# SIMULTANEOUS MULTI-REGION LOCAL FIELD POTENTIAL RECORDINGS IN RESPONSE TO NOXIOUS STIMULI FROM FREELY MOVING RATS

by

ZHEN WANG

THESIS

Submitted in partial fulfillment of the requirements

for the degree of Master of Science in Psychology at

The University of Texas at Arlington

December, 2019

Arlington, Texas

Supervising Committee:

Yuan Bo Peng, Supervising Professor

Perry Fuchs

Qing Lin

Acknowledgements

I was fortunate enough to have Dr. Yuan Bo Peng as my mentor, who I would like to acknowledge for providing me the opportunity to work with him. I accumulated advanced techniques in this lab, and learned critical thinking from my mentor. All I gathered would guide me to move on not only in my continuing research, but also in my life.

I would also like to thank my thesis committee members, Dr. Perry Fuchs and Dr. Qing Lin, who gave me extremely profound and insightful responses to my thesis.

At the end, I would like to thank my considerate family and caring friends who always support me.

Abstract

SIMULTANEOUS MULTI-REGION LOCAL FIELD POTENTIAL RECORDINGS IN RESPONSE TO NOXIOUS STIMULI FROM FREELY MOVING RATS

Zhen Wang, M. S.

The University of Texas at Arlington, 2019

Supervising Professor: Dr. Yuan Bo Peng

Pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage” can be ignited by noxious chemical (e.g. acid), mechanical (e.g. pressure) and thermal (e.g. heat) stimuli and generated by activation of sensory neurons and their axonal terminals called nociceptors in the periphery. Nociceptive information transmitted from the periphery is projected to the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), insular, anterior cingulate cortex (ACC), amygdala, periaqueductal grey (PAG), and prefrontal cortex (PFC), etc. Local field potential (LFP) attracts an increasing number of attentions as a neurophysiological tool to investigate the combined neuronal activity, ranging from several hundred micrometers to few millimeters (radius) located around the embedded electrode.

In this study, LFPs from the contralateral anterior cingulate cortex (ACC), ventral tegmental area (VTA) and bilateral amygdala, were simultaneously recorded, and differential LFP activities from multiple regions of the brain in response to peripheral noxious stimuli were determined in both anesthetized and freely moving animals. In detail, within the ACC region, LFP powers (intensity) of delta, theta, alpha, beta, and gamma bands increased significantly after formalin injection in both anesthetized animals and freely moving animals. Within the bilateral amygdala regions, LFP powers (intensity) of delta, theta, alpha, and beta bands increased significantly after formalin injection in both anesthetized animals and freely moving animals. However, LFP changes of gamma band could be detected in freely moving animals merely. Within the VTA regions, LFP powers (intensity) of delta, theta, and beta bands increased significantly after formalin injection in both anesthetized animals and freely moving animals. However, LFP changes of gamma band existed only in anesthetized animals. And difference of alpha band was not seen both. Besides, the ACC responded more strongly than that of bilateral amygdala after formalin injection in anesthetized animals only. The results of a Pearson's *r* correlation indicated that there was a correlation between behavioral and electrophysiology responses to pain. In detail, these significant relationships were showed in delta band from the ACC and left amygdala; delta, theta, and gamma bands from the right amygdala.

Table of Contents

List of Illustrations

# Chapter 1 INTRODUCTION

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Merskey et al., 1979). Pain can be ignited by noxious chemical (e.g. acid), mechanical (e.g. pressure) and thermal (e.g. heat or cold) stimuli and generated by the activation of sensory neurons and their axonal terminals called nociceptors in the periphery (Caterina et al., 1997). Nociception respond to perceived or actual tissue damage.

There are five ascending pathways for nociceptive information transmission from the spinal cord to the thalamus and cerebral cortex, including spinothalamic tract (the most prominent), spinoreticular tract, spinomesencephalic tract, cervicothalamic tract, and spinohypothalamic tract. Nociceptive information transmitted from thalamus is projected to primary somatosensory cortex (S1), secondary somatosensory cortex (S2), insular, and anterior cingulate cortex (ACC) (Apkarian et al., 2005). Information from the amygdala is projected to the basal ganglia.

Pain always remains a significant clinical issue worldwide. Pain impairs a variety of body system, such as increases of blood pressure, susceptibility to infection, increases of blood glucose, and exaggeration of mood disorders, etc. (Sheng et al., 2017; Swift, 2018). Significantly, pain burdens the economic costs, the annual cost of pain ranging from $560 to $635 billion is greater than the annual costs of heart disease ($309 billion), cancer ($243 billion),

and diabetes ($188 billion) in United States according to the Medical Expenditure Panel Survey (Gaskin and Richard, 2012).

Therefore, deciphering these underlying mechanisms is critical for developing effective and useful therapies to alleviate and suppress pain, and promoting well-beings.

### 1.1 Anterior cingulate cortex (ACC) and pain

The ACC contains two major subdivisions: a dorsal cognitive division that links with the prefrontal cortex, parietal cortex, motor system, and frontal eye fields; and a rostral ventral affective division that connects with the amygdala, nucleus accumbens, hypothalamus, hippocampus, and anterior insula (Bush et al., 2000).

The ACC is a part of the limbic system of the brain, and receives sensory signals from the somatosensory cortices and other cortical areas (Price, 2000; Vogt, 2005). Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) reveal that the ACC is involved in cognitive and emotional processing (Bush, 2000; Bush et al., 2013; Vogt, 2005; Zhuo, 2013). When receiving noxious stimuli, the ACC actively mediates and processes the affective component of pain (Fuchs et al., 2014; Vogt et al., 1992). In human, pain-related behaviors could be impeded by the lesions of the ACC. Using an animal model of formalin injection, the ACC has been demonstrated to mediate the pain response to formalin injection (Pastoriza, 1996).

The ACC not only responds to physical pain, but involves in psychological pain (Papini et al., 2015). Silencing the gene of the N-methyl-D-aspartate (NMDA) receptor NR2B subunit in ACC neurons could ease pain-related aversion (Guo et al., 2015). The balance of glutamate and GABA in the ACC regulates the response of affective-motivational pain in guinea pigs (Zugaib

et al., 2014). Optogenetic stimulation to the inhibitory neurons involving in the ACC expressing channelrhodosin-2 suppresses the pain behaviors, which indicates the ACC as a promising target to relieve the chronic pain through inhibition the specific neurons (Gu et al., 2015). The studies of ACC lesions reveal that the ACC manipulates the emotional response involving the incentive downshift event, which also attenuates the inflammatory instead of neuropathic pain behavior (Donahue et al., 2001; Ortega et al., 2011). Spinal cord dorsal horn neurons recording after mechanical stimulation also demonstrates that the electrical stimulated ACC possesses the ability to hinder the spinal cord dorsal horn responses to cope with mechanical stimuli (Senapati et al., 2005). The rats treated with L5 spinal nerve ligation studies indicates that activation of the ACC influences affective pain processing, but not for sensory processing (LaBuda and Fuchs, 2005).

Previous studies also present that the activity of local field potentials in the ACC changes after carrageenan injection (inflammatory pain) in hindpaw (Harris-Bozer and Peng, 2016). The decreased intensity of local field potentials in ACC can be recorded from the rats with varicella zoster virus injection (Kramer et al., 2017).

### 1.2 Amygdala and pain

The amygdala is located in the temporal lobe of the brain with a heterogeneous structure, containing around 12 nuclei (Balleine and Killcross, 2006; Pape and Pare, 2010). The amygdala receives inputs from olfactory bulbs, thalamus, cortical afferent, and brainstem, etc.

The amygdala plays an important role in emotional learning and memory (Cardinal et al., 2002; McGaugh, 2004). There is an increasing number of evidence showing that the amygdala is also involved in pain processing (Derbyshire et al., 1997; Jasmin et al., 1997). The lateral (LA), basolateral (BLA), and central nuclei (CeA) of the amygdala are revealed to be correlated with

the processing of nociceptive responses (Ji and Neugebauer, 2008; Neugebauer, 2006; Phelps and Ledoux, 2005).

The emotional pain reactions could be decreased by the lesion or inactivation of the amygdala (Calvino et al., 1982). The electrophysiological studies revealed that the neurons in the amygdala could be activated by the noxious stimuli (Neugebauer and Li, 2002).

### 1.3 Ventral tegmental area (VTA) and pain

The VTA contains dopaminergic neurons with projecting axons that transmit information to the nucleus accumbens, amygdala, hippocampus, cingulate gyrus, and prefrontal cortex, called "The mesolimbic dopamine systems" (Albanese and Minciacchi, 1983; Berridge and Kringelbach, 2015). The VTA is highly involved in the reward processes, and drug abuse, etc. (Oliva and Wanat, 2016; Ranaldi, 2014; Volkow and Morales, 2015). The signal of dopamine from the VTA to nucleus accumbens increases in the process of pleasure and reward, which relates the processing of pain response (Schultz, 2007; Scott et al., 2006).

The electrophysiological studies reveal that more than 30% of neurons in the VTA are inhibited after the mechanical stimulation to the skin, which demonstrates that the VTA also participates in the response of noxious mechanical stimuli (Hentall et al., 1991). The previous studies show that the electrical stimulation of the VTA can promote the mechanical and thermal paw withdrawal thresholds merely after carrageenan injection, but inhibit the neuron responses in spinal cord dorsal horn through single cell recording, which illustrates that VTA might mainly involve the descending pain pathway (Li et al., 2016). The increased activity of local field potentials in VTA is also recorded form the rats after cocaine injection (Harris-Bozer et al., 2016).

## 1.4 Local field potential (LFP) and pain

LFP, the electric potential in the extracellular space around neurons, attracts an increasing number of attentions as a neurophysiological tool to investigate the combined neurons activity. LFP consists of both action potentials and other membrane potential-derived fluctuations in a small neuronal volume ranging from several hundred micrometers to few millimeters (radius) located around the embedded electrode (Buzsáki et al., 2012; Kajikawa and Schroeder, 2011; Katzner et al., 2009). LFP is different from electroencephalogram (EEG) that tracks and records brain signals from the surface of scalp using macro-electrodes, and electrocorticogram (ECoG) that records signal from the cortical surface using subdural grid electrodes (Buzsáki et al., 2012).

LFP usually measures low-frequency activity of neuron (≤ 500 Hz) (Lindén et al., 2011). It can be further divided into delta (0.1-3 Hz), theta (3-7 Hz), alpha (7-12 Hz), beta (12-30 Hz), and gamma (30-100 Hz) bands (Marzbani et al., 2016). In this study, low frequency band (⩽ 100 Hz), which emerges mainly from slower transmembrane postsynaptic events instead of other non-synaptic events, such as glial activity, will be analyzed to demonstrate the neuron activity in response to noxious stimuli (Buzsáki et al., 2012).

## 1.5 Formalin pain model

Subcutaneous dilute formalin injection, as a continuous nociceptive stimulus, into the paw of the animals is widely conducted as a formalin model to the pain study (Dubuisson and Dennis, 1977).

Formalin-induced behavioral response is divided into the first phase, interphase, and second phase. The first phase of the response lasting around 5 minutes is evoked by direct

activation of peripheral nerve terminals distributed around the site chemical stimulation, followed by the interphase lasting 5-15 minutes, and finally the second phase comes out resulting mainly from the subsequent inflammation lasting 15-60 minutes (Lariviere et al., 2011; Porro and Cavazzuti, 1971).

## 1.6 Specific Aims

The purpose of this proposed study was to determine the differential LFP activities from multiple regions of the brain in response to peripheral noxious stimuli in both anesthetized and freely moving rats. The hypothesis was that different brain areas, which are involved in sensory components of pain [e.g. primary somatosensory cortex (S1) and ventral posterolateral thalamic nucleus (VPL)] and emotional components of pain [e.g. anterior cingulate cortex (ACC), amygdala, ventral tegmental area (VTA)], would show different responses to peripheral noxious input produced by formalin injection and the patterns of LFPs would be seen differently between anesthetized and freely moving animals. In this study, LFPs from the contralateral ACC, VTA, and bilateral amygdala, were simultaneously recorded. The rationale was that these four regions related to the processing of pain, that was, involve the pain pathway directly (ACC, amygdala) and indirectly (VTA). As well, it was feasible to implant four electrodes simultaneously according to the spatial distance among these four regions. There were three specific aims.

Aim 1: Record simultaneous multi-region LFPs in response to noxious stimuli in anesthetized animals.

Aim 2: Record simultaneous multi-region LFPs, measure pain behavioral changes in response to noxious stimuli in freely moving animals

Aim 3: Test the correlation between electrophysiological and behavioral responses.

# Chapter 2 LFP ACTIVITIES FROM MULTIPLE REGIONS OF THE BRAIN IN ANESTHETIZED RATS

## 2.1 Methods

### 2.1.1 Animals

11 male aged 3 months and 1 female aged 8 months Sprague Dawley rats were used in this study. All procedures were approved by the Institutional Animal Care and Use Committees of University of Texas at Arlington. The rats were housed in cages with a 12/12h light/dark cycle. Food and water were available ad libitum.

### 2.1.2 Electrode implantation

The rat was placed on a stereotaxic frame under 3% isoflurane inhaled anesthesia. Four electrodes (0.010 inch) were separately implanted into four regions: right ACC at 0 mm posterior to bregma, 0.70 mm lateral to the right, 3.20 mm deep; left and right amygdala at 2.00 mm posterior to bregma, 4.00 mm lateral to the left and right, 8.00 mm deep; and right VTA at 4.80 mm posterior to bregma, 0.90 mm lateral to the right, 8.35 mm deep (Paxinos & Watson, 1998). One screw was placed under the skull connecting to a cable as ground and reference. Dental cement was used to fix the four electrodes and screw on the skull.

### 2.1.3 Module setup and LFP recording

The four electrodes and screw cable were connected to the wireless module (designed by SiChuan NeoSource BioTektronics Limited) to receive the LFP signal from the brain. And a USB dongle paired with the module was inserted into a laptop to transmit the signal from the module to the recording software.

### 2.1.4 Formalin model induction

LFP signal was recorded under 3% isoflurane anesthesia for 10 minutes as baseline. 3% formalin was then injected for 0.1ml to the left hindpaw of rats after baseline recordings. Thereafter, recordings were continued for 60 minutes.

### 2.1.5 Data analysis

The raw data of LFPs (time-domain) recorded from the module was processed by power spectrum analysis in Spike2 and fast Fourier transform (FFT) in MATLAB. The power (frequency-domain) was calculated in MATLAB every 10 seconds. We then averaged the power intensity every 5 minutes. Finally, the power of each frequency band [delta (0.1-3 Hz), theta (3-7 Hz), alpha (7-12 Hz), beta (12-30 Hz), and gamma (30-100 Hz)] was normalized by the average power of the baseline.

SPSS was used to test statistical significance. Data were presented as mean ± SEM. A one-way repeated measures analysis of variance (ANOVA) with LSD post-hoc test was conducted to compare the difference before and after formalin injection. A repeated measures factorial ANOVA with LSD post-hoc test was conducted to test whether the LFP power responses were different among these four regions (unilateral ACC and VTA, and bilateral amygdala). A significance was determined at $p < .05$ level.

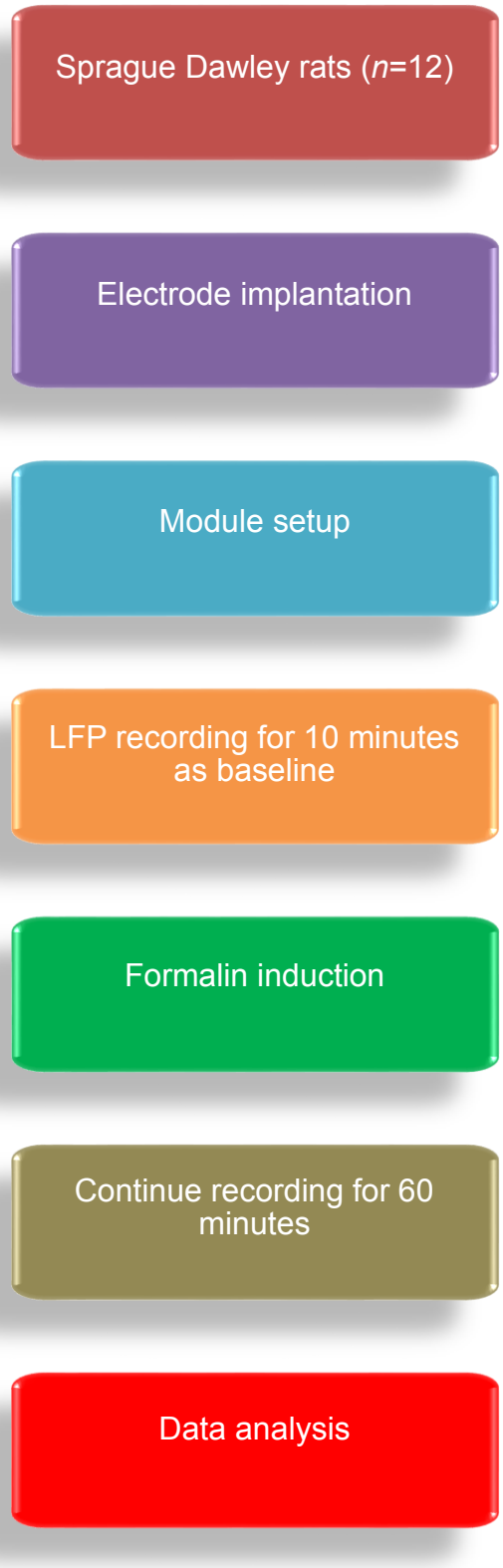


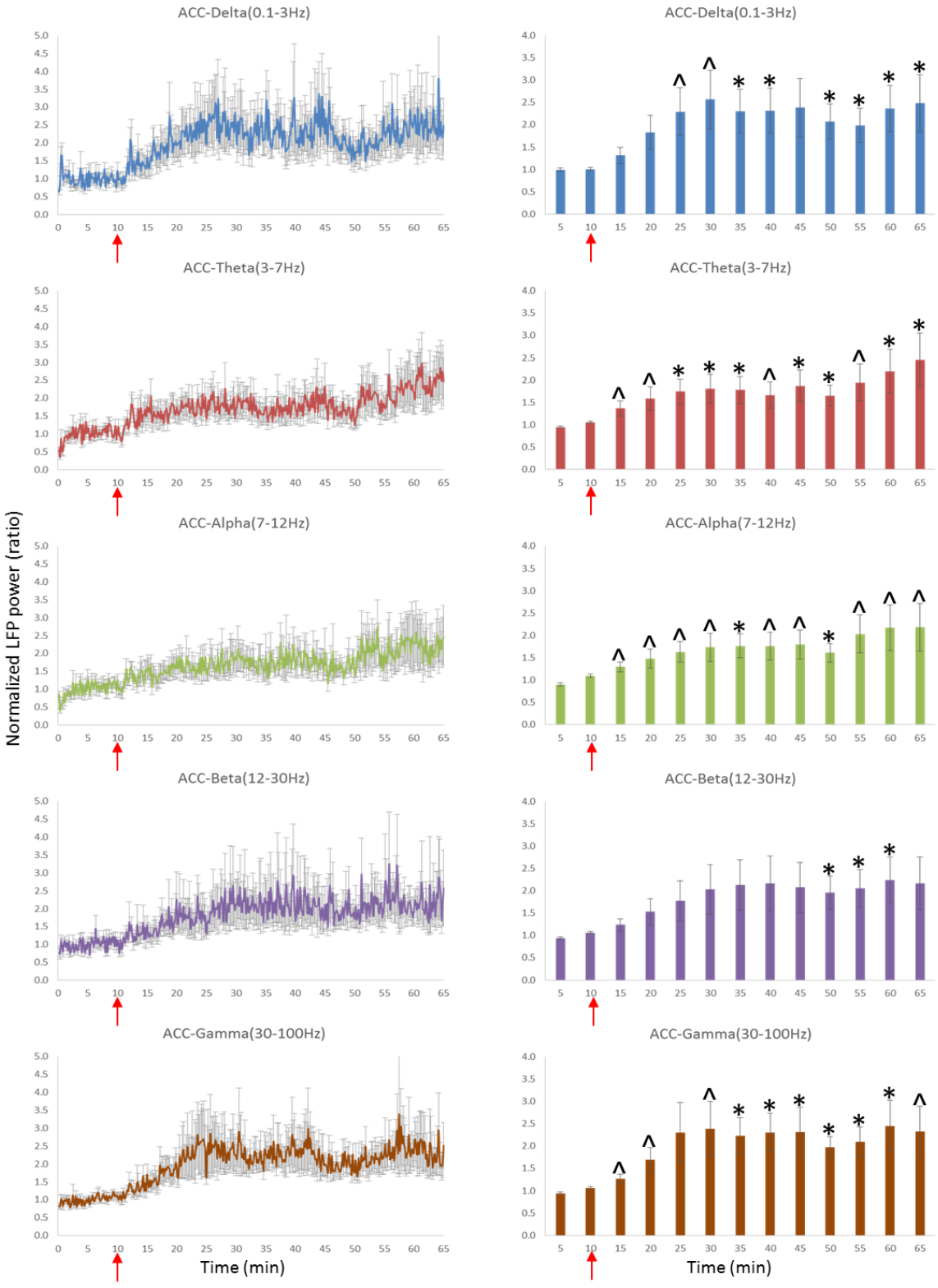

Figure 1. Procedures for anesthetized animals.

## 2.2 Results

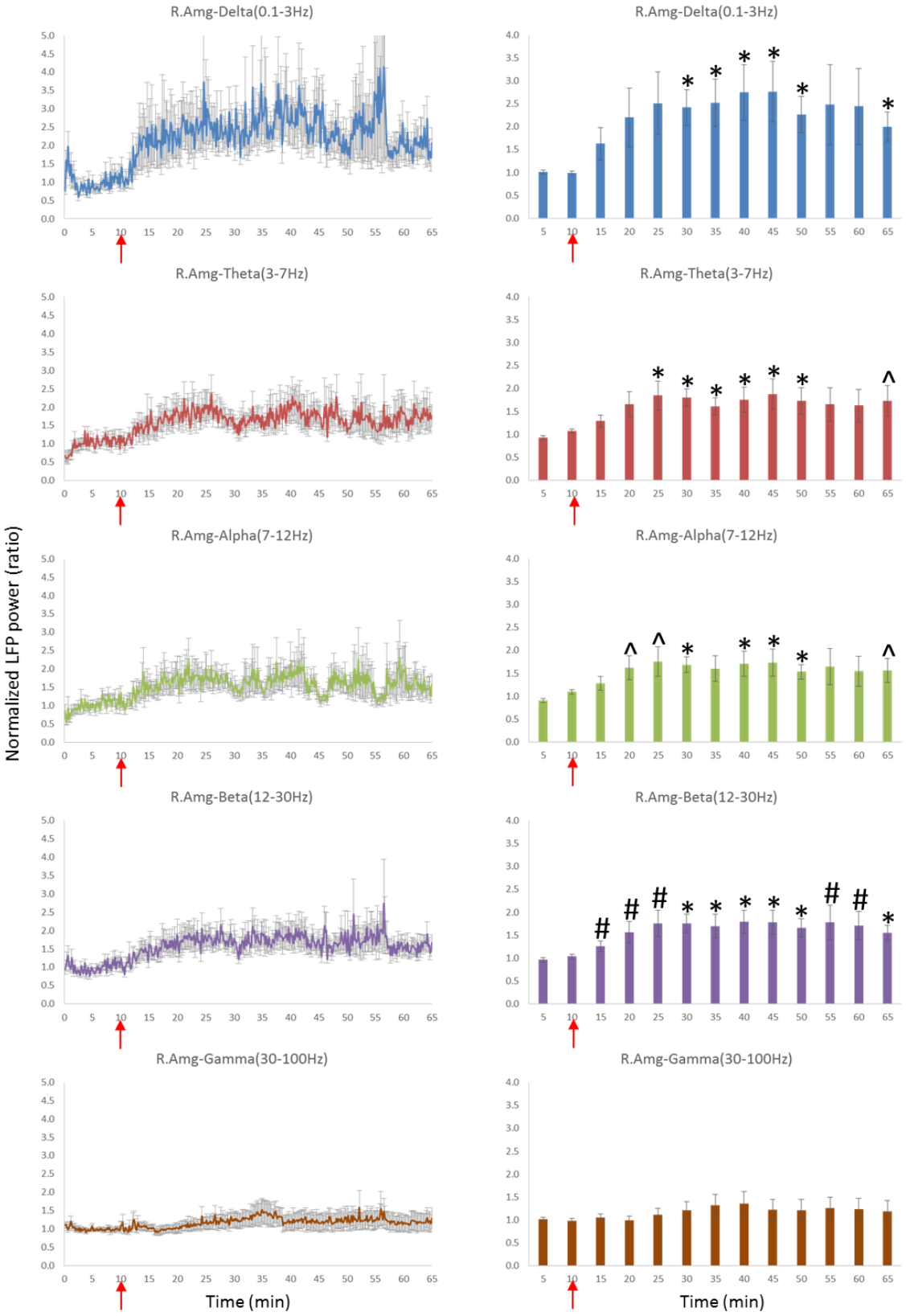
### 2.2.1 Different regions showed various increases in power at various frequency bands

At the ACC region, the LFP powers (intensity) of delta, theta, alpha, beta, and gamma bands increased significantly comparing with the baseline in Figure 2A. At the right amygdala region, the LFP powers (intensity) of delta, theta, alpha, and beta bands increased significantly comparing with the baseline, but not for gamma band, after formalin injection in Figure 2B. At the left amygdala region, the LFP powers (intensity) of delta, theta, alpha, and beta bands increased significantly comparing with the baseline, but not for gamma band, after formalin injection in Figure 2C. At the VTA region, the LFP powers (intensity) of delta, theta, and beta bands increased significantly comparing with the baseline, but not for alpha, and gamma bands, after formalin injection in Figure 2D.

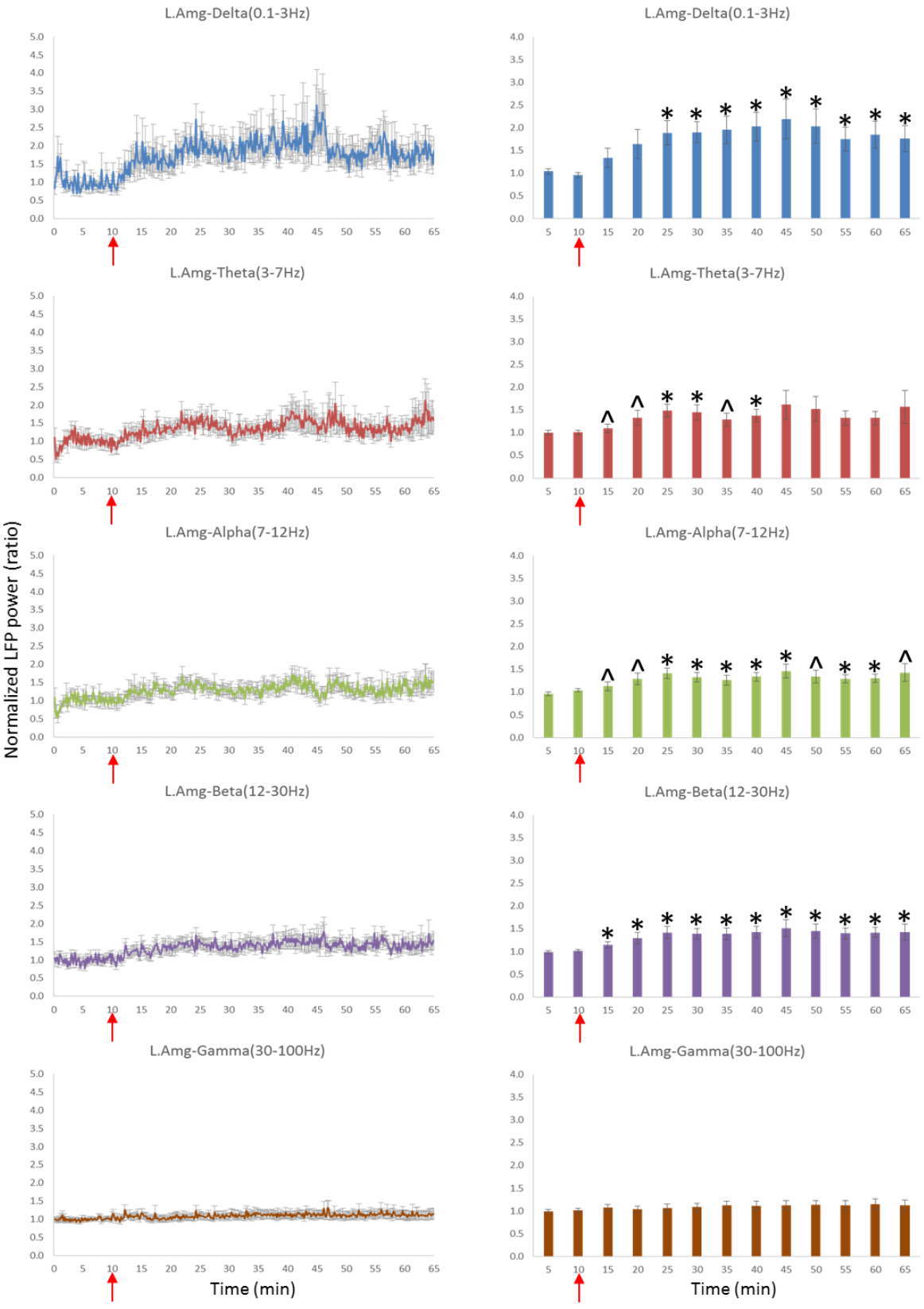
**A**


ACC-Delta(0.1-3Hz)
ACC-Delta(0.1-3Hz)
ACC-Theta(3-7Hz)
ACC-Theta(3-7Hz)
ACC-Alpha(7-12Hz)
ACC-Alpha(7-12Hz)
ACC-Beta(12-30Hz)
ACC-Beta(12-30Hz)
ACC-Gamma(30-100Hz)
ACC-Gamma(30-100Hz)
Normalized LFP power (ratio)
Time (min)
Time (min)

**B**


R.Amg-Delta(0.1-3Hz)
R.Amg-Delta(0.1-3Hz)
R.Amg-Theta(3-7Hz)
R.Amg-Theta(3-7Hz)
R.Amg-Alpha(7-12Hz)
R.Amg-Alpha(7-12Hz)
R.Amg-Beta(12-30Hz)
R.Amg-Beta(12-30Hz)
R.Amg-Gamma(30-100Hz)
R.Amg-Gamma(30-100Hz)
Normalized LFP power (ratio)
Time (min)
Time (min)

**C**


L.Amg-Delta(0.1-3Hz)
L.Amg-Theta(3-7Hz)
L.Amg-Alpha(7-12Hz)
L.Amg-Beta(12-30Hz)
L.Amg-Gamma(30-100Hz)
Normalized LFP power (ratio)
Time (min)

**D**

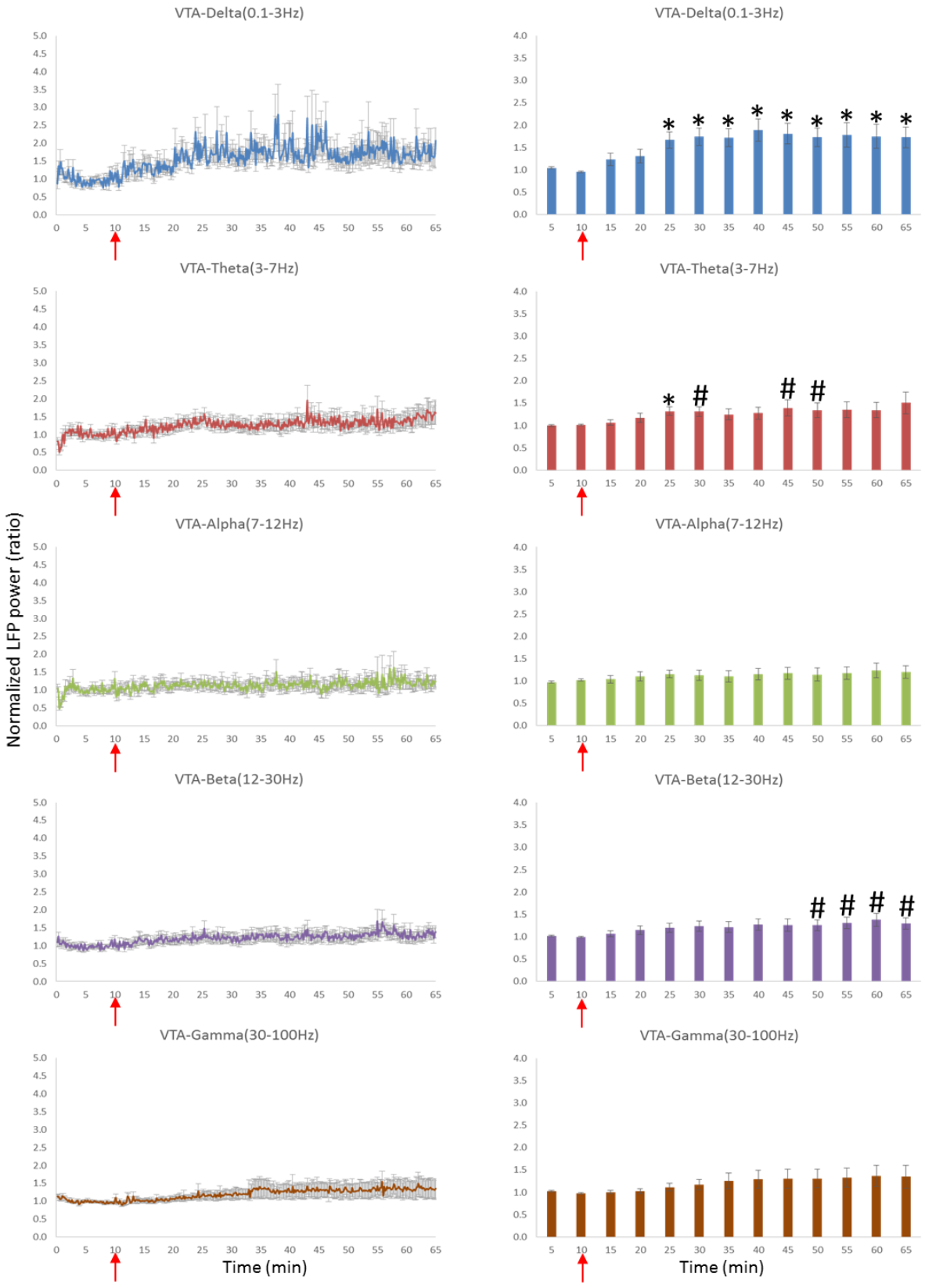

VTA-Delta(0.1-3Hz)
VTA-Delta(0.1-3Hz)
VTA-Theta(3-7Hz)
VTA-Theta(3-7Hz)
VTA-Alpha(7-12Hz)
VTA-Alpha(7-12Hz)
VTA-Beta(12-30Hz)
VTA-Beta(12-30Hz)
VTA-Gamma(30-100Hz)
VTA-Gamma(30-100Hz)
Normalized LFP power (ratio)
Time (min)
Time (min)

Figure 2. LFP intensity changes in delta, theta, alpha, beta, and gamma bands at (A) ACC, (B) right amygdala, (C) left amygdala, and (D) VTA in anesthetized rats. On the left column, the power was shown in every 10 s, whereas on the right column, it was the average of every 5 min. *: significantly different comparing with the baseline timepoints 5 and 10 minute ($p < .05$). ^: significantly different comparing with the baseline timepoint of 5 minute ($p < .05$). #: significantly different comparing with the baseline timepoint of 10 minute ($p < .05$). ↑: formalin injection.

### 2.2.2 No significant differences of frequency bands (except gamma band) among different regions

For delta band, there was no main effect for brain regions, $F(3, 30) = 1.005$, $p = .404$, suggesting no significant differences for the LFP power responses among these four regions. For theta band, there was no main effect for brain regions, $F(3, 30) = 1.671$, $p = .194$, suggesting no significant differences for the LFP power responses among these four regions. For alpha band, there was no main effect for brain regions, $F(2.215, 22.154) = 2.111$, $p = .141$, suggesting no significant differences for the LFP power responses among these four regions. For beta band, there was no main effect for brain regions, $F(1.632, 16.320) = 1.638$, $p = .225$, suggesting no significant differences for the LFP power responses among these four regions. For gamma band, there was a main effect for brain regions, $F(1.224, 12.243) = 4.630$, $p = .046$. In detail, the ACC responded ($M = 1.956$, $SE = .365$) more strongly than that of left amygdala ($M = 1.099$, $SE = .074$) and right amygdala ($M = 1.131$, $SE = .154$) after formalin injection. And there were no differences among remaining regions.

# Chapter 3 LFP ACTIVITIES FROM MULTIPLE REGIONS OF THE BRAIN IN FREELY MOVING RATS

In order to avoid the influence of isoflurane and mimic the clinical situation of pain, we recorded the LFP activities from freely moving animals, where we could also observe the behavioral pain scores to test the correlations behavioral and electrophysiological responses to pain.

## 3.1 Methods

### 3.1.1 LFP recordings from freely moving animals

10 male and 2 female aged 4-6 months Sprague Dawley rats were used in this study. All procedures [animal preparation, electrode implantation, module setup, LFP recording, formalin induction (here we injected formalin at 15 minutes), and data analysis] were the same as in 2.1 of chapter 2, except for recording LFPs and measuring pain behavior changes in freely moving animals one week after electrode implantation with 12 Sprague Dawley ages rats.

### 3.1.2 Formalin pain score measurement

Behavioral testing was assessed immediately after 3% formalin injection (0.05ml) using the software (designed by Dr. Fuchs's lab) and last for 120 minutes. Time bin was set as 5 minutes with 3 categories: paw licking, elevated, and down. Pain score was calculated with the

weighted formula: Pain score = [0(time spent with the paw down) + 1(time spent with the paw elevated) + 2(time spent with the paw licking)]/300 (Hentall et al., 2001; LaBuda et al., 2001).

### 3.1.3 Link between LFP bands and behavior

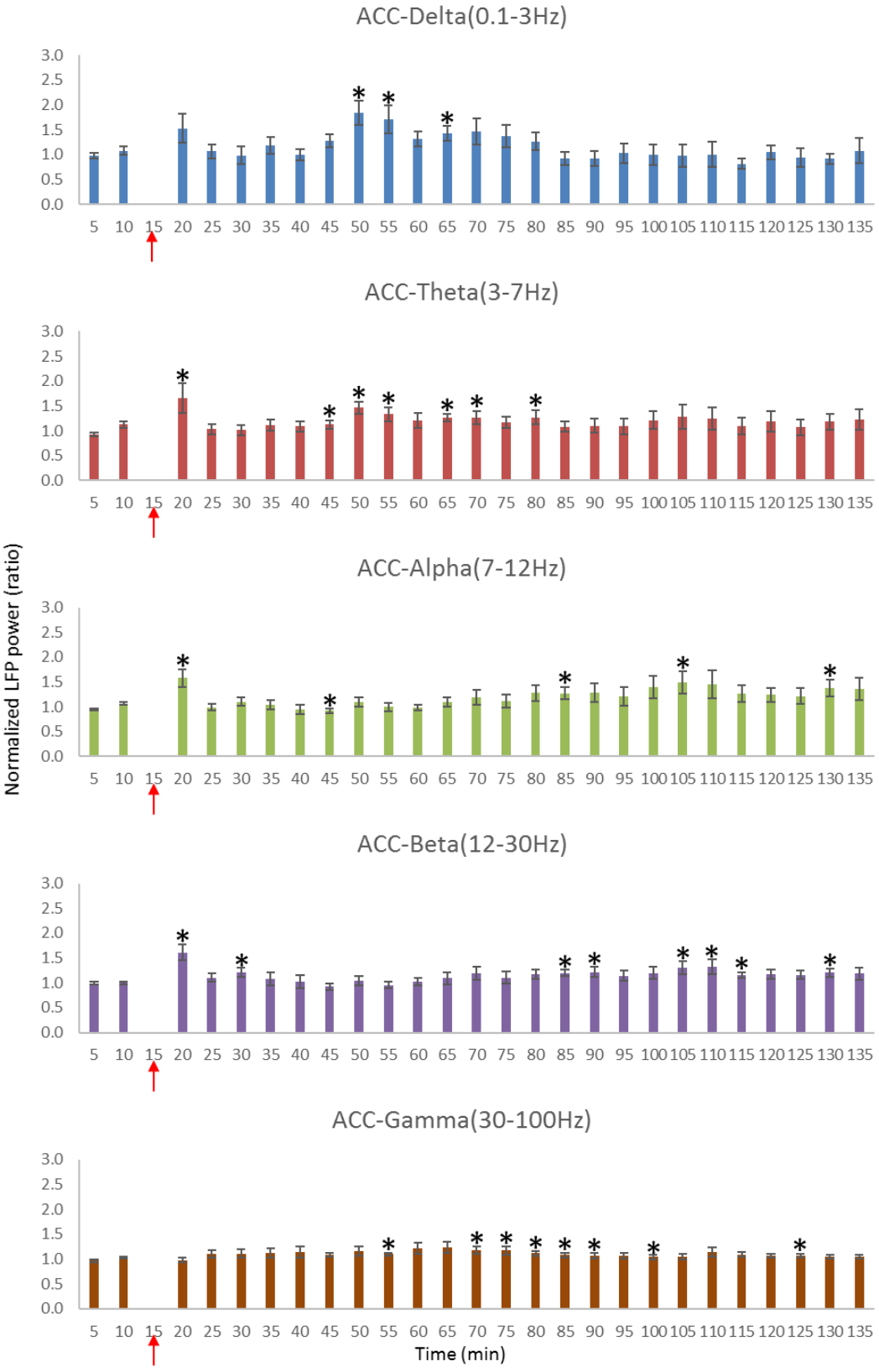
A Pearson's *r* correlation was conducted to test the relationship between behavioral and electrophysiology responses to pain. A significance was determined at $p < .05$ level.

## 3.2 Results

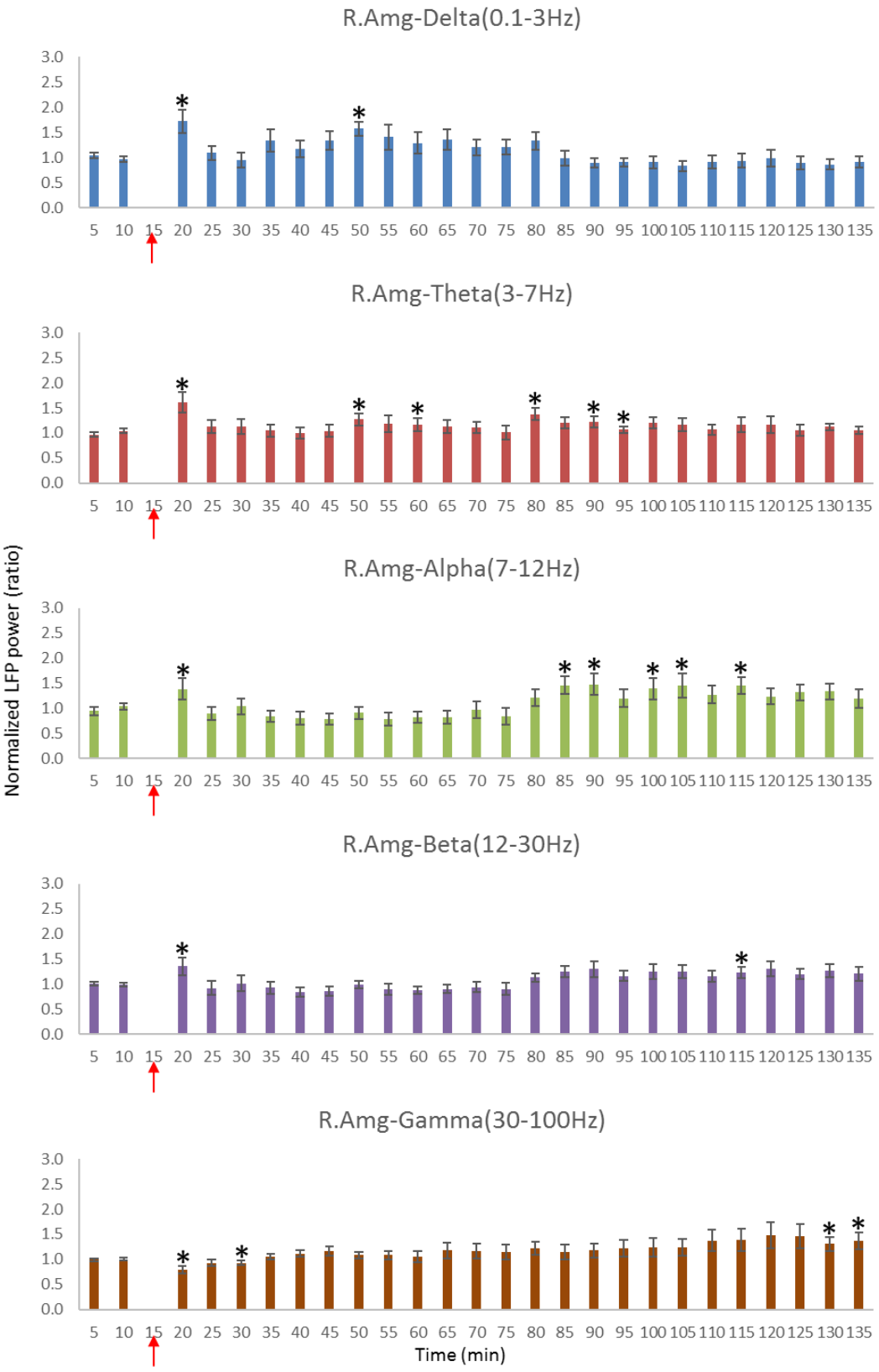
### 3.2.1 Various changes of power of frequency were observed in different regions

At the region of ACC, right amygdala, and left amygdala, the LFP powers (intensity) of delta, theta, alpha, beta, and gamma bands increased significantly comparing with the baseline after formalin injection in Figure 3A, 3B, and 3C. At VTA region, the LFP powers (intensity) of delta, theta, beta, and gamma bands increased significantly comparing with the baseline, but not for alpha band, after formalin injection in Figure 3D.

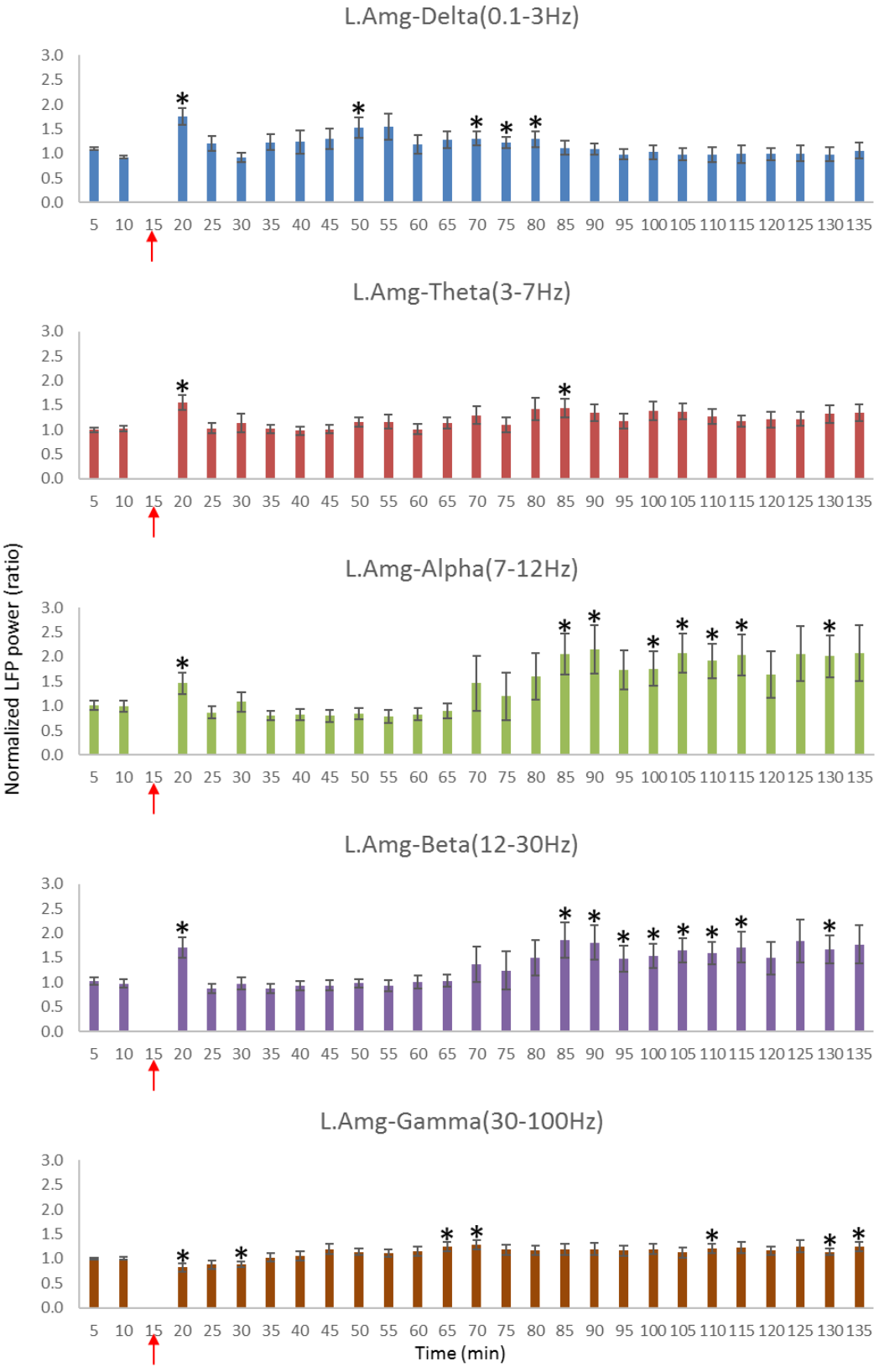
**A**


ACC-Delta(0.1-3Hz)
ACC-Theta(3-7Hz)
ACC-Alpha(7-12Hz)
ACC-Beta(12-30Hz)
ACC-Gamma(30-100Hz)
Normalized LFP power (ratio)
Time (min)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135

**B**


R.Amg-Delta(0.1-3Hz)
R.Amg-Theta(3-7Hz)
R.Amg-Alpha(7-12Hz)
R.Amg-Beta(12-30Hz)
R.Amg-Gamma(30-100Hz)
Normalized LFP power (ratio)
Time (min)

**C**


L.Amg-Delta(0.1-3Hz)
L.Amg-Theta(3-7Hz)
L.Amg-Alpha(7-12Hz)
L.Amg-Beta(12-30Hz)
L.Amg-Gamma(30-100Hz)
Normalized LFP power (ratio)
Time (min)

**D**

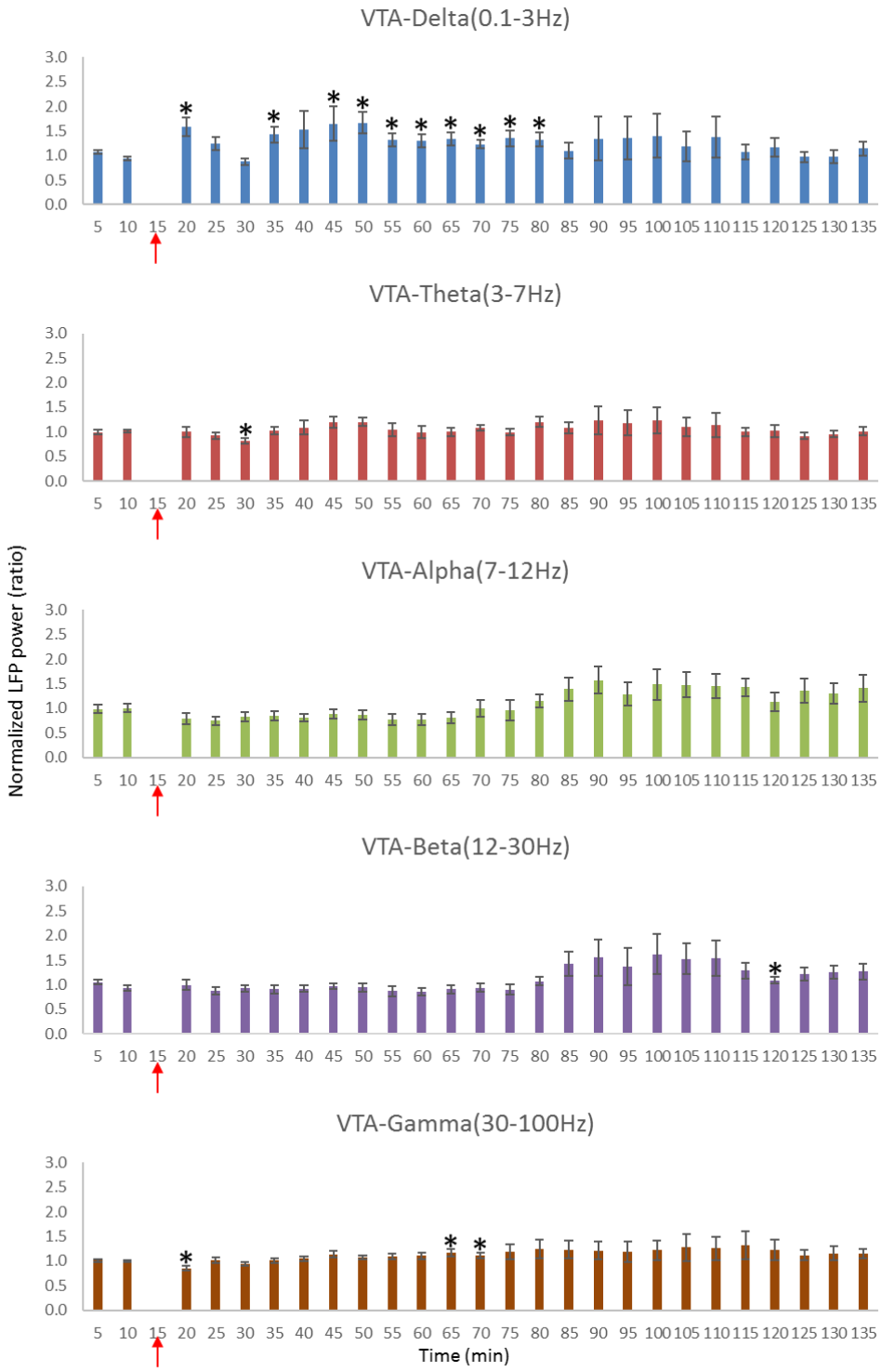

VTA-Delta(0.1-3Hz)
VTA-Theta(3-7Hz)
VTA-Alpha(7-12Hz)
VTA-Beta(12-30Hz)
VTA-Gamma(30-100Hz)
Normalized LFP power (ratio)
Time (min)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135

Figure 3. LFP intensity changes in delta, theta, alpha, beta, and gamma bands at (A) ACC, (B) right amygdala, (C) left amygdala, and (D) VTA in freely moving rats. The power was shown in every 5 min. *: significantly different comparing with the baseline timepoints 5 or 10 minute ($p < .05$). ↑: formalin injection.

### 3.2.2 No significant differences of frequency bands among different regions

For delta band, there was no main effect for brain regions, $F(3, 24) = .276$, $p = .842$, suggesting no significant differences for the LFP power responses among these four regions. Same situation for theta band, $F(3, 24) = .680$, $p = .573$; alpha band, $F(1.744, 13.951) = 2.496$, $p = .123$; beta band, $F(2.443, 19.545) = 2.070$, $p = .146$; and gamma band, $F(3, 24) = .029$, $p = .993$.

### 3.2.3 Formalin pain score

At 5-20 min after formalin injection, pain score went down gradually. After 20 minutes, it increased and reached the maximal point at 70 minutes, then kept decreasing until the end in Figure 4.

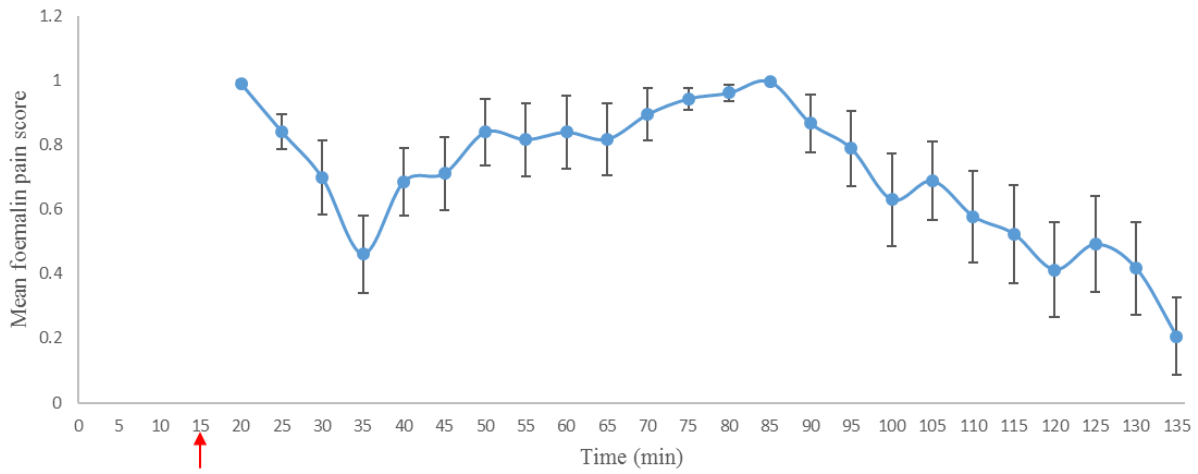



Figure 4. Mean formalin pain score after formalin injection for 120 minutes. ↑: formalin injection.

3.2.4 Formalin behavioral response was correlated with some frequency bands in some regions

In order to test the relationship between behavioral and electrophysiology responses to pain, a Pearson's *r* correlation was run. The results demonstrated (Figure 5) that:

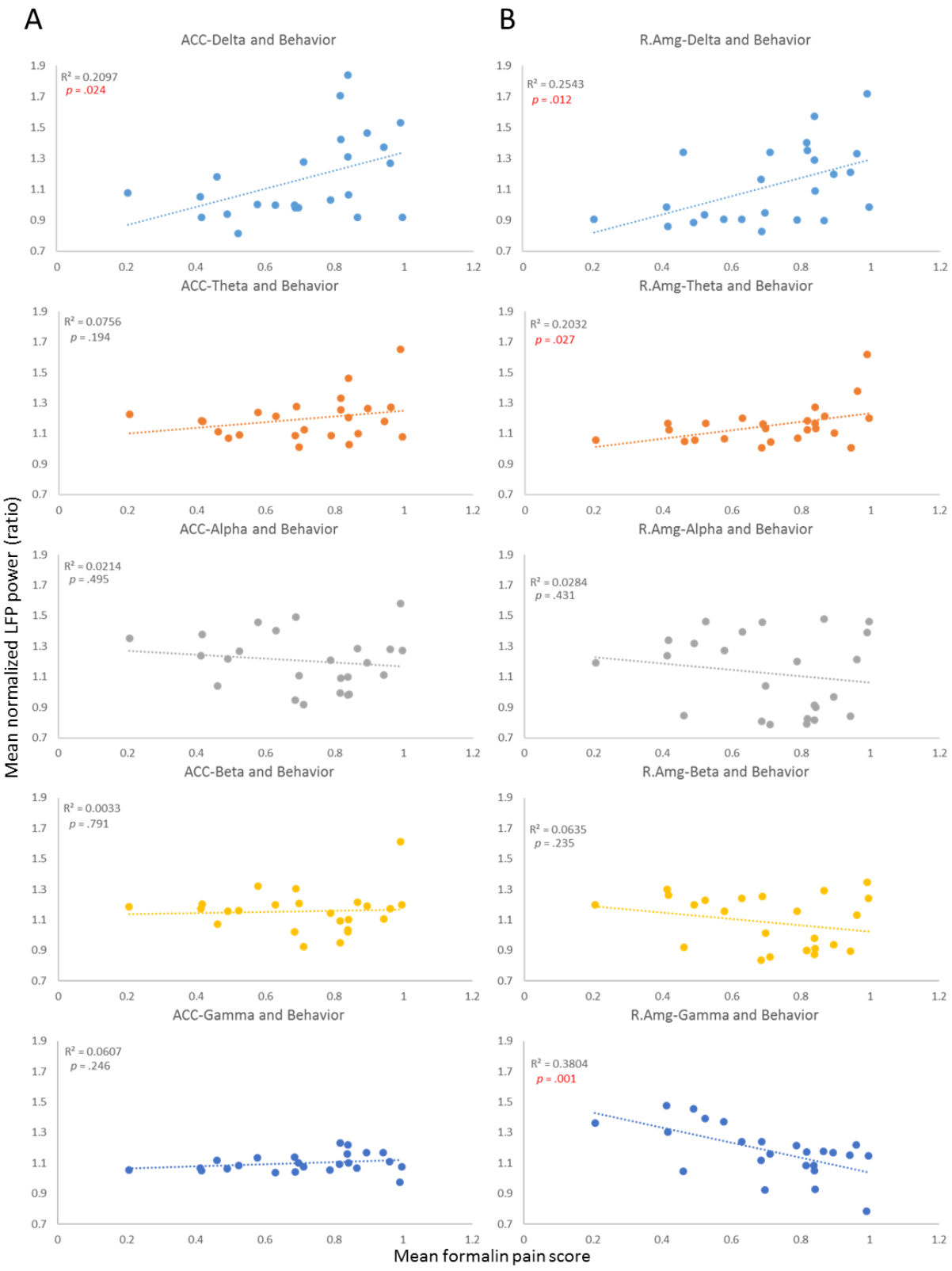
At the ACC region, there was a positive correlation between delta band and behavior, $r(22) = .458$, $p = .024$, which meant electrophysiology responses in delta band fluctuated along with the formalin pain. However, we did not detect the correlation in theta, $r(22) = .275$, $p = .194$; alpha band, $r(22) = -.146$, $p = .495$; beta band, $r(22) = .057$, $p = .791$; and gamma band, $r(22) = .246$, $p = .246$.

At the right amygdala region, there was a positive correlation between delta band and behavior, $r(22) = .504$, $p = .012$; between theta band and behavior, $r(22) = .451$, $p = .027$; and a negative correlation between gamma band and behavior, $r(22) = -.617$, $p = .001$, suggesting electrophysiology responses in delta, theta, and gamma bands changed along with the formalin

pain. However, there was no correlations between alpha band and behavior, $r(22) = .451$, $p = .431$; between beta band and behavior, $r(22) = -.252$, $p = .235$.

At the left amygdala region, there was a positive correlation between delta band and behavior, $r(22) = .544$, $p = .006$. In detail, electrophysiology responses in delta band changed along with the formalin pain. However, we did not get the correlation in theta band, $r(22) = .121$, $p = .573$; alpha band, $r(22) = -.307$, $p = .145$; beta band, $r(22) = -.194$, $p = .364$; and gamma band, $r(22) = -.216$, $p = .311$.

At the VTA region, there was no correlations in delta band, $r(22) = .366$, $p = .078$; theta band, $r(22) = .244$, $p = .250$; alpha band, $r(22) = -.350$, $p = .093$; beta band, $r(22) = -.236$, $p = .267$; and gamma, $r(22) = -.160$, $p = .455$.


A
B
ACC-Delta and Behavior
R² = 0.2097
p = .024
R.Amg-Delta and Behavior
R² = 0.2543
p = .012
ACC-Theta and Behavior
R² = 0.0756
p = .194
R.Amg-Theta and Behavior
R² = 0.2032
p = .027
ACC-Alpha and Behavior
R² = 0.0214
p = .495
R.Amg-Alpha and Behavior
R² = 0.0284
p = .431
ACC-Beta and Behavior
R² = 0.0033
p = .791
R.Amg-Beta and Behavior
R² = 0.0635
p = .235
ACC-Gamma and Behavior
R² = 0.0607
p = .246
R.Amg-Gamma and Behavior
R² = 0.3804
p = .001
Mean normalized LFP power (ratio)
Mean formalin pain score

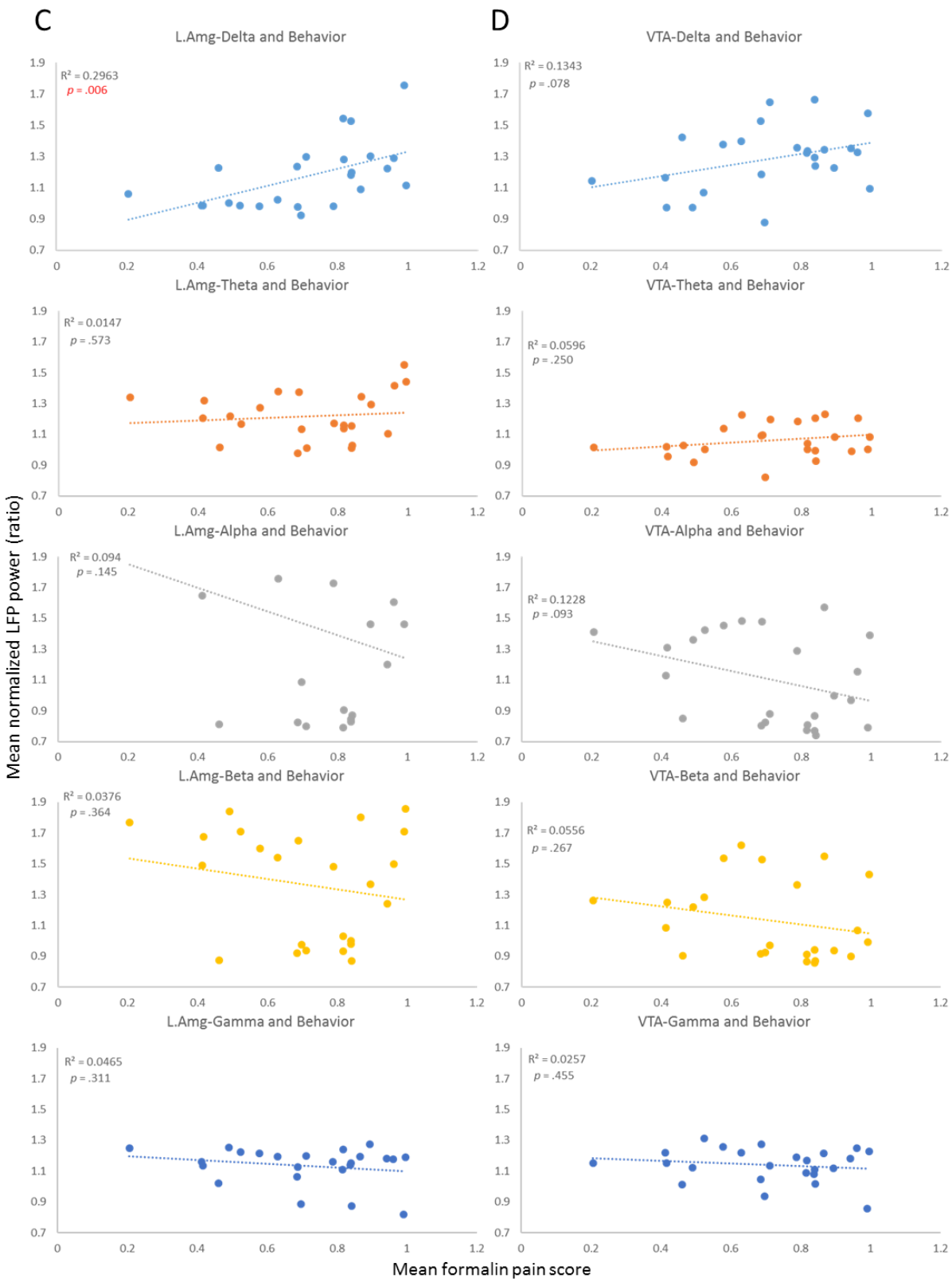

C
L.Amg-Delta and Behavior
R² = 0.2963
p = .006
L.Amg-Theta and Behavior
R² = 0.0147
p = .573
L.Amg-Alpha and Behavior
R² = 0.094
p = .145
L.Amg-Beta and Behavior
R² = 0.0376
p = .364
L.Amg-Gamma and Behavior
R² = 0.0465
p = .311
D
VTA-Delta and Behavior
R² = 0.1343
p = .078
VTA-Theta and Behavior
R² = 0.0596
p = .250
VTA-Alpha and Behavior
R² = 0.1228
p = .093
VTA-Beta and Behavior
R² = 0.0556
p = .267
VTA-Gamma and Behavior
R² = 0.0257
p = .455
Mean normalized LFP power (ratio)
Mean formalin pain score

Figure 5. Correlations between behavior and delta band, theta band, alpha band, beta band, gamma bands at (A) ACC, (B) right amygdala, (C) left amygdala, and (D) VTA.

# Chapter 4 DISCUSSION

Local field potential power changes were observed from the ACC, bilateral amygdala, and VTA simultaneously both in anesthetized animals (Figure 2) and freely moving animals (Figure 3). It matched well with existing knowledge that these regions contributed to the emotional aspect of pain (Bushnell et al., 2013). Differential LFP activities from brain regions in the response to noxious stimulus (formalin injection) could be used in generating neural signature for pain in the future.

When comparing the LFP power responses among these four regions (unilateral ACC and VTA, and bilateral amygdala), we only detected that ACC showed higher responses in gamma band comparing to other regions in anesthetized animals. More analysis methods should be conducted to compare the different responses to pain. For example, phase-amplitude coupling (PAC), one of kinds of cross-frequency coupling, could not only demonstrate the brain activity in each frequency band, but also reveal the interaction between oscillations in different bands (Samiee and Baillet, 2017).

For the previous study of formalin pain, there were three phases after formalin injection: the first phase which lasts around 5 minutes, the interphase which lasts 5-15 minutes, and the second phase which lasts 15-60 minutes (Dubuisson and Dennis, 1977). However, in our study, the results did not match well which exhibited the prolonged phases than previous results (Figure 4). This phenomenon could be led to by some reasons. First, we carried out the surgery and injected buprenorphine one week before formalin pain measurement. When testing, the animals

worn the recording module on backpack, which caused difference pain behavior responses because of the extra burden.

When testing the relationship between electrophysiology and behavioral responses to pain, we did gather the significant correlations between behavior and delta band in the ACC and left amygdala region, and delta, theta, gamma band in the right amygdala (Figure 5). The correlation in VTA was not seen through Pearson's *r* correlation method. Maybe because the variable of "Time" was not considered in this analysis. In the future, we therefore would run the dynamics of neural-behavioral correlations which include the variable of time to measure the dynamics of the functional link between LFP bands and behavior (Smith et al., 2011). Cross correlation might also be conducted to decipher the various connectivity or connectome (Magrans et al., 2018).

Activation of ACC and VTA (Friedman et al., 2012) may provide a negative feedback, as demonstrated in our lab that electrical stimulation of ACC or VTA induced antinociceptive effect (Li et al., 2016; Senapati et al., 2005).

In the future, we would keep the same dose of formalin to compare different patterns of formalin response between anesthetize and freely moving animals, which could infer the influence of isoflurane to brain signal processing as well.

The LFP power changes in alpha and gamma bands from VTA, and gamma band from bilateral amygdala regions in anesthetized animals; alpha band from VTA in freely moving animals were not significant, suggesting a possible differential response pattern among these regions. With future study involving more brain regions, these neural signatures could be used to guide clinical evaluation for treatment of pain.

# Chapter 5 CONCLUSION

Differential LFP activities from brain regions in response to noxious stimulus (formalin injection) could be used in generating neural signature for pain in the future. With future study involving more brain regions, these neural signatures could be used to guide clinical evaluation for treatment of pain.

REFERENCES

Albanese A, Minciacchi D (1983) Organization of the ascending projections from the ventral tegmental area: a multiple fluorescent retrograde tracer study in the rat. J Comp Neurol 216:406-420.

Apkarian AV, Bushnell MC, Treede RD, Zubieta JK (2005) Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 9:463-484.

Balleine BW, Killcross S (2006) Parallel incentive processing: an integrated view of amygdala function. Trends Neurosci 29:272-279.

Berridge KC, and Kringelbach ML (2015) Pleasure systems in the brain. Neuron 96:646-664.

Bush G, Luu P, Posner MI (2000) Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 4:215-222.

Bushnell MC, Ceko M, Low LA (2013) Cognitive and emotional control of pain and its disruption in chronic pain. Nat Rev Neurosci 14:502-511.

Buzsáki G, Anastassiou CA, Koch C (2012) The origin of extracellular fields and currents--EEG, ECoG, LFP and spikes. Nat Rev Neurosci 13:407-420.

Calvino B, Levesque G, Besson JM (1982) Possible involvement of the amygdaloid complex in morphine analgesia as studied by electrolytic lesions in rats. Brain Res 233:221-226.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321-352.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816-824.

Derbyshire SW, Jones AK, Gyulai F, Clark S, Townsend D, Firestone LL (1997) Pain processing during three levels of noxious stimulation produces differential patterns of central activity. Pain 73:431-445.

Donahue RR, LaGraize SC, Fuchs PN (2001) Electrolytic lesion of the anterior cingulate cortex decreases inflammatory, but not neuropathic nociceptive behavior in rats. Brain Res 897:131-138.

Dubuisson D, Dennis SG (1977) The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4:161-174.

Friedman A, Lax E, Abraham L, Tischler H, Yadid G (2012) Abnormality of VTA local field potential in an animal model of depression was restored by patterned DBS treatment. European Neuropsychopharmacology 22: 64-71.

Fuchs PN, Peng YB, Boyette-Davis JA, Uhelski ML (2014) The anterior cingulate cortex and pain processing. Front Integr Neurosci 8:35.

Gaskin DJ, Richard P (2012) The economic costs of pain in the United States. J Pain 13:715-724.

Gu L, Uhelski ML, Anand S, Romero-Ortega M, Kim YT, Fuchs PN, Mohanty SK (2015) Pain inhibition by optogenetic activation of specific anterior cingulate cortical neurons. PLoS One 10.

Guo SG, Lv XH, Guan SH, Li HL, Qiao Y, Feng H, Cong L, Wang GM (2015) Silencing the NR2B gene in rat ACC neurons by lentivirus-delivered shRNA alleviates pain-related aversion. Int J Clin Exp Med 8:6725-6734.

Harris Bozer AL, Li AL, Sibi JE, Bobzean SA, Peng YB, Perrotti LI (2016) Local field potentials in the ventral tegmental area during cocaine-induced locomotor activation: Measurements in freely moving rats. Brain Res Bull 121:186-191.

Harris-Bozer AL, Peng YB (2016) Inflammatory pain by carrageenan recruits low-frequency local field potential changes in the anterior cingulate cortex. Neurosci Lett 632:8-14.

Hentall ID, Kim JL, Gollapudi L (1991) Responses of neurons in the ventromedial midbrain to noxious mechanical stimuli. Neurosci Lett 133:215-218.

Jasmin L, Burkey AR, Card JP, Basbaum AI (1997) Transneuronal labeling of a nociceptive pathway, the spino-(trigemino-)parabrachio-amygdaloid, in the rat. J Neurosci 17:3751-3765.

Ji G, Neugebauer V (2008) Pro- and anti-nociceptive effects of corticotropin-releasing factor (CRF) in central amygdala neurons are mediated through different receptors. J Neurophysiol 99:1201-1212.

Kajikawa Y, Schroeder CE (2011) How local is the local field potential? Neuron 72:847-858.

Katzner S, Nauhaus I, Benucci A, Bonin V, Ringach DL, Carandini M (2009) Local origin of field potentials in visual cortex. Neuron 61:35-41.

Kramer PR, Strand J, Stinson C, Bellinger LL, Kinchington PR, Yee MB, Umorin M, Peng YB (2017) Role for the Ventral Posterior Medial/Posterior Lateral Thalamus and Anterior Cingulate Cortex in Affective/Motivation Pain Induced by Varicella Zoster Virus. Front Integr Neurosci 11:27.

LaBuda CJ, Donahue R, Fuchs PN (2001) Enhanced formalin nociceptive responses following L5 nerve ligation in the rat reveals neuropathy-induced inflammatory hyperalgesia. Pain 94:59-63.

LaBuda CJ1, Fuchs PN (2005) Attenuation of negative pain affect produced by unilateral spinal nerve injury in the rat following anterior cingulate cortex activation. Neuroscience 136:311-322.

Lariviere WR, Fiorenzani P, Ceccarelli I, Massafra C, Sorda G, Canio CD, Aloisi AM (2011) Central CRH administration changes formalin pain responses in male and female rats. Brain Res 1383:128-134.

Lee IO, Kong MH, Kim NS, Choi YS, Lim SH, Lee MK (2000) Effects of different concentrations and volumes of formalin on pain response in rats. Acta Anaesthesiol Sin 38:59-64.

Li AL, Sibi JE, Yang X, Chiao JC, Peng YB (2016) Stimulation of the ventral tegmental area increased nociceptive thresholds and decreased spinal dorsal horn neuronal activity in rat. Exp Brain Res 234:1505-1514.

Lindén H, Tetzlaff T, Potjans TC, Pettersen KH, Grün S, Diesmann M, Einevoll GT (2011) Modeling the spatial reach of the LFP. Neuron 72:859-872.

Magrans de Abril I, Yoshimoto J, Doya K (2018) Connectivity inference from neural recording data: Challenges, mathematical bases and research directions. Neural Netw 102:120-137.

Marzbani H, Marateb HR, Mansourian M (2016) Neurofeedback: A Comprehensive Review on System Design, Methodology and Clinical Applications. Basic Clin Neurosci 7:143-158.

McGaugh JL (2004) The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 27:1-28.

Merskey H, Albe Fessard D, Bonica JJ, Carmon A, Dubner R, Kerr FWL, Lindblom U, Mumford JM, Nathan PW, Noordenbos W, Pagni CA, Renaer MJ, Sternbach RA, Sunderland S (1979) Pain terms: a list with definitions and notes on usage. Recommended by the IASP subcommittee on taxonomy. Pain 6:249–252.

Neugebauer V (2006) Subcortical processing of nociceptive information: basal ganglia and amygdala. Handb Clin Neurol 81:141-158.

Neugebauer V, Li W (2002) Processing of nociceptive mechanical and thermal information in central amygdala neurons with knee-joint input. J Neurophysiol 87:103-112.

Oliva I, Wanat MJ (2016) Ventral Tegmental Area Afferents and Drug-Dependent Behaviors. Front Psychiatry 7:30.

Ortega LA, Uhelski M, Fuchs PN, Papini MR (2011) Impairment of recovery from incentive downshift after lesions of the anterior cingulate cortex: emotional or cognitive deficits? Behav Neurosci 125:988-995.

Pape HC, Pare D (2010) Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev 90:419-463.

Papini MR, Fuchs PN, Torres C (2015) Behavioral neuroscience of psychological pain. Neurosci Biobehav Rev 48:53-69.

Pastoriza LN, Morrow TJ, Casey KL (1996) Medial frontal cortex lesions selectively attenuate the hot plate response: possible nocifensive apraxia in the rat. Pain 64:11-17.

Price DD (2000) Psychological and neural mechanisms of the affective dimension of pain. Science 288:1769-1772.

Porro CA, Cavazzuti M (1993) Spatial and temporal aspects of spinal cord and brainstem activation in the formalin pain model. Prog Neurobiol 41:565-607.

Ranaldi R (2014) Dopamine and reward seeking: the role of ventral tegmental area. Rev Neurosci 25:621-630.

Samiee S, Baillet S (2017) Time-resolved phase-amplitude coupling in neural oscillations. Neuroimage 159:270-279.

Schultz W (2007) Behavioral dopamine signals. Trends Neurosci 30:203-210.

Scott DJ, Heitzeg MM, Koeppe RA, Stohler CS, Zubieta JK (2006) Variations in the human pain stress experience mediated by ventral and dorsal basal ganglia dopamine activity. J neurosci 26:10789-10795.

Senapati AK, Lagraize SC, Huntington PJ, Wilson HD, Fuchs PN, Peng YB (2005) Electrical stimulation of the anterior cingulate cortex reduces responses of rat dorsal horn neurons to mechanical stimuli. J Neurophysiol 94:845-851.

Sheng J, Liu S, Wang Y, Cui R, Zhang X (2017) The Link between Depression and Chronic Pain: Neural Mechanisms in the Brain. Neural Plast.

Smith JET, Zhan CA, Cook EP (2011) The functional link between area MT neural fluctuations and detection of a brief motion stimulus. J Neurosci 31: 13458-13468.

Swift A (2018) Understanding pain and the human body's response to it. Nursing Times 114:22-26.

Vogt BA (2005) Pain and emotion interactions in subregions of the cingulate gyrus. Nat Rev Neurosci 6:533-544.

Vogt BA, Finch DM, Olson CR (1992) Functional heterogeneity in cingulate cortex: the anterior executive and posterior evaluative regions. Cereb Cortex 2:435-443.

Volkow ND, Morales M (2015) The Brain on Drugs: From Reward to Addiction. Cell 162:712-725.

Zhuo M (2013) Long-term potentiation in the anterior cingulate cortex and chronic pain. Philos Trans R Soc Lond B Biol Sci 369.

Zugaib J, Coutinho MR, Ferreira MD, Menescal-de-Oliveira L (2014) Glutamate/GABA balance in ACC modulates the nociceptive responses of vocalization: an expression of affective-motivational component of pain in guinea pigs. Physiol Behav 126:8-14.