The Effects of Acute Fluoxetine treatment on Hippocampal Spectral Power Density

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Abstract

The hippocampus is one of the stress-sensitive limbic structures implicated in the pathophysiology of mood-associated disorders, including depression. Convergent lines of research implicate the hippocampus in the pathogenesis of major depression disorders (MDD). Fluoxetine, a selective serotonin inhibitor (SSRI), has been effectively used in the clinical management of MDD. However, little is known about the effects of antidepressant fluoxetine on the electrical brain wave of the hippocampus. This study aimed to investigate the effect of fluoxetine on hippocampal local field potential (LFP). Male Swiss Albino (ICR) mice were implanted with an intracranial electrode into the hippocampus for LFP recording. Following fluoxetine (20 mg/kg) treatment, hippocampal LFP signals were recorded and analyzed in comparison to the control levels. Raw LFP signals were then transformed into power spectral densities (PSD) and expressed as the percentages of total power. The results revealed that fluoxetine significantly decreased alpha2 (9.8 – 12.7 Hz) and beta1 (13.2 – 18.1 Hz) powers and pronouncedly increased gamma1 (30.8 – 43.0 Hz) power in particular with the maximum response at 1 hour following the treatment. Suppression of slow frequency and enhancement of specific gamma power spectrum indicated the hippocampal activation status, which might be associated with the increasing of monoamine neurotransmission. In conclusion, the results from this study suggested that fluoxetine may involve neurotransmitter systems that produce an alteration of hippocampal local field potential.

Keywords*: *Depression, Major Depressive Disorders, Fluoxetine, Antidepressant, EEG, Hippocampus

1. Introduction

Acute exposure to selective serotonin inhibitors (SSRIs) has been used as standard positive controls of antidepressant screening model, including forced swimming test (FST) and tail suspension test (TST) for comparison with unknown substances or herbal extracts. Moreover, animal models of anxiety have also been sensitive to the acute administration of SSRIs. Increasing monoamine levels is associated with the antidepressant effect of SSRIs as well as fluoxetine. However, the underlying anxiolytic mechanism of fluoxetine is not fully understood. It is generally accepted that activation of 5-HT1A, 5-HT2A, or 5-HT2C receptor subtypes is associated with anxiolytic-like effects, whereas activation of 5-HT3 induces anxiogenesis (Griebel, 1996). Fluoxetine has been proposed to produce whether anxiogenic- or anxiolytic-like effects in animal models (Borsini, Podhorna, and Marazziti 2002). However, its action remained unclear.

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter that plays essential roles in mood, stress, and anxiety-anxiolytic regulations. The serotonergic enhancer, fluoxetine, is one of the most widely used medications for the treatment of major depression disorders (MDD) (Schoevers et al., 2008). Fluoxetine has also prescribed for the treatment of anxiety with or without co-morbid depression. (Vaswani, Linda, and Ramesh, 2003). Fluoxetine can rapidly increase the extracellular serotonin levels by preventing the reuptake of serotonin into presynaptic neurons. Maintaining the increased serotonin levels in the synaptic region enhances the stimulation of postsynaptic serotonin receptors. However, it usually takes weeks to reveal their antidepressant therapeutic effects. Adaptive changes of central serotonergic plasticity have been thought to be involved with chronic fluoxetine treatment (Taylor et al., 2005). The most remarkable effects of fluoxetine on neural plasticity are the increases in adult neurogenesis after chronic treatment (2-4 weeks) in several brain areas including the hippocampal dentate gyrus (Segi-Nishida, 2017) and cerebral cortex (Ohira et al., 2013).

The hippocampus is a part of the limbic system, which connects with various brain areas including emotional-related brain regions; prefrontal cortex and amygdala, and the reward-related brain region; nucleus accumbens. The hippocampus is the brain area that is highly sensitive to stress stimuli. This brain region contains high levels of glucocorticoids and glutamate receptors that regulate the hypothalamus-pituitary-adrenal (HPA) axis, which make it more susceptible to stress, anxiety, and depression. In the study in animal,

long-term exposure to stress-associated stimuli, including restraint stress, chronic unpredictable stress or psychosocial stress, results in impairment of hippocampal network plasticity, for example, apical dendritic atrophy or reduced dendritic arborization (Magariños et al., 1996; Pittenger and Duman, 2008; Vyas et al., 2002; Watanabe, Gould and McEwen, 1992). Therefore, the hippocampus is the most studied brain region in depression and anxiety research.

2. Objectives

This study aimed to investigate the effects of acute fluoxetine treatment on LFP oscillation in the hippocampus.

3. Materials and Methods

3.1. Animals

Male Swiss albino ICR mice (7–8 weeks old) were used in this study. Animals were housed in the individual stainless-steel cage (17 x 28.5 x 17 cm) at the standard environment (12/12 h light/dark cycle, 22 ± 1°C and 55 ± 10% relative humidity). Commercial food pellets and water were available ad libitum. All experiments were carried out between 8 a.m. and 4 p.m. In order to acclimatize and minimize animal stress, all animals were handled for one week before the initiation of the experiment at the Southern Laboratory Animal Facility, Prince of Songkla University. An effort was made to minimize the animal suffering during experimental procedures. All experimental protocol was performed according to the guidelines of the European Science Foundation (Use of Animals in Research, 2001) and International Committee on Laboratory Animal Science, ICLAS (2004) on the protection of animals used in scientific research. The experimental procedures and protocols were approved by the Institute Animal Care and Use Committee, Prince of Songkla University (MOE0521.11/1376).

3.2. Electrode implantation surgery and LFP recording

The method of electrode implantation was described previously. Briefly, animals were anesthetized by pre-injection intramuscularly with 16 mg/kg xylazine and followed by 50 mg/kg Zoletil® 100 (Virbac, Thailand Co. Ltd.). Then the animal's head was mounted in a stereotaxic frame (Figure 1). Local analgesic, lidocaine (Locana, L.B.S. Laboratory Ltd., Part., Thailand) was applied before an incision was made at the midline to expose the skull. The silver wire electrodes (diameter = 203.2µm; DC resistance = 8.7 Ω, A.M. system Inc., USA) was stereotaxically positioned on the left side of the dorsal CA1 hippocampus (AP: 2.5 mm, ML: 2mm, DV: 1.5 mm. The neutral reference and ground electrode were also implanted on the skull at midline over the cerebellum (AP: -6.0, ML: 0.0 mm, DV: 1.5 mm). All electrode positions were stereotaxically positioned according to the mouse brain atlas (Franklin and Paxinos, 2008). Additional holes were drilled for fixing the stainless-steel anchor screws. Dental acrylic was then applied to secure and fix all electrodes on the skull. After surgery, animals were placed in a clean cage with a heating pad and monitored until ambulatory behavior was seen. Antibiotics ampicillin (100 mg/kg) was applied intramuscularly every 12 hours for three days to prevent infection. They were allowed to recover for at least 7 – 10 days fully.

During three consecutive days before the test, animals were placed in the recording chamber (3 hours in each day) to get accustomed to the novelty of the experimental condition. The hippocampal signals were amplified with a low-pass 2 kHz and high-pass 0.3 Hz, digitized at 2 kHz by a PowerLab 16/35 system (AD Instruments, Castle Hill. NSW, Australia) with 16-bit A/D, and stored in a PC through the LabChart 7 pro. Software. Recorded files were overviewed by using visual inspection, and only noise-free signals were selected and used for the off-line analysis.

3.3. Experimental procedure

The timeline of the experimental protocol is shown in Figure 1. After fully recovered, animals were individually placed in the recording chamber (3 hours each day) for three consecutive days before the test to get accustomed to the novelty of the lab environment. The cylindrical recording chamber used is made of black laminate material with 35-cm diameter. The animals were divided into two groups (control and fluoxetine groups) to receive either oral gavage of distilled or fluoxetine (20 mg/kg), respectively. During the testing day, the hippocampal signal was recorded for 30 minutes as a baseline activity. Therefore, the animals received either distilled water or fluoxetine and the hippocampal signal was recorded for 180 minutes as post-treatment data. All experiments were carried out during the light period from 9.00 a.m. to 4.00 p.m.

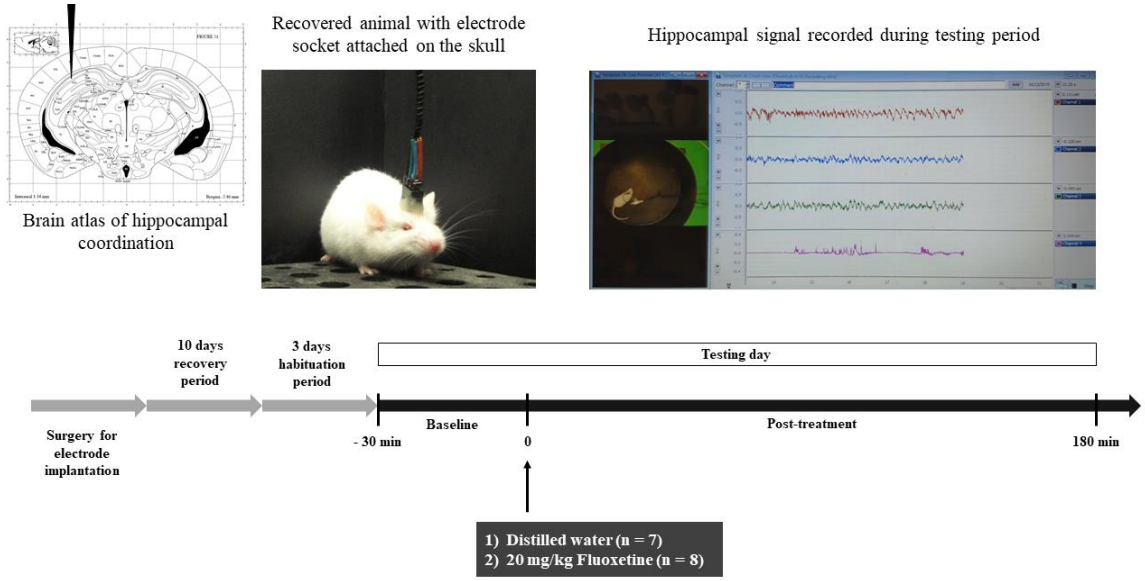

Figure 1 An experimental procedure.

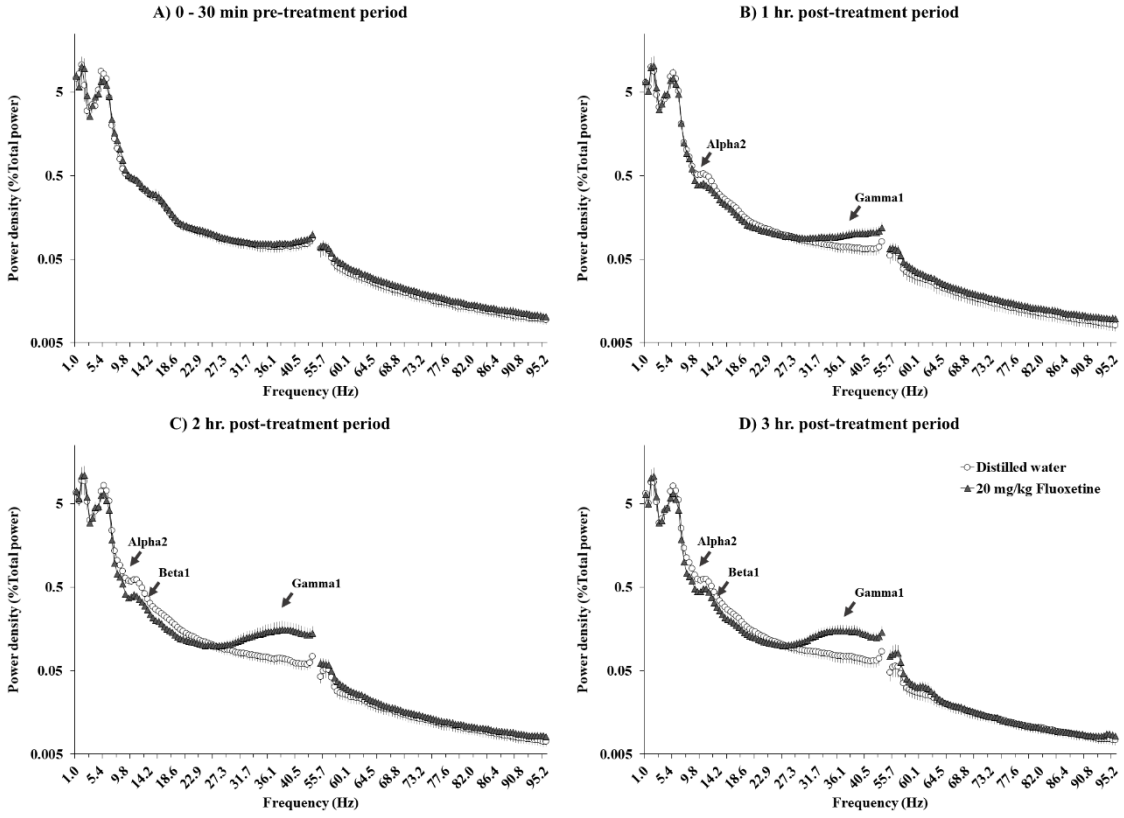

Figure 2 Hippocampal LFP power spectral density recorded in open field chamber distilled water and fluoxetine-treated mice; The power spectral densities were expressed as a percentage of total powers (%Total power) during 30-minute pre-treatment or baseline period (A) and 1-hour (B), 2-hour (C), and 3-hour (D) post-treatment period.

3.4. Power spectral analysis

All LFP signals were processed through 1 - 100 Hz band-pass digital filter. For spectral power analysis, power spectral density (PSD) was generated by LabChart software using fast Fourier transforms (FFT) algorithm (2048 FFT size, 50% overlap, Hanning window cosine). The PSD in each frequency bin was expressed as the percentage of total power (1-100 Hz). The average spectral power was constructed in discrete frequency bands of each group and averaged within specific frequency ranges; delta: 1.0 – 3.9 Hz; theta: 4.4 – 6.3 Hz; alpha1: 6.8 – 9.3 Hz; alpha2: 9.8 – 12.7 Hz; beta1: 13.2 – 18.1 Hz; beta2: 18.6 – 30.3 Hz; gamma1: 30.8 – 43.0, and gamma2: 55.2 – 95.2 Hz. Frequency ranges in this study were overlapped with those used in the previous study by Dimfel (2009), which examined the electroencephalogram patterns of antidepressive substances.

3.5. Statistics

EEG data were normalized with that of the total power to obtain % total power values. They were subsequently averaged and expressed as mean ± S.E.M for each frequency band within for each group. Statistical analyses were performed to compare data of treated and control (distilled water and 20 mg/kg of fluoxetine) groups. All datasets that passed the normality test were therefore analyzed by Two-way analysis of variance (ANOVA) to determine the influences of treatment and time of the treatment factors. Multiple comparisons using Turkey's post hoc test was performed to indicate specific points of difference considered to be statistically significant at $P<0.05$.

4. Results

The power spectral density (PSD) was analyzed and expressed as the percentage of total power (%Total power). In order to observe the general effect of fluoxetine treatment, the PSD of both distilled water and fluoxetine groups are displayed separately during the baseline pre-treatment and post-treatment period (Figure 1A). Data were shown every 1 hour for 3 hours following the treatment (Figure 1B – D). It was found that both groups showed similar levels of baseline PSD. However, treatment with 20 mg/kg fluoxetine clearly appeared to affect the PD of alpha1, beta1, and gamma1 frequency ranges.

According to the effects of fluoxetine on PSD of particular frequency ranges, then the PSD (%Total power) levels of specific frequency bands including delta, theta, alpha1 alpha2 beta1, beta2 gamma1, and gamma2 were averaged. The mean PSD of each frequency band was plotted and shown in Figure 2A – H. On the other hand; two-way ANOVA was also performed to examine the influence of either treatment factor (distilled water x 20 mg/kg fluoxetine), time factor of the treatment period (baseline pre-treatment, 0 - 1 hr., 1 – 2 hr. and 2 – 3 hr.) or the interaction between these two factors. The *F* values for the two-way ANOVA test are shown in Table 1.

The statistical analyses in Figure 3 revealed that only treatment factor, but not the time of the treatment, had significant effect on PSD. Multiple comparisons indicated significant differences observed in alpha 2 [$F(1, 59) = 20.768$, $P < 0.001$], beta1 [$F(1, 59) = 5.771$, $P < 0.05$], and gamma1 [$F(1, 59) = 16.645$, $P < 0.001$] frequency bands. In addition, significant interaction between both factors are also seen in alpha2 frequency band [$F(3, 59) = 3.847$, $P = 0.015$].

Table 1 The *F*-values of two-way repeated ANOVA

Factors	Treatment $F(1, 59)$	Time of the treatment $F(3, 59)$	Interaction between both factors $F(3, 59)$
Delta	0.217	0.030	0.011
Theta	0.786	0.088	0.014
Theta	0.786	0.088	0.014
Alpha2	**20.768 ($p < 0.001$)**	2.274	**3.847 ($p = 0.015$)**
Beta1	**5.771 ($p = 0.020$)**	0.433	1.439
Beta2	0.096	0.359	0.105
Gamma1	**16.645 ($p < 0.001$)**	2.171	2.335
Gamma2	2.003	2.007	0.001

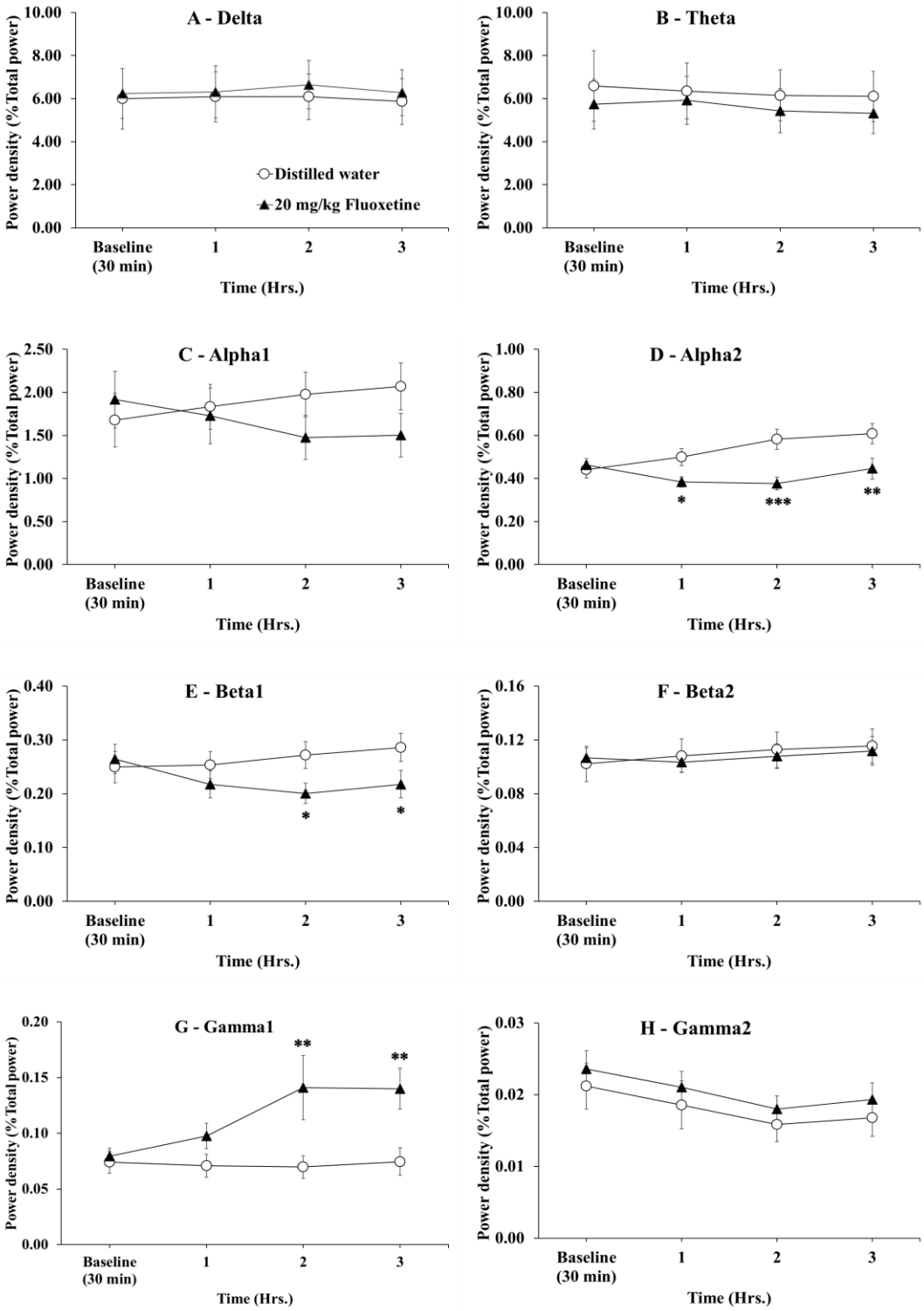

Figure 3 Data were analyzed during baseline (30-minutes) and every 1 hour during the post-treatment period. Data were expressed as mean ± S.E.M. (A - G). Differences between groups were determined by using two-way repeated-measures ANOVA followed by Tukey's post hoc test. *p < 0.05, **p < 0.01.

5. Discussion

Due to the long-term therapeutic effect of SSRIs on MDD treatment, most studies tend to focus on the chronic effects of these drugs. However, a behavioral study in rodents indicated that SSRIs and related drugs are found to exhibit acute therapeutic effects in the depression-related tests including forced swim test and tail suspension test (Porsolt, Castagné and Moser, 2008). The present study was designed to identify and characterize the effects of treatment with fluoxetine, SSRIs drugs, on hippocampal LFP signals by using the power spectral density analysis. It is based on the fact that the oscillatory patterns of the hippocampal signal are ending physiological events driven by underlying mechanisms produced by the net effect of fluoxetine's action on the hippocampal network circuits. It was found that 20 mg/kg fluoxetine affected the powers of specific frequency activities that resulted in decreased powers of alpha1 and beta1 and increased power of gamma1.

Previously, there was no direct evidence of fluoxetine effect on hippocampus spectral power in rodents. Dimpfel (2009) had created Electropharmacogram, pharmaco-specific electroencephalogram (EEG) fingerprints to examine the effect of various substances, including antidepressant drugs on LFP signals of 4 different brain areas, including the dorsal hippocampus. Alpha1 (6.0-9.50 Hz) and beta1 (12.75-18.50 Hz) frequency ranges defined in those studies were overlapped with the present study. However, the study by Dimpfel covered only the frequency ranges within 0.8 – 35.0 Hz but not gamma. Various classes of antidepressant drugs have been tested to produce Electropharmacogram. It has been found that most of the antidepressant drugs decrease the powers of broad frequency components (Dimpfel, 2003). Some drugs such as mianserin obviously decreased alpha1 frequency while 2.5 mg/kg moclobemide (monoamine oxidase A inhibitor) significantly decreases alpha2 and beta1 frequency powers in the hippocampus during the first hour of the treatment period. These results indicated that changes of alpha1 and beta1 powers are associated at least with the enhancement of monoamine neurotransmission as a major underlying mechanism of antidepressant drugs.

Hippocampal neural signaling shows a rhythmic oscillation in various frequency ranges which is dependent on behavioral states. In rodents, slow frequency within 5–10 Hz (theta) and fast frequency, gamma (30–100 Hz) are observed mostly during active exploration and rapid eye movement (REM) sleep. Besides, these gamma and theta oscillations also appear throughout the neocortex and subcortical regions in both animals (Bragin et al., 1995) and human (Fell et al., 2001). These two rhythmic oscillations often coexist but can also occur separately and have been proposed to constitute a fundamental mechanism underlying various activities, including sleep-wake state and cognitive-related tasks. Numerous *in vitro* models have been deployed to gain insight into the cellular and synaptic mechanisms of theta and gamma oscillations (Gloveli, Kopell, and Dugladze, 2010).

However, very few studies have examined the effects of fluoxetine on the hippocampal circuit using *in vitro* hippocampal brain slice electrophysiological recording. Suppression of theta oscillations (range: 4.5 – 6.5 Hz) in the EEG of the rabbit hippocampus by intracerebroventricular administration of fluoxetine was previously reported (Kudina et al., 2004). The effect of fluoxetine on fast rhythmic activity or gamma frequency range was examined in hippocampal slices (Krause and Jia, 2005). It was found that changes in gamma activity produced by acute fluoxetine were selectively modulated by two 5-HT receptor subtypes; 5-HT_{1A} and 5-HT_2 receptors. These studies strongly implicated the serotonergic neurotransmission in the modulation of hippocampal circuit gamma oscillation. Surprisingly, recent work from Me´ndez and colleagues found that acute administration of fluoxetine strongly and directly alters GABA-mediated (GABAergic) hippocampal neurotransmission independently of their effects on amine reuptake systems that result in the alteration of gamma oscillation (Méndez et al., 2012).

Serotonergic projections from the midbrain raphe nuclei (the origin of serotonergic neurons) to the neocortex play a vital role in the maintenance of neocortical electroencephalographic (EEG) activation, which produces a high frequency, low amplitude EEG activation in rodent (Vanderwolf, Baker, and Vanderwolf, 1986). This electrophysiological recording has a consistent result of a single acute administration of fluoxetine which increases extracellular serotonin (5-HT) levels in the forebrain. Decreases in the power of hippocampal alpha1 and also beta1 waves shown in this study might be due to cortical activation of 5-HT. Decreasing of slow frequency component (1-25 Hz) of spectral power was likely to reflect a low amplitude activity both in

the neocortex and hippocampus. The previous study confirmed a reduction in alpha band amplitude by fluoxetine that raises the possibility that alpha activity is the most sensitive EEG index of serotonergic stimulation (Dringenberg and Diavolitsis, 2002).

Convergent lines of research implicate the hippocampus in the pathogenesis of MDD. The hippocampus is one of the most highly stress-sensitive brain regions. Long-term stress is associated with structural changes in the hippocampus which leads to the pathogenesis of MDD (MacQueen and Frodl, 2011). Many electrophysiological recording studies established that raphe nucleus, a primary site of origin of serotonergic forebrain projection neurons, might involve with the site of action of antidepressant drugs (Czachura and Rasmussen, 2000; O'Leary et al., 2007). The previous report also demonstrated that dorsal hippocampal CA1 receive serotonergic input from median raphe nucleus which mediate adaptive or coping responses to aversive events and that dysfunction of this system is related to symptoms of depression (Guimarães et al., 1993; Graeff et al., 1996). However, little is known about the effect of antidepressant drugs on the hippocampus, especially in the patterns of electrophysiology. Further studies are needed to characterize neural signaling biomarkers of standard antidepressant drugs.

5. Conclusion

This study demonstrated the effect of fluoxetine treatment on LFP powers of alpha2, beta1, and gamma1 frequency ranges in the dorsal hippocampus of mice. In terms of mechanism, multiple neurotransmitter systems are hypothesized to be involved. Most of the drugs interact with more than one receptor. Basically, brain circuits consist of multiple neurotransmitter systems linked to neural chains and networks. Stimulation or inhibition of one neurotransmitter system always affects the overall function of the network. Therefore, this study was designed to identify and characterize the effects of fluoxetine treatment on the hippocampus by using power spectral profiles. It is based on the fact that the oscillatory patterns of neural signals are ending physiological events driven by underlying mechanisms produced by the net effect of drug's action on multiple neurotransmitter systems. In this study, the analyses of electrical brain activity offered profound advantages to investigate the net effects of the treatments and distinguish the antidepressant effect.

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