Acute and chronic pain affects local field potential of the medial prefrontal cortex in different band neural oscillations

Bo Fu[1], Shao-nan Wen[1], Bin Wang[1], Kun Wang[2], Ji-yan Zhang[3], and Shao-jun Liu[1]

Abstract

The medial prefrontal cortex is involved in the process of sensory discrimination. In this study, we examined the local field potential activity response to the different stages of pain in the prelimbic cortex (PrL) which is a sub-region of the medial prefrontal cortex. Recent studies revealed extensive information about neural oscillations, but there is limited information on the local field potential profiles for acute or chronic pain, particularly in freely moving animals. This study showed that acute mechanical pain increases alpha oscillation and decreases beta and gamma oscillations before spared nerve injury surgery. Delta oscillation was decreased by chronic pain and gamma oscillation varied with time. However, acute mechanical pain stimulus had no effects on local field potential in rats under mechanical allodynia. Together, our findings provide novel insights into the role of medial prefrontal cortex local field potential activity response to pain stimulus.

Keywords

Pain, medial prefrontal cortex, local field potential

Introduction

Acute and chronic pain causes different changes in physiological and psychological processes. During acute pain, the variety of motivation, cognition, or emotion is adapted to block future damage. However, mood disorders or other decreased motivational symptoms arise during chronic pain.[1] Previous studies showed that neuropathic pain caused by peripheral nerve injury leads to maladaptive changes in brain structures[2,3] like the medial prefrontal cortex (mPFC), and functional abnormalities appear in the mPFC in chronic pain animal model.[4] Moreover, the mPFC exhibits hyperactivity after acute and chronic pain stimuli.[5]

Accordingly, some case studies reported that a neuropathic pain model (spared nerve injury, SNI) could induce anxiety and depression-like behaviors at 14 days after surgery.[6,7] The mPFC activity is decreased by the progression of pain from acute to chronic stage.[8] However, the underlying mechanisms are poorly understood, especially in neural electrophysiology like local field potential (LFP). The LFP is classified into different frequency ranges and is linked to cognitive functions.[9] It reflects low-frequency extracellular activity,[10] such as EPSPs, IPSPs, and potentials of somatodendritic spikes.[11,12] In this study, we used von Frey filaments to create acute pain stimulus; a chronic neuropathic pain model to assess the changes in the PrL, a

[1]Department of Neurobiology, State Key Laboratory of Proteomics, Institute of Military Cognitive and Brain Sciences, Academy of Military Medical Sciences, Beijing, China
[2]Department of occupational medicine, Tianjin Institute of Environmental and Occupational Medicine, Tianjin, China
[3]Department of Molecular Immunology, Institute of Military Cognitive and Brain Sciences, Academy of Military Medical Sciences, Beijing, China

Corresponding Authors:
Shao-jun Liu, Department of Neurobiology, State Key Laboratory of Proteomics, Institute of Military Cognitive and Brain Sciences, Academy of Military Medical Sciences, 27 Taiping Road, Beijing 100850, China.
Email: Liusj@nic.bmi.ac.cn
Ji-yan Zhang, Department of Molecular Immunology, Institute of Military Cognitive and Brain Sciences, Academy of Military Medical Sciences, 27 Taiping Road, Beijing 100850, China.
Email: Zhangjy@nic.bmi.ac.cn

sub-region of the mPFC; and an LFP activity response to assess long-term pain in freely moving rats. The purpose of this study was to investigate the LFP profiles in the mPFC during acute and chronic pain to delineate the mPFC response to pain.

Materials and methods

Animals

A total of 16 male Sprague–Dawley (SD) rats (weighing 200–250 g, 2.5 months of age) were housed in a vivarium under a 12-h light–dark cycle at constant temperature (25°C) and humidity (50%–60%), with free access to food and water. Animals were acclimatized to the laboratory for one week before starting the experiments. In addition, the Laboratory Animal Welfare and Ethics Committee of our institute approved all the experimental procedures.

Surgical procedure for electrode implantation

Briefly, the SD adult male rats were initially sedated by 1% Nembutal (60 mg/kg). The rat's head was fixed in a stereotaxic frame (Kopf Instruments, USA). The skull surface was exposed, the connective tissue was removed, and the skull surface was cleaned. One craniotomy, slightly larger than the electrode array, was made above the mPFC (AP = 3.0 mm; ML = 0.8 mm; depth = 2.5 mm from the dura). A 4×4 electrode array (16 channels) made with isonel-coated nickel–cadmium alloy wires (diameter of 35 microns) was slowly lowered into the brain. Electrodes were fixed in place using dental cement, leaving only the connectors exposed.

In vivo electrophysiological recording

All the rats were left undisturbed for one week to recover from electrode implantation. LFP recording was conducted 3 days before SNI surgery in all rats (for baseline measurements), and then 14 days and 28 days after SNI surgery. In addition, 8.0 g von Frey filaments were used to inflict acute mechanical pain stimulus (4 s duration, "paw withdrawal" reflex) during LFP recording before and 28 days after the surgery. All recordings were conducted while the rats were in their home cages, conscious, freely moving, and relaxed, with white noise in the background. The implanted electrode was connected by cable to an electro-swivel attached to a multichannel acquisition processor (MAP) system (Plexon Inc). Only frequencies below 100 Hz were further analyzed. The electrophysiological information detected by the recording electrode was used to analyze LFP if its channel could test neural spike information.

Nissl staining. The rats were deeply anesthetized with 1% sodium pentobarbital (60 mg/kg) on day 28 after SNI surgery. The heart was perfused with 0.9% NaCl (100 ml) followed by 4% paraformaldehyde in phosphate-buffered saline (PBS, 500 ml). Perfusion-fixed brain tissues were further fixed for about 2–4 h in the solution and tissue fluids were dehydrated by 30% sucrose in PBS. Frozen brain tissue was cut into 20-μm-thick serial sections. For Nissl staining, the sections were hydrated in 1% toluidine blue at 37°C for 5 min and immersed in 0.1 mol/L PBS three times for 5 min according to the manufacturer's instructions (Beyotime Institute of Biotechnology).

Data collection and analysis. Neural activity was amplified, bandpass filtered at 150–8000 Hz, and continuously sampled at 40 kHz using a MAP system (Plexon Inc). LFPs were first band-pass filtered into six bands[12]: delta (1–4 Hz), theta (4–7 Hz), alpha (7–12 Hz), beta (13–30 Hz), low gamma (30–45 Hz), and high gamma (60–100 Hz), and then the data were analyzed by neuro-explorer. For each rat, the data of LFP were recorded for at least 5 min.

Behavioral tests

Acute mechanical stimulus. The 8 g von Frey filament was used to stimulate each rat's paw for 4 s, which led to paw withdrawal reflex during in vivo electrophysiological recording before and 28 days after SNI surgery to explore the relationship between acute mechanical stimulus and mPFC electrical activity.

Mechanical hypersensitivity test. Mechanical hypersensitivity was measured by von Frey filaments and Dixon up-down method. Briefly, the rats were individually placed into Plexiglas chambers over a mesh table and acclimated for 20 min before examination. Beginning with 2.0 g, von Frey filaments in a set with logarithmically incremental stiffness (0.8, 1.0, 1.2, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, and 15.0 g) were applied to the lateral one-third of right paws of rats.

Surgery for SNI. All 16 rats were divided into two groups, one group with 10 rats were anesthetized by Nembutal (1%, 60 mg/kg), the skin of the right thigh was incised, and the biceps femoris muscle was dissected to expose the sciatic nerve's three branches: sural, common peroneal, and tibial nerves. A 5-0 silk suture was used to ligate the common peroneal and tibial nerves and then excised distal to each knot to remove 3–5 mm of the distal ends. In the sham surgery group, the above nerves were exposed but not excised.
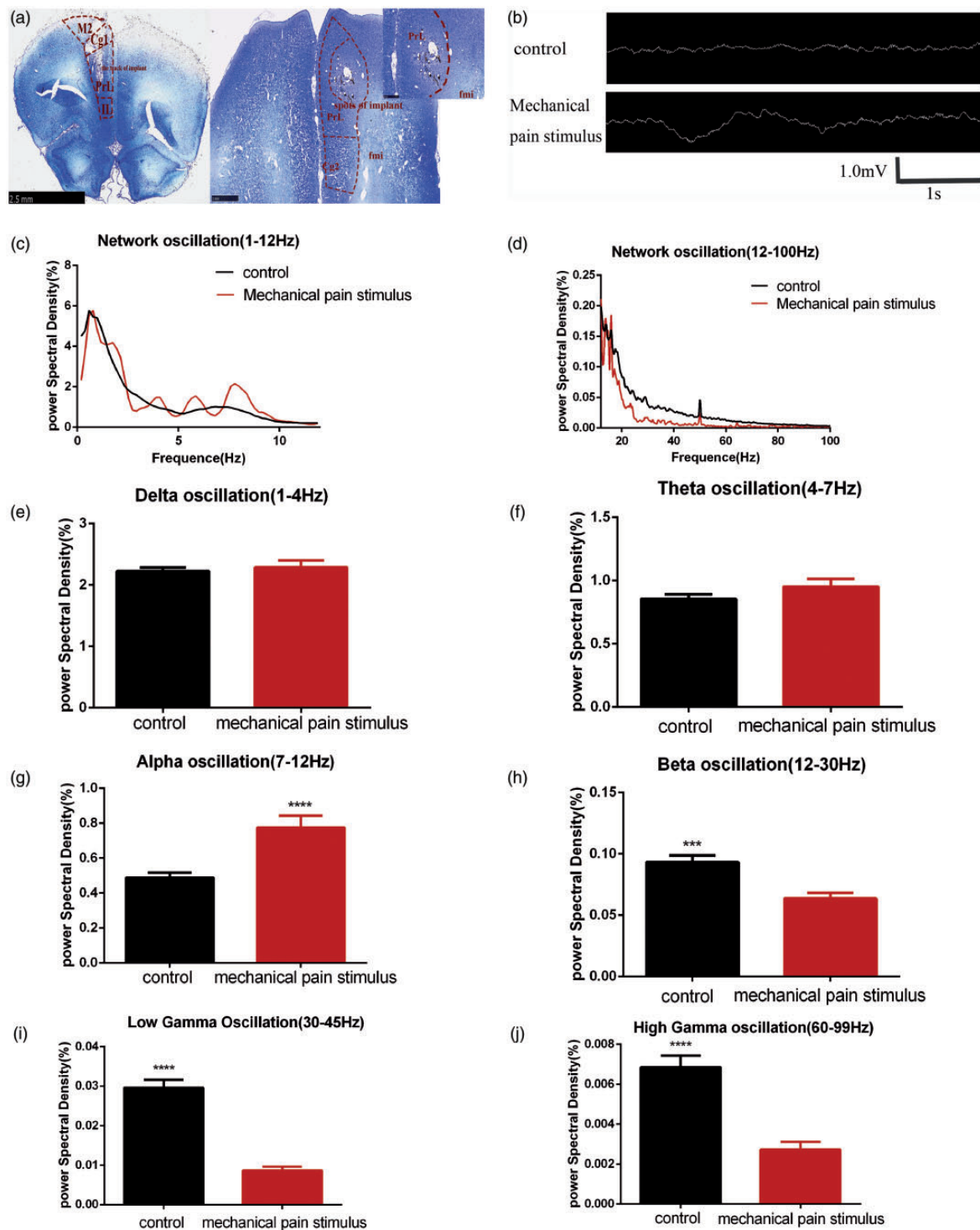
Figure 1. Acute mechanical pain stimulus affected mPFC-averaged LFP power spectra. (a) Location of the recording electrode in mPFC, the tracks of implant in coronary slice, and the spots of implant in horizontal slice (20×, Hamamatsu Photonics). (b) Examples of actual LFP data in mPFC in free-moving rats for control and mechanical pain stimulus (4 s). (c, d) Linear graphs show averaged LFP power spectra (1–12 Hz, 12–100 Hz). Bar graphs represent mean total LFP power in control and acute mechanical pain stimulus rats within six frequency band ranges: delta and theta oscillation, alpha oscillation, beta oscillation, low gamma, and high gamma oscillation. Acute mechanical pain stimulus significantly increased power spectral density (%) of mPFC in alpha band ((g) unpaired t-test, t = 4.433, p < 0.0001), and decreased in beta ((h) unpaired t-test, t = 3.869, p = 0.0001), low gamma ((i) unpaired t-test, t = 7.767, p < 0.0001) and high gamma oscillation ((l) unpaired t-test, t = 5.203, p < 0.0001) as compared to control rats; (e, f) no difference appeared in delta and theta oscillations. n = 144 (LFP channels), 9 (control rats); n = 96 (LFP channels), 6 (mechanical pain stimulus rats). Values are reported as mean ± SEM; ***p < 0.001, ****p < 0.0001.
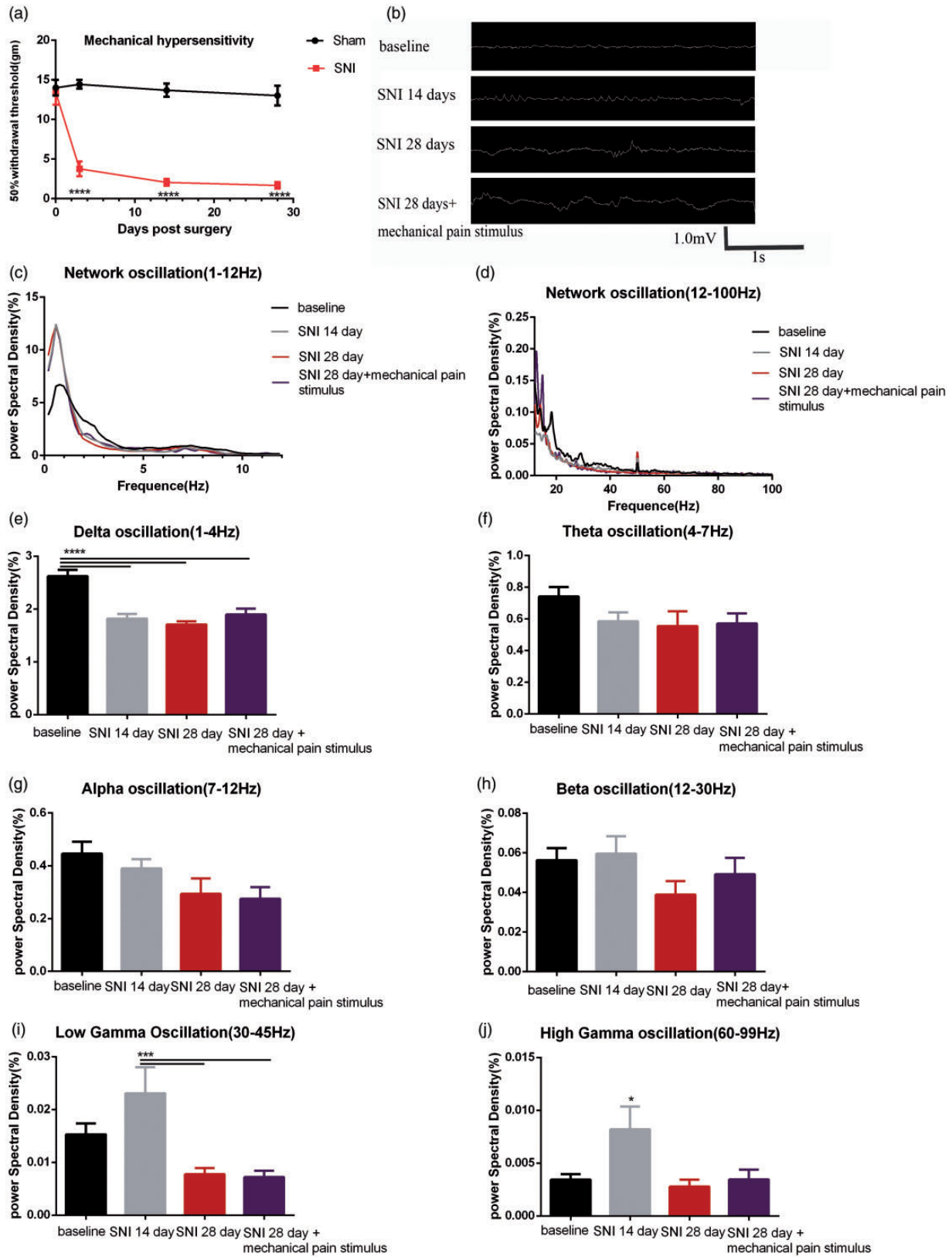
Figure 2. Chronic neuropathic pain stimulus affected mPFC-averaged LFP power spectra. (a) SNI decreased mechanical withdrawal threshold (n = 6 for each group, repeated measure two-way ANOVA followed by Bonferroni *post hoc* test; effect of time, $F_{(1, 20)}$ = 147.5; effect of group, $F_{(3, 20)}$ = 20.53; effect of interaction, $F_{(3, 20)}$ = 14.31, p < 0.0001, posttest ****p < 0.0001). (b) Examples of actual LFP

Statistical analysis

The unpaired t-test was used for comparisons of LFP power spectral density (%) between the two groups. The mechanical withdrawal threshold was analyzed by two-way analysis of variance (ANOVA) with Bonferroni post hoc test. One-way ANOVA with Bonferroni post hoc test was used for analysis of the LFP after SNI surgery. All the data are presented as the mean ± standard error of the mean. Graph pad PRISM 6.0 was used for statistical analysis.

Results

To study the electrophysiological mechanism between pain and mood disorder, we measured the mPFC LFP after acute and chronic pain stimuli in free-moving rats (the spots of electrode in mPFC, Figure 1(a); examples of LFP data, Figure 1(b)). First, we examined the LFP of mPFC by average power spectra (Figure 1(c) and (d)) when the rat withdrew its paw after pain stimulus of 4 s with 8.0 g von Frey filaments. The results showed that acute mechanical stimulus significantly increased the power spectral density (%) in the alpha oscillation (Figure 1(g)) and decreased the power spectral density in beta, low gamma, and high gamma oscillations (Figure 1 (h) to (i), and (l)) as compared to the control rats but no difference in delta and theta oscillation (Figure 1(e) and (f)). These data revealed that acute mechanical pain stimulus affected mPFC-averaged LFP power spectra.

Next, we tested whether chronic neuropathic pain affects mPFC LFP power spectra using a neuropathic model SNI. We measured the basal mechanical withdrawal threshold before SNI surgery and then retested at day 3, 14, and 28 after surgery. The results showed that SNI significantly decreased the mechanical withdrawal threshold at day 3 after surgery, which persisted till day 28 (Figure 2(a)). Then, we analyzed the power spectral density (%) of mPFC LFP before surgery and at day 14 and 28 after surgery. In addition, at day 28 after surgery, an 8.0 g von Frey filament was used to inflict acute mechanical pain stimulus, which resulted in paw

withdrawal reflex during in vivo *electrophysiological* recording to examine how acute mechanical pain stimulation affects mPFC LFP when rats were under mechanical allodynia (Figure 2(b) to (d)). The results showed that chronic neuropathic pain significantly decreased the power spectral density (%) in delta band oscillation (Figure 2(e)). However, the power spectral density (%) in low gamma oscillation was also decreased at day 28 after SNI surgery as compared to day 14 after SNI but with no difference from baseline. Meanwhile, 14 days' neuropathic pain stimulus increased the low gamma band power spectra, without statistical difference (Figure 2(i)). High gamma band power spectral density (%) was increased at day 14 after SNI surgery and significantly decreased at day 28 by chronic neuropathic pain (Figure 2(l)), with no significant difference in theta, alpha, and beta oscillations (Figure 2(f) to (h)).

Finally, we examined how acute mechanical pain stimulation affects mPFC LFP when rats were sensitive to pain. On day 28 after SNI surgery, acute mechanical pain stimulus had no effect on the mPFC LFP (Figure 2(e) to (i) and l). However, as compared to the baseline data, the mPFC LFP power spectra density (%) after acute mechanical stimulation was lower in delta oscillation (Figure 2(e)). Moreover, the gamma oscillation had a remarkable difference as compared to day 14 after SNI(Figure 2(i) and (l)).

Discussion

The mPFC is involved in multiple functional networks. This study showed that acute mechanical pain increased alpha oscillation and decreased beta and gamma oscillation in freely moving rats. The acute pain appeared to be a negative stress stimulation and affected mPFC alpha, beta, and gamma oscillations, which led to adverse emotional experiences. According to recent studies, the delta band (1–4 Hz) is a representation of slow-wave sleep. The amplitude of theta and alpha oscillations (4–12 Hz) are related to spatial memory in animal models.[13,14] Beta and gamma oscillations (12–100 Hz)

Figure 2. Continued.
data in mPFC in free-moving rats for baseline, SNI 14 days, SNI 28 days, and SNI 28 days +mechanical pain stimulus (4 s). (c, d) Linear graphs show averaged LFP power spectra (1–12 Hz, 12–100 Hz). Bar graphs represent mean total LFP power in the group of baseline, SNI 14 days, SNI 28 days, and mechanical pain stimulus within six frequency band ranges: delta and theta oscillation, alpha oscillation, beta oscillation, and low gamma and high gamma oscillation; (e) chronic neuropathic pain decreased the power spectral density (%) in delta band oscillation ($p < 0.0001$, $F(3, 252) = 17.19$, $p < 0.0001$), the mPFC LFP power spectra density (%) was lower in delta oscillation after acute mechanical pain stimulus at day 28 after SNI surgery ($p < 0.0001$); (i) the power spectral density (%) in low gamma oscillation was decreased at day 28 after SNI surgery as compared to day 14 after SNI ($p = 0.009$, $F(3, 252) = 7.044$, $p = 0.0001$) but with no difference from baseline ($p = 0.3553$) as well as allodynia rats after acute pain stimulation ($p = 0.005$). (l) High gamma band power spectral density was increased at day 14 after SNI surgery ($p = 0.0450$), and significantly decreased at day 28 after SNI ($p = 0.0145$) as compared to day 14 after SNI, as well as allodynia rats after acute pain stimulation ($p = 0.0469$, $F(3, 252) = 4.023$, $p = 0.0081$); (f, g, h) no significant differences appeared in theta, alpha, and beta oscillations. n = 64 (LFP channels), 4 (rats). Values are reported as mean±SEM; one-way ANOVA with Bonferroni *post hoc* test, *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$. SNI: spared nerve injury.

are involved in episodic memory,[15] and recent studies indicated that the beta oscillation is more relevant for long-range synchronization involving long transmission delays[16] and the gamma oscillation is more evident in the surrounding communication.[17] This study showed that the changes of mPFC LFP followed by acute pain stimulation are adapted to prevent further damage from physiological and psychological processes.

In this study, chronic neuropathic pain decreased delta oscillation, and both low gamma and high gamma oscillations also decreased with time, which suggested that the connectivity of mPFC and hippocampus or other brain areas was decreased. Clinically, patients suffering from chronic pain experience negative mood symptoms such as insomnia, depression, and anxiety. The alteration of delta and gamma oscillations may be responsible for comorbidity of pain and mood disorders.

Finally, we also tested if acute mechanical pain affects mPFC LFP when rats are under allodynia. No significant differences were found in all six band oscillations of mPFC LFP when rats suffered from acute mechanical pain stimulus at day 28 after SNI surgery. In summary, acute mechanical pain stimulus increased alpha and decreased beta and gamma oscillations; delta oscillation decreased and gamma oscillation dynamically decreased during chronic pain stimulus. Further studies are needed to reveal how acute and chronic pain affects mPFC neural activity at the levels of neurons and neural circuits to induce anxiety, depression, or other mood disorders.

Author contributions

BF performed all the experiments and prepared the manuscript outline, designed the studies, and wrote the manuscript. SNW, BW, and KW wrote the manuscript. JYZ and SJL supervised the experiments and wrote the manuscript. All authors read and approved the final manuscript.

Declaration of conflicts of interests

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