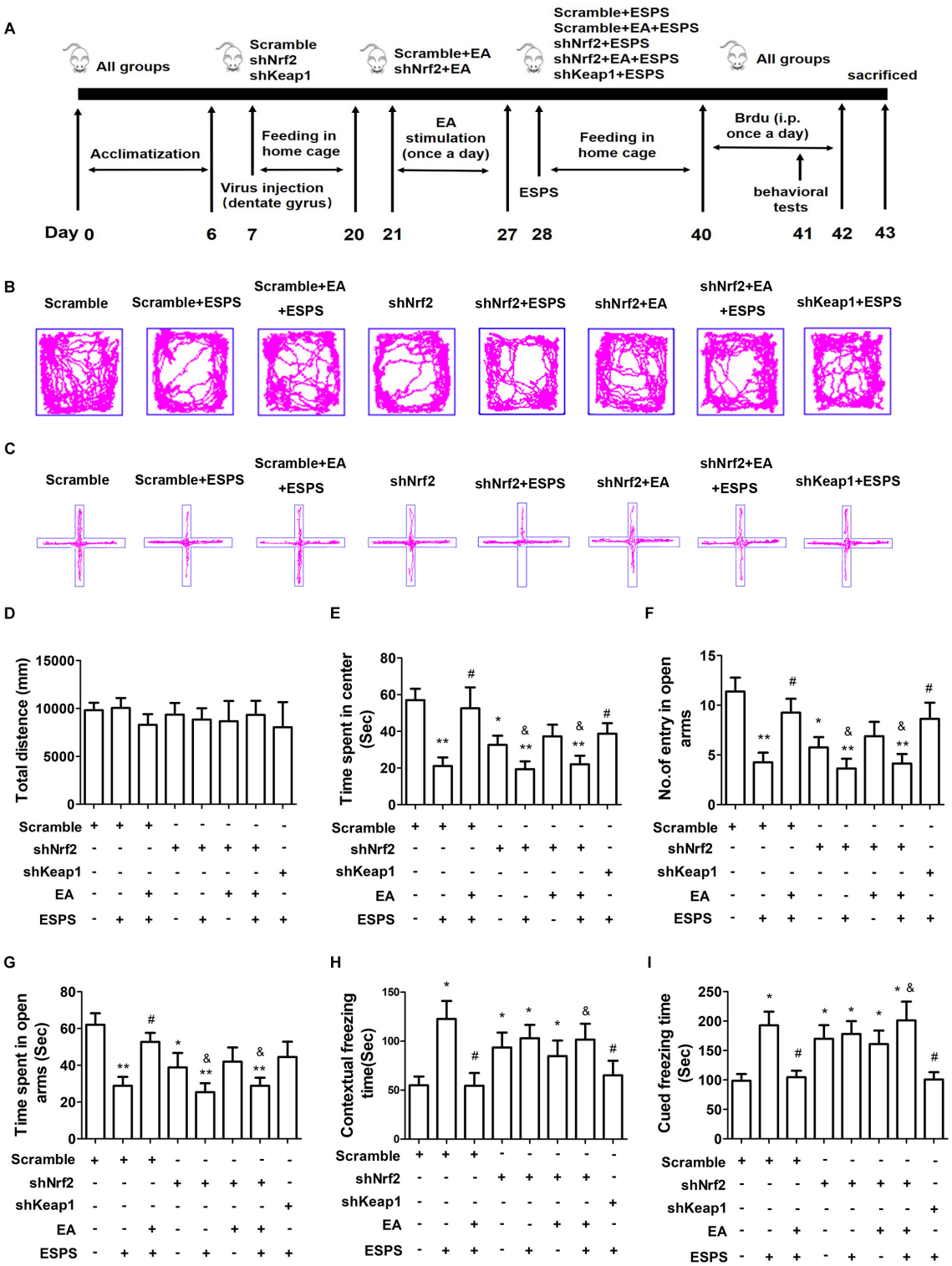

FIGURE 4 | Nrf2 knockdown in the adult hippocampus inhibits the protective effects of electroacupuncture pretreatment on behavior of ESPS-treated rats. **(A)** Timeline of the stereotactic injection, EA pretreatment, ESPS administration and behavioral testing in Experiment II. Lentivirus with shRNA (Scramble or shNrf2 or shkeap1) were stereotaxically injected into the bilateral hippocampal dentate gyrus on day 7, EA was administered once a day from day 21 to 27. ESPS treatment was performed at day 28. BrdU was administered once a day from day 40 to 42 and behavioral alterations were measured from day 41 to 43 before rats were sacrificed. **(B)** Real-time movement traces in the open field. **(C)** Real-time movement traces in the Elevated plus maze. **(D)** Quantification of the total distance traveled in the open field test. **(E)** The time spent in center of the open filed box. **(F)** Numbers of entries in the open arms of the elevated plus maze test. **(G)** The time spent in the open arms of the elevated plus maze test. **(H)** Freezing times in the contextual fear. **(I)** Freezing times in the cued fear conditioning tests. $^{*}P < 0.05$ vs. Scramble, $^{**}P < 0.01$ vs. Scramble, $^{\#}P < 0.05$ vs. Scramble + ESPS, $^{\&}P < 0.05$ vs. Scramble + EA + ESPS.

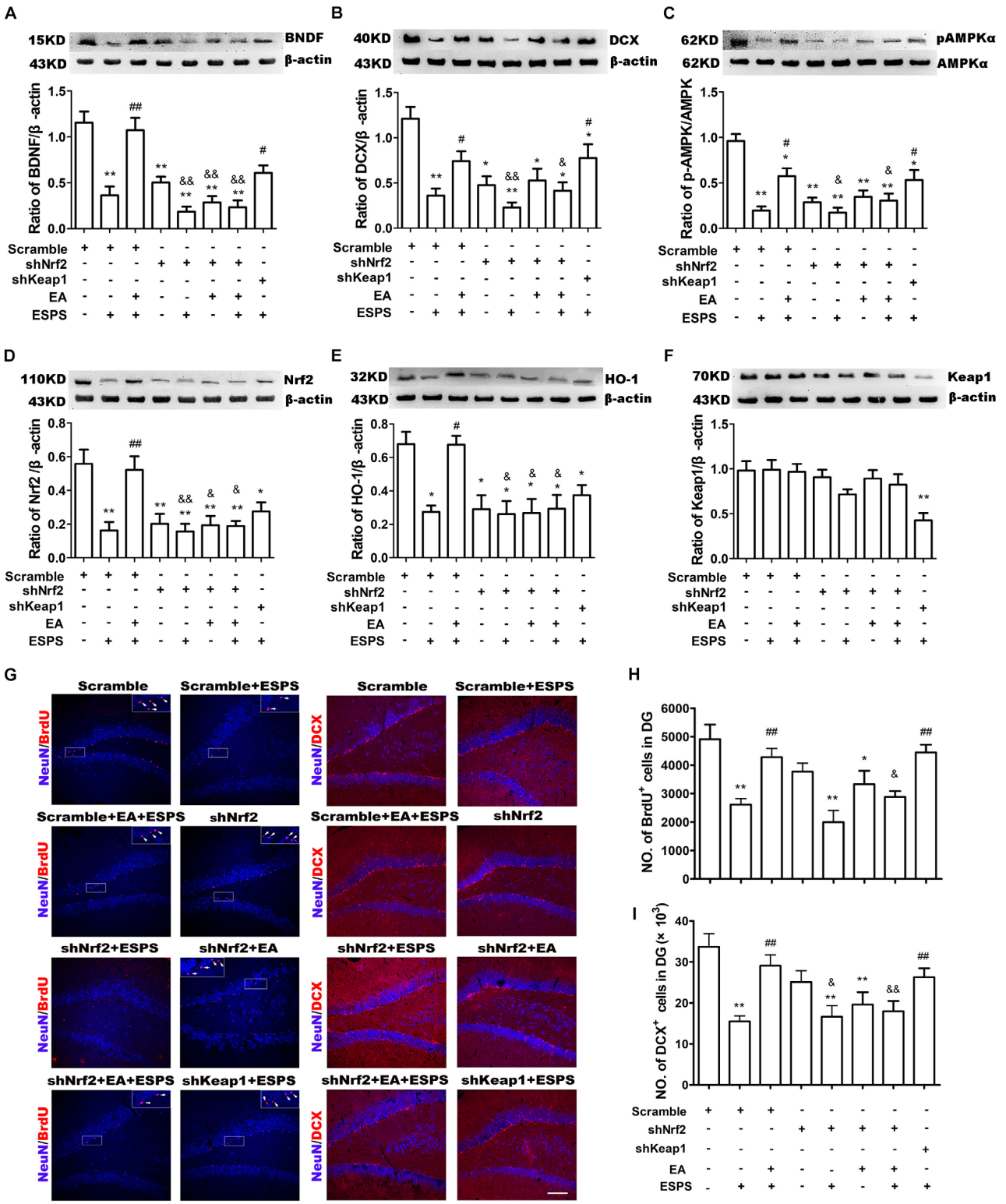

FIGURE 5 | Knockdown of Nrf2 with shRNA lentivirus in the hippocampus dampened the neuroprotective effect of EA pretreatment on ESPS-treated rats. **(A–F)** Representative immunoblots and densitometry analysis of **(A)** BDNF, **(B)** DCX, **(C)** p-AMPK, **(D)** Nrf2, **(E)** HO-1, and **(F)** keap1 in the total hippocampus of Scramble, Scramble + ESPS, Scramble + EA + ESPS, shNrf2, shNrf2 + EA + ESPS, and shkeap1 + ESPS treated groups. **(G)** Microphotographs and **(H,I)** histograms of the BrdU-positive proliferation cells and of the DCX-positive immature neurons in the dentate gyrus. $^{*}P < 0.05$ vs. Scramble; $^{**}P < 0.01$ vs. Scramble; $^{\#}P < 0.05$ vs. Scramble + ESPS; $^{\#\#}P < 0.01$ vs. Scramble + ESPS; $^{\&}P < 0.05$ vs. Scramble + EA + ESPS; $^{\&\&}P < 0.01$ vs. Scramble + EA + ESPS. Bar: 100 μm.

It is well known that hippocampal neurogenesis is involved in anxiety. Factors impairing hippocampal neurogenesis may induce disruption of mood and anxiety (Hill et al., 2015; Miller and Hen, 2015). In addition, hippocampal neurogenesis seems to be involved in anti-anxiety drug effects and promotion of hippocampal neurogenesis have been shown to hold the potential to alleviate anxiety and mood disorders (Jin et al., 2016; Mohammad et al., 2017). Recent anatomical and functional evidence indicates a dissociation of the dorsal and ventral regions of the hippocampus. It was found that the dorsal hippocampus is critical for learning and memory performance, while the ventral hippocampus is involved in anxiety and behavioral inhibition (Bannerman et al., 2014; Kempadoo et al., 2016; Floriou-Servou et al., 2018). In line with this, a growing body of evidences also supports a role for adult hippocampal neurogenesis in both the cognitive functions that are thought to be mediated by the dorsal hippocampus and emotional regulation that has been attributed to the ventral hippocampus (Wu and Hen, 2014; Zhang et al., 2018). Thus, neurogenesis in both the dorsal and ventral DG might be involved in the pathogenesis of PTSD. However, it should also be recognized that dorsal and ventral DG are not completely isolated from each other. Instead, they can interact via several routes (Fanselow and Dong, 2010). Meanwhile, it has been shown that hippocampal neurogenesis can be modulated indirectly by altering the *in vivo* hippocampal microenvironment (Monje et al., 2003; Seki, 2003). In our present study, although viral shRNA interference was delivered to dorsal DG, the decreased expression of Nrf2 or Keap1 was observed in the whole hippocampus (**Supplementary Figure S1**). The effects of viral shRNA interference that was delivered to other regions of the hippocampus were still needs further investigations.

Since Nrf2 is involved in the regulation of hippocampal neurogenesis and in the neuroprotective effects of EA (Wang X. R. et al., 2015; Robledinos-Anton et al., 2017), we further measured the activity of AMPK and expression of Nrf2 and HO-1 in the hippocampus. We also observed the involvement of Nrf2 or Keap1 in the neuroprotective effects of EA pretreatment by shRNA knockdown experiments. We showed that EA pretreatment promoted neurogenesis, as suggested by an increase in the number of $BrdU^+$ cells and DCX^+ immature neurons, and increased Nrf2/HO-1 and AMPK activity in the hippocampus of ESPS-treated rats. Downregulation of Nrf2 not only dampened the effects of EA pretreatment on PTSD-like behaviors but also reduced EA-induced increased neurogenesis in the hippocampus, indicating that the neuroprotective effects of EA pretreatment on PTSD rats may involve the Nrf2/HO-1 pathway. Previous studies have shown that the antioxidant effects of Nrf2 are exerted by disassociation from keap1, and treatment with a keap1 inhibitor exhibits protection in several diseases of the central nervous system (Quinti et al., 2017). In line with these results, we found that keap1 knockdown increased the expression of HO-1, improved hippocampal neurogenesis, and alleviated PTSD-like behaviors of ESPS-treated rats, replicating the therapeutic effects of EA. On the other hand, we found that Nrf2 knockdown alone induces effects that are similar to those of ESPS alone. Thus, it is a possible scenario that keap1/Nrf2 and its downstream antioxidative cascade elements play a role in the anti-PTSD effects of EA pretreatment. In addition, "Bai hui" is located on the skin incision line and the incision might interfere with the following EA treatment even after 2 weeks of recovery. Although the results in Experiment II indicated that ESPS induced a significant PTSD-like behavior in rats that received virus injection (Scramble vs. Scramble + ESPS), and this PTSD-like behavior was ameliorated by EA (Scramble + ESPS vs. Scramble + EA + ESPS), the interference of skin incision on the effects of EA pretreatment is still unrevealed. A comparison between EA + ESPS and Scramble + EA + ESPS is required in the future. In addition, Nrf2 is ubiquitously expressed in the central nervous system. Indeed, astrocytic-derived extrinsic support is known to play an important role in protecting neurons against oxidative stress (Shih et al., 2003). Previous studies further found that Nrf2-mediated glutathione biosynthesis and release from astrocytes protects neurons from oxidative stress, and Nrf2 overexpression specifically in astrocytes confers non-cell autonomous protection to surrounding neurons and leads to neuroprotection in *in vivo* models (Vargas et al., 2008; Chen et al., 2009). Recent work also indicated that developmental epigenetic Nrf2 repression weakens neuronal antioxidant defenses but is necessary to create an environment that supports neuronal development (Bell et al., 2015), and astrocytic Nrf2 signaling could be regulated by neuronal activity (Habas et al., 2013). Thus, based on the available literature, the regulation both on the expression of Nrf2 either in neurons or glial cells is meaningful. The present study found that Nrf2 was widely expressed in the nuclei of neurons and astrocytes, and ESPS induced a significant reduction in the double labeling of NeuN and Nrf2 or GFAP and Nrf2, which was ameliorated by EA pretreatment. The precise cellular mechanism still calls for further investigation.

In addition to reduced hippocampal neurogenesis, a growing body of evidence has been indicating that disruption of the brain derived neurotrophic factor (BDNF) may be also involved in the pathophysiology of PTSD (Cohen et al., 2018; Hou et al., 2018). Interestingly, recent studies have also reported that BDNF is involved in the biological effects of EA (Lin et al., 2017; Pak et al., 2018). The interplay between Nrf2 and BDNF has also been investigated. A previous study indicated that BDNF protein levels are decreased in the Nrf2 knockout mice (Martin-de-Saavedra et al., 2013). However, another study reported that Nrf2 activation was regulated by the TrkB-BDNF pathway (Bouvier et al., 2017) and the Nrf2 antioxidant axis was upregulated by BDNF overexpression in a rat model of traumatic brain injury (Chen et al., 2017; Ishii et al., 2018). In line with these observations, we found that EA pretreatment normalized BDNF expression in the hippocampus of ESPS rats, and this effect was blocked by Nrf2 shRNA knockdown. In addition, basal hippocampal expression of BDNF was also decreased in rats injected with Nrf2 shRNA, while down-regulation of keap1 up-regulated the expression of BDNF in the hippocampus of PTSD-like rats. Based on these data, we suggest that regulation of BDNF may also be involved in the anti-PTSD effects of EA pretreatment and that Nrf2 may be an upstream regulator of BDNF. However, we do not provide evidence clarifying the potential mechanism by which Nrf2 activation may regulate BDNF expression.

In summary, our results show that EA pretreatment has neuroprotective effects against ESPS-induced anxiety-like behaviors and hippocampal neurogenesis defects in rats. We also found that the neuroprotective effect of EA pretreatment was associated with an upregulation of the molecular mechanism associated with protection against oxidative damage and of BDNF expression. This effect of EA may involve the activation of the keap1/Nrf2/HO-1 pathway. Additionally, we found that Nrf2 is an upstream regulator of BDNF during EA-induced neuroprotection. Altogether, our findings provide new insights regarding the possibility of using EA in the prevention of PTSD and the mechanisms by which this protective effect may occur. However, the effects of different parameters of EA treatment on the activation of Nrf2 antioxidant pathway as well as the direct influence of Nrf2 knockout on PTSD-like behaviors remain unclear. Further studies are required to explore the detailed signaling cascades and cellular mechanisms involved in the regulation of keap1/Nrf2 after EA treatment.

ETHICS STATEMENT

The experiment procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Use and Protection Committee of the Animal Center of Fourth Military Medical University.

AUTHOR CONTRIBUTIONS

Z-wP, H-nW, Q-rT, and Z-JZ were involved in conception and design of the study. C-hZ, FX, and S-sX conducted the final data analyses and drafted the manuscript. C-hZ, FX, LL, and S-sX acquired and analyzed the data. YW and MC performed Western blotting assay and analysis. Z-wP, H-fS, and Z-JZ reviewed the article. All authors have approved the final drafts of this manuscript for its publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fncel.2019.00275/full#supplementary-material

FIGURE S1 | Lentivirus screening and microinjections. The silencing efficiency of different lentiviruses carrying different shRNA sequences were tested in astrocyte cultures by real-time PCR analysis. **(A)** Representative microphotographs of GFAP staining (red), DAPI staining (blue), and lentivirus infection (green) in astrocytes. **(B,C)** Histograms showing the effect of different shRNA sequences on the levels of Nrf2 and keap1 mRNAs. **(D)** Microphotographs of the NeuN (blue) and lentivirus infection (green) signals in the dentate gyrus. **(E)** Illustration of the bilateral injection of the lentivirus in the dentate gyrus of rats. **(F,G)** Histograms showing the change of Nrf2 and keap1 mRNAs in the hippocampus of rats after lentivirus infection. **(H,I)** Representative immunoblots and densitometry analysis the change of Nrf2 and keap1 protein level in the hippocampus of rats after lentivirus infection. $^{*}P < 0.05$ vs. Scramble; $^{**}P < 0.01$ vs. Scramble. Bar: 100 m.

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frontiers in Human Neuroscience

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Background Music Dependent Reduction of Aversive Perception and Its Relation to P3 Amplitude Reduction and Increased Heart Rate

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Music is commonly used to modify mood and has attracted attention as a potential therapeutic intervention. Despite the well-recognized effects of music on mood, changes in affective perception due to music remain majorly unknown. Here, we examined if the perception of aversive stimuli could be altered by mood-changing background music. Using subjective scoring data from 17 healthy volunteers, we assessed the effect of relaxing background music (RelaxBGM), busy background music (BusyBGM), or no background music (NoBGM) conditions on response to aversive white noise stimulation. Interestingly, affective response to the white noise was selectively alleviated, and white noise-related P3 component amplitude was reduced in BusyBGM. However, affective responses as well as P3 amplitude to reference pure tone stimuli were similar regardless of background music conditions. Interestingly, heart rate (HR) increased in BusyBGM, whereas no increase in HR was found in similar distress, NoBGM condition. These findings suggest that increase in HR, which happens during BusyBGM exposure, can be a reflecting feature of music that ameliorates the affective response to aversive stimuli, possibly through selective reduction in neurophysiological responses.

Keywords: background music, affective response, mood changes, event-related potentials, modulation of affective perception

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INTRODUCTION

From majestic operas to a casual humming, music plays an indispensable and extensive role in human life. One reason for the ubiquity of music is its ability to change mood (Sloboda and Juslin, 2001). For example, the choice of background music in a movie can dramatically change the impact of visual scenery perception, even if the music itself is not being consciously

Abbreviations: NoBGM, no background music; RelaxBGM, relaxing background music; BusyBGM, busy background music; ANOVA, analysis of variance; ANS, autonomic nervous system; CRT, cathode ray tube; HR, heart rate; POMS, Profile of Mood States; TMD, Total Mood Disturbance; SAM, Self-Assessment Manikin; ERP, event-related potential; EPN, early posterior negativity; LORETA, low resolution brain electromagnetic tomography; PCC, posterior cingulate cortex.

listened to Boltz (2004). The mood adjusting effects of background music are not only applicable to movies, but are widely used in environments, such as shopping malls and restaurants, to enhance the behavior of customers (Milliman, 1982, 1986). Background music is not limited to composed melodies: several online music streaming services provide artificial mixtures of daily noise claimed to serve as "concentration helping" background music (Zhang et al., 2013).

Recently, an increasing number of studies have elucidated the efficacy of music therapy (Bradt et al., 2013; Aalbers et al., 2017; van der Steen et al., 2018). Interestingly, a relaxing effect is not the only expected outcome of some music therapies. For example, in music therapy for pain, the main outcome is the alleviation of pain perception (Lee, 2016). This suggests that music can regulate cognitive perception, beyond a direct effect on mood. Another interesting aspect of music therapy is continuous attention to music is not necessary because passive listening is as effective as active listening in several cases (Mercadie et al., 2015; Millett and Gooding, 2018). Indeed, the music used in music therapy is played at normative loudness; it need not be loud or boisterous as in a concert hall. These findings suggest that unconscious listening to background music in daily life can affect mood and consequently modify affective perception.

Such change in affective perception can be assumed as a kind of affective bias. In relation to the clinical consideration, the affective perceptions are negatively biased in patients with depression, which is paralleled by reduced P3 amplitudes related to happy-face perception (Cavanagh and Geisler, 2006). In the basic cognitive science study, it is reported that auditory-induced pleasant mood enhances the cognitive inhibition that is paralleled with pronounced amplitudes in event-related potential (ERP) components between 150 and 550 ms (Yuan et al., 2011). In addition, these early components of ERP are thought to be involved in the mechanism of affective bias (Huang and Luo, 2006). For example, P3 component is related to the valence perception (Conroy and Polich, 2007). Early posterior negativity (EPN) is also considered to be the early stage of affective process, mirroring the fast and effortless detection of emotional stimuli (Olofsson et al., 2008; Ullrich et al., 2016). Consistently, in a recent article, we reported that the sounds of different aversiveness were associated with different neuroelectric activities in this time range. Briefly, aversive white noise stimuli involved more activity in the parietal region than pure tone stimuli in the time range corresponding to EPN and P3 components (Masuda et al., 2018).

Intriguingly, the effects of background music on cognitive function are not conclusive. For example, one study reported that background music has beneficial effect on reasoning or memory performance (Rauscher et al., 1993) whereas another study found detrimental effects on memory and comprehension tasks (Furnham and Strbac, 2002). These contrasting results could be attributable to the difference in the methodologies, targeted cognitive function, or applied choice of music. Choice of music type is important because it is reported that music interferes with the learning process depending on the congruency of the learning material and the kind of background music (Sousou, 1997). Similarly, music with increased arousal and positive affect can improve the performance of certain tests of spatial abilities (Thompson et al., 2001). The difference in the autonomic nervous system (ANS) might be involved in the inconsistent results because the autonomic nervous activity is related to type of music (Zatorre, 2015).

Thus, to establish the basis of affective bias caused by daily loudness music therapy, we performed a multimodal study that examined neurocognitive responses as well as the ANS changes. As an ANS measure that is closely related to mood, we examined changes in heart rate (HR) along with the subjective measurement of mood (Sammler et al., 2007).

We hypothesized that calming music can alleviate the aversive perception paralleled with reduced amplitudes of aversive-related EPN/P3 and reduction of corresponding neural activity in parietal region. In addition, we expected such music to have a soothing effect on ANS activity, such that it would be observable as decreased HR. This report is an extension of our recent work that reported the appraisal mechanism of white noise and pure tone (Masuda et al., 2018).

MATERIALS AND METHODS

Subjects

Advertisements were used to recruit 17 healthy adult participants for this study (10 men; mean age ± standard deviation, 21.6 ± 2.06 years). The participants were compensated with a gift card with a value equivalent to ¥2,500. Interview by a psychiatrist confirmed that the participants had no psychiatric disorders, hearing problems, or smoking history and did not habitually take medication or consume caffeine on the day of the study. All the subjects were right-handed, which was confirmed using Edinburgh handedness score being not <50 (Oldfield, 1971). All subjects had normal hearing ability. Four subjects had experience of taking music lessons in their childhood, but no subject was taking music education at the time of the experiment. This study was in accordance with the recommendations of the ethical committee at Shiga University of Medical Science with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the ethical committee at Shiga University of Medical Science (Approved #26-227).

Experimental Design and Settings

Background Music and Sound Stimuli

Two types of background music were used to induce changes in mood. Relaxing background music (RelaxBGM) was a privately composed music that was spacious and ethereal, such as music typically played for yoga or meditation. Busy background music (BusyBGM) was an artificial mixture of traffic noise that was reminiscent of a busy highway. The background music was played at the same loudness in all conditions, at an average loudness of 40 dB[A]. In addition, no background music (NoBGM) condition [<30 dB(A) silence] was used.

A 500-ms burst of 50 dB[A] white noise with instantaneous (10 ms) rise/fall times was used as an aversive stimulus. The white noise included all frequency bands within the audible range. A 1,000-Hz pure tone was used as a reference. Pure tone was used as a reference because sounds with 1,000 Hz peaks are most ubiquitously observed (Kim et al., 2012) and less affected by age-related losses in hearing sensitivity (Cruickshanks et al., 1998). We presented the stimuli in a passive task context, where subjects were instructed to simply view a presented fixation point without special attention to sound or background music.

Subjective Measures of Mood and Affective Response

The subjects were asked to score their subjective emotional responses using the Self-Assessment Manikin (SAM), a two-dimensional subjective scoring system developed for assessing affective stimuli of the International Affective Picture System (Bradley and Lang, 1994). This is a nine-point rating scale comprising sets of figures to measure valence (1 = unpleasant; 9 = pleasant) and arousal responses (1 = arousing; 9 = calming). We used the Profile of Mood States (POMS), which has high levels of reliability and validity, to measure psychological distress. In the scale, participants were required to rate 65 mood-related adjectives on a 5-point scale (0 = not at all to 4 = extremely). The scores of 65 adjectives were combined to make six sub-scale scores, and Total Mood Disturbance (TMD) was then calculated based on the sub-scale scores. A larger TMD score indicates an increased state of distress.

Procedures and Settings

Subjects underwent three background music experiments, in which one of the three background music options (RelaxBGM, BusyBGM, or NoBGM) was played (**Figure 1**, upper panel). The background music was selected following randomized counterbalanced crossover design. During the experiment, subjects remained seated on a chair placed 70 cm in front of a cathode ray tube (CRT) display in a sound proof and electromagnetic shield room. The illumination in the room was maintained at 80 lux. The subjects were instructed to look at a white-cross fixation point that appeared against the black CRT background during the entire experiments. Auditory stimulation was provided through headphones (AKG closed-back headphones, K404, Vienna, Austria).

One experiment was consisted with two major blocks (**Figure 1**, lower panel). In the first block, participants were only exposed to the background music. The effect of background music on mood was examined by TMD before and after a 5-min exposure to the background music. In addition, the participants were asked to use SAM to rate their appraisal evaluation of the background music. After completion of the ratings, the subjects immediately proceeded to the second block.

In the second block, the participants were exposed to the same background music for 5 min as in the first block, followed by the administration of pure tone and white noise sound stimuli with background music lasting approximately 5 min. The pure tone and white noise sound stimuli were programmed to randomly produce each frequency 75 times, with randomized stimulus intervals of 2,000 ± 200 ms using E-Prime v 2.0 software (Psychology Software Tools, Pittsburgh PA, USA). The participants completed SAM for both white noise and pure tone stimuli immediately after each experiment.

Electroencephalography Data Acquisition

Electroencephalography (EEG) signals were recorded using NetStation software [Electrical Geodesics Inc (EGI), Eugene, OR, USA] with 64-channel recordings made through a HydrocCel Geodesic Sensor Net v.1.0. gel cap. Data were sampled using a high-input impedance amplifier (200 MΩ, EGI Inc., Model: GES 300), at 500 Hz and referenced to Cz. Electrode impedances were kept at <60 kΩ throughout the experiments, following the guideline recommending the electrode impedance to be less than the input impedance of the amplifier by a factor of at least 100 (Picton et al., 2000). The participants were asked to remain awake, and a vigilant state was qualitatively confirmed by online observation of the EEG signal by a somnologist during the study.

Event-Related Potential (ERP) Data Processing

EEG data processing was performed using EEGLAB (version 14.1.2; Delorme and Makeig, 2004), an open source toolbox that runs on MATLAB version 2017a (Mathworks Inc. Natick, MA, USA). Briefly, EEG data were re-referenced to the average of the left and right mastoids, and bandpass filtered offline by 0.1–50 Hz using linear finite impulse response filtering method. Gross artifacts were visually rejected following independent component analysis based artifact correction embedded in EEGLAB, excluding 1–3 components produced by eye movement or muscle activity. We epoched all data segments 500 ms prior to and 1,500 ms post stimulations, and baseline corrections were done by subtracting the average of 100 ms prior to stimulation using ERPLAB (version 7.0.0; Lopez-Calderon and Luck, 2014). Epochs for ERP calculation were first selected using the simple voltage threshold function of ERPLAB using 100 μV as the threshold. Finally, an examiner, without the knowledge of the experiment conditions, visually confirmed artifact-free epochs for ERP calculation. The average number of epochs used for ERP calculation was as follows: 55.76 ± 2.57 for pure tone and 53.94 ± 2.44 for white noise in NoBGM; 54.29 ± 2.69 for pure tone and 51.88 ± 2.77 for white noise in RelaxBGM; and 48.59 ± 3.54 for pure tone and 48.76 ± 3.43 for white noise in BusyBGM. To compare component amplitudes, we calculated the mean relative-to-baseline amplitude value between the specified time range from each electrode. To compare ERP component amplitudes on region-of-interest basis, we averaged the potentials of four electrodes (Pz, Cz, C3, and C4). We focused on these four electrodes because EPN/P3 related potentials were most pronounced in these electrodes (**Supplementary Figure S1**). To investigate the regions involved in the differential processes between pure tone and white noise, we used time series standardized low resolution brain electromagnetic tomography analysis (sLORETA) every 2 ms to estimate the current source density distribution for each ERP component (Pascual-Marqui et al., 1994).

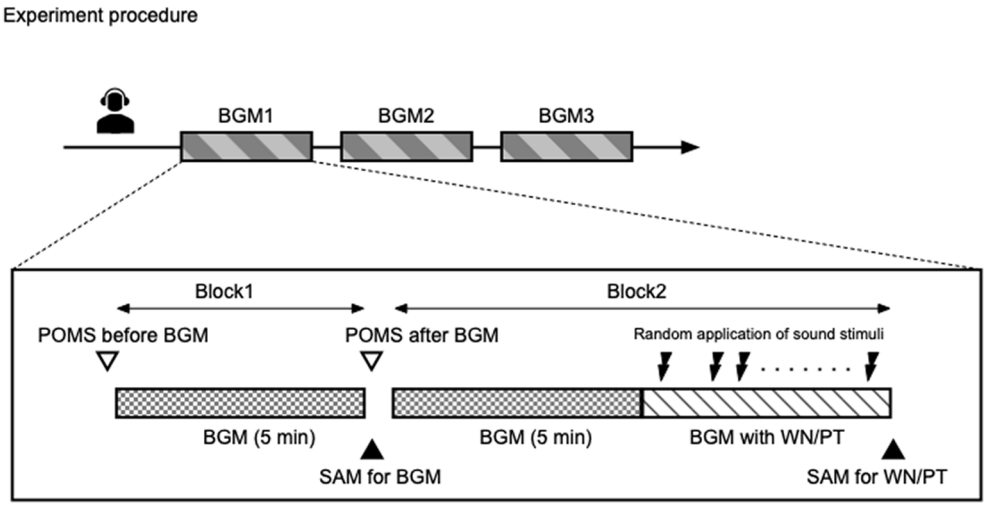

FIGURE 1 | Schematic representation of the study. The study was conducted following randomized counterbalanced crossover design. Each participant underwent three experiments with different background music conditions. In each background music condition, two blocks of experiments were conducted. In block 1, participants were exposed to selected background music for 5 min. In block 2, participants were exposed to the background music for 5 min, followed by exposure to the same background music with intermittent sound stimuli.

Heart Rate and Calculation of Autonomic Features

Kubios HRV (version 2.1, Kubios, Finland) was used for HR detection (Tarvainen et al., 2014). The type II lead of electrocardiogram was simultaneously recorded during EEG recordings, and the data were later processed by Kubios HRV. HR was calculated from 20 s data at the beginning or end of BGM exposure.

Statistical Analyses

Data are shown as mean ± standard error of mean, unless otherwise stated. For the examination of effects by background music and sound stimuli, a two-way repeated measures (3 × 2) analysis of variance (ANOVA) with three background music conditions and two sound stimuli as the within-subjects factors was conducted unless otherwise described. Greenhouse-Geisser correction was used when the sphericity was violated. Bonferroni pairwise comparison was used to adjust for multiple comparisons. SPSS statistics software Version 22 (IBM, Armonk, NY, USA) was used to perform statistical analysis. sLORETA images were statistically compared between sound conditions using the voxel-by-voxel *t*-test, which was corrected by Statistical non-Parametric Mapping (SnPM) randomization (number of randomizations = 5,000). The threshold of statistical significance was set at $P < 0.05$.

Comparisons of Components of Event-Related Potentials

To examine the changes in sound stimulation related potentials in each background music condition, we focused on 200–300 and 300–450 ms because these time ranges were pivotal in the aversive process of pure tone and white noise (Masuda et al., 2018).

RESULTS

Subjective Ratings of Appraisal Response and Consequential Mood by Background Music

First, to investigate how participants experienced the background music, the two-way repeated measures (2 × 2) ANOVA within the subject factors background music (RelaxBGM and BusyBGM) and subjective evaluations (SAM scores in valence and arousal) was performed. This analysis showed significant effect of background music ($F_{(1,16)} = 94.298$, $P < 0.01$, partial $\eta^2 = 0.855$), and SAM ($F_{(1,16)} = 28.810$, $P < 0.01$, partial $\eta^2 = 0.643$) as well as their interaction between background music and SAM ($F_{(1,16)} = 24.800$, $P < 0.01$, partial $\eta^2 = 0.608$). BusyBGM was perceived as more aversive than RelaxBGM (valence score: BusyBGM, 2.71 ± 0.24; RelaxBGM, 6.24 ± 0.22, **Figure 2A**, $t_{(16)} = 13.631$, $P < 0.01$). In addition, BusyBGM was more arousing than RelaxBGM (arousing score: BusyBGM, 5.18 ± 0.33; RelaxBGM, 6.88 ± 0.32, $t_{(16)} = 6.5884$, $P < 0.01$).

Thereafter, we examined the mood changes caused by 5-min exposure to RelaxBGM, BusyBGM, and NoBGM using TMD calculated from POMS questionnaire. TMD score had high internal consistency in our study sample (Cronbach's Alpha = 0.915). The two-way repeated measures ANOVA with background music and the time (before or after background music exposure) as within subject factors found simple main effect by time ($F_{(1,16)} = 11.437$, $P < 0.01$, partial $\eta^2 = 0.417$) and no effect of background music ($F_{(2,32)} = 1.251$, $P = 0.300$, partial $\eta^2 = 0.073$), and significant interaction between background music and time ($F_{(2,32)} = 10.159$, $P < 0.01$, partial $\eta^2 = 0.388$). Following planned comparisons, to check background music specific change of the mood, found worsening of TMD in

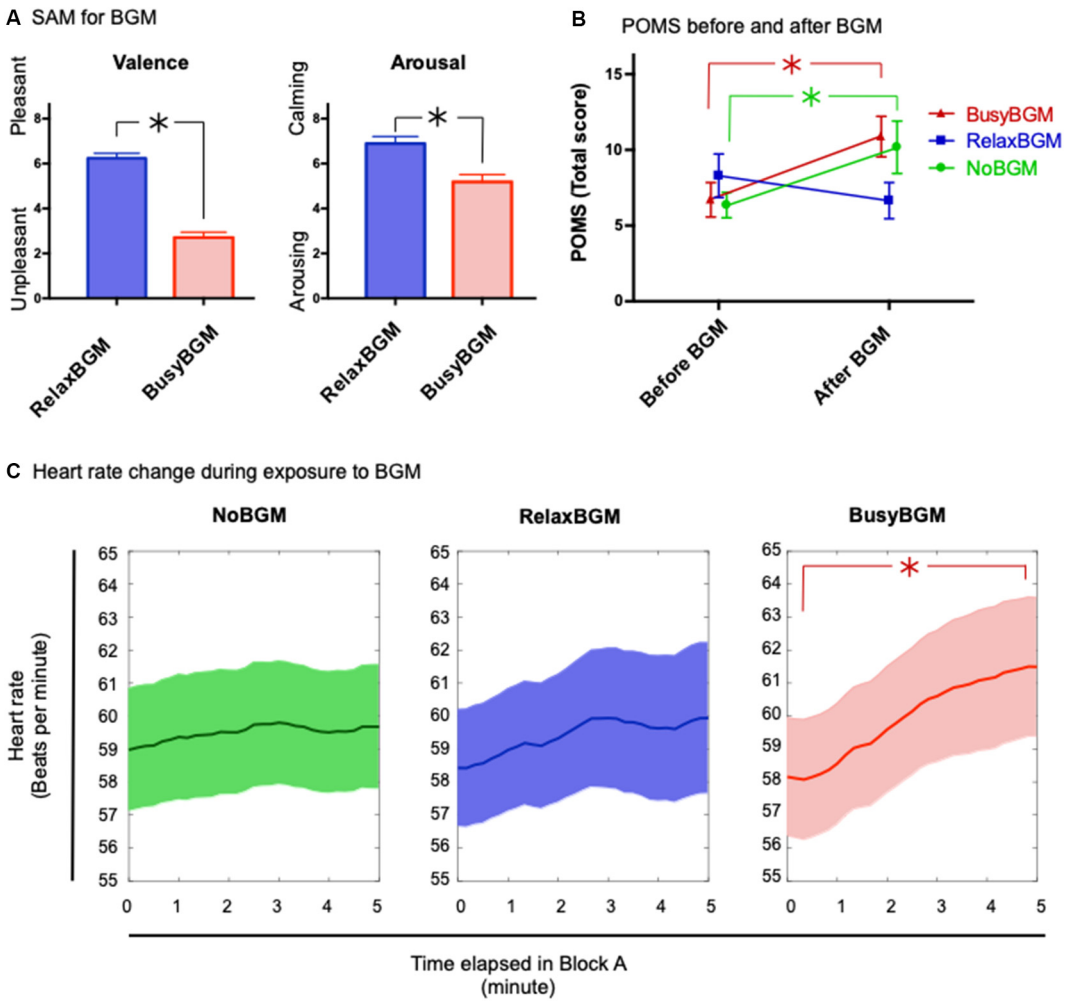

FIGURE 2 | Affective responses and consequential mood for each background music condition. **(A)** Affective responses to relaxing background music (RelaxBGM) and busy background music (BusyBGM) are shown based on two-dimensional evaluation: valence and arousal. Note that RelaxBGM was more pleasant and calming than BusyBGM. **(B)** Emotional consequences of listening to each background music for 5 min are shown. Note that mood worsened in BusyBGM and no background music (NoBGM) conditions, whereas RelaxBGM prevented the worsening. **(C)** Heart rate (HR) changes were noted during exposure to each background music. HR increased in BusyBGM, while HRs at the beginning were similar in all conditions. Asterisk shows significant difference between the indicated pair.

BusyBGM (pre vs. post: 6.71 ± 1.14 vs. 10.88 ± 1.34, $t_{(32)} = 4.077$, $P < 0.01$, **Figure 2B**) and NoBGM (pre vs. post: 6.35 ± 0.84 vs. 10.18 ± 1.73, $t_{(32)} = 3.733$, $P < 0.01$), although no worsening was observed in RelaxBGM (pre vs. post: 8.29 ± 1.44 vs. 6.65 ± 1.19, $t_{(32)} = 1.608$, $P = 0.353$).

Heart Rate Changes Due to the Background Music

Similar to the analysis of the mood changes due to background music, we performed the two-way repeated measures ANOVA on HR with background music and time as within subject factors. We used two representative average HR from two time windows, the first and the last 20 s of BGM exposure (**Figure 2C**). This analysis found significant main effect of the time ($F_{(1,16)} = 9.061$, $P = 0.008$, partial $\eta^2 = 0.362$). However, no significant effect of background music was found ($F_{(2,32)} = 0.319$, $P = 0.729$, partial $\eta^2 = 0.020$) and marginal interaction was found ($F_{(2,32)} = 3.014$, $P = 0.063$, partial $\eta^2 = 0.159$). In following planned comparisons, an increase in HR was found to be specific to BusyBGM condition (pre vs. post: 58.16 ± 1.79 vs. 61.49 ± 2.10, $t_{(32)} = 4.291$, $P < 0.01$), and HR remained constant in NoBGM (pre vs. post: 58.97 ± 1.86 vs. 59.68 ± 1.89, $t_{(32)} = 0.901$, $P > 0.900$) and RelaxBGM conditions (pre vs. post: 58.43 ± 1.77 vs. 59.94 ± 2.29, $t_{(32)} = 1.945$, $P = 0.182$).

Changes in Affective Response to White Noise/Pure Tone in the Three Background Music Conditions

We examined if pure tone and white noise caused different affective responses in the three background music conditions using the two-way repeated measures ANOVA. The analysis

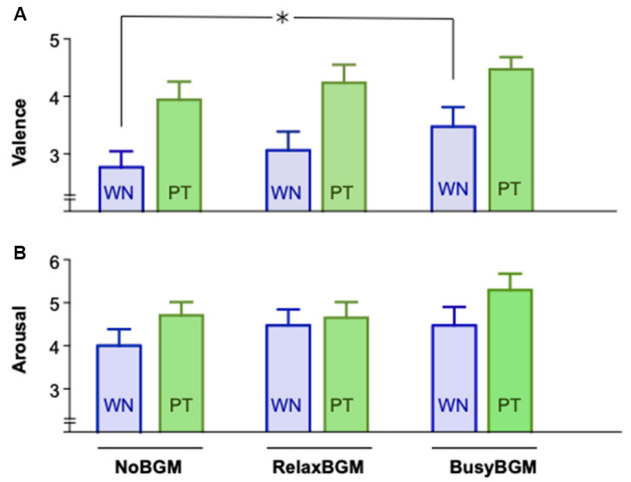

FIGURE 3 | Affective response to white noise and pure tone for each background music condition. Self-Assessment Manikin (SAM) scores for white noise and pure tone are graphically presented. Panel **(A)** shows the valence scores, whereas Panel **(B)** shows the arousal scores. Significant difference due to background music condition is shown by asterisk.

on valence found significant main effect for the sound stimuli ($F_{(1,16)}$ = 16.812, P = 0.001, partial η^2 = 0.512) and marginal significance for background music ($F_{(2,32)}$ = 2.744, P = 0.079, partial η^2 = 0.146), although the interaction between them was not significant ($F_{(2,32)}$ = 0.232, P = 0.794, partial η^2 = 0.014). Planned comparisons within the same sound stimulus found aversive response to white noise significantly reduced in BusyBGM compared to those in NoBGM (BusyBGM vs. NoBGM: 3.47 ± 0.34 vs. 2.76 ± 0.28, $t_{(32)}$ = 3.339, P < 0.01, **Figure 3A**), although no significant reduction of aversiveness was found in RelaxBGM compared to NoBGM (RelaxBGM vs. NoBGM: 3.06 ± 0.33 vs. 2.76 ± 0.28, $t_{(32)}$ = 1.391, P = 0.521). Interestingly, aversive response to pure tone was comparable to NoBGM in both RelaxBGM (RelaxBGM vs. NoBGM: 4.24 ± 0.32 vs. 3.94 ± 0.31, $t_{(32)}$ = 1.391, P = 0.521) and BusyBGM (BusyBGM vs. NoBGM: 4.47 ± 0.21 vs. 3.94 ± 0.31, $t_{(32)}$ = 2.504, P = 0.052).

The same analysis on arousal found marginal effect of sound stimuli ($F_{(1.000,16.000)}$ = 4.176, P = 0.058, partial η^2 = 0.207) but no significant effect of background music ($F_{(1.916,30.656)}$ = 1.171, P = 0.322, partial η^2 = 0.068) as well as no significant interaction ($F_{(1.471,23.539)}$ = 2.368, P = 0.127, partial η^2 = 0.129).

Planned comparisons found no significant difference in arousal responses to white noise, which was similar to NoBGM condition in both RelaxBGM (RelaxBGM vs. NoBGM: 4.47 ± 0.37 vs. 4.00 ± 0.38, $t_{(16)}$ = 1.095, P = 0.869) and BusyBGM (BusyBGM vs. NoBGM: 4.47 ± 0.43 vs. 4.00 ± 0.38, $t_{(16)}$ = 1.000, P > 0.900 in BusyBGM, **Figure 3B**). Similarly, comparable arousal responses were found for pure tone (arousal response to pure tone: RelaxBGM vs. NoBGM: 4.65 ± 0.36 vs. 4.71 ± 0.31, $t_{(16)}$ = 0.203, P > 0.900) and in BusyBGM (BusyBGM vs. NoBGM: 5.29 ± 0.38 vs. 4.71 ± 0.31, $t_{(16)}$ = 2.163, P = 0.138).

Neurophysiological Response to Sound Stimulations

The two-way ANOVA analysis on P3 component amplitude found simple main effect of background music ($F_{(2,32)}$ = 7.601, P < 0.01, partial η^2 = 0.322) and sound stimuli ($F_{(1,16)}$ = 77.962, P < 0.01, partial η^2 = 0.830), although there was no significant interaction ($F_{(2,32)}$ = 0.929, P = 0.405, partial η^2 = 0.055). Following comparison within each sound stimuli found that white noise-related amplitude was significantly smaller in BusyBGM (BusyBGM vs. NoBGM: 3.36 ± 0.58 vs. 5.64 ± 0.64 μV, $t_{(32)}$ = 3.906, P < 0.01; **Figure 4**), although amplitude was comparable between RelaxBGM and NoBGM (RelaxBGM vs. NoBGM: 4.54 ± 0.42 vs. 5.64 ± 0.64 μV, $t_{(32)}$ = 1.885, P = 0.21). Interestingly, the pure tone–related amplitude in BusyBGM was comparable to NoBGM (BusyBGM vs. NoBGM: 0.98 ± 0.34 vs. 2.15 ± 0.58 μV, $t_{(32)}$ = 1.999, P = 0.16) as well as in RelaxBGM (RelaxBGM vs. NoBGM: 1.47 ± 0.42 vs. 2.15 ± 0.58 μV, $t_{(32)}$ = 1.175, P = 0.75).

For EPN component, simple main effect of sound stimuli ($F_{(1,16)}$ = 31.617, P < 0.01, partial η^2 = 0.664) was found, although there was no main effect of background music ($F_{(2,32)}$ = 0.361, P = 0.700, partial η^2 = 0.022) and no significant interaction ($F_{(2,32)}$ = 0.591, P = 0.560, partial η^2 = 0.036). Planned comparisons of amplitudes within each sound stimuli found EPN in BusyBGM (BusyBGM vs. NoBGM: −0.64 ± 0.38 vs. −0.89 ± 0.72 μV, $t_{(32)}$ = 0.587) and RelaxBGM (RelaxBGM vs. NoBGM: −1.17 ± 0.69 vs. −0.89 ± 0.72 μV, $t_{(32)}$ = 0.634) were comparable to those in NoBGM for white noise. Pure tone–related EPN in BusyBGM (BusyBGM vs. NoBGM: −2.69 ± 0.40 vs. −3.35 ± 0.60 μV, $t_{(32)}$ = 1.524, P = 0.412) and RelaxBGM (RelaxBGM vs. NoBGM: −2.96 ± 0.45 vs. −3.35 ± 0.60 μV, $t_{(32)}$ = 0.890, P > 0.900) were also comparable to those in NoBGM.

Source Localization of ERP

Time series analysis using sLORETA between white noise and pure tone revealed significantly greater electrical activity induced by white noise than pure tone under NoBGM and RelaxBGM, whereas no difference was found for BusyBGM (**Figure 5**). In the NoBGM condition, significant difference between white noise and pure tone were found for the time window between 294 and 328 ms after sound stimulation, as previously reported (Masuda et al., 2018). During this time range, significantly increased electrical activity was found in the parietal lobe centering at the left inferior parietal lobule, for white noise compared with pure tone (left parietal lobe, BA 40, **Figure 5A**). In RelaxBGM, the difference between white noise and pure tone at the same time range was not found. However, there was a significantly increased electrical activity in the posterior cingulate cortex (PCC) in white noise compared with pure tone (Brodmann 40) at 340 ms in RelaxBGM (**Figure 5B**).

DISCUSSION

In this study, we examined the effect of mood-changing background music on affective perception. Because music

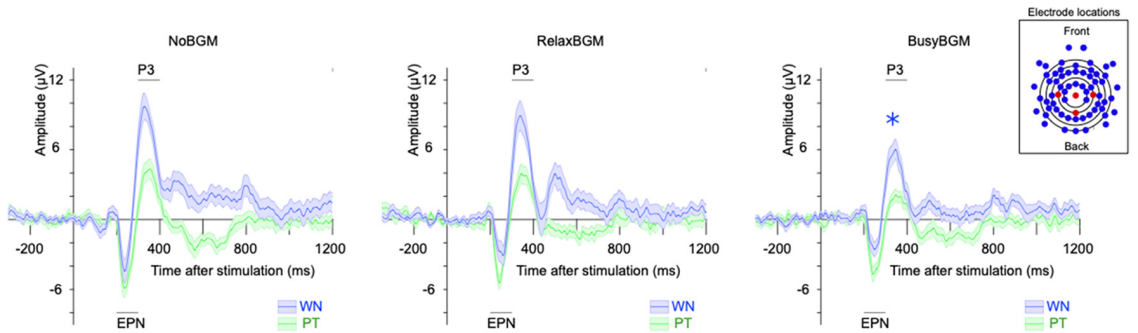

FIGURE 4 | Event-related potentials (ERPs) to white noise and pure tone for each background music condition. Each panel shows the grand average ERP for each BGM condition. ERPs evoked by white noise (blue line), and pure tone (green line) are presented. Shaded colors represent the mean ± standard error. (The inset box) The electrodes used in the calculation of ERP (Pz, Cz, C3, and C4) are shown in red, while others are in blue.

therapy is reportedly used in pain clinics to reduce pain perception, we predicted that calming background music would help to reduce aversive reaction to white noise.

We used two newly composed background music, aiming to change the mood. The initial assessment of appraisal response to RelaxBGM and BusyBGM revealed that they had intended appraisal effects. As we expected, BusyBGM had mood worsening effect. Slightly different from our initial expectation, the mood was worsened in NoBGM condition possibly because of the stress of sitting still. RelaxBGM appeared to prevent such mood worsening, if not improved the mood. The mood in BusyBGM or NoBGM was not at an evidently stressful level, as the POMS total disturbance scores in the present study were low compared to that in studies assessing a stressful condition using the same measure (Rosenzweig et al., 2003). Intriguingly, even with the similar level of mood worsening in BusyBGM and NoBGM, ANS activity selectively changed in BusyBGM as shown by the increased HR. Considering that the initial HR was similar at the beginning of background music exposure, HR increase was specifically attributable to BusyBGM. The result is consistent with a previous study that showed HR changes depend on music (Koelsch and Jäncke, 2015), particularly in the presence of discomforting music (Sammler et al., 2007). Thus, the absence of HR increase in the NoBGM condition suggests that ANS activity could be differentially modulated even in the same subjective distress level. This is consistent with a report that showed differential ANS modulation by different type of stressors (Hu et al., 2016).

In examination of the background music effect on affective perception of white noise and pure tone, we found unexpected reduction in aversive response to white noise in BusyBGM. The reduction was specific to white noise, suggesting that low level aversiveness, as that found against pure tone, was less prone to the background music effect. One possible psychological explanation for these unexpected results is that moderate distress can reduce the effect of aversive stimulation. It is often indicated that moderate stress is more facilitating for human performance than no stress because the presence of stress often leads to improved performance (Smeets et al., 2008; Hupbach and Fieman, 2012) and emotion (Marin et al., 2010).

In this study, the neurophysiological responses showed the effect of background music similar to subjective response: white noise-related P3 amplitude was reduced in BusyBGM, whereas that in RelaxBGM remained comparable to NoBGM. The reduction in P3 amplitudes in BusyBGM was not due to simple phonic masking effects of the stimulus sounds by background music because the same loudness RelaxBGM did not show the same effects. In addition, P3 amplitude related to pure tone was comparable in all background music, suggesting that the observed amplitude change was white noise-specific. The reduction in P3 amplitude could be assumed as a reflection of reduced cognitive capacity to the sound stimulation, according to the processing capacity model (Kok, 2001). A study using a similar sound stimulation technique reported reduced ERP amplitudes in a state with increased mental concentration (Ullsperger et al., 2001). The amplitude reduction in sound-related ERP due to mental state is reminiscent of distress-dependent ERP changes in the present study. Thus, it is suggested that passive hearing of BusyBGM continuously consumes cognitive capacity, thereby reducing the white noise-related P3 component, although RelaxBGM did not have such an effect. In addition, a similar sound probe experiment showed the P3 amplitude related to startling loud sound was reduced while subjects were looking at emotional pictures (Keil et al., 2007). Because our study used normative loudness sound (50 dB) stimulation, instead of loud sound (95 dB) as in Keil's study, the present results expand the knowledge that appraisal response to everyday level loudness sound could also be modulated by background music. Contrary to our findings, a study addressing the effects of similar background music conditions (excited background music, relax background music, and NoBGM) on cognitive inhibitory function reported that N2d and P3 component amplitudes are not affected (Burkhard et al., 2018). These findings suggest that the interference of background music might be a cognitive function specific, although this idea awaits further examination.

The time range corresponding to P3 (300–650 ms) is thought to be involved in linking sound stimuli and emotion (Koelsch, 2010). Our previous findings also support the involvement of

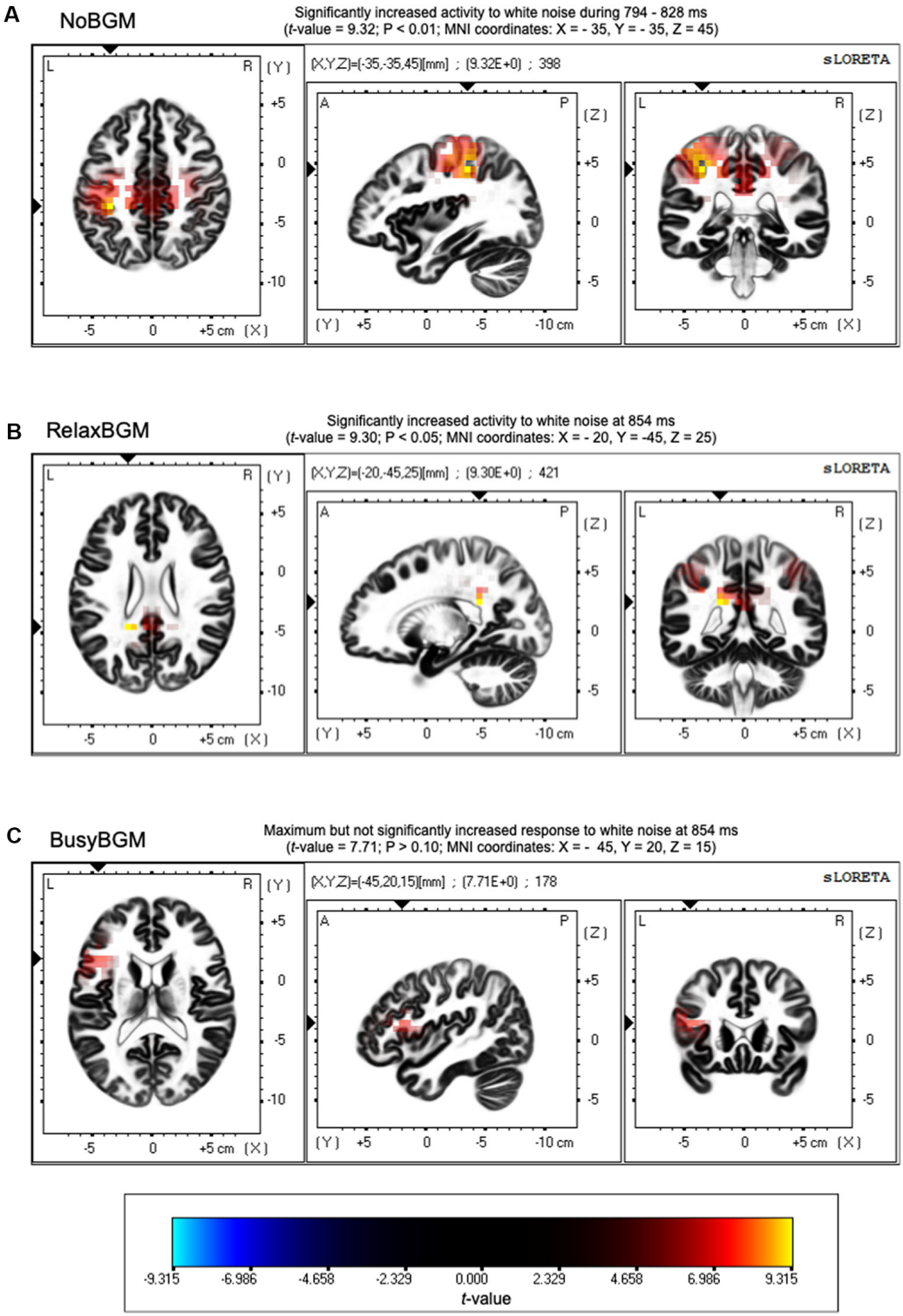

FIGURE 5 | Source localization of differences in ERPs. Differences in neuroelectric activity induced by white noise and pure tone are presented. Regions with significantly increased neuroelectric activity responding to white noise stimulus are shown in yellow to red gradations. **(A)** Increased activities were found around Brodmann area 40 in no background music (NoBGM) condition. **(B)** Increased activities were found around Brodmann area 31 in relaxing background music (RelaxBGM) condition. **(C)** No statistically different responses were found in busy background music (BusyBGM) condition. Color bars show the locations of extreme *t*-values that close to the significant level ($P < 0.05$, corrected for multiple comparisons). Maximum *t*-value and corresponding *P*-value are described above in corresponding figures, and the regions of maximum difference are shown as Montreal Neurological Institute (MNI) coordinates.

P3 in the valence determination for white noise perception (Masuda et al., 2018). To further examine the mechanism of reduced P3 amplitude, we performed the current source density analysis on aversive perception related potentials. This analysis showed significantly different neuroelectric activities between white noise and pure tone in NoBGM and RelaxBGM, whereas no significant difference was found in BusyBGM.

The absence of significantly different neuroelectric activity in BusyBGM was consistent with the decreased P3 amplitude difference in BusyBGM, whereas significantly different neuroelectric activities were consistent with the significant ERP amplitudes difference in NoBGM and RelaxBGM. The increased neuroelectric activity of the parietal region in NoBGM may have resulted from the additional process associated with white noise, presumably resolving the sound feature (Masuda et al., 2018). In RelaxBGM, increased neuroelectric activity was found in the PCC. The PCC is generally believed to function as one of the nodes in default mode network (Buckner et al., 2008), and thus, increased activity in white noise compared with pure tone process is unexpected. However, a report mentioned that the PCC also plays a role in the cognitive perception of tintius (Vanneste and De Ridder, 2012), suggesting that the activity in the area may be involved in the perception of discomfort auditory experience. These results suggested that background music exert effects on process later than 300 ms, explaining why we did not find background music effect on EPN.

Considering that the HR increase was the difference between NoBGM and BusyBGM, HR increase could be a physiological feature predicting reduced perception of aversiveness. Because increased HR is associated with increased mental workload (Liu et al., 2017), HR increase in BusyBGM may reflect increased mental workload related to continuous auditory processing of busy noise. This assumption fits aforementioned cognitive capacity model. Thus, the P3 amplitude reducing effect may be exerted by background music that accompanies an increase in HR possibly through increased mental activity, which was typically found in BusyBGM in this study.

Although we have discussed the results of the present study in relation to music and possible link to music therapy in general, there are several limitations. First, we used white noise as aversive stimulation, although it was only shown to be aversive relative to 1,000 Hz pure tone. Thus, we should be careful when interpreting the findings of the present study as a common mechanism underlying all aversive stimuli. In addition, we used two background music, as a representative of busy music and relaxing music. However, it is not warranted that all music within one category will have the same effect. In fact, RelaxBGM did not improve the mood in this study, although it apparently prevented mood worsening. Thus, it is inappropriate to conclude the effect of the mood improving music based on the results of the present study. It should also be noted that we used city noise as BusyBGM. Further studies in this field are required to generalize the effects of background music on affective perception. Regarding the statistics, we reported the results of planned comparisons in all analyses based on the suggestion that comparisons are meaningful even when interaction was not significant (Wei et al., 2012). Last, our study sample included four participants who had history of music education. We included these participants to keep statistical power, but we also confirmed that results were largely similar even after excluding these participants (**Supplementary Material**).

Overall, this study concluded that background music can modulate affective perception *via* changes in neuroelectric response to certain stimulation. We hope these findings facilitate the optimization of music therapy and cognitive control.

ETHICS STATEMENT

All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the ethical committee at Shiga University of Medical Science (Approved #26-227).

AUTHOR CONTRIBUTIONS

HK and MM conceived the study. FM and YS recruited participants and conducted the study, under management by NY. AY conducted the mathematical analysis, and MT conducted the statistical analysis. MM prepared the manuscript and HK confirmed the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnhum.2019.00184/full#supplementary-material

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frontiers in Human Neuroscience

SYSTEMATIC REVIEW
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Current Status of Neurofeedback for Post-traumatic Stress Disorder: A Systematic Review and the Possibility of Decoded Neurofeedback

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Background: Post-traumatic stress disorder (PTSD) is a neuropsychiatric affective disorder that can develop after traumatic life-events. Exposure-based therapy is currently one of the most effective treatments for PTSD. However, exposure to traumatic stimuli is so aversive that a significant number of patients drop-out of therapy during the course of treatment. Among various attempts to develop novel therapies that bypass such aversiveness, neurofeedback appears promising. With neurofeedback, patients can unconsciously self-regulate brain activity via real-time monitoring and feedback of the EEG or fMRI signals. With conventional neurofeedback methods, however, it is difficult to induce neural representation related to specific trauma because the feedback is based on the neural signals averaged within specific brain areas. To overcome this difficulty, novel neurofeedback approaches such as Decoded Neurofeedback (DecNef) might prove helpful. Instead of the average BOLD signals, DecNef allows patients to implicitly regulate multivariate voxel patterns of the BOLD signals related with feared stimuli. As such, DecNef effects are postulated to derive either from exposure or counter-conditioning, or some combination of both. Although the exact mechanism is not yet fully understood. DecNef has been successfully applied to reduce fear responses induced either by fear-conditioned or phobic stimuli among non-clinical participants.

Methods: Follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, a systematic review was conducted to compare DecNef effect with those of conventional EEG/fMRI-based neurofeedback on PTSD amelioration. To elucidate the possible mechanisms of DecNef on fear reduction, we mathematically modeled the effects of exposure-based and counter conditioning separately and applied it to the data obtained from past DecNef studies. Finally, we

conducted DecNef on four PTSD patients. Here, we review recent advances in application of neurofeedback to PTSD treatments, including the DecNef. This review is intended to be informative for neuroscientists in general as well as practitioners planning to use neurofeedback as a therapeutic strategy for PTSD.

Results: Our mathematical model suggested that exposure is the key component for DecNef effects in the past studies. Following DecNef a significant reduction of PTSD severity was observed. This effect was comparable to those reported for conventional neurofeedback approach.

Conclusions: Although a much larger number of participants will be needed in future, DecNef could be a promising therapy that bypasses the unpleasantness of conscious exposure associated with conventional therapies for fear related disorders, including PTSD.

Keywords: PTSD, real-time functional magnetic resonance imaging, multi-voxel decoding, fMRI decoded neurofeedback (DecNef), neural reinforcement, neuromodulation

INTRODUCTION

Post-traumatic stress disorder (PTSD) is a debilitating condition following life-threatening traumatic events. PTSD is characterized by four symptom clusters, namely, re-experiencing of the traumatic event, avoidance of trauma-related stimuli, general changes in mood and cognition, and hyperarousal (DSM-5). While exposure therapy is one of the most effective treatments for PTSD (Foa and Kozak, 1986; Schnurr et al., 2007), it involves exposure to trauma-related stimuli and is itself an excruciating process. In exchange for its effectiveness, the distress of exposure therapy renders the patients with difficulties in engagement and with a considerable rate of early drop-out (i.e., 20–40% within the first 2 months of the treatment period), which may lead to suboptimal outcomes (Hembree et al., 2003; Schnurr et al., 2007). Furthermore, another limitation of exposure therapy is that 30–50% of PTSD patients do not respond to this treatment (Bradley et al., 2005). Therefore, a novel therapy for PTSD is necessary from a clinical perspective.

Neurofeedback is a promising alternative approach to ameliorate PTSD symptoms without unnecessary distress. Neurofeedback can modulate brain activity via real-time monitoring and feedback of EEG or fMRI signals, which are used to self-regulate brain functions. Repeatedly induced PTSD-related brain activity during feedback session may change its frequency of spontaneous appearance after feedback session (Kluetsch et al., 2014; van der Kolk et al., 2016). As reviewed in this article, the conventional neurofeedback mainly regulates the average EEG or fMRI signals from specific brain region in a univariate way: either up- or down-regulate the average activity of a specific region. So far, these effects are promising, but are yet to replace conventional therapy. Decoded Neurofeedback (DecNef) has recently grown rapidly as a novel neurofeedback procedure for clinical applications (Watanabe et al., 2017; Shibata et al., 2018). Instead of the average fMRI BOLD signals, DecNef allows patients to implicitly regulate multivariate voxel patterns of BOLD signals which has been decoded in advance. By targeting the multivariate patterns representing feared stimuli, DecNef has been shown to change symptom-related brain activity in subclinical phobia (Taschereau-Dumouchel et al., in submission).

Since DecNef regulates multivariate brain activity, it has three advantages over the conventional univariate neurofeedback. First, DecNef can regulate neural representation for specific stimuli, which allows one to design neurofeedback to directly intervene them. This particularly benefits the treatment of PTSD, since traumatic episodes and the related neural representations differ across individual patients. Second, it allows patients to induce ideal brain activation patterns which are likely to be observed during or after an effective exposure therapy. This might especially benefit the exposure therapy-resistant patients. For example, using a method called hyperalignment (Haxby et al., 2011; Taschereau-Dumouchel et al., 2018), the exposure therapy-resistant patients may learn to induce the neural representations which would be observed following successful exposure therapy, when such representations are inferred from the "surrogate" therapy responders. Third, DecNef can infer the causality of brain activity pattern associated with PTSD (Watanabe et al., 2017). The change of PTSD causative brain activities should change PTSD-related behavior. If DecNef only changes brain activity without affecting behavior, the seemingly PTSD causative brain activities might not be really causative. It might just be observed as a confounder: it might arise as a result of other true causative brain activity. In this regard, DecNef allows one to carefully test whether the targeted changes in brain activity accompanies the intended changes in behavior.

Despite such advantages of DecNef, whether DecNef is effective on actual PTSD symptoms is yet to be determined. To determine the future direction to developing neurofeedback for PTSD therapy, it is essential to compare the effects across different neurofeedback strategies: EEG, fMRI neurofeedback, and DecNef. Furthermore, to efficiently develop a novel

treatment method based on DecNef, it is desirable to understand the exact mechanism underlying its effects.

In this review, we first discuss recent challenges in application of both EEG and fMRI neurofeedback to PTSD treatment as well as state-of-art technique that can be applied to PTSD. Second, we illustrate the potential and power of fMRI-based neurofeedback methods for PTSD treatment including DecNef. Thirdly, we discuss the possible mechanisms of DecNef on fear reduction. We hope that this review will aid the researchers who try to develop novel neurofeedback therapy on PTSD by selecting the most promising strategy among EEG or fMRI, or DecNef.

MATERIALS AND METHODS

Systematic Literature Search

A systematic literature search was undertaken in line with the search conducted by Reiter et al. (2016). Briefly, the PubMed, PsychoInfo, and Cochrane databases were used on dates between October 5 and October 24, 2018. The following keywords were used in our search: "Neurofeedback" OR "EEG biofeedback" OR "neurotherapy" combined by AND with "PTSD" OR "post-traumatic stress disorder." Case studies were excluded. The present systematic review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The inclusion criteria are presented in the PRISMA flow chart (**Figure 1**). Neurofeedback trials were included if they fulfilled the following criteria: (1) PTSD patients according to relevant classification systems (e.g., DSM-IV/V or ICD-10), (2) published in English, (3) comparing EEG or fMRI neurofeedback effects with regard to (a) pre vs. post-neurofeedback interventions, (b) neurofeedback vs. waiting list, (c) neurofeedback vs. sham/active neurofeedback, and (d) neurofeedback vs. conventional treatment. (4) Trials had to report (a) symptom severity or (b) brain activity at the time of the follow up. Here, a participant assigned to waiting list receives intervention after the active treatment group. In sham feedback, participants are provided with brain signal of another participant or with an artificially generated signal. In active neurofeedback, participants are provided with feedback of an alternative aspect of brain function. Titles and abstracts were screened for eligibility by one assessor (TC) (screening phase, $n = 48$). All studies not excluded in this process were examined in detail on a full text and included in this review independently by two assessors (KI, TC; $n = 13$). All reference lists of review papers and potentially eligible studies were reviewed to identify any additional papers. The risk of bias in each study was assessed by the Oxford Centre for Evidence-based Medicine, Levels of Evidence (Ellis et al., 1995). We additionally review the state-of-art studies derived from hand search during the systematic literature search.

Decoded Neurofeedback for PTSD

We conducted a DecNef experiment for 4 individuals with PTSD with approval from the Ethics Committee of Osaka Medical College. Signed, informed consent was obtained before all procedures. Inclusion criteria were: diagnosed with DSM-IV PTSD as determined by the Clinician-Administered PTSD Scale, age of 20–55 years, traumatized by angry human males (i.e., they are victims of domestic violence or child abuse), having strong fear for passive viewing of angry face picture, which was confirmed with a score of >60 on the self-report subjective units of distress (SUDs). SUDs scale is continuum from 0 (no stress) to 100 (maximum load), and 50 is regarded as strong but barely endurable load. Exclusion criteria were: moderate or severe head injury, and/or a current diagnosis of psychosis or active suicidality in addition to general contraindication to MRI. Participants were scanned in a 3T MRI scanner (Prisma, Siemens) with a head coil at the ATR Brain Activation Imaging Center. fMRI signals were acquired using a gradient EPI sequence. During the experiments, we obtained 33 contiguous slices (TR = 2 sec, voxel size = $3 \times 3 \times 3.5$ mm^3, 0 mm slice gap) oriented parallel to the AC-PC plane, which covered the entire brain. We also obtained T1-weighted MR images (MP-RAGE; 256 slices, voxel size = $1 \times 1 \times 1$ mm^3, 0 mm slice gap).

Session for Decoder Construction

We first conducted a decoding session to quantify neural representations of traumatic stimuli, i.e., angry male-face pictures. The decoder was constructed so as to classify the fMRI bold signal pattern in superior temporal sulcus (STS) evoked by angry male faces from those evoked by happy female faces. Here, STS is known to represent facial emotions (Peelen et al., 2010). A modified continuous flash suppression (CFS) method was applied to render face presentation subjectively less distressing. The whole experiment comprised of 88 trials of each condition and was subdivided in 11 runs of 5 min duration. Whole exemplars (i.e., 16 exemplars) were shown once in each run in a randomized order. The obtained BOLD signals were preprocessed with mrVista software developed at Stanford University (http://vistalab.stanford.edu/software/). The functional images went through 3D motion correction without spatial and temporal smoothing. Then, the images went through rigid-body transformations to be aligned to the structural image for each participant. The BOLD signals from only the gray matter were extracted using a gray matter mask. Following preprocessing, the BOLD signals from the STS was further processed in the following steps: After removing a linear trend, the time-course in each voxel was z-score transformed within each run to minimize the baseline differences across the runs. The BOLD signal was averaged across 3 TRs which corresponded to the image presentation period at the maximum contrast (6 s). The signals were shifted by 6 s (3TRs) to compensate for the hemodynamic delay. The preprocessed fMRI signals from the STS were then used to construct a decoder to classify the activation patterns for angry vs. neutral faces. We used sparse logistic regression (SLR) (Yamashita et al., 2008) to automatically select the voxels that were relevant for classification. We trained the decoder using 176 data points obtained from 176 trials (across all 11 fMRI runs). The decoder was used in the following DecNef training to evaluate the trial-by-trial likelihood that participants could induce brain activation patterns for the angry faces.

DecNef Session

DecNef was conducted for 3 consecutive days following previous procedures (Koizumi et al., 2016; Taschereau-Dumouchel et al.,

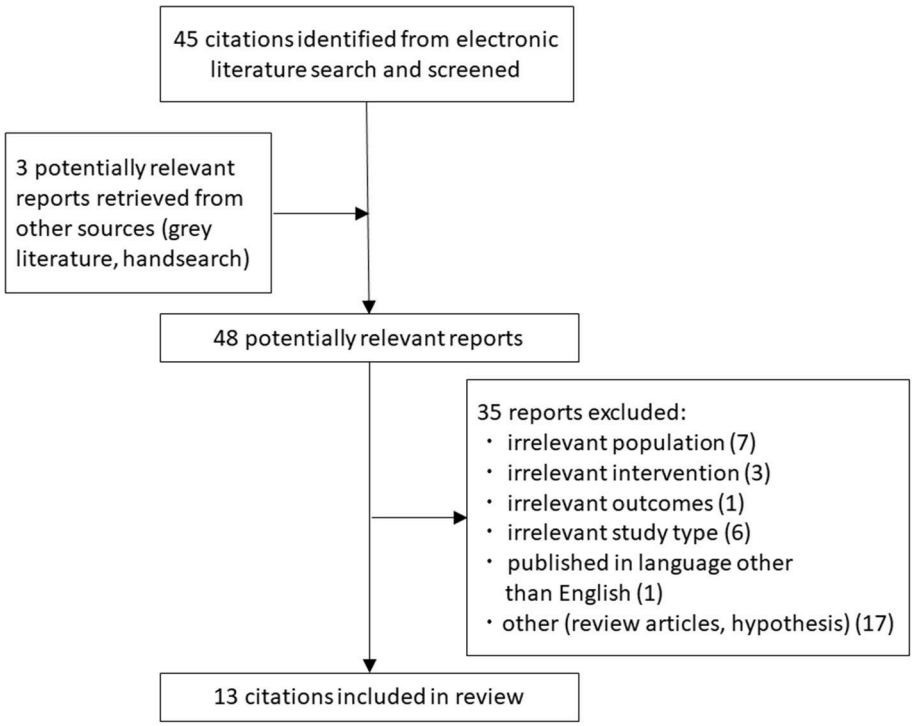

FIGURE 1 | PRISMA flow-chart illustrating the results of the search strategy.

2018). During the DecNef training stage of our experiment, STS neural patterns of activity related to angry male faces occurred repeatedly, without the participants' awareness of their doing so. Such, successful activation of this multi-voxel pattern was reinforced with monetary reward. On each day, participants went through 11 fMRI runs with 15 trials each (20 sec per trial). Each trial had a sequence of an induction period (6 s), a fixation period (7 s), a feedback period (1 s), and an inter-trial interval (6 s). Participants were instructed to "somehow" regulate their brain activity during induction period so as to maximize the feedback score. Feedback was calculated based on how similar the induced neural pattern was to that related to angry faces. Feedback was presented as a size of a disc after 6 s of the fixation period following the induction period. A hemodynamic delay of 6 s was taken into account. Participants were not informed as to what the feedback score represented (that is, likelihood of angry face activation in the STS). The size of the disc was determined as follow: First, the functional images obtained from Induction period underwent 3D motion correction with the Turbo BrainVoyager software (Brain innovation). Second, we extracted the time-course of BOLD signals from the voxels selected during decoder construction (see decoder construction), and shifted the signals by 3TRs (i.e., 6 s) to adjust for the hemodynamic delay. Third, after removing a linear trend, the BOLD signal time-course was z-score transformed for each voxel using the BOLD signals obtained during the 20 s period following the initial 10 s period from each fMRI run. Fourth, the processed BOLD signals for each voxel were averaged across the 3 TRs corresponding to the induction period from each trial. Lastly, we calculated the likelihood that the patterns of averaged BOLD signals represented angry faces using a decoder constructed with the data from decoder construction session. The disc size (i.e., radius) was proportional to the calculated likelihood of angry faces (0–100%). The feedback disc was presented inside a ring with 5° radius, which indicated the possible maximum size of the disc. After each run, texts were presented on the monitor to inform the amount of monetary reward earned from the current run as well as the accumulated amount from all the completed runs on that day. The reward corresponded to the sum of trial-by-trial likelihoods of angry faces, scaled to yield maximum amount of 300 yen (US $2.5) per run. After completing DecNef training each day, participants received the total monetary reward in cash.

The Mechanism of Decoded Neurofeedback

We hypothesized that the DecNef effects on fear reduction were either exposure-based (EB) or depend more on counter conditioning (CC), two common fear reduction effects achieved with the behavioral procedures to present feared objects alone without aversive outcome or to associate feared objects with positive outcome, respectively (Dickinson and Dearing, 1979; Foa and Kozak, 1986). To clarify the mechanism underlying DecNef, we mathematically modeled the effects of EB and CC separately, on the basis of the Rescorla-Wagner model (Rescorla and Wagner, 1972), and synaptic plasticity rules (Hebb, 1949).

Based on this framework, we re-analyzed data from Koizumi et al. (2016) and Taschereau-Dumouchel et al. (2018).

Rescorla-Wagner Model

In the Rescorla-Wagner model, degree of learning is quantified in terms of associations between conditioned (CS) and unconditioned (US) stimuli. Here, CS usually means emotionally neutral stimuli which will be paired with (CS+: target stimuli) or not paired with (CS–: control stimuli) US in the fear conditioning session. US is itself aversive stimuli such as pain or loud noise. After presented with US, CS+ presentation alone would evoke fear response, which are not observed before paired with US. This model casts the conditioning processes into discrete trials, during which stimuli may be either present or absent. This model defines ΔV_X as the change in the strength of the association between the CS (labeled "X") and the US:

$$\Delta V_{\mathrm{X}}^{n+1} = \alpha_X \beta(\lambda - V_{tot})$$
$$\mathrm{V}_{\mathrm{X}}^{n+1} = V_{\mathrm{X}}^{n} + \Delta V_X^{n+1}$$

where α is the salience of X, β is the learning rate parameter for the US, λ is the maximum conditioning possible for the US, and Vtot is the total associative strength of all stimuli present, that is, X plus any others. That is, $(\lambda - V_{tot})$ indicates the prediction error for the US. Vx is the current associative strength of X and is used to predict the associative strength of the next trial V_X^{n+1} using the expected change in the association ΔV_X^{n+1}.

Estimation of Effect Based on Simple Exposure

In exposure-based therapy, V_X^n can be considered as prediction error while α_X can be considered as likelihood for the target stimuli during induction period. Overall, part of ΔV_X^{n+1} results from EB effect is calculated as follows:

$$\Delta V_{X(EB)}^{n+1} = -\beta \ \mathrm{threshold}\left(L_{target(n)} - L_{control(n)}\right) \ V_X^n$$
$$\Delta V_{X(EB)}^{n+1} = -\beta_{sp} \ \mathrm{threshold}\left(L_{target(n)} - L_{control(n)}\right) \ V_X^n$$

where the *threshold(X)* $= X$ if $X > 0$, and 0 otherwise. The β_{sp} is the parameter for synaptic plasticity, that is, the learning rate of conditioning with positive value. $L_{target(n)}$ is the likelihood for the target information at the *n-th* trial, while $L_{control(n)}$ is the likelihood for the control information at the same trial. The extinction learning generally occurs after repeated exposure (Milosevic and Radomsky, 2008; Maren et al., 2013), therefore the expected change in the association ΔV_X^{n+1} through single exposure trial is postulated to be small in comparison with the strength of the association V_x. According to this postulation, V_X^n can be approximated to be constant throughout the session. Given the linear decrease in V_x across exposure therapy (Milosevic and Radomsky, 2008), we also assumed that the $\Delta V_{X(EB)}$ across trials are almost constant when the likelihood is higher than the chance level. Thus, the equation above can be approximated as follows:

$$\Delta V_{X(EB)}^{n+1} = -\beta'_{sp} H(L_{target(n)} - L_{control(n)})$$

H(X) is the Heaviside step function, which is 1 if $X > 0$ and 0 otherwise. Overall, to estimate EB in line with Rescorla-Wagner model, we assumed that EB effect is linearly proportional to the total number of trials in which induction of brain activation pattern resemble the one of the target stimuli. The trial was defined as successful when likelihood of brain activation pattern for target is higher than chance level, that is, higher than 50% in Koizumi et al. (2016), and higher than the likelihood for the control animal category in Taschereau-Dumouchel et al. (2018). Thus, DecNef effect based on EB throughout the session is approximated as follows:

$$\sum\nolimits_i \Delta V_{X(EB)}^i = -\beta'_{sp} \sum\nolimits_i H(\mathrm{L_{target(i)}} - \mathrm{L_{control(i)}})$$

Estimation of Effect Based on Counter Conditioning

Regarding the CC effect, the difference between Reward and V_X^n can be considered as prediction error. To estimate CC, we assumed that the trial has a fear reduction effect when the brain activation pattern for target was associated with a reward. The target brain activity is assumed to be induced when the likelihood of brain activation pattern for target was higher than chance level; i.e., 50%. We also assumed that the CC effect is a product of the two factors, namely success in induction of the neural activity pattern for the target stimuli and the amount of the reward. Because both factors are in proportion to likelihood for target pattern, the part of ΔV_X^{n+1} resulting from CC effect is calculated as follows:

$$\Delta \mathrm{V}_{\mathrm{X(CC)}}^{\mathrm{n+1}} = -\beta_1 \ threshold\left(\mathrm{L_{target(n)}} - 0.5\right)(\mathrm{Reward} - \mathrm{V}_{\mathrm{X}}^{\mathrm{n}})$$

where Reward is κ $threshold\left(L_{target(n)} - 0.5\right)$. The κ is a coefficient of the reward. Under the assumption that V_X is much smaller than Reward, the equation above can be approximated as follows:

$$\Delta \mathrm{V}_{\mathrm{X(CC)}}^{\mathrm{n+1}} = -\beta_1 \kappa \ threshold\left(\mathrm{L_{target(n)}} - 0.5\right)^{\wedge 2}$$

Thus, DecNef effect derived from CC throughout the session is calculated as follows:

$$\sum_i \Delta V_{X(CC)}^i = -\beta_1 \kappa \ \sum_i threshold(\mathrm{L_{target(i)}} - 0.5)^{\wedge 2}$$

Separate Estimation of the Effects by EB and CC

Finally, to separately estimate the effect of EB and CC on fear reduction, we assumed that the DecNef effect is weighted linear summation of $V_{X(EB)}$ and $V_{X(CC)}$ using mixed effect model to adjust the clustering from study type, that is either experimentally conditioned fear (Koizumi et al., 2016) or naturalistic animal phobia (Taschereau-Dumouchel et al., 2018). The mixed effect was used to adjust the difference in strength between the experimental vs. natural association with fear. Tests for absence of influential data points and independence did not reveal any violation of the assumptions for mixed effect models. The total effect is given as follows:

$$V_{X(amg)} = \beta_{EB} \ V_{X(EB)}{}' + \beta_{cc} \ V_{X(CC)}{}' + \ (1|\mathrm{paper})$$
$$V_{X(EB)}{}' = V_{X(EB\,)}/\beta_{sp}{}'$$
$$V_{X(CC)}{}' = V_{X(EB\,)}/\beta_1 \kappa$$

where $V_{X(amg)}$ is a subtraction of amygdala response to control stimuli at post-DecNef from those to target stimuli at post-DecNef. The β_{EB} and β_{CC} is the coefficient of EB effect and CC effect, respectively.

RESULTS

Thirteen published articles were identified that met the criteria for this review. Ten studies adopted the EEG neurofeedback approach, while 3 studies adopted the fMRI neurofeedback approach.

Neurofeedback

EEG Based Neurofeedback on PTSD

EEG neurofeedback was performed to alter the power spectrum of certain filtered frequencies of activity. In line with that for other anxiety disorders (Hammond, 2005a,b, 2011; Schoenberg and David, 2014), EEG neurofeedback for PTSD is mainly used to regulate the power of either alpha waves alone or of both alpha and theta waves. Alpha activity is targeted because it is generally associated with a calm, relaxed state. PTSD patients have both decreased power and accelerated frequency of the alpha rhythm (Jokić-begić and Begić, 2003; Wahbeh and Oken, 2013). Six studies were designed to up-regulate the power of alpha rhythms either by combining rewards with alpha wave (Gapen et al., 2016; van der Kolk et al., 2016; Askovic et al., 2017) or by alpha desynchronization (Kluetsch et al., 2014; Nicholson et al., 2016; Ros et al., 2017). Alpha/theta training has been adopted in three studies (Peniston and Kulkosky, 1991; Peniston et al., 1993; Smith, 2008). Contrary to typical EEG neurofeedback for PTSD which targets alpha and/or theta waves, several studies have instead adopted sensorimotor rhythm (SMR) training (Pop-Jordanova and Zorcec, 2004; Askovic et al., 2017). SMR training was associated with enhanced attention performance and less motor activity (Sterman, 1996; Egner and Gruzelier, 2001). In one of these studies (Askovic et al., 2017), the therapists selected a neurofeedback protocol to specifically target each individual's specific maladaptive EEG patterns. Probably the most reliable empirical evidence for the success of EEG neurofeedback for PTSD came out from a study, reported above (van der Kolk et al., 2016), that was performed in the randomized, waitlist-controlled manner (van der Kolk et al., 2016). In this study, individuals with chronic PTSD in the neurofeedback group, compared with the control group, showed significant PTSD symptom improvement, as well as improvement in affect regulation capacities as measured by the Inventory of Altered Self-Capacities.

fMRI-Based Neurofeedback on PTSD

Conventional fMRI neurofeedback for PTSD was mainly used for modulation of amygdala activity levels (**Table 1**). Two studies downregulated amygdala activity during symptom provocation (Gerin et al., 2016; Nicholson et al., 2017a,b), while one study upregulated amygdala activity during happy emotion induction (Zotev et al., 2011). In one of these studies (Gerin et al., 2016), 2 of 3 patients had clinically meaningful improvement in PTSD severity as measured by CAPS, while the third patient had almost no improvement. In another of these studies (Zotev et al., 2011), a consummate technique called emotion regulation was used. In this technique, participants learn to upregulate their amygdala activity while recalling happy autobiographical memories. This technique was originally developed in the research field on depression, in which it was found to show sizable effects with a double-blind placebo control design (Young et al., 2017). In Zotev's PTSD study, however, the effect was found modest.

Neurofeedback Using EEG Fingerprint

EEG is mobile and low cost but with limited spatial resolution, while MRI has a high spatial resolution but with low accessibility and low cost-effectiveness. To overcome these limitations of both equipments, simultaneous EEG-fMRI was introduced to estimate the amygdala fMRI-bold signal from EEG data, which is termed the amygdala electrical fingerprint (Keynan et al., 2019). Based on this fingerprint, amygdala activity was calculated using EEG only during the neurofeedback session, which was fed back to the participants.

This procedure is applied successfully to stress management in healthy soldiers and its effectiveness was demonstrated in double blinded manner. In comparison with participants assigned to either control neurofeedback group or with no neurofeedback group, participants assigned to experimental group showed significant reduction in alexithymia and faster emotional stroop which was regarded as activating a resilience process.

Decoded Neurofeedback (DecNef) for Fear Memory

DecNef can be used to modify brain activity specific to different pathogeneses. Specifically, using this approach the multi-voxel activation patterns of fMRI signal within specific region of interests (ROIs) that represent designated mental experiences and states can be targeted. **Figure 2** shows a conceptual schema of DecNef. Prior to DecNef training, participants first go through a fMRI decoder construction session. In this session, fMRI multi-voxel patterns for specific stimuli (e.g., red circle and green circle) are recorded. This fMRI signal is subsequently examined by a machine learning technique to decode brain activity on the basis of the presented stimuli (e.g., to decode the two fMRI signal patterns that correspond to when viewing a red and a green circle, respectively). This decoded multi-voxel pattern is used to create the target for induction in the participants brain during subsequent DecNef training in the MRI scanner (e.g., the target might be to induce brain activity related to a red, rather than a green circle). During DecNef training, real-time fMRI signal is processed immediately and the similarity between this signal and that of the target, within a predefined brain activity, is calculated online. Roughly speaking, feedback is given based on this similarity and participants aim to unconsciously and/or volitionally manipulate their own brain activity so that this similarity is increased. The feedback approximately represents the "similarity" between the target fMRI signal pattern evoked by the real stimulus (e.g., red circle or animal pictures) and a current fMRI signal pattern observed in the absence of the real stimulus. In this article, we use the term "similarity" for the sake of simplicity. Rigorously, however, the feedback is not the similarity of a current fMRI signal pattern for specific stimuli.

TABLE 1 | Applications of neurofeedback for PTSD patients.

	References	Sample	Design							
		N	%male	Age (years)	Medicated (yes/no)	Randomized (yes/no)	NF approach	Control group	Risk of bias	Outcome measures and measures used
DecNef	Chiba, this manuscript	4	0	40 (mean)	Yes, $n = 3$	No	Multivariate pattern for angry face	No	C	CAPS:97.8–>54.5
f-MRI-nf	Zotev et al., 2018	23 (15 NF vs. 8 sham)	100		30.8 vs. 36.8 (mean)	Yes	Amygdala upregulation during a happy emotion induction	sham	B	CAPS: 55–>41
	Nicholson et al., 2017a,b	10	40	49.6 (mean)	Yes, $n = 9$	No	Amygdala downregulation	No	C	A shift in amygdala complex connectivity
	Gerin et al., 2016	3	100	37.3 (mean)	Yes, $n = 3$	No	Amygdala downregulation	No	C	CAPS: 65–>37
EEG-nf	Askovic et al., 2017	2	100	31(mean)	Yes, $n = 2$	No	Enhance either the SMR or alpha rhythm	No	C	HTQ:3.15–>1.85 HSCL-D: 3.30>2.1 HSCL-A: 3.2–>1.95
	van der Kolk et al., 2016	28	89	46 (mean)	Yes, $n = 16$ (NF) $n = 10$ (WL)	Yes	Enhance alpha activity	WL	B	NF: CAPS:80.1–> 44.1 DTS:67.3–>55.7 WL: CAPS:75.2–> 65.8 DTS:63.0–>60.6
	Nicholson et al., 2016	21	14	39.9 (mean)	Yes, $n = 11$;	No	Alpha desynchronization		C	A shift in amygdala complex connectivity
	Ros et al., 2017	21	14	39.9 (mean)	Yes, $n = 11$;	No	Alpha desynchronization	No	C	Decrease in TAC correlated with increases in Hurst exponent at the feedback channel Increase in Alpha amplitude
	Gapen et al., 2016	17	12	32–64 (Range)	Yes, $n =$	Yes (T4-P4 or T3-T4)	Enhance alpha activity	Active		DTS: 69.14–>49.26
	Kluetsch et al., 2014	21	14	39.9 (mean)	Yes, $n = 11$; no, $n = 10$	No	Alpha desynchronization	No	C	A shift in functional connectivity
	Smith, 2008	10	100	26–63 (Range)	Yes, $n = 3$	No	Two phased: (1) bipolar uptraining (15–18 Hz and 12–15 Hz) + theta (4–7 Hz) suppression and (2) alpha/theta (5–8 Hz) training followed by bipolar uptraining	No	C	PTSD induced symptoms of depression and attention measured by HAMD and TOVA
	Pop-Jordanova and Zorcec, 2004	10	70	9 (mean)	No	No	SMR	No	C	Skin electric resistance Brainwave changes PTSD symptoms
	Peniston et al., 1993	20	100	37.2 (mean)	Not reported	No	Alpha/theta	No	C	Synchronization, Brainwave amplitude changes, PTSD symptoms reported by monthly telephone contact
	Peniston and Kulkosky, 1991	29 (15 NF vs. 14 TAU)	100	36.1 vs. 37.25 (mean)	Yes	Yes	Alpha/theta	TAU	B	MMPI-indexed personality changes Medication consumption PTSD symptoms reported by monthly telephone contact

TAC, Thayer Activation Checklist; NF, neurofeedback condition; WL, waitlist condition; CAPS, Clinician-Administered PTSD Scale; HTQ, Harvard Trauma Questionnaire; HSCL-D, Hopkins Symptom Checklist Depression Scale; HSCL-A, Hopkins Symptom Checklist Anxiety Scale; DTS, the Davidson Trauma Scale; HAMD, Hamilton Depression Rating Scale; TOVA, Test of Variables of Attention; SMR, The sensorimotor rhythm; MMPI, Minnesota Multiphasic Personality Inventory; TAU, Treatment-as-usual.

The feedback is based on how much the decoder classifies the current fMRI signal into a target class, that is, likelihood of the target class. More concretely, the decoder was constructed to identify a stimulus (e.g., a snake picture) that is presented to a participant among different stimuli (e.g., animal pictures other than the snake) based on fMRI signal patterns. The feedback reflects the output of the decoder that represents likelihood of the target stimulus. Consequently, the feedback could be derived from hundreds or even hundreds of millions of brain activity patterns, and is an abstract index of a specified information by the decoder. This is a unique characteristic of DecNef compared with other causal methods such as optogenetics reproducing only once-occurred brain activity.

DecNef has been applied to manipulations of brain activity patterns corresponding to various mental states such as perceptual learning (Shibata et al., 2011), face preference (Shibata et al., 2016), meta-cognition (Cortese et al., 2016), color-orientation association (Amano et al., 2016), and reduction in physiological fear responses (Koizumi et al., 2016; Taschereau-Dumouchel et al., 2018).

During DecNef for reduction in physiological fear responses, participants could be trained to associate with a reward the decoded brain representation of given traumatic/distressful events. This approach might be more effective than conventional neurofeedback because it is somewhat akin to exposure therapy, which is the most effective therapy for phobia and PTSD, but does not cause the conscious awareness of the fearful event that so many people find so aversive during exposure therapy.

Recent studies have shown that DecNef can reduce physiological fear responses to both fear conditioned stimuli (Koizumi et al., 2016) and feared animals (Taschereau-Dumouchel et al., 2018; **Figure 3**). There was particularly strong evidence for the effect of DecNef in the study with feared individuals, because this study utilized a double-blind, placebo-controlled, randomized paradigm (Taschereau-Dumouchel et al., 2018).

In the study where DecNef was used to reduce fear to fear conditioned stimuli (Koizumi et al., 2016) the multi-voxel activation pattern of activity related to the fear conditioned stimuli was paired with a reward. As a result, a significant reduction of participants' physiological fear response to these stimuli was observed. Specifically, in this study participants were told that during each trial of the DecNef training they should "somehow" self-regulate their neural activity. Unbeknown to the participants, the target was for them to induce the multi-voxel pattern of fMRI signal related to one of the two fear conditioned stimuli. On each trial, if the participant successfully induced the target pattern of fMRI signal, then they received a large reward. Thereby, via trial and error, participants learned to induce this particular pattern of neural activity, resulting in a reduced fear response to this stimulus when it was presented after DecNef training. However, this approach contains a fundamental problem for clinical application. Using this approach, prior to DecNef training, the target multi-voxel pattern of fMRI signal has to first be determined in a decoding session. This requires the explicit and repeated presentation of the target stimulus. In a laboratory setting, it is possible to decode the fMRI signal patterns for the to-be-feared conditioned stimuli a priori; i.e., ahead of fear conditioning. However, such a priori decoding is difficult in the clinical setting where patients will come in with the fear associations already strongly formed. Exposure to fear-relevant stimuli during the decoding session is likely to be highly distressful for the patients with phobia/PTSD.

This problem was overcome in a study by Taschereau-Dumouchel et al. (2018). Using a method called hyperalignment, the relevant neural representations of feared animals were inferred based on data from "surrogate" participants. Briefly, in an fMRI experiment, participants were presented with images of multiple animals and objects. In order to create the decoder of an animal feared by a designated participant, hyperalignment was used to create a "common representational space" using the neural representations of the non-fearful animals. Through this common space, it was then possible to use only the data of the "surrogate" participants to train a multi-voxel decoder of the feared animal. As such, the decoders could be trained without presenting the designated participant with aversive pictures. By subsequently using these decoders in a DecNef training, a significant reduction in the physiological fear response to the feared animals was found (**Figure 3**).

In summary, participants unconsciously induced brain activity for stimuli that they feared. Of importance, in contrast to conventional exposure-based therapy, these procedures evoked no distress in the participants.

Decoded Neurofeedback for PTSD: A Preliminary Result

Recently, we conducted a DecNef experiment for 4 individuals with PTSD. After DecNef training, all 4 patients exhibited a clinically significant reduction (Krystal et al., 2011) (10-point decrease) in scores on the Clinician-Administered PTSD scale for DSM-4 (CAPS-4), which represents PTSD severity. **Figure 4** shows the CAPS total scores before and after the intervention. After the intervention, 1 patient no longer even met the PTSD diagnosis criteria, which is defined as a total score of below 20 on CAPS (Weathers et al., 2001).

Mechanisms of Decoded Neurofeedback (DecNef) Effect

DecNef seems to be a promising approach to treat fear-related diseases such as anxiety disorder, phobia, and PTSD. However, how DecNef reduces the fear responses is not fully understood. Two possible mechanisms have been previously postulated (Koizumi et al., 2016; Taschereau-Dumouchel et al., 2018), namely exposure-based (EB) effect and counter conditioning (CC) effect. The EB effect is consistent with the idea in conventional exposure-based therapy. That is, simple exposure to feared target under the safe condition reduces fear response to the target. This idea is also consistent with fear extinction learning. The CC effect is to change the association of the stimuli with fear by associating the stimuli with a reward (Dickinson and Dearing, 1979). That is, presentation of fearful stimuli together with reward reduces the fear response to the target.

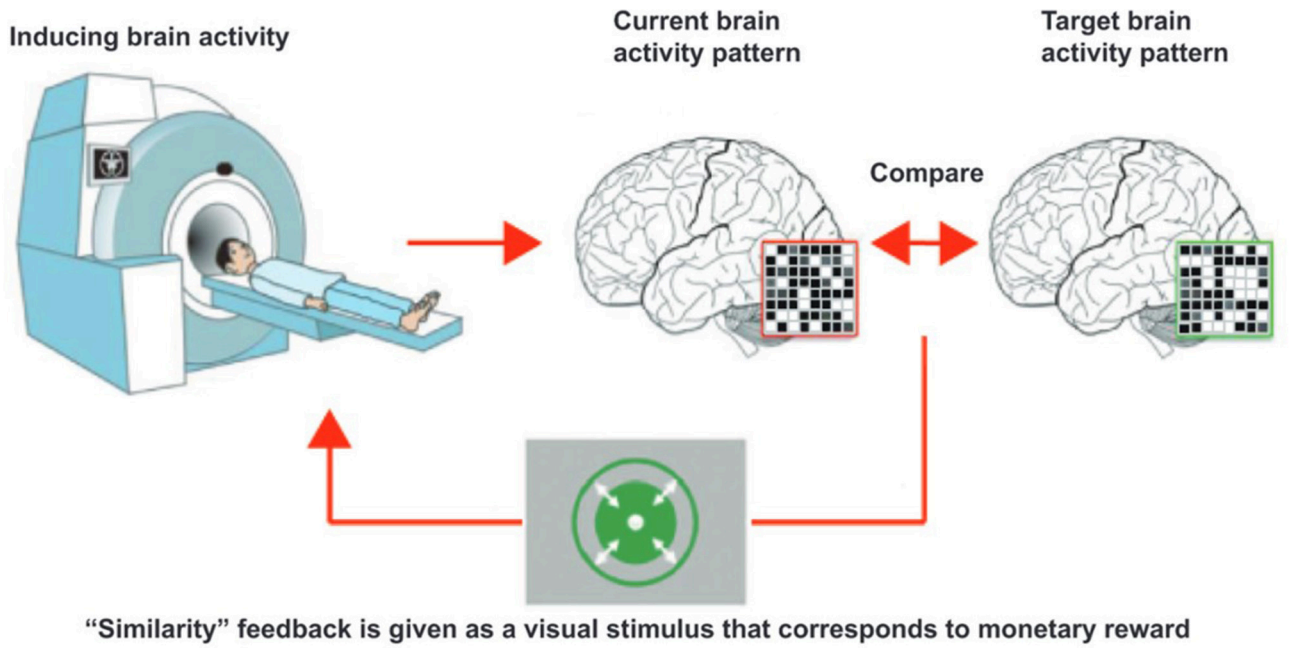

FIGURE 2 | Schema of decoded neurofeedback. The participant, in the scanner, is instructed to "somehow" regulate their brain activities so that the feedback is maximized. Then, "somehow" manipulated brain activity pattern are processed as a fMRI signal and compared with target brain activity pattern. Finally, the participants are presented with a disk whose size is in proportion to the likelihood, which is also in proportion to the amount of reward the participant will gain from that trial. This cycle is then repeated. The figure is adopted from Yamada et al. (2017), with no permission required.

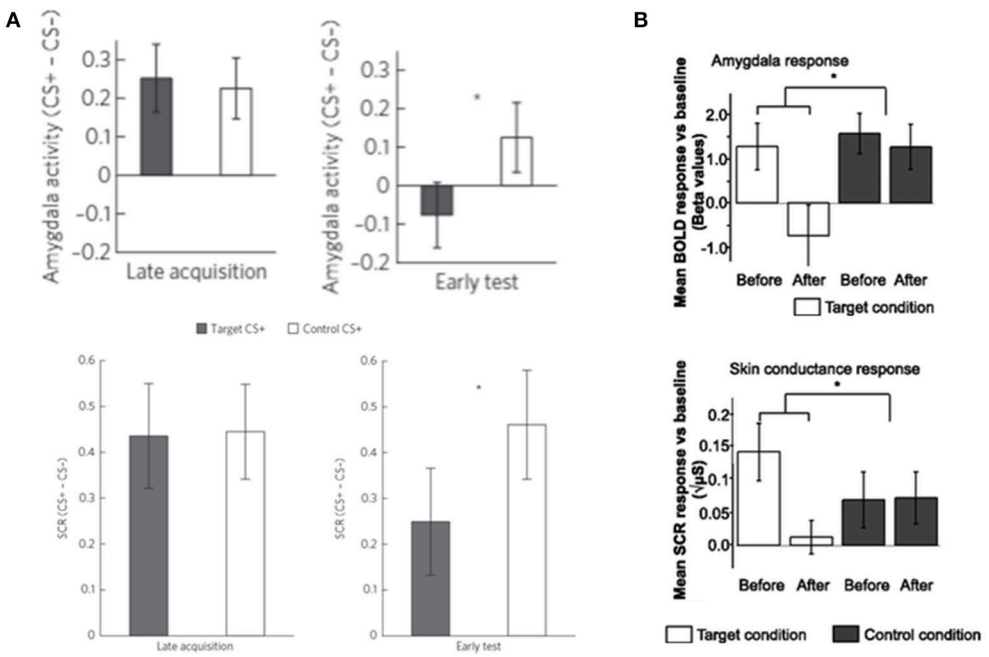

FIGURE 3 | DecNef effects on fear reduction in **(A)** fear-conditioned stimuli and **(B)** feared animals. The response to target stimuli was reduce compared to control stimuli in both **(A)** fear-conditioned stimuli and **(B)** feared animals as measured by both amygdala activity and skin conductance response (SCR). Error bars represent standard errors. **(A)** Modified from Koizumi et al. (2016), with permission from the authors. **(B)** Modified from Taschereau-Dumouchel et al. (2018), with no permission required. *$p < 0.05$.

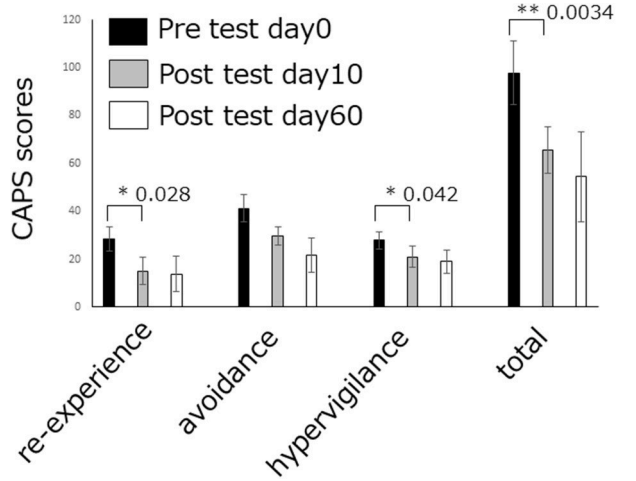

FIGURE 4 | DecNef effects on PTSD amelioration. PTSD symptom cluster (i.e., re-experiencing, avoidance, and hypervigilance) and total severity scores as measured by the past week version of the CAPS-4. The re-experiencing symptoms and hypervigilance symptoms, as well as total PTSD severity at pre DecNef session reduced significantly compared at post-DecNef session. Error bars represent standard errors.

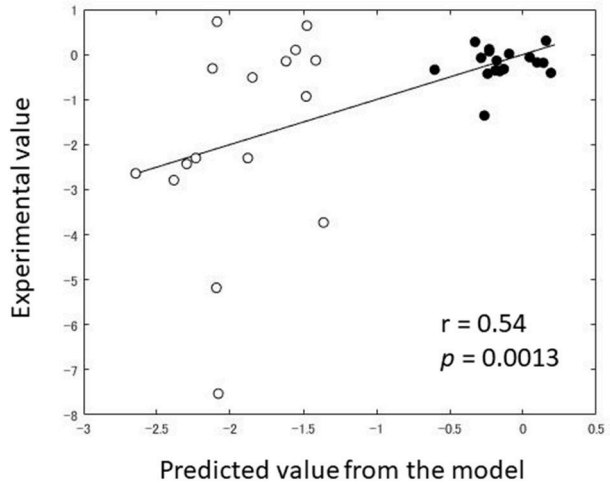

FIGURE 5 | Comparison between predicted value and experimental value of Amygdala reactivity post-DecNef (target–control). Black dot indicates the individual data from Koizumi et al. (2016) while the white dot indicates the individual data from Taschereau-Dumouchel et al. (2018).

This effect is known to be larger than simple exposure effect (Newall et al., 2017).

In order to dissociate the effects of EB from those of CC on fear reduction via DecNef, we mathematically modeled the DecNef effects as those derived from EB and CC separately, on the basis of Rescorla-Wagner model and synaptic plasticity rules. Briefly, we assumed that EB effect is linearly proportional to the numbers of the trials in which the target activity pattern was successfully induced (likelihood above chance). We also assumed that CC effect of each trial is proportional to the induction likelihood of the target pattern multiplied by the amount of reward, which the participant obtains the trial. This model can predict the DecNef effect ($\beta_{EB} = -0.016$, $p = 0.0069$, $df = 28$, $\beta_{CC} = 0.014$, $p = 0.0017$, $df = 28$) with a non-significant estimated intercept for the paper ($1|\text{paper} = -0.692$, $p = 0.55$, $df = 28$). The predicted values from the model were correlated with the experimental values ($r = 0.54$, $p = 0.0013$; **Figure 5**). Since negative value of $V_{X(amg)}$ indicates the reduction of physiological reactivity to target stimuli, the smaller value of beta indicates that the corresponding variables are more effective. Therefore, this result suggests that EB effect, the negative coefficient, is the key component for DecNef effect on the reduction of fear response observed from Koizumi et al. (2016), and Taschereau-Dumouchel et al. (2018). The $V_{X(EB)}$ and $V_{X(CC)}$ have a significant effect only when data from two studies were combined. No statistically significant effect has been observed for them from a single study. With each study, the predicted values from the model were not significantly correlated with the experimental values [$r = 0.36$, $p = 0.17$ for Koizumi et al. (2016); $r = 0.36$, $p = 0.16$ for Taschereau-Dumouchel et al. (2018)], however, the effect sizes were of intermediate magnitude, in the direction expected.

DISCUSSION

We reviewed current status of neurofeedback trials for PTSD amelioration intended to be informative for neuroscientists in general as well as practitioners planning to use neurofeedback as a therapeutic strategy for PTSD. Despite promising results are derived from both EEG and fMRI neurofeedback (**Table 1**), the efficacies of these approaches have not yet been warranted.

We show preliminary data indicating that DecNef ameliorated PTSD symptoms through 3 days of feedback training. Although tentative, this result was comparable to conventional exposure therapy and conventional neurofeedback approach. Together with a short intervention period required, the results so far are encouraging to suggest that DecNef could be a promising procedure to alleviate actual PTSD symptoms. In the future, a larger sample of participants and a double-blind placebo control design are needed to demonstrate the effectiveness of this novel method for treating PTSD.

To further clarify the underlying mechanisms of DecNef, we demonstrated that the previously reported effect of DecNef in fear response reduction (Koizumi et al., 2016; Taschereau-Dumouchel et al., 2018) is estimated by the amount of successful induction of the target brain activity patterns. Whether the predominant contribution of EB effect is intrinsic to DecNef or specific to the previous two studies awaits further investigation. For example, it is worth testing the possibility that the effect of CC became noisier in the two studies because of the temporal delay of reward by several seconds. Here, we assumed that the linear term of the degree to which the targeted neural representation is successfully induced (i.e., likelihood for target pattern) corresponds to EB effects, while the quadratic term corresponds to counter conditioning effects. Although these

assumptions are tentative, the results still hold that the DecNef effect in fear reduction is explained by the likelihood for successful induction of activation pattern linearly rather than by the quadratic polynomial of it. The current model should be applied to a much larger sample size for further validation in a future study.

In clinical application, DecNef has a limitation in that it can induce only specific brain activation patterns which can be decoded via multivariate pattern analysis. However, DecNef can directly access the representation for feared stimuli without eliciting conscious aversive experience if combined with procedures such as hyperalignment or CFS. This means that DecNef allows patients to be implicitly exposed to extreme traumatic stimuli with little distress, which could be advantageous to conventional exposure based therapy which can deal with only moderate traumatic stimuli.

In addition to DecNef, three promising alternative approaches have been proposed in research areas other than PTSD. First, conventional univariate fMRI-based neurofeedback can be used more effectively with deep understanding of disease. With deep understanding of Major depressive disorder, Young et al. demonstrated its efficacy utilizing a double-blind, placebo-controlled, randomized clinical paradigm (Young et al., 2017). Patients with depression show blunted amygdala hemodynamic activity to positive stimuli, and amygdala engagement appears to be critical for emotional processing and responding to both negative and positive stimuli. Based on these knowledges, they increased the amygdala's hemodynamic response to positive memories in patients with depression. Specifically, participants were instructed to retrieve positive memories while attempting to increase the hemodynamic activity in the left amygdala which was feedback to the participant as a blue bar (Young et al., 2017). This neurofeedback significantly decreased depressive symptoms and increased the percent of specific memories recalled on an autobiographical memory test. Second, EEG-fingerprint has been shown to be a feasible approach (Keynan et al., 2019). One of the fundamental problems in applications of neurofeedback for PTSD treatment arises from equipment characteristics: EEG is mobile and low cost but with limited spatial resolution, while MRI has a high spatial resolution but with low accessibility and low cost-effectiveness. To overcome these limitations, EEG-fingerprint technique enables us to estimate the amygdala fMRI-bold signal from EEG data. It can confer a participant stress resilience (Keynan et al., 2019). In the future, prospective cohort study may be needed to verify the effectiveness of this novel method for preventing PTSD development. Lastly, Functional Connectivity Neurofeedback (FCNef) (Fukuda et al., 2015; Yamashita et al., 2017) has been applied to patients of major depressive disorder and schizophrenia, and autistic participants, and its preliminary but encouraging effects have been shown (Yamada et al., 2017). Instead of brain activity patterns in specific region, FCNef manipulates the functional connectivity which is defined as synchronicity of activation between spatially apart two brain regions. FCNef allows patients to induce brain activity so as to normalize disease specific resting state functional connectivity patterns which are objectively determined using machine learning technique (Yahata et al., 2016, 2017; Yamada et al., 2017). Further development of these alternative approaches as well as of DecNef should bring more effective treatment options for wider clinical populations.

CONCLUSION

In this review, we discussed recent advances in neurofeedback therapy for PTSD and presented the findings of a DecNef experiment that we conducted on patients with this disorder. While neurofeedback therapy is still in the initial stages of development, approaches such as DecNef have the potential to provide an alternative to the conventional method of PTSD treatment by preventing PTSD patients from feeling distress during the course of treatment. One limitation of this review is that since it is the dawn period of neurofeedback development, we cannot draw a conclusion from current literature what type of neurofeedback is most promising for PTSD amelioration. However, in the future, using neurofeedback approaches such as DecNef may allow for more targeted pathogenesis-based treatment of a variety of other psychiatric disorders as well.

AUTHOR CONTRIBUTIONS

TC, TK, AK, KI, VT-D, HL, and MK designed the research. TC, KI, and MS performed the research. TC, AK, and VT-D analyzed the data. TC, TK, AK, VT-D, SB, AH, IS, HL, HY, and MK wrote the paper.

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frontiers in Human Neuroscience

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A Transcranial Stimulation Intervention to Support Flow State Induction

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Background: Flow states are considered a positive, subjective experience during an optimal balance between skills and task demands. Previously, experimentally induced flow experiences have relied solely on adaptive tasks.

Objective: To investigate whether cathodal transcranial direct current stimulation (tDCS) over the left dorsolateral prefrontal cortex (DLPFC) area and anodal tDCS over the right parietal cortex area during video game play will promote an increased experience of flow states.

Methods: Two studies had participants play Tetris or first-person shooter (FPS) video games while receiving either real tDCS or sham stimulation. Tetris recruited 21 untrained players who infrequently played video games while the 11 FPS participants played FPS frequently. Flow experience was assessed before and after stimulation.

Results: Compared to sham stimulation, real stimulation increased flow experience for both untrained Tetris and trained FPS players. Improved performance effects were only seen with untrained groups.

Conclusion: Cathodal and anodal tDCS over the left DLPFC and right parietal areas, respectively may encourage flow experiences in complex real-life motor tasks that occur during sports, games, and everyday life.

Keywords: flow, psychophysiology, tDCS, neuromodulation, decision making

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INTRODUCTION

Flow, or optimal experience is a "holistic response" which results from a harmony found between all the states of consciousness and the individuals' skills matching their goals (Csikszentmihalyi, 1990). According to Csikszentmihalyi's (1990, 1997) flow theory, the flow state relates to the skill set perceived to be possessed by the individual relative to the perceived challenges of the activity. Challenges can be considered as "opportunities for action" thus flow is produced by any situation that requires skill (Csikszentmihalyi and Nakamura, 1999; Nakamura and Csikszentmihalyi, 2014). One of the leading neurocognitive theoretical models of flow purported by Dietrich (2004) denotes a state of transient hypofrontality, which enlists the full support of the implicit system to execute a task at optimal output (maximum skill/maximum efficiency) while the majority of the online executive function of the prefrontal cortices are inhibited (Dietrich, 2004, 2006). Implicit memory has been identified as a key functional region in flow states as it reduces verbal-analytical involvement in motor control by encouraging limited dependence on working memory

(Masters, 1992; Maxwell et al., 2001; Liao and Masters, 2002) enabling performance with higher neural efficiency than explicit motor tasks relying on working memory (Zhu et al., 2011). Whereas, the automaticity reached in implicit memory is fast, effortless and free from distraction (Shiffrin and Schneider, 1977).

Specifically, the left dorsolateral prefrontal cortex (DLPFC) has been shown to modulate working memory (Barbey et al., 2013). Sharing Brodmann's area 8 (BA8) and close proximity to the frontal left is the medial prefrontal cortex (MPFC) which has been associated with self-monitoring and reflective processing employed during explicit processes which limit the efficiency of the system (Shiffrin and Schneider, 1977; Gusnard et al., 2001; Northoff et al., 2006; Yarrow et al., 2009). More recently, Ulrich et al. (2014) identified certain neural underpinnings that help explain part of the flow paradigm, in particular, a decrease in frontal activity around the MPFC.

Furthermore, the flow system is proposed to be a reflexive system guided by the preceding input (Dietrich, 2003). Therefore, it is believed that a basic level of skill acquisition is needed to have a flow experience, as the implicit system requires a series of learnt specialized and independent response patterns to output (Csikszentmihalyi and Csikszentmihalyi, 1988). These automated stimulus response procedures are believed to require many hours of highly dedicated practice. Learning of automated responses takes time because of the limited ability of the explicit working memory to transfer specialized and reflexive response patterns to the implicit system due to capacity restrictions (Mishkin et al., 1984; Dietrich, 2004). Experts are expected to have more automaticity available as the implicit system requires a series of specialized and independent response patterns to output, free from buffering other properties of the information in a higher order representation (Masters, 1992; Ohlsson, 2012). Flow is considered to increase in intensity on the continuum of experiential quality of the activity as the participant learns to utilize more of their dedicated facilities required for the task (Csikszentmihalyi and Csikszentmihalyi, 1988).

It has been shown that the brain makes use of an internal model which provides a sensorimotor representation of oneself with the world around (Jordan, 1996). Forward and inverse models can be utilized to explain the role of implicit processing by identifying the role of the network connecting the cerebellum, parietal and frontal regions to explain this control of high level processes such as decision making (Ito, 2008). These models consider that the prefrontal regions construct the mental model, but this mental model, used to explain and anticipate reality, exists in the parietal regions (Penfield and Perot, 1963), enabling the prefrontal region to be bypassed (Atherton et al., 2003; Chen et al., 2003). In one of the few neuroimaging studies on flow, an increase in activation was shown in the parietal regions as well as a decrease in prefrontal activity during a math task (Ulrich et al., 2014). Additionally, it has been shown that implicit bottom-up visual attention receives greater control from the parietal regions whereas top down control of more explicit processes are related to the frontal regions (Li et al., 2010). Furthermore, a long-range circuit has been found between these two regions that appears anatomically connected to guide choices toward movement goals (Sasaki et al., 1976; Pesaran et al., 2008).

To further test flow states and how it emerges, and possibly induced, is essential to better understand the flow state in practice. Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique that alters cortical excitability and activity in a polarity-dependent way. Anodal stimulation increases excitability (Liebetanz et al., 2002), whereas cathodal decreases it (Nitsche and Paulus, 2001). Stimulation for a few minutes has been shown to induce plastic alterations of cortical excitability and more specifically has shown to influence cognitive functions such as working memory by stimulating the left DLPFC (Fregni et al., 2005; Chrysikou et al., 2013; Zhu et al., 2015). Cathodal DLPFC tDCS has been shown to improve implicit learning outcomes for high-level motor tasks such as golf putting (Zhu et al., 2015) and cognitive flexibility (Chrysikou et al., 2013). Furthermore, it has been shown that tDCS has helped improve learning outcomes for implicit motor tasks, in which right parietal anodal stimulation resulted in greater neural efficiency through an improved task learning performance (Clark et al., 2012), as well as mental activities such as numerical competence (Cohen et al., 2010), network connectivity (Hunter et al., 2015) object detection during visual search (Bolognini et al., 2010; Clark et al., 2012; Tseng et al., 2012), spatial attention (Roy et al., 2015), and non-verbal material (Manuel and Schnider, 2016). Additionally, tDCS influence on parietal regions has shown a balance between the working memory capacity (skill) and the working memory task (Jones and Berryhill, 2012). More recently, Ulrich et al. (2018) used anodal tDCS over the forehead Fpz to stimulate the medial prefrontal cortex (MPFC) and found higher flow experiences for people experiencing low flow. Therefore, tDCS learning enhancement could increase the level of visual attention skill in order that the participant could reach the skill-challenge balance (Clark et al., 2012) and limit the role of the prefrontal monitoring in order to allow for greater movement into flow states (Zhu et al., 2015).

While flow states require a certain level of previous skill to be automatized into their implicit memory, tDCS has been shown to result in ceiling effects for experts compared to novice performers (Bullard et al., 2011; Tseng et al., 2012; Furuya et al., 2014; Rosen et al., 2016). Therefore, two groups of trained and untrained video gamers were selected for the study to explore the contrasting effects of the required skill acquisition and expertise to move into flow states with tDCS ceiling effects of expertise. The Tetris game paradigm has proved easy to quantify performance and level of difficulty in both flow (Keller and Bless, 2008; Keller et al., 2011; Harmat et al., 2015) and tDCS studies (Spiegel, 2013). First person perspective video games have also shown to operationalize a good balance of skill and challenge with immersive experiences for both flow (Kivikangas, 2006; Nacke L. and Lindley C., 2008; Nacke L. and Lindley C. A., 2008; Nacke and Lindley, 2010; Nacke et al., 2010; Klasen et al., 2011) and tDCS studies (Bullard et al., 2011; Clark et al., 2012; Coffman et al., 2012; Falcone and Parasuraman, 2012). Therefore, both experimental paradigms were used to determine the mediating role tDCS will have in supporting the induction of flow states.

The focus of this study was to observe the inductive role of tDCS on flow states using two different paradigms. It was hypothesized that right parietal anodal tDCS and cathodal tDCS

of the left prefrontal area would result in a shift in the subjective experience toward higher intensity experiences of flow states for both trained and untrained users of video games.

MATERIALS AND METHODS

Participants

Two experiments were ethically approved (by University Committee) to study the effects of tDCS on flow states during video game play. All participants were recruited by word of mouth or from advertisements in game forums. Experiment 1 inclusion requirement was trained gamers played 1st person shooter videogames (FPS) on average several times a week. Eleven right-handed males ($M = 29$ years, $SD = 7.15$) played a FPS across two sessions within a week using randomized active and sham tDCS conditions.

Experiment 2 inclusion requirement was untrained gamers who on average played videogames once a month or less. Twenty-three participants were originally tested but two were corrupted due to their being initial pilot tests, therefore only 21 right handed participants were tested; 11 females ($M = 30.18$ years, $SD = 6.14$) and 10 males ($M = 31.8$ years, $SD = 3.61$), played TETRIS® (Tetris Holding). Tetris was used for the untrained group as it is an easy game to learn and all participants were familiar with how to play it. Participants were randomly assigned between active and sham conditions.

Inter-game Flow Questionnaire

At the end of each trial, participants were asked to retroactively assess their experience from their recent game trial and respond to a Flow State Scale (Jackson and Marsh, 1996) with two additional core questions of the flow state: "Everything Clicked" and "I was 'in the zone'."

Game Play

In Experiment 1, participants were given the choice to play two different FPS games: "Counter Strike: Global Offensive" (Valve) or "Battlefield 4" (EA). Both games had the same settings of competing against live online players, most kills wins and played only in a single map environment. Due to different map, weapon and control settings, two games were used to allow players to participate in the FPS game they felt most proficient in to give them the best chance to enter into flow.

In Experiments 2, three versions of TETRIS were used: slow (bored), adaptive (flow), and fast (anxious). The slow round was set to a speed of 2 and the drop button was disabled, forcing the person to sit around and wait for the piece to reach the bottom of the screen. The anxious round started at speed level 8 and would go up once a person made 5 lines. The adaptive condition started at 4 and went up in score if the player made 5 lines in 20 moves, but it would slow a level down if they had not met this criterion.

Stimulation

tDCS stimulation was applied using an NeuroConn DC-Stimulator (NeuroConn GmbH) machine with a montage of left prefrontal cathode and right parietal anode. tDCS was administered via two 5×5 cm electrodes covered with saline-soaked sponges. The stimulation site was determined by means of the 10/20 system, in which the cathode and anode were positioned over the F3 area and P6 area, respectively. Whilst tDCS excitability changes have been shown to last up to 60 min (Nitsche and Paulus, 2001), results have shown performance effects dwindle after 30 min of stimulation (Iyer et al., 2005). Therefore, stimulation condition was set for 20 min (including 10 s ramp-up and 10 s ramp-down time) at 2 mA while sham condition also lasted 20 min but was set for 30 s of stimulation at 1 mA. Participants are shown typically unable to determine whether receiving real or sham stimulation (Gandiga et al., 2006).

Procedure

In Experiment 1, participants were told they were receiving tDCS stimulation over two separate sessions. In the first session, participants chose their FPS game and entered an online game room with 16 or more online players. The games' objective is to stop the other team therefore game scores were based on number of kills. Participants played a warm up round of free play without testing for about 20 min while the experiment set-up occurred. Participants would then be informed that testing would begin. A trial would last until the participant lasted longer than 3 min and completed two kills in a row without dying. They then would be notified the trial had finished with a flashing light controlled by the researcher to fill out the Inter-Game Flow Questionnaire. The participant would press a button to acknowledge the light flash before answering the questionnaire.

The participant was randomly assigned a stimulation or sham condition which lasted 20 min of either 2 mA for the active stimulation condition or 30 s of 1 mA over the 20 min period for sham condition. Participants would continue to play during that time without testing. Participants would then begin another testing session after stimulation following the previous testing procedure. Experiment 1 participants would return a week later and participate again with the same experimental protocol but receiving the opposite stimulation condition.

In Experiment 2, participants played a 15 min warm up of the balanced condition prior to testing. Then the participants would be informed about a change in the gaming condition and they would complete two trials of the slow, fast, and then adaptive TETRIS games for ∼3 min. The researcher would then request they complete the Inter-Game Flow Questionnaire after each trial. The participant was randomly assigned a stimulation or sham condition which lasted 20 min of either 2 mA for the active stimulation condition or 30 s of 1 mA over the 20 min period for sham condition. Participants would continue to play the adaptive condition during that time, and complete subsequent Inter-Game Flow Questionnaires. Participants would then begin another testing session after stimulation but only complete the adaptive and fast conditions.

Statistical Analysis

The research explored different hypotheses around performance ceilings as well as flow induction for the different training level of the groups to reduce learning effects and therefore enlisted different group design in the analysis.

Experiment 1

A repeated measures analysis of variance (ANOVA) was used to assess the significant main effect of the dependent variable, perceived state of flow score, during the first person video game before and after the two trials (tDCS and sham).

Experiment 2

A mixed ANOVA was used to determine a significant main effect of the dependent variable, perceived state of flow score, during the events associated with each of the trials and games; e.g., this was compared to lines completed in TETRIS during different conditions. Similarly, a mixed ANOVA was used to determine a significant main interaction effect for tDCS stimulation with each of the trials and games.

RESULTS

No participant reported experiencing adverse effects during or after tDCS. A slight itching sensation during approximately the first 30 s of stimulation was reported. The sham condition reported the same initial itching sensation, and when explicitly asked, believed to have undergone real stimulation.

An overall positive effect was observed for all participants from both experiments, in which participants from both experiments resulted in a significantly higher experience of flow states after tDCS compared to sham or control conditions. Experiment 1 hypothesized specifically that tDCS would modulate the experience of flow states for trained players of first-person shooter videogames. A repeated measures ANOVA determined a significant main effect of [$F_{(1,54)} = 5.82$, $p < 0.02$, $\eta_p{}^2 = 0.10$; see **Figure 1**]. As hypothesized, simple main effects revealed that participants rated higher experiences of flow states after tDCS stimulation on average by ($M = 0.37$, $p < 0.001$, $\eta_p{}^2 = 0.24$) compared to sham which increased non-significantly on average by $M = 0.08$.

Additionally, there were non-significant effects for main effects of kill performance [$F_{(1,54)} = 0.214$, $p = 0.645$; see **Figure 2**], with greater performance improvements after tDCS on average by $M = 0.45$ compared to sham which reduced on average by $M = -0.2$.

Experiment 2 also resulted in the expected modulation pattern of flow states for untrained players of the puzzle game TETRIS. A mixed ANOVA was used to determine a significant main interaction effect for tDCS stimulation [$F_{(1,48)} = 7.24$, $p < 0.01$, $\eta_p{}^2 = 0.13$; see **Figure 3**]. As hypothesized, planned simple main effects revealed participants in the flow condition rated higher experiences of flow states after tDCS stimulation on average by $M = 0.27$ ($p < 0.02$, $\eta_p{}^2 = 0.22$) compared to sham which reduced non-significantly by $M = -0.13$. While there was no main effect for the interaction of tDCS over time for the anxious condition, a significant effect showed higher flow states after tDCS stimulation by M = 0.27 ($p < 0.05$, $\eta_p{}^2 = 0.2$) compared to a non-significant effect for sham that increased flow scores on average by $M = 0.17$. Note that tDCS was not tested in the boredom condition.

Additionally, as expected there was a significant main interaction effect for performance in TETRIS based on number of completed lines [$F_{(1,48)} = 7.41$, $p < 0.01$, $\eta_p{}^2 = 0.13$; see **Figure 4**], with greater line completion performance after tDCS on average by $M = 3.54$ ($p < 0.001$, $\eta_p{}^2 = 0.4$), compared to a non-significant effect for sham that increased line completion by $M = 0.31$.

DISCUSSION

As hypothesized, the results of this study indicate that tDCS can modulate an induction into flow states for video game players using a montage of prefrontal left cathode and right parietal anode. Additionally, as expected the trained FPS players performance was not improved by tDCS while the untrained TETRIS players improved due to tDCS stimulation compared to sham. While the results across both trained and untrained players of video games presented higher flow states after tDCS, the authors did find this interesting because it was unknown whether the performance ceiling effect might also effect the experienced intensity of flow states. While tDCS ceilings effects were present

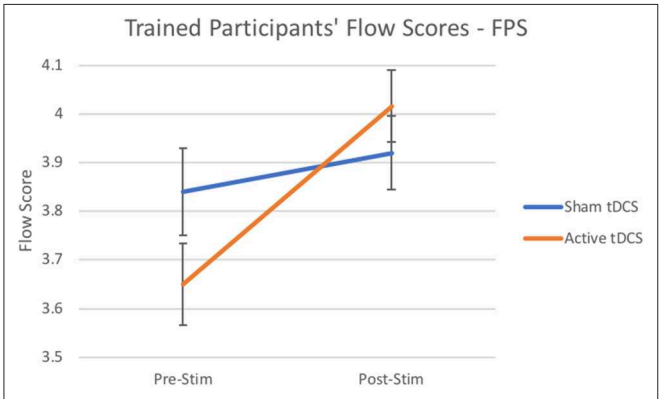

FIGURE 1 | Flow scores from trained participants after Active Stimulation and Sham Stimulation. Bars—Standard Error.

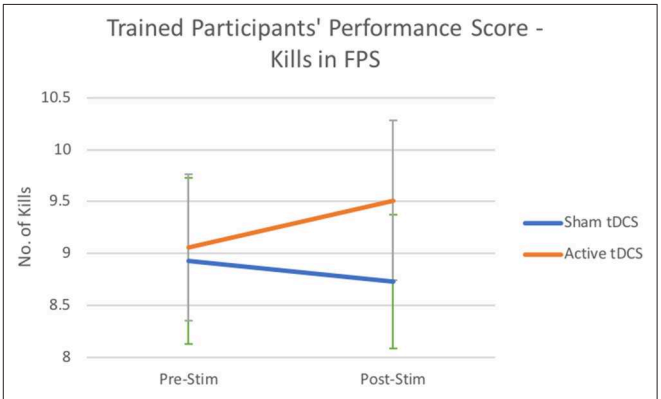

FIGURE 2 | Number of kills performance scores from trained participants after Active Stimulation and Sham Stimulation. Bars—Standard Error.

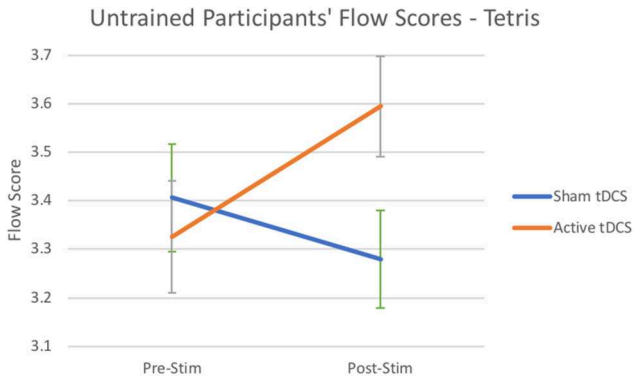

FIGURE 3 | Flow scores from untrained participants playing TETRIS after Active Stimulation and Sham Stimulation. Bars—Standard Error.

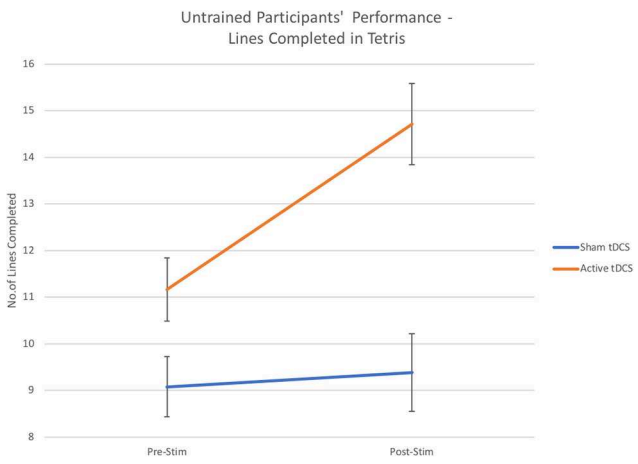

FIGURE 4 | Number of completed lines—performance scores from untrained participants after Active Stimulation and Sham Stimulation. Bars—Standard Error.

in the performance results of this study, which has been shown previously to apply to expert compared to novice performers (Bullard et al., 2011), studies have typically observed this from the perspective of motor skill tasks and not for psychological states. Perhaps psychological states may not be limited in the realm of performance by tDCS, i.e., tDCS studies have been shown to improve mood (Nitsche et al., 2009) and maybe further worth exploring the difference in limits tDCS modulation has for psychological states compared to motor skills. Another reason for the lack of ceiling effect may be that the high frequency of game play in the trained vs. the untrained group was not high enough to denote expertise and thus diminish the modulating effects of tDCS on flow states.

Whilst, to the authors knowledge, there has only been one prior research paper published on tDCS for flow states, which used a different montage of anodal stimulation over Fpz (Ulrich et al., 2018), the findings in this study could therefore be considered foreshadowed by previous papers documenting effects of tDCS in learning and working memory. The current findings align with previous research indicating that cathodal left prefrontal tDCS stimulation, as shown by Zhu et al. (2015), results in the reliance of improved implicit motor learning which could be considered to increase the modulation of the intensity of the flow experience as more resources are freed up for experiential processing (Dietrich, 2003). Inhibiting DLPFC has been shown to increase motor learning by disrupting the explicit motor system (Galea et al., 2010), as well as a dynamic balance with resources between explicit and implicit systems (Eichenbaum and Cohen, 2004; Kantak et al., 2012). The current study aimed to take advantage of this disruption of explicit executive functions to enhance the role of implicit processing and hence enable easier movement into elevated intensity of flow states. Furthermore, Zhu et al. (2015) reported a reduction in verbal working memory after the application of left prefrontal cathodal tDCS which Dietrich (2003) considers a requirement of his hypofrontality hypothesis to describe flow due to the reduction of high level buffering and maintenance.

Furthermore, the current findings also align with previous right anodal parietal research indicated in Clark et al. (2012) which resulted in positive learning effects in visual attention, thereby possibly reducing the amount of resources required to dedicate to the task to facilitate flow through implicit systems. Furthermore, the fronto-parietal attention network has been shown as a brain network relevant to attention activation during target detection tasks (Posner and Petersen, 1990). A review by Andersen and Cui (2009) indicated the role that the posterior parietal cortices (PPC) plays in the frontal parietal network through sensorimotor transformations including planning, decision making, forward model estimation and attentional faculties. Additionally, the tDCS has been shown to influence parietal regions based on a balance between the working memory capacity (skill) and the working memory task (Jones and Berryhill, 2012) which appears quite similar to the principle antecedents of flow states (Csikszentmihalyi and Csikszentmihalyi, 1988). In this study, we suspect that as attentional resources continue to increase during visual search elements of a task, such as video games, it may lead to a greater probability of noticing target objects, enhanced encoding of the location of the target object within the image and, therefore, greater accuracy and less buffering. This reduction in processing requirement could possibly open up the processing capacity to increase the perception of skill and thereby result in higher flow states ratings.

Dietrich (2004) originally considers flow states a reflexive system however from these results a new understanding maybe beginning to unfold as flow states may better be considered a predictive system that has developed and implemented through "forward and inverse models" which are considered neurological attempts at predicting the outcome of each action (Kawato, 1999). Ito (2008) describes the forward model through the prefrontal, temporal-parietal, and cerebellar network, in which the prefrontal area as the "controller" creates and transmits command signals that modify activities encoded while the temporo-parietal areas are considered the "mental model" which converts a command into an output action. Parietal anodal stimulation

appeared to increase within network connectivity between key elements of the forward and inverse models including the inferior and superior parietal along with the cerebellar intrinsic networks, key for enhanced learning outcomes (Hunter et al., 2015).

This forward model could help explain the modulatory impact of the tDCS in inducing flow states as the system becomes less reliant on the moderating effects of the prefrontal controller whilst encouraging the ability to output commands fed in from the cerebellar network. This freedom from higher order interference enables the action output of the temporal-parietal regions the ability to more easily implement the memory model. This smoother activation free from frontal modulation may have resulted in the experience of less thinking and concern with the surroundings while the parietal excitation may have felt like an easier implementation of the memory models.

Additionally, the inverse model affords the prefrontal area to be bypassed and instead processing relies more heavily on the anterior cingulate cortex (ACC). The ACC has also been shown to be involved in flow states such as an EEG game study testing the difference between boredom, frustration and flow states (Nuñez Castellar et al., 2016). The ACC was determined as an actor in engaging the fronto-parietal network as well as monitoring conflicts in the focus of attention (Walsh et al., 2011). However, more recently Ulrich et al. (2014) found in a similar three level (boredom, flow, overload) arithmetic fMRI study of flow that the ACC reduced in activity. Nonetheless, while more study is needed to ascertain its role in attentional focus and flow states, the pattern of decreased prefrontal activity and increased parietal activity reported in Ulrich et al. (2014), found flow state results that mirrored the fronto-parietal network tDCS montage used in this study. It would be interesting to replicate this current study with a mirrored montage as the forward model appears to be supported by bilateral activation of the fronto-parietal network.

Limitations

Whilst the results are indicative of a positive intervention of tDCS toward flow states, it would also be advantageous to consider the vast range of tDCS impacts. TDCS's effects have been shown as distributed rather than local (Keeser et al., 2011) and thus could impact unintended areas such that placing the prefrontal cathodal could influence multiple areas such as the DLPFC and the MPFC. Therefore, it may be worth considering using High Definition-tDCS in order to more accurately target locations associated with flow states in order to understand which areas specifically are responsible.

Furthermore, it is difficult to assess the full comparative impact of tDCS on flow and the ceiling effects between the trained and untrained players because the experimental design used a different methodology of a repeated experiment, alternating sham and tDCS for trained players while for untrained players they were only exposed one time to the experiment with a random allocation of tDCS or sham. This testing methodology in addition to testing between two different gaming paradigms are contributing factors to confounding the results. Therefore, for future testing it would be worth testing the role of tDCS ceiling effects on flow scores between trained and untrained players using the same experimental and gaming paradigm.

Additionally, it would be interesting to test different tDCS montages for modulating flow states. Flow states had been found in neuroimaging studies with both left and right parietal activation (Ulrich et al., 2014). Additionally, forward model neuroimaging studies have shown bilateral activation of parietal regions (Heinzel et al., 2017; Sokolowski et al., 2017).

CONCLUSIONS

In the present study, we explored the subjective experiences of flow states for video gamers at different level of training after a tDCS intervention with a montage of a left prefrontal cathode and right parietal anode. Results revealed a subjective change toward higher intensity of flow experiences and an expected ceiling on task performance for trained and an improvement in task performance for untrained participants. With more research, tDCS could prove to be an effective tool to uncover more of the functional pathways involved in flow states and promote more positive subjective experiences for complex tasks including greater levels of immersion and enjoyment. By improving performance and states, tDCS could assist people to become more diligent, motivated and effective in tasks for occupational and rehabilitative efforts.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Swinburne University Human Experimentation Committee (SUHREC) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the SUHREC.

AUTHOR CONTRIBUTIONS

JC and JG: conceptualization and experimentation. JG: manuscript. JC: review and supervision.

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Pre-stimulus Brain Activity Is Associated With State-Anxiety Changes During Single-Session Transcranial Direct Current Stimulation

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Transcranial direct current stimulation is a promising neuromodulation method for treating depression. However, compared with pharmacological treatment, previous studies have reported that a relatively limited proportion of patients respond to tDCS treatment. In addition, the neurophysiological mechanisms underlying tDCS treatment remain unclear, making it difficult to identify response predictors for tDCS treatment based on neurophysiological function. Because treatment effects are achieved by repetitive application of tDCS, studying the immediate effects of tDCS in depressive patients could extend understanding of its treatment mechanisms. However, immediate changes in a single session of tDCS are not well documented. Thus, in the current study, we focused on the immediate impact of tDCS and its association with pre-stimulus brain activity. To address this question, we applied anodal tDCS to the left dorsolateral prefrontal cortex (DLPFC) or dorsomedial prefrontal cortex (DMPFC) in 14 patients with major depressive disorder (MDD) and 19 healthy controls (HCs), at an intensity of 1.0 mA for 20 min in a single session. To evaluate anxiety, the state trait anxiety inventory was completed before and after tDCS. We recorded resting electroencephalography before tDCS, and calculated electrical neuronal activity in the theta and alpha frequency bands using standardized low-resolution electromagnetic tomography. We found that, during application of left DLPFC tDCS to patients with MDD, the anxiety reduction effect of tDCS was related to higher baseline theta-band activity in the rostral anterior cingulate cortex (rACC) and no medication with benzodiazepine used as hypnotic. For DMPFC stimulation in MDD, the anxiety reduction effect was associated with lower baseline alpha-band activity in the left inferior parietal lobule. In contrast, in HCs, the anxiety reduction effect was associated with higher baseline alpha activity in the precuneus during DMPFC stimulation. The current results suggest that the association between pre-tDCS brain activity and the anxiety reduction effect of

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tDCS depends on psychopathology (depressed or non-depressed) as well as the site of stimulation (DMPFC or left DLPFC) and insomnia. Furthermore, the results suggest that tDCS response might be associated with baseline resting state electrophysiological neural activity.

Keywords: transcranial direct current stimulation, left dorsolateral prefrontal cortex, dorsomedial prefrontal cortex, anterior cingulate, anxiety, depression

INTRODUCTION

Transcranial direct current stimulation is a widely used neuromodulation technique for basic neurocognitive research in healthy subjects as well as clinical applications in major depression and other psychiatric disorders (Fregni et al., 2015; Martin et al., 2018) In clinical practice, the development of new treatment approaches without medication is important for patients, who show low tolerance to pharmacotherapy because of substantial side effects (Brunoni et al., 2012). tDCS provides a potentially useful approach because the tDCS stimulator is a mechanically simple device, with a lower cost than other non-invasive brain stimulation devices.

In recent decades, major depressive disorder (MDD) has become one of the most serious lifetime diseases in many countries (Murray and Lopez, 1996). Although treatments for MDD have improved, current treatment options have limitations (Kupfer et al., 2012).

In treatment methods involving non-invasive brain stimulation for MDD, the left dorsolateral prefrontal cortex (DLPFC) has been found to play a major role in executive functioning, and is widely recognized as a suitable target for anodal tDCS to recover executive control and emotion regulation.

A recent meta-analysis supports the application of tDCS to the DLPFC in MDD (Mutz et al., 2018). Furthermore, a recent large-scale study reported no inferiority of tDCS treatment compared with escitalopram (Brunoni et al., 2017). However, the specific treatment effects of tDCS remain controversial (Tremblay et al., 2014; Mondino et al., 2015; Brunoni et al., 2017; Martin et al., 2018). A recent study by Brunoni and colleagues reported that response rates to tDCS were significantly higher than placebo, but the remission rate was not significantly different between tDCS and placebo groups (Brunoni et al., 2017). Furthermore, the treatment mechanisms of tDCS remain unclear.

Recently, several studies proposed additional targets for treatment of MDD, suggesting non-invasive brain stimulation of the dorsomedial prefrontal cortex (DMPFC) as one potential approach (Downar and Daskalakis, 2013). This proposal is based on the finding that the DMPFC, including the anterior cingulate cortex, is involved in regulation of emotions (Bush et al., 2000), and is anatomically connected with the amygdala and nucleus accumbens, which have both been implicated in MDD.

More recent studies have confirmed the feasibility of rTMS on the DMPFC for MDD (Downar et al., 2014; Kreuzer et al., 2015; Schulze et al., 2018). However, we know little evidence of the mechanism of DMPFC stimulation even beyond the context of major depression (Bakker et al., 2015; Colzato et al., 2015; Kreuzer et al., 2015).

There are several limitations of the current evidence supporting further implementation of tDCS into clinical practice. First, better understanding of the neurophysiological mechanisms underlying the effects of tDCS is needed. Second, biomarkers are needed for predicting tDCS treatment responders. One possible approach for addressing these current limitations is to examine the neurophysiological signatures of patients. Specifically, the pre-stimulus state of the brain may explain the variability in responses to tDCS. Recent studies have reported that pre-treatment electroencephalography (EEG) predicts changes in cognition after 15 sessions of tDCS in the left DLPFC in depressive patients, and that frontal electrodes exhibit predictive power for changes in cognition (Al-Kaysi et al., 2016, 2017).

Although predicting treatment effects with pre-treatment neurophysiological activity would have direct implications for clinical practice, the neurophysiological mechanisms underlying treatment effects may remain obscured because treatment effects are achieved by repeated application of single-session tDCS, and the accumulation of immediate neural responses to single-session tDCS may modify the stable state of brain activity and eventually improve depressive symptoms. Therefore, we assumed that examining the neural mechanisms of a single-session of tDCS intervention might provide a first step for disentangling the complex treatment mechanisms of tDCS for MDD. Among symptomatic problems of MDD, single session of tDCS is hard to change sustained symptoms such as depressive mood, anhedonia, agitation or loss of motivation, while anxiety is relatively volatile across time. In the current study, we therefore focus on state anxiety to look at the effect of single-session tDCS.

In the current study, we set two main aims. First, we investigated the immediate effects of prefrontal tDCS on brain activity and state anxiety. Second, we compared the effects between stimulation of the left DLPFC, the canonical target of tDCS for MDD, and the DMFPC, the potential target predicted by neuroanatomical architecture. To this end, we applied anodal tDCS to the left DLPFC or DMPFC in MDD patients and healthy controls (HCs), with 20 min of stimulation in a single session. We measured state anxiety before and after tDCS and neural activity with EEG before tDCS. Finally, we examined association between neural activity and state anxiety to investigate neural predictors of the change in anxiety induced by tDCS.

MATERIALS AND METHODS

Participants

We recorded a total of 20 patients with MDD, assessed by the DSM-IV and evaluated with the Hamilton Depression Rating Scale (HAM-D), and a total 24 HCs subjects recruited for this study. After eliminating data corresponding to subjects that were left-handed, or unavailable EEG, or psychological evaluation, 14 patients with MDD and 19 HCs were finally included in this study. All participants were right-handed, and were graduates of high school or higher education. All participants were diagnosed by experienced psychiatrists using a structured interview and physical examination. We excluded patients with history of dementia, schizophrenia, substance dependence, epilepsy or head trauma. Participants do not have anxiety disorder comorbidities, such as generalized anxiety disorder, panic disorder, and phobia. Thirteen patients have received antidepressant. 10 patients were medicated by benzodiazepine as sleeping medication and 2 patients medicated by a mood stabilizer. Chi-squared test with Yates's correction between gender did not show significant difference ($x^2(1) = 3.076$, p (0.08). All HCs had no history of psychiatry disorders. This study was carried out in accordance with the recommendations of "Safety of transcranial direct current stimulation, tDCS by Japanese Journal of Clinical Neurophysiology 2011."

The study protocol was approved by the Institutional Ethics Review Committee of Kansai Medical University (UMIN000015046). We obtained written informed consent from all participants in accordance with the Declaration of Helsinki. Participants were recruited from September 2014 to April 2017. Details of participants are shown in **Table 1**.

TABLE 1 | Demographic data.

Group	MDD		HC	
Session	**Left DLPFC**	**DMPFC**	**Left DLPFC**	**DMPFC**
Sample size	14		19	
Sex: Male/female	12/2		12/7	
Drug treatment				
No	1		0	
One antidepressant	11		0	
Two antidepressants	2		0	
Benzodiazepine	10		0	
Mood stabilizer	2		0	
Age-years: mean ± SD	44.93 ± 14.68		48.94 ± 15.80	
Education period	15.36 ± 1.55		15.63 ± 1.34	
Number of previous episodes: mean ± SD	2.36 ± 0.93		0.00 ± 0.00	
HAM-D17 score on the day of the session: mean ± SD	14.07 ± 5.40	13.79 ± 4.82	0.21 ± 0.42	0.21 ± 0.54

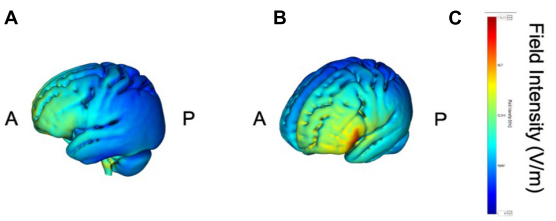

FIGURE 1 | Modeling of electric field distribution for the montage of left DLPFC stimulation. **(A)** Sagittal view, **(B)** side view, **(C)** above view, A: anterior, P: posterior.

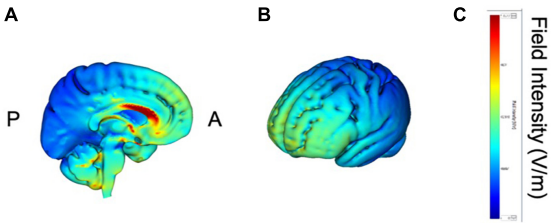

FIGURE 2 | Modeling of electric field distribution for the montage of DMPFC stimulation. **(A)** Sagittal view, **(B)** side view, **(C)** above view, A: anterior, P: posterior.

tDCS

tDCS was administered with a battery-driven stimulator (DC Stimulator Plus, Neuroconn, Ilmenau, Germany). The electrical current was applied at 1 mA via electrically conductive rubber electrodes (20 cm^2, circular in shape) attached with an adhesive conductive EEG paste. Anodal stimulation was administered over the left-DLPFC (F5, 10–10 EEG international electrode placement, **Figure 1**) or the DMPFC (AFz, 10-10 EEG international electrode placement, **Figure 2**) with the cathodal electrode placed on the left shoulder. Direct current was administered for 20 min during the resting state. We also simulated the current flow of our montage with a simulation software using a finite element model (HD-explore, Soterix Medical, New York, United States) (**Figures 1**, **2**).

Procedure

We adopted a between-subjects cross-over design (**Figure 3**). The order of stimulation was counterbalanced. Each subject was randomly assigned to receive left DLPFC or DMPFC tDCS in the first session. The participant received tDCS on the other site in the second session. There was an interval of at least 1 week between tDCS sessions.

Psychological Test

We measured the STAI (state-trait anxiety inventory), which consists of two subscales, STAI-SA for state anxiety to

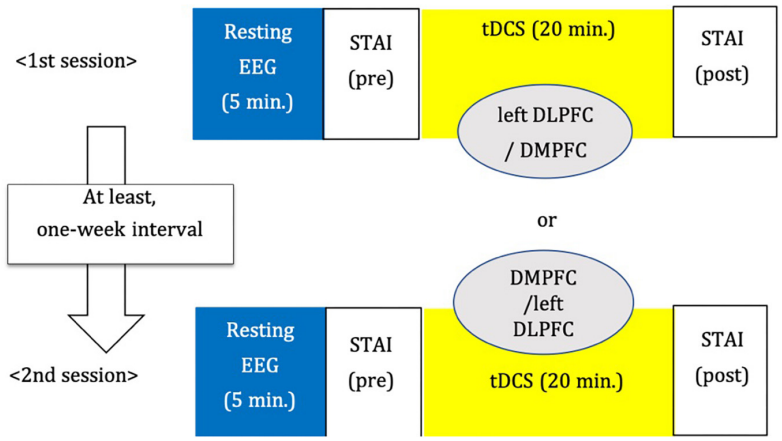

FIGURE 3 | Study design.

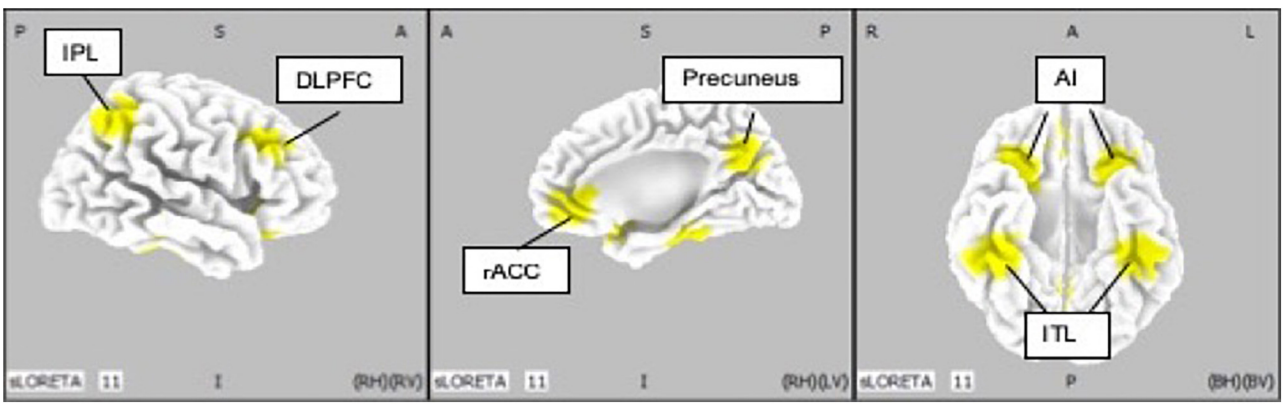

FIGURE 4 | Location of regions of interest (IP: inferior parietal lobe; DLPFC: dorsolateral prefrontal cortex; rACC: rostral anterior cingulate cortex; AI: anterior insula; ITL: inferior temporal lobe).

assess anxiety before and after tDCS and STAI-TA for trait anxiety before tDCS.

EEG Recording

Resting and eyes-closed EEG was recorded with an EEG-1200 Nihon Kohden (Tokyo, Japan) system. A 64 ch Ag/AgCl sintered Waveguard Original EEG cap from ANT Neuro (Netherland) was used for the recordings. It was necessary to use a subset of the electrodes comprising of 19 EEG electrodes corresponding to the international 10–20 system for analyses, because the tDCS electrodes placed under the EEG cap interfered with substantial number of EEG electrodes in the frontal area. We recorded EEG before and after tDCS. However, only EEG recordings before tDCS were used in the analyses described below.

EEG Analysis

Signals of cortical electric neuronal activity were computed from the baseline, pre-stimulation EEG recordings using standardized low resolution electromagnetic tomography (sLORETA) (Pascual-Marqui, 2002). In its current implementation (free academic software package available at https://www.uzh.ch/keyinst/loreta), this method produces signals of appropriately standardized current density from 6239

TABLE 2 | Regions of interest (ROIs) and their coordinates in the MNI space.

	x	*y*	*z*
rACC	0	45	0
Left DLPFC	−40	26	34
Right DLPFC	40	26	34
Left AI	−30	24	−13.5
Right AI	30	24	−13.5
Left ITL	−42	−33	−25.5
Right ITL	42	−33	−25.5
Left IPL	−45	−52	48
Right IPL	45	−52	48
Precuneus	0	−66	34

rACC, rostral anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; AI, anterior insula; ITL, inferior temporal lobe; IPL, inferior parietal lobe.

TABLE 3 | STAI-SA pre tDCS and STAI-SA post tDCS.

Group	MDD		HC	
Session	**Left DLPFC**	**DMPFC**	**Left DLPFC**	**DMPFC**
STAI-SA_pre	47.29 (±9.10)	44.71 (±8.11)	36.32 (±7.02)	35.84 (±6.80)
STAI-SA_post	44.64 (±9.96)	43.14 (±9.81)	38.42 (±6.40)	37.16 (±6.52)
Post–Pre change of STAI-SA	−2.64 (±5.23)	−1.57 (±6.01)	2.11 (±5.86)	1.32 (±4.35)

DLPFC, dorsolateral prefrontal cortex stimulation; DMPFC: dorsomedial prefrontal cortex stimulation, MDD: major depressive disorder, HC: healthy controls, STAI-SA: State-Trait Anxiety Inventory - state anxiety.

cortical gray matter voxels, sampled on a 5 mm resolution grid, using the MNI152 anatomical template (Mazziotta et al., 2001; Fuchs et al., 2002). sLORETA has received both theoretical (Greenblatt et al., 2005; Sekihara et al., 2005; Pascual-Marqui, 2007) and experimental validation (Pascual-Marqui et al., 2009).

The sLORETA signals were then further processed to produce values of cortical spectral power in two classical EEG frequency bands: theta (4–8 Hz) and alpha (8–12 Hz). We chose these two frequency bands because they have been repeatedly reported to be associated with MDD and response to treatment (Klimesch, 1999; Moore et al., 2000; Nishida et al., 2015; Kitaura et al., 2017).

Regions of Interest

Ten regions of interest (ROIs) were chosen based on previous studies investigating neurophysiological mechanisms in patients with MDD (McGrath et al., 2013; Kaiser et al., 2015; Pizzagalli et al., 2018) (**Figure 4**). Pizzagalli et al., investigated the importance of current density in rACC for improvement of depression symptoms with EEG-LORETA. The meta-analysis by Kaiser et al., showed significant difference in resting state

TABLE 4 | Single regression analysis about STAI-SA.

Session	Dependent variable	Independent variable	β	SE β	*t*-value	*p*-value	Standard β	*R2*
MDD	Change of STAI-SA	STAI-SA pre	−0.064	0.127	−0.507	0.616	−0.099	−0.028
HC	Change of STAI-SA	STAI-SA pre	−0.339	0.111	−3.047	0.004	−0.453**	0.183

MDD, major depressive disorder; HC, healthy controls; STAI-SA, State-Trait Anxiety Inventory-state anxiety; β, the regression coefficient; SE β, standard error of the regression coefficient; R^2, the squared multiple correlation coefficient.

TABLE 5 | Log-transformed current density power at 10 ROIs in alpha and theta bands.

Group	MDD				HC			
Session	**Left DLPFC**		**DMPFC**		**Left DLPFC**		**DMPFC**	
Current density	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***
Theta_rACC	0.186	0.518	0.133	0.570	0.521	0.519	0.400	0.454
Theta_leftDLPFC	−0.380	0.459	−0.386	0.411	−0.079	0.526	−0.153	0.417
Theta_rightDLPFC	−0.342	0.512	−0.258	0.480	−0.214	0.492	−0.162	0.462
Theta_leftInsula	0.072	0.528	0.070	0.437	0.478	0.491	0.346	0.361
Theta_rightInsula	0.138	0.585	0.215	0.531	0.276	0.516	0.279	0.447
Theta_lrftITP	−0.350	0.413	−0.246	0.247	−0.003	0.437	−0.084	0.366
Theta_rightITP	−0.328	0.467	−0.199	0.376	−0.115	0.438	−0.264	0.466
Theta_leftIPL	−0.531	0.481	−0.667	0.378	−0.216	0.426	−0.119	0.618
Theta_rightIPL	−0.543	0.486	−0.637	0.433	−0.253	0.481	−0.254	0.606
Theta_precuneus	−0.412	0.669	−0.613	0.521	−0.175	0.467	−0.013	0.582
Alpha_rACC	−0.172	0.399	−0.190	0.331	−0.017	0.406	−0.111	0.358
Alpha_leftDLPFC	−0.720	0.468	−0.661	0.242	−0.484	0.331	−0.508	0.310
Alpha_rightDLPFC	−0.525	0.453	−0.432	0.315	−0.636	0.409	−0.681	0.341
Alpha_leftInsula	−0.056	0.545	0.009	0.230	0.157	0.273	0.039	0.292
Alpha_rightInsula	0.116	0.590	0.185	0.376	−0.028	0.311	−0.099	0.257
Alpha_lrftITP	0.233	0.794	0.535	0.494	0.381	0.614	0.299	0.592
Alpha_rightITP	0.404	0.925	0.621	0.633	0.316	0.616	0.064	0.725
Alpha_leftIPL	−0.010	0.793	0.290	0.705	0.140	0.706	0.327	0.763
Alpha_rightIPL	0.173	0.857	0.460	0.706	0.210	0.612	0.179	0.793
Alpha_precuneus	0.254	0.881	0.483	0.758	0.281	0.710	0.357	0.826

DLPFC, dorsolateral prefrontal cortex; DMPFC, dorsomedial prefrontal cortex, rACC, rostral anterior cingulate cortex; AI, anterior insula; ITL, inferior temporal lobe; IPL, inferior parietal lobe; MDD, major depressive disorder; HC, healthy controls.

functional connectivity in patients with depression and HCs. In addition, McGrath et al. have shown that anterior insula and inferior temporal lobe were candidates of biomarkers of treatment by cognitive behavior therapy by using positron emission topography.

We defined rACC from average coordinates (i.e., centroid) of the atlas which Pizaggali et al. used in their paper. Atlas of DLPFC and Inferior temporal lobe in our study was obtained from Kaiser's literature, and the coordinates used for the anterior insula and inferior temporal lobe originate from the work of McGrath (McGrath et al., 2013; **Table 2**).

Statistical Analysis

For each stimulation session, the change in anxiety scores was defined as the STAI-SA score at post tDCS minus the STAI-SA score at pre tDCS (baseline). Thus, a negative value of the change indicates a reduction of state anxiety. In the current study, we aimed to investigate the association between these dependent variables and cortical activities in 10 ROIs. We also included the "with" or "without" administration of benzodiazepines as an independent variable, and baseline STAI-SA scores for considering the effect of the diversity of participants.

Firstly, we applied a least absolute shrinkage and selection operator (LASSO) for selecting appropriated variables and regularization. The set of independent variables consisted of the cortical spectral power for the theta and alpha bands, at 10 ROIs calculated from baseline, pre-stimulation EEG-sLORETA for each DLPFC or DMPFC session, plus the medication about with/without benzodiazepines and STAI-SA scores at pre-tDCS in patients and controls separately. Cross-Validation leave-one-out was performed to determine the optimal tuning penalty parameter (λ) for each session. Finally, variable selection was performed by using the estimated λ value. We performed LASSO with R (3.6.0), RStudio (1.2.1335), and glmnet package (2.0–18).

Next, forced entry multiple regression analyses were conducted for changes in STAI-SA scores as dependent variables, with the set of cortical activity in each theta and alpha band at selected independent variable, for both left DLPFC and DMPFC stimulation session, in the MDD group and in the control group. SPSS version 26 was used for this multiple regression analysis.

Adverse Events

Six of 23 participants reported headaches, tingling sensation, itching, or experiencing the taste of iron. Because all reported events were mild, all participants continued the experiments and recovered from the adverse effects immediately after the sessions.

RESULTS

Change in STAI Scores

We first examined overall changes in state anxiety. **Table 3** shows the baseline and the Post–Pre tDCS changes in STAI-SA scores (**Table 3**).

Analysis of variance revealed that the baseline STAI-SA score was significantly higher in the MDD group than that in the HC group ($F[3,62] = 8.943$, $p < 0.001$). However, analysis of covariance revealed no significant difference in changes of STAI-SA score between the two groups ($F[1,63] = 1.562$, $p = 0.215$), and within each group (MDD: $F[1,25] = 0.180$, $p = 0.675$; HC: $F[1,35] = 0.397$, $p = 0.533$). The paired t-test did not show the significantly between the score of pre-tDCS and the one of post-tDCS in both MDD (left DLPFC session: $t = 1.67$, $df = 14$, $p = 0.12$, DMPFC session: $t = 0.83$, $df = 14$, $p = 0.42$) and HC (left DLPFC session: $t = -1.57$, $df = 18$, $p = 0.14$, DMPFC session: $t = -1.32$, $df = 18$, $p = 0.20$) groups.

In order to examine the influence of baseline STAI-SA score on tDCS-induced changes of STAI-SA, we performed single regression analysis where the independent variable is the baseline STAI-SA score for each group, and the dependent variable is as STAI-SA change (**Table 4**). We did not find significant association between pre-tDCS STAI-SA score and tDCS-induced changes of STAI-SA score in the MDD group ($p = 0.61$), while it was significant in the HC group ($\beta = -0.339$, $p = 0.004$). We also examined whether pre tDCS STAI-SA score was different between the subjects treated with benzodiazepine and those without benzodiazepine. Mann–Whitney U-tests did not show the significant difference between the two groups ($p = 0.72$).

TABLE 6 | Least absolute shrinkage and selection operator (LASSO) for selecting appropriated variables and regularization.

Sessions	Dependent variable	Independent variable	λ	β
MDD on left DLPFC	Change of STAI-SA	Theta rACC	0.199	−0.375
		Benzodiazepine		−0.752
MDD on left DLPFC	Change of STAI-SA	Alpha left ITL	0.227	0.285
		Alpha precuneus		0.187
		Benzodiazepine		−0.414
MDD on DMPFC	Change of STAI-SA	Theta left DLPFC	0.262	−0.199
		Theta right insula		−0.036
		Theta precuneus		0.205
MDD on DMPFC	Change of STAI-SA	Alpha rACC	0.156	−0.018
		Alpha left DLPFC		−0.184
		Alpha right ITL		0.114
		Alpha left IPL		0.544
HC on left DLPFC	Change of STAI-SA	Theta n.s.	0.503	n.s.
HC on left DLPFC	Change of STAI-SA	Alpha n.s.	0.503	n.s.
HC on DMPFC	Change of STAI-SA	Theta n.s.	0.244	n.s.
HC on DMPFC	Change of STAI-SA	Alpha rACC	0.220	0.129
		Alpha precuneus		−0.403

rACC, rostral anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; ITL, inferior temporal lobe; IPL, inferior parietal lobe; DLPFC, dorsolateral prefrontal cortex stimulation; DMPFC stimulation, dorsomedial prefrontal cortex; MDD, major depressive disorder; HC, healthy controls; β, the regression coefficient; λ, tuning penalty parameter; n.s, no significance.

TABLE 7 | Regression analysis in each theta and alpha bands at on left DLPFC and MDD in patients and HC.

Session	Dependent variable	Independent variable	β	SE β	*t*-value	*p*-value	Standard β	*R*
MDD on left DLPFC	Change of STAI-SA	Theta rACC	−5.912	1.809	−3.268	0.007**	−0.586	0.583
		benzodiazepine	−6.281	1.998	−3.144	0.009**	−0.563	
MDD on left DLPFC	Change of STAI-SA	Alpha left ITL	2.571	1.915	1.343	0.209	0.390	0.598
		Alpha Precuneus	1.728	1.725	1.002	0.340	0.291	
		benzodiazepine	−4.181	2.039	−2.051	0.067	−0.375	
MDD on DMPFC	Change of STAI-SA	Theta left DLPFC	−6.600	6.328	−1.043	0.321	−0.451	0.327
		Theta right Insula	−0.428	5.022	−0.085	0.934	−0.038	
		Theta Precuneus	5.299	2.768	1.914	0.085	0.460	
MDD on DMPFC	Change of STAI-SA	Alpha rACC	−2.237	3.497	−0.640	0.538	−0.123	0.679
		Alpha left DLPFC	−6.678	4.630	−1.442	0.183	−0.268	
		Alpha right ITL	2.259	1.735	1.302	0.225	0.238	
		Alpha left IPL	5.210	1.587	3.283	0.009**	0.611	
HC on left DLPFC	Change of STAI-SA	Theta n.s.	–	–	–	–	–	–
HC on left DLPFC	Change of STAI-SA	Alpha n.s.	–	–	–	–	–	–
HC on DMPFC	Change of STAI-SA	Theta n.s.	–	–	–	–	–	–
HC on DMPFC	Change of STAI-SA	Alpha rACC	3.416	2.349	1.454	0.165	0.282	0.481
		Alpha precuneus	−2.923	1.019	−2.869	0.011*	−0.556	

*rACC, rostral anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; ITL, inferior temporal lobe; IPL, inferior parietal lobe; MDD, major depressive disorder, HC; healthy controls. *$p < 0.05$, **$p < 0.01$.*

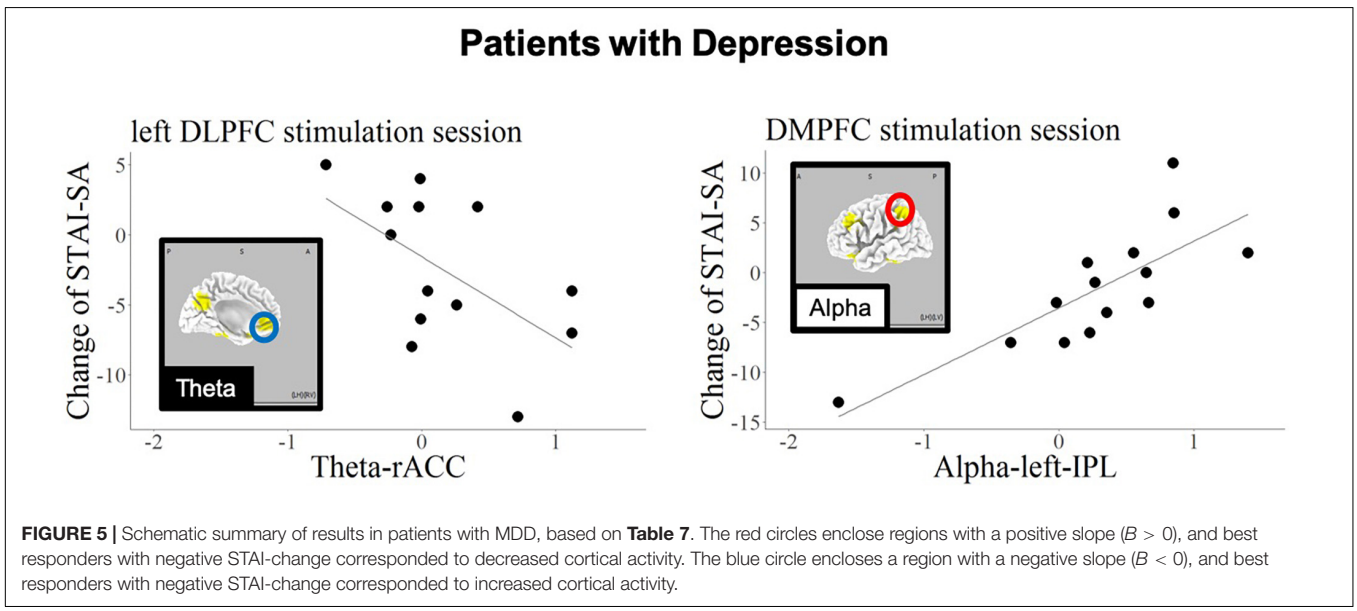

FIGURE 5 | Schematic summary of results in patients with MDD, based on **Table 7**. The red circles enclose regions with a positive slope ($B > 0$), and best responders with negative STAI-change corresponded to decreased cortical activity. The blue circle encloses a region with a negative slope ($B < 0$), and best responders with negative STAI-change corresponded to increased cortical activity.

Brain Activity in ROIs

We further examined pre-tDCS brain activity estimated by sLORETA. **Table 5** shows the values of brain activity calculated by LORETA. Paired *t*-tests revealed no significant difference in log-transformed current density power between the DLPFC and DMPFC sessions, in each of the MDD and HC groups (MDD: $p = 0.299$; HC: $p = 0.255$).

Multiple Linear Regression Models

To examine whether pre-tDCS brain activity can be associated with anxiolytic effect, we first performed Lasso regression to select predictor variables of each set of theta or alpha activity (**Table 6**). Here we also included benzodiazepine medication as a predictor variable to control effect of benzodiazepine medication. We then further performed multiple regression analysis if selected variables was associated with tDCS-induced STAI-SA changes. **Table 7** shows the results of forced entry multiple linear regression models. Negative values for STAI change corresponded to a reduction of state anxiety after tDCS.

Figures 5, **6** show the schematic summaries of the MDD and HC group with significantly difference ($P < 0.05$). We modeled the change in STAI-SA scores in vertical axis, and the set of cortical theta and alpha activity in 10 ROIs in horizonal axis for left DLPFC and DMPFC stimulation, and in each group.

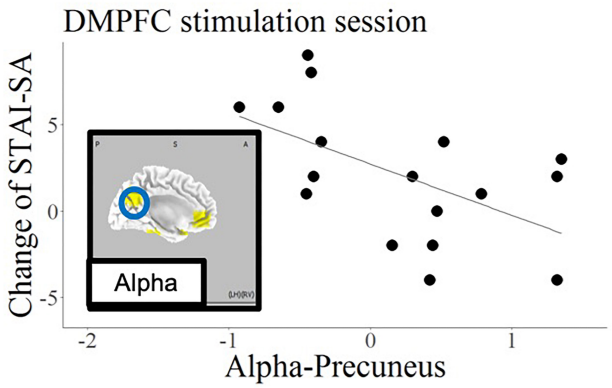

FIGURE 6 | Schematic summary of results in the HC group, based on **Table 7**. The blue circle encloses a region with a negative slope ($B < 0$), and best responders with negative STAI-change corresponded to increased cortical activity.

DISCUSSION

The current findings confirmed that, regarding the left DLPFC stimulation site in patients with MDD, the anxiety reduction effect of tDCS was related to higher baseline theta-band activity in the rostral anterior cingulate cortex (rACC). In contrast, the anxiety reduction was associated with higher baseline alpha activity in the precuneus in the HC group.

In the current study, we have specifically focused on immediate anxiolytic effect of tDCS, and we did not expect to change sustained depressive symptoms. We indeed consider accumulation of immediate anxiolytic effects will eventually lead to long-term improvement of depressive symptoms.

The association of the anxiolytic effect of left DLPFC tDCS with high baseline theta-band activity in the rACC is in accord with the findings of previous studies (Arns et al., 2014; Li et al., 2014; Bailey et al., 2018). A large scale meta-analysis studies have further shown that functional and structural alterations in the rACC are associated with broad spectrum of psychiatric disorders (Goodkind et al., 2015; Sha et al., 2019). Patients with MDD have been reported to exhibit dysfunction in the left DLPFC as well as the rACC (Pizzagalli et al., 2002; Mayberg et al., 2005). A study for treatment resistant depression patients who were administered with rTMS for 4 to 7 weeks showed the antidepressive effect was predicted by functional connectivity between stimulation site and the subgenual cingulate (Weigand et al., 2018). Liston and colleagues also reported that activation of the subcallosal cingulate cortex was a main predictor for the effect of transcranial magnetic stimulation (Liston et al., 2014). Pizzagalli and colleagues reported that LORETA current density of theta-band in the rACC was a predictor of response to antidepressants. The current findings are in accord with these previous studies, and further suggest that the rACC, including the subcallosal cingulate cortex, is involved in the anxiolytic effects of tDCS applied to the left DLPFC, and may appear to be important for predicting the response of MDD patients to tDCS.

The current findings also revealed a correlation between baseline alpha-band activity in the IPL and state anxiety reduction during DMPFC stimulation in the MDD patient group. Several previous studies have examined the relationship between anxiety and functional brain imaging in IPL (Hasler et al., 2007; Goldin et al., 2009). Importantly, the current results revealed opposite prediction patterns in patients with MDD and HCs; the best responding HCs (exhibiting negative STAI change) were those with high alpha activity in precuneus in response to DMPFC stimulation.

A study by Fox et al. suggested that the region for stimulation by neuromodulation can be selected not only by the direct effect of the stimulation, but also by the propagation effect, depending on the interconnected regions of the resting state networks (Fox et al., 2014).

As the precuneus and ACC constitute the default mode network (Sheline et al., 2009), applying tDCS to the DMPFC might affect activity in the precuneus, which is functionally densely connected with the DMPFC. This association was only found in the HC group, presumably reflecting intact functionality of the default mode network in healthy individuals. Importantly, other remote effects of tDCS have been reported in previous studies. tDCS applied to the left DLPFC was reported to increase functional connectivity in the fronto-parietal network, while decreasing connectivity in the default mode network (van der Werf et al., 2010; Eldaief et al., 2011). It should be noted that anxiety disorder and depression are likely to be related to dysfunction of this frontal-parietal network (Sylvester et al., 2012). And this dysfunction also may yield the opposite changing of STAI-SA; decreasing the mean score of STAI-SA in MDD and increasing STAI-SA mean score in HCs.

Regarding the prediction of responses to tDCS treatment in patients with MDD, Al-Kay et al. conducted a prediction analysis with EEG data for treatment outcomes in response to prefrontal tDCS. The results revealed that frontal EEG channels were important for predicting mood improvement after treatment sessions (Al-Kaysi et al., 2017). In contrast to previous studies, the current study involved a single tDCS application, and did not examine predictors of overall treatment, but immediate responses to a single session of treatment. We believe determining the immediate neurophysiological effects of tDCS is particularly important for understanding the treatment mechanisms of tDCS, because the accumulation of immediate changes may eventually lead to long-term plasticity underlying the overall treatment effects. It should also be emphasized that sLORETA can localize activity in deeper subcortical regions, whereas scalp EEG electrodes provide limited information about the underlying cortical activity due to cortical surface orientation and volume conduction effects.

Interestingly, the patients taking no benzodiazepine medication had apparently an anxiety reduction effect for left DLPFC stimulation; however, there was no significantly difference pre-STAI scores between patients with and without benzodiazepine. The results is indeed consistent with a previous clinical trial. The clinical trial comparing the treatment response

between tDCS and sertraline showed that benzodiazepine decreases the effect only in the tDCS treatment group (Brunoni et al., 2013). Benzodiazepine is used as hypnotics in this study, thus, it might be interesting to investigate interactions between tDCS and benzodiazepine relating to insomnia in future research.

Additionally, previous studies have reported slow EEG power changes before and after tDCS under the tasks, which would support further studies using slow frequencies (Keeser et al., 2011; Wirth et al., 2011; Jacobson et al., 2012; Ulam et al., 2015).

LIMITATIONS

Our current results, demonstrating the anxiety-reducing effects of tDCS in patients with MDD, will be of interest to researchers and clinicians who seek to use neuromodulation techniques as a novel treatment for depression. However, several limitations of the current study should be noted. First, the relatively small number of participants and no placebo stimulations may warrant some caution in the interpretation of these results. Second, because we applied only one stimulation, we did not examine the therapeutic effects of tDCS on depressive symptoms. Third, chi-squared test for gender imbalance between the MDD and HC groups tended to be imbalanced, suggesting gender effect may account for the difference between MDD and HC. However, this study design also has several strengths. First, we adopted with-in subject cross over design. Second, by adopting single-session tDCS, we were able to reduce the total experiment time and burden on the subjects compared with experiments involving multiple stimulation sessions. In future research, a randomized controlled trial of tDCS intervention with a large number of participants would be helpful for addressing these limitations.

CONCLUSION

The current results revealed that the immediate anxiolytic effect of left DLPFC tDCS was associated with activity in the rACC and the left IPL, whereas DMPFC stimulation was correlated with activity in the precuneus. These findings suggest that the effects of tDCS are not only directly related to the stimulation area, but also to other brain areas involved in the same resting state networks. Further, we propose that pre-stimulus EEG, in combination with the LORETA source estimation analysis, provides a promising tool for predicting the outcome of treatment intervention, including tDCS.

DATA AVAILABILITY

The datasets generated for this study can be found in Kansai Medical University, KAKEN 26860950 and KAKEN 19K08056.

ETHICS STATEMENT

The study protocol was approved by the Institutional Ethics Review Committee of Kansai Medical University.

AUTHOR CONTRIBUTIONS

KN and YM designed the study. KN, KK, SI, and MY recruited the participants. YK, SU, and RP-M analyzed the data. KN wrote the first draft. YM, RP-M, RI, and TK wrote the final manuscript.

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Safety and Feasibility of Transcranial Direct Current Stimulation for Cognitive Rehabilitation in Patients With Mild or Major Neurocognitive Disorders: A Randomized Sham-Controlled Pilot Study

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Introduction: Transcranial direct current stimulation (tDCS) is a potentially novel strategy for cognitive enhancement in patients with mild or major neurocognitive disorders. This study aims to assess the safety and efficacy of tDCS during cognitive training on cognitive functioning in patients with mild or major neurocognitive disorders.

Methods: This study was primarily a single arm for safety, secondary a two-arm, parallel, randomized, and sham-controlled trial for potential efficacy. Patients with mild or major neurocognitive disorders were recruited. The participants and raters were blinded to the group assignment. The participants in the active arm received tDCS (anodal; F3, cathodal, Fp2, 2A, 20 min) twice daily for five consecutive days, whereas those in the sham arm received the same amount of sham-tDCS. Calculation and reading tasks were conducted in both arms as a form of cognitive intervention for 20 min during tDCS. The primary outcome was the attrition rate during the trial in the active arm, which is expected to be less than 10%. The secondary outcomes were the between-group differences of adjusted means for several cognitive scales from baseline to post-intervention and follow-up.

Results: Twenty patients [nine women (45%)], with a mean (standard deviation) age of 76.1 years participated; nine patients (45%) with minor neurocognitive disorders and 11 (55%) with major neurocognitive disorders were randomized, and 19 of them completed the trial. The attrition rate in the active arm was 0%, with no serious adverse events. Further, in the Intention-to-Treat analysis, patients in the active arm showed no statistically significant improvement compared with those who received the sham in the mean change scores of the mini-mental state examination [0.41; 95% CI (−1.85; 2.67) at day five, 1.08; 95% CI (−1.31; 3.46) at follow-up] and Alzheimer's disease assessment

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scale – cognition subscale [1.61; 95% CI (−4.2; 0.98) at day 5, 0.36; 95%CI (−3.19; 2.47) at follow-up].

Conclusion: These findings suggest that tDCS is safe and tolerable but causes no statistically significant cognitive effects in patients with mild or major neurocognitive disorders. Additional large-scale, well-designed clinical trials are warranted to evaluate the cognitive effects of tDCS as an augmentation to cognitive training.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier NCT03050385.

Keywords: transcranial direct current stimulation, cognitive training, neurocognitive disorder, dementia, mild cognitive impairment

INTRODUCTION

Dementia is a disorder characterized by cognitive decline that interferes in patients' daily living and social functioning. Alzheimer's disease (AD), is the most common cause of developing dementia, and its progression is usually insidious and slow. Often, a prodromal and transitional state exists, without loss of independence, called mild cognitive impairment (MCI) (Petersen et al., 1999; Gauthier et al., 2006). The progression of dementia not only causes functional impairment in patients, it also degrades the caregiver's quality of life or social functioning due to caregivers' burden (Gill et al., 2017). Currently approved pharmacotherapies, cholinesterase inhibitors, and memantine are not disease-modifying and cannot revert the course of the disease, although they show a slight improvement in cognitive scales (Birks, 2006). Therefore, increasing focus has been placed on delaying deterioration or conversion from MCI to AD and other forms of dementia.

Recent studies have gradually revealed a few potentially modifiable factors, such as physical inactivity, social isolation, and depression (Gill et al., 2017). Further, a few cognitive interventional studies have also been conducted. Cognitive training generally includes guided practice on a set of standardized tasks designed to reflect specific cognitive domains. A recent meta-analysis indicated that the overall effect of cognitive training on cognition in MCI was moderate (Hedges' g = 0.35) and that in dementia was small (g = 0.26) (Hill et al., 2017). However, many of these trials assessed short-term cognitive outcomes. Moreover, based on the results from randomized trials that lasted for at least 6 months, cognitive training in patients with MCI suggested no effects on performance, with low strength, and insufficient evidence (Butler et al., 2018). Therefore, further strategies are awaited to combat cognitive decline in such patients. Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulation technique, which involves passing a direct electrical current through the cerebral cortex, usually *via* two electrodes placed on the scalp. One electrode serves as an anode and the other functions as the cathode. The tDCS device generates and delivers a small electric current (usually 1 to 2 mA) to different areas of the brain (Yokoi et al., 2018). The basic mechanism of tDCS is that the anodal tDCS increases neuronal excitability by causing a depolarization of the resting potential, while the cathodal tDCS hyperpolarizes the resting potential, thereby suppressing neuronal excitability (Philip et al., 2017). The change in neuronal excitability may lead to alteration in brain functioning in the vicinity of the stimulated area (Meinzer et al., 2015). Further, the alteration in brain functioning may also be explained by the hypothesis that prolonged membrane polarization by tDCS changes neuroplasticity through N-Methyl-D-aspartic acid (NMDA) receptors, thereby leading to long-lasting aftereffects of tDCS (Nitsche et al., 2003).

A recent meta-analysis of randomized controlled trials indicated that tDCS may be effective on cognition in healthy participants (Dedoncker et al., 2016); however, a meta-analysis of randomized controlled trials on the effect of tDCS on cognition in patients with dementia and MCI shows that tDCS is not always effective overall (Inagawa et al., 2018). The discord among the findings of these studies may be due to differences in the electrode montage, stimulation parameters, and timing of tDCS in the training tasks (Liu et al., 2017). Further, according to our systematic review and meta-analysis (Inagawa et al., 2018), only one study among the 11 previous studies on the effects of tDCS on cognition in dementia and MCI described a plan to provide sample size calculation. In addition, although we had planned to estimate sample size from previous studies, no study assessed the effect of tDCS combined with cognitive rehabilitation in patients with MCI at July 2016, when we started this study. Therefore, it was impossible to calculate sample size from the results of previous studies. It is important to provide *a priori* sample size calculation in order to gain sufficient statistical power to detect a difference in clinical trials. However, many previous studies did not provide sample size calculations and, thus, the results from these studies may be false-negatives, when the results are actually positive. Further, according to our meta-analytic review (Inagawa et al., 2018), the quality of study designs in these previous studies seems to be poor. In fact, many of them did not clearly state allocation concealment, blinding of personnel, or any method of handling missing data. These problems may overestimate the effects of tDCS, although tDCS is actually not effective.

Abbreviations: AD, Alzheimer's disease; ADAS-Cog, Alzheimer dementia assessment scale – cognitive subscale; CDR-J, clinical dementia rating-Japanese version; CI, confidential interval; DLPFC, dorsolateral prefrontal cortex; FAB, frontal assessment battery; LBD, lewy body disease; LTP, long-term potentiation; MCI, mild cognitive impairment; MMRM, mixed-effect model repeated measurement; MMSE, mini-mental state examination; SD, standard deviation; tDCS, transcranial direct current stimulation.

Further, previous studies on tDCS revealed the beneficial cognitive/anti-depressant effects of anodal tDCS in AD patients undergoing simultaneous cognitive training (Hsu et al., 2015), in depressive patients taking antidepressants (Brunoni et al., 2013), and in MCI patients receiving physical therapy (Manenti et al., 2016). Further, a randomized trial demonstrated that active tDCS, but not sham, over DLPFC combined with a working memory task showed greater improvement in healthy participants in terms of the performance of an attention and working memory test 1 month after the final treatment, compared with tDCS alone (Martin et al., 2013). These studies indicate the possibility of augmentation strategies of tDCS simultaneously with the conduct of cognitive training for improving cognition in patients with dementia and MCI.

The objectives of the proposed study are to assess the safety and feasibility of tDCS during cognitive rehabilitation, as well as to estimate potential efficacy applicable for further confirmatory studies in patients with neurocognitive disorders. Because combining tDCS with cognitive training may enhance the benefits of tDCS, we hypothesize that tDCS will improve, particularly when administered during cognitive tasks in patients with these disorders.

MATERIALS AND METHODS

Trial Design

This exploratory study was a single-arm study in terms of safety, while this was a two-arm, parallel, randomized, sham-controlled trial for the assessment of potential efficacy, and feasibility among 20 participants with a diagnosis of major neurocognitive disorder or mild neurocognitive disorder based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013). This study is reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement of information to include when reporting a randomized trial, which was developed to provide guidance in the form of a checklist of recommended items to help improve the quality of a study design (Moher et al., 2010; **Supplementary Table S1**).

Participants were supposed to be randomly assigned in the ratio of 1:1 to one of two groups – an active group or a sham group – using the order of entry into the study and a computer-generated randomization list obtained using a computer-generated randomization method, MUJINWARI (IRUKA System Cooperation, Tokyo, Japan). This was done to ensure a balanced allocation of the following factors across groups: age range (55–60 years, 61–70 years, 71–80 years, or 81–90 years), sex (male or female), and diagnosis (major neurocognitive disorder or mild neurocognitive disorder). This randomization method includes stratification for all of those factors. The allocation sequence was concealed until they completed the follow-up evaluations. tDCS device was always kept at the back of participants' visibility during its administration so that participants did not recognize allocation concealment. tDCS administrators only obtained access to a computer-generated randomization list, and tDCS administrators kept the allocation secret so that outcome raters did not recognize allocation concealment. Both the participants and raters were blinded to the group allocation; however, the investigators and those administering the tDCS were not blinded to the group allocation. In order to assess the quality of the blinding, after completing the study on day 5, the participants were asked to guess whether they were allocated to the active or sham group of the study. Further, we evaluated demographic and clinical characteristics, and used these data to provide descriptive characteristics of the population, and to analyze whether these characteristics could predict the outcomes. This study was registered on ClinicalTrials.gov (NCT03050385).

Participants

Both inpatients and outpatients were recruited by referrals from psychiatrists in a single academic hospital: National Center of Neurology and Psychiatry in Tokyo, Japan; both male and female patients were selected. The principal investigators or sub investigators assigned participants to interventions. The following were the key inclusion criteria: (a) subjects aged between 55 and 90 years and diagnosed with either major neurocognitive disorder or mild neurocognitive disorder, as defined in DSM-5; (b) subjects taking a stable dose of anti-dementia medications, such as cholinesterase inhibitors or memantine, for at least 2 weeks preceding enrollment; and (c) subjects who are able to walk independently, with or without an aiding device. The following were the key exclusion criteria: (a) subjects with severe psychotic symptoms requiring antipsychotic treatment, (b) subjects estimated to be in need of hospitalization within 6 weeks because of severe depression and/or suicidal ideation, (c) subjects who have a clinical contraindication to electroconvulsive therapy or tDCS, (d) subjects with an MMSE score less than 18 or a clinical dementia rating-Japanese version (CDR-J) global score of more than two, (e) subjects who were unable to attend more than 2 days of the trial, and (f) subjects for whom MMSE subscales of either "write a sentence" or "copy a figure" was zero. The patients were carefully assessed by a specialized psychiatrist before the trial. Because of the safety of tDCS to date (Bikson et al., 2016), no specific exclusion criteria were applied. Patients who were receiving anti-dementia medications were not excluded, but they were required to be receiving stable doses of these medications for at least 2 weeks prior to the first day of the administration of stimulation.

Intervention

Transcranial direct current stimulation was performed using a specially developed battery-driven constant 1 × 1 low-intensity tDCS (Model 1300A; Soterix Medical Inc., New York, NY, United States) that delivers direct current through two saline-soaked surface sponge electrodes (35 cm2) with a maximum output of 2 mA. This device also has a switch off allocation. If the administrator turned the switch on, the sham stimulation was delivered; if the administrator turned the switch off, active stimulation was delivered. During the stimulation, the device was placed behind the participants, and their allocation was kept secret so that they would not know which group they were randomized to. The anode electrode was placed over the left

dorsolateral prefrontal cortex (DLPFC) (F3) and the cathode was placed over the contralateral supraorbital ridge (Fp2), using the 10/20 electrode placement system in both active and sham arms. This method of DLPFC placement has been established by neuro-navigation techniques, as this method is relatively accurate for localization (Herwig et al., 2003). The DLPFC was selected because a previous study on healthy participants indicated that tDCS over DLPFC had beneficial effects on working memory (Martin et al., 2013). The cathode electrode was placed over the contralateral supraorbital area, which was similar to recent tDCS studies on cognitive functioning in patients with MCI (Manenti et al., 2016), Alzheimer's dementia (Khedr et al., 2014), and schizophrenia (Narita et al., 2017). It must be noted that for aging populations, stimulation with 2 mA has been shown to be safe. Actually, no severe adverse events due to tDCS with 2 mA have been reported (Bikson et al., 2016). The participants in the active arm received active tDCS at a constant current with an intensity of 2 mA for 20 min per session, with two sessions per day for 5 consecutive days. Those in the sham arm received the same treatment as those in the active arm, but the overall active stimulation period was only 60 s, including the 30 s for both the fade-in and fade-out periods. For the other periods, the stimulator remained active but did not generate current for 20 min in each session. Therefore, those in the sham arm usually experienced an initial itching sensation but received no current for the remainder of the session. All the participants received both tDCS (either active or sham) and cognitive training task for 20 min per session. On each day, the second active or sham tDCS session was conducted at least 20 min after the end of the first tDCS session in order to take into consideration the aftereffects of tDCS. In other words, the interval between the first and second tDCS session was 20 min.

These tasks comprised an initial 10 min calculation task, followed by a 10 min language task in Japanese. During the calculation task, participants solved basic arithmetic questions – such as single-digit addition, subtraction, and multiplication – as quickly and accurately as possible. During the language tasks, which included the Kanji writing task and the Kanji connecting task, participants answered questions related to Japanese Kanji letters. All the questions were printed on A4 sheets (210 mm × 297 mm). In the Kanji writing task, each participant was asked to interpret the meaning of hiragana characters and to write letters in Kanji. The participants performed a Kanji connecting task on a 10 × 10 grid, which contained a Kanji letter in each grid. In a separate table, there was a list of 20 different Kanji letters. In this task, the participants began from the first Kanji letter in the upper-left corner of the 10 × 10 grid (**Figure 1a**). Next, the participants were instructed to look at the Kanji letters on the right and toward the bottom of the first Kanji letter (**Figure 1b**) and match it with a Kanji letter included in the list of 20 different Kanji letters in the table (**Figure 1c**). When one of them would match, they connected this Kanji letter to the first Kanji by drawing a line from one to another (**Figure 1d**). In the next step, the new Kanji letter would take the place of the first Kanji letter (**Figure 1e**). The participants were asked to repeat this process for all the Kanji letters until they reached the Kanji letter in the bottom-right corner of the grid (**Figure 1f**).

The difficulty and complexity of the cognitive tasks were the same across sessions, but the content was different. We did not calculate the scores that could be obtained from the tasks. A previous randomized controlled trial indicated that similar

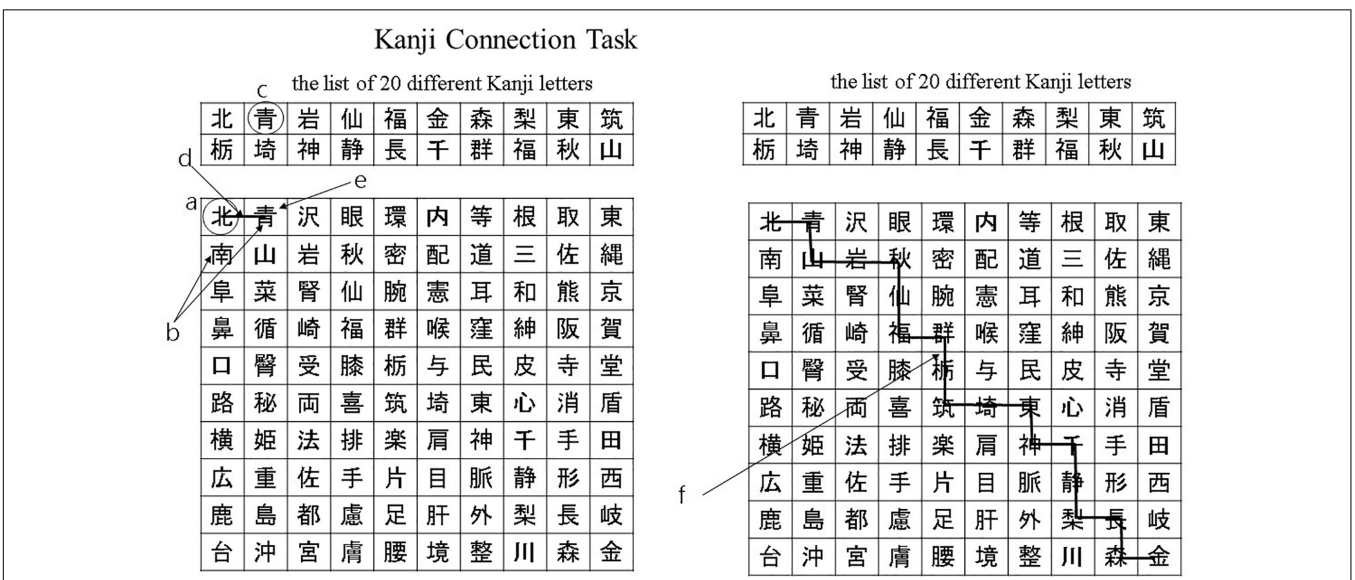

FIGURE 1 | Kanji connection task. **(a)** The participants began from the first Kanji letter in the upper-left corner of the 10 × 10 grid. **(b)** The participants were instructed to look at the Kanji letters on the right and toward the bottom of the first Kanji letter. **(c)** The participants were instructed to match it with a Kanji letter included in the list of 20 different Kanji letters in the table. **(d)** When one of them would match, they connected this Kanji letter to the first Kanji by drawing a line from one to another. **(e)** The new Kanji letter would take the place of the first Kanji letter. **(f)** The participants were asked to repeat this process for all the Kanji letters until they reached the Kanji letter in the bottom-right corner of the grid.

working memory tasks (reading and simple arithmetic problems) as the one in our pilot study improve executive functions, verbal episodic memory, focus attention, and processing speed in elderly patients who are healthy (Nouchi et al., 2016). The rationale for using these working memory tasks is that the training activates the bilateral prefrontal cortex (Arsalidou and Taylor, 2011). We also chose this task because the task was familiar to Japanese elderly population and feasible to be conducted among patients with dementia (Kawashima et al., 2005). We were interested in investigating the effects of tDCS on global cognition when combined with the cognitive training task commonly used for the elderly population in Japan, in order to ensure whether clinically meaningful effects can be obtained by adding multisession tDCS to cognitive training using common cognitive measures of MMSE and ADAS-Cog.

Outcomes

As this was the first phase of an exploratory feasibility trial, a sample size of 20 individuals, including sham, was adapted. In the intervention group, the primary outcome was the attrition rate during the trial, which is expected to be less than 10%. We selected the attrition rate as the primary outcome because it was important to first assess the safety and feasibility of tDCS in Japanese patients with neurocognitive disorders due to the fact that, to the best of our knowledge, no previous studies were found in such patients receiving tDCS while simultaneously being engaged in cognitive rehabilitation in Japan on July, 2016 when we started this study. The secondary outcomes were between-group differences in mean ADAS-Cog, MMSE, frontal assessment battery (FAB), and CDR-J scores from the baseline. We selected ADAS-Cog (whose score ranged from 0 to 70) and MMSE (whose score ranged from 0 to 30) for the assessment of global cognition, FAB (whose score ranged from 0 to 18) for the evaluation of frontal lobe functions, and CDR (whose score ranged from 0 to 3) for the estimation of the severity of dementia. All the above-mentioned outcome measures were scored by a psychologist after a clinical interview, who was blinded to group allocation. The outcome measures were assessed at the baseline, at the end of the final stimulation, and 2 weeks after the final stimulation (**Supplementary Table S2**).

Data Collection Methods and Data Monitoring

The assessments were conducted at the baseline, immediately after the intervention, and 2 weeks after the end of the intervention (**Supplementary Table S2**). Baseline and follow-up evaluations were conducted by experienced psychologists, who were blinded to the group assignments. The outcome data was sent to an independent data monitor, and neither the investigators nor the raters handled any data directly throughout the study. The data were initially recorded on paper files, with each participant assigned to a code number. These files were stored in a locked security box. Upon completion of the follow-up data collection, the data was sent to an independent data tester for cleaning up. The data monitor center also oversaw and reviewed the progress of the trial. If a participant decided to withdraw their consent, we allowed that participant to stop at any time. We also ceased the intervention if we observed any severe adverse events like burning. In this pilot study, the Efficacy and Safety Assessment Committee, which comprised members that were independent of the research, in the National Center of Neurology and Psychiatry checked and assessed whether or not this clinical trial was conducted safely and appropriately. The committee was called upon to decide whether it is possible to continue the trial or whether the research protocol must be revised in cases of either severe adverse events or protocol violations. The committee also performed this procedure by checking the documents of this trial in the intermediate period when five participants completed or discontinued their participation in this trial. The safety questionnaire on adverse events was established at the time according to the guidelines published in a recent consensus paper (Brunoni et al., 2011).

Statistical Analysis

We conducted an intention-to-treat analysis for patients who were randomized to either the active or sham arms; in addition, we summarized demographic data for all patients. Further, we calculated the point estimate of tDCS-related dropout proportion in the intervention group, where we checked whether the estimate was less than 10%. The exact confidential intervals (CIs) of this binomial proportion (Clopper and Pearson, 1934) were assessed. In order to evaluate the mean treatment effect, we conducted mixed models for repeated measures (MMRM) analysis to detect changes from baseline in ADAS-Cog, MMSE, FAB, and CDR-J at day 5 and follow-up. The MMRM analysis models included the covariates of age (55–60 years, 61–70 years, 71–80 years, or 81–90 years), sex (male or female), and disease (dementia or MCI), which were the stratification factors of dynamic allocation. This MMRM analysis models had treatment groups, time, group-by-time interaction, age, sex, diagnosis, and baseline as a fixed effect and unstructured covariance structure. Fisher's exact test was used to assess the integrity of blinding. We used STATA 14 (StataCorp LP, College Station, TX, United States) and SAS version 9.4 to conduct the statistical analysis.

RESULTS

Participants

Of the 21 participants who agreed to provide written consent, 20 were randomized to either the active or sham arms. Seven patients (five patients had AD, one had lewy body disease (LBD), and the other had the other type of etiology) were allocated to the active arm, while 13 patients (11 patients had AD, one had LBD, and the others had unspecified types of neurocognitive disorders) were allocated to the sham arm. Further, 19 participants (seven in the active arm and 12 in the sham arm) received all 10 tDCS sessions and completed the final assessment. All patients in tDCS group were outpatients. Two participants withdrew from the study: one withdrew during the intervention phase, and one before randomization. **Figure 2** depicts a flow chart on participants' selection, and **Table 1** presents baseline characteristics. Recruitment and follow-up were

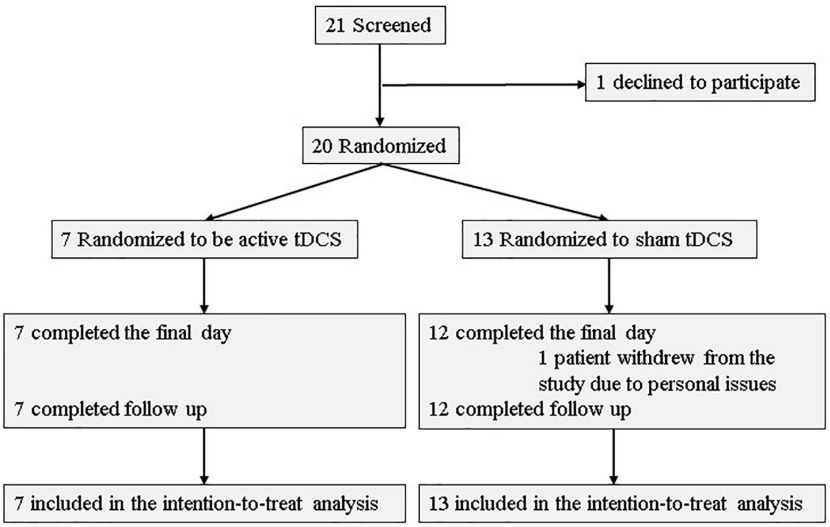

FIGURE 2 | Flow chart of participant selection.

conducted from July 2016 until July 2017 because more than 20 participants finished follow-up examinations. 20 participants were included for statistical analysis.

Primary Outcome

The primary outcome was an attrition rate in the intervention group. The attrition rate in the group was 0%, with no requirements of hospitalization, trial discontinuation, or any specific treatment in the active arm. The CIs of this proportion were from 0.0 to 41.0%.

TABLE 1 | Demographics and clinical characteristics (n = 20).

	Active group	Sham group
	Mean ± SD or n (%)	
	7 (100%)	13 (100%)
Age (year)	76.6 ± 5.7	76.2 ± 7.7
Female	4 (57.1%)	6 (46.2%)
Major neurocognitive disorder	3 (42.9%)	7 (53.8%)
Right-handed	7 (100%)	13 (100%)
Duration since diagnosis (year)	0.9 ± 1.2	1.2 ± 1.5
Family history		
Dementia	3 (42.9%)	3 (23.1%)
Mental disorder	0 (0%)	0 (0%)
Neurological disorder	1 (14.3%)	1 (7.7%)
Medication over the past 6 months		
Antidepressant, antipsychotics	1 (16.7%)	3 (23.1%)
Benzodiazepine	3 (42.9%)	4 (30.8%)
Cholinesterase inhibitors	4 (57.1%)	10 (76.9%)
Past history		
Substance abuse disorder	1 (14.3%)	0 (0%)
Schizophrenia	0 (0%)	0 (0%)
Mood disorder	0 (0%)	0 (0%)
Neurologic disorder	0 (0%)	2 (15.4%)
Head trauma	1 (14.3%)	2 (15.4%)
Visits to day care center for seniors	1.3 ± 2.6	0 ± 0

SD, standard deviation.

Secondary Outcomes

MMSE

The differences in adjusted means between groups for MMSE scores were 0.41 [95% CI: −1.85 to 2.67] (p = 0.705) at day five, and 1.08 [95% CI: −1.31 to 3.46] (p = 0.352) at follow-up, respectively (**Table 2**). There was no statistical significance in the between-group difference between the active and sham groups. **Supplementary Table S3** presents the change scores in adjusted mean difference from the baseline in each group. **Figure 3** illustrates the mean values and standard deviations (SDs) at each point.

TABLE 2 | The differences in adjusted means between groups for each cognitive scale in the MMRM analysis.

Clinical studies	Active tDCS vs. Sham							
	Post-treatment from baseline				Two-weeks follow up from baseline			
	Difference	95% CI		p	Difference	95% CI		p
MMSE	0.41	−1.85	2.67	0.705	1.08	1.31	3.46	0.352
ADAS-Cog	−1.61	−3.19	2.47	0.205	−0.36	−3.19	2.47	0.791
FAB	−2.27	−6.17	1.63	0.233	−3.01	−6.46	0.45	0.083
CDR					0.06	−0.09	0.22	0.404

MMRM, mixed-effect model repeated measurement; tDCS, transcranial direct current stimulation; 95% CI, 95% confidential intervals; MMSE, mini-mental state examination; ADAS-Cog, Alzheimer dementia assessment scale – cognitive subscale; FAB, frontal assessment battery; CDR-J, clinical dementia rating-Japanese version.

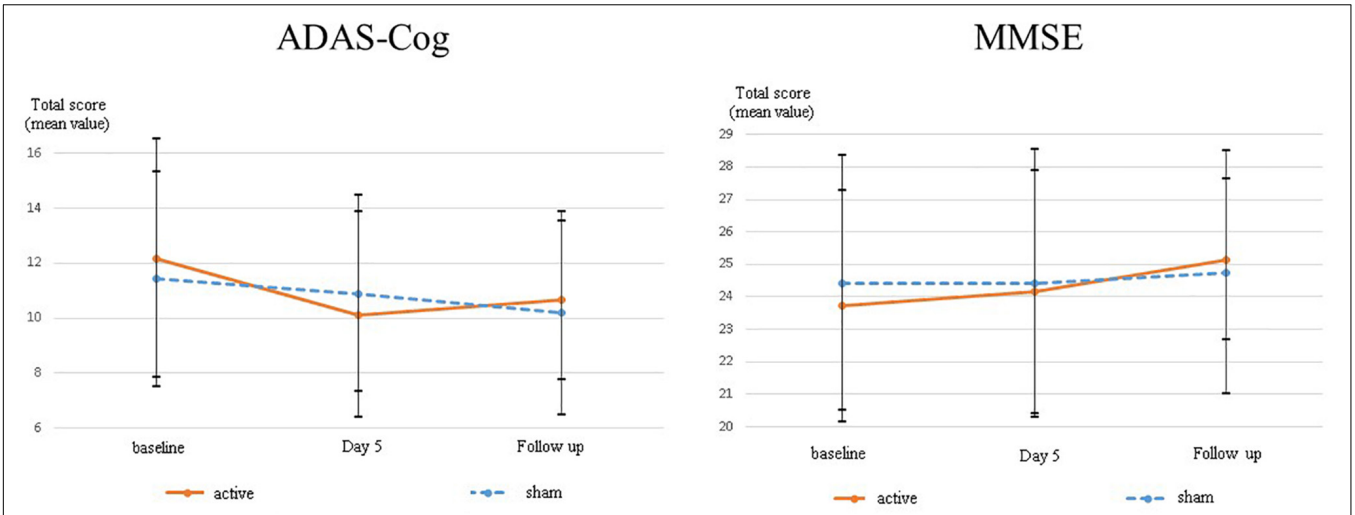

FIGURE 3 | The change scores in adjusted mean difference from baseline on ADAS-Cog and MMSE. In order to understand the mean change from baseline in each group easily, baseline scores in each group were shown as zero in this graph.

ADAS-Cog

The differences in adjusted means between groups for ADAS-Cog scores were 1.61 [95% CI: −4.2 to 0.98] ($p = 0.205$) at day five and 0.36 [95% CI: −3.19 to 2.47] ($p = 0.791$) at follow-up (**Figure 3**). There was no statistically significant difference between the active and sham arms. **Supplementary Table S3** presents the change scores in the adjusted mean difference from baseline in each group. **Figure 3** illustrates the mean values and SDs at each point.

FAB

The differences in adjusted means between groups for FAB scores were −2.27 [95% CI: −6.17 to 1.63] ($p = 0.233$) at day five and −3.01 [95% CI: −6.46 to 0.45] ($p = 0.083$) at follow-up. FAB showed a dip in the active arm at both day five and follow-up, but no statistically significant differences were found between the groups. **Supplementary Table S3** presents the change scores in the adjusted mean difference from baseline in each group.

CDR

The differences in adjusted means between groups for CDR scores were 0.06 [95% CI: −0.09 to 0.22] ($p = 0.404$) at follow-up. **Supplementary Table S3** presents the change scores in the adjusted mean difference from baseline in each group.

Adverse Events

We found neither severe adverse events nor the need for medications caused by adverse events in each group. **Table 3** presents adverse events related to tDCS.

Integrity of Blinding

In the sham and active groups, seven of 12 participants (58.3%) and three of seven participants (42.9%), respectively, correctly identified the allocation group ($p = 1.000$, as assessed by Fisher's exact test). Thus, participants were unable to guess their actual group beyond that by chance.

DISCUSSION

Using data from a small sized sample, no cognitive effects of tDCS were detected in this pilot study; however, this is certainly not definitive because of the insufficient sample size used in this study. Further studies using a larger sample size are warranted in order to arrive at a clear conclusion regarding whether or not tDCS is effective for improving cognition in patients with mild or major neurocognitive disorders and also to evaluate the generalizability of this pilot study. Moreover, we did not adjust for multiplicity because the primary objective of this study was to assess the safety and feasibility of tDCS in Japanese elderly patients with neurocognitive disorders, and the secondary aim was to exploratorily estimate potential efficacy applicable for further proof-of-concept studies to demonstrate the effects of tDCS on cognition in patients with neurocognitive disorders. Apart from that, the augmentation strategy in this pilot study should have been more sophisticated. Although proof of concept has been indicated, whereby anodal stimulation over DLPFC during a working memory task led to enhanced performance the

TABLE 3 | Adverse effects related to tDCS reported by patients in each group.

	tDCS	Sham	*p*
Headache (n, %)	1 (14.3%)	5 (38.5%)	0.354
Neck pain (n, %)	0 (0%)	2 (15.4%)	0.521
Scalp pain (n, %)	0 (0%)	4 (30.8%)	0.249
Tingling (n, %)	3 (42.9%)	9 (69.2%)	0.428
Itching (n, %)	1 (14.3%)	3 (30.8%)	1.000
Burning sensation (n, %)	2 (28.6%)	4 (30.8%)	1.000
Skin redness (n, %)	2 (28.6%)	2 (15.4%)	0.587
Sleepiness (n, %)	1 (14.3%)	2 (15.4%)	1.000
Trouble concentrating (n, %)	1 (14.3%)	1 (7.7%)	1.000
Acute mood change (n, %)	0 (0%)	1 (7.7%)	1.000
Others (n, %)	0 (0%)	0 (0%)	1.000

next day (Martin et al., 2013), the cognitive training task used in this pilot study is not exactly the same as that employed in previous studies. Moreover, the Kanji connection task is short and tough as compared with that in previous studies. A previous study indicates that those trainees who put more effort into training were more anxious or depressed, and showed lesser improvement in cognition (McAvinue et al., 2013). Therefore, it may be necessary not only to modify the tDCS protocol but also to optimize the cognitive task in future trials.

Further, the target population may not have been optimal. A previous randomized controlled trial indicates that tDCS is not effective on global cognition assessed by ADAS-Cog in patients with moderate and severe dementia with apathy. The authors discuss that in order to gain cognitive benefits of anodal repetitive tDCS, it is necessary to have at least some remaining neuronal function to promote plasticity, which may not be possible in aged patients with AD. In this study, the target population may have been patients in an early stage of the disease, like MCI. Moreover, the difference between mild neurocognitive disorders and major neurocognitive disorders is the interference with independence in daily activities. Then, cognitive deficits in mild neurocognitive disorders may be stable or even reversible, but those in major neurocognitive disorders may be continuous or even progressive. These differences between the mild and major disorders especially for the potential difference in the course of cognitive change may have confounded the results presenting the after-treatment cognitive changes from baseline although we did include the variable as a covariate in the analysis. A preliminary randomized sham-controlled trial indicated that comparisons of anodal tDCS for the DLFPC group (2 mA, 25 min daily for 10 days) vs. the sham group revealed significant interaction between time and treatment for MMSE scores post-stimulation, 1 month later, and 2 months later in 22 patients (11 in each group) with mild-to-moderate Alzheimer's dementia ($p = 0.04$) (Khedr et al., 2014). Another preliminary randomized sham-controlled trial indicated that anodal tDCS over the DLPFC group (2 mA, 25 min daily for 10 days) vs. the sham group showed a significant interaction between time and treatment ($p = 0.0041$) on the Parkinson's disease cognitive rating scale at the post-stimulation point and 3-month follow-up period in 20 patients (10 in each group) with MCI in Parkinson's disease (Manenti et al., 2016). On the other hand, no significant effect of anodal tDCS over DLPFC was found on the Apathy scale ($p = 0.55$ for repeated measures) or ADAS-Cog ($p > 0.40$) in 40 patients (20 in each group) with moderate-to-severe AD compared to the sham group (Suemoto et al., 2014). Moreover, tDCS may not be effective in global cognition in these patients. Although a few studies show potential cognitive benefits, a functional trade-off has been suggested in which improvement in a single cognitive domain comes at the cost of decline in another one (Philip et al., 2017). In addition, the effect of tDCS appears to be site-specific; thus, the effect of tDCS in itself may not sufficiently transfer to other brain regions to improve global cognition (Kim et al., 2014).

In order to determine whether or not the effect of tDCS on cognition in neurocognitive disorders is clinically meaningful, one of the possible options is to compare the effect of tDCS with that of the first-line standard treatment. Although Cholinesterase inhibitors (ChE-Is) have never been approved for standard treatment in patients with MCI in Japan, they are considered to be the first-line pharmacological agent for mild to moderate AD. ChE-Is work by inhibiting the breakdown of acetylcholine, an important neurotransmitter related to memory, by blocking the enzyme acetylcholinesterase. The between-group difference in mean changes of MMSE was 1.37, according to a systematic review and meta-analysis of unconfounded, double-blinded, randomized, placebo-controlled trials designed to evaluate the efficacy of patients with dementia due to AD, in which treatment with a ChE-I was administered for approximately 6 months (Birks, 2006). If the effect size obtained from the ongoing phase-II randomized trial is similar to that in our pilot study, which was 1.08 at follow-up, tDCS could be a potential tool for alleviating cognitive deficits in those patients. Further, the differences in adjusted means between groups for ADAS-Cog scores at day five was −1.61 ($p = 0.205$) (**Figure 3**). Further trials are warranted to evaluate whether these cognitive benefits can be generalized to the larger population in MCI and mild dementia in this tDCS protocol.

The strength of this study is that it has a relatively low bias risk as compared with previous studies. Although tDCS administrators were not blinded, both participants and raters were blinded. A random sequence was generated through computers, and allocation was concealed until the disclosure of the data; blinding was well integrated. Further, all pre-specified outcomes were shown after registration on ClinicalTrials.gov. The dropout rates were low in both groups: 0% in the active group and 7.69% in the sham group. These indicate the quality of this study. Another strength of our study is the novelty that, to the best of our knowledge, this is the first study to assess optimized tDCS protocol combined with cognitive training in patients with both MCI and mild dementia. Further, our pilot study indicated that tDCS combined with cognitive training was safe and feasible. In addition, we selected ADAS-Cog and MMSE because these scales are the screening tools that are most commonly used to measure cognitive deficits in clinical settings.

This study has a few additional limitations. First, the follow-up period is too short to evaluate changes in disease progression and to test whether additional interventions are needed over time. Second, cognitive training tasks used in this pilot study are not entirely the same as those used in previous studies, which indicates that calculation tasks and reading tasks improve executive functions, verbal episodic memory, focus attention, and processing. Third, our cognitive training protocol is short and tough compared to that of previous studies. This may have caused psychological stress among participants, which may have decreased the effect of cognitive training (McAvinue et al., 2013). Fourth, our pilot study only selected MMSE and ADAS-Cog total scores for the assessment of global cognition and FAB for the evaluation of frontal lobe cognitive function. In future studies, a standard scale, like the repeatable battery for the assessment of neuropsychological status (RBANS), is an appropriate choice to comprehensively assess the global cognition and cognitive domains separately and to gain statistical power enough to assess the meaningful cognitive benefits in patients with early stages of neurocognitive disorders. For example, RBANS may

be well suited because it is a brief and comprehensive battery consisting of the following indices: memory, attention, language, visuospatial/constructional, and total scores. This scale enables raters to simultaneously assess global cognition and several other domains of cognition, including memory, attention, and language. Fifth, we selected MMRM analysis for multiple outcome measures and several time points, so the results obtained from those measures should be interpreted carefully. Future trials with a proper sample size calculation for one primary outcome may provide meaningful results. Sixth, a small sample size may lead to lack of statistically significant power. It is important to provide *a priori* sample size calculation in order to gain sufficient statistical power to detect a difference in clinical trials. However, we did not provide it because it was necessary to first assess the safety and feasibility of tDCS in Japanese patients with neurocognitive disorders, as, to the best of our knowledge, this is the first pilot study of tDCS in such patients. Therefore, the results obtained from this study may well lack statistical power to detect a difference between the active and sham arms. If the primary outcome of a future trial lies in the differences of adjusted means between groups for ADAS-Cog scores at day five, the minimal sample size with statistical power over 0.8 is estimated to be 46 in each group; this is based on the assumption that the between-group difference in ADAS-Cog scores is −1.61 and its SD is 2.70. Based on this sample size calculation, we have initiated a phase-II randomized, sham-controlled trial of tDCS on cognition in MCI and mild dementia. This trial has been registered in the Japan Registry of Clinical Trials (protocol number: jRCTs032180016). Seventh, the patients were supposed to be randomized to the treatment groups with a 1:1 ratio but the outcome of the allocation was 7:13. That was because too many factors were included in the factors for minimization techniques in spite that the sample size was small. Eighth, the results may be interpreted carefully because we conducted statistical tests for a variety of outcome groups without taking multiplicity into consideration because this trial was an exploratory trial.

In conclusion, tDCS is safe and tolerable in the context of cognitive rehabilitation. We found no statistically significant cognitive effects of tDCS in patients with mild or major neurocognitive disorders. Further trials with larger samples may clarify the efficacy of tDCS on global cognition and several cognitive domains in such patients.

ETHICS STATEMENT

This study was conducted in accordance with the Declaration of Helsinki and is based on the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. The protocol was presented to the institutional review board of the National Center of Neurology and Psychiatry Ethics Committee for approval (A2016-048). The principal investigator and the research coordinator were responsible for conducting the informed consent process with all the study participants. All the participants were required to provide written consent to participate in the trial. They were assessed only after being informed of the objectives of the study and providing written informed consent. Any relevant changes in the study protocol and/or the informed consent process were sent to the Ethical Committee as protocol amendments. The identities of all the participants were protected using individual codes, which were accessible only by the principal investigator. The protocol was also registered on ClinicalTrials.gov.

AUTHOR CONTRIBUTIONS

TI and YY developed the original concept of the trial. ZN and KN advised and reviewed the design and methodology of the original protocol. YY established the protocol. TI and ZN conducted the tDCS. KM conducted the statistical analysis of the results. TI wrote the manuscript and all other authors reviewed and commented on the draft. MO contributed to the safety monitoring and management of our research progress. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnhum.2019.00273/full#supplementary-material

TABLE S1 | CONSORT statement.

TABLE S2 | Study Procedure. The outcome measures were assessed at the baseline, at the end of the final stimulation, and two weeks after the final stimulation.

TABLE S3 | The change scores in adjusted mean difference from baseline in each group.

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