

LOCAL FIELD POTENTIAL SIGNATURES IN THE ANTERIOR CINGULATE  
AND PRIMARY SOMATOSENSORY CORTICES  
DURING PAIN PROCESSING

by

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Abstract

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Pain is the primary motive to seek physician care and animal research is informative for investigating the brain activity that underlies pain. Extensively studied brain areas involved in pain processing include the anterior cingulate cortex (ACC) and primary somatosensory cortex (S1) which contribute to the emotional and sensory dimensions of pain processing, respectively. Rodent studies have implicated these areas using a variety of methods including: examining behavioral effects of lesions, post-mortem tissue recording or biomarking, and recording or stimulation of intact target areas. However, information about the broad-range, low-frequency neural activity known as local field potential (LFP) in those areas has yet to be extensively investigated in real time. A series of experiments were designed to elucidate the local field potential activity in the ACC and S1 areas during carrageenan inflammation and by peripheral nerve stimulation using the novel cuff stimulating electrode (CSE) model implanted at the L5 nerve site. Results indicated that the S1 responded similarly with no significant changes to a variety of mechanical and electrical stimulations whereas the ACC responded in a stimulus intensity-dependent fashion to resting inflammation and suprathreshold mechanical stimulation. A trend towards a graded, intensity-dependent relationship between electrical

stimulation and ACC LFP emerged, albeit was not significant. Further examination of the CSE model in a separate experiment explored the possibilities of using the model as a spontaneous pain model. Peripheral nerve stimulation by CSE yielded spontaneous pain behaviors at the high (100 Hz, .5v) stimulation parameter. Aversiveness of the stimulation measured with an avoidance paradigm did not yield significant avoidance of high vs. low stimulation over time, yet avoidance scores were consistent with previously published data for other pain conditions. Taken together, these findings elucidate the low-frequency brain activity in the ACC and S1 and demonstrate potential for the CSE model for use in investigating spontaneous pain.

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## Chapter 1

### INTRODUCTION

Pain is one of the great, universal mysteries of the world and although it has been studied for centuries, much about it still eludes us. Enigmas abound in the study of pain including the reports of severely wounded soldiers in wartime who feel no pain, patients reporting pain in the absence of any physical explanation, or individuals that are incapable of experiencing pain altogether (Beecher, 2012; Fields, 2004; Melzack & Wall, 2004). There is no satisfactory definition of pain. Nevertheless, the International Association for the Study of Pain has defined pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (2002, 210). The nebulous nature of this definition reflects the complexity of pain, and highlights the indispensable need for further research (Anand & Craig, 1996; Derbyshire, 1999; Kopelman, 1999; Wright, 2011).

Pain is adaptive because it can raise the alarm that there is potential for danger, but when the alarm becomes chronic, excessive, or disproportionate to the hazard, the adaptive advantage is diminished (Barrot, 2012). Pain is the top reason that patients seek medical attention each year (McCarberg & Billington, 2006). When including direct healthcare costs and costs related to loss of worker productivity, the total expenditure annually in the U.S. is estimated to be upwards of \$635 billion (Gaskin & Richard, 2012; Gereau et al., 2014; Mavandadi, Rook, & Newsom, 2007; Penney, 2011; Pizzo, 2011). In addition, pain can result in indirect costs such as reduced time spent at work, reduced quality of life, and with comorbidities such as depression (McCarberg & Billington, 2006; Stewart, Ricci, Chee, Morganstein, & Lipton, 2003; Strassels, 2006).

Pain can result from a variety of factors and there is no blood examination, genetic indicator, or other measure that can assist in the definitive diagnosis of chronic

pain (Borsook & Becerra, 2006). Even further, pain involves the complex integration of sensory, affective/emotional, and quality of life components (Melzack & Casey, 1968). Pain is organized by a neural network that has been referred to as the “body-self neuromatrix” (Loeser & Melzack, 1999; Melzack, 1999). The neuromatrix architecture is influenced by genetic and sensory mechanisms and the network is comprised of somatosensory, thalamocortical, and limbic processing structures. Various inputs converge to stimulate the network including sensory and emotional inputs and stress-regulatory and modulation systems (Melzack, 1999). Ultimately, pain is a perception which occurs in the brain. Bushnell, Čeko, and Low have detailed the brain pathways involved in ascending pain processing (2013) (Figure 1-1).

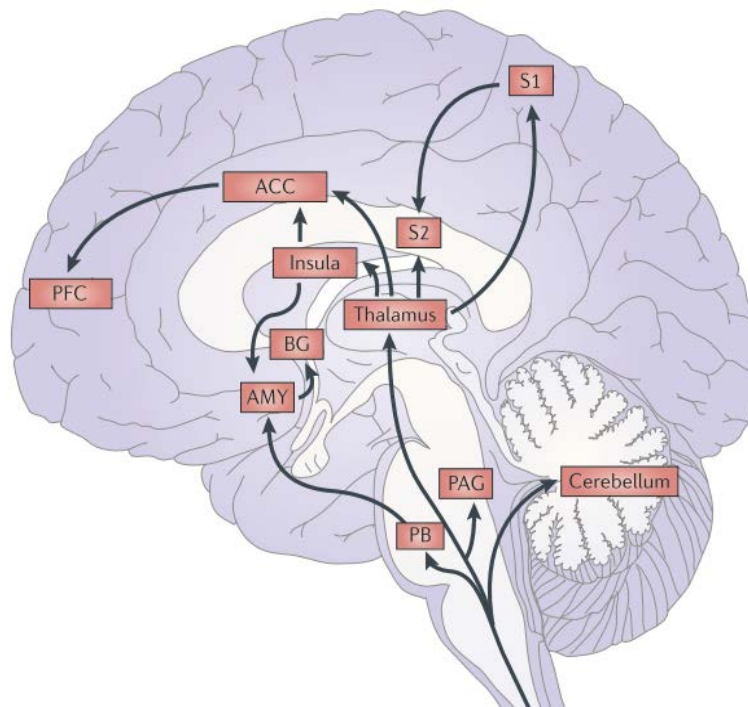


Figure 1-1 Ascending Pain Pathways in the Brain (Bushnell, Čeko, & Low, 2013)  
 Reprinted by permission from Macmillan Publishers Ltd: [NATURE REVIEWS NEUROSCIENCE] (Bushnell, M. C., Čeko, M., & Low, L. A. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. *Nature Reviews Neuroscience*, 14(7), 502–11) copyright (2013).

Imaging studies spanning the last three decades have yielded a multitude of data about how the human brain processes pain using a variety of techniques such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and the electroencephalogram (EEG) (Apkarian, Bushnell, Treede, & Zubieta, 2005; Borsook & Becerra, 2003). Imaging reveals that noxious input travels through the spinal cord into the thalamus (Bushnell et al., 2013). One major conduit for information is the spinothalamic pathway wherein the thalamus projects information to the anterior cingulate cortex (ACC) which projects to the prefrontal cortex. The thalamus also projects to the insular cortex as well as the primary (S<sub>1</sub>) and secondary somatosensory cortices (Bushnell et al., 2013). The areas in this pathway are some of the most frequently discussed in the literature for pain processing (Wall & Melzack, 2005). The scope of this work was focused on the ACC and the S1 areas.

### 1.1. Anterior Cingulate Cortex (ACC) and Pain

#### *Structure*

The ACC is located in the medial portion of the frontal lobes and is bordered by the cingulate sulcus and callosal sulcus (Yücel et al., 2001). The ACC is part of the limbic system and receives nociceptive projections from the medial and lateral thalamic regions (Bushnell et al., 2013; Wall & Melzack, 2005). Specifically, projections to the area are received from thalamic nuclei such as the nucleus parafascicularis (Pf), nucleus medialis dorsalis (MDvc), ventroposterior nucleus (VPvss) and the ventroposterior inferior nucleus (VPI) (Harte, Spuz, & Borszcz, 2011; Wall & Melzack, 2005). The ACC also receives projections from the insula and subsequently projects to the prefrontal cortex (Bushnell et al., 2013; Devinsky, Morrell, & Vogt, 1995). The ACC contains nociceptive specific neurons, the activation of which occurs bilaterally (Wall & Melzack, 2005). Cell types in

the ACC are widely heterogeneous (Vogt & Sikes, 2000; Vogt, 2009), and vary across species including by a dense population of spindle neurons in humans and apes that is lacking in other animals (Allman, Hakeem, Erwin, Nimchinsky, & Hof, 2001).

The ACC may also play a role in the descending pain inhibition system through multiple sites including the periaqueductal gray (PAG), rostral ventromedial medulla, and locus coeruleus (Fields, 2004; Ikeda, Takasu, & Murase, 2014; Senapati, Lagraize, et al., 2005). The PAG and RVM are major players in the descending inhibition system and both regions are influenced by the ACC (Heinricher, Tavares, Leith, & Lumb, 2009).

### *Function*

The sub-components of the ACC are functionally specialized, with some evidence to suggest that the dorsal portion is recruited for cognitive factors and the rostral-ventral portion is for emotional factors (Allman et al., 2001; Bush, Luu, & Posner, 2000; Devinsky et al., 1995; Etkin, Egner, & Kalisch, 2011; Gasquoine, 2013). Specifically, the ACC is involved in the affective-motivational dimension of pain and has often been cited in the development of pain affect in humans (Bentley, Derbyshire, Youell, & Jones, 2003; Coghill et al., 1994; Price, 2000; Rainville, Duncan, Price, Carrier, & Bushnell, 1997; Vogt, 2005).

The affective dimension of pain is generally described as the perceived unpleasantness and is correlated with intensity (Price, 2000). Cingulotomy/Cingulectomy results in reduced emotional but not sensory response to pain in some patients while most neurocognitive functions are remain intact (Bernad & Ballantine, 1987; Cohen et al., 2001; Foltz & White, 1962; Yen et al., 2005; Yen et al., 2009) and deep brain stimulation to the area targets the affective dimension of pain (Boccard et al., 2014). Interestingly, deCharms and his colleagues found that subjects can learn to control activity in the rostral portion of the ACC using real time rtfMRI neurofeedback, and subsequently report

a change in their perception of pain (deCharms et al., 2005). Psychophysical investigations have indicated that the affective dimension of pain can be dissociated from sensory pain (Price, 2000). This dissociation was highlighted when Corkin and Hebben found that the removal of the ACC attenuated the emotional component of pain, without disruption of the sensory component (Wall & Melzack, 2005). Rainville and his peers also determined that pain affect is encoded in the ACC but not in the sensory processing areas (1997). An illustration of the dimensions of pain related to their respective brain processing areas was provided by Price in Figure 1-2.

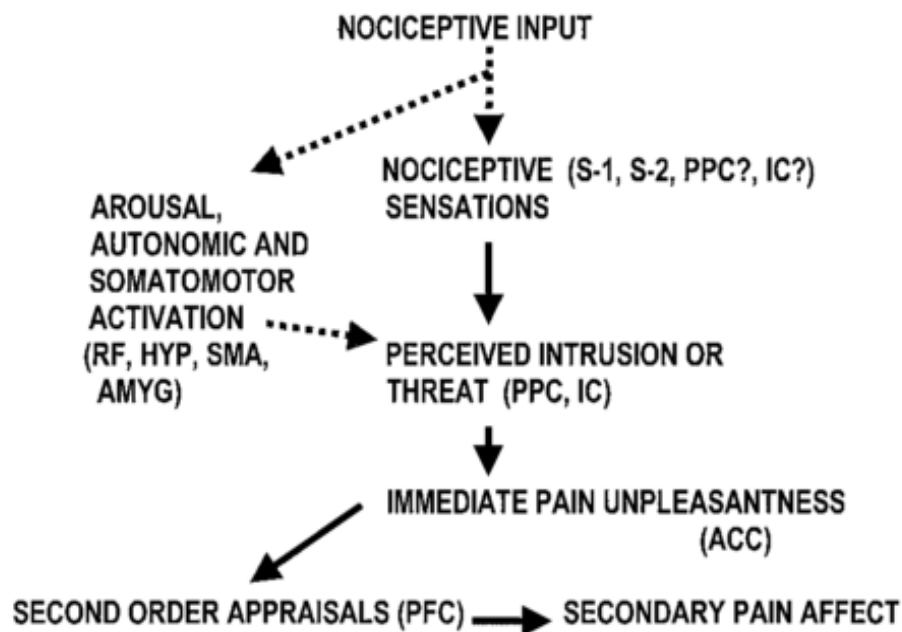


Figure 1-2 Relationship Between Brain Areas and Pain Dimensions (Price, 2000)  
 "From [Price, D. D. (2000). [Psychological and neural mechanisms of the affective dimension of pain. Science, 288\(5472\), 1769–1772.](#)]. Reprinted with permission from AAAS."

In primates, single cell recording activity is increased in the ACC during pain avoidance tasks (Koyama, Kato, Tanaka, & Mikami, 2001), and lesions of the ACC impeded the development of avoidance behaviors in Long Evans rats (Johansen, Fields,



& Manning, 2001) and in Sprague Dawley rats (Donahue, LaGraize, & Fuchs, 2001). In fact, the importance of the area has been demonstrated with a variety of noxious stimulation in rodents. This includes evidence from patch clamp recordings of enhanced synaptic excitation in slices of the ACC after injections of noxious bee venom (Gong et al., 2010) and increases in cholecystokinin in the ACC after a carrageenan-induced arthritis model (Erel, Arborelius, & Brodin, 2004). Animal studies including biomarker and lesion methods have implicated activation of the ACC during several commonly used rodent pain conditions (Fuchs, Peng, Boyette-Davis, & Uhelski, 2014; LaGraize, Labuda, Rutledge, Jackson, & Fuchs, 2004).

Numerous studies have indicated the role of the NMDA receptors in the ACC in nociceptive behavior (Fan et al., 2009; Lei, Sun, Gao, Zhao, & Zhang, 2004; T.-T. Li et al., 2009; López-Avila, Coffeen, Ortega-Legaspi, del Angel, & Pellicer, 2004; Wu et al., 2005). For example, formalin injection can induce ACC Fos activity and conditioned place avoidance behaviors that can be eliminated by administration of an NMDA receptor antagonist in the ACC (Lei et al., 2004). Sensory processing remained intact after lesions to the ACC while anxiodepressive effects were diminished in rats with unilateral sciatic nerve damage (Barthas et al., 2014). Likewise, sensory processing was not effected after lesion to the ACC in rats with L5 nerve ligation, yet late-phase formalin licking behaviors were reduced (Donahue et al., 2001). Pain avoidance behaviors can be attenuated with microinjection of morphine into the area (LaGraize, Borzan, Peng, & Fuchs, 2006), stimulation (LaBuda & Fuchs, 2005), or with lesions to the area (LaGraize et al., 2004). Measures of cFOS expression have confirmed activity in the area during pain avoidance (Uhelski, Morris-Bobzean, Dennis, Perrotti, & Fuchs, 2012). Additionally, stimulation of the ACC in rats inhibited the response of noxious stimulation in dorsal horn neurons in the spinal cord implicating the role of the area in the descending inhibitory pain pathway

(Senapati, Lagraize, et al., 2005). Taken together, converging behavioral and electrophysiological findings from these studies demonstrate the importance of the ACC in the processing of pain.

## 1.2. Primary Somatosensory Cortex (S1) and Pain

### *Structure*

The primary somatosensory cortex receives projections from the thalamus that contain spatial and temporal information about noxious input (Hofbauer, Rainville, Duncan, & Bushnell, 2001). The S1 projects information to the secondary somatosensory cortex (Bushnell et al., 2013). Unlike the ACC, specific input to the S1 is processed contralaterally (Coghill, Sang, Maisog, & Iadarola, 1999). The S1 has been suggested to be the only somatotopically organized brain area that is involved in processing the sensory dimension of pain while also demonstrating laterality of function (Wall & Melzack, 2005).

### *Function*

A relationship has been established between primary somatosensory cortex (S1) activity and pain processing in many studies using a variety of methods (Backonja, 1996). Imaging studies spanning three decades have indicated the importance of the S1 in processing pain (Backonja, 1996; Bushnell, Duncan, & Hofbauer, 1999). Extensively used methods include fMRI, PET, EEG, and MEG imaging techniques (Apkarian et al., 2005).

For example, control of pain intensity resulted in changes in S1 activity measured by PET imaging (Hofbauer et al., 2001). Additionally, painful stimulation by laser resulted in increased local field potential in the S1 in human subjects that were preparing to undergo surgical treatment for epilepsy (Liu, Franaszczuk, Crone, Jouny, & Lenz, 2011). It was also found that electrical stimulation to multiple regions of the body

resulted in MEG recorded somatotopic representation of noxious input in the S1 region (Omori et al., 2013b). Chronic pain results in an increase of power at the theta band in the S1 in humans and animals (Leblanc, Lii, Silverman, Alleyne, & Saab, 2014).

The S1 encodes the location of noxious input and has been related to mechanical allodynia in humans (Eto et al., 2011; Omori et al., 2013a; Petrovic, Ingvar, Stone-Elander, Petersson, & Hansson, 1999). This relationship was established in rats; lesions to the hind limb region of the somatosensory cortex resulted in asomaesthesia measured by an attenuation of sensory pain elicited by mechanical stimulation (Uhelski, Davis, & Fuchs, 2012). These examples also serve to bolster the aforementioned evidence that there are specific anatomical and functional neural mechanisms for processing sensory and affective dimensions of pain.

The role of the S1 has been demonstrated using other techniques as well. This includes research by Kalliomäki and colleagues who demonstrated that noxious thermal stimulation to the hindpaw of anesthetized resulted in a robust change in field potentials recorded in the S1 area (1993). During progressive stimulation at the sciatic nerve in anesthetized rats, optical imaging showed increased activity and spatial activation in the S1 with increasing intensity of stimulation, and surrounding inhibition in areas nearby (Luo, Li, Chen, & Luo, 2005). Increased spontaneous activity was also found in the mouse S1 in an inflammation model of pain (Eto et al., 2011). Furthermore, stimulation of the S1 region resulted in inhibition of noxious input transmission through the dorsal horn of the spinal cord (Senapati, Huntington, et al., 2005). For this reason, there is also evidence to suggest the importance of the S1 the descending inhibition of pain. Taken together, converging behavioral and electrophysiological findings from these studies demonstrate the importance of the S1 in the processing of pain.

### 1.3. Local Field Potential for Pain Studies

The aforementioned research methods aimed at investigating the brain mechanisms of pain processing have included techniques such as lesions, post-mortem tissue evaluation, biomarkers of activity, and recording or stimulation of brain areas in anesthetized animal experiments. However, the real time local field potential activity in these areas in freely moving animals has not been extensively investigated which provides an opportunity to the researcher to study the activity as it occurs.

Local field potential recording is a useful behavioral electrophysiology tool for studying brain activity and has gained increasing favor in recent years (Einevoll, Kayser, Logothetis, & Panzeri, 2013). Measurement is accomplished by inserting electrodes into the tissue and recording the low-frequency activity ranging from 0 to ~100 Hz, reflecting the spatially weighted sum of activity nearest to the electrode (Buzsáki, 2004, 2006; Mazzoni, Logothetis, & Panzeri, 2012).

#### *Contributing Constituents of LFP*

Local field potential is a measure of extracellular activity that is a result of multiple cellular influences (Buzsáki, Anastassiou, & Koch, 2012). Neuronal membranes contain a lipid bilayer that hold ions in or out of the cell (Bedard & Destexhe, 2012). When there is incoming input to a cell there is a subsequent response of bidirectional ion flow across the membrane, resulting in excitatory post-synaptic (EPSPs) or inhibitory post-synaptic potentials (IPSPs) (Pipa, 2006). Current spikes are caused by excitatory input and current sinks represent inhibitory input (Pipa, 2006). The prevailing perspective for some time was that recording of neural activity was principally influenced by excitatory input (Oren & Paulsen, 2010). However, synaptic currents that are inhibitory also result in recordable field potentials (Bazelon, Dinocourt, Cohen, & Miles, 2010).

The primary contributing components to LFP recording are EPSPs and IPSPs (Gyorgy Buzsáki, 2011). Low frequency activity such as non-synaptic calcium spikes, glial cell fluctuations and other subthreshold membrane oscillations, somatodendritic afterpotentials, and GABA<sub>A</sub> receptor inhibitory input also contribute (Berens, Keliris, Ecker, Logothetis, & Tolias, 2008; Buzsáki et al., 2012). Therefore, LFP provides a wider range of spatial recording than traditional measurement of single unit action potentials (Buzsáki, 2006; Mazzoni et al., 2012).

#### *Contribution of Action Potential Spiking*

The influence of action potentials on LFP measurement is negligible. High frequency action potentials are subject to attenuation over space (Buzsáki, 2006), so only those that occur very near to the electrode are recorded (Bédard, Kröger, & Destexhe, 2004). Additionally, the extracellular medium is composed of tissue and fluid creating a low pass filter that attenuates the high frequency activity of action potential spikes (Bédard, Kröger, & Destexhe, 2004). Specifically, local field potential activity is attained through application of a low pass filter in the range of 100-300 Hz (Mazzoni et al., 2012). Single unit activity, on the other hand, is attained through application of a high pass filter at frequencies larger than 500 or 600 Hz (Brette & Destexhe, 2012; David, Malaval, & Shamma, 2010; Rasch, Gretton, Murayama, Maass, & Logothetis, 2008; Waldert, Lemon, & Kraskov, 2013).

LFP detects sub-threshold activity in the vicinity of the electrode, providing a more comprehensive view of local network activity and synaptic dynamics than single-unit recording (Barbieri, Mazzoni, Logothetis, Panzeri, & Brunel, 2014; Mazzoni et al., 2012). Berens has postulated that the large generator LFP signals that occur at lower frequencies demonstrate coherent, large network communication (2008).

### *Spatial resolution*

Although there is agreement that local field potential represents the spatially weighted sum of this activity nearest the electrode, the actual contributing area has been a source of some debate (Kajikawa & Schroeder, 2011; Pesaran, 2009). The spatial reach of the recording is contingent upon multiple factors including the morphology of the cells in the region of the electrode (Kajikawa & Schroeder, 2011; Lindén et al., 2011; Zheng et al., 2012) and the electrode materials used (Csicsvari et al., 2003). Studies have suggested that local field potential activity is spatially recorded from hundreds of micrometers all the way up to 5mm (Einevoll et al., 2013; Kajikawa & Schroeder, 2011; Lindén et al., 2011). However, it has been determined recently that the local field potential reflects recordings within an average range of 200-400 micrometers (Katzner et al., 2009; Xing, Yeh, & Shapley, 2009).

### *LFP for Pain Studies*

Recent advancements in technology have allowed the recording of neural activity in small freely moving animal experiments (Ativanichayaphong et al., 2008; Chestek et al., 2006; Chien & Jaw, 2005; Greger, Kateb, Gruen, & Patterson, 2007; Grohrock, Häusler, & Jürgens, 1997; Hampson, Collins, & Deadwyler, 2009; Hawley, Hargreaves, Kubie, Rivard, & Muller, 2002; Heredia-López, Bata-García, Góngora-Alfaro, Alvarez-Cervera, & Azpiroz-Leehan, 2009; Nieder, 2000; Obeid, Nicoletis, & Wolf, 2004; Pinkwart & Borchers, 1987; Roy & Wang, 2012; Schregardus et al., 2006; Shaw, Chen, Tsao, & Yen, 1999; Wise et al., 2004; Ye et al., 2008; Zuo et al., 2012). Although local field potential has been widely applied in some areas of research, it has not been used much in freely moving rodent pain research. Recording LFP will contribute to the field in the following ways: 1) to confirm pain processing in the brain in real time, 2) to expand knowledge by revealing how activity changes over time and 3) revealing the brain activity that underlies

pain behaviors. There are numerous experimental models meant to map onto a myriad of human pain conditions, and LFP is a window into differential activity across conditions.

### *LFP during Pain Models*

Animal models of pain have been imperative for studying how pain is processed in the nervous system and provide research opportunities that require more intrusive methods than can be used in humans, including investigating how pain is processed in the brain (Mogil, Davis, & Derbyshire, 2010). Pain models in rodents consist of application of stimulation to the nervous system providing the researcher with an opportunity to study the subsequent processing in the brain. There are many rodent models that mimic clinical pain conditions (see Table 1-1) (Barrot, 2012; Gregory et al., 2013; Le Bars, Gozariu, & Cadden, 2001; Wang & Wang, 2003). Providing an extensive overview of all models available is outside of the scope of this research, but the table demonstrates a sample of the variety of models available to the researcher.

Table 1-1 Murine Pain Models

<b>Pain Model</b>	<b>Method</b>	<b>Reference</b>
<b>Inflammation</b>		
Subcutaneous Tissue	Carrageenan into Plantar Hind Paw	(Morris, 2003)
	Adjuvant into Plantar Hind Paw	(Millan et al., 1988)
Deep Tissue	Intramuscular Capsaicin into Plantar Hind Paw	(Sluka, 2002)
	Intra-articular Capsaicin into Ankle	(Sluka, 2002)
Musculoskeletal/Joint	Carrageenan into Knee Joint or Muscle	(Radhakrishnan, Moore, & Sluka, 2003)
Spontaneous (Acute)	Subcutaneous Formalin into the Hind Paw	(Dubuisson & Dennis, 1977)
Brain (M.S.)	Experimental Autoimmune Encephalomyelitis	(Pender & Sears, 1986)
<b>Cancer</b>		
Facial	Injection of Cancer Cells into the Face	(Hidaka et al., 2011)
Bone	Injection of Cancer Cells into the Tibia	(Walker et al., 2002)
	Injection of Cancer Cells into the Femur	(Schwei et al., 1999)
<b>Postoperative</b>		
Skin and Muscle	Lateral Incision of Skin, Fascia, and Muscle	(Brennan, Vandermeulen, & Gebhart, 1996)
<b>Visceral</b>		
	Intraperitoneal Acetic Acid	(Koster, Anderson, and DeBeer)
	Colorectal Distention	(Ness & Gebhart, 1990) (Ness & Gebhart, 1988)
<b>Neuropathy</b>		
Mechanical Injury	Loose Ligature of the Sciatic Nerve	(Bennett & Xie, 1988)
	Partial Ligation of the Sciatic Nerve	(Seltzer, Dubner, & Shir, 1990)
	Tight Ligation of the Spinal Nerve	(Kim & Chung, 1992) (Kim, Yoon, & Chung, 1997)
Diabetic	Intraperitoneal Streptozotocin (STZ) Injection	(Courteix, Eschalier, & Lavarenne, 1993)
Chemotherapeutic	Intravenous Vincristine Infusion	(Nozaki-Taguchi et. al, 2001)
Post-Herpetic Neuralgia	Injection of Herpes Varicella Zoster	(Dalziel et al., 2004)

### *Carrageenan Inflammation Model*

The carrageenan inflammation model was initially developed as a method for analyzing the efficacy of anti-inflammatory compounds (Winter, Risley, & Nuss, 1962) and it has been extensively investigated in rodent pain research. Inflammation can reliably be created by subcutaneous injection of carrageenan lambda, a plant polysaccharide (Morris, 2003; Necas & Bartosikova, 2013). This results in edema of the paw that maximizes at 3-5 hours post injection (Morris, 2003). The cardinal signs of inflammation are redness, swelling, heat, and pain which results from pressure on nerves (Wall & Melzack, 2005).

The biphasic inflammatory response to carrageenan begins with a first phase of edema caused by the release of histamine, serotonin, and bradykinin which cannot be attenuated with NSAIDS (Necas & Bartosikova, 2013). The second phase of edema includes a release of prostaglandins and Cox-2 which results in augmented vascular permeability and can be blocked by NSAIDS (Necas & Bartosikova, 2013). These neurotransmitters or cytokines will bind on their specific receptors on the sensory nerve terminals to induce pain. There are other mechanisms contributing to the development of edema such as introduction of neutrophils to the area which produce oxygen-derived free radicals. At the level of the spinal cord, primary afferents and other neurons activate glial cells through glutamate and neuropeptide release (Necas & Bartosikova, 2013).

Behavioral displays of thermal hyperalgesia (Hargreaves, Dubner, Brown, Flores, & Joris, 1988), mechanical hyperalgesia, vocalizations (Kayser & Guilbaud, 1987), and aversion to mechanical stimulation at the site also develop (LaBuda & Fuchs, 2001). Although the amount of carrageenan that is injected can be manipulated, the level of hyperalgesia or edema cannot be controlled or manipulated by the experimenter.



### *Cuff Electrode Model*

Experimental pain conditions can be graded at introduction, but the end result cannot be changed without treatment, application of external stimuli, or letting the natural course intensify. Based on initial discussion (Dr. Peng and Dr. Fuchs), we developed a novel pain model, the cuff- stimulating electrode (CSE), which addresses these issues. We hypothesize that one new experimental option is to provide the experimenter with the ability to control the pain condition in vivo, including the ability to confirm the response of brain areas to variable painful stimulation in real time. An additional benefit will be providing a reliable way to test spontaneous pain. In this cuff- stimulating electrode model (CSE), electrical stimulation of the peripheral nerve mimics spontaneous or shooting pain that is experienced by some neuropathic pain patients.

### *Correlating LFP with Pain Behaviors*

After the introduction of a pain model in rodents, behavioral testing is conducted to measure the efficacy of the model to develop a pain condition. Behavioral testing usually includes the application of electrical, mechanical, or thermal stimuli and the resulting behaviors are then quantified (Le Bars et al., 2001). Because pain is multidimensional, behavioral tests have been specifically related to the sensory or affective dimensions of pain (Gregory et al., 2013). There are many measurements of pain in animals (for extensive reviews see Barrot's from 2012, Gregory et al. from 2013 and Le Bars et al. from 2001). In this series of experiments, the mechanical paw withdrawal test (MPWT) and place escape avoidance paradigm (PEAP) will be applied.

### *Sensory Testing*

A well-established, commonly applied reflex-based test involves the application of Von Frey stimulus hairs which consist of filaments of varying diameter attached to an applicator stick (Barrot, 2012; Von Frey, 1896). The Mechanical Paw Withdrawal

Threshold test (MPWT) was initially created for use in humans, but the test was altered to be used in lab rodents (Barrot, 2012; Gregory et al., 2013). In rodents, withdrawal of the hind paw in response to mechanical stimulation by a Von Frey filament signals the reflexive withdrawal response. The MPWT is commonly used in rodent pain experiments to test for the development of allodynia and hyperalgesia. In a naïve animal, application of the filaments should not result in withdrawal. In an animal with an ongoing pain condition (such as the inflammation condition previously described), application of the filaments should elicit a withdrawal response (Barrot, 2012; Gregory et al., 2013). The hind limb region of the S1 area in rats has been associated with the MPWT sensory test, as lesion to the area impedes the development of withdrawal behavior during a pain condition in rodents (Uhelski, Davis, et al., 2012).

#### *Affective Testing*

Pain in research animals cannot be established solely by application of reflexive based measures (Chapman et al., 1985). Affect related to pain has been described as the perceived unpleasantness of the pain condition (Craig, 2003). Rodent tests for the affective dimension of pain focus on the aversive reactions of the animals that indicate unpleasantness. One measure of pain unpleasantness in rodents is the Place Escape Avoidance Paradigm (PEAP) (LaBuda & Fuchs, 2000). The test chamber is divided into light and dark sides of a chamber allowing the animal to roam freely inside the box and select the desired side of the chamber. Rats prefer the dark side of the chamber until it is paired with a noxious stimulus causing a shift in preference to the light side of the chamber. The test differs from sensory measures because rodents make a conscious decision about their placement in a box that is assigned to either non-noxious or noxious stimulation (LaBuda & Fuchs, 2000).

Lesions to the ACC result in decreased PEAP scores but not MPWT scores in animals with a pain condition indicating that the area is imperative for the development of pain affect but not pain sensation in rodents (LaGraize et al., 2004). A separate study indicated that excitotoxic lesions to the ACC did not influence the mechanical hypersensitivity induced by sciatic nerve cuff implantation, yet attenuated anxiodepressive effects of pain (Barthas et al., 2014).

Microinjection of GABA and muscimol (a GABA<sub>A</sub> agonist) into the rostral portion of the ACC similarly attenuated PEAP avoidance but not MPWT hypersensitivity (LaGraize & Fuchs, 2007). Furthermore, the expected underlying cellular activity was confirmed in the ACC during the PEAP test but was not related to the MPWT test when c-FOS was measured (Uhelski, Davis, et al., 2012). These studies offer further evidence that differential brain activity underlies the sensory and affective dimensions of pain subserved by the S1 and ACC areas, respectively.

#### 1.4. Purpose of This Research

The primary objective of this research was to examine the contribution of low frequency activity in the ACC and S1 brain areas underlying pain. The **central hypothesis** was that LFP activity in the ACC and S1 will be contribute differentially to pain. Specifically, the ACC will respond to higher intensity stimulation and the role of the ACC in processing affective behavioral testing will be confirmed in real time during freely moving animal testing. The S1 will respond to a broader range of stimulation parameters and the role of the S1 in processing sensory behavioral testing will be confirmed in real time during freely moving animal testing. This will be accomplished by implementing the following four specific aims:

Aim 1: Determine the LFP ACC activity during behavioral displays of hyperalgesia elicited by repeated suprathreshold mechanical stimulation after carrageenan inflammation.

Procedures: Record LFP in the ACC area of freely moving rodents during carrageenan inflammation and suprathreshold mechanical stimulation.

**H<sub>1</sub>:** Injection of carrageenan will result in an increase in LFP activity in the ACC.

**H<sub>2</sub>:** After injection of carrageenan, repeated suprathreshold mechanical stimulation will further increase the LFP activity.

Aim 2: Examine ACC and S1 activity during inflammatory pain and further expand their contributions to the affective and sensory dimensions of pain, respectively.

Procedures: Record LFP activity in the ACC and S1 in freely moving rats during carrageenan inflammation and during standard behavioral tests of hyperalgesia (MPWT) and pain affect (PEAP).

**H<sub>3</sub>:** Injection of carrageenan will result in an increase in LFP activity in the ACC that will be significantly increased during the standard mechanical paw withdrawal threshold test (MPWT).

**H<sub>4</sub>:** ACC LFP will increase during the PEAP test of pain affect. Specifically, ACC activity will be highest when in the dark side of the PEAP chamber.

**H<sub>5</sub>:** The S1 LFP will respond to stimulation in the MPWT and PEAP tests similarly.

**H<sub>6</sub>:** S1 LFP activity will not differ in the light vs. dark side of the chamber.

Aim 3: Determine the dynamic LFP response profiles of ACC and S1 to peripheral nerve stimulation using a cuff electrode.

Procedures: Record LFP activity in the ACC and S<sub>1</sub> in anesthetized rats during variable and progressive electrical stimulations to the L5 nerve.

**H<sub>7</sub>:** The ACC will increase in response to high intensity stimulation.

**H<sub>8</sub>:** The S1 will respond similarly to a wide range of stimulation parameters.

Aim 4: Determine the effectiveness of cuff electrode model as a model of spontaneous pain.

Procedures: The cuff electrode will be implanted in freely moving animals and measures of mechanical hyperalgesia, pain affect, and spontaneous pain behavior will be recorded during progressive electrical stimulation to the L<sub>5</sub> nerve.

**H<sub>9</sub>**: The cuff model will result in spontaneous pain behaviors.

**H<sub>10</sub>**: The cuff model stimulation will result in the development of pain affect.

## Chapter 2

### LFP IN THE ACC DURING SUPRATHRESHOLD MECHANICAL STIMULATION

#### 2.1. Background

It is well known that the ACC is involved in processing pain. However, our current knowledge of how local field potential (LFP) activity changes in the ACC in real time during commonly used pain models such as carrageenan inflammation is limited. Furthermore, our understanding of the underlying brain activity for behavioral displays of hypersensitivity as it occurs is inadequate. The use of our custom-designed wireless recording module (Ativanichayaphong et al., 2008) presents an opportunity to assess changes in neuronal activity during pain conditions freely moving rats. Moving forward, we want to use our device to investigate the neuronal activity underlying pain in freely moving animals and the sensory tests of hyperalgesia that are standard measures in rodent pain research present a logical place to begin creating LFP response profiles during pain. The aim (***Aim 1***) of the first experiment was to determine the LFP ACC activity during behavioral displays of hyperalgesia elicited by repeated suprathreshold mechanical stimulation after carrageenan inflammation. The first hypothesis (***H<sub>1</sub>***) was that introduction of the carrageenan inflammation will result in an increase of activity in the ACC. This would confirm the role of the ACC during inflammatory pain in freely moving animals. The second hypothesis (***H<sub>2</sub>***) was that after injection of carrageenan, application of suprathreshold mechanical stimuli will further increase the LFP activity in the ACC, over and above activity from the carrageenan alone.

#### 2.2. Subjects

Twenty-three adult Sprague Dawley rats aged 4-7 months were taken at random from the University of Texas at Arlington vivarium. Subjects were placed into a separate room on a 7:30 a.m. to 7:30 p.m. light/dark cycle and housed in cages of 2-3. Animals

were given access to food and water ad libitum. Testing occurred during the light cycle. All procedures had the approval of the UT Arlington Institutional Care and Use Committee and followed the ethical guidelines for pain experiments in animals (Zimmermann, 1983). Animals were placed into carrageenan and saline groups. Seven animals were excluded after histology revealed that the electrode was not in the target area leaving 8 in each group.

### 2.3. Methods

Baseline measures of mechanical sensitivity were assessed to ensure that there was no preexisting hypersensitivity. Electrodes were implanted into the ACC region and 7-10 days of recovery were allotted. On the test day, baseline MPWT data were collected again and LFP was recorded for 3 minutes first while animals were under anesthesia and then while awake. Injections of carrageenan or saline were administered and 3 hours were allotted for the development of the inflammation condition before LFP was recorded again for 3 minutes. Next, LFP was recorded during repeated stimulation of the injected hind paw with a suprathreshold Von Frey mechanical stimulus (100g / 476 mN) for 3 minutes. Overall procedures are summarized in Figure 2-1 and specific procedures are addressed in the following sections.

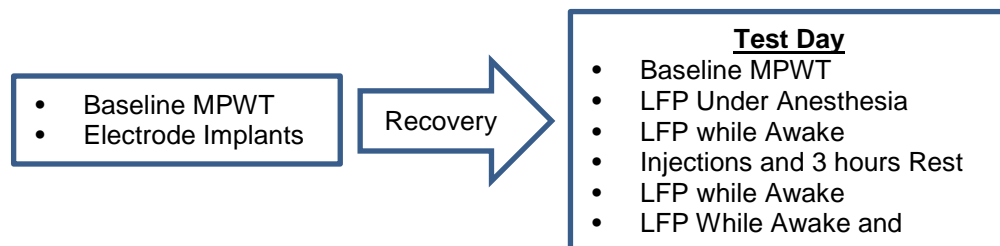


Figure 2-1 Procedural Flowchart for Experiment One

#### *Electrode Implantation*

Recording electrodes were surgically placed for chronic implantation. All tools and equipment were autoclaved before surgery and the head was shaved and skin was

prepared with aseptic scrub before surgery. Under 3% Isoflurane inhaled anesthesia, the head was fixed into ear bars on a stereotaxic frame. The skin was incised on the top of the skull. Bregma was located using coordinates from "The Rat Brain in Stereotaxic Coordinates" and a burr hole was made over the region of interest before the tungsten bipolar recording microelectrode (PlasticsOne) was placed in the ACC (Figure 2.2: circle 4) at .7 mm lateral, -3.2 mm dorsal from Bregma at a 15 degree angle (Paxinos & Watson, 1998). Three additional burr holes were created in the skull for the placement of three stainless steel screws (1.57 mm shaft diameter, Plastics One, Roanoke VA). One screw was placed anterior to the electrode site as an anchor (Figure 2.2: circle 1). Two hind screws were placed posterior to the electrode site on the left and right sides of the midline. Pliable wire was placed underneath the 2 hind screws to be connected later to the recording module ground and reference wires (Figure 2-2: circles 2 and 3).

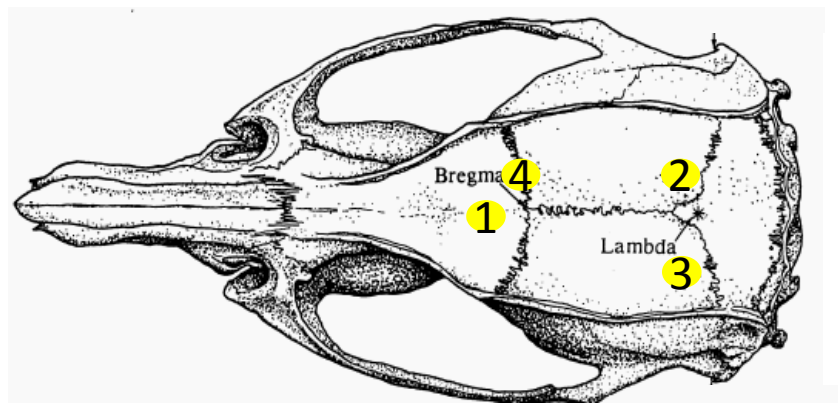


Figure 2-2 Placement of Electrodes and Screws (Paxinos & Watson, 1998)  
Circle 1 represents placement for the anterior anchor screw, circles 2 and 3 represent anchor screws with pliable wire connections for ground and reference, and circle 4 represents the ACC electrode placement. Skull diagram portion was reprinted with permission from Macmillan Publishers Ltd (Paxinos, G., & Watson, C. (1998). The rat brain in stereotaxic coordinates. Fourth edition. Academic Press.) Copyright (1998).

The electrode and screws were glued to the skull using dental cement. Simple interrupted sutures were used to close the skin around the implant with only wire and the



electrode protruding. Lidocaine was topically applied to the skin to treat incisional pain. Animals were monitored daily for distress or discomfort.

#### *Recording LFP*

Subjects were placed under 3% Isoflurane anesthesia and the implanted electrode was connected to the recording module. Reference and ground wires on the module were attached to the pliable wires that were attached to the implanted screws. The microcontroller module (specifications in Appendix A) was attached to a backpack that the rat could wear while freely moving, without restriction. Subjects were placed into a recording chamber and 20 minutes of recovery time was allotted before awake recordings began. After recording, animals were placed back under anesthesia to safely remove the electrode, reference, and ground connections from the microcontroller module.

#### *Carrageenan Inflammation Model*

The carrageenan model (Morris, 2003) was used to produce a unilateral inflammation condition. Animals received a subcutaneous injection of .05 mL of 1% carrageenan lambda (Sigma-Aldrich) dissolved in saline in the left hind paw. Animals assigned to the control group received an injection of .05 mL of saline. Three hours were allotted for the development of the condition before testing procedures began.

#### *MPWT Procedures*

Subjects were habituated for 10 minutes to a Plexiglas chamber atop a mesh floor platform. Von Frey monofilaments ranging from 9.71 to 205.94 mN (.6 to 26 grams) of force were applied to the plantar surface of the hind paw until the filament bended, beginning with the lowest force filament and ascending through the series. Responses or lack thereof, determined the pattern of application of the following stimuli. Responses included licking, flicking, raising, or any removal of the paw that resulted from filament

application. Responses resulted in application of a filament of a higher force, and no response resulted in application of a lower force stimulus in the series.

Calculation of MPWT scores took into account the pattern of responses, the force of the filament eliciting the first response, and the force of the final filament applied. The Dixon up-down method was used, with the modification of using 5 total stimulations after the first response during each trial (1980). Three trials were averaged for each paw. Lowered scores indicate hypersensitivity (referred to as a “drop in threshold”). The expected outcome was that all animals at baseline and after saline injection will show a maximum threshold, and animals injected with carrageenan will display a drop in threshold indicating sensitivity to mechanical stimuli.

#### *Euthanasia*

Animals were euthanized with carbon dioxide gas following the guidelines of the American Veterinary Medical Association’s guidelines for euthanizing rodents (AVMA Panel on Euthanasia, 2007). Animals were administered carbon dioxide gas in their home cage for several minutes past the time when reflexive respiration terminated.

#### *Histology*

Brains were extracted and fixed in a 33% formaldehyde solution for at least 48 hours. Brains were moved to a 30% sucrose solution for at least 48 hours and then sliced using a microtome (American Optical Corporation, Buffalo NY, Model 860) at 80µm. Slices were mounted to slides coated with gelatin and stained with Thionine. Slides were cover slipped using Shur/Mount Toluene based liquid mounting media (Triangle Biomedical Sciences, Durham NC). Placement was confirmed under the microscope by 2 observers and data was excluded for 8 subjects that had electrode placement that was not in the target region defined by “The Rat Brain in Stereotaxic Coordinates” (Paxinos & Watson, 1998) (See Appendix B).

## 2.4. Results

### *Injection of Carrageenan Reduced MPWT Threshold*

Mixed repeated measures ANOVAs were run with injection as the between subjects variable (carrageenan or saline) and MPWT scores over time (baseline and post-injection) as the dependent variable for both left and right paw scores to assess the efficacy of the injections. LSD post hoc tests were run to investigate significant effects when there was an interaction. The expectation was that carrageenan would result in a significant drop in MPWT threshold and that the saline injection would not.

Results for the **left injected paw** MPWT scores mixed repeated measures ANOVA indicated that there was a significant main effect for time  $F(1, 14) = 36.751, p < .001$ , for injection  $F(1, 14) = 24.297, p < .001$ , and a significant interaction effect  $F(1, 14) = 21.482, p < .001$ . Significant post hoc tests revealed that carrageenan group scores dropped significantly after injection ( $p < .001$ ), and carrageenan scores ( $n=8$ ) were significantly lower than saline scores ( $n=8$ ) after injection ( $p < .001$ ). Results for the left paw are summarized in Figure 2-3.

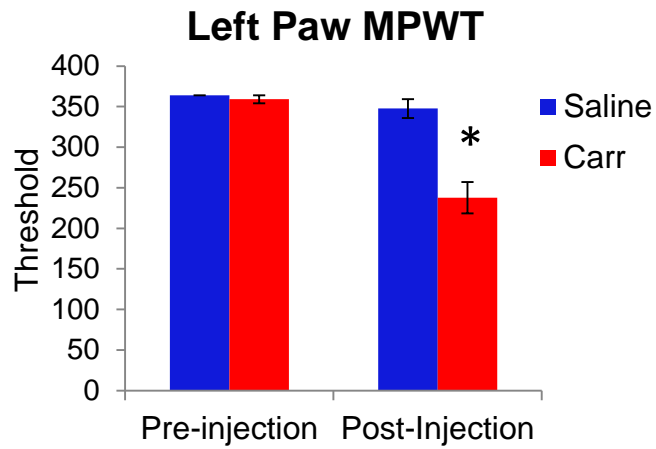


Figure 2-3 Left Paw MPWT Scores by Group

The asterisk represents a significant drop in threshold from baseline to injection scores in the Carrageenan group as well as a significant difference from the saline control group at the post-injection time point. carrageenan n=8, saline= 8.

Results for the **right paw** MPWT scores indicated that there was no significant main effect for time  $F(1, 14) = .154, p = .700$ , for injection  $F(1, 14) = .182, p = .676$ , or interaction effect  $F(1, 14) = 1.846, p = .196$ . Results for the right paw are summarized in Figure 2-4.

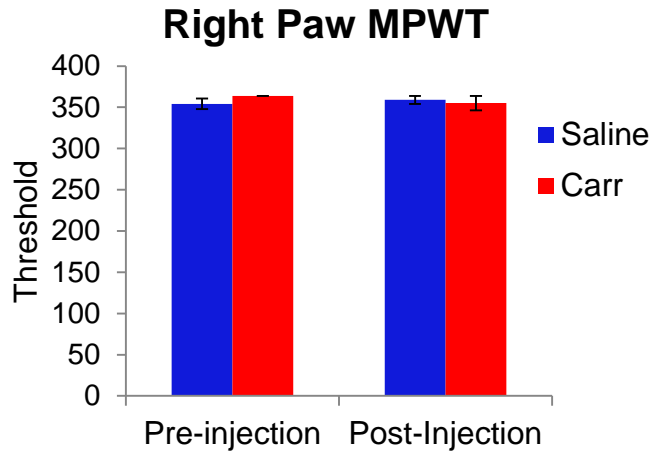


Figure 2-4 Right Paw MPWT Scores by Group

There were no significant differences between saline and carrageenan group scores in the right paws that received no injection. Additionally, there were no significant differences from pre-injection to post-injection. carrageenan n=8, saline= 8.

*Carrageenan and Suprathreshold Mechanical Stimulation Increased ACC LFP*

After recording in custom software, raw local field potential data were packaged in a text file that was imported into Spike 2 (Cambridge Electronic Design) for analysis using a sampling rate of 4096 Hz. Using Spike 2, sixty-four spectrograms of the local field potential data were viewed for all of the LFP time points (Isoflurane, awake, carrageenan, and carrageenan with stimulation).

Sixty-four power spectrum analyses using fast Fourier transform were computed (Spike software) using an FFT block size of 4096 and the Hanning window to display the power at each frequency for each animal at each of the time points (16 Isoflurane, 16 awake, 16 after injection, and 16 during stimulation). For each power spectrum, a histogram was constructed between 0 -100 Hz. A sample of raw data spectrograms and power spectrum histograms are available in Figure 2-5.

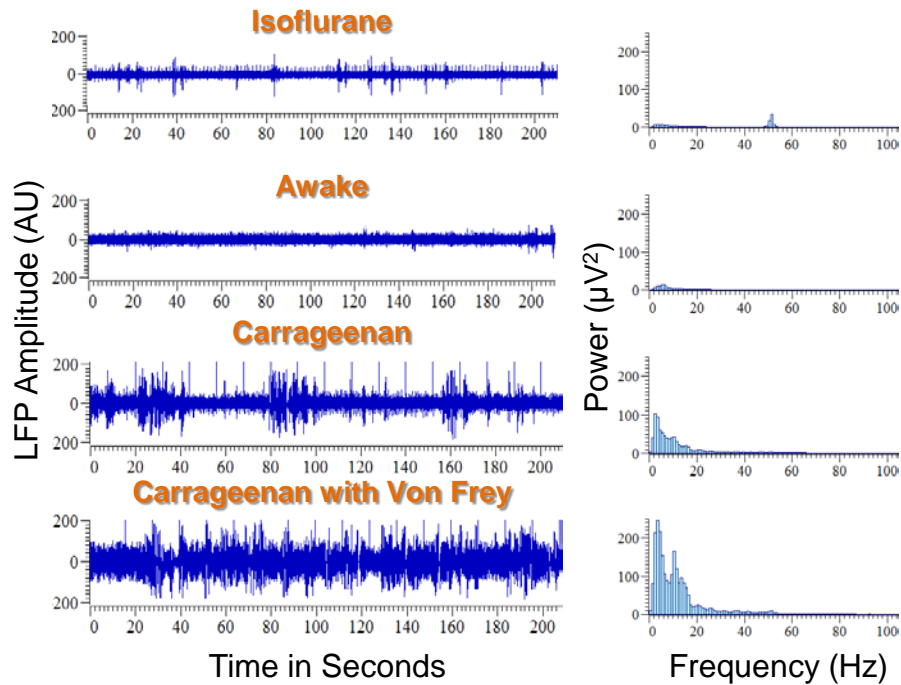


Figure 2-5 Raw Data Sample Spectrograms with Power Spectrums (n=1)  
 The left side of the diagram depicts sample LFP raw traces during recording time points (Isoflurane, awake, carrageenan, and carrageenan with Von Frey stimulation). The right side of the diagram depicts corresponding sample power spectrum analyses in histogram format (from frequencies 0-100).

Each power spectrum analysis was saved as a text file that displayed the power at each frequency. This data was imported into an excel file and organized by animal, brain recording area, and frequency band (Delta 0 - 3 Hz, Theta 4 - 8 Hz, Alpha 9 - 13 Hz, Beta 14 - 30 Hz, and Gamma 31 - 100 Hz). The mean (average of all subjects) and standard error of the mean (SEM) for power at each frequency band was computed in Excel, graphed, and imported into SPSS for analysis. The overall power spectrum at each band is available in Figure 2-6.

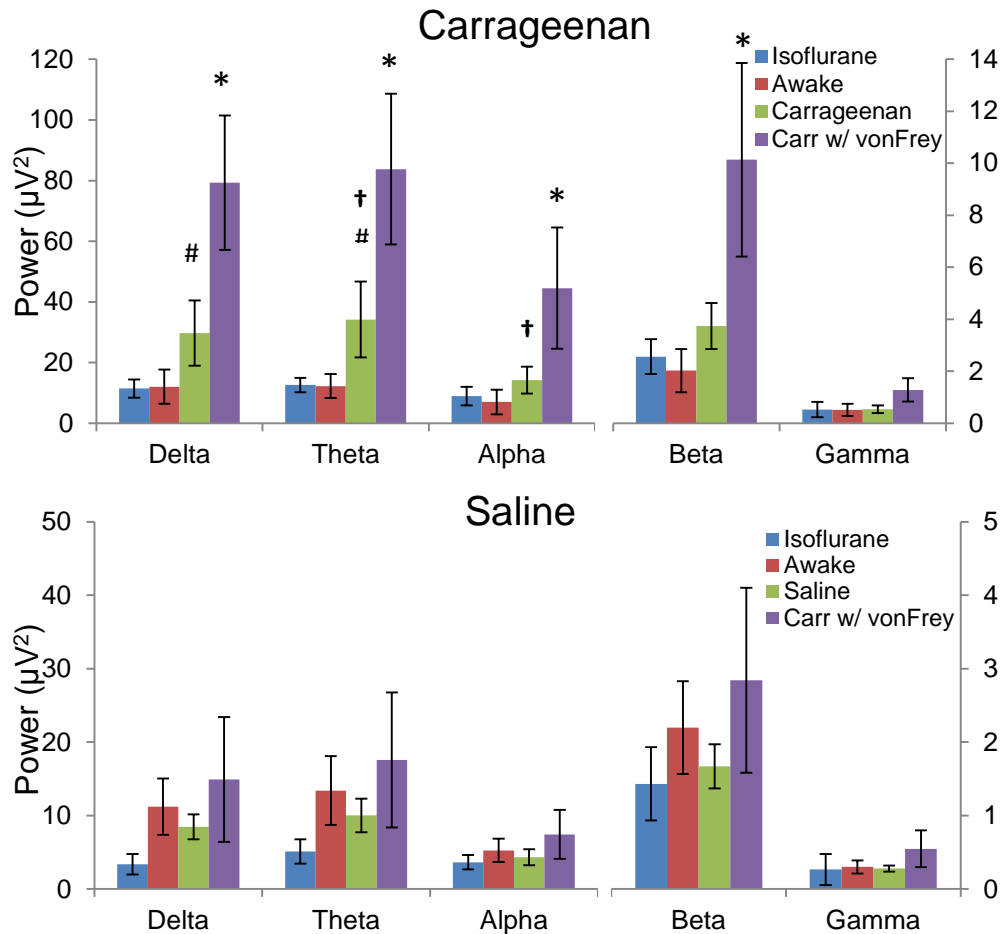


Figure 2-6 Summary of ACC LFP Data in Frequency Bands

LFP data in each frequency band are demonstrated for the saline group (top) and carrageenan group (bottom). There were no significant differences in the saline group. In the carrageenan group, significant differences are denoted by # Higher than Isoflurane † Higher than Awake \* Higher than Isoflurane, awake, and carrageenan. carrageenan n=8, saline= 8.

In SPSS, overall analyses of the power at the predefined frequency bands was conducted using repeated measures ANOVAs and LSD post hoc tests. A separate ANOVA for each frequency band was computed to determine where the power of the signal was distributed across frequency bands.

Delta band analyses included frequencies from 0 – 3 Hz. There was a significant main effect of time  $F(3, 42) = 8.836, p < .001$ , a significant main effect for injection  $F(1,$

14) = 7.334,  $p = .017$ , and a significant interaction effect  $F(3, 42) = 5.606$ ,  $p = .003$ . Post hoc tests revealed no significant differences in the saline group data. Carrageenan was higher than Isoflurane ( $p = .028$ ). Carrageenan with stimulation was higher than Isoflurane ( $p = .001$ ), awake ( $p = .001$ ), and carrageenan ( $p = .002$ ).

Theta band analyses included frequencies from 4 - 8 Hz. There was a significant main effect of time  $F(3, 42) = 7.093$ ,  $p < .001$ , a significant main effect for injection  $F(1, 14) = 7.591$ ,  $p = .015$ , and a significant interaction effect  $F(3, 42) = 4.457$ ,  $p = .008$ . Post hoc tests revealed no significant differences in the saline group data. Carrageenan was higher than Isoflurane ( $p = .031$ ) and awake ( $p = .040$ ). Carrageenan with stimulation was higher than Isoflurane ( $p = .002$ ), awake ( $p = .001$ ), and carrageenan ( $p = .016$ ).

Alpha band analyses included frequencies from 9 – 13 Hz. There was a significant main effect of time  $F(3, 42) = 4.342$ ,  $p = .009$ , no significant main effect for injection  $F(1, 14) = 3.437$ ,  $p = .085$ , and a significant interaction effect  $F(3, 42) = 3.114$ ,  $p = .036$ . Post hoc tests revealed no significant differences in the saline group data. Carrageenan was higher than awake ( $p = .041$ ). Carrageenan with stimulation was higher than Isoflurane ( $p = .017$ ), awake ( $p = .009$ ), and carrageenan ( $p = .026$ ).

Beta band analyses included frequencies from 14 – 30 Hz. There was a significant main effect of time  $F(3, 42) = 5.015$ ,  $p = .005$ , no significant main effect for injection  $F(1, 14) = .3654$ ,  $p = .077$ , and a significant interaction effect  $F(3, 42) = 2.961$ ,  $p = .043$ . Post hoc tests revealed no significant differences in the saline group data. Carrageenan with stimulation was higher than Isoflurane ( $p = .015$ ), awake ( $p = .007$ ), and carrageenan ( $p = .020$ ).

Gamma band analyses included frequencies from 30-100 Hz (excluding noise from frequencies 50, 51, & 52). There was no significant main effect of time  $F(3, 42) =$



2.499,  $p = .073$ , no significant main effect for injection  $F(1, 14) = 3.154$ ,  $p = .097$ , and no significant interaction effect  $F(3, 42) = .573$ ,  $p = .636$ .

## 2.5. Discussion

Results from the left paw MPWT data demonstrated that injection of carrageenan yielded the expected significant drop in the mechanical sensitivity threshold and saline did not. Right paw data did not yield any effects, eliminating the concern for a contralateral hypersensitivity confound. Therefore, LFP data could be analyzed with the understanding that the injections resulted in the expected effect on sensory thresholds.

The aim (**Aim 1**) of the first experiment was to determine the LFP ACC activity during behavioral displays of hyperalgesia elicited by repeated suprathreshold mechanical stimulation after carrageenan inflammation. There were no significant changes in LFP power after injection of saline at any frequency band. However, LFPs in the ACC were significantly higher after injection of carrageenan across delta, theta, and alpha frequency bands (higher than Isoflurane and/or awake with no significant difference between Isoflurane and awake) partially supporting the hypothesis (**H<sub>1</sub>**) that introduction of the carrageenan inflammation model would result in an increase of activity in the ACC. However, carrageenan did not result in an increase in the highest LFP frequency bands beta and gamma. Changes in LFP in the ACC after injection of carrageenan were most robust for lower frequency activity.

Furthermore, repeated application of the suprathreshold Von Frey stimulus resulted in a multi-band increase in LFP over and above carrageenan alone in Delta, Theta, Alpha, and Beta bands. This was frequency band-dependent support of the second hypothesis (**H<sub>2</sub>**) that after injection of carrageenan, application of suprathreshold mechanical stimuli would further increase the LFP activity in the ACC over and above activity from the carrageenan alone. Interestingly, mechanical stimulation to the saline

injected paw did not result in any significant increase in LFP, demonstrating that the mechanical stimulation in the absence of a pain condition does not change LFP.

The significant increase in ACC activity after carrageenan injection confirms the role of the ACC in painful inflammation in real time. While it is well known that carrageenan inflammation results in hypersensitivity to mechanical stimuli, this study demonstrates a simultaneous increase in ACC activity. This suggests a graded relationship between intensity of sensory input (chemical with mechanical) and ACC LFP activity in the lowest frequency bands.

These data provide converging behavioral and electrophysiological evidence that low-frequency broad-band ACC activity is involved in processing carrageenan inflammation during rest as well as evoked mechanical stimulation in freely moving animals. The increase in ACC activity mapped onto the increase in stimulation to the system and mirrored the classic hyperalgesia behavioral effect demonstrating a psychophysical isomorphism. These findings support the further use of the custom-designed wireless recording module (Zuo et al., 2012) to further explore neural activity during pain behaviors in freely moving animals. Therefore, a series of studies were designed using LFP recordings in freely moving animals to construct profiles of ACC and S1 activity in response to painful stimuli.

## Chapter 3

### LFP PROFILES IN THE ACC AND S1 DURING INFLAMMATION

#### 3.1. Background

The aim of the second experiment (**Aim 2**) was to examine ACC and S1 activity during inflammatory pain in freely moving animals and further expand on their contributions to the affective and sensory dimensions of pain, respectively. LFP activity was recorded in the ACC and S1 after carrageenan injection and during behavioral tests of hyperalgesia and pain affect. The third hypothesis (**H<sub>3</sub>**) was that injection of carrageenan will result in an increase in LFP activity in the ACC that will be significantly increased during the standard mechanical paw withdrawal threshold test (MPWT). The fourth hypothesis (**H<sub>4</sub>**) was that ACC LFP will increase during the PEAP test of pain affect. Specifically, ACC activity will be highest when in the dark side of the PEAP chamber. The fifth hypothesis (**H<sub>5</sub>**) was that S1 LFP will respond to stimulation in the MPWT and PEAP tests similarly. The sixth hypothesis (**H<sub>6</sub>**) was that S1 LFP will not differ in the light vs. dark side of the chamber.

#### 3.2. Subjects

Forty-one adult Sprague Dawley male rats aged 4-6 months old were taken at random from the UT Arlington vivarium. Rats were kept on a 12 hour light/dark cycle and testing occurred during the light cycle from 7:30 a.m. to 7:30 p.m. Subjects had access to food and water ad libitum and were housed in cages of 2-4. All procedures had the approval of the UT Arlington IACUC and followed the ethical guidelines for pain experiments in animals (Zimmermann, 1983). Seven animals were excluded from the final analyses due to histology, and two were excluded in comparative ACC/S1 analyses due to incomplete data for repeated measures.

### 3.3. Methods

Animals were randomly pulled from the UT Arlington vivarium and placed into groups. Animals were handled and housed in a small colony room for the duration of the experiment. Baseline MPWT recording was conducted to ensure that there was no preexisting hypersensitivity. Electrodes were implanted in a brain site based on placement in groups (ACC or S1). Ten days of recovery were allotted and animals were monitored daily for health (4 animals were given 11 days of recovery). After recovery, baseline LFP was recorded for 10 minutes and then MPWT testing was performed. Saline and carrageenan injections were administered and three hours of time was allotted for rest. Ten minutes of LFP were recorded before MPWT and PEAP testing were recorded along with LFP. A second test day was conducted 24 hours later with LFP, MPWT, and PEAP testing. Euthanasia and histology were performed using the same methods as previously described. Overall methods are summarized in Figure 3-1.

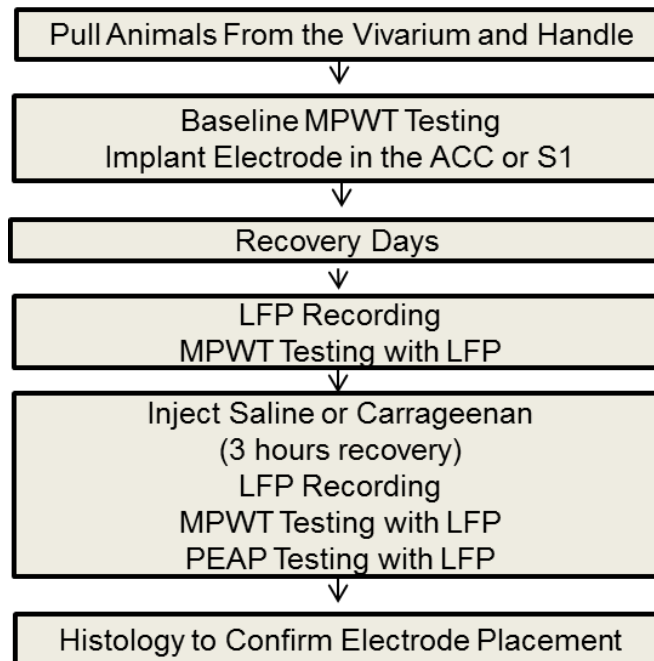


Figure 3-1 Procedural Flowchart for Experiment Three

### *Electrode Implantation*

Recording electrodes were surgically implanted for chronic recording using methods previously described. ACC electrodes were implanted at .7 mm lateral, -3.2 mm dorsal from Bregma at a 15 degree angle while S1 electrodes were implanted at -1.5 posterior, -2.1 lateral, -2.1 dorsal from bregma (Paxinos & Watson, 1998) (See Appendix B).

### *Modified MPWT Procedures*

Mechanical paw withdrawal threshold (MPWT) testing was applied as a measure of hyperalgesia. Procedures for MPWT were the same as described in Chapter 2, but LFP was also recorded during the test. The recording device was turned on immediately after the animals were placed in the chamber for testing. A marker in the recording file was made to signal the beginning and completion of each MPWT trial. MPWT data was collected at the start of the experiment and after electrode implantation to ensure there was no pre-existing hypersensitivity.

### *PEAP Procedures*

To address the affective dimension of pain, the Place Escape Avoidance Paradigm (PEAP) was run. LFP was simultaneously recorded to examine the brain activity activated during this test in real time. Subjects were placed into a half dark/half light chamber measuring 40 x 30 x 15 cm. The chamber was placed atop a wire mesh platform. For each 15 seconds during the 30 minute testing period, a suprathreshold Von Frey filament (476 mN of force) was applied to either the injected left hind paw when the rat was in the dark side of the chamber or the right hind paw when in the light side of the chamber. Handwritten tallies were made to indicate which stimulation was applied at each 15 second time bin. The LFP recording module was turned on immediately after placement in the testing chamber. A marker was made in the file each time stimulation

was applied to demonstrate which side of the box the animal was on (and which paw was stimulated). The box and mesh were cleaned after each test to eliminate residual scent cues.

### 3.4. Results

Day 2 data were excluded from statistical analyses due to 10 animal exclusions that occurred as a result of recording device malfunctions and/or extreme noise in the facility. This resulted in uneven group sizes that were too small to analyze statistically.

#### *Injection of Carrageenan Reduced MPWT Threshold*

MPWT averages across 3 trials for each animal at each time point were calculated and placed into SPSS for analysis. Mixed repeated measures ANOVAs were run for each brain area (S1 or ACC) with the between subjects factor as injection (saline or carrageenan) and the within subjects factor as MPWT over time (baseline, post-implant, and post injection). Post hoc LSD tests were computed to investigate significant differences.

Results for the **left paw** MPWT scores for animals in the **S1 group** indicated that there was a main effect for time (baseline, after implant, and post-injection)  $F(2, 32) = 14.008, p < .001$ , no significant main effect for injection (saline or carrageenan)  $F(1, 16) = 2.246, p = .153$ , and a significant interaction effect  $F(2, 32) = 4.080, p = .026$ . Post hoc tests revealed that there were no significant differences in the saline group across time points ( $p > .05$ ). Although carrageenan and saline group scores were not significantly different after injection ( $p = .079$ ), there was a significant drop in threshold scores in the carrageenan group from baseline and post-implant to post-injection ( $p = .001$ ).

Results for the **left paw** MPWT scores for animals in the **ACC group** indicated that there was a significant main effect of time (baseline, after implant, and post-injection)  $F(2, 28) = 16.265, p < .001$ . There was a significant main effect of injection (saline or

carrageenan)  $F(1, 14) = 11.847, p = .004$ . There was a significant interaction effect  $F(2, 28) = 13.855, p < .001$ . Post hoc tests revealed that there were no significant differences in the saline group across time points ( $p > .05$ ). Significant LSD post hoc tests for the carrageenan group revealed that threshold scores at post-injection were significantly lower than at baseline and post-implant,  $p < .05$ . Additionally, carrageenan scores were significantly lower at post-injection than saline scores,  $p = .002$  (left paw results are summarized in Figure 3-2).

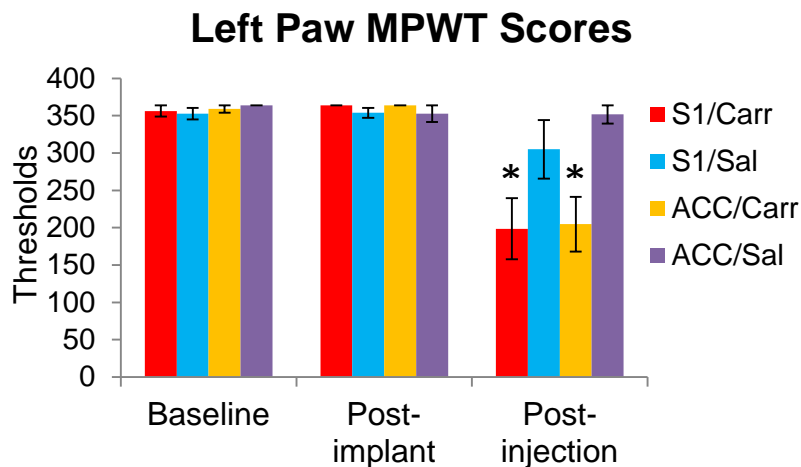


Figure 3-2 Summary of Left Paw MPWT Scores by Group  
 Asterisks represent a significant decrease in threshold after carrageenan injection compared to baseline and post-implant time points. ACC/Saline n=8, ACC/Carrageenan n=8, S1/Saline n=9, S1/Carrageenan n=9

Results for the **right paw** MPWT scores for animals in the **S1 group** indicated that there was no significant main effect of time  $F(2, 32) = .410, p = .667$ . There was no significant main effect of injection (saline or carrageenan)  $F(1, 16) = .441, p = .516$  and no significant interaction effect  $F(2, 32) = 1.165, p = .325$ .

Results for the **right paw** MPWT scores for animals in the **ACC group** indicated that there was no significant main effect of time  $F(2, 28) = .516, p = .603$ . There was no significant main effect of injection (saline or carrageenan)  $F(1, 14) = 2.183, p = .162$  and

no significant interaction effect  $F(2, 28) = .516, p = .603$  (all right paw results are summarized in Figure 3-3).

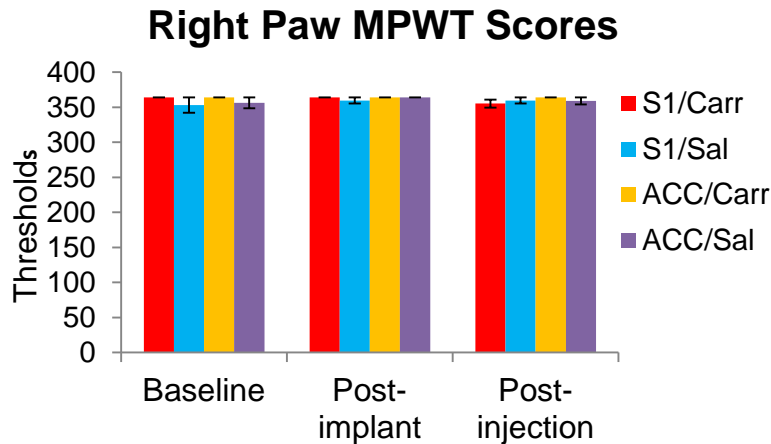


Figure 3-3 Summary of Right Paw MPWT Scores by Group  
There were no significant drops in threshold in the right paw data at any time point.  
ACC/Saline n=8, ACC/Carrageenan n=8, S1/Saline n=9, S1/Carrageenan n=9

#### *Carrageenan Injection did not Induce Avoidance of Mechanical Stimulation*

To analyze PEAP data within the test, mixed repeated measures ANOVAs were run for each brain area (S1 or ACC) with injection as the between subjects factor (saline or carrageenan) and preferences over time (PEAP scores within one trial in 5 minute time bins) as the dependent factor. For animals with **ACC implants**, there was no significant main effect of time  $F(5, 60) = .083, p = .995$ , no significant main effect of injection (saline or carrageenan)  $F(1, 12) = .216, p = .650$  and no significant interaction effect  $F(5, 60) = 1.309, p = .272$ . For animals with **S1 implants**, there was no significant main effect of time  $F(5, 80) = .075, p = .996$ . There was no significant main effect of injection (saline or carrageenan)  $F(1, 16) = .036, p = .851$  and no significant interaction effect  $F(5, 80) = 1.887, p = .106$ . All PEAP scores for each group are available in Figure 3-4.



## Preference Scores in the PEAP Chamber

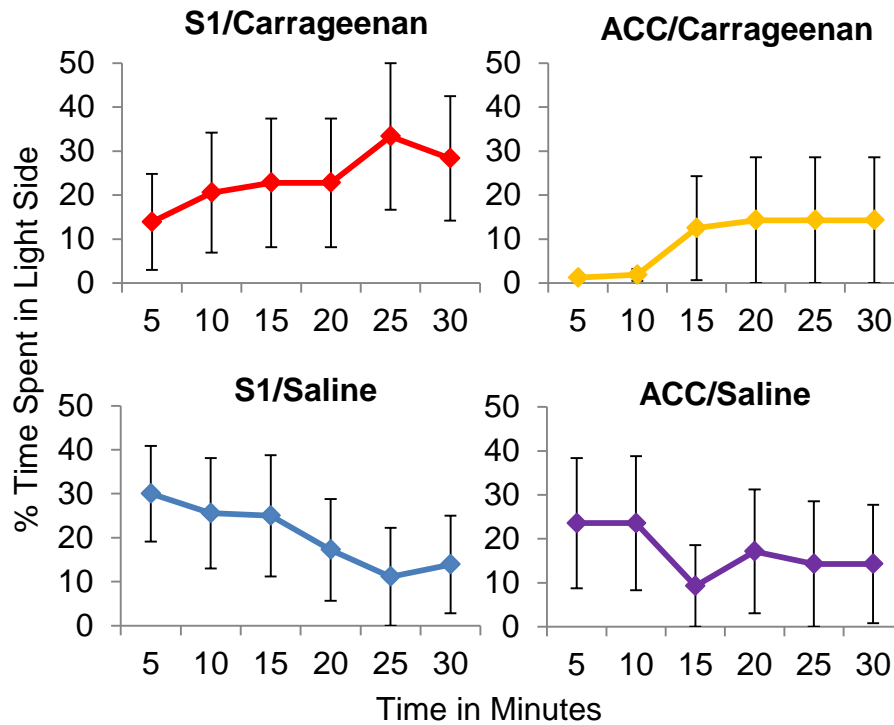


Figure 3-4 PEAP Scores (Percent Time Spent in Light Side) by Group  
 The percent time spent in the light side of the chamber during the PEAP test receiving right (non-injected) paw stimulation was collapsed into 5 minute time bins. There were no significant differences over time or across groups. ACC/Saline n=8, ACC/Carr n=8, S1/Saline n=9, S1/Carr n=9

To analyze crossing behaviors within the test, mixed repeated measures ANOVAs were run for each brain area (S1 or ACC) with injection as the between subjects factor (saline or carrageenan) and crossing behaviors over time (5 minute time bins) as the dependent factor. For animals with **ACC implants**, there was no significant main effect of time  $F(5, 60) = 1.140, p = .349$ . There was no significant main effect of injection  $F(1, 12) = 2.101, p = .173$  and no significant interaction effect  $F(5, 60) = 1.452, p = .219$ . For animals with **S1 implants**, there was a significant main effect of time  $F(5, 80) =$

2.663,  $p = .028$ . There was no significant main effect of injection  $F(1, 16) = .390$ ,  $p = .390$  and no significant interaction effect  $F(5, 80) = 1.687$ ,  $p = .147$ . Results are summarized in Figure 3-5.

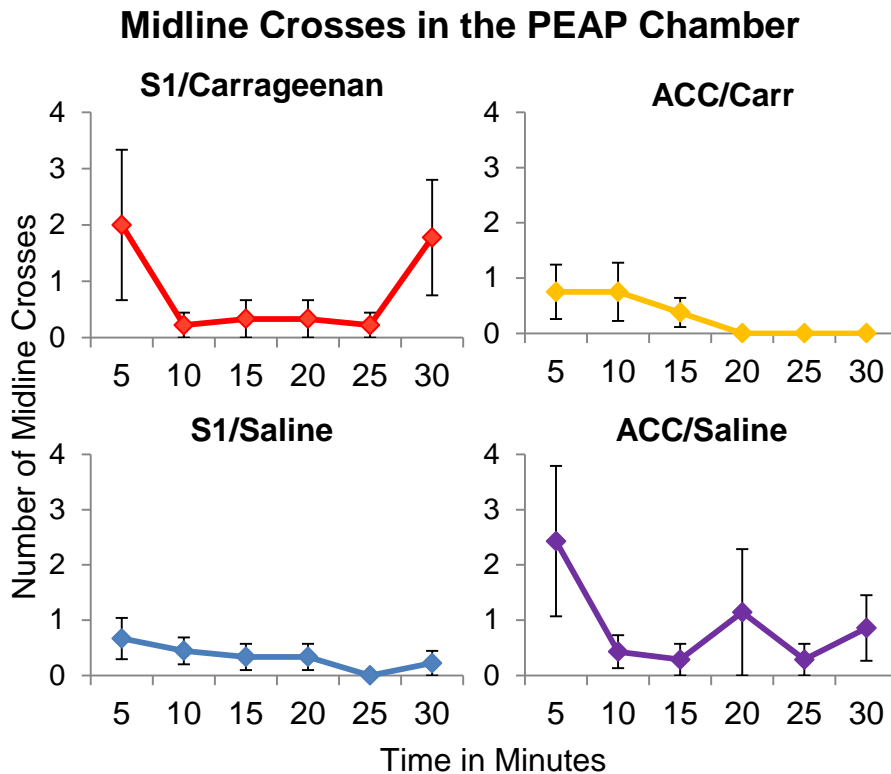


Figure 3-5 Midline Crosses in the PEAP Chamber in 5 Minute Time Bins by Group. The number of times the animals crossed from one side of the chamber to another within the PEAP test was recorded every 15 seconds. This was collapsed into 5 minute time bins. There were no significant differences over time or between groups for midline crossing behaviors. ACC/Saline  $n=8$ , ACC/Carr  $n=8$ , S1/Saline  $n=9$ , S1/Carr  $n=9$ .

#### *LFP Activity in S1 and ACC during MPWT and PEAP*

After recording in custom software, raw local field potential data was packaged in 2 files (recording and markers) that were imported from the custom made recording device into Spike 2 for analysis using a sampling rate of 4096 Hz. Using Spike 2, spectrograms of the local field potential data were viewed for each animal for all of the

LFP time points. Two hundred seventy-two spectrograms were visually inspected and power spectrum analyses were created for each using the same method as described before. This data was imported into an excel file and organized by animal, brain recording area, injection, and frequency band (Delta 0-4 Hz, Theta 4-8 Hz, Alpha 8-12 Hz, Beta 13-29 Hz, and Gamma 30-100 Hz). The mean (average of all subjects) and standard error of the mean (SEM) for power at each frequency band was computed in Excel, graphed, and imported into SPSS for analysis.

To investigate the effects of carrageenan inflammation on different frequencies of brain activity during different states (rest, during MPWT test, during PEAP test), mixed repeated measures ANOVAs were run for the average power spectrum at each frequency band of data using injection as the between subjects variable (carrageenan or saline) and LFP scores as the within subjects variable for both brain areas. Comparison of LFP activity in the ACC and S1 were run in each frequency band using mixed factorial ANOVAs comparing brain area (ACC or S1) by injection (carrageenan or saline) with LFP scores during variable stimulation (baseline, post-injection, during MPWT, and during PEAP) as the within subjects variable.

**Delta** band analyses included frequencies from 0 – 3 Hz. For **S1 animals** there was a significant main effect of stimulation  $F(3, 48) = 4.383, p = .008$ , no significant main effect for injection (saline or carrageenan)  $F(1, 16) = .493, p = .493$ , and no significant interaction effect  $F(3, 48) = .650, p = .587$ . For **ACC animals** there was no significant main effect of stimulation  $F(3, 36) = 1.159, p = .339$ , no significant main effect for injection  $F(1, 12) = .848, p = .375$ , and no significant interaction effect  $F(3, 36) = 2.097, p = .118$ . **When the ACC and S1 were compared**, there was a main effect of stimulation  $F(3, 84) = 3.695, p = .015$ , no main effect of brain area  $F(1, 28) = .369, p = .548$ , and no main effect of injection  $F(1, 28) = .068, p = .797$ , There was no interaction effect between

brain area and injection  $F(1, 28) = .827, p = .371$ . There was no interaction effect between brain area and injection  $F(3, 84) = .681, p = .566$ . The three-way interaction between brain area (ACC/S1), injection, and stimulation was not significant  $F(3, 84) = 2.638, p = .055$  (see Figure 3-6).

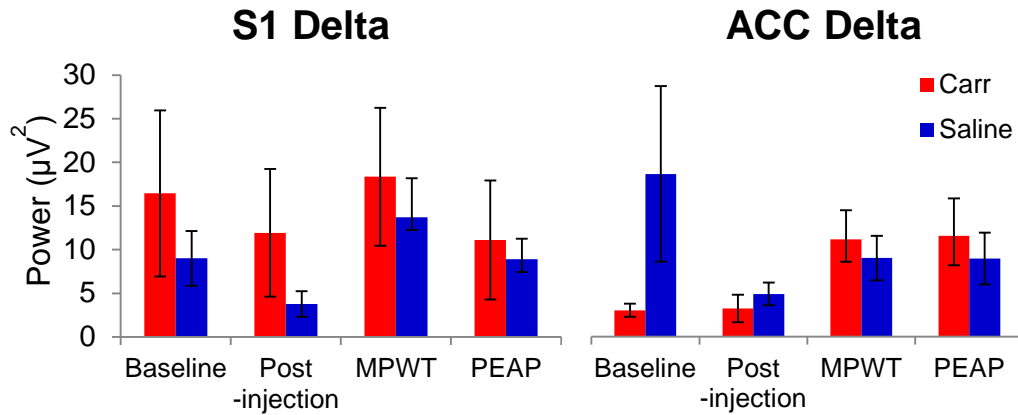


Figure 3-6 Comparison of ACC and S1 LFP Responses in Delta Band (0-3 Hz) There were no significant differences across injection type or brain area due to stimulation. ACC/Saline n=8, ACC/Carr n=8, S1/Saline n=9, S1/Carr n=9.

**Theta** band analyses included frequencies from 4 - 8 Hz. For **S1 animals** there was a significant main effect of stimulation  $F(3, 48) = 4.971, p = .004$ , no significant main effect for injection  $F(1, 16) = .562, p = .464$ , and no significant interaction effect  $F(3, 48) = .734, p = .537$ . For **ACC animals** there was no significant main effect of stimulation  $F(3, 36) = 1.609, p = .204$ , no significant main effect for injection  $F(1, 12) = 1.485, p = .246$ , and no significant interaction effect  $F(3, 36) = 1.129, p = .350$ . **When the ACC and S1 were compared**, there was a main effect of stimulation  $F(3, 84) = 5.134, p = .003$ , no main effect of injection  $F(1, 28) = .015, p = .902$ , no main effect of brain area  $F(1, 28) = .224, p = .639$ . There was no interaction effect between brain area and injection  $F(1, 28) = 1.594, p = .217$ . There was no interaction effect between brain area and stimulation  $F(3, 84) = .343, p = .795$ . The 3-way interaction between brain area, injection, and stimulation was not significant  $F(3, 84) = 1.609, p = .193$  (see Figure 3-7).

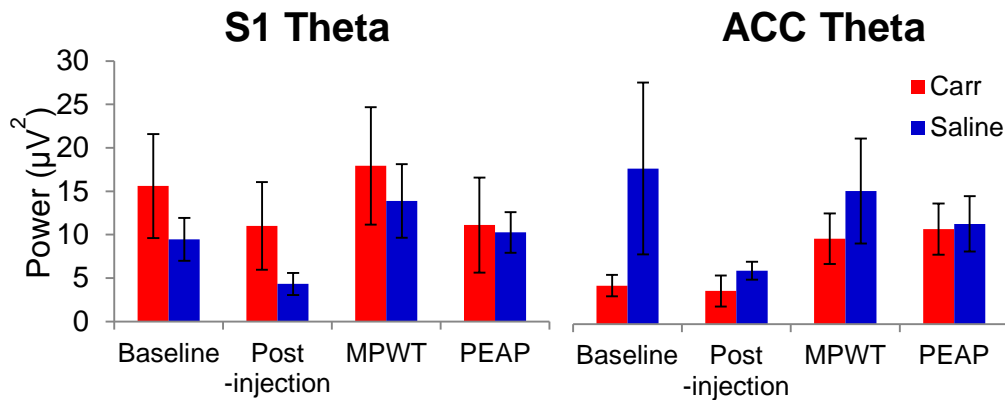


Figure 3-7 Comparison of ACC and S1 LFP Responses in Theta Band (4-8 Hz)  
 There were no significant differences across injection type or brain area due to stimulation. ACC/Saline n=8, ACC/Carr n=8, S1/Saline n=9, S1/Carr n=9.

**Alpha** band analyses included frequencies from 9 - 13 Hz. For **S1 animals** there was a significant main effect of stimulation  $F(3, 48) = 3.039, p = .038$ , no significant main effect for injection  $F(1, 16) = .207, p = .655$ , and no significant interaction effect  $F(3, 48) = .617, p = .607$ . For **ACC animals** there was no significant main effect of stimulation  $F(3, 36) = 1.444, p = .246$ , no significant main effect for injection  $F(1, 12) = 1.029, p = .330$ , and no significant interaction effect  $F(3, 36) = .866, p = .468$ . **When the ACC and S1 were compared**, there was a main effect of stimulation  $F(3, 84) = 3.993, p = .010$ , no main effect of injection  $F(1, 28) = .288, p = .596$ , no main effect of brain area  $F(1, 28) = .052, p = .821$ . There was no interaction effect between brain area and injection  $F(1, 28) = 1.213, p = .280$ . There was no interaction effect between brain area and stimulation  $F(3, 84) = .108, p = .955$ . There was no significant 3 way interaction between brain area, injection, and stimulation  $F(3, 84) = .998, p = .398$  (see Figure 3-8).

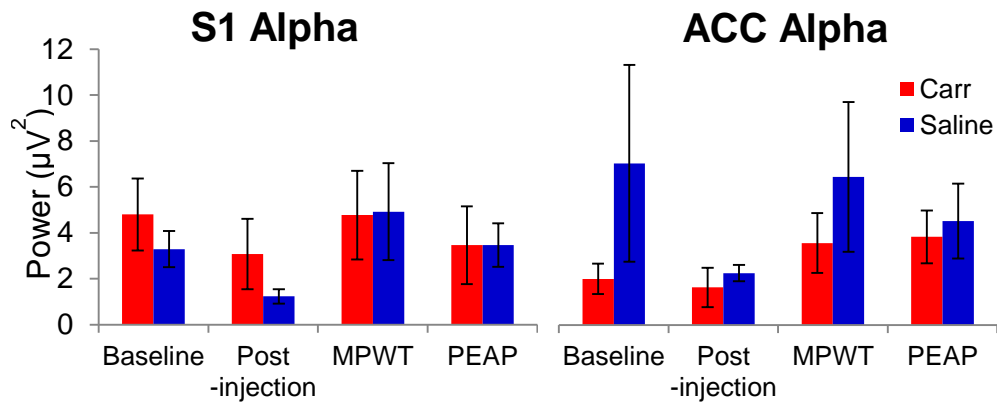


Figure 3-8 Comparison of ACC and S1 LFP Responses in Alpha Band (9-13 Hz)  
 There were no significant differences across injection type or brain area due to stimulation. ACC/Saline n=8, ACC/Carr n=8, S1/Saline n=9, S1/Carr n=9.

**Beta** band analyses included frequencies from 14 – 30 Hz. For **S1 animals** there was a significant main effect of stimulation  $F(3, 48) = 2.845, p = .047$ , no significant main effect for injection  $F(1, 16) = .554, p = .467$ , and no significant interaction effect  $F(3, 48) = .396, p = .757$ . For **ACC animals** there was no significant main effect of stimulation  $F(3, 36) = 1.230, p = .313$ , no significant main effect for injection  $F(1, 12) = 1.622, p = .227$ , and no significant interaction effect  $F(3, 36) = .701, p = .557$ . **When the ACC and S1 were compared**, there was a main effect of stimulation  $F(3, 84) = 3.436 p = .021$ , no main effect of injection  $F(1, 28) = .324 p = .574$ , no main effect of brain area  $F(1, 28) = .383 p = .541$ . There was no interaction effect of brain area and injection  $F(1, 28) = 2.224, p = .147$ . There was no interaction effect between brain area and stimulation  $F(3, 84) = .173 p = .914$ . There was no three way interaction between brain area, injection, and stimulation  $F(3, 84) = .970, p = .411$  (See Figure 3-9).

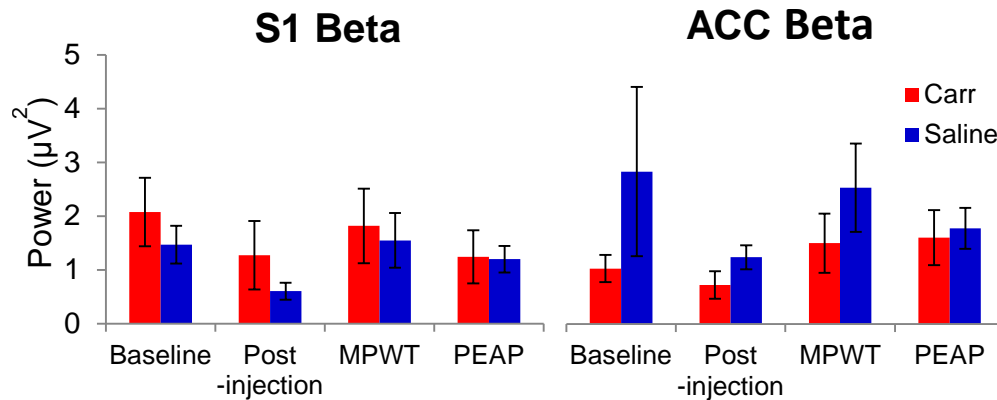


Figure 3-9 Comparison of ACC and S1 LFP Responses in Beta Band (14-30 Hz)  
 There were no significant differences across injection type or brain area due to stimulation. ACC/Saline n=8, ACC/Carr n=8, S1/Saline n=9, S1/Carr n=9

**Gamma** band analyses included frequencies from 30-100 Hz (excluding noise from frequencies 50, 51, & 52). For **S1 animals** there was no significant main effect of stimulation  $F(3, 48) = .917, p = .440$ , no significant main effect for injection  $F(1, 16) = 3.477, p = .081$ , and no significant interaction effect  $F(3, 48) = .994, p = .404$ . For **ACC animals** there was no significant main effect of stimulation  $F(3, 36) = 1.063, p = .377$ , no significant main effect for injection  $F(1, 12) = .363, p = .558$ , and no significant interaction effect  $F(3, 36) = .294, p = .830$ . **When the ACC and S1 were compared**, there was a main effect of stimulation  $F(3, 84) = .757, p = .521$ , no main effect of injection  $F(1, 28) = 3.018, p = .093$ , and a significant main effect of brain area  $F(1, 28) = 4.421, p = .045$ . There was no interaction effect of brain area and injection  $F(1, 28) = 2.2129, p = .156$ . There was no interaction effect between brain area and stimulation  $F(3, 84) = .666, p = .575$ . There was no three way interaction between brain area, injection, and stimulation  $F(3, 84) = .733, p = .535$  (see Figure 3-10).

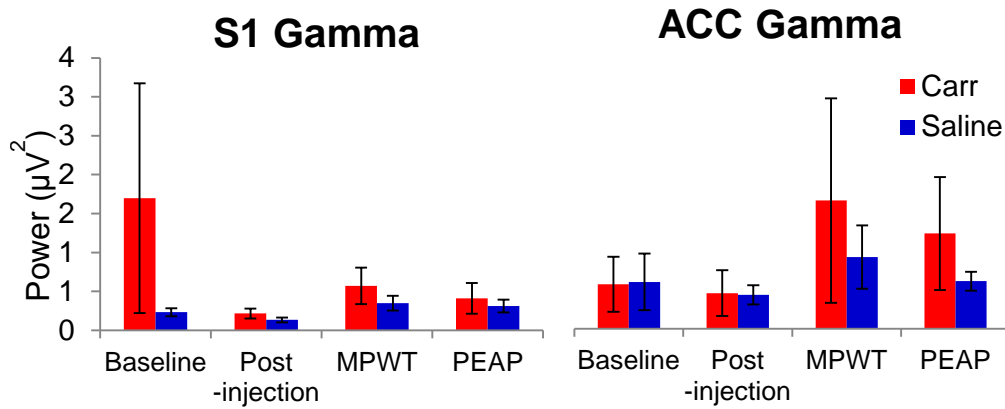


Figure 3-10 Comparison ACC and S1 LFP Response in Gamma Band (30-100 Hz)  
 There were no significant differences across injection type or brain area due to stimulation. ACC/Saline n=8, ACC/Carr n=8, S1/Saline n=9, S1/Carr n=9

### 3.5. Discussion

The aim of the second experiment (**Aim 2**) was to examine ACC and S1 activity during inflammatory pain in freely moving animals and further expand on their contributions to the affective and sensory dimensions of pain, respectively. Results from the MPWT data for the left paw revealed that injection of carrageenan yielded the expected significant drop in the mechanical sensitivity threshold over time where saline did not. There were no significant right paw effects. LFP and PEAP data could be analyzed with the understanding that the conditions resulted in the expected effect on sensory thresholds.

It was expected that during the PEAP test, carrageenan animals would spend significantly more time in the light side of the PEAP chamber while saline animals would not. There was a significant upward trend over time in the carrageenan groups and a significant downward trend in the saline groups. Yet, results did not indicate that animals in the carrageenan inflammation condition were significantly averse to the stimulation. One possible explanation for this could be that the recording back-pack interfered with



the PEAP effect. Although the backpack does not restrict the movement of the animals, animals may have chosen to move around less. The investigation of midline crossing behavior also did not yield any significant changes over time. In fact, the mean number of midline crosses for the first 5 minutes remained under 3. Previously published papers have indicated a much higher number of midline crosses throughout the test (for examples, see Uhelski & Fuchs, 2009, 2010).

The low number of midline crosses demonstrates that animals did not experience sufficient exploration of both sides of the box, and as a result did not experience the differential stimulation outcomes. Because the premise of the test is based on presentation of a dilemma between the choosing the light and dark sides of the box, it is imperative that animals experience both sides of the box before conclusions could be made about the test outcomes. Furthermore, as the expected shift to the light side for carrageenan animals did not occur, LFP analysis of the differential outcomes in the chamber sides could not be computed with meaning. Therefore examination of the hypotheses about how PEAP behavior relates to LFP was not completed (**H<sub>4</sub>** that ACC LFP would increase during the PEAP test of pain affect and specifically that ACC activity would be highest when in the dark side of the PEAP chamber as well as **H<sub>6</sub>** that S<sub>1</sub> LFP would not differ in the light vs. dark side of the chamber).

An alternative explanation for the lack of PEAP effect could be that the electrode left lesions in the areas of interest. However, the implants in this study were unilateral and previous studies indicated that lesions to the S1 attenuated mechanical hypersensitivity, but the PEAP avoidance was still present (Uhelski, Davis, et al., 2012), so this is not a likely explanation because in this study the S1 carrageenan animals did not experience asomaesthesia.

Local field potential data did not yield any significant differences within brain regions. Comparative analyses data did not reveal significantly different profiles for the S1 and ACC group data, which was surprising. The lack of significant change over time in the LFP data for the S1 animals lent support for the fifth hypothesis (**H<sub>5</sub>**) that the S1 LFP will respond to stimulation in the MPWT and PEAP tests similarly. This finding further supplements findings from experiment 3 (next chapter) that the S1 responds similarly to a wide variety of electrical stimulation. The findings in this study are in line with a study by Murell et al. that mechanical stimulation to the tail of rats did not change significantly change S1 EEG activity when compared to baseline (Murrell, Mitchinson, Waters, & Johnson, 2007).

However, there was a lack of significant change over time by group leading to a lack of support for the third hypothesis (**H<sub>3</sub>**) that injection of carrageenan would result in an increase in LFP activity in the ACC that would be significantly increased by mechanical stimulation in the standard mechanical paw withdrawal threshold test (MPWT). This was unexpected due to findings from experiment 1 that indicated that carrageenan injection and mechanical stimulation resulted in respective significant increases in LFP in the ACC. The same trend for mechanical stimulation was present in the current data set in the carrageenan group, however it is likely that the variance in the saline control group data occluded the effect of the increase in ACC LFP in the carrageenan data.

Using a combinatorial approach of recording local field potential and 2-dimensional current source density methods, Lu et. al (2014) recently found that an injection of inflammatory bee venom resulted in a significantly altered synaptic organization in slice preparations of the ACC. Spatial activation of the LFP as well as amplitude were stimulation intensity-dependent which supported the findings from

experiment 1 in this series of studies. For this study, another potential reason that the intensity-dependent relationship between ACC LFP and stimulation was not found to be significant in this study was the nature of the stimulation. In experiment 1, a suprathreshold Von Frey stimulus was used and repeatedly applied in temporal proximity whereas in this study, MPWT testing involved smaller filament sizes. Although the PEAP test used a larger diameter filament of a higher intensity than the MPWT test, stimulation was differentially applied at a minimum of 15 seconds apart in time. The differences in experimental methods are plausible explanations for the difference in findings.

In any case, the lack of the significant changes in the ACC group contributed to non-significant comparative analyses across brain regions. It should be noted that the lack of LFP effect in the ACC mirrors the lack of the PEAP behavioral effect, which further expands on the supposition from experiment 1 that there may be a psychophysical isomorphism for the ACC LFP pain profile. However, the results from experiment 1 indicate a positive isomorphism with an increase (behavioral increase mirrors LFP increase), while this study demonstrates a lack of LFP effect with a lack of behavioral effect.

## Chapter 4

### LFP PROFILES IN THE ACC AND S1 DURING PERIPHERAL NERVE STIMULATION

#### 4.1. Background

The aim of the third experiment (**Aim 3**) was to determine the dynamic LFP response profiles of the ACC and S1 during peripheral nerve stimulation using a cuff electrode. LFP activity was recorded in the ACC and S1 in anesthetized rats during variable and progressive electrical stimulations to the L5 nerve. The seventh hypothesis (**H<sub>7</sub>**) was the ACC will increase in response to high intensity stimulation. The eighth hypothesis (**H<sub>8</sub>**) was the S1 will respond similarly to a range of parameters.

#### 4.2. Subjects

Eighteen adult male Sprague Dawley rats were taken at random from the University of Texas at Arlington vivarium at 4-11 months old. Animals were kept on a 12 hour light/dark cycle, with testing occurring during the light cycle from 7:30 a.m. to 7:30 p.m. Subjects had access to food and water ad libitum. All procedures had the approval of the UTA IACUC and followed the ethical guidelines for pain experiments in animals (Zimmermann, 1983). Animals were designated to receive either ACC or S1 electrode implantation first (ACC then S1 recording or S1 then ACC recording). Eight animals were excluded due to: unknown illness (2), no histological confirmation (2), data overwritten (1), irregular anatomy not allowing proper nerve implantation (1), and mechanical damage to the L5 nerve (2), leaving 8 animals in each group.

#### 4.3. Methods

Animals were randomly pulled from the UT Arlington vivarium and placed under anesthesia with 3% isoflourane/97% oxygen inhaled gas in a stereotaxic frame for surgery. The head and back were shaved. Vitals were monitored every fifteen minutes for the duration of the surgery. The cuff-stimulating electrode was implanted at the L5 spinal

nerve location. Next, electrodes were implanted at designated LFP brain sites according to group assignment. During local field potential recording in each area, electrical stimulation at varying parameters was applied to the cuff electrode. After all recordings, animals were euthanized and brains were extracted. Histology was performed to determine electrode placement (see Appendix B). Specific procedures are detailed below.

#### *Cuff Implantation Procedures*

The cuff electrode was implanted at the L5 spinal nerve site. A lateral incision of 1-1.5 inches was made just to the left of the spinal cord. Paraspinal muscle tissue was removed as well as approximately one half of the transverse process to expose the L5 nerve. The L5 nerve was isolated and two soft insulated wires were threaded around the nerve with a small portion of the exposed wire touching only the dorsal side of the nerve. Wires were secured around the nerve with silk suture thread (Coated Vicryl, 3-0). The ends of the wires were uninsulated and connected to the stimulating module outside of the body (depicted in Figure 4-1).

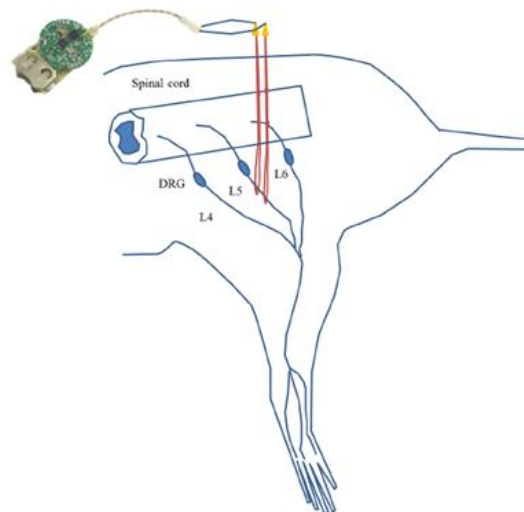


Figure 4-1 "Illustration of cuff electrode stimulation" (Li, 2014)  
Figure was reprinted with permission from (Li, 2014)

### *LFP and Cuff Stimulation*

After the electrode was implanted at either the ACC or S1, the nerve was stimulated at 6 different parameters while LFP was recorded. Stimulation parameters are provided in Table 4-1.

Table 4-1 Stimulation Parameters

Frequency	Intensity	Pulse Duration	Duration
50 Hz	.5 V	1 ms	10 sec
50 Hz	1 V	1 ms	10 sec
50 Hz	1.5 V	1 ms	10 sec
100 Hz	.5 V	1 ms	10 sec
100 Hz	1 V	1 ms	10 sec
100 Hz	1.5 V	1 ms	10 sec

Three minutes of LFP activity was recorded with no stimulation before and after each parameter was applied. A marker was made in the recording file to signal the beginning and end of stimulation. After all parameters were recorded at one brain area, the recording electrode was moved to the next brain area and stimulation procedures were repeated.

## 4.4. Results

### *Data Preparation and Power Analyses*

Raw local field potential data was packaged in two text files (one for stimulation markers called “stimulate” and one for local field potential data called “record”). The files were imported into Spike 2 software (CED) and spectrograms were viewed in alignment with stimulation markers.

Using markers that indicate where each stimulation parameter began and ended, 10 second sections of the signal were extracted to create time bins for each stimulation

and resting period, for each animal. Two-hundred and eight spectrograms were visually inspected and power spectrum analyses and histograms were computed for each of the time bins and saved in text file format. Text file data was imported into an excel file and organized by animal, brain recording area, stimulation or resting period, and frequency band (Delta 0-4 Hz, Theta 4-8 Hz, Alpha 8-12 Hz, Beta 13-29 Hz, and Gamma 30-100 Hz). The means (average power of all subjects) at each frequency band as well as an overall summary from 0-100 Hz were computed in Excel, graphed, and imported into SPSS for analysis.

*Statistical Analyses*

SPSS was used to compute repeated measures ANOVAs for each frequency band to determine if LFP activity was differential in response to increasing stimulation parameters. Repeated measures ANOVAs were also run to determine if LFP activity changed during the resting periods over time. Simple effects were investigated using LSD post hoc tests where there were significant effects.

*S1 LFP during Peripheral Nerve Stimulation*

Repeated measures ANOVAs for S1 LFP in each frequency band during stimulation did not yield any significant results. Results from the ANOVAs are available in Table 4-2 and graphed data are available in Figure 4-2 by frequency band with an average of all LFP band data (0-100 Hz).

Table 4-2 Summary of Repeated Measures ANOVA Results for S1 Stimulations Data

<b>Band</b>	<b>Frequencies</b>	<b>Statistical Result</b>
Delta	0 – 3 Hz	$F (6, 42) = .548, p = .768$
Theta	4 - 8 Hz	$F (6, 42) = 1.190, p = .331$
Alpha	9 – 13 Hz	$F (6, 42) = 1.090, p = .384$
Beta	14 – 30 Hz	$F (6, 42) = .975, p = .454$
Gamma	30 - 49 & 53 - 100 Hz	$F (6, 42) = 1.027, p = .421$

### S1 Local Field Potential During Stimulation

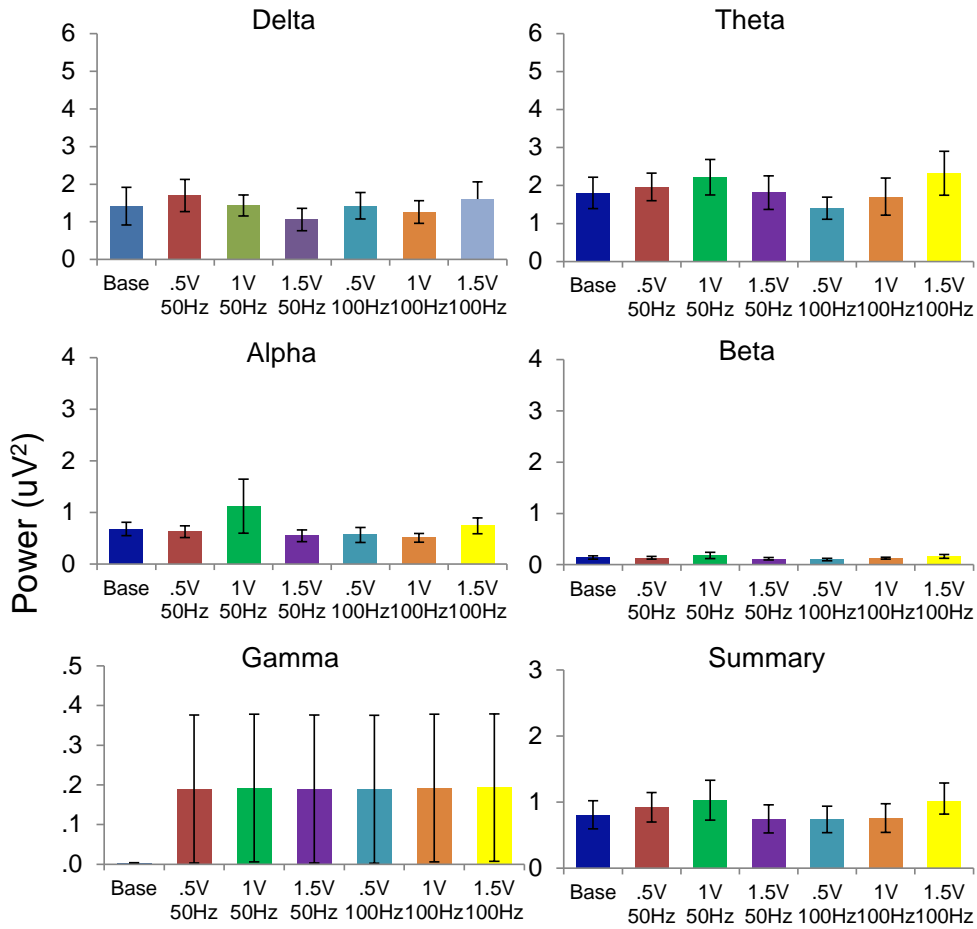


Figure 4-2 Summary of S1 LFP during Peripheral Nerve Stimulation  
 Average S1 LFP activity during progressive stimulation is represented within each frequency band as well as a summary of all local field potential (0-100 Hz). LFP did not change significantly over time in any frequency band (n=8).



Repeated measures ANOVA for LFP in each frequency band during resting periods did not yield any significant results. Results from the ANOVAs are available in Table 4-3 and graphed data are available in Figure 4-3 by frequency band as well as an average of all LFP band data (0-100 Hz).

Table 4-3 Summary of Repeated Measures ANOVA Results for S1 Resting Data

<b>Band</b>	<b>Frequencies</b>	<b>Statistical Result</b>
Delta	0 – 3 Hz	$F(6, 42) = .706, p = .646$
Theta	4 - 8 Hz	$F(6, 42) = .300, p = .933$
Alpha	9 – 13 Hz	$F(6, 42) = .662, p = .681$
Beta	14 – 30 Hz	$F(6, 42) = 1.541, p = .189$
Gamma	30 - 49 & 53 - 100 Hz	$F(6, 42) = 1.030, p = .419$

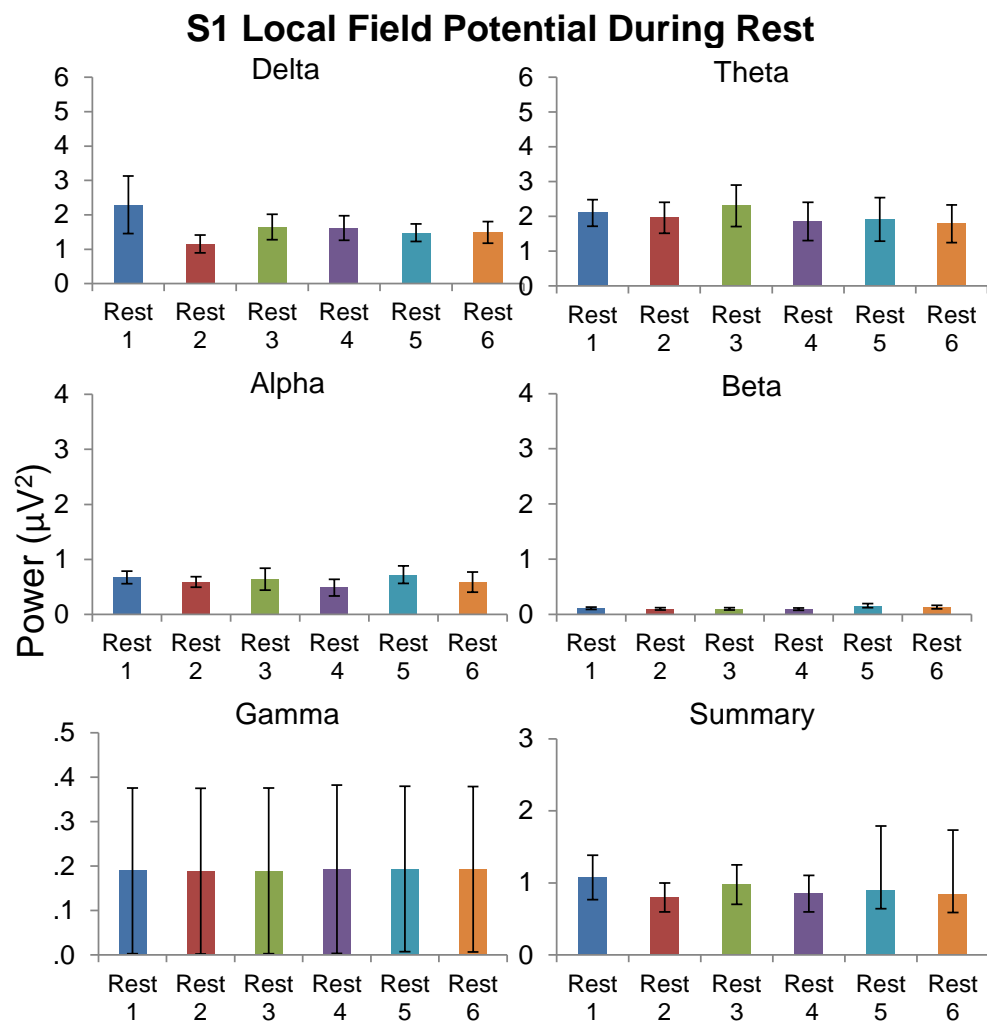


Figure 4-3 S1 LFP during End of 3 Minute Resting Periods  
 Average S1 LFP activity during the end of the resting periods is represented within each frequency band as well as a summary of all local field potential (0-100 Hz). LFP did not change significantly over time in any frequency band (n=8).

### *ACC LFP during Peripheral Nerve Stimulation*

Repeated measures ANOVA for ACC LFP in each frequency band during stimulation did not yield any significant results. Results from the ANOVAs are available in Table 4-4 and graphed data are available in Figure 4-4 by frequency band as well as an average of all LFP band data (0-100 Hz).

Table 4-4 Summary of Repeated Measures ANOVA for ACC Stimulations Data

<b>Band</b>	<b>Frequencies</b>	<b>Statistical Result</b>
Delta	0 – 3 Hz	$F (6, 42) = 2.188, p = .063$
Theta	4 - 8 Hz	$F (6, 42) = 1.507, p = .199$
Alpha	9 – 13 Hz	$F (6, 42) = .776, p = .593$
Beta	14 – 30 Hz	$F (6, 42) = .930, p = .483$
Gamma	30 - 49 & 53 - 100 Hz	$F (6, 42) = .993, p = .442$

## ACC Local Field Potential During Stimulation

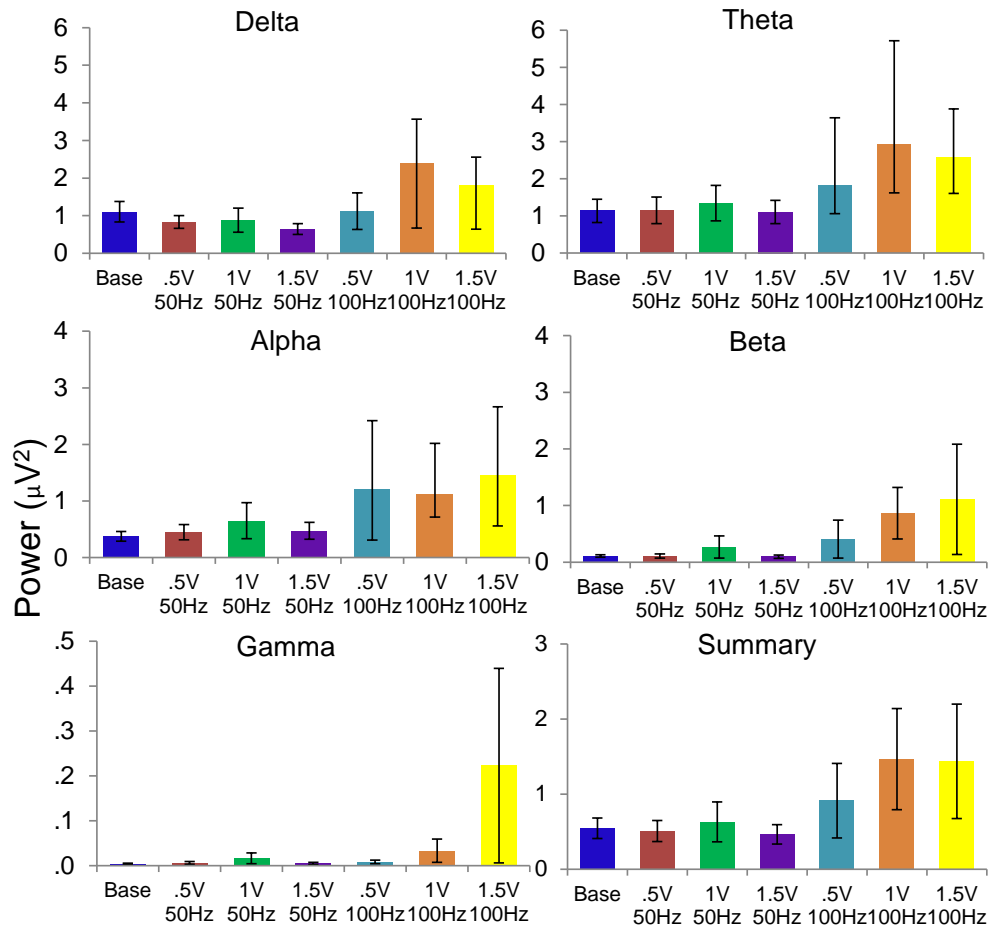


Figure 4-4 Summary of ACC LFP during Peripheral Nerve Stimulation  
 Average ACC LFP activity during stimulation is represented within each frequency band as well as a summary of all local field potential (0-100 Hz). LFP did not change significantly over time in any frequency band (n=8).

Repeated measures ANOVA for ACC LFP in each frequency band during resting periods did not yield any significant results. Results from the ANOVAs are available in Table 4-5 and graphed data are available in Figure 4-5 by frequency band as well as an average of all LFP band data (0-100 Hz).

Table 4-5 Summary of Repeated Measures ANOVA for ACC Resting Data

<b>Band</b>	<b>Frequencies</b>	<b>Statistical Result</b>
Delta	0 – 3 Hz	$F(6, 42) = 1.947, p = .095$
Theta	4 - 8 Hz	$F(6, 42) = 1.138, p = .357$
Alpha	9 – 13 Hz	$F(6, 42) = .895, p = .507$
Beta	14 – 30 Hz	$F(6, 42) = 1.055, p = .404$
Gamma	30 - 49 & 53 - 100 Hz	$F(6, 42) = 1.110, p = .373$

## ACC Local Field Potential During Rest

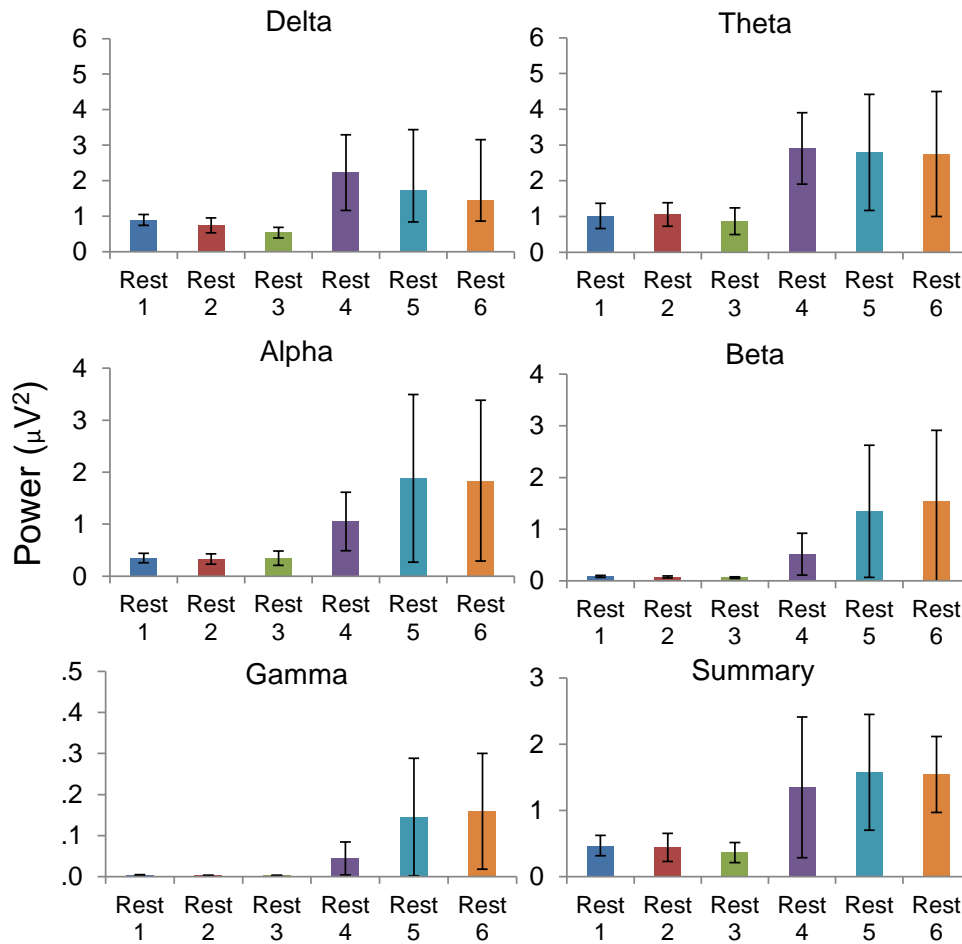


Figure 4-5 ACC LFP during End of 3 Minute Resting Periods  
Average ACC LFP activity during the end of resting periods is represented within each frequency band as well as a summary of all local field potential (0-100 Hz). LFP did not change significantly over time in any frequency band (n=8).

### 4.5. Discussion

The aim of the third experiment (**Aim 3**) was to determine the dynamic LFP response profiles of the ACC and S1 during peripheral nerve stimulation using a cuff electrode. Results indicated that the ACC did not respond significantly differently to different parameters; in contrast to the seventh hypothesis (**H<sub>7</sub>**) that the ACC activity

would increase in response to high intensity stimulation. However, ACC activity did increase at higher intensities and persisted after stimulation into the resting periods. Although the result was not significant, progressive high intensity stimulation led to a small increase in ACC sensitization that persists even after stimulation has ceased. The lack of statistical significance is not surprising given the amount of variance at higher parameters, as well as findings from experiment 2 indicating that the ACC did not respond differentially in the MPWT and PEAP tests.

Results indicated that LFP in the S1 was not significantly different across stimulation parameters, and there was no trend for otherwise, which confirmed the eighth hypothesis ( $H_8$ ) that the S<sub>1</sub> would respond similarly to a wide range of parameters. This is expected because the S1 has been closely linked to processing of incoming a wide variety of sensory information. Previous studies have indicated that the role of the S1 in the sensory processing of noxious input is localization based on a sensory homunculus, discrimination, and potentially coding stimulus intensity (Bushnell et al., 1999; Coghill et al., 1994; Hofbauer et al., 2001; Lin et al., 2003; Omori et al., 2013a; Vierck, Whitsel, Favorov, Brown, & Tommerdahl, 2013).

The S1 responds to a wide variety of noxious and non-noxious stimuli (Chang & Shyu, 2001; Lamour, Willer, & Guilbaud, 1982). For example, using fMRI in rats, activation of the S1 during application of non-noxious as well as noxious electrical sciatic nerve stimulation has been recorded (Chang & Shyu, 2001). In this study we did not see a linear relationship between S1 activity and peripheral nerve stimulation. There is an ongoing debate in the literature about the relationship between the S1 and processing the intensity of stimulation as some research has indicated that the intensity of stimulation can be dissociated from activity in areas of the pain matrix such as the S1 area yet other research has demonstrated that the S1 is imperative for interpretation of stimulation

intensity (Hofbauer et al., 2001; Kenshalo, Chudler, Anton, & Dubner, 1988; Legrain, Iannetti, Plaghki, & Mouraux, 2011; Peyron et al., 1999).

Furthermore, authors of a PET imaging study in humans suggested that the S1 area is not involved in intensity coding, but rather, the S1 is involved in the discrimination and localization of the stimulation (Peyron et al., 1999). One potential explanation for this discrepancy can potentially be extrapolated from a single cell study by Follett and Dirks in rats with visceral stimulation by colorectal distension (1994). The authors found that some cells in the S1 area yielded a flat response with varying intensity of stimulation while some cells responded in a graded fashion and appeared to code for the intensity of the stimulus (Follett & Dirks, 1994). Differential response profiles of cells in the S1 could be responsible for discrepant findings across studies.

Our research demonstrates that stimulation ranging from 50-100 Hz and .5 to 1.5 volts at the L5 location results in similar activation in the S1, as expected. However, it is important to keep in mind that the role of the S1 and ACC areas in processing noxious stimulation are modulated by cognitive factors. For example, EEG data from the human ACC has indicated that signals from 4-7 Hz are highest during concentration tasks and increase in amplitude alongside task difficulty (Allman et al., 2001). In addition, the local field potential power profiles for both the ACC and S1 were much lower in this experiment than the recordings in the freely moving animal experiments, particularly for the delta frequency band.



## Chapter 5

### CUFF STIMULATING ELECTRODE FOR A SPONTANEOUS PAIN MODEL

#### 5.1. Background

We propose to use the peripheral nerve stimulation set-up described in experiment 2 as the cuff- stimulating electrode (CSE), a model for spontaneous pain to examine the burning or shooting symptoms that may occur as a result of pain (Campbell & Meyer, 2006). An already established rodent model of spontaneous pain introduces an injection of the inflammatory substance formalin into the hind paw which results in a pain state marked by a unique set of spontaneous pain behaviors, yet the model is acute (Dubuisson & Dennis, 1977). There is also some evidence that the widely used spinal nerve ligation model induces spontaneous pain (King et al., 2009), yet it cannot be manipulated or controlled by the experimenter for study. The proposed CSE model will allow experimenters to introduce spontaneous pain that can be directly controlled over the long term and be manipulated to address a variety of experimental questions. The L<sub>5</sub> nerve location was chosen as the site of implantation to allow convenience of placement without inducing motor dysfunction. Parameters from the previous experiment were utilized.

The fourth aim (**Aim 4**) was to determine the effectiveness of the cuff stimulating electrode model as a model of spontaneous pain. The cuff electrode was implanted in freely moving animals and measures of mechanical hyperalgesia, pain affect, and spontaneous pain behaviors were recorded during progressive electrical stimulation to the L<sub>5</sub> nerve. The ninth hypothesis (**H<sub>9</sub>**) was the cuff model will result in spontaneous pain behaviors. The tenth hypothesis (**H<sub>10</sub>**) was the cuff model stimulation will result in the development of pain affect.

## 5.2. Subjects

Fifteen adult male Sprague Dawley rats were taken at random from the UT Arlington vivarium at 4-7 months old. Rats were kept on a 12 hour light/dark cycle and testing occurred during the light cycle from 7:30 a.m. to 7:30 p.m. Subjects had access to food and water ad libitum and were housed in cages of 2-4 (2 animals were housed separately due to fighting). All procedures had the approval of the UT Arlington Institutional Care and Use Committee and followed the ethical guidelines for pain experiments in animals (Zimmermann, 1983). Seven animals were excluded due to facility noise during testing or nerve damage, leaving 8 animals for analyses.

## 5.3. Methods

### *Overall Methods*

Animals were randomly pulled from the UT Arlington vivarium and placed into groups. Animals were handled and housed in a small colony room for the duration of the experiment. Baseline MPWT recording was conducted to ensure that there was no preexisting hypersensitivity. Animals were placed under anesthesia with 3% isoflourane/97% oxygen inhaled gas for surgery. The cuff electrode was implanted then three full days were allotted for recovery. The following day, animals were placed under light anesthesia and the stimulator was connected to the cuff implant pins. MPWT, PEAP, and spontaneous pain behavioral tests were conducted during peripheral nerve stimulation (stimulator specifications available in Appendix A). Euthanasia followed testing and a brief autopsy was performed to ensure that the cuff stimulating electrode was still placed securely around the L5 nerve location.

### *Cuff-stimulating Electrode Model*

To create the CSE model in freely moving animals, the cuff was implanted as previously described with slight modifications made for chronic placement (as detailed in

Li, 2014). All tools and equipment were autoclaved before surgery. The wire was prepared with betadine and surgical scrub before implantation. After the cuff was implanted and stabilized at the site the muscle tissue was sutured and the skin was stapled. To treat pain at the incision site, .1 ml of lidocaine was applied underneath the staples. During recovery, animals were monitored for eating, drinking, breathing, chromodachyhorrea, wound status, and changes in fur that may indicate distress.

#### *MPWT Procedures*

MPWT testing was applied at baseline and after implantation for the purpose of ensuring that there was no hypersensitivity due to the surgical implant. It was important to demonstrate that electrical stimulation was the source of pain indications, rather than a pain condition that was inadvertently created by cuff implantation.

#### *PEAP Procedures*

PEAP procedures were conducted as before, with the exception of a change in stimulation. When the animals were in the dark side of the chamber, a high electrical stimulation of 100 Hz 1V was applied through the cuff implant. When in the light side of the chamber a lower electrical stimulation at 50 Hz .5V was applied.

#### *Spontaneous Pain Testing*

The formalin test method for recording spontaneous pain behaviors was modified in this experiment as a measure of spontaneous pain behavior. Animals were placed into a testing chamber and paw up, paw down, and licking was quantified during stimulation. Each 10 second stimulation was administered followed by one minute of rest between each stimulation for 10 trials. The last 5 trials were recorded without any stimulation to investigate behaviors in the absence of stimulation and to measure any lingering spontaneous pain behaviors. The spontaneous pain test was measured twice for each animal, at both high (100 Hz, 1v) and low (50 Hz, .5V) stimulation parameters.

## 5.4. Results

### *CSE Implant did not Change MPWT Threshold*

Repeated measures ANOVAs were run for MPWT data on each paw (baseline and post-injection) for both left and right paw scores. Results for the **left paw** MPWT scores indicated that there was no significant effect for time,  $F(1, 7) = 3.791, p = .093$ . Results for the **right paw** MPWT scores did not change before and after surgery (the mean was 363.90 before and after surgery). Results are summarized in Figure 5-1.

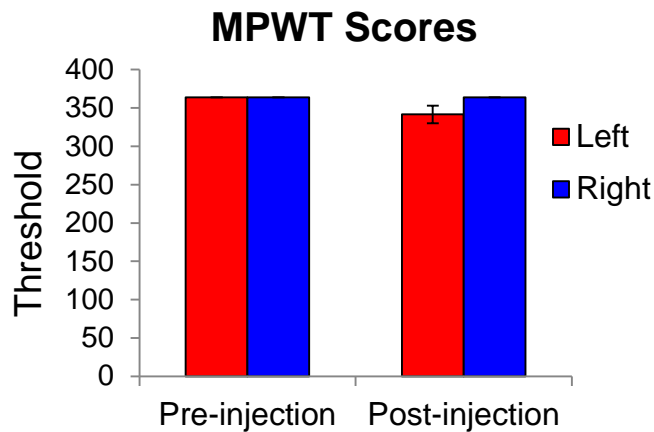


Figure 5-1 MPWT Scores Before and After CSE Implantation (N=8)  
There was no significant drop in MPWT threshold due to implantation.

### PEAP Avoidance during CSE Stimulation

To analyze PEAP data within the test, a repeated measures ANOVA was run for preferences over time. Data were presented in 5 minute time bins. Results indicated that there was no significant change in preferences over time  $F(5, 35) = .749, p = .592$  (See Figure 5-2).

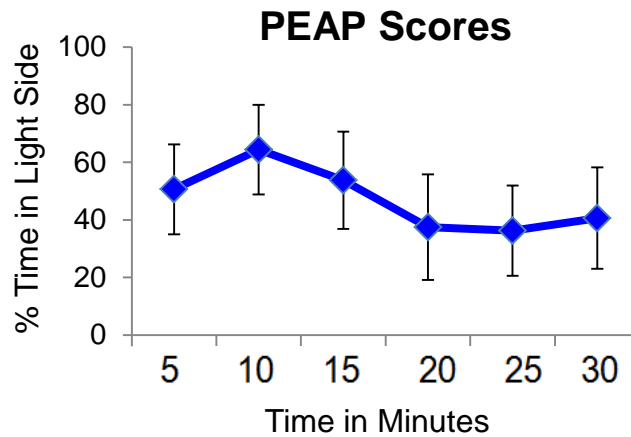


Figure 5-2 PEAP Scores in Five Minute Time Bins (N=8)  
 The percent time spent in the light side of the chamber during the PEAP test receiving right paw stimulation was collapsed into 5 minute time bins. There were no significant differences over time.

To assess exploratory behavior (crosses), a repeated measures ANOVA was run for crosses over time (5 minute time bins). Results indicated that there was a significant change in crosses over time  $F(5, 35) = 3.047, p = .022$  (See Figure 5-3). Post hoc tests revealed that crossing behaviors were significantly higher during the first 5 minutes than during 15-20 and 25-30 minutes of the test,  $p < .05$ .

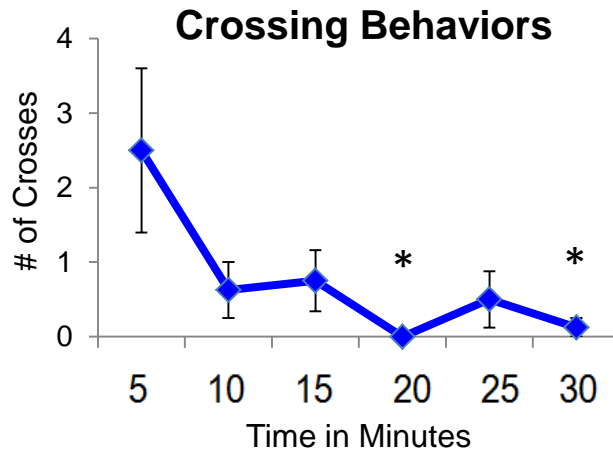


Figure 5-3 Midline Crosses During the PEAP Test in Five Minute Time Bins (N=8)  
 The number of times the animals crossed from one side of the chamber to another within the PEAP test was recorded every 15 seconds. This was collapsed into 5 minute time bins. \* The 15-20 and 25-30 minute time bins were significantly different from the first 5 minutes time bin

*Spontaneous Pain Behaviors were Present during CSE Stimulation*

Spontaneous behaviors were analyzed by taking average pain scores (the up, down, and flick behaviors) for each animal. Spontaneous behaviors were recorded in 1 minute time bins. Average scores for up, down, and lick were calculated. A pain score was then computed for each trial using the mean amount of time spent up, down, or licking the paw. The weighted pain score formula was calculated using the previously reported formula in the literature as follows:  $[(0 \times \text{down}) + (\text{up}) + (2 \times \text{lick})] / 300$ . Using this formula, lower pain scores indicate less spontaneous pain behavior and higher scores indicate more spontaneous pain behaviors.

A repeated measures ANOVA was run to compare baseline spontaneous pain behaviors during low stimulation (50 Hz, .5V) and the 5 minute resting period (after stimulation). Results indicated that there were no significant changes in spontaneous pain behavior,  $F = (2, 14) = 3.108, p = .076$ . A repeated measures ANOVA was run to compare baseline spontaneous pain behaviors during high stimulation (100 Hz, 1V) and

the 5 minute resting period (after stimulation). Results indicated that there was a significant increase in spontaneous pain behaviors,  $F = (2, 14) = 5.978$ ,  $p = .013$ . Spontaneous pain behaviors were higher than baseline during stimulation ( $p = .008$ ) and during rest following stimulation ( $p = .040$ ) (See Figure 5-4).

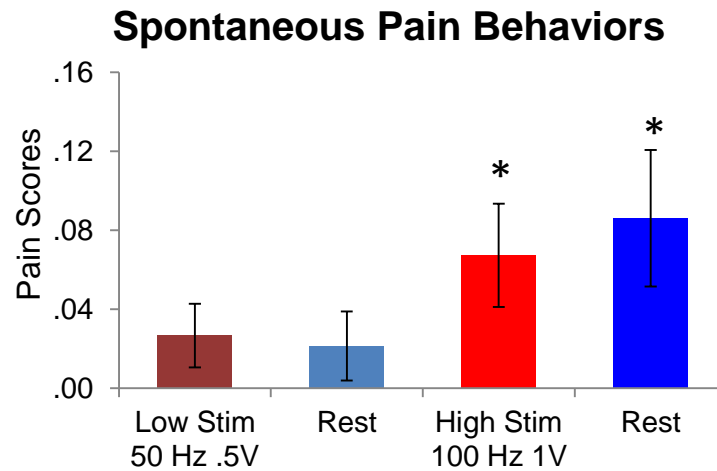


Figure 5-4 Spontaneous Pain Behaviors during Cuff Stimulation (N=8) Spontaneous pain behaviors during entire stimulation and resting periods. Spontaneous pain behaviors were significantly higher than baseline during the high stimulation and resting period following high stimulation.

Spontaneous pain behavior scores were further broken down into one minute time bins (10 minutes of stimulation followed by 5 minutes of rest) to assess changes in spontaneous pain behaviors over time. Repeated measures ANOVAs were run for each stimulation parameter (high or low stimulation) and scores over time using 0 (no spontaneous pain behaviors prior to stimulation) as the first time point (baseline) with LSD post hoc tests. Overall results were summarized in Figure 5-5.

Results from the repeated measures ANOVA for **high stimulation** revealed that there was a significant effect of time,  $F(15, 105) = 1.815$ ,  $p = .042$ . LSD post hoc tests revealed that scores during high stimulation at minutes 2, 7, 8, 9, & 10 were significantly higher than baseline ( $p < .05$ ). Scores during rest at minutes 1, 2, & 4 were significantly

higher than baseline ( $p < .05$ ). Results from the repeated measures ANOVA for **low stimulation** revealed that there was no significant effect of time,  $F(15, 105) = 1.682$ ,  $p = .066$ .

### Spontaneous Pain Behaviors

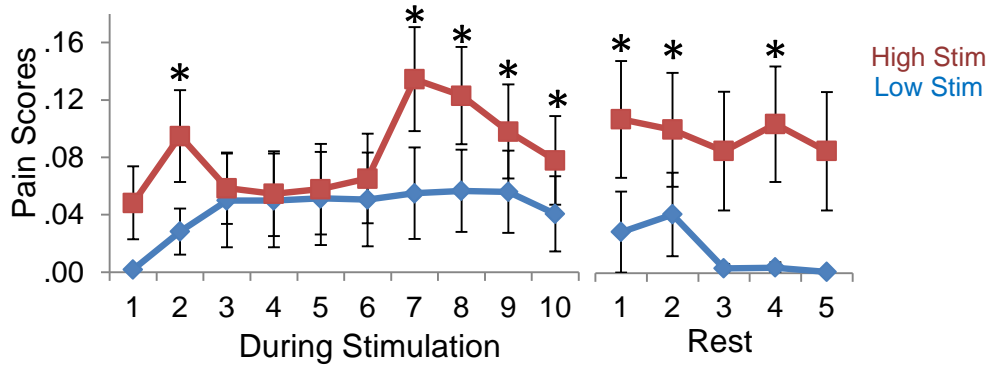


Figure 5-5 Spontaneous Pain Behaviors in One Minute Time Bins (N=8) Spontaneous pain behaviors in one minute time bins during low and high stimulations as well as during the five minute resting period with no stimulation. There were no significant changes from baseline during the low stimulation test and resting period. \*Represents a significant difference from baseline during the high stimulation test and resting period.

#### 5.5. Discussion

It was important to demonstrate that implantation of the CSE could be accomplished without significant nerve damage. Results indicated that there was a slight yet non-significant drop in left paw MPWT threshold and no change in right paw threshold which allowed for analysis of the CSE implant stimulation as a possible model for spontaneous pain rather than a neuropathic pain condition inadvertently created by surgical implantation at the nerve site.

The fourth aim (**Aim 4**) was to determine the effectiveness of the cuff stimulating electrode model as a model of spontaneous pain. The ninth hypothesis (**H<sub>9</sub>**) was that the cuff model would result in spontaneous pain behaviors. Overall, the low stimulation yielded less spontaneous pain behaviors than high stimulation. The high stimulation



resulted in a significant increase of spontaneous pain behaviors from baseline whereas the low stimulation did not. Analyses in one minute time bins indicated that spontaneous pain behaviors at five time points during high stimulation were significantly different from baseline whereas low stimulation scores were not different from baseline at any time point. Additionally, it is interesting to note that after the low stimulation there were less spontaneous pain behaviors during rest, yet conversely, after the high stimulation there was an increase in spontaneous pain behaviors during rest. In fact, 3 out of the 5 time points during high stimulation were significantly higher than baseline. This suggests that high intensity stimulation selectively results in behavioral wind-up which persists for minutes after the high stimulation is over. Overall, the cuff stimulation resulted in spontaneous pain behaviors at the high parameter, lending support for the ninth hypothesis.

It was expected that during the PEAP test, animals would spend increasingly more time in the light side of the chamber over time yet this was not supported by the results. One possible explanation for the lack of an increase of time spent in the light side of the chamber is the animals may not be able to discriminate between the two different stimulation parameters. It was expected that the dark and light sides of the chamber would provide sufficient exteroceptive cues for the animals to associate with a higher and lower stimulation, respectively. Classic literature on conditioning has indicated that animals may not be able to discriminate between varying electrical stimulations, and a generalized response to multiple stimuli may emerge (Honig & Slivka, 1964; Honig & Urcuioli, 1981). Moving forward, selecting a lower and higher intensity stimulation could be used to help animals discriminate between aversive and non-aversive options.

Providing additional exteroceptive cues or developing a stimulation specific novel paradigm could also be helpful. An alternative explanation for the lack of shift to the light

side of the box is that it is possible that both stimulation parameters were aversive/unpleasant such that learned helplessness played a role within the learning of the study. Learned helplessness has been demonstrated in dogs studies where electrical shock has been applied in shuttle box paradigms (Overmier & Seligman, 1967; Seligman & Maier, 1967).

Despite the lack of increase of time spent in the light side of the chamber avoiding higher stimulation, the overall PEAP result averages at each time bin were consistent with averages of animals in experimental groups with pain conditions shown in previously published papers (Uhelski & Fuchs, 2010; Uhelski, Morris-Bobzean, et al., 2012). For this reason, we can cautiously accept the tenth hypothesis ( $H_{10}$ ) that the cuff model stimulation would result in the development of pain affect. Determining the presence and level of pain affect in animals with CSE model may require a reframing of behavioral assessment within the test, as well as an investigation into the possibilities of removing generalization and/or learned helplessness in the model which may be contributing to a flat line of preferences over time bins.

This research expands upon the method of CSE stimulation described by Li (2014) by using lower parameters that do not excite motor reactions or intense vocalizations from the animals. However, additional research is needed to determine definitively if the model can be used as a model of spontaneous pain in research animals using a wide variety of stimulation parameters.

Moving forward, the CSE model could yield potential benefits for animal use and conservation. The proposed pain model can reduce the amount of pain and distress to the animals, as testing should include only the necessary amount of stimulation required for experimental methods. The model can also reduce the number of animal controls that are needed because varying degrees of pain can be studied within the same animal. It is

known that many experimental animals with nerve injury do not develop neuropathic pain conditions. Rather than excluding animals based on rigid guidelines for the development of a condition, we can cater the incoming stimulation to account for some inter-individual variance. Moreover, the experimental control over peripheral input can allow the experimenter to investigate the complex relationship between peripheral input and brain activity to understand the relationship more deeply.

## Chapter 6

### OVERALL CONCLUSION

The principal purpose of this research was to more deeply reveal the local field potential activity in the ACC and S1 areas during pain processing. It has been stated that there is a clear neural signature for pain constructed from these areas with clear sub-functions, however, studies like this demonstrate that the relationships between stimuli, brain activity, and the perception of pain may be more complicated than we previously imagined. Discussion in the literature about areas like the ACC and S1 in the “pain matrix” are moving from a consensus of a classic dissociation (the S1 is to sensory processing as the ACC is to emotional processing), to a view that the role of these areas in processing pain is more integrated than previously imagined (Legrain et al., 2011). For example, recent findings have indicated that the intensity of stimuli can be dissociated from activity in the pain matrix which could shift experimental emphasis on the intensity of the stimulation to a view that places a growing emphasis on the organism’s response to the threat and the salience detection system (Borsook, Edwards, Elman, Becerra, & Levine, 2013; Hayes & Northoff, 2012; Legrain et al., 2011; Sabatinelli, Bradley, Lang, Costa, & Versace, 2007). The ability of the organism to recognize, orient, and react to salient sensory stimulation is processed within the pain neuromatrix in a complex fashion and influences the multifarious nature of the processing (Legrain et al., 2011; Vogt & Sikes, 2000).

#### *Local Field Potential for Pain Research*

The addition of local field potential recording to pain research using freely moving animals provides a window to the researcher for understanding changes in the brain during manipulations of the nervous system. The added benefits of having local field potential profiles in the ACC and S1 include the following:

- Identifying differential low-frequency broad-band activity in brain areas
- Investigating brain activity related to varying peripheral inputs / pain models
- Revealing the brain activity that underlies pain behaviors and organism detection and responses to threat
- Examining how neural activity changes with pain over time
- Examination of large synaptic network communication

#### *Cuff Stimulating Electrode (CSE) Model*

Another goal of this research was to propose and validate the CSE pain model using converging evidence from behavioral and electrophysiological experiments. The implications of having a pain model that can be manipulated in a controlled way in freely moving animals include:

- Introducing variable peripheral input while recording LFP activity to reveal dynamic response profiles for brain areas
- A controllable method for the experimenter to investigate spontaneous pain
- Reducing animal distress by applying only the stimulation necessary to accomplish experimental methods
- Reducing animal numbers required for experimentation

#### *Final Conclusion*

In the past, translation of preclinical knowledge of pain has sometimes failed to effectively translate into clinical efficacy (Berge, 2011; Gereau et al., 2014). The underlying neurobiology of immediate and long-term changes in chronic pain is inarguably complex (Zhuo, 2014). A more comprehensive view of pain using a combinatorial approach of simultaneous behavioral and electrophysiological recordings will help us to further understand the complex mechanisms we encounter in preclinical studies. We hope that by using local field potential in freely moving animals, we can

improve the understanding of pain mechanisms including the role of the ACC and S1 areas.

## Appendix A

### Specifications for Wireless Recording and Stimulating Modules

The wireless recording system contains both recording and stimulating modules. The hardware mounts easily to a custom rodent backpack and allows free movement of the animals (see hardware in Figure A-1) (Zuo et al., 2012).

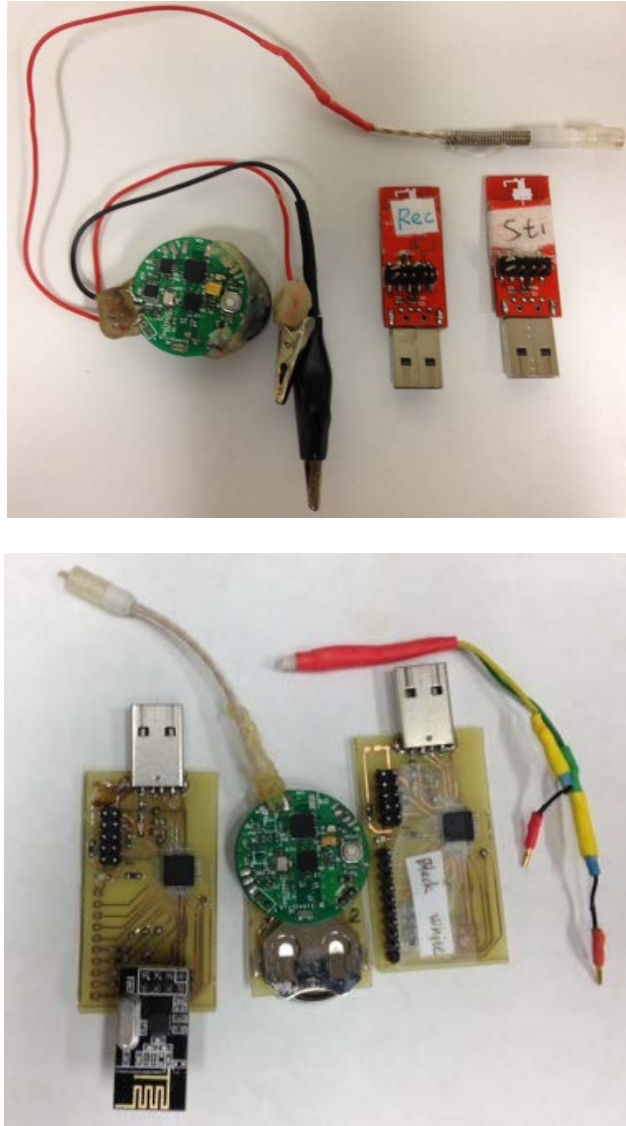


Figure A-1 Custom Designed System Hardware (As described in Zuo et al., 2012)



For local field potential recording, signals from the electrode are amplified and changed from volts to digital form by an Analog-to-Digital Converter (ADC) within the recording microcontroller module (Zuo et al., 2012). Signals are then transmitted to a receiver on a USB dongle in a computer. For stimulation, the computer is pre-programmed with stimulation parameters, and signals are transmitted to the stimulating device. Signals are translated by a Digital-to-Analog converter (DAC) in the microcontroller unit (MCU) located in the stimulator module. Signals are amplified and then stimulation is applied (Zuo et al., 2012).

Appendix B  
Histological Confirmation

Placement was confirmed using Thionine staining and microscope observation with reference to "The Rat Brain in Stereotaxic Coordinates" (Paxinos & Watson, 1998). An example of the insertions of the electrode in the target areas is demonstrated below:

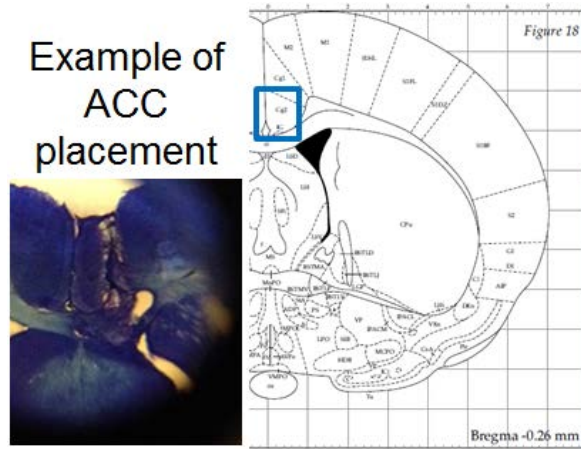


Figure B-1 Representative ACC Placement

Atlas portion of the figure reprinted by permission from Macmillan Publishers Ltd (Paxinos, G., & Watson, C. (1998). The rat brain in stereotaxic coordinates. Fourth edition. *Academic Press.*) Copyright (1998).

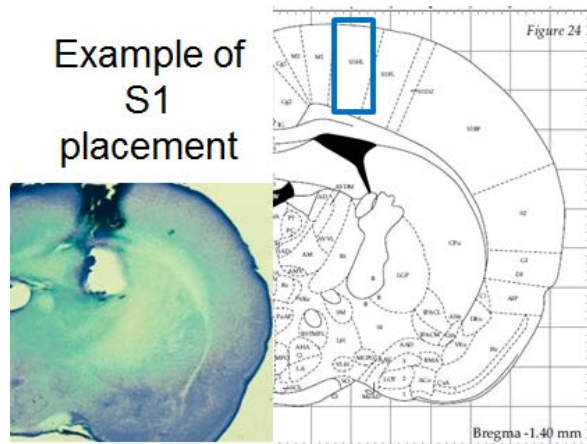


Figure B-2 Representative S1 Placement

Atlas portion of the figure reprinted by permission from Macmillan Publishers Ltd (Paxinos, G., & Watson, C. (1998). The rat brain in stereotaxic coordinates. Fourth edition. *Academic Press.*) Copyright (1998).

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### Biographical Information

Amber received her Bachelor of Science in Psychology with a minor in Social Work from Tarleton State University in 2008. She obtained a Master of Science in Educational Psychology with an emphasis in Experimental Psychology from Tarleton in 2010 while doing research with humans on cerebral lateralization and handedness and beginning her teaching skills. She came to the University of Texas at Arlington in 2010 to participate in the Health Psychology PhD program with an emphasis in Neuroscience and continued teaching. She received her Master of Science in Health Psychology in 2013 while working in the behavioral neuroscience of pain lab under Dr. Perry Fuchs. Her PhD work is being done in the electrophysiology pain lab under mentorship from Dr. Yuan Bo Peng. While at UT Arlington, Amber has had the opportunity to work on a variety of projects. After graduation, Amber plans to continue her career in research in neuroscience and teaching in the fields of biology, psychology, and education.