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Early- and late-phase changes of brain activity and early-phase neuromodulation in the posttraumatic stress disorder rat model

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ABSTRACT

Posttraumatic stress disorder (PTSD) is a complex syndrome that may occur after life-threatening events. Fear memory abnormalities may play vital roles in the pathogenesis of PTSD. Previous work has found that fear memories are not rigid; the retrieval of fear memories may change over time. Furthermore, prior studies suggest that theta wave (4 Hz) activity is highly correlated with fear expression in an animal model. However, the relationship between pathological fear memory and potential brain wave features in PTSD remains largely uncharacterized. Here, we hypothesized that after traumatic stress exposure, the longitudinal dynamics of abnormal fears in PTSD animal models could be reflected by the measurement of local field potentials (LFPs). Using a well-established modified single-prolonged stress and footshock (SPS & FS) PTSD rat model, animals were restrained for 2 h and subsequently subjected to 20 min of forced swimming, then exposed to diethyl ether until they lost consciousness and placed in a conditioning chamber for fear conditioning. To characterize the temporal changes, we characterized freezing behavior brain wave features during the conditioning chamber re-exposure in the early (10 and 30 min; 2, 4, and 6 h) and late (day 1, 3, 7, and 14) phases after traumatic stress exposure. Our results indicate that SPS & FS rats showed co-morbid PTSD phenotypes including significantly higher levels of anxiety-, depression-, and anhedonia-like behaviors, and impaired fear extinction. Delta wave (0.5–4 Hz) suppression in the medial prefrontal cortex, amygdala, and ventral hippocampus occurred 10 and 30 min after traumatic stress, followed by continuous delta wave activity from 2 h to day 14, correlating with fear levels. tDCS reduced delta activity and alleviated PTSD-like phenotypes in the SPS & FS group. In this study, profiling abnormal fears with brain wave correlates may improve our understanding of time-dependent pathological fear memory retrieval in PTSD and facilitate the development of effective intervention strategies.

1. Introduction

Posttraumatic stress disorder (PTSD) is a complex syndrome that may occur after exposure to life-threatening events. PTSD is a stress-related disorder. According to the DSM-5 (The Diagnostic and Statistical Manual of Mental Disorders, 5th Edition, American Psychiatric Association, 2013), patients with PTSD generally experience heterogeneous and persistent affective, cognitive, somatic, and behavioral alternations (van der Kolk et al., 1996). Fear conditioning may play an important role in PTSD maintenance (Shin and Liberzon, 2010). At the time of trauma exposure, strong emotional responses associated with environmental factors (such as objects or loud sounds) and formed fear conditioning may cause the development of PTSD (Careaga et al., 2016). The abnormal fears associated with PTSD, including enhanced fear consolidation and impaired fear extinction (Pitman et al., 2012; VanElzakker et al., 2014; Yehuda and LeDoux, 2007), may enhance the core symptom—intrusive re-experiencing of trauma—and the maintenance of PTSD for months or years after the traumatic events (Milad et al., 2006; Quirk et al., 2006; Rauch et al., 2006).

Dysregulation of inter-regional neuronal activity is thought to be associated with long-term biological changes in PTSD. In PTSD animal models, several key neural correlates have been characterized. These stress-associated neural correlates include the medial prefrontal cortex (mPFC), anterior cingulate gyrus (ACC), nucleus accumbens, thalamus,

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hypothalamus, hippocampus (Hipp), amygdala (AMY), and periaqueductal grey (Hales et al., 2014; Ritov et al., 2016). In addition, loss of brain volume in specific regions such as the Hipp, AMY, and ACC has also been found in PTSD patients (O'Doherty et al., 2015). It has been suggested that abnormal associations between the Hipp and AMY cause traumatic memories to be improperly contextualized. Moreover, uncontrollable AMY-dependent fear reactions associated with the dorsal visual stream and insula may contribute to traumatic event flashbacks and pathological hypermnesia (Desmedt et al., 2015). Finally, the dysfunction of related fear circuits may cause persistent re-experiencing of traumatic events and the maintenance of PTSD (Misic et al., 2016).

Memory encoding is a longitudinal process with both spatial and temporal properties. A review article by Do Monte et al. (2016) indicated that fear memories are not rigid; the retrieval of fear memory changes over time. Brain wave features have been considered closely related to memory formation (Duzel et al., 2010; Hanslmayr and Staudigl, 2014; Tyng et al., 2016). With respect to global brain activities, distinct phase synchronies (synchronous and asynchronous activity) occur during different stages of memory encoding (Burke et al., 2013; Guderian et al., 2009). The measurement of brain wave activities, such as via local field potentials (LFPs) or electroencephalography (EEG), may help explore the temporal synchrony of brain activity and inter-regional neuronal activities. A previous animal study indicated that 4-Hz oscillations in the dorsomedial PFC were strongly correlated with the length of freezing episodes, potentially reflecting fear levels (Karalis et al., 2016). Additionally, PTSD severity was found to correlate with frontal asymmetry (Kemp et al., 2010). Moreover, PTSD patients experienced reduced alpha power (Sheridan et al., 2012) and high gamma hyper-connectivity during states of trauma re-experiencing, hypervigilance, and functional block (Misic et al., 2016).

Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulatory technique that involves the application of weak direct current between an anode and a cathode. The modulatory effects of tDCS can be affected by current strength and the direction of current flow (Nitsche et al., 2007). In clinical studies, tDCS has been shown to improve a range of emotional and cognitive performances (Saunders et al., 2015). Currently, tDCS is still not FDA-approved for PTSD treatment (Bikson et al., 2008; Novakovic et al., 2011). Thus, efforts to understand the proper application and underlying mechanisms of tDCS in PTSD animal models may help find effective PTSD interventions.

The single-prolonged stress (SPS) rat model (Liberzon et al., 1997) is a well-established and frequently used animal model of PTSD. Several lines of evidence indicate that the SPS model exhibits the wide range of neuroendocrine and behavioral abnormalities observed in PTSD patients (Liberzon et al., 1997, 1999). Specifically, phenotypes such as enhanced hypothalamic-pituitary-adren (HPA) axis negative feedback (Liberzon et al., 1999), enhanced contextual freezing (Iwamoto et al., 2007), hyperarousal responses (Khan and Liberzon, 2004), and increased anxiety and depressive levels (Imanaka et al., 2006; Serova et al., 2013) have been observed in the model. In the present study, we applied an enhanced SPS paradigm in rats, named as SPS & FS model, consisting of both SPS and fear conditioning (footshock; FS) (Wang et al., 2008, 2015). This enhanced SPS model displays persistently conditioned and sensitized fears that increase with prolonged times (Wang et al., 2008).

This study addresses the temporal network alternations and enhanced fears that occur after trauma exposure. A better understanding of the longitudinal dynamics of abnormal fears in PTSD animal models during early and late time phases may help explain the pathological trajectory of PTSD and shed new light on the treatment of this complex disorder.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (weight: 300–400 g; BioLASCO Taiwan Co., Ltd., Taipei, Taiwan) were housed in ventilation cages with food and water access *ad libitum*. The temperature of the colony room was maintained at 20 ± 2 °C with a 12-hr light/dark cycle (light on from 7 a.m. to 7 p.m.). All of the experiments were performed during the light phase and followed the standard ethical guidelines of the Academia Sinica Institutional Animal Care and Utilization Committee (IACUC; IACUC ethical protocol ID: 19-12-1386). All efforts were made to minimize the animals' suffering and reduce the numbers of animal use.

2.2. Preparation of SPS & FS animal model

We prepared the SPS & FS model as previous literature (Wang et al., 2008, 2015). Briefly, rats were individually restrained for 2 h in a restraint tube (inner diameter 7 cm, length 20 cm), we then immediately introduced each rat individually to forced swimming for 20 min in a cylindrical tank (diameter 40 cm, height 60 cm) filled with water to a depth of 30 cm at 25 ± 1 °C. After a 15-min recovery period, rats were exposed to ether until loss of consciousness, which was defined as a lack of response to a foot pinch. Before the animals awoke, they were placed into a shock cage (50 cm × 50 cm × 50 cm) equipped with a speaker for delivering the auditory tone. Immediately after they awoke, a modified FS protocol was employed (Mikics et al., 2008). Specifically, we delivered two shock trains of 1.5 mA for 1 s (unconditioned stimulus, US) and each shock was paired with a 30-sec, 80-dB, 1 kHz neutral auditory tone (conditioned stimulus, CS). The FS and auditory neutral tones were offset together. Altogether, 10 shocks were delivered.

2.3. Freezing behavior measurement

2.3.1. Conditional contextual fear extinction measurement for FS and SPS & FS models

For the fear extinction experiment, animals were randomly assigned to the FS (n = 5) or SPS & FS group (n = 6). In the FS group, animals only received an FS (two shock trains of 1.5 mA for 1 s; total 10 shocks) for contextual fear conditioning. In the SPS & FS groups, the SPS procedure was conducted as described in section 2.2 "Preparation of SPS & FS animal model" above. However, in the present fear extinction experiment, animals received FS (two shock trains of 1.5 mA for 1 s; 10 shocks) for contextual fear conditioning without a subsequent auditory cue. Subsequently (day 1–10 for fear extinction; day14 and day 40 for fear recall), all animals were repeatedly re-exposed to situational reminders for 5-min measurements of freezing behavior.

2.3.2. Conditioning chamber re-exposure with auditory neutral tones for the FS and SPS & FS groups

To characterize the temporal dynamics of brain wave activities after traumatic stress exposure or FS, animals were exposed to a FS (n = 5) or SPS & FS (n = 5) on day 0 and returned to the FS cage for the conditioning chamber re-exposure with six auditory neutral tones (80 dB for 30 s; CS^+) at early (10 and 30 min; 2, 4, and 6 h) and late (day 1, 3, 7, and 14) time points after FS or SPS & FS exposure. During the FS cage re-exposure, we measured fear levels in response to six CS^+. Tones were delivered at 30-sec intervals (periods without auditory neutral tone are designated CS^-), for a total of six CS^+ and six CS^- periods. Freezing behavior was defined as a lack of animal movement for 2 s. Freezing episodes during CS^+ were detected and scored by the FreezeScan system (Clever Sys, Inc., Reston, VA, USA).

2.4. PTSD phenotype measurement

Behavioral tests for evaluating PTSD phenotypes were performed on day 8, 9, and 10. We performed open field (OF) and elevated plus maze (EPM) tests to measure anxiety levels, forced swimming tests (FSTs) to measure depressive levels, and sucrose preference (SP) tests to assess levels of anhedonia.

2.4.1. OF test

The OF test leverages an 80 cm × 80 cm × 40 cm black Plexiglass apparatus. In each test, rats were placed in the center of the OF arena with dim light and allowed to explore for 5 min. Animal behavior was videotaped during the 5-min test, and the amount of time spent in the center, inner zone, and outer zone was scored by the TopScan TM1.0 system (Clever Sys, Inc., Reston, VA, USA). The apparatus was cleaned with 70% alcohol before introducing the next subject. A reduced percentage of time spent in the center zone during each 5-min session is indicative of higher anxiety levels.

2.4.2. EPM test

The EPM is a plus-shaped maze with four arms (each arm: 50 cm long × 10 cm wide). The EPM platform is elevated by 50 cm and contains two open arms and two closed arms (wall height: 40 cm). At the beginning of each test, animals were placed in the center zone facing one of the open arms and allowed to explore the platform for 5 min. The EPM was used to assess anxiety-like behavior; the amount of time spent in the center zone and open and closed arms was quantified by the TopScan TM1.0 system (Clever Sys, Inc.). An increased percentage of time spent in the closed arms during a 5-min session is indicative of higher anxiety levels.

2.4.3. Forced swim test (FST)

Animals were individually introduced to an open cylindrical container (diameter: 20 cm, height: 60 cm), and forced to swim for 5 min. During each test, freshwater was filled to a height of 40 cm and animal behavior was videotaped. The total durations of swimming, immobility, and climbing during the 5-min test were scored by video analysis.

2.4.4. SP test

After 23 h of food and water deprivation, animals were exposed to both drinking water and a test solution (20% sucrose) for 1 h. The preference for sucrose was calculated as (sucrose intake/total fluid intake) × 100%, which is a reliable index for anhedonia.

2.5. Surgery and recording

2.5.1. Electrode implantation

Electrodes for LFP measurements were constructed with formvar-coated stainless steel wires (100-μm diameter, 304 HFV, California Fine Wire, Grover Beach, CA, USA). All electrode wires were attached to a 20-pin connector. A stainless-steel grounding electrode (coordinates: 9 mm anterior to the bregma) and a stainless-steel reference electrode (coordinates: 12 mm posterior to the bregma) were anchored to the skull. The reference electrodes were connected to the 20-pin connector via polyimide-coated silver wires.

For electrode implantation, animals were anesthetized with 3% isoflurane in O_2 and maintained with 1.5% isoflurane in O_2 during surgery. During surgery, body temperature was maintained at 37 °C with a homeothermic blanket system (model 50–7079, Harvard Apparatus, Holliston, MA, USA). Animals were secured in a stereotaxic apparatus, the fur on the scalp was shaved, and a midline incision was made to expose the skull. A total of six individual craniotomies were made and electrodes were bilaterally implanted in the targeted sites, including the mPFC (coordinates: 3.25 mm anterior to the bregma, 0.5 mm lateral to the midline, 2.0 mm ventral to the cortical surface), AMY (coordinates: 3.15 mm posterior to the bregma, 5.25 mm lateral to the midline, 8.6 mm ventral to the cortical surface), and ventral Hipp (vHipp; coordinates: 5.15 mm posterior to the bregma, 5.2 mm lateral to the midline, 8.0 mm ventral to the cortical surface). All electrodes were fixed to the skull with adhesive dental cement (Super-bond C&B, Sun Medical Co, Ltd. Moriyama, Japan). Next, two polyimide-coated silver wires were wrapped individually around grounding and reference electrodes. After implantation, the electrodes and skull surface were thoroughly covered with Lang's Jet liquid acrylic and powder (Lang Dental Manufacturing Corp., Inc., Chicago, IL, USA). Following surgery, animals were administered antibiotics and allowed to recover for one week.

2.5.2. LFP recordings and analysis

During the conditioning chamber re-exposure, animal behavior was videotaped and free-moving LFP activities were recorded at the same time, the headstage was connected to a preamplifier and linked to a multi-channel data acquisition system (Tucker-Davis Technologies, Alachua, FL, USA), in case channels were damaged during the procedure of electrode implantation, the LFP signals from each brain region were recorded using two channels. In addition, one channel was used for reference and another for grounding, resulting in a total of 14 recorded channels. The sampling rate of recorded analog signals was 3 kHz and LFPs were filtered with a band-pass filter at 0.5–50 Hz. Bandwidths were defined as delta (0.5–4 Hz), theta (5–8 Hz), alpha (9–12 Hz), beta (13–20 Hz), high-beta (21–30 Hz), and gamma (31–50 Hz) waves.

LFP traces were analyzed using custom MATLAB (MathWorks, Natick, MA, USA) scripts. The Fast Fourier Transformation (FFT) was applied to reflect a time-frequency representation. We then calculated the spectral power (SP) ratio of each freezing episode in each frequency band.

$$\mathrm{SP}_i = \mathrm{norm}[\mathrm{SP}(f_i)], \text{where } i = 1 - 6 \tag{1}$$

We also computed the ratio of SP (%) in each frequency band to total power.

$$\mathrm{SP}\ (\%) = \frac{\mathrm{norm}[\mathrm{SP}(f_i)]}{\mathrm{norm}[\mathrm{SP}(f_T)]} \times 100, \text{where } 0.5 < f_i < 4, 5 < f_i < 8, 9 < f_i < 12, \\ 13 < f_i < 20, 21 < f_i < 30, 31 < f_i < 50, 0.5 < f_i < 50 \tag{2}$$

The SP (%) across all the frequency bands for each freezing episode (FS and SPS & FS groups) was calculated and averaged for plotting the brain wave temporal changes for early and late time points. For the control group, we collected the immobile episodes, and calculated and plotted with the same calculation procedure.

Additionally, the multi-channel LFP coherence coefficient (R) between two frequency power spectra, $X_a(t)$ and $X_b(t)$, was computed to describe the synchronous states among different brain regions using the Pearson correlation coefficient method:

To analyze correlations between freezing behavior and frequency band power, the length (sec) of individual freezing episodes and the ratio of spectral power (%) were cross-correlated with the Pearson correlation coefficient method. To calculate the asymmetry of LFP activities, we compared the SP ratio (%) of delta and alpha activities between the right and left mPFCs.

2.6. tDCS application

2.6.1. tDCS delivery and setting

tDCS stimulation was delivered with an animal tDCS device (Soterix Medical Inc., Woodbridge, NJ, USA). A guide cannula (diameter: 2.5 mm, length: 8.0 mm, center: 5.3 mm anterior to the bregma) was attached to the frontal region of the skull and fixed with dental cement during electrode implantation surgery (described in 2.6.1). During tDCS stimulation, animals were anesthetized (1.5% isoflurane in O_2), the guide cannula was filled with conductive gel, and the cathode was inserted into the guide cannula. The anode, a circular metal plate (diameter: 3.7 mm), was placed under the chest, fixed with a jacket, and attached to the skin with conductive gel.

2.6.2. tDCS application after SPS & FS exposure

To understand the effects of tDCS modulation on SPS & FS models, tDCS modulation was used from day 0–7; repeated cathodal tDCS (200 μA, 30 min) (Wu et al., 2017) and sham (200 μA, 30 s) stimulation was

applied once daily for eight days. PTSD phenotypes were evaluated on day 8, 9, and 10 post-SPS & FS with the OF test, FST, EPM, and SP test. As previously described in section 2.5.2 (LFP recordings and analysis), freezing behavior and brain wave activities were recorded during the conditioning chamber re-exposure. To measure changes in delta activity (0.5–4 Hz) after tDCS application, the average delta activity area under the curve (AUC) for six CS^{+} was calculated for each animal. Mean delta activity AUCs for each group were presented as mean ± SEM.

2.7. Data analysis

All data were presented as mean ± SEM. Data from fear extinction assessment was compared between FS and SPS & FS groups by two-way repeated-measures analysis of variance (ANOVA). Data from the OF test, EPM, FST, SP test, and ELISAs were analyzed via one-way ANOVA with Bonferroni post-hoc tests. Freezing levels between FS and SPS & FS groups at multiple time points were compared using two-way repeated-measures and freezing levels between sham and SPS & FS + tDCS groups on D1, D3, and D14 were compared by using unpaired Student's t-tests. Brain wave measurements at specific time points (e.g., D1), bandwidths (e.g., delta activity), and brain regions (e.g., L-mPFC and R-mPFC) between groups (e.g., control vs. SPS & FS) were compared with the magnitude of habituation state by two-way repeated-measures ANOVA followed by post hoc Bonferroni t-tests. For the measurement of asymmetric activities, the magnitudes of delta activity between two hemispheres were compared between right and left brain regions by Student's t-tests. For the quantification of delta activity AUC between sham and SPS & FS + tDCS groups, data were compared by Student's t-tests. All data were analyzed with GraphPad Prism (GraphPad Software, San Diego, CA, USA). Values of $p < 0.05$ were considered statistically significant. The neural network heatmap was generated with MATLAB.

3. Results

3.1. SPS & FS animal model preparation and impaired fear memory extinction after SPS & FS exposure

To understand how PTSD arises from network dysregulation, we used the SPS & FS PTSD animal model (Fig. 1A). This model represents an enhanced SPS paradigm (Wang et al., 2008, 2015) that combines SPS stress and fear conditioning, which was described in section 2.2 "Preparation of SPS & FS animal model".

To investigate abnormal fears in the PTSD model, we performed a fear memory extinction assessment (Fig. 1B). PTSD model was compared with the conditioned fear model, specifically, after administration of SPS & FS, or FS solely, on day 0, rats were repeatedly returned to the shock cage once per day from day 1–10 for fear memory extinction. The fear level of the FS group was significantly higher (Time, $F_{11, 99} = 3.96$, $p < 0.0001$; Group, $F_{1, 9} = 3.13$, $p = 0.11$; Group x Time, $F_{11, 99} = 9.70$, $p < 0.0001$) than that of the SPS & FS group on day 1, but gradually decreased on the subsequent days. SPS & FS animals exhibited gradually increasing freezing levels after traumatic stress from day 1–3 and impaired fear extinction from day 6–10. For fear recall tests, the SPS & FS group continued to exhibit higher freezing levels than the FS group on day 15 (Time, $F_{11, 99} = 3.96$, $p < 0.0001$; Group, $F_{1, 9} = 3.13$, $p = 0.11$; Group x Time, $F_{11, 99} = 9.70$, $p < 0.05$) and day 40 (Time, $F_{11, 99} = 3.96$, $p < 0.0001$; Group, $F_{1, 9} = 3.13$, $p = 0.11$; Group x Time, $F_{11, 99} = 9.70$, $p < 0.05$). However, the FS group exhibited gradually decreasing freezing levels with continuous fear extinction.

3.2. Assessment of various behaviors in the PTSD model

To characterize behavioral responses after SPS & FS exposure, we performed behavioral tests that are considered representing the anxiety, depression, and anhedonia levels in rats on day 8–10 after traumatic stress exposure (Fig. 2A).

In the OF test (Fig. 2B), the representative traces of were shown in the left panel of Fig. 2B. Compared to the control group, both the FS ($F_{2, 14} = 13.40$, $p < 0.01$) and SPS & FS ($F_{2, 14} = 13.40$, $p < 0.001$) groups spent significantly less time in the central zone which represents higher anxiety-like levels in rats. However, no significant differences in locomotion between groups. In the EPM test (Fig. 2C), the representative traces of EPM were shown in the left panel of Fig. 2C, the SPS&FS group spent significantly more time in the closed arms ($F_{2, 15} = 6.32$, $p < 0.05$) and less time in the open arms ($F_{2, 15} = 4.95$, $p < 0.05$) compared to the FS group, and less time in the center ($F_{2, 15} = 4.20$, $p < 0.05$) compared to the control group. We found SPS & FS group exhibited significantly higher anxiety-like levels in both the OF and EPM tests. In contrast, the FS animals showed higher anxiety levels in the OF test but not in the EPM test.

Results from the FST (Fig. 2D) show that the SPS & FS animals exhibited higher depression-like levels with significant less climbing ($F_{2, 12} = 25.18$, $p < 0.0001$) and more swimming ($F_{2, 12} = 11.57$, $p < 0.01$) and immobility ($F_{2, 12} = 85.60$, $p < 0.0001$) after traumatic stress exposure. However, the FS group exhibited no significant differences in immobility compared to the control but did spend significantly more time swimming ($F_{2, 12} = 11.57$, $p < 0.01$) and less time climbing ($F_{2, 12} = 25.18$, $p < 0.01$).

Anhedonia-like levels were assessed with the SP test (Fig. 2E). The SPS & FS animals exhibited significantly less sucrose preference when compared to the control ($F_{2, 12} = 1.41$, $p < 0.0001$) and FS ($F_{2, 12} = 1.41$, $p < 0.0001$) groups, which is considered as higher anhedonia-like levels. However, there were no significant differences in total intake volume.

We also assessed the levels of plasma corticosterone, CRH, and ACT (Material and Methods A.1, Results B.1, and Fig. C1).

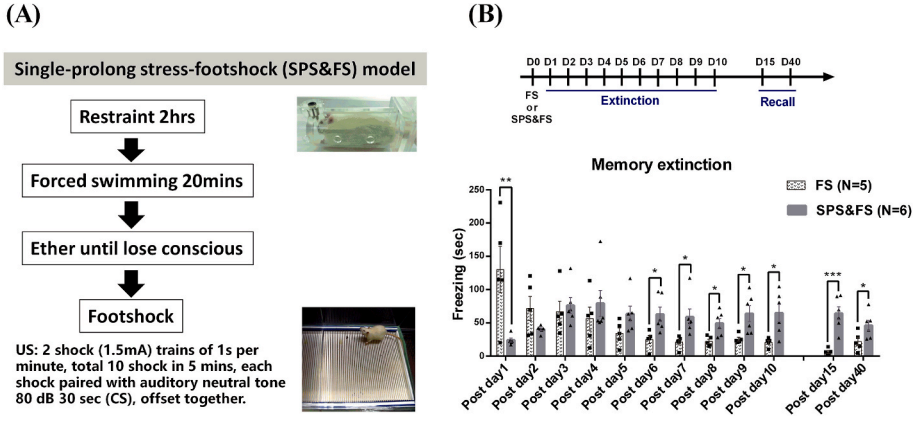
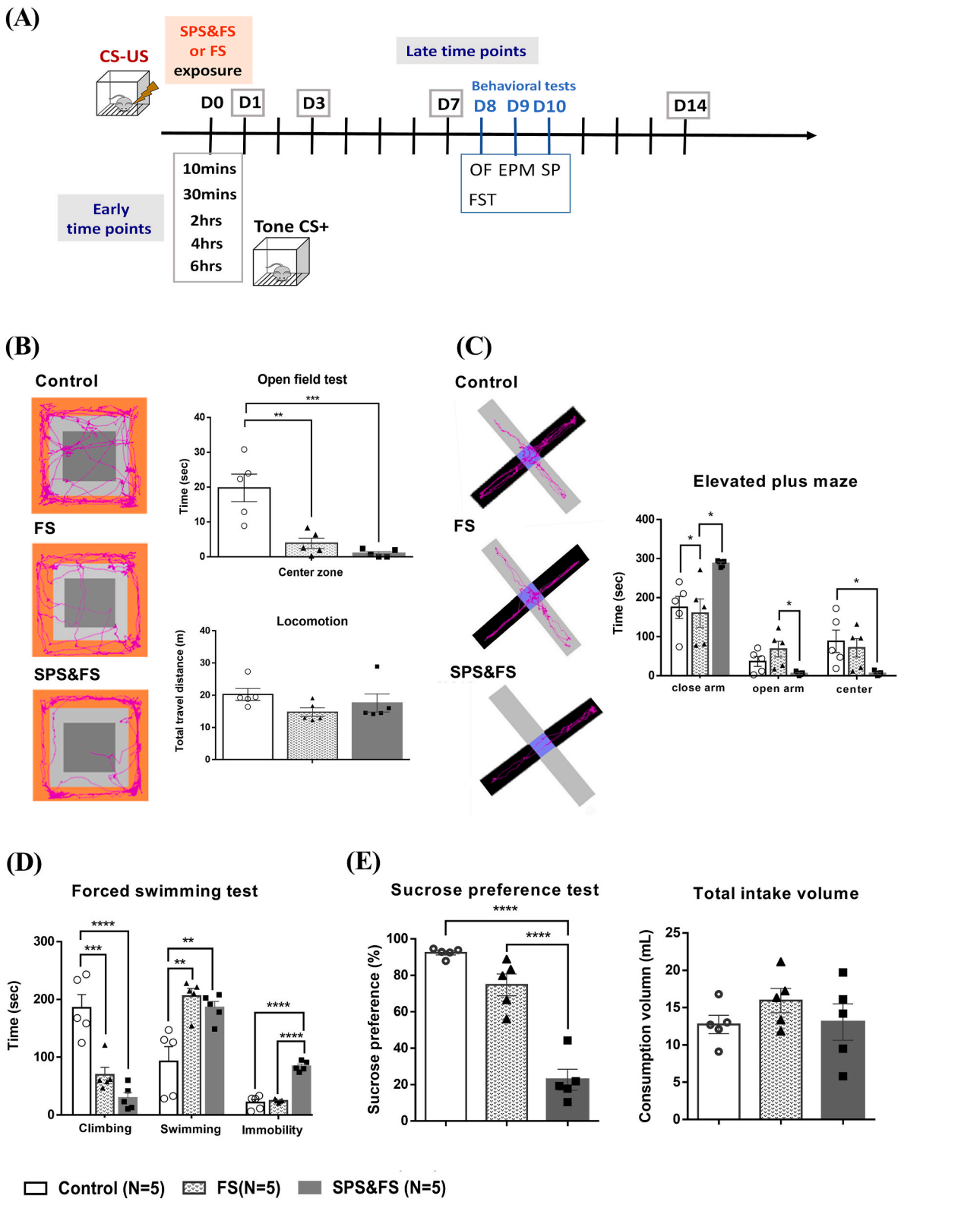


Fig. 1. Single-prolonged stress-footshock (SPS & FS) rat model and fear extinction assessment. (A) SPS & FS rat model. (B) Fear extinction assessment comparing the fear (n = 5) and SPS & FS models (n = 6). The fear level of the FS group was significantly higher (Time, $F_{11, 99} = 3.96$, $p < 0.0001$; Group, $F_{1, 9} = 3.13$, $p = 0.11$; Group x Time, $F_{11, 99} = 9.70$, $p < 0.0001$) than that of the SPS & FS group on day 1, but gradually decreased. For fear recall tests, SPS & FS animals exhibited impaired fear extinction on day 15 (Time, $F_{11, 99} = 3.96$, $p < 0.0001$; Group, $F_{1, 9} = 3.13$, $p = 0.11$; Group x Time, $F_{11, 99} = 9.70$, $p < 0.05$) and day 40 (Time, $F_{11, 99} = 3.96$, $p < 0.0001$; Group, $F_{1, 9} = 3.13$, $p = 0.11$; Group x Time, $F_{11, 99} = 9.70$, $p < 0.05$) after sequential context re-exposure. Values are mean ± SEM, $^{*}p < 0.05$, $^{***}p < 0.001$, two-way repeated-measures ANOVA.



(caption on next page)

Fig. 2. The study schematic and behavioral tests in the SPS & FS and fear models. (A) Study schematic for characterizing the temporal dynamics of brain wave activities and evaluating behavioral phenotypes after SPS & FS or FS exposure. (B) Open field (OF) test, the representative traces of test (Orange area: outer zone, light grey area: inner zone, grey area: center zone) were shown in the left panel and the right panel shows time spent in the center zone and total travel distance. Compared to the control group, both the FS ($F_{2, 14} = 13.40$, $p < 0.01$) and SPS & FS ($F_{2, 14} = 13.40$, $p < 0.001$) groups spent significantly less time in the central zone which represents higher anxiety-like levels in rats. However, no significant differences in locomotion between groups. (C) Elevated plus maze (EPM) test, the representative traces of EPM were shown in the left panel (Black area: close arm, grey area: open arm, purple area: center), amount of time spent in the open, close arms and center during the EPM test shown in the right panel. The SPS&FS group spent significantly more time in the closed arms ($F_{2, 15} = 6.32$, $p < 0.05$) and less time in the open arms ($F_{2, 15} = 4.95$, $p < 0.05$) compared to the FS group and less time in the center ($F_{2, 15} = 4.20$, $p < 0.05$) compared to the control group. (D) Forced swimming test (FST). The SPS & FS animals exhibited significantly less climbing ($F_{2, 12} = 25.18$, $p < 0.0001$) and more swimming ($F_{2, 12} = 11.57$, $p < 0.01$) and immobility ($F_{2, 12} = 85.60$, $p < 0.0001$), which is considered as higher depression-like levels. However, the FS group exhibited no significant differences in immobility compared to the control but did spend significantly more time swimming ($F_{2, 12} = 11.57$, $p < 0.01$) and less time climbing ($F_{2, 12} = 25.18$, $p < 0.01$). (E) Sucrose preference (SP) test and total intake volume. The SPS & FS animals exhibited significantly less sucrose preference when compared to the control ($F_{2, 12} = 1.41$, $p < 0.0001$) and FS ($F_{2, 12} = 1.41$, $p < 0.0001$) groups, which is considered as higher anhedonia-like levels. Control (n = 5), FS group (n = 5), and SPS & FS group (n = 6). Values are mean ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, one-way ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Temporal changes in brain wave features and freezing behavior after SPS & FS exposure

3.3.1. Temporal changes in freezing behavior

After animals received SPS & FS or FS on day 0, we returned them to the FS chamber and six CS^+ were delivered to remind the animals of conditioned fears. Time points for brain wave recording were divided into the early (10 and 30 min; 2, 4, and 6 h) and late (day 1, 3, 7, and 14) phases.

First, we assessed patterns of freezing behavior during re-exposure in the FS chamber with six CS^+. The SPS & FS group (Fig. 3A) generally exhibited higher freezing levels than the FS group during the early phase; these levels were significantly higher at the following time points: 10 min (Time, $F_{9, 72} = 6.41$, $p < 0.0001$; Group, $F_{1, 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9, 72} = 3.74$, $p < 0.0001$), 30 min (Time, $F_{9, 72} = 6.41$, $p < 0.0001$; Group, $F_{1, 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9, 72} = 3.74$, $p < 0.05$), 2 h (Time, $F_{9, 72} = 6.41$, $p < 0.0001$; Group, $F_{1, 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9, 72} = 3.74$, $p < 0.0001$), 4 h (Time, $F_{9, 72} = 6.41$, $p < 0.0001$; Group, $F_{1, 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9, 72} = 3.74$, $p < 0.0001$), and 6 h (Time, $F_{9, 72} = 6.41$, $p < 0.0001$; Group, $F_{1, 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9, 72} = 3.74$, $p < 0.0001$). Additionally, the freezing levels of the SPS & FS group were significantly higher than those of the FS group on day 3 (Time, $F_{9, 72} = 6.41$, $p < 0.0001$; Group, $F_{1, 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9, 72} = 3.74$, $p < 0.01$) and day 14 (Time, $F_{9, 72} = 6.41$, $p < 0.0001$; Group, $F_{1, 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9, 72} = 3.74$, $p < 0.0001$). However, there were no significant differences in the immobility of the control group across different time points compared to the habituation state (Fig. C2).

3.3.2. Characteristics of temporal brain wave activities

Bilateral LFP recording sites, including the mPFC, AMY, and vHipp, are shown in Fig. 3B. Representative raw traces recorded from the FS group are shown in Fig. 3C, the freezing episodes in the FS group correlated with a higher brain wave frequency (~4 Hz). In addition, representative raw traces from the SPS & FS group are shown in Fig. 3D, which show the freezing episodes after SPS & FS exposure correlated with a lower frequency (<4 Hz). When the temporal spectrograms of the FS and SPS & FS groups are compared (Fig. 3E), low-frequency correlations emerged 2 h after SPS & FS exposure and were maintained until the late phase (day 14). However, in the FS group, low-frequency correlations emerged immediately after FS exposure (10 min post-FS) and shifted to higher frequencies around 4–5 Hz 4 h after FS, and exhibited the strongest magnitudes during the late phase (day 1–3, 7, and 14). Thus, our results indicate distinct temporal spectrogram patterns between these two models.

Besides, we calculated the magnitudes of different frequency bands that correlated with freezing episodes during CS^+ in the two models. In the FS group (Fig. 4), the delta activities increased immediately after FS exposure (10 min post-FS) and gradually declined from 10 min to 6 h. Similar patterns were observed in the mPFC, AMY, and vHipp. However, in the SPS & FS group (Fig. 5), delta activities were first inhibited during the early phase (10 and 30 min post-SPS & FS) but started to predominate 2 h post-SPS & FS and were sustained until day 14, the delta magnitudes of the left-side mPFC show significantly inhibited while compared with the delta activities of habituation (Time, $F_{9, 160} = 2.50$, $p < 0.05$; Group, $F_{3, 160} = 4.85$, $p < 0.01$; Group x Time, $F_{27, 160} = 0.43$, $p < 0.05$). Additionally, the other frequency bands (theta and alpha) in the FS group were first inhibited at the early time point (10 min post-FS) and gradually increased from 30 min until the late phase. The higher frequency bands (beta, high-beta, and gamma) in the FS group gradually increased from the early to the late phase (Fig. C3). However, the higher frequency bands in the SPS & FS group were maintained at low levels in all recording sites across different time points (Fig. C4).

In addition, we correlated brain activities with fear expression levels during CS^+ to understand time-dependent inter-regional changes and progressive network development after SPS & FS exposure (Table C.1 and Fig. C5). Delta activities were highly correlated with freezing behavior at the beginning of the assessment (10 min post-SPS & FS), but the correlation shifted to higher frequency activities (high-beta and gamma) 30 min post-SPS & FS. The correlation then shifted back to delta activity 2 h after traumatic stress exposure and was maintained until day 14.

3.3.3. Temporal changes in regional correlations and asymmetric delta activity

The regional correlations between different brain areas at 2 h are shown in Fig. 6. The results and correlation patterns for other time points (4, 6 h, day 1, 3, 7 and 14) were concluded in Appendix section B.2 and Fig. C6. At the time point of 2 h post-exposure (Fig. 6), we found highly regional synchronized activities among all frequency bands in the SPS & FS group, especially the regional synchronization in delta activities, in comparison, the control and FS groups show relatively less synchronized in delta and also lower regional correlation in higher frequency bands (theta-gamma), the correlation pattern of the SPS & FS group differs from the control and FS groups.

In addition, when compared with the FS group (Fig. 7A), we observed stronger asymmetric delta activity in the right mPFC ($t = 2.31$, $p < 0.05$) of the PTSD group on day 14 (Fig. 7B). However, other frequency bands such as alpha activities showed no significant differences. Besides, we also observed the temporal patterns in c-Fos expression post-SPS & FS exposure (Material and Methods A.3, Results B.3, and Fig. C7). These results indicate the temporal differences in the effects of traumatic stress.

3.4. PTSD maladaptive symptoms were improved by tDCS

3.4.1. Fear levels and brain wave features after tDCS application in SPS & FS animals

Before applying tDCS in the PTSD model, we evaluated (1) the

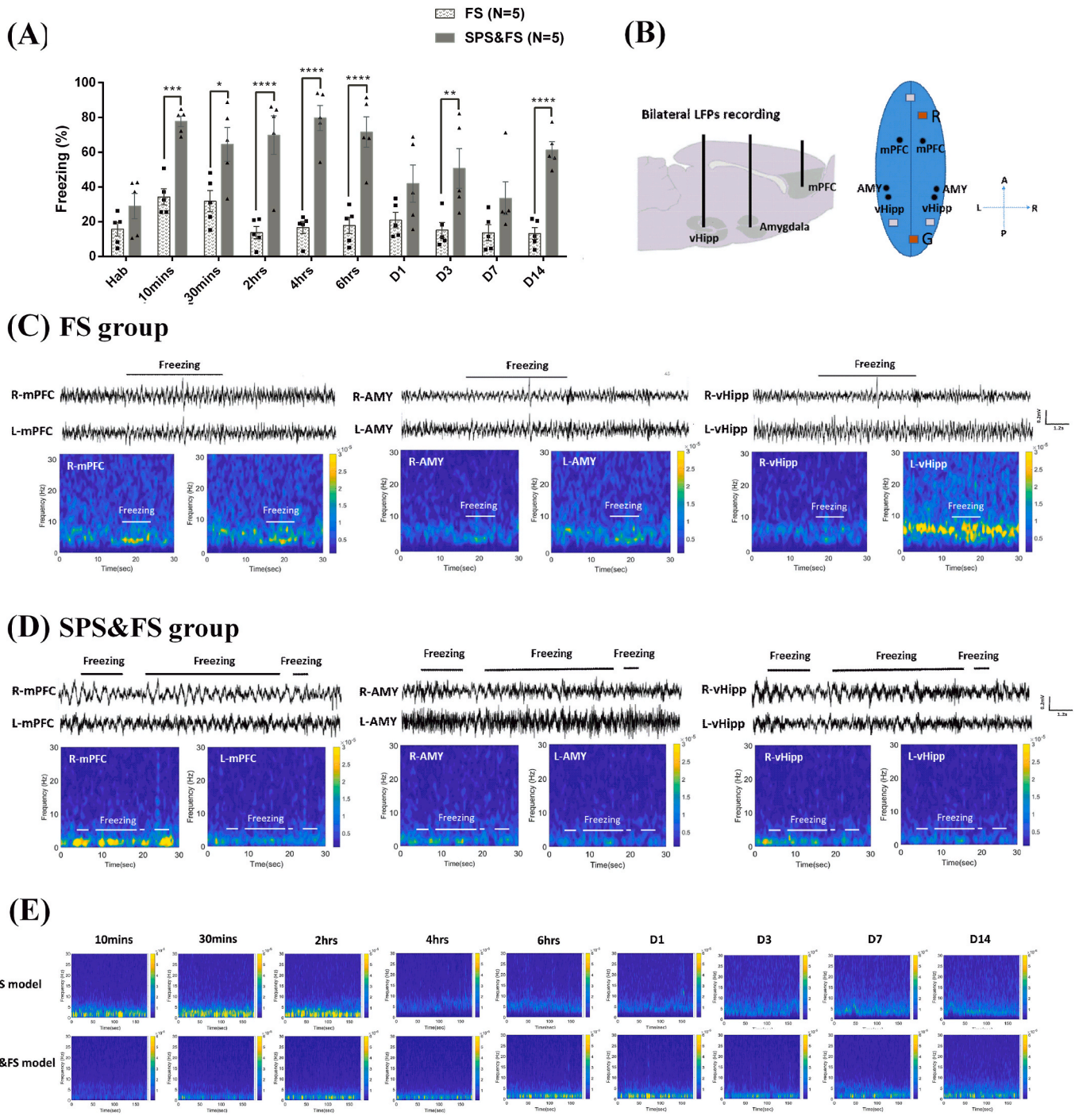


Fig. 3. Temporal changes in freezing levels and representative LFP activities in the FS and SPS & FS groups. (A) Temporal changes in freezing levels in the FS and SPS & FS groups. The SPS & FS group generally exhibited higher freezing levels than the FS group during the early phase; these levels were significantly higher at the following time points: 10 min (Time, $F_{9,\ 72} = 6.41$, $p < 0.0001$; Group, $F_{1,\ 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9,\ 72} = 3.74$, $p < 0.0001$), 30 min (Time, $F_{9,\ 72} = 6.41$, $p < 0.0001$; Group, $F_{1,\ 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9,\ 72} = 3.74$, $p < 0.05$), 2 h (Time, $F_{9,\ 72} = 6.41$, $p < 0.0001$; Group, $F_{1,\ 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9,\ 72} = 3.74$, $p < 0.0001$), 4 h (Time, $F_{9,\ 72} = 6.41$, $p < 0.0001$; Group, $F_{1,\ 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9,\ 72} = 3.74$, $p < 0.0001$), and 6 h (Time, $F_{9,\ 72} = 6.41$, $p < 0.0001$; Group, $F_{1,\ 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9,\ 72} = 3.74$, $p < 0.0001$). Additionally, the freezing levels of the SPS & FS group were significantly higher than those of the FS group on day 3 (Time, $F_{9,\ 72} = 6.41$, $p < 0.0001$; Group, $F_{1,\ 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9,\ 72} = 3.74$, $p < 0.01$) and day 14 (Time, $F_{9,\ 72} = 6.41$, $p < 0.0001$; Group, $F_{1,\ 8} = 56.17$, $p < 0.0001$; Group x Time, $F_{9,\ 72} = 3.74$, $p < 0.0001$). Values are mean ± SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ (n = 5 per group), two-way repeated-measures ANOVA. (B) The bilateral positions of recording sites, including the bilateral mPFC, AMY, and vHipp. Coordinates are specified in section 2.5.1 of the **Materials and Methods** section. Representative 30-sec low-frequency raw LFP traces were recorded from the bilateral mPFC, AMY, and vHipp during freezing behavior from (C) the FS group and (D) the SPS&FS groups. (E) Representative 3-min spectrograms of LFP activities were recorded from the right mPFC of FS and SPS & FS animals. Time points included early (10 and 30 min, and 2, 4, and 6 h) and late time points (day 1, 3, 7, and 14).

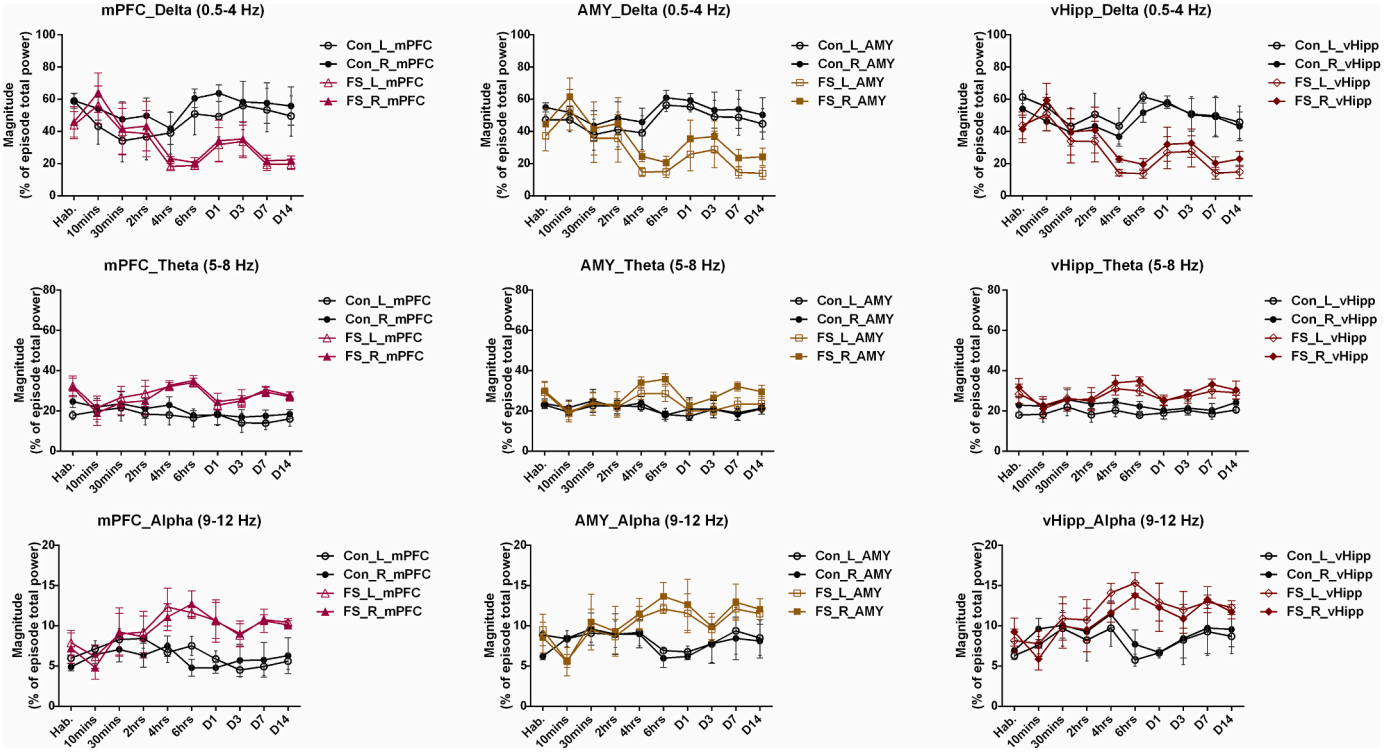


Fig. 4. The temporal dynamics of LFPs (delta, theta, and alpha activities) during freezing episodes during CS + exposure in the FS group. The mean power magnitude of freezing episodes was quantified across different time points in the FS group. In the FS group, the delta activities increased immediately after FS exposure (10 min post-FS) and gradually declined from 10 min to 6 h. Similar patterns were observed in the mPFC, AMY, and vHipp. Pink line: mPFC activity in the FS group; golden line: AMY activity in the FS group; brown line: vHipp activity in the FS group; black line: control group. [R]: right hemisphere; [L]: left hemisphere. Values are mean ± SEM, (n = 5 per group), two-way repeated-measures ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

modulational effects of cathodal and anodal tDCS on evoked LFPs (Material and Methods A.4, Results B.4 and 5, and Fig. C8 and 9) (2) the effective electric field of tDCS (Material and Methods A.5, Results B.6, and Fig. C10). After confirming these two parameters, we hypothesized that the excitatory effects of 0.2 mA cathodal tDCS can enhance LFPs and suppress low-frequency correlates. Hence, on day 0, we applied 0.2 mA cathodal tDCS for 30 min immediately after SPS & FS exposure, and then again once per day from day 0–7 (Fig. 8A), brain wave activities were recorded on day 1, 3, 7, and 14.

When compared with the sham group (Fig. 8B), the representative mPFC spectrogram of SPS & FS + tDCS group (Fig. 8C) showed reduced low-frequency correlates on day 1, 3, 7, and 14 after cathodal tDCS application. Additionally, the LFPs power spectra of mPFC showed reductions in delta activities. Thus, we found that the application of cathodal tDCS from day 0–7 could effectively reduce the magnitudes of low-frequency correlates. Besides, we also assessed the average delta activity AUC for six CS^+ in the sham and SPS & FS + tDCS groups. Significantly reduced delta activity AUCs were observed in the SPS & FS + tDCS group on day 3 ($t = 3.55$, $p < 0.01$), day 7 ($t = 5.64$, $p < 0.001$), and day 14 ($t = 6.35$, $p < 0.05$) (Fig. 8D).

3.4.2. PTSD maladaptive symptoms were alleviated by tDCS

After the sequential tDCS cathodal application from day 0–7, we compared fear levels during the re-exposure in the FS chamber with CS^+ reminders (Fig. 9A). The SPS & FS + tDCS group showed significant reductions in freezing levels on day 1 ($F_{1, 12} = 15.23$, $p < 0.01$), day 3 ($F_{1, 12} = 15.23$, $p < 0.01$), and day 14 ($F_{1, 12} = 15.23$, $p < 0.01$). However, due to a decline in the freezing levels of the sham group on day 7, there were no significant differences between the sham and SPS & FS + tDCS groups at this time point. However, the freezing levels of SPS & FS + tDCS animals remained low (~10%).

PTSD phenotypes were evaluated by behavioral tests after tDCS application. In the OF test Fig. 9B, the representative traces are shown the left panel of Fig. 9B, the SPS & FS + tDCS group spent significantly more time in the central zone than the sham group ($t = 2.97$, $p < 0.05$), which was considered as reduced anxiety-like levels in rats. However, no significant differences in locomotion between the two groups. In the EPM test (Fig. 9C), the representative traces are shown the left panel of Fig. 9C, we found that the SPS & FS + tDCS group spent significantly less time in the closed arms than the sham group ($t = 2.25$, $p < 0.05$), but no significant differences were observed in the open arms and center. These lines of evidence indicate that after stimulation with 0.2 mA tDCS, the SPS & FS + tDCS group exhibited significantly lower anxiety-like levels. The FST results (Fig. 9D) show that the SPS & FS + tDCS animals exhibited lower depression-like levels, with significantly less immobility ($t = 2.67$, $p < 0.05$), and no significant changes in climbing or swimming. Furthermore, after tDCS application, we found that SPS & FS + tDCS animals demonstrated significantly reduced anhedonia-like levels ($t = 2.63$, $p < 0.05$) with increased preferences for sucrose (Fig. 9E). However, no significant differences in total intake volume were observed between the sham and SPS & FS + tDCS groups. Thus, the application of 0.2 mA tDCS could alleviate the various behaviors associated with PTSD, including fear, anxiety-like, depression-like, and anhedonia-like phenotypes.

A schematic (Fig. 10) summarizes the temporal changes that occurred after traumatic stress exposure. With exposure to extreme stress, SPS & FS caused changes in brain wave activity and temporal dynamics. These changes contributed to the development of fear after traumatic stress exposure. Additional comorbid symptoms of PTSD, including anxiety, depression, and anhedonia, were observed in the SPS & FS model and were distinguishable from those in the FS model. Furthermore, by using tDCS to target the frontal region, these symptoms

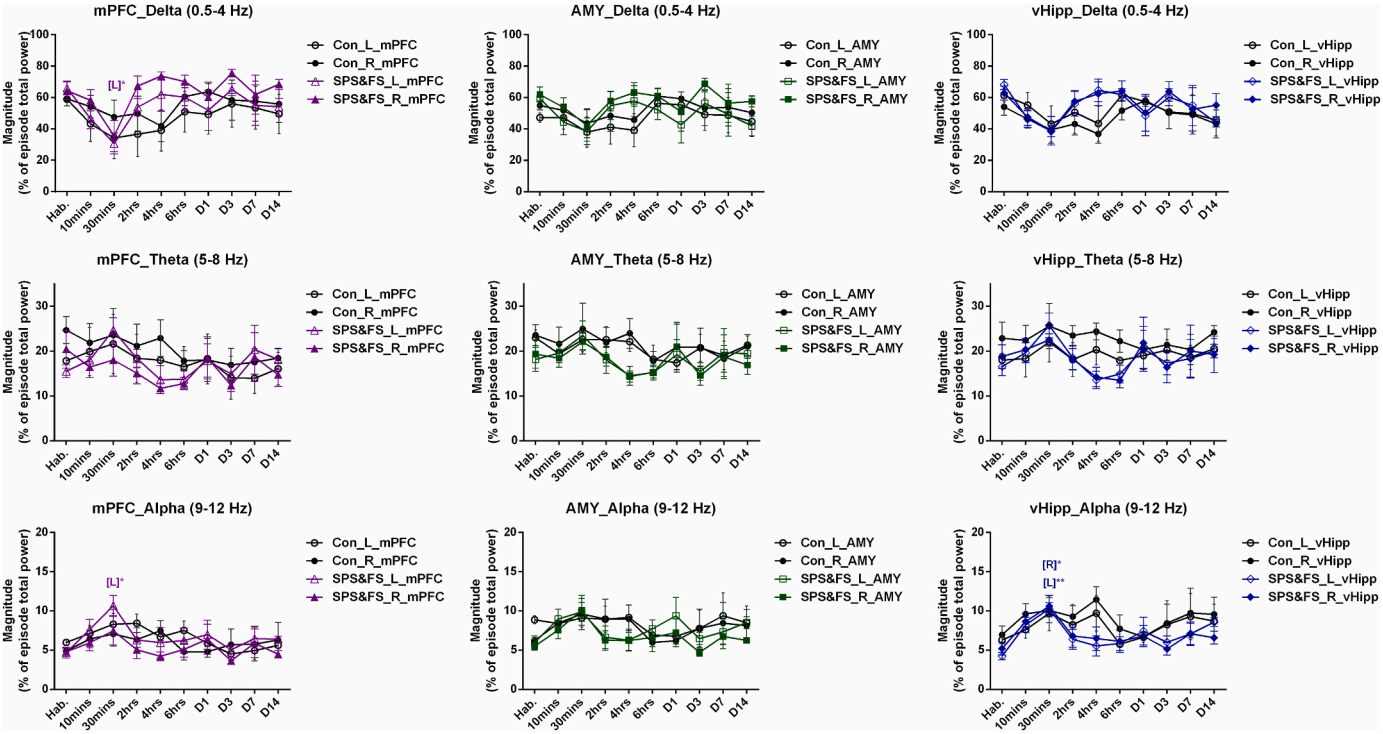


Fig. 5. The temporal dynamics of LFPs (delta, theta, and alpha activities) during freezing episodes during CS^{+} in the SPS & FS group. The mean power magnitude of freezing episodes was quantified across different time points in the SPS & FS group. In the SPS & FS group, delta activities were first inhibited during the early phase (10 and 30 min post-SPS & FS) but started to predominate 2 h post-SPS & FS and were sustained until day 14, the delta magnitudes of the left-side mPFC show significantly inhibited while compared with the delta activities of habituation (Time, $F_{9,\ 160} = 2.50$, $p < 0.05$; Group, $F_{3,\ 160} = 4.85$, $p < 0.01$; Group x Time, $F_{27,\ 160} = 0.43$, $p < 0.05$). Purple line: mPFC activity in the SPS & FS group; green line: AMY activity in the SPS & FS group; blue line: vHipp activity in the SPS & FS group; black line: control group. [R]: right hemisphere; [L]: left hemisphere. Values are mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$ (n = 5 per group), two-way repeated-measures ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

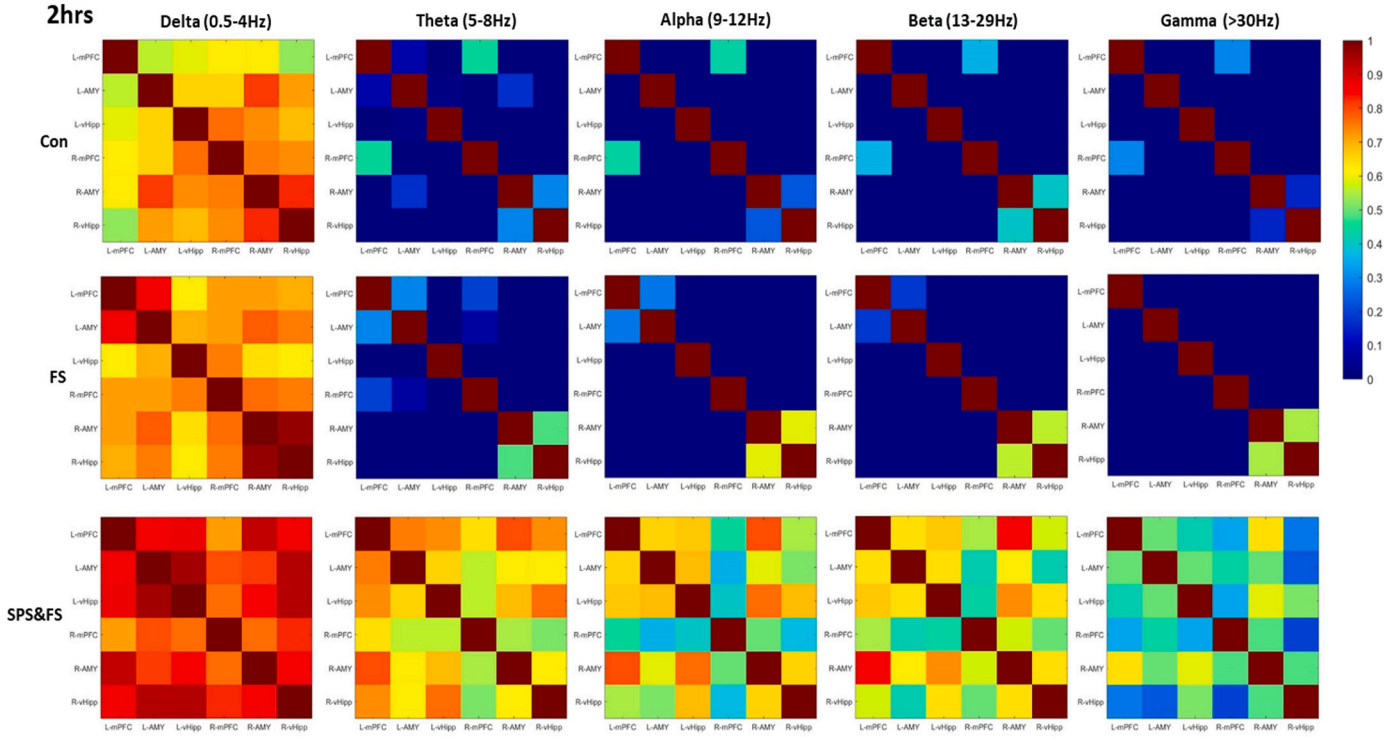


Fig. 6. Temporal changes in regional correlations 2 h post-exposure. Two hrs post-SPS & FS, the regional correlations between the bilateral mPFC, AMY, and vHipp which were calculated in delta, theta alpha, beta, and gamma activities (n = 5 per group). Delta activities across bilateral mPFC, AMY, and vHipp were highly correlated with freezing behavior at the time point 2 h after traumatic stress exposure.

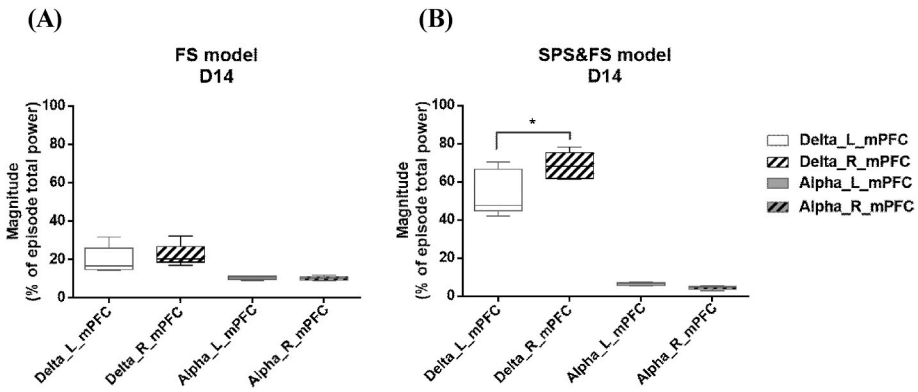


Fig. 7. The SPS & FS model shows stronger asymmetric delta activity in the right frontal region. (A) Delta and alpha magnitudes of the left and right mPFCs on day 14 in the FS group show no asymmetric activity. (B) Asymmetric delta activity in the right frontal region in the SPS & FS group on day 14. When compared with the FS group, the SPS&FS group shows stronger asymmetric delta activity in the right mPFC ($t = 2.31$, $p < 0.05$) on day 14. R: right hemisphere; L: left hemisphere. Values are mean ± SEM, $^*p < 0.05$ (n = 5 per group), Student's t-tests.

were alleviated in the SPS & FS model.

4. Discussion

PTSD is a complex syndrome that may occur after extreme traumatic stress exposure. Several studies characterizing physiological, emotional, and cognitive dysregulations have revealed the heterogeneity of PTSD. Besides, fear memory abnormalities may play a vital role in the pathogenesis of PTSD. In the present study, we utilized the SPS & FS PTSD animal model to investigate temporal brain activity and PTSD-like symptoms. We observed impaired fear memory extinction and increased freezing levels in the SPS & FS group, while the FS group exhibited decreased freezing levels with fear extinction. In addition, rats in the SPS & FS group exhibited high anxiety, depression, and anhedonia levels and dysregulated HPA axis function. Our findings indicate that the SPS & FS animals exhibited dysregulation of the HPA axis, with low levels of corticosterone and high levels of CRH and ACTH, which is consistent with previous research on the blunted cortisol response in individuals with PTSD (Yehuda, 2002). However, the FS model did not exhibit any HPA axis dysregulation.

Brain wave recordings revealed that SPS & FS exposure increased delta waves from the time point of 2 h during the early phase, which correlated with freezing levels and was maintained to the late phase on day 14. Furthermore, the application of tDCS caused a reduction in delta activity and helped alleviate PTSD-like phenotypes in the SPS & FS group. Overall, our findings suggest that SPS & FS exposure induces temporal changes in brain activity and PTSD-like symptoms that can be modulated by tDCS.

In previous studies, dysrhythmia has been identified in PTSD patients, with some studies revealing increased theta activity in the central regions of the brain, as well as increased beta activity in the frontal, central, and left occipital regions (Begic et al., 2001). Increased frontal beta activity powers have also been observed following complex trauma (Jang et al., 2017). In contrast, other studies of PTSD subjects have reported decreased delta, theta, and low-beta frequency bands (Shim et al., 2017), reduced alpha activity power (Sheridan et al., 2012), and decreased alpha and increased beta activity powers in the frontal and central regions (Jokic-Begic and Begic, 2003). The heterogeneous rhythm dysregulation observed in PTSD patients may imply the existence of progressive pathological stages in this disorder.

Our study revealed that frontal delta activity was significantly reduced in the early phases after traumatic stress exposure (30 min post-SPS & FS), started to increase after 2 h, and was sustained until day 14. The magnitudes of these constant, low-frequency delta activities correlated with fear expression levels. Specifically, analysis of LFPs in the PTSD animal model revealed the existence of temporal, inter-regional changes, and the development of a progressive network from early to late time points. Hence, the conflicting EEG results observed in previous studies (Begic et al., 2001; Jang et al., 2017; Jokic-Begic and Begic, 2003; Sheridan et al., 2012; Shim et al., 2017) may be due to the progression of PTSD. Identifying and clarifying temporal brain wave features in the clinic may help differentiate the pathological stages of PTSD.

The biology of stress-induced longitudinal memory encoding proposed by Diamond et al. (2007), as well as by the staging model of PTSD (McFarlane et al., 2017), may provide insights into establishing a model of longitudinal PTSD in the clinic. The stress-induced changes in the spatial and temporal dynamics of LFPs, which may be correlated with a previously proposed temporal dynamics model (Diamond et al., 2007), include an enhancement of LTP in the Hipp that lasts for seconds to minutes after stress (Ahmed et al., 2006), and involves the activation of AMPA and NMDA receptors and glutamatergic transmission (Kole et al., 2002; Venero and Borrell, 1999). Stress exposure may lead to an increase in glucocorticoid levels within minutes (Wiegert et al., 2006); however, in the hours to days after stress exposure, there may be desensitization of calcium-induced NMDA receptors in the Hipp (Nakamichi and Yoneda, 2005). Furthermore, the temporal dynamics model proposed by Diamond et al. (2007) suggests inhibition of the PFC; however, the AMY has a similar pattern as the Hipp. In the clinical staging model (McFarlane et al., 2017), it was proposed that there are mainly two categorizes in the development and maintenance of PTSD symptoms: early stages of developing disorder and clinical disorder stages. Early stages of developing disorder comprise individuals who have an increased risk of developing symptoms following trauma exposure, with stage 1a and 1b representing different levels of symptom severity. It is essential to identify both risk and protective factors that influence symptom progression at this stage. The clinical disorder stages comprise individuals who meet the diagnostic criteria for PTSD, with stage 2 and 3 representing acute and persistent symptoms, respectively. Symptoms may fluctuate depending on ongoing stressors, and comorbidities such as major depressive disorder and substance abuse in this stage. In stage 4, this stage is a prolonged period, increasing the likelihood of an unremitting illness with increasing chronicity. The findings of our study can be possibly fitted into this stage model, in our study, SPS & FS exposure can trigger the stage as the preclinical stage of PTSD indicated in the staging model, with subclinical symptoms present during the acute phase (stage 1). Dysfunction of the HPA axis and comorbid PTSD-like phenotypes were observed on day 7, which may align with stage 3, a stage with long-standing symptoms. However, the persistence of fear during fear recall tests on day 15 and day 40 may align with the chronic phase in stage 4 of the staging model. In turn, the establishment of such a staging model may help provide more precise treatments and a time-dependent basis for this complex disease. In addition, it's important to note that when interpreting the results of the animal study in the context of the proposed stage model for PTSD, it is crucial to acknowledge that the animal model has limitations in reproducing the complete spectrum of symptoms experienced by patients in clinical settings. Moreover, the study mainly investigated the effects of SPS & FS exposure in animal models, which offers valuable knowledge

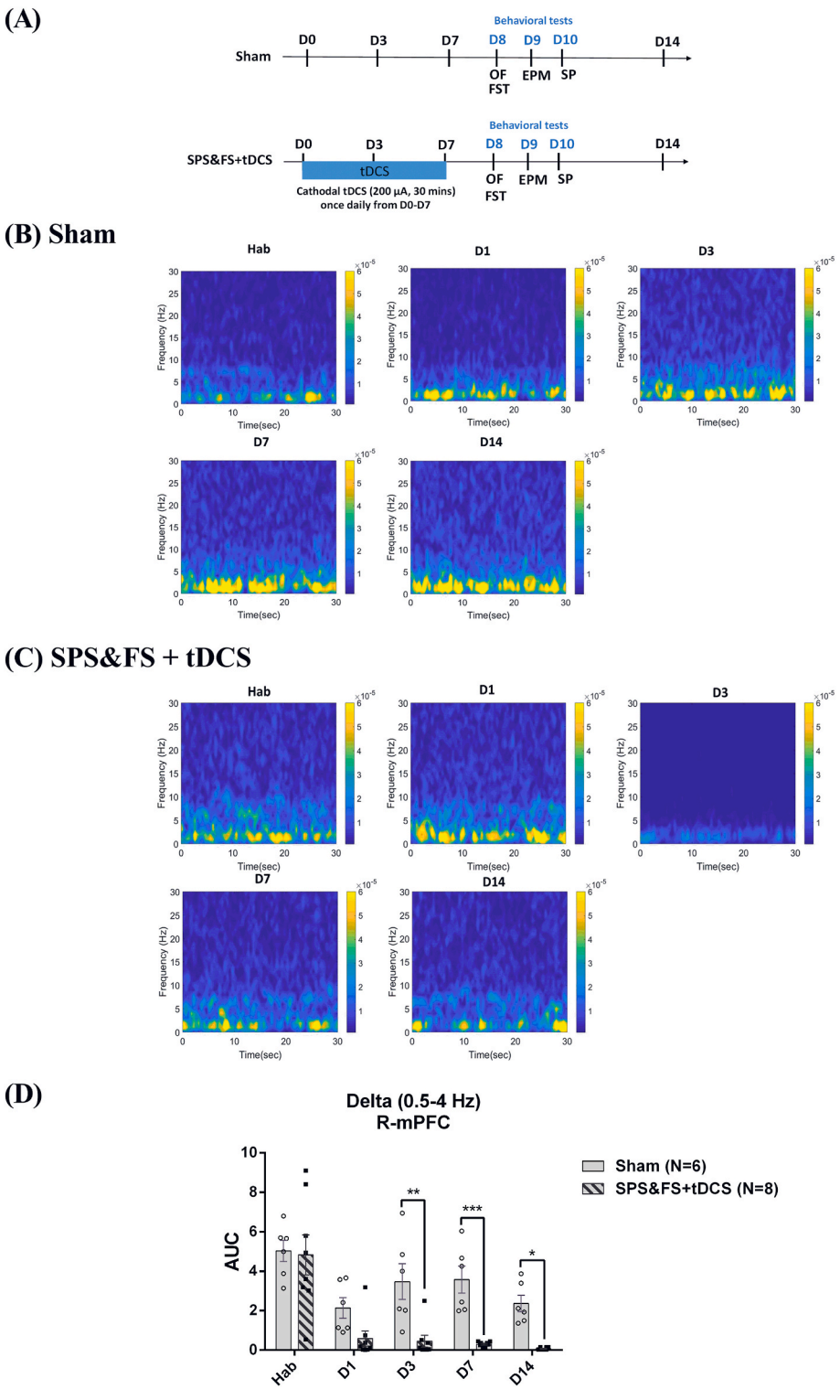


Fig. 8. The application of tDCS modulation from day 0–7 once daily after SPS & FS exposure. (A) The study schematic for tDCS modulation from day 0–7 after SPS & FS exposure. Behavioral phenotypes were evaluated on day 8, 9, and 10 post-SPS & FS exposure. (B, C) Representative spectrograms of LFP activities were recorded from the right mPFC in (B) the sham group and (C) the SPS & FS + tDCS group on day 1, 3, 7, and 14. (D) Quantification of delta activity (0.5–4 Hz) area under the curve (AUC) during 30-sec CS^+ exposures. The application of cathodal tDCS from days 0–7 could effectively reduce the magnitudes of low-frequency correlates. Besides, we also assessed the average delta activity AUC for six CS^+ in the sham and SPS & FS + tDCS groups. Significantly reduced delta activity AUCs were observed in the SPS & FS + tDCS group on day 3 ($t = 3.55$, $p < 0.01$), day 7 ($t = 5.64$, $p < 0.001$), and day 14 ($t = 6.35$, $p < 0.05$). Values are mean ± SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Sham group n = 6, SPS & FS + tDCS group n = 8), Student's t-tests.

into the underlying biological mechanisms and potential interventions. However, in clinical settings, PTSD can arise from various traumatic experiences. Although the proposed stage model provides a theoretical framework, it is important to emphasize that additional research is required to validate and provide potential guidance for the treatment of this disorder.

In this study, we also compared temporal brain wave alternations in both the FS and SPS & FS models. Analysis of the resulting spectrograms revealed that the temporal patterns of the FS model are different from those of the SPS & FS model, potentially implying that the dynamics of fear development are different in the setting of general fears vs. psychological trauma-related fears.

Currently, FDA-approved treatments for PTSD mainly target the serotonin system; they include the two selective serotonin reuptake inhibitors sertraline and paroxetine, however, 30% of patients are treatment-resistant (McFarlane et al., 2017; Murphy and Smith, 2018). In the present study, we considered that tDCS may have utility for modulating traumatic stress exposure-associated dysrhythmia in PTSD.

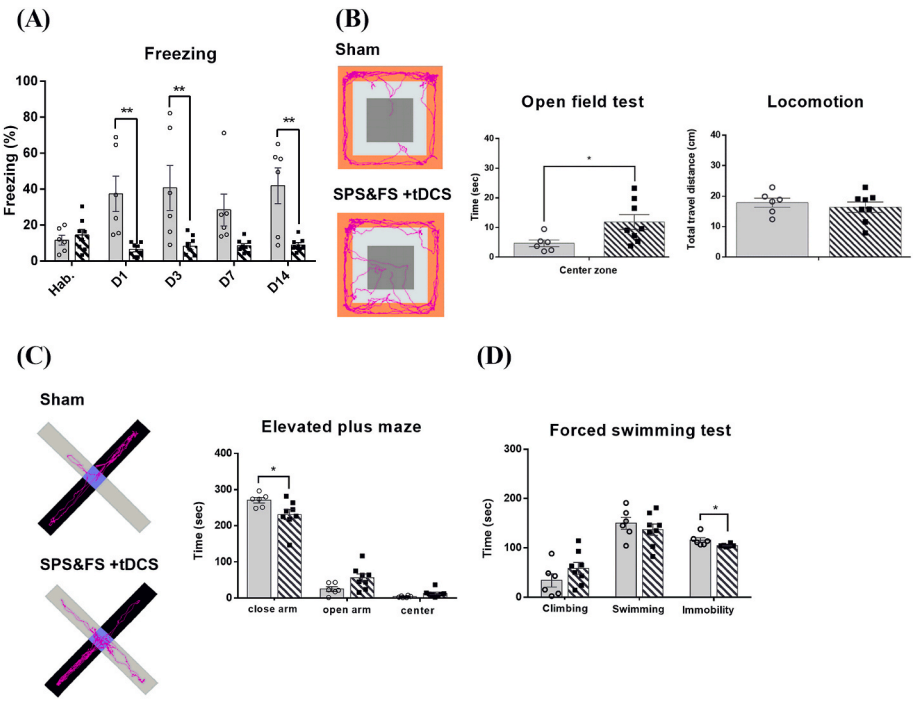


Fig. 9. tDCS modulation from day 0–7 after SPS & FS exposure helps alleviate various PTSD behavioral phenotypes. (A) Freezing levels were reduced in the SPS & FS + tDCS group on D1 ($F_{1, 12} = 15.23$, $p < 0.01$), D3 ($F_{1, 12} = 15.23$, $p < 0.01$), and D14 ($F_{1, 12} = 15.23$, $p < 0.01$) compared to the sham group. (B) Open field (OF) test, the representative traces of test (Orange area: outer zone, light grey area: inner zone, grey area: center zone) were shown in the left panel and the right panel shows time spent in the center zone and total travel distance. The SPS & FS + tDCS group spent significantly more time in the central zone than the sham group ($t = 2.97$, $p < 0.05$), which was considered as reduced anxiety-like levels in rats. However, no significant differences in locomotion between the two groups. (C) Elevated plus maze (EPM) test, the representative traces of EPM were shown in the left panel (Black area: close arm, grey area: open arm, purple area: center), amount of time spent in the open, close arms and center during the EPM test shown in the right panel. The SPS & FS + tDCS group spent significantly less time in the closed arms than the sham group ($t = 2.25$, $p < 0.05$), which represents significantly lower anxiety-like levels in the model, but no significant differences were observed in the open arms and center. (D) Forced swimming test (FST). The SPS & FS + tDCS animals exhibited lower depression-like levels, with significantly less immobility ($t = 2.67$, $p < 0.05$), and no significant changes in climbing or swimming. (E) Sucrose preference (SP) test and total intake volume. The SPS & FS + tDCS animals demonstrated significantly reduced anhedonia-like levels ($t = 2.63$, $p < 0.05$) with increased preferences for sucrose. However, no significant differences in total intake volume were observed between the sham and SPS & FS + tDCS groups. Values are mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ (Sham group n = 6, SPS & FS + tDCS group n = 8), one-way ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

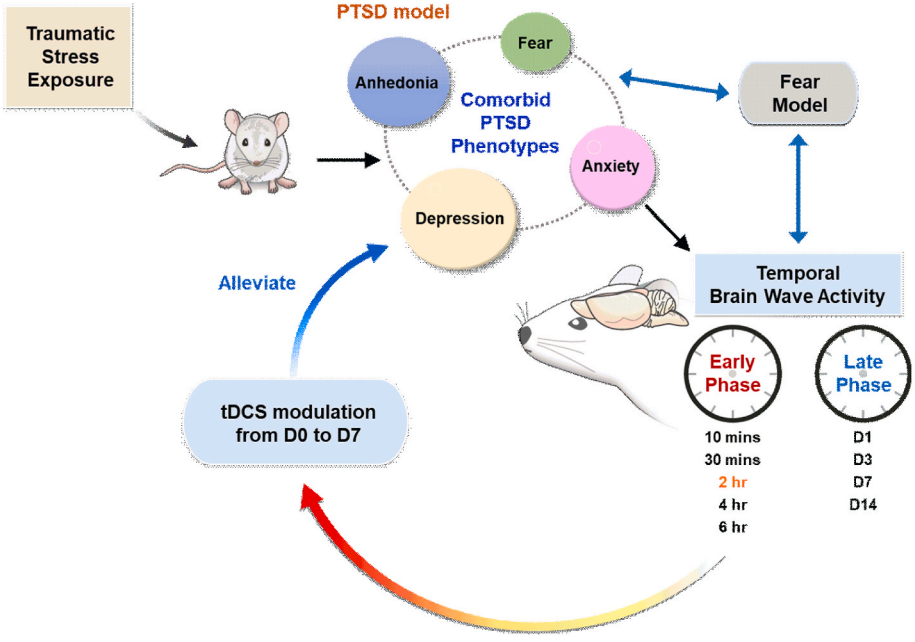


Fig. 10. Schematic summarizing the temporal changes that occurred after traumatic stress exposure. Exposure to extreme stress in the form of SPS & FS leads to changes in brain wave activity and contributes to the development of fear after traumatic stress exposure. Delta activity was enhanced 2 h after SPS & FS exposure and maintained until D14. Other co-morbid symptoms of PTSD, such as anxiety, depression, and anhedonia, were observed in the SPS & FS model and distinguishable from the FS model. tDCS modulation helped alleviate fear levels and other behaviors.

Our results indicate that tDCS application could effectively reduce low-frequency correlates associated with fear expression and the levels of anxiety-, depression-, and anhedonia-like phenotypes after traumatic stress exposure. These modulatory effects may be explained by the induction of synaptic plasticity and modulation of N-methyl-D-aspartic acid (NMDA) receptors (Nitsche et al., 2003). At the level of gene transcription, the induction of LFPs by tDCS modulation is associated with the expression of Zif268 (zinc finger protein 225) and c-Fos (Kimoto et al., 2014; Vaisanen et al., 2004); the activation of Zif268 is particularly critical for the induction and maintenance of LTPs (Ranieri et al., 2012). Moreover, the expression of neuroplasticity-related genes such as brain-derived neurotrophic factor and catechol-O-methyltransferase has been directly linked to the neuroplasticity induced by tDCS (Ira et al., 2013; Nieto et al., 2013; Wiegand et al., 2016). Besides, the application of tDCS has been shown to improve PTSD-associated behaviors such as impaired fear extinction, poor working memory, and excessive vigilance (Abend et al., 2016; Fregni and Pascual-Leone, 2007; Ironside et al., 2016). Furthermore, tDCS has no serious adverse effects, low cost and is easier to handle (D'Urso et al., 2018). For disorders charactered with dysrhythmia such as PTSD, the availability of non-invasive neurostimulation could be considered as a promising alternative for treatments.

5. Conclusion

In conclusion, our results indicate the time-dependent development of delta activity that they emerged 2 h after traumatic stress exposure and lasted until day 14. Additionally, the correlation between the magnitudes of different bandwidth activities and fear expression levels shifted in a stage-dependent manner. Moreover, these temporal dynamics are different between the FS and SPS & FS models, as evidenced by a fear memory extinction test and correlation between brain wave features and freezing levels. Based on these lines of evidence, traumatic stress-induced fears are different from the fears in the fear model. In the present study, we found that after traumatic stress exposure, the longitudinal dynamics of abnormal fears in PTSD animal models can be reflected by LFP measurements and can be used to distinguish between early and late phases. Moreover, modulation of tDCS caused a reduction in delta activity and helped alleviate PTSD-like phenotypes. The findings suggest that obtaining brain wave measurements could be an effective method for monitoring the pathological fear progression in PTSD models and could also be used to distinguish the pathological state of PTSD from the nonpathological state of learned fears. Furthermore, the modulation of tDCS could be considered a promising intervention strategy.

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CRediT authorship contribution statement

Shao-Han Chang: Writing – original draft, Data collection, Formal analysis, and, Conceptualization. **Huan-Yuan Chen:** Technical support. **Fu-Zen Shaw:** Conceptualization, Validation, and, Supervision. **Bai-Chuang Shyu:** Conceptualization, Validation, and, Supervision.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2023.100554.

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