PAIN MODULATION BY THE AMYGDALA: HEMISPHERIC LATERALIZATION OF PRO-
AND ANTI-NOCICEPTIVE PROPERTIES DURING THE
PROGRESSION OF INFLAMMATORY PAIN

by

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Abstract

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Inflammatory pain is a feature of various diseases, and a leading cause of disabling discomfort among patients. Despite the extensive and costly neurophysiological and pharmacological research, even the most potent of the currently available treatments do not work for all patients, and cause adverse side effects that hinder effective pain management. In an attempt to identify potential target sites for alternative pain alleviation procedures, the current study investigated the role of the central nucleus of the amygdala (CeA) in the onset and progression of inflammatory pain. This is the first in vivo study to investigate pain-related changes in neural oscillations, as a basis for demonstrating the lateralization of the pro- and anti-nociceptive properties of the CeA. The main goal of this study was to determine pain-related changes in the left and right CeA activity, and the effect of unilateral lesion of the CeA on the onset and progression of inflammatory pain. The study used adult Sprague Dowley rats with: 1) right CeA lesions, 2) left CeA lesions, and 3) no lesions (control). Rats in the right and left CeA lesion groups had a bipolar electrode chronically implanted into the spared CeA. The non-lesion (control) group had two subgroups of
rats with either a right or left CeA electrode implant. Inflammatory pain was induced by subcutaneous injection of 2 mg of carrageenan (100µl of 2% (w/v) in saline) into either the left or right hind paw. Each of the three study groups had two sub-categories of animals based on the side of the carrageenan injection. Local field potential (LFP) oscillations were recorded at 6 time points; that is, baseline, immediately, 1, 3, 5, and 48-72 hours after the carrageenan injection. The paw withdrawal thresholds and latencies, as well as the neural oscillations induced by thermal and mechanical stimuli were recorded at all the time points, except immediately after the injection. Overall, the results revealed that: 1) there was lateralized increase and/or decrease in the CeA oscillatory activity in some, but not all, frequency bands and conditions; 2) the response patterns and differential changes in the right and left CeA neural oscillations were consistent with the known trend of pain-related changes during persistent pain; 3) the right, but not left, CeA lesions induced early onset and enhancement of mechanical, but not thermal, sensitivity; and 4) both the right and left CeA neural oscillations strongly correlated with pain sensitivity, but the right CeA oscillations were the prominent predictors of mechanical and thermal sensitivity. This is the first in vivo study to demonstrate lateralization of pain-related changes in the neural oscillations of the CeA, and provides numerous implications, and insight into directions for future research.
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Chapter 1

Introduction

1.1 Background

Inflammatory pain is a feature of many diseases and illnesses including arthritis, ulcerative colitis, irritable bowel syndrome, multiple sclerosis, and cancer, and is one of the major public health concerns. Over 100 million people in the United States suffer from chronic pain conditions and between $560 to 635 billion is annually spent on pain treatment (Fishman, 2012; Gaskin & Richard, 2012). However, despite this high expenditure even the most potent of the currently available treatments do not work for all patients or pain conditions, and cause adverse side effects that range from mild to life threatening (Fishman, 2012). The fear of the adverse side effects hinders the effective use of pain medication, as demonstrated by 72.6% of clinicians who could not prescribe the maximum dose of opioid analgesics required to relieve cancer pain (Chang et al., 2005). Severe pain is thus undertreated, and the unrelieved pain causes disabling and prolonged discomfort among patients. This has resulted in an increasing need for alternative treatment strategies.

One of the important steps toward achieving this goal is identification of the brain sites involved in the development and progression of inflammatory pain conditions, which could be potential targets for alternative surgical, pharmaceutical or electrophysiological treatment procedures. The amygdala is one of the major sites involved in the integration and processing of both the emotional and sensory dimensions of pain (Carrasquillo & Gereau, 2007; Crock et al., 2012; Jeong S Han,
Bird, & Neugebauer, 2004; G. Ji & Neugebauer, 2010; Neugebauer, Li, Bird, & Han, 2004; Neugebauer, 2007; H.-C. Pape & Pare, 2010). The amygdala is not only the neural substrate for the integration of the emotional and sensory dimensions of nociceptive input, but also plays a central role in the facilitation and inhibition of pain.

1.2 The Amygdala Nuclei involved in Pain Modulation

The amygdala has over twelve nuclei (Pape & Pare, 2010) which are anatomically and functionally distinct (Ren & Neugebauer, 2010). Among all the amygdala nuclei, the lateral (LA), basolateral (BLA), and central nucleus (CeA) are of particular interest in pain research because they are involved in the processing and integration of sensory information (Ji & Neugebauer, 2008; Phelps & LeDoux, 2005) including nociceptive input and pain-related emotions. The reciprocal relationship between pain and negative emotions is believed to be mediated by these nuclei of the amygdala (Ikeda et al., 2007; Langevin, 2012; Li & Neugebauer, 2004a, 2004b; Neugebauer et al., 2004; Veinante et al., 2013a) which have projections to various brain sites involved in the processing of emotions and endogenous pain control.

1.2.1 The Lateral (LA) Nucleus

The lateral (LA) nucleus is the initial site of sensory convergence and integration in the amygdala (G. Ji & Neugebauer, 2010; Neugebauer et al., 2004; H.-C. Pape & Pare, 2010). It receives polymodal sensory information from the thalamus and cortical regions such as the insular cortex, anterior cingulate cortex and association areas (Kulkarni et al., 2005; Sowards & Sowards, 2002). This sensory
information is processed by the LA before it is projected to the CeA (G. Ji & Neugebauer, 2010; Phelps & LeDoux, 2005; Sah, Westbrook, & Lüthi, 2008; Zschenderlein, Gebhardt, von Bohlen Und Halbach, Kulisch, & Albrecht, 2011). The LA also indirectly projects to the CeA via the BLA, but it is still unclear whether the LA sufficiently influences the CeA activities through the direct connection or via the indirect LA-BLA-CeA link (Phelps & LeDoux, 2005).

1.2.2 The Basolateral (BLA) Nucleus

The BLA and CeA are the two amygdala nuclei that play crucial roles in pain modulation, with the BLA being mainly involved in regulation of the emotional dimension of pain (Finnegan, Chen, & Pan, 2006). The BLA has GABA, glutamate, all types of opioid (mu, kappa, and delta) (Finnegan et al., 2006), and CGRP receptors (Li et al., 2008): all of which are involved in pain modulation through regulation of the excitatory glutamatergic projections to the CeA (Tappe-Theodor, Fu, Kuner, & Neugebauer, 2011). The BLA is involved in mediating fear-conditioned analgesia (Finnegan et al., 2006; Rea, Lang, & Finn, 2009), which is regulated by cannabinoid1 (CB1) receptors that are highly expressed on GABAergic interneurons in the BLA but not present in the CeA (Roche, O’Connor, Diskin, & Finn, 2007).

The CeA activity is directly influenced by the BLA through glutamatergic projections, and indirectly through the GABAergic intercalated cell masses (ITC) which if activated inhibit the CeA neurons (Pape & Pare, 2010; Ren & Neugebauer, 2010; Tappe-Theodor et al., 2011). The inhibition of the CeA neurons via the BLA-mediated activation of the GABAergic ITC neurons (Tappe-Theodor et al., 2011) is an underlying mechanism for loss of fear expression (Pape & Pare, 2010; Ren &
Neugebauer, 2010; Ren, Palazzo, Maione, & Neugebauer, 2011), but it is not yet clear whether it can result in pain inhibition (Ren & Neugebauer, 2010). There is however, evidence suggesting that excessive emotional arousal induces the BLA-mediated inhibition of the CeA activity via the GABAergic ITC neurons, which can lead to freezing behavior and emotion-induced analgesia (Rea et al., 2009).

1.2.3 The Central Nucleus of the Amygdala (CeA)

The CeA is the major output nucleus of the amygdala (Fudge & Tucker, 2009; Sah et al., 2008) and it is anatomically and physiologically associated with pain modulation (Han et al., 2006; Kolber et al., 2010). Through the LA and BLA, the CeA receives information of various modalities (Carrasquillo & Gereau, 2007; Finnegan et al., 2006; Neugebauer et al., 2004; Ren & Neugebauer, 2010) including emotional and nociceptive input (Neugebauer, 2007). The CeA also receives purely nociceptive information from the spinal cord via the parabrachial area (PB). This purely nociceptive projection is the spino-parabrachio-amygdaloid pain pathway, which originates in the spinal cord dorsal horn and terminates in the laterocapsular division of the CeA (CeLC) (Bourgeais, Gauriau, & Bernard, 2001; Carrasquillo & Gereau, 2007; Han et al., 2004a; Watabe et al., 2013). Thus, the CeA receives direct nociceptive projections from the spinal cord and brainstem (Carrasquillo et al., 2007; Gauriau & Bernard, 2002; Han et al., 2010, 2006; Neugebauer et al., 2004) and indirectly receives projections from the thalamus and cortex through the LA and BLA nuclei (Fu et al., 2008; Han et al., 2006; LeDoux, 2000; LeDoux, 2003; Neugebauer et al., 2004; Ren, Palazzo, Maione, & Neugebauer, 2011).
The CeA has projections to various brain sites involved in the modulation of emotions and endogenous pain control (Han et al., 2006; Neugebauer et al., 2004; Phelps & LeDoux, 2005). It regulates pain through multisynaptic projections to the basal forebrain, substantia innominata dorsalis, brainstem, and periaqueductal grey (PAG) (Li & Neugebauer, 2004a) as well as the rostral ventromedial medulla (RVM) (Gebhart, 2004; Han et al., 2006; Palazzo et al., 2011). Through its projection to the periaqueductal gray (PAG), the CeA modulates the descending pain inhibitory (Gauriau & Bernard, 2002; Palazzo et al., 2011) and descending pain facilitatory or disinhibition (Ji, Fu, Adwanikar, & Neugebauer, 2013) systems of the PAG, RVM and spinal cord (see Figure 1-1).

![Figure 1-1: The amygdala nociceptive nuclei and circuitry](image)

The polymodal sensory information received by the LA (red dotted arrow) undergoes associative processing in the LA-BLA circuitry and an affective component is added to this sensory information before it is projected to the CeA (blue arrows). The CeA also receives a direct purely nociceptive input via the spino-parabrachial-amygdaloid pain pathway (red solid arrow), and integrates the sensory
and emotional dimensions of pain. It has projections to various supraspinal sites including the thalamus, hypothalamus, and brainstem (purple arrow), which are involved in the modulation of emotions and endogenous pain control. The CeA also modulates the descending pain pathways through its projections to the PAG (black arrow).

1.3 The Pro-nociceptive and Anti-nociceptive Properties of the Amygdala

Among the amygdala nuclei, the CeA is particularly important in pain modulation (Han, Fu, Bird, & Neugebauer, 2006; Kolber et al., 2010). Most (80%) of the neurons of the laterocapsular division of the CeA (CeLC) exclusively respond to painful stimuli (Neugebauer et al., 2004) and this portion of the CeA is referred to as the “nociceptive amygdala” (Bird et al., 2005; Gonçalves & Dickenson, 2012; Hamlin, McNally, & Osborne, 2007; Han, Bird, & Neugebauer, 2004; Han et al., 2006; Ikeda, Takahashi, Inoue, & Kato, 2007; Li & Neugebauer, 2004a, 2004b; Min, Yang, Yen, Chen, & Cheng, 2011; Nakao, Takahashi, Nagase, Ikeda, & Kato, 2012).

Research evidence indicates that the CeA plays dual pain-inhibitory (Nandigama & Borszcz, 2003; Neugebauer et al., 2004; Palazzo et al., 2011) and pain-facilitatory roles (G. Ji & Neugebauer, 2008; Neugebauer et al., 2004; Veinante et al., 2013; Zhang & Hammond, 2009). For example, electrical and chemical stimulation of the CeA induces both inhibition and activation of PAG neurons (da Costa Gomez & Behbehani, 1995; Neugebauer et al., 2004). Similarly, bilateral lesions of the CeA inhibit shock-induced hyperalgesia but also prevent shock-induced anti-nociception in the tail-flick test (Crown, King, Meagher, & Grau, 2000; Neugebauer et al., 2004).
1.3.1 The Dual Pain-Facilitatory and Pain-Inhibitory Roles of the CeA

The pain-related roles of the CeA have been attributed to its involvement in the modulation of the descending pain inhibitory (Gauriau & Bernard, 2002; Palazzo et al., 2011) and facilitatory (Ji et al., 2013) systems of the PAG, RVM and spinal cord, as well as its pro-nociceptive and anti-nociceptive receptor mechanisms.

1.3.2 The Pain-Facilitatory Mechanisms of the CeA

The CeA has a number of receptors which when activated increase pain sensitivity. For example, persistent pain induces activation of Group I metabotropic glutamate receptors (mGluR1 and mGluR5) (Ansah, Gonçalves, Almeida, & Pertovaara, 2009; Crock et al., 2012; Han, Li, & Neugebauer, 2005; Han & Neugebauer, 2005; Neugebauer, Li, Bird, Bhave, & Gereau, 2003), calcitonin gene-related peptide (CGRP1) receptors (Han et al., 2004a, 2005; Han & Neugebauer, 2005), N-methyl-D-aspartate (NMDA) receptors (Han et al., 2006), and corticotropin-releasing factor (CRF1) receptors (Ji & Neugebauer, 2008). The activation of these pro-nociceptive receptors induces changes in synaptic plasticity and increases the excitability of the CeA neurons (Gonçalves & Dickenson, 2012; Neugebauer et al., 2004).

Previous evidence indicates that mGluR5 postsynaptically enhance both the excitatory and inhibitory transmission of the CeLC neurons during both normal and painful conditions (Ren & Neugebauer, 2010). On the other hand, the activation of mGluR1 which occurs during pain conditions may result in the disruption of the GABAergic inhibitory mechanisms that control the excitatory synaptic transmission under normal circumstances (Ren & Neugebauer, 2010). The disruption of the
GABAergic inhibitory mechanisms of the CeA can result in increased excitatory synaptic transmission, and subsequent activation of the descending pain facilitatory pathways. This is demonstrated by the mGluR1-mediated changes in synaptic plasticity and sensitization of the right CeA neurons (Ren & Neugebauer, 2010) which are associated with an increase in nociceptive transmission along the CeA-PAG projection (Carrasquillo & Gereau, 2008). This projection is largely GABAergic and its activation can induce a GABA-mediated inhibition of the opioid and serotonin receptors of the PAG. The inhibition of these receptors in the PAG results in disruption of the descending inhibitory, and activation of the descending facilitatory mechanisms. The activation of the descending pain facilitatory pathway is demonstrated by infusion of a mGluR1 agonist (DHPG) into the CeA which activates the ON-cells in the RVM and enhances pain sensitivity (Ansah et al., 2009).

The CeA-dependent descending pain facilitation is also demonstrated by an increase in the levels of glucocorticoids in the CeA, which is characterized by sensitization of lumbosacral spinal neurons that results in colorectal hypersensitivity, and spontaneous activity in the spinal neurons with nociceptive input from the colon and rectum (Neugebauer et al., 2004). The increased sensitivity of the spinal neurons in absence of injury or damage to the colon and rectum receptors suggests that the changes in the CeA can disrupt the descending inhibitory (Neugebauer et al., 2004; Porreca, Ossipov, & Gebhart, 2002) and activate the facilitatory mechanisms (Ansah et al., 2009). The sensitization of spinal neurons and subsequent hypersensitivity to pain in absence peripheral of damage or injury, illustrates the central role played the CeA in the modulation of pain.
Although the CeA neurons are activated by both thermal and mechanical stimuli (Gonçalves & Dickenson, 2012; Li, Zhang, Xu, & Yu, 2012; Neugebauer & Li, 2002, 2003) the increase in their sensitivity during chronic pain is observed in response to mechanical, but not thermal, stimuli (Carrasquillo & Gereau, 2007; Neugebauer & Li, 2003). This suggests that the CeA might be less involved in the modulation of thermal pain. However, a recent study found that microinjection of opioids and a GABA<sub>A</sub> receptor antagonist (bicuculline) reduced thermal sensitivity, while the infusion of a GABA<sub>A</sub> receptor agonist (mucinol) increased thermal sensitivity (Rashvand, Khajavai, Parviz, Hasanein, & Keshavarz, 2014). These results suggest that the CeA might also be involved in the modulation of thermal pain, but due to still unknown mechanisms it has a greater effect on mechanical, compared to thermal, sensitivity.

1.3.3 The Pain-Inhibitory Mechanisms of the CeA

Some of the receptors of the CeA play a role in pain inhibition. For example, direct activation of CRF2 (Ji & Neugebauer, 2008), group II mGluRs (Han et al., 2006) and opioid (Chieng, Christie, & Osborne, 2006; Nakamura et al., 2013) receptors suppresses the sensitization of the CeA neurons and inhibits nociceptive transmission. Infusion of opioid agonists results in analgesia that is expressed through the PAG and RVM, which are part of the descending pain pathways (Carrasquillo & Gereau, 2007; Helmstetter, Tershner, Poore, & Bellgowan, 1998; McGaraughty & Heinricher, 2002; Shane, Acosta, Rossi, & Bodnar, 2003). Thus stimulation of the CeA activates the descending inhibitory pain pathway via the PAG (Oka, Tsumori, Yokota, & Yasui, 2008) and induces analgesia (Gebhart, 2004; Han et
al., 2004a; Palazzo et al., 2011; Rea et al., 2009; Tye et al., 2011). This descending pain inhibitory effect occurs as a result of increased release of spinal cord inhibitory mediators including serotonin, noradrenaline, opioids (Peng, Lin, & Willis, 1996; Peng, Wu, Willis, & Kenshalo, 2001) and GABA (Peng et al., 2001; Peng, Ling, Ruda, & Kenshalo, 2003).

The pain inhibitory mechanisms of the CeA also involve the activation of group II and III mGluRs. Activation of Group II mGluRs with a selective agonist (LY354740) inhibits synaptic transmission in normal rats as well as pain-related synaptic plasticity in the CeA of arthritic rats (Han et al., 2006). Similarly, the activation of presynaptic group III mGluRs inhibits the pain-induced changes synaptic plasticity in the CeA (Han et al., 2004, 2006). Stimulation of mGluR8 in the CeA with a selective mGluR8 agonist ((S)-3, 4-DCPG) inhibits pain through activation of the CeA-PAG-RVM descending pain inhibitory pathway, and could be used in the alleviation of chronic pain (Palazzo et al., 2011).

1.3.4 Counterbalancing Effect of the Pain Inhibitory and Facilitatory Mechanisms

The dual pain-inhibitory and pain-facilitatory mechanisms of the CeA can counterbalance each other, and result in either suppression or facilitation of the onset and progression of inflammatory pain. Both the left and right CeA are activated by acute pain but due to still unknown mechanisms, there is increased pain-related activity in the right, but not left, CeA during persistent pain. Since the CeA has both pro- and anti-nociceptive properties, it can hypothesized that the pain inhibitory mechanisms of the left CeA prevail over the pain facilitatory mechanisms. On the other hand, due to still unknown mechanisms the pain inhibitory
mechanisms of the right CeA are disrupted by the continued nociceptive input which results into sensitization of the right CeA neurons and increased sensitivity to pain.

The inter-linkage between the different receptors in the CeA with the GABAergic system is among the underlying mechanisms for the pro- and anti-nociceptive properties of the CeA. For example, the activation of mu-opioid receptors in the CeA induces inhibition of the CeA-PAG GABAergic projection (Bajo, Roberto, Madamba, & Siggins, 2011; B. Chieng & Christie, 2009; Oka et al., 2008), which in turn results in reduced GABA-mediated inhibition of opioid and serotonin receptors in the PAG. This disinhibition or activation of the opioid (Tortorici, Aponte, Acevedo, Nogueira, & Vanegas, 2009) and serotonin (Palazzo et al., 2011) receptors in the PAG results in activation of the OFF-cells, and inhibition of the ON-cells in the RVM, and subsequently induces pain inhibition (Tortorici et al., 2009). Similarly, the stimulation of mGluR8 in the CeA results in a decrease in GABA, and an increase in serotonin and glutamate release, as well as inhibition of the ON-, and activation of the OFF-cells in the RVM, and subsequently induces anti-nociception (Palazzo et al., 2011).

On the other hand, a reduction in GABA-mediated inhibition of the CeA-PAG projection, results in activation of the ON-cells and pain facilitation. For example, the activation of mGluR1 in the CeA is associated with a disruption of the GABAergic inhibitory mechanisms which control excitatory synaptic transmission (Ren & Neugebauer, 2010), and can increase nociceptive transmission along the CeA-PAG projection (Carrasquillo & Gereau, 2008). Since the CeA-PAG projection is largely GABAergic, its activation results in inhibition of opioid and serotonin receptors of
the PAG and subsequent disruption of the descending pain inhibitory, and activation of the facilitatory mechanisms. This is demonstrated by direct activation of mGluR1 in the CeA which enhances the firing of the ON-cells in the RVM and increases pain sensitivity (Ansah et al., 2009). These CeA-dependent descending pain facilitatory mechanisms can induce sensitization of spinal cord neurons which results into the hypersensitivity to pain (Neugebauer et al., 2004) and may contribute to the development of chronic pain through inducing or sustaining central sensitization (Neugebauer et al., 2004; Porreca et al., 2002). The dual pain inhibitory and facilitatory mechanisms of the CeA are illustrated in Figure 1-2.

![Figure 1-2: The dual pain facilitatory and inhibitory roles of the CeA](image)

Peripheral pain activates the dual pain inhibitory and facilitatory mechanisms of the CeA (red solid arrow). These inhibitory and facilitatory mechanisms can counterbalance each other and result in a net effect of either activation of the descending pain inhibitory or facilitatory pathways (blue arrows). It could be hypothesized that if the pain inhibitory mechanisms outweigh the facilitatory mechanisms, the descending inhibitory pathway is more activated; resulting in suppression of pain sensitivity (black arrow). On the other hand, if the pain facilitatory mechanisms outweigh the pain inhibitory mechanisms, the
descending pain facilitatory pathway is more activated; thereby resulting in hypersensitivity to pain (green arrows). If the nociceptive input is persistent, the CeA neurons undergo sensitization and nociceptive facilitation (red dotted arrow).

1.4 The Amygdala and Emotion-induced Analgesia and Hyperalgesia

The dual pain-inhibitory and pain-facilitatory roles of the amygdala are also associated with its involvement in the reciprocal interaction between pain and emotional states. Some emotional states (e.g. anxiety, and depression) are associated with changes in amygdala pain-related mechanisms as well as increased pain intensity and duration (Han et al., 2010; Strobel, Hunt, Sullivan, Sun, & Sah, 2014) while other emotional states (e.g. fear, acute stress, and strong shock) inhibit pain (Crown et al., 2000; Neugebauer et al., 2004).

The association between emotions and the pain facilitatory roles of the CeA is demonstrated by previous evidence indicating that corticosterone-induced activation of glucocorticoid and mineralocorticoid receptors in the CeA increases both anxiety and sensitivity to colorectal distension (Meerveld et al., 2001; Neugebauer et al., 2004). The enhanced anxiety observed following the infusion of corticosterone into the CeA is believed to result from the increased secretion of corticotropin releasing factor (CRF) (Neugebauer et al., 2004), which has heterogeneous effects on pain sensitivity.

Previous evidence indicates that infusion of CRF into the CeA can induce both inhibition and facilitation of pain (G. Ji et al., 2013; G. Ji & Neugebauer, 2008). The pain inhibitory and facilitatory effects of CRF have been attributed to the properties of the CRF1 and CRF2 receptors which are involved in the CRF-mediated pain
modulation in the CeA (Ji & Neugebauer, 2008). The amygdala contains more CRF1 than CRF2 receptors, and CRF has higher affinity for CRF1 compared to CRF2 receptors (G. Ji & Neugebauer, 2008). Thus, low amounts of CRF facilitate nociceptive processes through activation of CRF1 receptors, but higher concentrations of CRF results in increased activation of CRF2 receptors and consequently induce an inhibitory effect; which may serve to suppress or reverse the facilitatory effect evoked by the CRF1 receptors (G. Ji & Neugebauer, 2008). This might account for acute stress or fear-induced analgesia and the pain hypersensitivity during chronic stress. It could be hypothesized that acute stress or fear induces excessive release of CRF which activates CRF2 receptors and inhibits pain, but with the decrease in CRF levels and/or the neuroadaptations that occur during chronic stress result in increased activation of the CRF1 receptors and enhanced pain sensitivity.

1.5 Lateralization of Pain Modulation by the CeA

1.5.1 Lateralization of Pain Processing and Changes in Pain Sensitivity

Although both the left and right CeA receive, and are activated by nociceptive input (Carrasquillo & Gereau, 2007) the pain processing and pain-related changes in synaptic plasticity and sensitization are lateralized in the right CeA (G. Ji & Neugebauer, 2009). First reported by Carrasquillo and Gereau (2007; 2008) the lateralization of the pain-related mechanisms of the CeA has been found by several studies using different pain models [S.-J. Cheng et al., 2011; Fu et al.,
The magnitude of evoked activity in the left and right CeA neurons does not significantly differ under normal conditions (G. Ji & Neugebauer, 2009) but due to still unknown mechanisms, persistent pain-induced changes in synaptic plasticity and sensitization occur in the right, but not left, CeA (Gonçalves & Dickenson, 2012; G. Ji & Neugebauer, 2009). These pain-related changes in the right CeA occur regardless of the side of the peripheral injury (Carrasquillo & Gereau, 2007; G. Ji & Neugebauer, 2009; Kolber et al., 2010; Neugebauer & Li, 2003) which demonstrates that the CeA has bilateral receptive fields (G. Ji & Neugebauer, 2009).

Direct activation of the right CeA neurons results in increased sensitivity to both ipsilateral and contralateral tactile (Carrasquillo & Gereau, 2008) and painful stimuli (G. Ji & Neugebauer, 2009). Direct activation of the left CeA also induces hypersensitivity to peripheral stimuli (Min et al., 2011) but due to still unknown mechanisms the left CeA neurons do not undergo pain-induced changes in synaptic plasticity and sensitization (G. Ji & Neugebauer, 2009). Recent research evidence suggests that the underlying mechanisms that promote the activation of the right, but not left, CeA during persistent pain, are linked to receptor signaling mechanisms involving protein kinases.

1.5.2 Lateralization of Pain-Related Receptor Signaling Mechanisms

Studies using different pain models have demonstrated that persistent pain increases the levels of extracellular signal-related kinase (ERK) in the CeA (Chen et al., 2010; S.-J. Cheng et al., 2011; Min, Yang, Yen, Chen, & Cheng, 2011). The
activation of ERK is greater in the right compared to the left CeA (Carrasquillo & Gereau, 2008; Kolber et al., 2010) and is associated with enhanced PBA-CeA synaptic transmission (Min et al., 2011). On the other hand, blocking of ERK activation in the right, but not left, CeA significantly reduces hypersensitivity to peripheral pain (Carrasquillo & Gereau, 2007, 2008; Fu et al., 2008; G. Ji & Neugebauer, 2009). Blocking of formalin-induced ERK activation completely inhibits hypersensitivity in the non-injured contralateral hind paw (Carrasquillo & Gereau, 2007) which suggests that the spinal circuitry involved in contralateral hyperalgesia is modulated by the ERK signaling mechanisms in the CeA.

Inhibition of ERK signaling blocks the effect of PKA and PKC on spinal transmission (Fu et al., 2008) and significantly reduces inflammation-induced tactile hypersensitivity, but does not have an effect on the sensitivity to acute noxious stimuli (Carrasquillo & Gereau, 2007). Similarly, under normal conditions the activity of both the left and right CeA is increased by Forskolin-induced activation of protein kinase A (PKA), but a PKA inhibitor only decreases right CeA activity during persistent pain (G. Ji & Neugebauer, 2009). These results demonstrate that pain-induced activation of the receptor signaling mechanisms is lateralized, and the right CeA modulates persistent, but not acute, pain.

Carrasquillo and Gereau (2007) found that in the absence of peripheral inflammation, pharmacological activation of ERK in the CeA results in tactile, but not thermal, hypersensitivity, and correspondingly, ERK inhibitors suppressed tactile, but not thermal, hypersensitivity. This suggests that either the CeA does not
modulate thermal sensitivity or the sensitivity to thermal stimuli is modulated by different mechanisms other than ERK activation (Carrasquillo & Gereau, 2007).

ERK activation is involved in the sensitization of mGluR5, which when directly activated induce peripheral hypersensitivity in absence of injury (Kolber et al., 2010). Mechanical hypersensitivity is significantly reduced by both pharmacological inhibition and global disruption of mGluR5 expression in the right CeA (Kolber et al., 2010). Under normal conditions, the expression of mGluR5 is significantly higher in the right, compared to the left, CeA (Gonçalves & Dickenson, 2012; Kolber et al., 2010) which demonstrates a basis for lateralization of the pain-related roles of these receptors.

Whereas a number of studies using different pain models have found that pain-related changes in synaptic plasticity and sensitization are lateralized in the right CeA, some of the recent studies have not found lateralization of function. For example, a study by Min et al. (2011) found that acid-induced muscle pain caused an increase in pERK levels of both the right and left CeA, and was associated with contralateral mechanical hypersensitivity without lateralization. Tran and Meerveld (2012) found that unilateral activation of either the left or right CeA with corticosterone (CORT) induced anxiety-like behavior and somatic allodynia while visceral hypersensitivity was induced by bilateral activation of the CeA. There is evidence to suggest that direct stimulation of the left CeA neurons induces changes in their activity but due to still unknown mechanisms they do not undergo pain-induced changes in synaptic plasticity and sensitization (G. Ji & Neugebauer, 2009). It is thus possible, to induce peripheral hypersensitivity through direct activation of
the left CeA but pain-induced changes in activity have consistently been found to be lateralized in the right CeA.

Using a neuropathic pain model, Goncalves and Dickenson (2012) found that the lateralization of pain-related processes in the CeA is lateralized in a molecular and time-related manner. The neuronal activity was higher in the left CeA at 2 and 6 days after the spinal nerve legation, but declined as the pain progressed, while the right CeA activity persisted beyond 14 days, regardless of the side of injury. These results demonstrate that lateralization of pain-related processes in the CeA occurs in time based manner, but it is still not known whether the trend of lateralization is consistent or similar for different pain models.

1.5.3 *Lateralization of the Emotional Dimension of Pain*

Fear-conditioned analgesia is modulated by the descending pain inhibitory pathway, which originates in the CeA and projects to the PAG, then to the RVM, and ends in the dorsal horn of the spinal cord (Finn et al., 2006). Prolonged stress is associated with NMDA receptor-mediated changes in synaptic plasticity in the CeA, and an increase in neural transmission along the CeA-PAG projection, which is largely ipsilateral (Adamec, Blundell, & Burton, 2005; G. Ji & Neugebauer, 2009). The increase in transmission along the CeA-PAG projection during stress, is observed in the right, but not left, hemisphere (Adamec et al., 2005; Carrasquillo & Gereau, 2008; G. Ji & Neugebauer, 2009). Thus, the increased pain sensitivity associated with prolonged stress might be linked to the pain-related changes in synaptic plasticity and sensitization, which are lateralized in the right CeA (G. Ji & Neugebauer, 2009).
The CeA-PAG projection is largely GABAergic and thus an increase in neural transmission along this pathway can result in inhibition of the opioid (Tortorici et al., 2009) and serotonin (Palazzo et al., 2011) receptors and suppression of the OFF-, and activation of the ON-cells of the PAG and RVM, which consequently activates the descending facilitatory pathways and increases pain sensitivity. This is evidenced by direct activation of mGluR1 in the CeA, which is associated enhanced firing of the ON-cells in the RVM, and subsequent increase in pain sensitivity (Ansah et al., 2009).

1.5.4 Brain Imaging Evidence for Lateralization in Humans

There is increasing evidence indicating that the amygdala pain-related and emotional processes are also lateralized in humans. The evidence for lateralization of pain processing in the human amygdala is still limited, but it has been observed in different regions. For example, regardless of the side of the receptive field, both innocuous and painful stimuli induce activation of some sites of the right hemisphere including the thalamus, and frontal cortex (Coghill, Gilron, & Iadarola, 2001). Thus pain-related processes might also be lateralized in humans.

The evidence for lateralization of emotions in humans has been inconsistent with some brain imaging studies indicating left, and others right amygdala dominance in emotional processing (Tran & Greenwood-Van Meerveld, 2012). A functional Magnetic Resonance Imaging (fMRI) study found sex-related differences in emotionally arousing memories with significantly higher activation of the right amygdala for males and left amygdala for females (Cahill, Uncapher, Kilpatrick, Alkire, & Turner, 2004). Negative emotions tend to be lateralized in the right, while
positive emotions are more predominant in the left amygdala (Canli, Desmond, Zhao, Glover, & Gabrieli, 1998; Yoshimura et al., 2009).

Lanteaume et al. (2007) found that electrical stimulation of the amygdala produced both positive and negative emotional states, but the positive emotions only resulted from stimulation of the left amygdala, while negative emotions (e.g. fear, anxiety, and sadness) were induced by stimulation of both the left and right amygdala. The negative emotions were however, more frequent during right amygdala stimulation, which suggests that whereas the left amygdala is involved in the modulation of both positive and negative emotions, the right amygdala plays a greater role in regard to the negative emotions (especially fear and anxiety) (Lanteaume et al., 2007). Since negative emotions such as depression and anxiety are associated with increased pain sensitivity, their lateralization in the right hemisphere suggests possible asymmetry of pain modulation in the human amygdala.

1.5.5 *Rationale of the Current Study*

The partly inconsistent results from previous studies indicate that whereas both the left and right CeA receive, and are activated by nociceptive input, the pain-related changes in synaptic plasticity and sensitization occur in the right, but not, left CeA. Whereas these changes in the right CeA are associated with increased sensitivity to peripheral stimuli, the extent to which they contribute to the onset and progression of inflammatory pain is still unknown. This has in part, been a result of the fact that almost all of the previous studies which investigated the role of the CeA
in pain modulation were in vitro, and the few in vivo studies used anesthetized animals.

Thus the previous studies have extensively examined the underlying mechanisms, while the topic of the pain-related changes and lateralization of function in the CeA in awake or freely moving animals is still understudied. A major limitation of in vitro studies and the use of anesthetized animals is that the perceptual component and the emotional dimension of pain are eliminated and not adequately explored, and thus the results may not reflect the complete pain experience. It is thus important to use awake and freely moving animals to record the pain-related changes in the CeA, and also examine the exclusive roles of the left and right CeA in the onset and progression of inflammatory pain.

One of the effective methods for examining pain-related changes in the CeA activity in freely moving animals is the recording of local field potentials (LFPs). LFPs are a summation of the extracellular neuronal activity, and indicate the unique oscillatory mechanisms around the tip of a recording electrode (R.-K. Cheng, Williams, & Meck, 2008) implanted in a given brain site. In other words, if several neurons are activated in a regular and synchronized manner, the summation of the generated signals is recorded as LFPs, which may represent an increase or decrease in neuronal activity (Bartos, Vida, & Jonas, 2007). The recording of LFPs includes both supra and sub-threshold events and thus captures more neuronal processes than the recording of spike activity (Magri, Mazzoni, Logothetis, & Panzeri, 2012).

The LFP oscillations are portioned into different frequency bands, which are believed to indicate different neuronal events (Buzsáki, 2006; Magri et al., 2012). A
given LFP frequency band may thus be indicative of sensory, motor, and/or cognitive processes (Knyazev, 2012). The LFPs are categorized on the basis of the rate of oscillation which ranges from the slow delta and theta, to the fast gamma frequency bands (Bartos et al., 2007). The exact demarcations of the frequency bands are still unknown and partitioning of the LFPs varies depending on the impression of the recorded data or previous research (Magri et al., 2012). Therefore, the frequency bands used vary from study to study but the most frequently used by previous studies include; delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and gamma (30-100 Hz). The gamma frequency band is usually subdivided into the low-gamma (30-55 Hz) and high-gamma (65-100) frequency bands.

The main objective of the current study was to examine the changes that occur in the left and right CeA neural oscillations as the pain progresses, and the effect of unilateral lesion of the left and right CeA on the onset and progression of inflammatory pain. On the basis of the current literature, this is the first study to investigate pain-related changes in the CeA neural oscillations, and to use them as evidence for lateralization of pro- and anti-nociceptive properties.

1.5.6 Specific Aims

1) **To determine the differences in the right and left CeA neural oscillatory activity during the onset and progression of inflammatory pain.**

   **Hypothesis:** It was hypothesized that both the left and right CeA would initially respond to peripheral pain, but the activity would increase in the right CeA, and decrease in the left CeA as the pain progressed.
2) To determine the effect of unilateral lesion of the left and right CeA on the onset and progression of inflammatory pain.

Hypothesis: It was hypothesized that unilateral lesion of the right, but not left, CeA would prevent or inhibit the onset and progression of inflammatory pain.

3) To determine the predictive power of the correlations between the left and right CeA neural oscillations and inflammatory-pain induced thermal and mechanical sensitivity.

Hypothesis: It was predicted that the inflammatory pain-induced changes in the right, but not left, CeA oscillatory activity would be positively correlated with, and significantly predictive of the peripheral mechanical, but not thermal, sensitivity.
Chapter 2

Methods

2.1 Animals

The study used a total of 82 Sprague-Dawley rats (64 males and 18 females; Age: 3 – 7 months; wt: 275 – 420g). The females were used to identify the electrolytic lesion parameters, while the males were used in the experiments. Histological analysis reduced the number of the male rats used in the experiments to 43. The study had a total of three groups; control ($N = 15$) and experimental ($N = 28$) groups. Each of the animals in the experimental groups underwent a unilateral left ($N = 13$) or right ($N = 15$) CeA lesion. In each of the groups, there were two subcategories of animals that received either a left or right hind-paw carrageenan injection to induce the inflammatory pain condition. Thus overall the animals were divided into a total of six subgroups denoted as having either a left or right paw carrageenan-induced injury. That is: 1) control (non-lesion) group with a left paw injury; 2) control (non-lesion) group with a right paw injury; 3) right CeA lesion group with a left paw injury; 4) right CeA lesion group with a right paw injury; 5) left CeA lesion group with a left paw injury; and 6) left CeA lesion group with right paw injury (Figure 2-1).
The figure shows the distribution of the animals used in the study. Each of the three study groups (i.e. the right and left CeA lesion, and control groups) had two subgroups categorized on the basis of whether they had a left or right paw carrageenan-induced injury.

2.2 Experimental Protocol

The experimental protocol was approved by the University of Texas at Arlington Institutional Animal Care and Use Committee (IACUC). All the experimental procedures conformed to the National Institutes of Health (NIH) as well as those of the International Association for the Study of Pain (IASP). All the animals used in the study were bred and raised in the UTA Animal Care Facility.

2.3 Surgical Procedures

All the animals in the experimental and control groups underwent stereotaxic surgery. The procedure involved implant of a bipolar recording electrode (MS 303/1-B, Plastics One Inc. Roanoke, VA) into either the right or left
CeA. For the experimental groups, an electrolytic lesion was made in the CeA that was not implanted with a recording electrode.

2.3.1 CeA Lesions

Rats were anesthetized with 1% pentobarbital sodium (50 mg/kg i.p.) and then placed on a stereotaxic frame. For the animals in the CeA lesion groups, bilateral blurr holes were drilled at the location of the CeA (2.0 mm posterior to bregma, 4.0 mm lateral to the midline, and 8.0 mm ventral from the skull). For the reference and grounding electrode connections, two stainless screws were driven into the skull at 2mm posterior to the lambda. These screws also served as anchors for the bone cement used to hold the recording electrode in a firm position after the surgery, in addition to two other anchorage screws driven into the skull, 2mm anterior to the bregma. Two wires (MIL-W-16878E/1 Type B, dia., .012 In, Alpha Wire, Elizabeth, NJ) were connected to the two stainless screws posterior to the lambda and these wires later served as the connections for the reference and ground terminals of the LFP recording module.

A monopolar electrode was lowered into the left or right CeA and a current of +0.7 mA for 12 seconds was delivered to create a lesion using a lesion maker device (model 53500, Ugo Basile, Italy). These lesion parameters were determined in preliminary lesion trials using three groups of female rats (N = 18). Each rat in each of the trial lesion groups (N = 6) respectively received bilateral lesions at different current intensities of +.5 mA, +.7mA, and +1.0 mA, for 12 seconds. The lowest trial lesion parameters (+.5 mA; 12 sec) were based on a previous study (Campese et al., 2014) which created electrolytic lesions of the CeA using the same type of the lesion
making device (model 53500, Ugo Basile, Italy). The lesions at +.5 and +.7 mA caused moderate damage while the lesions at +1.0 mA caused extensive damage that for some of the animals included portions of the amygdala nuclei that are adjacent to the CeA. On the other hand, the lesions at +.5 mA caused minimal damage that in some animals appeared to spare parts of the CeA. On the other hand, the +.7 mA current intensity cause damage that eliminated the CeA, while sparing the surrounding amygdala nuclei.

Thus, in the current study the +.7 mA at 12 sec were used as the adequate parameters for creating CeA lesions for all the animals. Some of the previous studies have reported that for procedures involving complete lesion of the CeA, the electrolytic lesions carried out at adequate parameters are more restricted to the target sites compared to the excitotoxic lesions which damage neurons in the adjacent amygdala nuclei (Lázaro-Muñoz, LeDoux, & Cain, 2010).

2.3.2 Electrode Implantation

After the lesion was made, a bipolar recording electrode was lowered into the spared left or right CeA of all the experimental group animals. All the control (non-lesion) group animals also had a recording electrode implanted into either the left or right CeA. After the lowering the electrode into the CeA, it was glued into position using cranioplast cement (Plastics One, Inc.) with four stainless screws driven into the skull (2mm anterior to the bregma, and 2mm posterior to the lambda) serving as anchors. The animals were then kept in the recovery room with routine inspection of surgical site for possible infection. All the animals were left to recover for at least 10 days before conducting the experiment.
2.4 *Inflammatory Pain Model*

Inflammatory pain was induced by injecting 2 mg of carrageenan (100µl of 2% (w/v) in saline) into the plantar region of the left or right hind paw. Carrageenan-induced rat paw edema has all the features of an inflammatory response (Posadas et al., 2004). The carrageenan pain model mimics the key features of human inflammatory pain, and is one of the most widely used methods in the evaluation of anti-inflammatory effect of drugs (Chakraborty, 2012; Fayazuddin, Ahmad, Kumar, & Yunus, 2013; Namita, Mukesh, & Tirath, 2012). An injection of carrageenan into a rat’s paw induces a biphasic inflammatory response; with an early phase that reaches a peak at 6 hours followed by a second phase that develops after 24 hours, peaks between 48 to 72 hours and reduces at 96 hours after the carrageenan injection (Posadas et al., 2004).

The edema that occurs during the first phase has low intensity and is independent of the concentration of the carrageenan used while the second phase is associated with more substantial edema (Posadas et al., 2004). The first phase of carrageenan induced-inflammation is due to release of histamine, and serotonin (Agrahari & Panda, 2010) during the first hour (Fayazuddin et al., 2013; Namita et al., 2012; Shah et al., 2010), followed by kinins (Chauliya, Haldar, & Mukherjee, 2012) up to around 2.5 hours. The second phase is characterized by the release of prostaglandins and slow reacting substances (Kumari, Lincy, Muthukumarasamy, & Mohan, 2012; Namita et al., 2012). The kinins maintain the edema between the first and second phases (Chauliya et al., 2012) and thus mediate the continuity between the two phases (Agrahari & Panda, 2010), while the second phase is promoted by
prostaglandin, cyclooxygenase, and lipoxygenase products like substances (Chaulya et al., 2012). The expected changes during the progression of a carrageenan-induced inflammatory response are represented in Figure 2-2.

Figure 2-2: A schematic representation of the trend of carrageenan-induced biphasic inflammatory response

Carrageenan induces a biphasic inflammatory response. The first phase starts immediately after the carrageenan injection and reaches a peak at 6 hours. The second phase develops after 24 hours, peaks between 48 to 72 hours and reduces at 96 hours.

2.5 Assessment of Inflammatory Pain Progression

The development and progression of inflammatory pain was assessed using: 1) the electrophysiological changes in the CeA as the pain progressed (at baseline, immediately, 1, 3, 5, and 48-72 hours), 2) the changes in the CeA neural oscillatory sensitivity to thermal and mechanical stimuli as the pain progressed; and 3) the changes in peripheral sensitivity to thermal and mechanical stimuli applied to both
the injured and non-injured paws at baseline, 1, 3, 5, and 48-72 hours after the carrageenan injection. All the electrophysiological and behavioral testing procedures were carried out at the same time points during the night period of the circadian cycle (5:00 PM – 7:00 AM).

2.5.1 **Electrophysiological Assessment**

The LFP oscillations were recorded using MC Rack wireless module and software (Multi Channel Systems, Reutlingen, Germany) at a sampling rate of 10 kHz, with the cutoff frequency of the low pass Butterworth filter set to 200 Hz. To determine the changes in the CeA activity that could be exclusively associated with inflammatory pain, a 10 minutes recording of LFP oscillations without any peripheral stimulation was the first to be obtained at each of the 6 time points: that is, baseline, immediately, 1, 3, 5, and 48-72 hours after the carrageenan injection.

The thermal and mechanical stimuli-induced LFP oscillations used to assess the changes in the CeA neural sensitivity to peripheral stimuli, were also recorded at all the time points, except immediately after the injection. The immediate time point was excluded in order to avoid the possible confounding effects of thermal and mechanical stimuli on the onset of the carrageenan-induced inflammatory response. The thermal-induced LFPs were recorded during thermal stimulation of the hind paws using the Hargreeves method, while the mechanical-induced LFPs were recorded during the process of poking the paws using a high force Von Frey filament (100g, Filament size 610; Stoelting Co., Wood Dale, IL).
2.5.2  *Mechanical Sensitivity Assessment*

Each rat was placed in a transparent plastic chamber placed on top of a wire mesh that allowed easy access to the hind paws during the testing of mechanical sensitivity. The rats were left to habituate for at least 10 minutes. After the cessation of cage exploratory behavior, the mechanical sensitivity of both hind paws was determined using a series of 8 calibrated von Frey filaments. The von Frey filaments have an approximate scale of the actual force (threshold) at which the hind paw is withdrawn and thus indicate the extent of mechanical sensitivity.

In the current study, the paw withdrawal thresholds were determined using the up-down method (Dixon, 1980) with a series of eight Touch Test von Frey filaments ranging from 3.85 to 251.34 mN (Stoelting Co., Wood Dale, IL). As described in previous studies the von Frey filaments were applied perpendicular to the plantar region and pressed against the surface until a filament bent (Bonin, Bories, & De Koninck, 2014; Chaplan, Bach, Pogrel, Chung, & Yaksh, 1994) and then held steadily for a maximum of 3 seconds (Bonin et al., 2014). A response was considered to be positive if the paw was sharply withdrawn. The procedure was repeated in situations where the response appeared to be a random movement.

Each testing started with a filament size of 4.56 (39.42 mN) which was in the middle of the series of the filaments. After applying this filament, the next filament presented was up or down the series, depending on whether or not the previously presented filament evoked a response. During each testing interval, the filaments were applied to the left and right paws in an alternating order, which ensured that there was sufficient time for each paw to adapt or resolve the effect of the previous
stimulation before the presentation of the next filament. Thus, after registering a response for a particular paw, the next filament in the series (with a higher or lower force), was presented to that paw after testing the opposite paw.

The error in the measurement of the withdrawal thresholds was minimized by registering a total of 15 responses for each paw at each of the study time points. The 15 responses were categorized as three sets of trials: each comprising of 5 responses that made patterns such as, XOOXX, XXOX, and XXX00 for the left hind paw; and XOX00, X00, and 0 for the right hind paw in Figure 2-3.

In each of the 3 sets of trials, the first response to be registered was based on the filament that induced the first positive response (X), except in situations where there was no response to the filament with the highest force, in which case a no response (O) was registered. Thus, a no response score (0) in the first last column of the score sheet (Figure 2-3) implies that the respective paw did not respond to the filament with the highest force (251.34 mN) and the presentation of filaments for that trial stopped (as indicated in Figure 2-3; trials 2 and 3 for the right hind paw). The paw withdrawal threshold (PWT) score for each of the three sets of trials was computed using the formula below as described in a previous study (Uhelski, Boyette-Davis, & Fuchs, 2011).

\[
PWT \text{ Score} = 10 \log_{10} (F_{LR}) + (K* LR_{Seq})
\]

Where: \( F_{LR} \) is the force of the last response filament (mN); \( K = 0.2593 \), is the average interval between von Frey filaments in Log units; and \( LR_{Seq} \) is a standardized value determined basing on the pattern established at a given trial.
(e.g. XOOXX) and the sequence of the filament at the last response. The withdrawal threshold for each paw at each of the study time points was the average of the computed withdrawal threshold scores for the three sets of trials (Figure 2-3).

<table>
<thead>
<tr>
<th>Filament size</th>
<th>3.61</th>
<th>3.64</th>
<th>4.08</th>
<th>4.31</th>
<th>4.56</th>
<th>4.93</th>
<th>5.18</th>
<th>5.46</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force (mN)</td>
<td>3.85</td>
<td>5.68</td>
<td>9.74</td>
<td>18.39</td>
<td>39.42</td>
<td>77.3</td>
<td>135.3</td>
<td>251.34</td>
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<tr>
<td>Trial 1</td>
<td>X</td>
<td>O</td>
<td>O</td>
<td>X</td>
<td>O</td>
<td>O</td>
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<td>Trial 2</td>
<td>X</td>
<td>O</td>
<td>O</td>
<td>X</td>
<td>O</td>
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<tr>
<td>Trial 3</td>
<td>X</td>
<td>O</td>
<td>O</td>
<td>X</td>
<td>O</td>
<td>O</td>
<td>X</td>
<td>O</td>
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<tr>
<td></td>
<td>X = Right paw response</td>
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<td></td>
<td>O = No Right paw response</td>
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<tr>
<td></td>
<td>X = Left paw response</td>
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<td>O = No Left paw response</td>
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</table>

Figure 2-3: A sample PWT score sheet indicating a pattern of responses at 3 hours after the carrageenan injection

At each of the time points (baseline, 1, 3, 5, and 48 – 72 hours) fifteen responses were recorded for each paw (5 responses in each of 3 sets of trials: red for left paw and black for the right paw). The PWT score for each paw in each of the 3 sets of trials was computed using a log formula on the basis of the response pattern (e.g. XOOXX) and then the 3 values were averaged to obtain the final score at a given time point.
For scaling purposes, in the current study the final PWT Score at each time point was expressed as a percentage of the maximum score (456.63) expected when there is no response to the highest force in all the sets of trials at a given time point.

2.5.3 Thermal Sensitivity Assessment

The development of thermal hyperalgesia was determined using the Hargreaves method (Hargreaves, Dubner, Brown, Flores, & Joris, 1988) which measures thermal sensitivity by automatically detecting the hind paw withdrawal latency after exposure to radiant heat. The Hargreaves method uses a chamber with an automated glass floor surface, which detects and records the time taken for the paw to be withdrawn in response to the radiant heat aimed at the plantar region. Rats were placed in a transparent plastic chamber, which was on the automated glass floor and left to habituate for 5 to 10 minutes. After the exploration behavior stopped, a radiant heat source (Plantar test, Ugo Basile) was directed at the plantar surface of the hind paw and a digital timer registered the paw withdrawal latency.

Before the start of the experiment, the I.R intensity was set at 60 and the mean withdrawal latency was 17 seconds for normal rats that had not been injected with carrageenan. Because a number of normal rats had withdrawal latencies above 20 s, the cutoff time for preventing tissue damage was set at 30 seconds which was the set time in some of the previous studies (e.g. Detloff, Smith, Quiros Molina, Ganzer, & Houlé, 2014; Detloff, Wade, & Houlé, 2013). In situations where a rat failed to withdraw its paw (i.e. no response), the radiant heat source was removed at the 30 seconds and this time was entered as withdrawal latency for that paw at
the respective trial. After recording the withdrawal latency for one paw, the radiant heat source was directed at the opposite paw to determine its withdrawal latency.

To minimize errors of measurement, three values of withdrawal latencies were recorded for each paw at each of the study time points. After recording the withdrawal latencies of both paws, a waiting time interval of 5 minutes was observed between each of the three trials. The order of the testing of the two paws was alternated at each of the three trials, such that the paw that was first to be tested during one trial was the second to be tested in the next trial and vice versa. The withdrawal latency of each paw, at a given time point was calculated as the average of the three values recorded at the three trials.

2.6 Data Acquisition Procedure

2.6.1 Baseline Data

At the beginning of the experiment, the rats were anesthetized with isoflurane and dressed in a vest that allowed them to freely move while carrying a light head-stage of the MC Rack wireless module on the back. After the head-stage was firmly attached to the vest, the recording terminal was firmly connected to the chronically implanted electrode. The electrode has a plastic cylindrical mold with screw threads, around which a male-female connector is fastened to firmly attach to the recording terminal. This firm connection ensured that the rat movements did not interfere with the recording of the LFP signals. All the 16 channels of the MC Rack wireless module were connected to the terminals of the bipolar electrode and thus all the channels recorded the same brain signals. The reference and ground terminals had soldered alligator clips which were firmly attached to the wires
connected to the two stainless screws driven into the skull (2 mm posterior to the lambda) during surgery.

After ensuring that the recording, reference, and ground electrodes were firmly connected, the rats were placed in a transparent plastic cage placed on top of a wire mesh. After the rats had fully recovered from the anesthesia, they were left to habituate for 10 to 15 min. The first step after the exploratory behavior stopped was recording of baseline LFP activity for 10 min. This was followed by testing of the mechanical sensitivity using the von Frey filaments. After the last (third) trial of the von Frey filaments presentation, the mechanical stimuli-induced LFP oscillations were recorded during poking of each of the hind paws with a high force von Frey filament (Size 6.10; 100g) which was not among the series of the 8 filaments used in mechanical sensitivity testing. Each paw was repeatedly poked (10 times) in order to obtain an estimation of 8 to 10 s duration of LFP recording. In situations of high hyperalgesia the intervals between the pokes were less regular due to frequent paw withdrawals, and thus the recording had to be repeated to obtain LFP activity with pokes delivered in close succession.

After recording of the LFP oscillations induced by mechanical stimuli, the rats were placed in the transparent chamber on the Hargreaves (Plantar Test) and allowed a 5 to 10 min habituation period. After the cessation of the exploratory behavior thermal sensitivity of each paw was tested using the paw withdrawal latencies as explained under the thermal sensitivity assessment section. At each of the study time points, the thermal stimuli-induced LFP oscillations were recorded during the first trial of detecting the withdrawal latencies of each paw. To ensure
accuracy in the recording of the thermal-induced changes in LFP activity, the recording of the oscillations was stopped at the point where the Hargreaves digital timer stopped. This ensured that the recording of the thermal-induced LFP activity stopped at the moment where the rats were beginning to withdrawal the hind paw from the radiant heat.

2.6.2 Carrageenan-induced Neural Activity Data

After recording of the baseline LFP oscillations, and the thermal and mechanical sensitivity, the rats were briefly exposed to isoflurane to render them less active during the injection of carrageenan. The carrageenan injections were administered into either the left or right hind paw as the rats were regaining consciousness. Rats were then placed in the transparent plastic chamber on the wire mesh and allowed a 10 min recovery period before recording the immediate effect of carrageenan on the LFP oscillations. The 10 min waiting period after the carrageenan injection was intended to minimize the possible confounding effect of isoflurane on the CeA activity. The immediate time-point only involved the recording of LFP activity because application of mechanical and thermal stimuli immediately after the carrageenan injection could interfere with the onset of the inflammatory response. However, all the recording and assessment procedures described at baseline were repeated at 1, 3, 5, and 48-72 hours after the carrageenan injection.
2.7 Histology

After the conclusion of the experiments the rats were euthanized by asphyxiation with carbon dioxide. The brains were extracted and stored in 10% formalin for at least 10 days, and then soaked in 30% sucrose solution for approximately 24 -72 hours. The brains were removed for slicing after sinking to the bottom of the sucrose solution. They were placed on a microtome, frozen using dry ice (solid CO$_2$), and sliced into 80µm sections. The slices were soaked in 70% ethanol for 10 to 15 min and then mounted onto gelatin-coated slides. Upon drying, the slices were stained using Thionin solution, and observed under a microscope to verify the sites of electrode placement and the lesions.

The study consisted of 64 rats including the control and experimental groups. Of these rats, 3 died in the process of recovery while the brain slices of 18 rats were identified as having the electrode tip and/or lesions in areas adjacent to the CeA. All the rats with an electrode tip or lesion outside the CeA were eliminated from the study.

2.8 Lesion verification

The location and extent of the lesion were determined by reconstruction of the damaged site on the templates of the stereotaxic atlas (Paxinos & Watson, 1998). This lesion verification method involves mapping out of the smallest and largest lesions on a series of coronal templates (Campeau & Davis, 1995; Choi & Brown, 2003; Choi, Cain, & LeDoux, 2010; Koo, Han, & Kim, 2004) (Figure 2-4). Rats were excluded from the study if the lesions were away from the target site or caused extensive damage to the areas surrounding the CeA.
Figure 2-4: Histological reconstruction of the CeA lesions

The extent of the lesions was determined by reconstruction of the damage to the CeA on a series of coronal plates of the stereotaxic atlas (Paxinos & Watson, 1998). The minimal (dark) and maximal (light) damage to the left CeA, and electrode tips in the right CeA were reconstructed on each coronal plate (A). On the right is a representative lesion of the right CeA (B) and electrode trace to the left CeA (C). The negative values indicate the distance posterior to the bregma.
Chapter 3

Results

3.1 Data analysis

3.1.1 Local Field Potential Signal Analysis

The LFP signals were sampled at 10 kHz and low pass filtered at 200 Hz using MC Rack Software (Multi Channel Systems, Reutlingen, Germany), and analyzed off-line using Spike 2 software (CED, Inc.). The LFPs were extracted via 2\textsuperscript{nd}-order high- and low-pass Butterworth filters, at cutoff frequencies of 1 Hz and 100 Hz, respectively. The 60 Hz line noise peak was removed using a notch filter. The LFP signal was down-sampled to 200 Hz (Johnson, Euston, Tatsuno, & McNaughton, 2010) and power spectrum analyses were run at an FFT size of 256 Hz, Hanning window, at time and frequency resolutions of 1.28 s and of 0.78 Hz, respectively.

The power spectra displayed from 0 to 100 Hz in 128 bins, and were normalized to unity using the Arrdiv([], A\textsubscript{CR}) Spike 2 script function; where A\textsubscript{CR} is the area of the cursor region between 0 and 100 Hz. The power spectral densities were then saved as a text file, for extraction of the numerical values for the different frequency bands: Delta (1-4 Hz), Theta (5-7 Hz), Alpha (8-13 Hz), Beta (14-30 Hz), Low-gamma (31-55Hz) and High-gamma (65-100Hz). Due to the of small values in the low frequency ranges, the normalized data for all the frequency bands were multiplied by 10\textsuperscript{3} for scaling purposes.
For the carrageenan-induced LFP activity a 3 min time window was selected as the segment of analysis from the 10 min recording of activity at each of the study points. For the thermal-induced activity, the LFPs were extracted from the last 3 seconds to withdrawal of each hind paw. The last 3 seconds of the paw withdrawal latency were considered to be the time when the animal was sensing the highest thermal stimulation. Thus the LFP activity in this time window was more likely to include of nociceptive responses in the CeA. The mechanical (poke) stimuli-induced LFP activity was extracted from a time window of 3 seconds; that is, between the 3rd to 6th second of the recorded raw trace. This time window was selected as the appropriate data segment in order to minimize error due to a 1 to 2 s lapse between the task of poking and switching on and off of the recording module at the beginning and end of the stimulation. Before the analysis the raw traces were visually inspected and in situations where there was noise in the LFP signal, the noisy segments were excluded from the analyses. Representative raw traces are shown in Figure 3-1.
Figure 3-1: Representative raw data traces

The figure shows representative raw traces of the right CeA oscillatory activity at baseline (A – C) and 5 hours after the carrageenan injection (D – F). At each of the study time-points (i.e. baseline, 1, 3, 5, and 48 – 72 hours) the CeA oscillations were recorded without peripheral stimulation (A), and during mechanical (B) and thermal (C) stimulation.

The activity observed in the LFP raw traces cannot be meaningfully interpreted until the low, high, and notch filters are applied to exclude noise, which can have a confounding effect on the results. The results of the data extracted from the filtered raw traces are illustrated in the sample power spectrum (Figure 3-2) and the spectrograms (Figure 3-3) which are visual representations of the changes in neural oscillatory power at different frequencies ranges.
Figure 3-2: A representative power spectrum

The figure shows a power spectrum obtained through power spectrum analysis of the waveform (raw trace) data and normalized to unity. The power spectra were saved as text files to obtain the numerical values of the power for the different frequency bands (i.e. delta, theta, alpha, beta, low-gamma, and high-gamma).
Figure 3-3: Representative spectrograms

The figure shows spectrograms generated using the Spike 2 sonogram mode, on a logarithmic (dB) scale. They are visual representations of the right CeA oscillatory activity of a rat with a right paw injury; at baseline and during the progression of the inflammatory response. The extent of activity (power) of a given
frequency at a particular time is indicated by the color intensity in the spectrograms (A-I). In reference to the color scale at the bottom of the figure, there was progressive increase in oscillatory power especially in the low frequency range (dark grey/blue) during mechanical (D–F) and thermal (G–I) stimulation, and in the no-stimulation (carrageenan-alone) condition (B & C). The spectrograms on the right are indicating baseline (A) and peripheral stimulation before the carrageenan injection (D & G). The rest of the spectrograms indicate activity recorded without stimulation (B-C) and responses to peripheral stimuli (E–F; H–I) at 5 and 48 – 72 hours after the carrageenan injection.

3.1.2 Statistical Analysis

The data were analyzed using IBM SPSS Statistic 22.0 (Armonk, NY: IBM Corp.). To test the effect of inflammatory pain on the CeA activity (hypothesis 1) the analyses were twofold: 1) 2 (Injury Side) x 6 (Time) mixed factorial ANOVAs were used to compare the groups with contralateral and ipsilateral carrageenan-induced injuries on the time dependent changes in the LFP oscillations of the right or left CeA; 2) two way repeated measures ANOVAs were run to determine the changes in the right and left CeA neural sensitivity to mechanical and thermal stimuli as the pain progressed.

To test the effect of the CeA lesions on the onset and progression of pain (hypothesis 2) the analyses were also twofold: 1) two-way repeated measures ANOVAs with all the study time points were run to examine the effect of the right and left CeA lesions on both mechanical and thermal sensitivity of the injured and non-injured paws of each group; and 2) 2 (Group) x 6 (Time) mixed factorial
ANOVAs were used to compare the changes in the mechanical and thermal sensitivity of each of the CeA lesion groups with that of the control group with an injury on the side of the body. These mixed factorial models included all the time points and offered insight into the effect of a lesioned and spared (intact) left or right CeA on the progression of pain. In all the analyses the significance level was $p < .05$, and all significant main effects were followed by Fisher’s least significant difference (or T-test) post hoc comparisons. One of the important assumptions of repeated measures ANOVA models is that the data has sphericity, such that the variance or covariance of the data follows a specific pattern. Mauchly’s Test was used to assess Sphericity in all the repeated measures ANOVA models. In situations where the assumption of Sphericity was not met ($p < .05$), Greenhouse-Geisser and Huynh-Feldt epsilon corrections were used with corrective coefficients of $\epsilon < .75$ and $\epsilon > .75$, respectively.

To test the correlation between the inflammatory pain-induced LFP oscillations and peripheral thermal and mechanical sensitivity (Hypothesis 3) Pearson correlation coefficients were used for data at each of the time points. The significant correlations were further explored using hierarchical multiple regression analyses. The mechanical (poke) and thermal-induced LFP oscillations were the predictor variables in the first step, while the oscillations induced by carrageenan without peripheral stimulation (carrageenan-alone) were entered in the second step of the hierarchical multiple regression models. The dependent (outcome) variables were the paw withdrawal thresholds or latencies that significantly correlated with the neural oscillations at a given time point. These analyses
provided an insight into whether each of the categories of LFP oscillations significantly was a predictor of peripheral thermal and/or mechanical sensitivity. The multiple regression analysis also indicated whether the inflammatory pain-induced (carrageenan-alone) CeA oscillations were significant predictors of peripheral thermal and mechanical sensitivity, after controlling for the effect of the oscillations associated with thermal and mechanical stimulation.

3.2 The Effect of Inflammatory Pain on the Right and Left CeA Oscillatory Activity

In order to determine if there were significant changes in the right and left CeA activity as the inflammatory pain progressed, the effect of carrageenan on the LFP oscillations was examined in the groups that received contralateral and ipsilateral injuries. The analyses focused on six different LFP oscillations including delta, theta, alpha, beta, low-gamma, and high-gamma frequency bands.

3.2.1 Carrageenan-induced Changes in Delta Oscillations

The results of a 2 (Injury Side) x 6 (Time) mixed factorial ANOVA comparing the two right CeA groups (right paw injury vs. left paw injury) on the changes in delta oscillations, revealed a significant main effect of time, \( F(5, 55) = 3.32, p = .011 \). Post hoc comparisons revealed that compared to baseline the carrageenan-induced left paw injury caused significant increase in the right CeA delta oscillations at 1 \( (p = .045) \), and 48 - 72 \( (p = .020) \) hours after the carrageenan injection. The right paw injury induced significant increase in delta oscillations at 3 \( (p = .019) \) hours after the carrageenan injection. There was however, no significant main effect injury side, \( F(1, 11) = .01, p = .877 \), and the Injury Side x Time interaction was also not significant,
\[ F(5, 55) = .38, \ p = .858. \] There were thus no significant time dependent differences in the effects of the ipsilateral and contralateral carrageenan-induced injuries on the right CeA delta activity (Figure 3-4A).

In the case of the left CeA activity, the results of a 2 (Injury Side) x 6 (Time) mixed factorial ANOVA revealed a significant main effect of injury side, \( F(1, 13) = 14.40, \ p = .002 \), such that the activity induced by the contralateral (right paw) injury \( (N = 8) \) was significantly greater than the activity due to the ipsilateral (left paw) injury \( (N = 7) \). There was also a main effect of time, \( F(5, 65) = 2.51, \ p = .038 \), but there was no significant the Injury Side x Time interaction, \( F(5, 65) = 1.38, \ p = .243 \). Post hoc analysis probing the significant main effect of time, revealed that there was a significant increase in left CeA delta activity at 3 hrs after the right paw carrageenan injection \( (p = .007) \) but no significant changes in activity at any of the other time points (Figure 3-4B).

![Figure 3-4: Carrageenan-induced changes in delta oscillations](image)

Both the ipsilateral \( (N = 7) \) and contralateral \( (N = 6) \) carrageenan injections induced significant increase in right CeA delta oscillations. The left CeA delta
oscillations significantly increased in response to the contralateral ($N = 8$), but not the ipsilateral injury ($N = 7$). *significantly higher than baseline ($p < .05$). The asterisk color matches the color of the respective side of the carrageenan injection.

3.2.1.1 *Control Group vs. Lesion Group on Delta Activity*

To examine whether the lesions had an effect on the activity of the non-lesioned CeA, 2 (Control vs. Lesion Group) x 6 (Time) mixed factorial ANOVAs were used to compare the time-dependent differences in CeA activity. A comparison of the right CeA delta oscillations of the lesion ($N = 7$) and control ($N = 8$) groups that had a left paw injury did not reveal significant main effects of condition, $F(1, 13) = .39$, $p = .544$, and time, $F(5, 65) = .82$, $p = .538$. The Condition x Time interaction was also not significant, $F(5, 65) = .49$, $p = .779$. Furthermore, the comparison of the left CeA delta oscillations of the lesion ($N = 8$) and control ($N = 7$) groups with a right paw injury, did not reveal a significant main effect of condition, $F(1, 13) = 2.61$, $p = .130$, but there was a significant main effect of time, $F(5, 65) = 2.84$, $p = .022$. There was a significant increase in the control group’s left CeA delta oscillations immediately ($p = .006$) and at 5 hours ($p = .038$), and those of the lesion group at 3 hours ($p = .002$) after the carrageenan injection. However, the Condition x Time interaction was not significant, $F(5, 65) = 2.12$, $p = .075$. Thus, there were no time dependent differences in the delta activity of the lesion and control groups, which suggests that the lesions did not significantly affect the responses of the non-lesioned CeA.
Figure 3-5: Control vs. Lesion groups on delta oscillatory activity

The comparison of the delta oscillations of the control and lesion groups which had contralateral injuries did not reveal significant time-dependent differences between the oscillations in all the conditions. However, there was a significant increase in the left CeA delta oscillations of the lesion group at 3 hours and of the control group, immediately and at 5 hours after the carrageenan injection (B). *significantly higher than baseline ($p < .05$).

3.2.1.2 Delta Mechanosensitivity

Two way repeated measures ANOVAs were conducted to examine the changes in the CeA delta oscillatory sensitivity to peripheral mechanical stimuli. The analysis compared delta activity recorded without (carrageenan-alone) and during poking of the injured and non-injured paws at all the study time points. For the right CeA group with contralateral injury ($N = 7$), there were no significant main effects of the poke applied to both the injured left paw (condition, $F (1, 6) = 2.32, p = .178$; time, $F (4, 24) = 2.00, p = .127$; Condition x Time interaction, $F (4, 24) = .99, p = .431$), and non-injured right paw, (condition, $F (1, 6) = .27, p = .621$; time, $F (4, 24) = .202$).
2.91, \( p = .077 \); Condition x Time interaction, \( F(4, 24) = 3.12, p = .065 \) (Figure 3-6A). Similar non-significant results were obtained for the right CeA group with ipsilateral injury (\( N = 6 \)) for the poke applied to the non-injured left paw, (condition \( F(1, 5) = 3.51, p = .120 \), and time, \( F(4, 20) = 1.95, p = .142 \), and Condition x Time interaction, \( F(4, 20) = 3.34, p = .113 \)), and injured right paw (condition \( F(1, 5) = 2.08, p = .209 \); time, \( F(4, 20) = 1.03, p = .415 \); Condition x Time interaction, \( F(4, 20) = .44, p = .777 \)) (Figure 3-6B). Thus the right CeA delta oscillations did not significantly change in response to peripheral mechanical stimulation regardless of whether the stimulus was applied to the injured or non-injured paws (Figure 3-6A-B).

For the left CeA group with contralateral injury (\( N = 8 \)), there were no significant main effects of the poke administered to the non-injured left paw (Condition, \( F(1, 7) = .31, p = .597 \); time, \( F(4, 28) = 3.88, p = .055 \); Condition x Time interaction, \( F(4, 28) = 1.58, p = .207 \)) and the injured right paw (condition, \( F(1, 7) = .28, p = .615 \); time, \( F(4, 28) = 1.68, p = .184 \); Condition x Time interaction, \( F(4, 28) = 1.91, p = .137 \)). There were also no significant main effects for Left CeA ipsilateral injury group (\( N = 7 \)) for poke administered to the injured left paw (Condition, \( F(1, 6) = 1.83, p = .225 \); time, \( F(4, 24) = 2.72, p = .053 \); Condition x Time interaction, \( F(4, 24) = 2.26, p = .093 \)), and the non-injured right paw (condition, \( F(1, 6) = 1.21, p = .313 \); time, \( F(4, 24) = .41, p = .798 \); Condition x Time interaction, \( F(4, 24) = .74, p = .574 \)) (Figure 3-6 C-D). Therefore, regardless of the side of the injury there were no significant changes in the CeA delta oscillatory sensitivity to mechanical stimuli as the pain progressed.
The figure demonstrates the trend of the changes in both the left and right CeA delta oscillations in response to mechanical (poke) stimuli at different time points after the carrageenan injection. A comparison of the oscillatory responses to mechanical stimuli with the carrageenan-alone activity (recorded without stimulation) did not reveal significant differences between the two conditions for both the left and right CeA delta oscillations ($p > .05$).

Figure 3-6: Delta oscillatory sensitivity to mechanical stimuli
3.2.1.3 *Delta Thermosensitivity*

A two way repeated measures ANOVA model comparing the right CeA delta activity recorded without, and during thermal stimulation of the injured left paw (*N* = 7) revealed a significant main effect of condition, *F*(1, 6) = 10.12, *p* = .019, with an overall increase in activity during thermal stimulation, but there was no significant main effect of time, *F*(4, 24) = 1.92, *p* = .140. There was also no significant Condition x Time interaction, *F*(4, 24) = 2.41, *p* = .077. For thermal stimuli applied to the non-injured right paw, there was a significant main effect of condition, *F*(1, 6) = 8.97, *p* = .024 with an overall increase in delta activity during thermal stimulation. There was no significant main effect of time, *F*(4, 24) = 1.97, *p* = .132 and the Condition x Time interaction was also not significant, *F*(4, 24) = 1.93, *p* = .138 (Figure 3-7A).

The results of the right CeA group with ipsilateral injury (*N* = 6) also did not indicate significant main effects of the thermal stimuli applied to the non-injured left paw (condition, *F*(1, 5) = .052, *p* = .828; time, *F*(4, 20) = 2.35, *p* = .089; Condition x Time interaction, *F*(4, 20) = 1.21, *p* = .339) and injured right paw (condition, *F*(1, 5) = 1.35, *p* = .298, and time, *F*(4, 20) = 2.42, *p* = .082; Condition x Time interaction, *F*(4, 20) = 1.84, *p* = .160) (Figure 3-7B). Thus the right CeA delta oscillations did not significantly change over time in response to thermal stimuli applied to the non-injured and injured hind paws.

For the left CeA group with contralateral injury (*N* = 8), there was no significant main effect of thermal stimulation of the non-injured ipsilateral paw on the left CeA delta activity, *F*(1, 7) = 1.79, *p* = .223, but there was a significant main effect of time, *F*(4, 28) = 3.51, *p* = .019; with no significant Condition x Time
interaction, $F(4, 28) = 1.95, p = .130$. There were also no significant main effects of thermal stimulation of the injured right paw (condition, $F(1, 7) = .06, p = .819$; time, $F(4, 28) = 2.07, p = .112$; Condition x Time interaction, $F(4, 28) = 1.21, p = .331$) (Figure 3-7C).

In the case of the left CeA group with ipsilateral injury ($N = 7$) thermal stimulation of the injured left paw did not indicate significant main effects of condition, $F(1, 6) = .05, p = .840$, and time, $F(4, 24) = 2.39, p = .079$; and there was a significant Condition x Time interaction, $F(4, 24) = 2.02, p = .124$. Thermal stimulation of the non-injured right paw, did not have significant main effects of condition, $F(1, 6) = 1.85, p = .223$, and time, $F(4, 24) = .39, p = .814$. There was also no significant Condition x Time interaction, $F(4, 24) = .57, p = .688$ (Figure 3-7D).
Figure 3-7: Delta oscillatory sensitivity to thermal stimuli

The figure demonstrates the trend of the changes in both the left and right CeA delta oscillations in response to thermal stimuli at different time points after the carrageenan injection. There were no statistically significant time-dependent changes in both the left and right CeA responses to thermal stimulation of the injured and non-injured paws as the pain progressed ($p > .05$).

3.2.2 Carrageenan-induced Changes in Theta Oscillations

The results of a 2 (Injury Side) x 6 (Time) mixed factorial ANOVA comparing the changes in theta oscillations of the two right CeA groups (right paw injury vs. left
paw injury) did not reveal significant main effects of injury side, $F(1, 11) < .001, p = .993$, and time, $F(5, 55) = 2.57, p = .062$, and the Injury Side x Time interaction was also not significant, $F(5, 55) = .82, p = .543$. Thus there were no significant carrageenan-induced changes in right CeA theta oscillations regardless of the side of the injury (Figure 3-8A). In the case of the left CeA theta activity, there were also no significant main effects of injury side, $F(1, 13) = 4.66, p = .050$, and time, $F(5, 65) = 1.40, p = .235$. The Injury Side x Time interaction was also not significant, $F(5, 65) = .53, p = .755$. Thus like the right CeA, the left CeA theta activity did not significantly change over time, regardless of the side of the peripheral injury (Figure 3-8B).

![Graph of theta oscillations](image)

Figure 3-8: Carrageenan-induced changes in theta oscillations

Both the right ($N = 6$) and left paw ($N = 7$) carrageenan injections did not induce significant changes in the right CeA theta oscillations. Similarly the left CeA theta oscillations did not significantly change in response to both the right ($N = 8$), and left paw injury ($N = 7$) ($p > .05$).
3.2.2.1 **Control Group vs. Lesion Group on Theta Activity**

The results of a 2 (Group) x 6 (Time) mixed factorial ANOVA comparing the right CeA theta oscillations of the lesion (N = 7) and control (N = 8) groups that had a left paw injury did not reveal significant main effect of condition, $F(1, 13) = .008, p = .929$. There was a significant main effect of time, $F(5, 65) = 4.07, p = .003$, with a significant increase in the control groups activity at hour 1 ($p = .040$) compared to baseline activity. The Condition x Time interaction was not significant, $F(5, 65) = .74, p = .599$. For the left CeA theta oscillations of the lesion (N = 8) and control (N = 7) groups, which had a right paw injury, there were no significant main effects of condition, $F(1, 13) = .77, p = .397$, and time, $F(5, 65) = .76, p = .583$. The Condition x Time interaction was also not significant, $F(5, 65) = 1.02, p = .415$. Thus, there were no time dependent differences in the theta activity of the lesion and control groups, which suggests that the lesions did not significantly affect the responses of the non-lesioned CeA.

![Figure 3-9: Control vs. Lesion groups on theta oscillatory activity](image-url)
The comparison of the theta oscillations of the control and lesion groups which had contralateral injuries revealed a significant increase in the activity of the control group at 1 hour after the carrageenan injection. There were however, no significant time-dependent group differences in theta activity (p > .05).

*Significantly higher than baseline.

3.2.2.2 Theta Mechanosensitivity

The results of two way repeated measures ANOVAs comparing the right CeA theta activity due to carrageenan alone with activity during the poke (mechanical) stimulation, did not indicate significant main effects for both the injured left paw (condition, F (1, 6) = 3.09, p = .129; time, F (4, 24) = .92, p = .468; Condition x Time interaction, F (4, 24) = .83, p = .519), and non-injured right paw (condition, F (1, 6) = .33, p = .588, and time, F (4, 24) = .48, p = .753; and Condition x Time interaction, F (4, 24) = .49, p = .746). For the right CeA group that received an ipsilateral carrageenan injection, there was a significant main effect of mechanical stimulation of the non-injured left paw, F (1, 5) = 17.61, p = .009, with an overall increase in activity during stimulation. There was also a significant main effect of time, F (4, 20) = 3.02, p = .042. The activity in the carrageenan-alone condition significantly increased at 3 hours (p = .001) after the carrageenan injection. The Condition x Time interaction was not significant, F (4, 20) = 2.24, p = .101. The mechanical stimuli applied to the injured right paw did not have significant main effects of the condition, F (1, 5) = 5.69, p = .063, and time, F (4, 20) = .38, p = .818. The Condition x Time interaction was also not significant, F (4, 20) = .23, p = .917. Thus regardless of whether the paw was injured or not, the right CeA theta activity during
mechanical stimulation did not significantly differ from the activity associated with carrageenan-alone condition (Figure 3-10A-B).

For the left CeA group with contralateral (right) paw injury, a two way repeated measures ANOVA comparing the theta activity due to carrageenan alone with the activity during mechanical stimulation did not indicate significant main effects for both the non-injured left paw (condition, \(F(1, 7) = .03, p = .858\); time, \(F(4, 28) = 1.82, p = .154\); Condition x Time interaction, \(F(4, 28) = 1.92, p = .134\)) and injured right paw (condition, \(F(1, 7) = .52, p = .496\); time, \(F(4, 28) = 1.12, p = .373\); Condition x Time interaction, \(F(4, 28) = 1.83, p = .152\)). For the left CeA ipsilateral injury group (\(N = 7\)) there was a significant main effect of mechanical stimulation of the injured left paw, \(F(1, 6) = 15.38, p = .008\); with an overall decrease in activity during mechanical stimulation. However, there was no significant main effect of time, \(F(4, 24) = 1.05, p = .405\), and the Condition x Time interaction was not significant, \(F(4, 24) = 2.04, p = .121\). There were also no significant main effects of mechanical stimuli applied to the non-injured right paw (condition, \(F(1, 6) = 1.40, p = .282\); time, \(F(4, 24) = 1.52, p = .229\), and Condition x Time interaction, \(F(4, 24) = .66, p = .626\)). Thus regardless, of whether the mechanical stimulus was applied to the injured or non-injured paws, it did not induce significant changes in left CeA theta oscillations compared to the activity attributed to the carrageenan alone condition (Figure 3-10C-D).
Figure 3-10: Theta oscillatory sensitivity to mechanical stimuli

The figure illustrates the trend of the changes in both the left and right CeA theta oscillations in response to mechanical stimuli at different time points after the carrageenan injection. The only statistically significant effect was an increase in the right CeA theta oscillations during mechanical stimulation of the left paw at 3 hours after the carrageenan injection (B) \((p < .05)\). *Significantly higher than baseline.

3.2.2.3 Theta Thermosensitivity

The results of a two way repeated measures ANOVA comparing the right CeA theta activity recorded without, and during thermal stimulation of the non-injured
right paw did not reveal significant main effects of condition, \( F(1, 6) = 3.46, p = .112 \), and time, \( F(4, 24) = .39, p = .812 \); and the Condition x Time interaction was also not significant, \( F(4, 24) = .44, p = .777 \). For thermal stimuli applied to the injured left paw, there were no significant main effects of condition, \( F(1, 6) = 1.15, p = .325 \), and time, \( F(4, 24) = 1.53, p = .226 \), but there was a significant Condition x Time interaction, \( F(4, 24) = 4.10, p = .011 \). The results of simple main effects analysis probing the significant Condition x Time interaction indicated that the right CeA theta activity significantly decreased during thermal stimulation of the injured left paw, at 1 \((p = .019)\) and 5 \((p = .029)\) hours after the carrageenan injection (Figure 3-11A).

For the right CeA group with ipsilateral injury, there were no significant main effects of thermal stimuli applied to the non-injured left paw (condition, \( F(1, 5) = .01, p = .915 \); time, \( F(4, 20) = 2.06, p = .125 \); Condition x Time interaction, \( F(4, 20) = 2.97, p = .126 \)) and injured right paw (condition, \( F(1, 5) = 2.01, p = .215 \); time, \( F(4, 20) = .56, p = .694 \); Condition x Time interaction, \( F(4, 20) = 1.17, p = .354 \)) (Figure 3-11B).

The two way repeated measures ANOVA comparing the left CeA theta activity recorded without, and during thermal stimulation of the non-injured ipsilateral (left) paw indicated a significant main effect of condition, \( F(1, 7) = 8.28, p = .024 \); with an overall decrease in activity during thermal stimulation. There was no significant main effect of time, \( F(4, 28) = 1.55, p = .215 \), and Condition x Time interaction, \( F(4, 28) = 2.24, p = .091 \). There were also no significant main effects of thermal stimuli applied to the injured right paw (condition, \( F(1, 7) = .01, p = .922 \);
time, $F(4, 28) = .70, p = .600$; and Condition x Time interaction, $F(4, 28) = .54, p = .705$) (Figure 3-11C). For the left CeA group with ipsilateral injury, the results of thermal stimulation of the injured left paw did not reveal significant main effects of condition, $F(1, 6) = 1.33, p = .293$ and time, $F(4, 24) = 23, p = .917$. The Condition x Time interaction was also not significant, $F(4, 24) = .75, p = .566$. The results for thermal stimulation of the non-injured right paw, did not reveal a significant main effect of condition, $F(1, 7) = 4.19, p = .087$, but there was a significant main effect of time, $F(4, 24) = 4.95, p = .005$; and a significant Condition x Time interaction, $F(4, 24) = 3.29, p = .028$. The results of simple main effects analysis probing the significant Condition x Time interaction indicated that the left CeA theta activity significantly decreased during thermal stimulation of the non-injured right paw between 48 – 72 hours ($p = .009$) after the carrageenan injection. The results further revealed that theta activity during thermal stimulation was significantly lower than activity recorded without stimulation at that time-point (Figure 3-11D).
Figure 3-11: Theta oscillatory sensitivity to thermal stimuli

There was a trend of decreased theta oscillations in both the left and right CeA during thermal stimulation of the injured and non-injured left (red) and right (blue) paws. This effect was statistically significant in the right CeA during thermal stimulation of the injured left paw (A) and in the left CeA during stimulation of the non-injured right paw (D). *significantly lower than the carrageenan-alone condition; **Significantly lower than baseline (p < .05).
3.2.3  *Carrageenan-induced Changes in Alpha Oscillations*

The mixed factorial ANOVA model comparing the alpha activity of the two right CeA groups (right paw injury vs. left paw injury) did not reveal a significant main effect of injury side, $F(1, 11) = 4.20, p = .065$, but there was a significant main effect of time, $F(5, 55) = 3.75, p = .005$, with a trend of decreased activity. The Injury Side x Time interaction was not significant, $F(5, 55) = .70, p = .623$. The post hoc analysis probing the significant main effect of time revealed that compared to baseline, there was significant decrease in right CeA alpha oscillations immediately ($p = .018$), and 48 – 72 ($p = .022$) hours after the right paw carrageenan injection, and between 48 – 72 hours ($p = .012$) after the left paw injection (Figure 3-12A).

For the left CeA groups (right paw injury vs. left paw injury) there was a significant main effect of injury side, $F(1, 13) = 6.68, p = .023$, with lower overall activity in response to the contralateral compared to the ipsilateral injury. There was however, no significant main effect of time, Greenhouse-Geisser $F(2.40, 31.17) = .90, p = .485$; and Injury Side x Time interaction, $F(2.40, 31.17) = .64, p = .562$. Thus regardless of the side of the peripheral injury, the left CeA alpha activity did not significantly change over time (Figure 3-12B).
Figure 3-12: Carrageenan-induced changes in alpha oscillations

Compared to baseline there was significant decrease in right CeA alpha oscillations immediately, and at 48–72 hours after the right paw \((N = 6)\) and 48–72 hours after the left paw \((N = 7)\) carrageenan injection. The left CeA alpha oscillations did not significantly change in response to both the right \((N = 8)\), and left paw injuries \((N = 7)\). *Significantly lower than baseline \((p < .05)\).

3.2.3.1 Control Group vs. Lesion Group on Alpha Activity

The results of a 2 (Group) x 6 (Time) mixed factorial ANOVA comparing the right CeA alpha oscillations of the lesion \((N = 7)\) and control \((N = 8)\) groups that had a left paw injury did not reveal significant main effects of condition, \(F(1, 13) = 1.08, p = .317\), and time, \(F(1, 13) = 2.52, p = .136\). The Condition x Time interaction was not also significant, Greenhouse-Geisser \(F(1, 13) = .12, p = .734\). For the left CeA alpha oscillations of the lesion \((N = 8)\) and control \((N = 7)\) groups, which had a right paw injury, there were no significant main effects of condition, \(F(1, 13) = .03, p = .874\), and time, \(F(5, 65) = .72, p = .608\). The Condition x Time interaction was also not significant, \(F(5, 65) = .37, p = .870\). Thus, there were no time dependent
differences in the alpha activity of the lesion and control groups, which suggests that
the lesions did not significantly affect the responses of the non-lesioned CeA.

![Graph of alpha oscillations](image)

Figure 3-13: Control vs. Lesion groups on alpha oscillatory activity

The comparison of the alpha oscillations of the control and lesion groups did not reveal significant changes in activity compared to baseline. There were also no significant time-dependent group differences in alpha activity ($p > .05$).

### 3.2.3.2 Alpha Mechanosensitivity

The results of the two way repeated measures ANOVA comparing the right CeA alpha activity recorded without, and during mechanical stimulation of the injured left paw ($N = 7$) did not reveal significant main effects of condition, $F(1, 6) = 5.28, p = .061$, and time, $F(4, 24) = .95, p = .455$. The Condition x Time interaction was also not significant, $F(4, 24) = 1.09, p = .386$. The results of mechanical stimulation of the non-injured right paw did not reveal significant main effects of condition, $F(1, 6) = 1.21, p = .314$, and time, $F(4, 24) = 2.20, p = .100$. However, there was a significant Condition x Time interaction, $F(4, 24) = 2.94, p = .042$. The results of the simple main effects analysis probing the significant Condition x Time
interaction revealed that compared to the activity recorded without stimulation, the right CeA alpha activity significantly decreased during mechanical stimulation of the non-injured right paw at 3 hours \((p = .004)\) after the carrageenan injection (Figure 3-14B).

For the right CeA group with ipsilateral carrageenan-induced injury, there were no significant main effects of mechanical stimulation of the non-injured left paw (condition, \(F (1, 5) = .06, p = .812\); time, \(F (4, 20) = .61, p = .659\); Condition x Time interaction, \(F (4, 20) = .34, p = .846\)). The results of mechanical stimulation of the injured right paw did not reveal a significant main effect of condition, \(F (1, 5) = .002, p = .969\). There was a significant main effect of time, \(F (4, 20) = 4.47, p = .010\) with a decrease in alpha activity at 1 \((p = .028)\) and 48 – 72 \((p = .014)\) hours, without peripheral stimulation (carrageenan-alone condition), and during mechanical stimulation of the injured right paw at 1 \((p = .047)\), 5 \((p = .025)\), and 48 – 72 \((p = .038)\) hours after the carrageenan injection (Figure 3-14B).

For the left CeA group with a contralateral injury, the two way repeated measures ANOVA model comparing the alpha activity recorded without, and during mechanical stimulation of the non-injured ipsilateral paw indicated a significant main effect of condition, \(F (1, 7) = 16.85, p = .005\), with an overall decrease in alpha activity during the poking of the paw. However, there was no significant main effect of time, \(F (4, 28) = 1.07, p = .388\), and the Condition x Time interaction was not significant, \(F (4, 28) = .49, p = .741\). Mechanical stimulation of the injured right paw, also had a significant main effect of condition, \(F (1, 7) = 5.93, p = .045\), with an overall decrease in alpha activity during poking of the paw. There was however, no
significant main effect of time, $F(4, 28) = 1.84, p = .152$, and the Condition x Time interaction was not significant, $F(4, 28) = 1.74, p = .169$. For the left CeA group with ipsilateral injury, there were no significant main effects of mechanical stimulation of the injured left paw (condition, $F(1, 6) = 1.63, p = .249$; time, $F(4, 24) = 1.13, p = .368$; Condition x Time interaction, $F(4, 24) = .63, p = .643$). The results of mechanical stimulation of the non-injured right paw revealed a significant main effect of condition, $F(1, 6) = 8.62, p = .026$; with an overall decrease in alpha activity during stimulation of the paw. There was however, no significant main effect of time, $F(4, 24) = .55, p = .704$; and the Condition x Time interaction was also not significant, $F(4, 24) = 1.27, p = .308$ (Figure 3-14C-D).
There was a trend of decreased alpha activity in both the left and right CeA during mechanical stimulation of the injured and non-injured paws. This suppression of alpha activity was significant in the right CeA during mechanical stimulation of the right paw at 3 hours after a contralateral paw carrageenan injection (A). The decrease in alpha activity was also significant in the right CeA in the carrageenan alone (no stimulation) condition, and during mechanical stimulation of the injured right paw (B). #significantly lower than the activity
recorded without stimulation at a given time-point; *Significantly lower than baseline \( p < .05 \).

### 3.2.3.3 Alpha Thermosensitivity

A two way repeated measures ANOVA comparing the right CeA alpha activity recorded without, and during thermal stimulation of the injured left paw indicated a significant main effect of condition, \( F (1, 6) = 21.87, p = .003 \), with an overall decrease in alpha activity during stimulation. There was however, no significant main effect of time, \( F (4, 24) = 1.45, p = .250 \), and the Condition x Time interaction was also not significant, \( F (4, 24) = 1.71, p = .180 \). Similarly, the results of thermal stimulation of the non-injured right paw revealed a significant main effect of condition, \( F (1, 6) = 6.48, p = .044 \), with an overall decrease in alpha activity during thermal stimulation. There was no significant main effect of time, \( F (4, 24) = 1.51, p = .230 \), and the Condition x Time interaction was also not significant, \( F (4, 24) = 1.88, p = .147 \) (Figure 3-15A).

For the right CeA group with an ipsilateral injury, there was no significant main effect of the thermal stimulation of the non-injured left paw, \( F (1, 5) = 2.74, p = .159 \). There was a significant main effect of time, \( F (4, 20) = 3.93, p = .016 \); with a significant decrease in activity recorded without peripheral stimulation at 1 \( p = .028 \), and 48 - 72 \( p = .014 \) hours, and during thermal stimulation of the paw at 1 \( p = .025 \), 3 \( p = .029 \), and 48 - 72 \( p = .002 \) hours after the carrageenan injection. The Condition x Time interaction was not significant, \( F (4, 20) = 2.16, p = .110 \). In the case of thermal stimulation of the injured right paw there was a significant main effect of condition, \( F (1, 5) = 13.83, p = .014 \), with an overall decrease in alpha
activity during thermal stimulation. There was also a significant main effect of time, \( F(4, 20) = 4.48, p = .010 \); with a significant decrease in activity recorded without peripheral stimulation at 1 (\( p = .028 \)), and 48 - 72 (\( p = .014 \)) hours, and during thermal stimulation of the paw at 3 (\( p = .025 \)) hours after the carrageenan injection. The Condition x Time interaction was not significant, \( F(4, 20) = 2.58, p = .068 \) (Figure 3-15B). These results indicate an overall decrease in right CeA alpha activity during the thermal stimulation of the injured hind paws.

For the left CeA group with contralateral injury (\( N = 8 \)), the results of the a two way repeated measures ANOVA comparing the left CeA alpha activity recorded without, and during thermal stimulation of the non-injured left paw indicated significant main effects of condition, \( F(1, 7) = 43.07, p < .001 \), with an overall decrease in alpha activity during thermal stimulation. There was however, no significant main effect of time, \( F(4, 28) = 1.85, p = .147 \), and there was no significant Condition x Time interaction, \( F(4, 28) = .92, p = .465 \). There were also no significant main effects in the case of thermal stimulation of the injured right paw (condition, \( F(1, 7) = .94, p = .365 \); time, \( F(4, 28) = 1.33, p = .284 \); Condition x Time interaction, \( F(4, 28) = 1.15, p = .355 \)). For the left CeA group with ipsilateral injury (\( N = 7 \)) there were no significant main effects of thermal stimulation of the non-injured right paw (condition, \( F(1, 6) = 3.08, p = .130 \); time, \( F(4, 24) = .35, p = .841 \); Condition x Time interaction, \( F(4, 24) = .28, p = .888 \)). The results of thermal stimulation of the injured left paw revealed a significant main effect of condition, \( F(1, 6) = 10.22, p = .019 \); with an overall decrease in alpha activity during thermal stimulation. There was however, no significant main effect of time, \( F(4, 24) = .55, p = .703 \); and the
Condition x Time interaction was also not significant, $F(4, 24) = .20, p = .936$ (Figure 3-15 C-D).

**Figure 3-15: Alpha oscillatory sensitivity to thermal stimuli**

There was a trend of decreased alpha activity in both the left and right CeA during thermal stimulation, which was significant in the right CeA (B). *Significantly lower than baseline ($p < .05$).
3.2.4 **Carrageenan-induced Changes in Beta Oscillations**

The results of a 2 (Injury Side) x 6 (Time) mixed factorial ANOVA comparing the effect of the two right CeA groups (contralateral vs. ipsilateral injury) on the changes in beta activity did not show a significant main effect of injury side, $F(1, 11) = .06, p = .805$, but there was a significant main effect of time, $F(5, 55) = 8.59, p < .001$; with a trend of decreased beta activity. Post hoc analysis revealed that there was a significant decrease in the right CeA beta oscillations immediately ($p = .020$), at 1 ($p = .009$), 3 ($p = .008$), and 5 ($p < .001$) hours after a carrageenan injection into the contralateral (left) paw. The right CeA beta activity also significantly decreased immediately ($p = .049$), at 3 ($p = .001$), and 5 ($p = .004$) hours after the carrageenan injection into the ipsilateral paw. The Injury Side x Time interaction was however, not significant, $F(5, 55) = 1.87, p = .114$; indicating that there were no time dependent differences in the effects of the ipsilateral and contralateral injuries on the right CeA beta activity (Figure 3-16A).

The results of a 2 (Injury Side) x 6 (Time) mixed factorial ANOVA comparing the effect of the contralateral and ipsilateral injuries on the left CeA beta activity did not show a significant main effect of injury side, $F(1, 13) = 4.05, p = .065$, but there was a significant main effect of time, Greenhouse-Geisser $F(2.63, 34.23) = 4.79, p = .009$; with an overall decrease in activity. Post hoc analysis revealed significant decrease in left CeA beta activity at 1 ($p = .014$), 3 ($p = .004$), 5 ($p = .001$), and 48 – 72 ($p = .036$) hours after the carrageenan injection into the contralateral paw. The Injury Side x Time interaction was not significant, Greenhouse-Geisser $F(2.63, 34.23) = 1.81, p = .123$. These results suggest that the left CeA beta oscillations
significantly decreased in response to a contralateral, but not ipsilateral, paw injury (Figure 3-16B).

![Figure 3-16: Carrageenan-induced changes in beta oscillations]

There was significant decrease in right CeA beta oscillations for both the ipsilateral ($N = 6$) and contralateral ($N = 7$) injury groups, during the first phase of inflammatory response. The beta oscillations of the left CeA group with contralateral ($N = 8$), but not ipsilateral ($N = 7$), paw injury significantly decreased during both the first and second phases of the inflammatory response. *Significantly lower than baseline ($p < .05$). The asterisk color corresponds with the color of the trend-line (Red-Left paw vs. Black-right paw injury)

3.2.4.1 Control Group vs. Lesion Group on Beta Activity

A comparison of the right CeA beta oscillations of the lesion ($N = 7$) and control ($N = 8$) groups that had a left paw injury did not reveal significant main effect of condition, $F (1, 13) = .16, p = .696$. There was a significant main effect of time, Greenhouse-Geisser $F(2.77, 36.03) = 4.29, p = .013$; with a significant decrease
in beta oscillations of the lesion group at hour 5 \((p = .025)\) compared to baseline (Figure 3-17A). The Condition x Time interaction was not significant, Greenhouse-Geisser \(F(2.77, 36.03) = .76, p = .512\). For the left CeA beta oscillations of the lesion \((N = 8)\) and control \((N = 7)\) groups that had a right paw injury, there was no significant main effect of condition, \(F(1, 13) = .593, p = .455\). There was a significant main effect of time, \(F(5, 65) = 9.02, p < .001\); and the Condition x Time interaction was also significant, \(F(5, 65) = 3.50, p = .007\). The results of a simple main effects analysis probing the significant Condition x Time interaction revealed that there was significant decrease in the control group’s left CeA beta oscillations, immediately \((p = .007)\), at 1 \((p = .018)\) and 5 \((p = .001)\) hours; and those of the lesion group, at 1 \((p = .017)\), 3 \((p = .009)\), and 5 \((p = .002)\) hours after the carrageenan injection. Furthermore, immediately after the carrageenan injection, the left CeA beta oscillations of the lesion group were significantly higher than those of the control group \((p = .042)\) (Figure 3-17B).
The comparison of the beta oscillations of the control and lesion groups revealed that the activity significantly decreased as the pain progressed, but the effect was more prominent in the left CeA (A - B). There were no time-dependent group differences in beta oscillations except for the activity of the control group which was significantly lower than that of the lesion group immediately after the carrageenan injection (B). *Significantly lower than baseline. #Significantly lower than the activity of the lesion group (p < .05).

3.2.4.2 Beta Mechanosensitivity

The results of a two way repeated measures ANOVA comparing the right CeA beta activity recorded without, and during mechanical stimulation of the injured left paw (N = 7) indicated a significant main effect of condition, $F(1, 6) = 7.75$, $p = .032$; with an overall decrease in beta activity during mechanical stimulation of the paw. However, there was no significant main effect of time, $F(4, 24) = 2.47$, $p = .072$, and the Condition x Time interaction was not significant, $F(4, 24) = 1.13$, $p = .364$. The results of mechanical stimulation of the non-injured right paw did not reveal a significant main effect of condition, $F(1, 6) = 4.90$, $p = .069$. There was no significant main effect of time, $F(4, 24) = 1.54$, $p = .222$, and the Condition x Time interaction was also not significant, $F(4, 24) = .85$, $p = .510$. These results indicated an overall decrease in the right CeA oscillations during mechanical stimulation, but there were no significant time dependent differences in the oscillations recorded without, and during the mechanical stimulation (Figure 3-18A).

For the right CeA group with ipsilateral injury, there were no significant effects of mechanical stimulation of the non-injured left paw (condition, $F(1, 5) =$
Mechanical stimulation of the injured right paw had a significant main effect of condition, $F(1, 5) = 13.09, p = .015$, with an overall decrease in beta oscillations during the poking of the paw. However, there was no significant main effect of time, $F(4, 20) = 2.22, p = .104$, and there was also no significant Condition x Time interaction, $F(4, 20) = 1.41, p = .266$. There was an overall decrease in the right CeA oscillations during mechanical stimulation of the injured right paw, but there were no significant time-dependent differences in the oscillations recorded without, and during the mechanical stimulation (Figure 3-18B).

The two way repeated measures ANOVA comparing the left CeA beta activity recorded without, and during mechanical stimulation did not indicate significant effects for both the non-injured left paw (condition, $F(1, 7) = .63, p = .453$; time, $F(4, 28) = 2.36, p = .077$; Condition x Time interaction, $F(4, 28) = 1.27, p = .304$) and injured right paw (condition, $F(1, 7) = .065, p = .805$; time, $F(4, 28) = 2.44, p = .071$; Condition x Time, $F(4, 28) = .85, p = .505$). For the left CeA group with ipsilateral injury, there was no significant main effect of mechanical stimulation of the injured left paw, $F(1, 6) = 1.24, p = .308$. There was a significant main effect of time, $F(4, 24) = 6.52, p = .001$, and Condition x Time interaction, $F(4, 24) = 4.47, p = .008$. The results of the simple main effects analysis probing the significant Condition x Time interaction revealed that compared to the carrageenan-alone condition, mechanical stimulation of the injured left paw induced significant increase in the left CeA beta activity at 1 hour ($p = .036$), and a significant decrease in activity at 5 hours ($p = .005$) after the carrageenan injection. The activity at 5 hours was significantly lower.
than baseline ($p = .028$). The results of mechanical stimulation of the non-injured contralateral paw did not reveal a significant main effects of condition, $F (1, 6) = 1.13, p = .330$, and time, $F (4, 24) = 2.82, p = .087$. The Condition x Time interaction was also not significant, $F(4, 24) = .85, p = .505$ (Figure 3-18C-D).

![Figure 3-18: Changes in beta oscillatory sensitivity to mechanical stimuli](image)

Mechanical stimulation of both the injured and non-injured paws induced a trend of decreased beta activity in both the left and right CeA. The effect was significant in the left CeA during stimulation of the injured left paw, which induced an increase in activity at 1 hour and a decrease in activity at 5 hours after the
carrageenan injection (D). *Significantly lower than baseline; #Significant increase or decrease compared to activity recorded without stimulation (carrageenan-alone condition) ($p < .05$).

3.2.4.3 Beta Thermosensitivity

A two way repeated measures ANOVA comparing the right CeA beta activity recorded without, and during thermal stimulation of the injured left paw indicated a significant main effect of condition, $F(1, 6) = 7.49, p = .034$, with an overall decrease in beta oscillations during thermal stimulation. However, there was no significant main effect of time, $F(4, 24) = .47, p = .755$, and the Condition x Time interaction was not significant, $F(4, 24) = .78, p = .551$. Stimulation of the non-injured right paw resulted in a significant main effect of condition, $F(1, 6) = 29.13, p = .002$; with an overall decrease in beta activity during thermal stimulation. There was a significant main effect of time, $F(4, 24) = 2.89, p = .044$ with a decrease in activity in the carrageenan-alone (no stimulation) condition at 1 ($p = .018$), 3 ($p = .006$), and 5 ($p < .001$) hours after the injection. The Condition x Time interaction was not significant, $F(4, 24) = .96, p = .450$. The results revealed an overall decrease in right CeA beta oscillations during thermal stimulation but no significant time-dependent differences in the activity recorded without, and during thermal stimulation (Figure 3-19A).

For the right CeA group with ipsilateral injury, there were no significant effects of the thermal stimulation of the non-injured left paw (condition, $F(1, 5) = .09, p = .772$; time, $F(4, 20) = 1.62, p = .208$; Condition x Time interaction, $F(4, 20) = 2.02, p = .130$). In the case of the injured right paw there was no significant main
effect of condition, $F(1, 5) = .08, p = .784$. There was however, a significant main effect of time, $F(4, 20) = 2.94, p = .046$; with a decrease in activity in the carrageenan-alone condition at 3 ($p = .018$) and 5 ($p = .046$) hours after the injection. There was no significant Condition x Time interaction, $F(4, 20) = 1.11, p = .378$ (Figure 3-19B).

The results of the a two way repeated measures ANOVA comparing the left CeA beta activity recorded without, and during thermal stimulation of the non-injured left paw did not indicate significant main effects of condition, $F(1, 7) = .06, p = .821$, and time, $F(4, 28) = 2.13, p = .104$, and there was no significant Condition x Time interaction, $F(4, 28) = .26, p = .902$. There was no significant main effect of thermal stimulation of the injured right paw condition, $F(1, 7) = .28, p = .614$, but there was a significant main effect of time, $F(4, 28) = 4.15, p = .009$. Post hoc analysis revealed that there was significant decrease in the left CeA beta oscillations at 1 ($p = .0132$), at 3 ($p = .002$), and 5 ($p = .011$) hours in the carrageenan-alone (no stimulation) condition. The Condition x Time interaction was however, not significant, $F(4, 28) = 1.80, p = .158$ (Figure 3-19C). For the left CeA group with ipsilateral injury, there were no significant main effects of thermal stimulation of the injured left paw (condition, $F(1, 6) = 1.10, p = .335$; time, $F(4, 24) = 3.71, p = .063$; Condition x Time interaction, $F(4, 24) = 2.12, p = .109$). There was also no significant main effect for the non-injured right paw condition, $F(1, 6) = .431, p = .536$, but there was a significant main effect of time, $F(4, 24) = 5.38, p = .014$; with an increase in activity during thermal stimulation of the paw at 48 – 72 hours ($p =$
.022) after the carrageenan injection. The Condition x Time interaction was not significant, $F(4, 24) = 3.04, p = .087$ (Figure 3-19D).

![Right CeA Beta Activity](image)

![Left CeA Beta Activity](image)

Figure 3-19: Changes in beta oscillatory sensitivity to thermal stimuli

Compared to baseline, there was a trend of decrease in beta activity of both the left and right CeA as the pain progressed, which was significant for the carrageenan-alone condition (A, B, and C). There was also an initial trend of decreased beta activity during thermal stimulation, but the stimulation induced an increase in activity at 48 – 72 hours after the carrageenan injection, which was
significant in the left CeA (D). *significantly lower or higher than baseline activity (p < .05).

3.2.5  Carrageenan-induced Low-Gamma Oscillations

The results of a 2 (Injury Side) x 6 (Time) mixed factorial ANOVA comparing the over time changes in right CeA low-gamma oscillations of the two groups (contralateral vs. ipsilateral injury) did not show a significant main effect of injury side, \( F (1, 11) = 1.15, p = .306 \). There was a significant main effect of time, Greenhouse-Geisser \( F (5, 55) = 5.21, p = .001 \); with a decrease in activity of the group with right paw injury at 5 hours (\( p = .037 \)) after the carrageenan injection. The Injury Side x Time interaction was also not significant, Greenhouse-Geisser \( F (5, 55) = .58, p = .715 \) (Figure 3-20A).

The results of a 2 (Injured Side) x 6 (Time) mixed factorial ANOVA comparing the changes in left CeA low gamma activity of the contralateral and ipsilateral injury groups, indicated a significant main effect of injury side, \( F (1, 13) = 5.16, p = .041 \); with lower overall activity in response to the contralateral compared to the ipsilateral injury. There was also a significant main effect of time, \( F (5, 65) = 2.62, p = .032 \). The activity of the left CeA significantly decreased at 3 hours (\( p = .042 \)) for the group with a contralateral injury, and increased at 48 – 72 hours (\( p = .019 \)) for the group with ipsilateral injury. The Injury Side x Time interaction was not significant, \( F(5, 65) = 1.32, p = .267 \) (Figure 3-20B).
There was a trend of decreased right CeA low-gamma oscillations for both the right ($N = 6$) and left paw ($N = 7$) injury groups, which was significantly lower than baseline for the left paw injury group. The low-gamma oscillations of the left CeA group with contralateral injury ($N = 8$), significantly decreased at 3 hours, while the activity of the group with ipsilateral injury ($N = 7$) significantly increased at 48-72 hours after the carrageenan injection ($p < .05$). *Significantly lower or higher than baseline.

3.2.5.1 Control Group vs. Lesion Group on Low-gamma Activity

A comparison of the right CeA low-gamma oscillations of the lesion ($N = 7$) and control ($N = 8$) groups that had a left paw injury did not reveal significant main effects of condition, $F(1, 13) = .69$, $p = .422$, and time, $F(5, 65) = 2.08$, $p = .080$. The Condition x Time interaction was also not significant, $F(5, 65) = .69$, $p = .634$ (Figure 3-21A). For the left CeA low-gamma oscillations of the lesion ($N = 8$) and control ($N = 7$) groups that had a right paw injury, there was no significant main effect of condition, $F(1, 13) = 1.61$, $p = .227$. There was a significant main effect of time, $F(5,$
with a significant decrease in the oscillations of the lesion group at 3 hours after the carrageenan injection \((p = .027)\). The Condition x Time interaction was however, not significant, \(F(5, 65) = 1.05, p = .397\) (Figure 3-21B).

**Figure 3-21: Control vs. Lesion groups on low-gamma oscillatory activity**

The comparison of the low-gamma oscillations of the control and lesion groups which had contralateral injury reveal that compared to baseline there was a decrease in activity as the pain progressed, which was significant in the left CeA of the lesion group at 3 hours after the carrageenan injection. There were however, no significant time-dependent group differences in low-gamma oscillations of both the control and lesion groups. *Significantly lower than baseline \((p < .05)\).

### 3.2.5.2 Low Gamma Mechanosensitivity

The results of a two way repeated measures ANOVA comparing the right CeA low-gamma activity recorded without, and during mechanical stimulation did not indicate significant main effects of both the injured left paw \((N = 7)\) (condition, \(F(1, 6) = .38, p = .560\); time, \(F(4, 24) = 1.36, p = .278\); Condition x Time interaction, \(F(4,
24) = .83, p = .519) and non-injured right paw (condition, F (1, 6) = .40, p = .550; time, F (4, 24) = 1.01, p = .420; Condition x Time interaction, F (4, 24) = .93, p = .466). For the group with ipsilateral injury (N = 6), there were also no significant effects of mechanical stimulation of the non-injured left paw (condition, F (1, 5) = 2.52, p = .173; time, F (4, 20) = .85, p = .511; Condition x Time interaction, F (4, 20) = .89, p = .487) and the injured right paw (condition, F (1, 5) = 1.81, p = .236; time, F (4, 20) = 1.71, p = .187; Condition x Time interaction, F (4, 20) = .37, p = .830) (Figure 3-22A-B).

The two way repeated measures ANOVA comparing the left CeA low-gamma activity recorded without, with the activity during mechanical stimulation did not indicate significant main effects for the non-injured ipsilateral (left) paw (N = 8) (condition, F (1, 7) = .55, p = .482; time, F (4, 28) = 2.06, p = .113; Condition x Time interaction, F (4, 28) = .23, p = .917). Mechanical stimulation of the injured right paw did not have a significant main effect of condition, F (1, 7) = .09, p = .777, but there was a significant main effect of time, F (4, 28) = 3.09, p = .032; with a significant decrease in low gamma oscillations in the carrageenan-alone condition at 3 hours (p = .021) after the injection. The Condition x Time interaction was however, not significant, F (4, 28) = .70, p = .600 (Figure 3-22C).

In the case of the left CeA group with ipsilateral injury (N = 7) the results of mechanical stimulation of the injured left paw did not reveal significant main effects of condition, F (1, 6) = 4.31, p = .083, and time, F (4, 24) = 2.40, p = .078, and the Condition x Time interaction was not significant, F (4, 24) = .74, p = .572. There were no significant main effects of mechanical stimulation of the non-injured
contralateral paw (condition, $F(1, 6) = .77, p = .413$; time, $F(4, 24) = 1.13, p = .367$; Condition x Time interaction, $F(4, 24) = 1.19, p = .341$) (Figure 3-22D).

Figure 3-22: Changes in low-gamma oscillatory sensitivity to mechanical stimuli

Mechanical stimulation of both the injured and non-injured paws did not induce significant changes in low-gamma oscillations of both the right and left CeA, compared to the carrageenan-alone activity. For the left CeA group with contralateral injury (C) there was a significant decrease in oscillations for the activity recorded without stimulation at 3 hours after the carrageenan injection. *Significantly lower than baseline ($p < .05$).
3.2.5.3 *Low Gamma Thermosensitivity*

The two way repeated measures ANOVA examining the changes in right CeA low-gamma activity recorded without, and during thermal stimulation did not indicate significant effects of both the injured left paw (condition, $F(1, 6) = .87, p = .387$; time, $F(4, 24) = 1.10, p = .380$; Condition x Time interaction, $F(4, 24) = 1.50, p = .233$) and non-injured right paw (condition, $F(1, 6) = .046, p = .837$; time, $F(4, 24) = 2.92, p = .074$; Condition x Time interaction, $F(4, 24) = 1.74, p = .174$). Similar non-significant results were obtained for the right CeA group with ipsilateral injury, during thermal stimulation of the non-injured left paw ($N = 7$) (condition, $F(1, 5) = 1.44, p = .283$; time, $F(4, 20) = .96, p = .451$; Condition x Time interaction, $F(4, 20) = .41, p = .799$) and the injured right paw (condition, $F(1, 5) = 13.28, p = .055$; time, $F(4, 20) = .67, p = .620$; Condition x Time interaction, $F(4, 20) = 1.17, p = .353$) (Figure 3-23A-B).

Similarly, in the case of the left CeA group with contralateral injury ($N = 8$) there were significant effects of thermal stimulation of both the non-injured left paw (condition, $F(1, 7) = .001, p = .993$; time, $F(4, 28) = 1.36, p = .275$; Condition x Time interaction, $F(4, 28) = .60, p = .663$), and the injured right paw (condition, $F(1, 7) = .31, p = .596$; time, $F(4, 28) = 2.10, p = .067$; Condition x Time interaction, Greenhouse-Geisser $F(4, 28) = .30, p = .878$) (Figure 3-23C).

For the left CeA group with ipsilateral injury, there were no significant main effect of thermal stimulation of the non-injured right paw, (condition, $F(1, 6) = 2.87, p = .141$; time, $F(4, 24) = 1.29, p = .300$; Condition x Time interaction, $F(4, 24) = .66, p = .625$) and the injured left paw (condition, $F(1, 6) = .65, p = .452$; time, $F(4, 24) =$
3.52, \( p = .052 \); Condition x Time interaction, \( F(4, 24) = 1.65, p = .195 \) (Figure 3-23D).

![Figure 3-23: Changes in low gamma oscillatory sensitivity to thermal stimuli](image)

There were no statistically significant time-dependent differences in the low-gamma activity recorded without, and during thermal stimulation (\( p > .05 \)).

3.2.6 *Carrageenan-induced High Gamma Oscillations*

The results of the 2 (Injury Side) x 6 (Time) mixed factorial ANOVA comparing the two right CeA groups (contralateral vs. ipsilateral injury) did not show significant main effects of injury side, \( F(1, 11) = 2.28, p = .160 \), and time,
Greenhouse-Geisser $F(2.24, 24.69) = 2.69, p = .069$. The Injury Side x Time interaction was also not significant, Greenhouse-Geisser $F(2.24, 24.69) = .95, p = .409$. Thus neither the ipsilateral nor the contralateral injuries had significant time-dependent effects on the right CeA high gamma oscillations (Figure 3-24A).

The results of a 2 (Injury Side) x 6 (Time) mixed factorial ANOVA examining the changes in left CeA high-gamma activity did not show a significant main effect of injury side, $F(1, 13) = 1.04, p = .327$. There was a significant main effect of time, Greenhouse-Geisser $F(5, 65) = 4.29, p = .012$; with a significant increase in activity immediately after the left paw injection ($p = .002$), and decrease in activity at 3 ($p = .004$), 5 ($p = .005$), and 48–72 ($p = .013$) hours after the right paw carrageenan injection. The Injury Side x Time interaction was not significant, Greenhouse-Geisser $F(5, 65) = 1.79, p = .168$ (Figure 3-24B).

Figure 3-24: Carrageenan-induced changes in high-gamma oscillations

There was a trend of decreased in right CeA high-gamma oscillations for both the right ($N = 6$) and left paw ($N = 7$) injury groups, but without significant changes
compared to baseline. The high-gamma oscillations of the left CeA group with ipsilateral injury ($N = 7$) significantly increased immediately after the carrageenan injection, while the activity of the group with contralateral paw injury ($N = 8$), significantly decreased at 3, 5, and 48 - 72 hours after the carrageenan injection.

*Significantly lower or higher than baseline ($p < .05$).

3.2.6.1 Control Group vs. Lesion Group on High-Gamma Activity

A comparison of the right CeA high-gamma oscillations of the lesion ($N = 7$) and control ($N = 8$) groups that had a left paw injury did not reveal significant main effects of condition, $F (1, 13) = .045, p = .836$, and time, Greenhouse-Geisser $F (3.41, 44.27) = 2.59, p = .058$. The Condition x Time interaction was also not significant, Greenhouse-Geisser $F (3.41, 44.27) = .24, p = .888$ (Figure 3-25A). For the left CeA high-gamma oscillations of the lesion ($N = 8$) and control ($N = 7$) groups that had a right paw injury, there was no significant main effect of condition, $F (1, 13) = 3.00, p = .107$. There was a significant main effect of time, Greenhouse-Geisser $F (2.24, 60) = 4.07, p = .026$; with a significant decrease in the high-gamma oscillations of the lesion group immediately ($p = .046$), at 3 ($p < .001$), 5 ($p = .007$), and 48 - 72 ($p = .041$) hours after the carrageenan injection. The Condition x Time interaction was however, not significant, $F (2.24, 24.69) = .95, p = .409$ (Figure 3-25B).
The comparison of the high-gamma oscillations of the control and lesion groups with contralateral injury revealed that compared to baseline there was a decrease in activity as the pain progressed. This decrease in high-gamma activity was significant in the left CeA of the lesion group immediately, at 3, 5, and 48 - 72 hours after the carrageenan injection. There were however, no significant time-dependent group differences in high-gamma oscillations of the control and lesion groups. *Significantly lower than baseline (p < .05).

### 3.2.6.2 High-Gamma Mechanosensitivity

The results of a two way repeated measures ANOVA examining the right CeA high-gamma activity recorded without, and during mechanical stimulation did not indicate significant effects for the injured left paw (N = 7) (condition, $F(1, 6) = .02$, $p = .888$; time, $F(4, 24) = 1.51$, $p = .231$; Condition x Time interaction, $F(4, 24) = .41$, $p = .797$). The results of stimulation of the non-injured right paw revealed significant a main effect of condition, $F(1, 6) = 6.89$, $p = .039$; with an overall increase in activity during stimulation of the paw. There was also a significant main
effect of time, $F(4, 24) = 3.10, p = .034$; with a decrease in activity in the carrageenan-alone condition at 1 ($p = .035$) and 5 ($p = .008$) hours, and during mechanical stimulation of the paw at 48 – 72 hours ($p = .031$) after the carrageenan injection. The Condition x Time interaction was not significant, $F(4, 24) = 2.60, p = .061$. For the right CeA group with ipsilateral injury ($N = 6$), there was a significant main effect of mechanical stimulation of the non-injured left paw condition, $F(1, 5) = 44.25, p = .001$; with an overall increase in high gamma activity during poking of the paw. There was however, no significant main effect of time, $F(4, 20) = 1.61, p = .210$, and the Condition x Time interaction was not significant, $F(4, 20) = .59, p = .675$. There was also a significant main effect of stimulation of the injured right paw, $F(1, 5) = 11.71, p = .019$, with an overall increase in activity during stimulation of the paw. There was however, on main effect of time, $F(4, 20) = 2.71, p = .060$, and the Condition x Time interaction was not significant, $F(4, 20) = .15, p = .959$ (Figure 3-26A-B).

The two way repeated measures ANOVA examining the left CeA high-gamma activity recorded without, and during mechanical stimulation did not indicate significant main effects for the non-injured ipsilateral (left) paw ($N = 8$) (condition, $F(1, 7) = .08, p = .783$; time, $F(4, 28) = 3.67, p = .051$; Condition x Time interaction, $F(4, 28) = .26, p = .901$). Mechanical stimulation of the injured contralateral paw did not have a significant main effects of condition, $F(1, 7) = .004, p = .953$, and time, $F(4, 28) = 1.48, p = .234$. The Condition x Time interaction was also not significant, $F(4, 28) = 1.02, p = .413$. For the left CeA group with ipsilateral injury ($N = 7$) mechanical stimulation of the injured left paw had no significant main effects of
condition, $F (1, 6) = 1.03, p = .350$, and time, $F (4, 24) = .65, p = .634$, and the Condition x Time interaction was not significant, $F (4, 24) = .75, p = .571$. There were also no significant effects of mechanical stimulation of the non-injured contralateral paw (condition, $F (1, 6) = 2.18, p = .191$; time, $F (4, 24) = .92, p = .468$; Condition x Time interaction, $F (4, 24) = 1.11, p = .375$) (Figure 3-26C-D).

![Image of graphs showing high-gamma activity](image)

Figure 3-26: Changes in high-gamma oscillatory sensitivity to mechanical stimuli

Compared to the oscillations recorded without peripheral stimulation, mechanical stimulation of the injured and non-injured paws did not induce
significant time-dependent changes in high-gamma oscillations of both the right and left CeA ($p > .05$).

3.2.6.3 High Gamma Thermosensitivity

The two way repeated measures ANOVA examining the changes in right CeA high-gamma activity recorded without, and during thermal stimulation did not indicate significant effects of stimulation of the injured left paw (condition, $F (1, 6) = 5.79, p = .053$; time, $F (4, 24) = .87, p = .497$; Condition x Time interaction, $F (4, 24) = .78, p = .549$), and non-injured right paw (condition, $F (1, 6) = 3.43, p = .114$; time, $F (4, 24) = 2.10, p = .112$; Condition x Time interaction, $F (4, 24) = 1.27, p = .308$) (Figure 3-27A). In the case of the right CeA group with ipsilateral injury there were no significant effects of thermal stimulation of the non-injured left paw ($N = 6$) (condition, $F (1, 5) = .82, p = .406$; time, $F (4, 20) = 2.79, p = .055$; Condition x Time interaction, $F (4, 20) = 2.44, p = .080$) and the injured right paw (condition, $F (1, 5) = 4.82, p = .079$; time, $F (4, 20) = 2.35, p = .089$; Condition x Time interaction, $F (4, 20) = 1.81, p = .167$) (Figure 3-27B).

For left CeA group with contralateral injury ($N = 8$) there was no significant effects of stimulation of the non-injured left paw, $F (1, 7) = .73, p = .422$, but there was a significant main effect of time, $F (4, 28) = 2.91, p = .039$; with a decrease in activity recorded without peripheral stimulation at 3 ($p = .001$), 5 ($p = .013$) and 48 – 72 ($p = .043$) hours after the carrageenan injection. There was no significant Condition x Time interaction, $F (4, 28) = 1.75, p = .167$. Similarly, the results of thermal stimulation of the injured right paw did not reveal a significant main effect of condition, $F (1, 7) = .38, p = .557$, but there was a significant main effect of time, $F
(4, 28) = 4.23, \( p = .008 \); with a decrease in activity recorded without peripheral stimulation at 3 (\( p = .001 \)), 5 (\( p = .013 \)) and 48 – 72 (\( p = .043 \)) hours, and during stimulation of the paw at 1 (\( p = .046 \)), 3 (\( p = .014 \)) and 5 (\( p = .031 \)) hours after the carrageenan injection. There was no significant Condition x Time interaction, \( F (4, 28) = 1.40, \ p = .259 \) (Figure 3-27C).

For the left CeA group with ipsilateral injury, there was a significant main effect of condition, \( F (1, 6) = 9.46, \ p = .022 \); with an overall increase in high gamma activity during thermal stimulation of the injured left paw. However, there was no significant main effect of time, \( F (4, 24) = .61, \ p = .657 \), and the Condition x Time interaction was not significant, \( F (4, 24) = .63, \ p = .643 \). For the non-injured right paw there were no significant main effects of condition, \( F (1, 6) = 1.84, \ p = .224 \), and time, \( F (4, 24) = .98, \ p = .436 \); and no significant Condition x Time interaction, \( F (4, 24) = .80, \ p = .540 \) (Figure 3-27D).
Although there was a trend of increased high gamma activity during thermal stimulation (A, B and D), the significant effect was a decrease in left CeA activity recorded without, and during thermal stimulation the right paw (C). *Significantly lower than baseline ($p < .05$).

3.3 CeA Lesions and the Progression of Inflammatory Pain

To determine the effect of the right and left CeA lesions on the onset and progression of inflammatory pain, the paw withdrawal thresholds data of the lesion
and non-lesion (control) groups were used, and the analyses were twofold: 1) Two-way repeated measures ANOVAs were run to examine the changes in mechanical sensitivity of the injured and non-injured paws of each of the study groups; 2) 2 (Group) x 6 (Time) mixed factorial ANOVAs were run to compare the mechanical sensitivity of the injured and non-injured paws of each CeA lesion group with that of the control group with injury on the same side of the body. Comparing the sensitivity of the right and left CeA lesion groups with that of the control group with the same side of injury ensured that all the conditions were similar, except the side of lesion or having no lesion. It also provided an illustration of the changes in peripheral pain sensitivity when a specific CeA is lesioned or left intact (spared).

3.3.1 CeA lesions and Left Injury-associated Mechanosensitivity

The two way repeated measures ANOVAs examining the changes in mechanical sensitivity of the injured and non-injured paws revealed that for the control group with left paw injury (N = 8) there were significant main effects of paw side, $F(1, 7) = 34.85, p = .001$, and time, $F(4, 28) = 20.40, p < .001$. The Paw Side x Time interaction was also significant, $F(4, 28) = 9.78, p < .001$. The significant interaction was investigated further using simple main effects analysis which indicated that compared to baseline, there was significant decrease in the paw withdrawal thresholds of the injured left paw at 3 ($p = .005$), 5 ($p < .001$) and 48 – 72 ($p < .001$) hours, and non-injured right paw at 48 – 72 ($p = .011$) hours after the carrageenan injection. The results further indicated that the withdrawal thresholds of the injured left paw were significantly lower than those of the non-injured right
paw at 3 \((p = .011)\), 5 \((p = .001)\) and 48 - 72 \((p = .014)\) hours after the carrageenan injection (Figure 3-28A).

For the right CeA lesion group with left paw injury \((N = 7)\) there were significant main effects of paw side, \(F(1, 6) = 21.04, p = .004\), and time, \(F(4, 24) = 10.02, p < .001\). The Paw Side x Time interaction was not significant, \(F(4, 24) = 1.69, p = .186\). The results of the post hoc analysis revealed that compared to baseline, the paw withdrawal thresholds of both the paws significantly decreased at all the time points; that is, for the injured left paw, at 1 \((p = .002)\), 3 \((p < .001)\), 5 \((p < .001)\), and 48 - 72 \((p = .003)\) hours, and non-injured right paw at 1 \((p = .022)\), 3 \((p = .002)\), 5 \((p = .004)\), and 48 - 72 \((p = .047)\) hours after the carrageenan injection. The results further revealed that the withdrawal thresholds of the injured left paw were significantly lower than those of the non-injured right paw \((p = .004)\), but the absence of a significant Paw Side x Time interaction suggests that there were no statistically significant time-dependent differences in the sensitivity of the injured and non-injured paws of the right CeA lesion group (Figure 3-28B).

For the left CeA lesion group with left paw injury \((N = 7)\) there were significant main effects of paw side, \(F(1, 6) = 16.84, p = .006\), and time, \(F(4, 24) = 3.29, p = .028\). The Paw Side x Time interaction was also significant, \(F(4, 24) = 10.58, p < .001\). The results of the simple main effects analysis probing the significant interaction revealed that compared to baseline, the paw withdrawal thresholds of the injured left paw significantly decreased at 5 hours after the carrageenan injection \((p < .001)\) and were significantly lower than the those of the non-injured (right) paw at that time point \((p = .001)\) (Figure 3-28C).
Figure 3-28: CeA lesions and left injury-associated mechanosensitivity

As indicated by the changes in the paw withdrawal thresholds, the control group experienced increased mechanical sensitivity in both paws, which reached statistical significance at 3 and 48 - 72 hours for the injured and non-injured paws, respectively (A). The right CeA lesions induced early onset and enhancement of mechanical sensitivity which was significant at all time points for both the injured and non-injured paws (B). The mechanical hypersensitivity of the left CeA lesion group was confined in the injured paw and reached statistical significance at only one time-point (5 hours) after the carrageenan injection (C). Except for the right
CeA lesion the condition, the injured paws were more sensitive to stimuli than the non-injured paws ($p < .05$). *significantly lower than baseline; #significantly lower than the thresholds of the non-injured paw.

3.3.2 Control vs. CeA Lesion on Left Injury-associated Mechanosensitivity

The 2 (Group) x 6 (Time) mixed factorial ANOVA comparing the right CeA lesion ($N = 7$) and control ($N = 8$) groups on the mechanical sensitivity of the injured left paw revealed significant main effects of group, $F(1, 13) = 6.34, p = .026$, and time, Greenhouse-Geisser $F(2.71, 35.18) = 29.34, p < .001$, and the Group x Time interaction was significant, $F(2.71, 35.18) = 3.94, p = .019$. The results of simple main effects analysis probing the significant Group x Time interaction revealed that compared to baseline, the paw withdrawal thresholds of the injured left paw of the right CeA lesion group significantly decreased at all the time points; that is, at 1 ($p < .001$), 3 ($p < .001$), 5 ($p < .001$), and 48 – 72 ($p < .001$) hours, while those the control group significantly decreased at 3 ($p < .001$), 5 ($p < .001$) and 48 – 72 ($p < .001$) hours after the carrageenan injection. The results further revealed that the withdrawal thresholds of the right CeA lesion group were significantly lower than those of the control group at 3 ($p = .008$) and 5 ($p = .033$) hours after the carrageenan injection (Figure 3-29A).

For the non-injured right paw comparison, there were significant main effects of group, $F(1, 13) = 7.94, p = .015$, and time, $F(4, 52) = 6.13, p < .001$; and a significant Group x Time interaction, $F(4, 52) = 5.27, p = .001$. The results of the simple main effects analysis probing the significant Group x Time interaction revealed that compared to baseline, the paw withdrawal thresholds of the non-
injured right paw of the right CeA lesion group significantly decreased at 1 \( (p = .001) \), 3 \( (p < .001) \), 5 \( (p < .001) \) and 48 - 72 \( (p = .009) \) hours, while those of the control group significantly decreased at 48 – 72 hours \( (p = .020) \) after the carrageenan injection. Furthermore, the withdrawal thresholds of the non-injured right paw of right CeA lesion group were significantly lower than those of the control group at 1 \( (p = .008) \), 3 \( (p = .003) \), and 5 \( (p = .004) \) hours after the carrageenan injection (Figure 3-29B).

The 2 (Group) x 6 (Time) mixed factorial ANOVA comparing the left CeA lesion \( (N = 8) \) and control \( (N = 7) \) groups on the mechanical sensitivity of the injured left paw did not reveal a significant main effect of group, \( F (1, 13) = 4.62, p = .051 \). There was however, a significant main effect of time, \( F (4, 52) = 18.90, p < .001 \). Compared to baseline there was a significant decrease in the paw withdrawal thresholds of left CeA lesion group at hour 5 \( (p < .001) \) and those of the control group at 3 \( (p = .002) \), 5 \( (p < .001) \), and 48 - 72 \( (p < .001) \) hours after the carrageenan injection. The Group x Time interaction was however, not significant, \( F (4, 52) = 2.45, p = .057 \). The mixed factorial ANOVA comparing the left CeA lesion group with the control group’s changes in the sensitivity of the non-injured right paw, did not reveal a significant main effect of group, \( F (1, 13) = 1.25, p = .284 \). There was a significant main effect of time, \( F (4, 52) = 5.65, p = .001 \); with a significant decrease in paw withdrawal thresholds of the control group at 48-72 hours \( (p = .017) \) compared to baseline. However, the Group x Time interaction was not significant, \( F (4, 52) = 1.02, p = .404 \) (Figure 3-29C-D).
As indicated by the paw withdrawal thresholds, the right CeA lesion group experienced an early onset of mechanical sensitivity in both the injured and non-injured paws, and both paws were more sensitive to stimuli than those of the control group (A-B). On the other hand, the control group’s paws were more sensitive to stimuli than those the left CeA lesion group (B-C). There were however, no significant time-dependent differences in the mechanical sensitivity of the left CeA lesion and control groups. *significantly lower than baseline; #significantly lower than the thresholds of the control group ($P < .05$).
3.3.3  *CeA lesions and Right Injury-associated Mechanosensitivity*

The results of the two-way repeated measures ANOVA examining the mechanical sensitivity of the injured right and non-injured left paws of the control group ($N = 7$), revealed significant main effects of paw side, $F (1, 6) = 23.31, p = .003$, and time, $F (4, 24) = 11.29, p < .001$. The Paw Side x Time interaction was also significant, $F (4, 24) = 8.11, p < .001$. The results of simple main effects analysis probing the significant interaction indicated that compared to baseline, the paw withdrawal thresholds of the injured right paw significantly decreased at $3 (p = .027), 5 (p < .001)$, and $48$ – $72 (p = .007)$ hours after the carrageenan injection. The results further revealed that the injured right paw had significantly lower withdrawal thresholds than the non-injured left paw ($p < .001$) at $5$ hours after the carrageenan injection. There were no significant changes in the withdrawal thresholds of the non-injured left paw (Figure 3-30A).

The right CeA lesion group with right paw injury ($N = 8$) also had significant main effects of paw side, $F (1, 7) = 24.65, p = .002$, and time, $F (4, 28) = 6.94, p = .001$; and the Paw Side x Time interaction was significant, $F (4, 28) = 3.64, p = .017$. The results of simple main effect analysis revealed that compared to baseline, the paw withdrawal thresholds of the injured right paw significantly decreased at $3 (p = .006), 5 (p < .001)$, and $48$ – $72 (p = .005)$ hours after the carrageenan injection. The results further revealed that the withdrawal thresholds of the injured right paw were significantly lower than those of the non-injured left paw at $3 (p = .021), 5 (p = .004)$ and $48$ – $72 (p = .023)$ hours after the carrageenan injection. The withdrawal
thresholds of the non-injured left paw did not significantly differ from baseline (Figure 3-30B).

The left CeA lesion group with right paw injury ($N = 6$) also had significant main effects of paw side, $F (1, 5) = 21.18$, $p = .006$, and time, $F (4, 20) = 11.03$, $p < .001$; and the Paw Side x Time interaction was significant, $F (4, 20) = 8.65$, $p < .001$. The results of the simple main effects analysis revealed that the paw withdrawal thresholds of the injured right paw significantly decreased at 3 ($p = .001$), 5 ($p = .004$), and 48 – 72 ($p = .013$) hours after the carrageenan injection, while the withdrawal thresholds of the non-injured left paw did not significantly change over time. The results further revealed that the withdrawal thresholds of the injured right paw were significantly lower than those of the non-injured left paw at 3 ($p = .001$), 5 ($p = .017$) and 48 – 72 ($p = .013$) hours after the carrageenan injection (Figure 3-30C).
Figure 3-30: CeA lesions and right injury-associated mechanosensitivity

As indicated by the paw withdrawal thresholds, in all the three conditions the right paw injury-associated mechanical hypersensitivity was confined in the injured paw, and started at 3 hours and lasted up to 48 – 72 hours (A, B, and C). In all the conditions the injured paws were more sensitive to stimuli than the non-injured paws. *significantly lower than baseline; #significantly lower than the thresholds of the non-injured paw (p < .05).
3.3.4  Control vs. CeA Lesion on Right Injury-associated Mechanosensitivity

The 2 (Group) x 6 (Time) mixed factorial ANOVA comparing the right CeA lesion group ($N = 8$) with the control group ($N = 7$) on the mechanical sensitivity of the injured right paw did not reveal a significant main effect of group, $F(1, 13) = .13, p = .729$. There was a significant main effect of time, $F(4, 52) = 25.92, p < .001$. The paw withdrawal thresholds of the injured right paw significantly decreased; at all time points for the right CeA lesion group, that is, at 1 ($p = .033$), 3 ($p = .001$), 5 ($p < .001$) and 48 – 72 ($p = .001$) hours; and for the control group decreased at 3 ($p = .014$), 5 ($p < .001$) and 48 – 72 ($p = .004$) hours after the carrageenan injection. However, the Group x Time interaction was not significant, $F(4, 52) = 1.10, p = .368$.

Similarly, a comparison of the sensitivity of the non-injured left paw did not reveal significant main effects of group, $F(1, 13) = .014, p = .909$, and time, Greenhouse-Geisser $F(2.61, 33.92) = 2.79, p = .062$; and the Group x Time interaction was not significant, $F(2.61, 33.92) = .40, p = .727$ (Figure 3-31A-B).

The 2 (Group) x 6 (Time) mixed factorial ANOVA examining the difference in the mechanical sensitivity of the injured right paw of the left CeA lesion group ($N = 6$) with that of the control group ($N = 7$), did not reveal a significant main effect of group, $F(1, 11) = .21, p = .653$. There was a significant main effect of time, $F(4, 44) = 31.51, p < .001$. Compared to baseline, the paw withdrawal thresholds of the injured right paw significantly decreased for the left CeA lesion group at 3 ($p = .001$), 5 ($p < .001$) and 48 – 72 ($p = .005$) hours, and control group at 3 ($p = .006$), 5 ($p < .001$) and 48 – 72 ($p = .002$) hours after the carrageenan injection. However, there was no significant the Group x Time interaction, $F(4, 44) = 1.78, p = .151$. The mixed
factorial ANOVA comparing the left CeA lesion group with the control group’s changes in the sensitivity of the non-injured left paw did not reveal a significant main effects of group, $F (1, 11) = 2.67, p = .131$, and time, Greenhouse-Geisser $F (2.21, 24.39) = 2.11, p = .139$. The Group x Time interaction was also not significant, $F (2.21, 24.39) = 1.44, p = .258$. These results suggest that there were no significant time-dependent differences in the control and left CeA lesion groups’ right injury associated mechanical sensitivity (Figure 3-31C-D).

![Figure 3-31: Control vs. CeA Lesion on right injury-associated mechanosensitivity](image)

Although there was significant decrease in the paw withdrawal thresholds in B, and D, there were no statistically significant time-dependent group differences in
the right paw injury-associated mechanical sensitivity (A-D). However, the right CeA lesion group had an early onset of mechanical sensitivity (B), which consistent with the observation among the rats with left paw injury. *Significantly lower than baseline (p < .05).

3.3.5  

**CeA lesions and Left Injury-associated Thermosensitivity**

The two-way repeated measures ANOVA comparing the changes in thermal sensitivity of the injured left and non-injured right paw of the control group (N = 8), revealed a significant main effect of paw side, $F(1, 7) = 11.42, p = .012$, with lower withdrawal latencies for the injured paw. There was no main significant effect for time, $F(4, 28) = 1.08, p = .385$, and the Paw Side x Time interaction was not significant, $F(4, 28) = .54, p = .710$. Thus there were no significant over time changes in the thermal sensitivity of the injured left and non-injured right paws of the non-lesion (control) group. Similarly, the results of the left CeA lesion group did not reveal significant main effects of paw side, $F(1, 6) = .02, p = .897$, and time, $F(4, 24) = 1.93, p = .139$; and the Paw Side x Time interaction was not significant, $F(4, 24) = 2.17, p = .104$. Thus both the control and left CeA lesion groups with left paw injury did not experience significant changes in thermal sensitivity (Figure 3-32A & C).

For the right CeA lesion group with left paw injury there were significant main effects of paw side, $F(1, 8) = 6.75, p = .041$, and time, $F(4, 24) = 18.49, p < .001$. The Paw Side x Time interaction was also significant, $F(4, 24) = 5.15, p = .004$. The results of the simple main effects analysis revealed significant decrease in the withdrawal latencies of both the injured left paw at 3 ($p = .024$), 5 ($p < .001$), and 48
- 72 ($p = .007$) hours, and non-injured right paw at 3 ($p = .01$), 5 ($p = .011$), and 48 – 72 ($p = .036$) hours after the carrageenan injection. The results further revealed that the withdrawal latencies of the left paw were significantly lower than those of the right paw at 5 ($p = .001$) and 48 – 72 ($p = .037$) hours after the carrageenan injection (Figure 3-32B).

![Figure 3-32: CeA lesions and left injury-associated thermosensitivity](image)

As indicated by the paw withdrawal latencies, the control (A) and left CeA lesion (C) groups did not experience significant changes in thermal sensitivity and the responses of the injured and non-injured paws did not significantly differ (A). The right CeA lesions were associated with a significant increase in the thermal
sensitivity of both the injured and non-injured paws, which started at 3 and lasted up to 48 – 72 hours (B). Furthermore, the injured paws of the right CeA lesion group were more sensitive to thermal stimuli than the non-injured paws. *Significantly lower than baseline; #Significantly lower than the thresholds of the non-injured paw (p < .05).

3.3.6 Control vs. CeA Lesion on Left Injury-associated Thermosensitivity

The 2 (Group) x 6 (Time) mixed factorial ANOVA examining the difference in the thermal sensitivity of the right CeA lesion and control groups, with left paw injury did not reveal a significant main effect of group, $F(1, 13) = 1.89, p = .192$, but there was a significant main effect of time, Greenhouse-Geisser $F(1.86, 24.16) = 7.86, p = .003$. Compared to baseline, the paw withdrawal latencies of the injured left paws significantly decreased for the right CeA lesion group at 3 ($p = .023$), 5 ($p = .001$), and 48 – 72 ($p = .005$) hours, and for the control group at 5 ($p = .048$) hours after the carrageenan injection. The Group x Time interaction was however, not significant, $F(1.86, 24.16) = 2.11, p = .146$ (Figure 3-33A).

For the non-injured right paws of the right CeA lesion and control groups there was a significant main effect of group, $F(1, 13) = 17.13, p = .001$, with the right CeA lesion group having lower latencies. There was also a significant main effect of time, $F(4, 52) = 3.76, p = .009$. Compared to baseline, the paw withdrawal latencies of the non-injured right paws significantly decreased for the right CeA lesion group at 5 ($p = .024$) hours. The Group x Time interaction was however, not significant, $F(4, 52) = .42, p = .796$. Thus, whereas the right CeA lesion group had lower
withdrawal latencies than the control group, there were no significant time dependent differences in the sensitivity of the two groups (Figure 3-33 B).

The comparison of the left CeA lesion and control groups’ thermal sensitivity, did not reveal a significant main effects of group, \( F (1, 13) = 1.39, p = .259 \) and time, \( F (4, 52) = 2.23, p = .078 \). The Group x Time interaction was also not significant, \( F (4, 52) = .42, p = .791 \). For the non-injured right paws, there were no significant main effects of group, \( F (1, 13) = .11, p = .742 \), and time, \( F (4, 52) = .97, p = .432 \). The Group x Time interaction was also not significant, \( F (4, 52) = 1.58, p = .194 \). Thus, there were no significant time dependent differences in the thermal sensitivity of the left CeA lesion and control groups (Figure 3-33 C-D).
The changes in thermal sensitivity were more prominent for the right CeA lesion compared to the group (A-B). There were no significant changes in the sensitivity of the left CeA lesion group (C-D). For all the conditions, there were no statistically significant group differences in left paw injury-associated thermal sensitivity (A-D). *Significantly lower than baseline ($p < .05$).

3.3.7 CeA lesions and Right Injury-associated Thermosensitivity

The two-way repeated measures ANOVA examining the thermal sensitivity of the injured right and non-injured left paw of the control group ($N = 7$), revealed a
significant main effect of paw side, \( F(1, 6) = 9.94, p = .020 \), and but not for time, Greenhouse-Geisser \( F(1.54, 9.26) = 3.56, p = .079 \). However, the Paw Side x Time interaction was significant, \( F(1.54, 9.26) = 7.10, p = .007 \). The results of the simple main effects analysis probing the significant Paw Side x Time interaction indicated that the paw withdrawal latencies significantly decreased for both the injured right paw (at 3 \( p = .042 \), 5 \( p = .021 \), and 48 – 72 \( p = .005 \) hours) and non-injured left paw (at 3 \( p = .004 \), and 48 – 72 \( p = .017 \) hours). Furthermore, the injured right paw had significantly lower withdrawal latencies than the non-injured left paw at 5 hours after the carrageenan injection \( (p < .001) \) (Figure 3-34A).

For the right CeA lesion group with right paw injury \( (N = 8) \) there were significant main effects of paw side, \( F(1, 7) = 6.08, p = .043 \), and time, \( F(4, 28) = 3.77, p = .014 \), and the Paw Side x Time interaction was significant, \( F(4, 28) = 6.32, p = .001 \). The results of the simple main effects analysis revealed that there was a significant decrease in the paw withdrawal latencies of the injured right paw at 5 \( p = .012 \), and 48 – 72 \( p = .006 \) hours, and non-injured left paw at 5 \( p = .033 \) hours after the carrageenan injection. The results further indicated that the withdrawal latencies of the injured right paw were significantly lower than those of the non-injured left paw at 5 \( p = .015 \) and 48 – 72 \( p = .012 \) hours after the carrageenan injection (Figure 3-34B).

In the case of the left CeA group with right paw injury \( (N = 6) \) there was no significant main effect of paw side, \( F(1, 5) = 3.56, p = .118 \). There was a significant main effect of time, \( F(4, 20) = 4.08, p = .014 \), and the Paw Side x Time interaction was also significant, \( F(4, 20) = 8.24, p < .001 \). The results of the simple main effects
analysis revealed that there was a significant decrease in the paw withdrawal latencies of the injured right paw at 3 ($p = .011$), 5 ($p = .003$), and 48–72 ($p = .038$) hours, and non-injured left paw at 3 ($p = .036$), and 48–72 ($p = .038$) hours after the carrageenan injection. The results further revealed that the withdrawal latencies of the injured right paw were significantly lower than those of the non-injured left paw at 3 ($p = .026$), and 5 ($p = .005$) hours after the carrageenan injection (Figure 3-34C).

Figure 3-34: CeA lesions and right injury-associated thermosensitivity

As indicated by the paw withdrawal latencies, the right side injury resulted in thermal hypersensitivity in both the injured and non-injured paws of the control
(A), right CeA lesion (B) and left CeA lesion (C) groups. For all the groups the injured paws were more sensitive to thermal stimuli than the non-injured paws. *significantly lower than baseline; #significantly lower than the thresholds of the non-injured paw (p < .05).

3.3.8 Control vs. CeA Lesion on Right Injury-associated thermosensitivity

The 2 (Group) x 6 (Time) mixed factorial ANOVA comparing the right CeA lesion and control groups on the thermal sensitivity of the injured right paw did not reveal a significant main effect of group, $F (1, 13) = .10, p = .761$. There was a significant main effect of time, $F (4, 52) = 9.92, p < .001$. Compared to baseline, the paw withdrawal latencies of the injured right paw significantly decreased for the right CeA lesion group at 3 ($p = .021$), 5 ($p = .002$) and 48 – 72 ($p < .001$) hours, and control group at 5 ($p = .025$) and 48 – 72 ($p = .017$) hours after the carrageenan injection. The Group x Time interaction was not significant, $F (4, 52) = .75, p = .562$. The comparison of these two groups on the thermal sensitivity of the non-injured left paw did not revealed significant main effects of group, $F (1, 13) = .04, p = .851$, and time, Greenhouse-Geisser $F (2.14, 27.79) = 1.39, p = .266$. However, there was a significant Group x Time interaction, Greenhouse-Geisser $F (2.14, 27.79) = 3.66, p = .036$. The significant Group x Time interaction was investigated using simple main effects analysis and the results revealed that there was a significant decrease in the paw withdrawal latencies of the non-injured left paw of the right CeA lesion group at 3 hours ($p = .005$), while those of the control group significantly decreased at 3 ($p = .023$), and 48 – 72 ($p = .001$) hours after the carrageenan injection. The control group’s paw withdrawal latencies were significantly lower than those of the right
CeA lesion group at 48 – 72 hours after the carrageenan injection \( (p = .008) \) (Figure 3-35A-B).

The 2 (Group) x 6 (Time) mixed factorial ANOVA examining the difference in the thermal sensitivity of the injured right paws of the left CeA lesion and control groups, did not reveal a significant main effect of group, \( F(1, 11) = .10, p = .762 \). There was a significant main effect of time, \( F(4, 44) = 11.74, p < .001 \). Compared to baseline, the paw withdrawal latencies of the injured right paw significantly decreased for the right CeA lesion group at 3 \( (p = .001) \), 5 \( (p = .001) \), and 48 – 72 \( (p = .009) \) hours, and control group at 3 \( (p = .051) \), 5 \( (p = .006) \) and 48 – 72 \( (p = .003) \) hours after the carrageenan injection. The Group x Time interaction was not significant, \( F(4, 44) = 1.07, p = .383 \). For the non-injured left paw withdrawal latencies, there was no significant main effect of group, \( F(1, 11) = .55, p = .473 \). There was a significant main effect of time, Greenhouse-Geisser \( F(2.33, 25.57) = 3.39, p = .043 \). Compared to baseline, the paw withdrawal latencies of the non-injured left paw significantly decreased for the left CeA lesion group at 3 \( (p = .002) \), and 48 – 72 \( (p = .029) \) hours, and control group at 48 – 72 \( (p = .005) \) hours after the carrageenan injection. The Group x Time interaction was not significant, Greenhouse-Geisser \( F(2.33, 25.57) = .78, p = .489 \). These results suggest that there were no significant time dependent differences in the thermal sensitivity of the control group and left CeA lesion group with right side injury (Figure 3-35C-D).
Figure 3-35: Control vs. CeA Lesion on right injury-associated thermosensitivity

As indicated by the paw withdrawal latencies, there was a significant increase in the thermal sensitivity of the non-injured left paws at 3 hrs for right CeA lesion group and at 3 and 48 – 72 hrs for the control group (A). The control group had significantly more sensitivity, than the right CeA lesion group at 48 – 72 hrs but overall the thermal sensitivity of control and CeA lesion groups did not significantly differ (A-D). *significantly lower than baseline; #significantly lower than the latencies of the non-injured paw ($p < .05$).
3.4 Correlations between CeA Oscillatory Activity and Mechanical Sensitivity

Correlation and hierarchical multiple regression analysis were conducted to examine the relationships between the inflammatory pain-induced changes in the CeA oscillatory activity and mechanical sensitivity at each of the time points. The first step of the analysis involved determining the correlation between the baseline and carrageenan-induced neural oscillations and the paw withdrawal thresholds and latencies recorded at the respective time points. Then hierarchical multiple regression analyses were run to further investigate whether the oscillations with significant correlation coefficients were significant predictors of peripheral mechanical and thermal sensitivity, after controlling for the effect of the neural activity induced by thermal and mechanical (poke) stimuli at the respective time points.

Each of the hierarchical multiple regression analyses indicated the unique contribution of the carrageenan, thermal, and mechanical (poke) induced oscillations to the thermal and mechanical sensitivity. The detailed results of the correlations are presented in Table A-1, A-2, A-3 and A-4 (Appendix A). In all the hierarchical multiple regression analyses scatter-plots were used to assess whether the variables had a problem of heteroscedasticity. In all the analyses, the variance of the residuals did not significantly differ along the regression lines, which implied that the error variance was not significantly uneven. Multicollinearity was also not a problem in the analyses, in that none of the predictors had a Tolerance value less than .10 and all of them had variance inflation factor (VIF) values that were less than 10.
3.4.1  *Delta Oscillations and Mechanical Sensitivity*

The right CeA delta oscillations significantly correlated with the withdrawal thresholds of the injured left, $r = -.86, p = .012$, and right paws, $r = .85, p = .034$ at 48-72 hours. The results of hierarchical regression analysis, revealed that the poke and thermal-induced right CeA delta activity accounted for 52.7% of the variance in the withdrawal thresholds of the injured left paw but the effect was not significant, adjusted $R^2 = .53; F (2, 4) = 2.23, p = .224$. The addition of the carrageenan-alone (no-stimulation) delta activity to the model explained only 3.4% of additional variance in the withdrawal thresholds, after controlling for the poke and thermal induced delta activity, $\Delta R^2 = 3.41, \Delta F (1, 3) = 8.73, p = .162$. In the final model, the delta activity associated with all the three conditions did not significantly predict mechanical sensitivity (carrageenan-alone, $\beta = -.71, p = .162$; thermal, $\beta = -.19, p = .590$; poke, $\beta = -.11, p = .774$). The negative correlation between delta activity and the paw withdrawal thresholds, suggests that an increase in delta oscillations was associated with an increased peripheral mechanical sensitivity (Figure 3-36A-C).

![Figure 3-36](image)

Figure 3-36: Right CeA delta oscillations correlated with thresholds of the injured left paw at 48 – 72 hrs
At 48-72 hours after the carrageenan injection, the delta oscillations negatively correlated with the paw withdrawal thresholds; suggesting an increase in delta activity with increasing mechanical sensitivity. However, none of the three stimuli-associated delta activity (A–C), was a significant predictor of mechanical sensitivity ($p > .05$).

For the right CeA group with a right paw injury, the results of hierarchical regression analysis revealed that the poke and thermal-induced right CeA delta activity accounted for only 5.7% of the variance in the withdrawal thresholds of the injured paw and the effect was not significant, adjusted $R^2 = .06$; $F (2, 3) = 1.15, p = .426$. The addition of carrageenan-alone delta activity to the model explained 55.5% of additional variance in the withdrawal thresholds, after controlling for the poke and thermal induced delta activity, $\Delta R^2 = .56, \Delta F (1, 2) = 105.11, p = .009$. In the final model the carrageenan-alone ($\beta = .80, p = .009$) and thermal-induced ($\beta = .40, p = .039$), but not the poke-induced ($\beta = .25, p = .090$) delta oscillations were significant predictors of the injured left withdrawal thresholds. These oscillations were positively correlated with the paw withdrawal thresholds, such that a decrease in delta oscillations was associated with an increased peripheral mechanical sensitivity.
Figure 3-37: Right CeA delta oscillations correlated with thresholds of the injured right paw at 48 – 72 hrs

The delta oscillations positively correlated with the paw withdrawal thresholds of the injured right paw, suggesting that as the mechanical sensitivity increased, the CeA delta activity decreased. The delta activity associated with the carrageenan alone (A) and thermal stimulation (C) conditions was a significant predictor of mechanical sensitivity ($p < .05$).

3.4.2 Theta Oscillations and Mechanical Sensitivity

Both the left and right CeA theta oscillations did not significantly correlate with mechanical sensitivity at all the time points. Since multiple regression analyses were run to further explore significant correlations, they were not conducted for the relationship between theta and mechanical sensitivity.

3.4.3 Alpha Oscillations and Mechanical Sensitivity

In all the experimental conditions, both the left and right CeA alpha oscillations did not significantly correlate with the paw withdrawal thresholds.
3.4.4  Beta Oscillations and Mechanical Sensitivity

The right CeA beta oscillations significantly correlated with the withdrawal thresholds of the non-injured right paw at hour 1, $r = .81, p = .012$. The results of hierarchical multiple regression analysis revealed that the poke and thermal-induced beta activity accounted for 80.8% of the variance in the withdrawal thresholds of the right paw and the effect was significant; adjusted $R^2 = .81; F(2, 4) = 13.60, p = .016$. The thermal stimuli-induced beta activity ($\beta = 1.17, p = .008$) was the significant predictor and had a higher Beta value than the poke-induced beta activity ($\beta = .459, p = .127$). The addition of the carrageenan-alone beta activity to the model only explained 1.4% of additional variance in the withdrawal latencies, and the effect was not significant, $\Delta R^2 = .01, \Delta F(1, 3) = .37, p = .588$. The beta oscillations positively correlated with the paw withdrawal thresholds (Figure 3-38A-C), which suggests that as mechanical sensitivity increased the right CeA beta activity decreased.

Figure 3-38: Right CeA beta oscillations correlated with thresholds of the non-injured right paw at hour 1

The beta oscillations positively correlated with the paw withdrawal thresholds of the non-injured right paw, suggesting that as the mechanical
sensitivity increased, the CeA beta activity decreased. The beta activity evoked by thermal stimulation (C) was a significant predictor of mechanical sensitivity ($p < .05$).

3.4.5 *Low Gamma Oscillations and Mechanical Sensitivity*

The right CeA low gamma oscillations significantly correlated with the withdrawal thresholds of the injured right paw at hour 3, $r = -.86$, $p = .027$. The results of hierarchical multiple regression analysis revealed that the poke and thermal-induced low gamma activity accounted for 25.9% of the variance in the withdrawal thresholds of the injured right paws, but the effect was not significant, adjusted $R^2 = .26$; $F(2, 3) = .49$, $p = .656$. The addition of the carrageenan-alone low gamma activity to the model explained 73.7% of additional variance in the withdrawal thresholds and the effect was significant, $\Delta R^2 = .74$, $\Delta F(1, 2) = 82.34$, $p = .012$. The low-gamma activity associated with all the three predictors significantly accounted for the variance in the injured right paw withdrawal thresholds in the final model (carrageenan, $\beta = -1.61$, $p = .012$; thermal, $\beta = -.68$, $p = .039$; poke, $\beta = -.78$, $p = .046$). Thus the right CeA low gamma activity was a significant predictor of mechanical sensitivity of the injured right paw at hour 3. The negative correlation between the right CeA low gamma oscillations and the paw withdrawal thresholds suggests that as these oscillations increased there was a corresponding increase in mechanical sensitivity (Figure 3-39A-B).
The right CeA low gamma oscillations negatively correlated with the paw withdrawal thresholds of the injured right paw at 3 hours after the carrageenan injection. The low gamma oscillations associated with all the three conditions (A, B, and C) were significant predictors of the withdrawal thresholds ($p < .05$).

For the right CeA group with left paw injury, the carrageenan-alone low gamma oscillations significantly correlated with the paw withdrawal thresholds of the injured left paw at 48-72 hours, $r = .79$, $p = .036$. The results of hierarchical multiple regression analysis revealed that the poke and thermal-induced low gamma activity accounted for only 2.0% of the variance in the withdrawal thresholds and the effect was not significant, adjusted $R^2 = .02$; $F (2, 4) = 1.06$, $p = .427$. The addition of carrageenan-alone low gamma activity to the model explained 27.8% of additional variance in the withdrawal thresholds, but the effect was not significant, $\Delta R^2 = .28$, $\Delta F (1, 3) = 2.23$, $p = .232$. These results suggest that whereas the right CeA low-gamma activity significantly correlated with the injured left paw withdrawal thresholds, it was not a significant predictor of the mechanical...
sensitivity. The positive correlation (Figure 3-40A-C) suggests that as the right CeA low gamma oscillations increased, there was a corresponding decrease in mechanical sensitivity of the injured left paw at 48 – 72 hours.

Figure 3-40: Right CeA low gamma oscillations correlated with thresholds of the injured left paw at 48 – 72 hours

During the second phase of the inflammatory response (48 -72 hrs), the low gamma oscillations positively correlated with the paw withdrawal thresholds. However, the low gamma activity associated with all the three stimuli (A, B, and C) was not a significant predictor of peripheral mechanical sensitivity ($p > .05$).

The left CeA low-gamma activity significantly correlated the withdrawal thresholds of the injured left paw at hour 5, $r = .84$, $p = .018$, and 48 – 72 hours, $r = .84$, $p = .019$. The results of hierarchical multiple regression analysis revealed that at hour 5, the poke and thermal-induced low gamma activity accounted for 94.1% of the variance in the withdrawal thresholds, and the effect was significant, adjusted $R^2 = .96$; $F(2, 4) = 49.16$, $p = .002$. Both the poke ($\beta = .70$, $p = .003$) and thermal ($\beta = .50$, $p = .009$) induced low gamma oscillations of the left CeA were significant predictors of the mechanical sensitivity of the injured left paw. The addition of carrageenan-
alone low gamma activity to the model only explained .4% of additional variance in the withdrawal thresholds after controlling for the poke and thermal induced low gamma activity, and the effect was not significant, $\Delta R^2 < .01$, $\Delta F(1, 3) = .35, p = .596$. The poke and thermal induced low gamma oscillations were still the significant predictors of the mechanical sensitivity in the final model (carrageenan-alone, .138, $p = .596$; poke, $\beta = .79, p = .025$; thermal, $\beta = .56, p = .034$). The positive correlations (Figure 3-41A-C) suggest that as the left CeA low gamma activity increased, there was a corresponding decrease in mechanical sensitivity of the injured left paw at hour 5.

![Figure 3-41: Left CeA low-gamma oscillations correlated with thresholds of the injured left paw at hour 5](image)

At hour 5, the left CeA low gamma activity positively correlated with the paw withdrawal thresholds (A-C). The animals were experiencing severe pain (as indicated by the low percent PWTs). The poke (B) and thermal (C) induced gamma oscillations were the significant predictors of peripheral mechanical sensitivity ($p < .05$).

The results of hierarchical multiple regression analysis revealed that at 48 – 72 hours, the poke and thermal-induced low gamma activity accounted for 11.8% of
the variance in the withdrawal thresholds of the injured left paw, and the effect was not significant, adjusted $R^2 = .12$; $F (2, 4) = 1.40, p = .346$. The addition of carrageenan-alone low gamma activity to the model explained 48.6% of additional variance in the withdrawal thresholds, and the effect was significant, $\Delta R^2 = .49, \Delta F (1, 3) = 14.20, p = .033$. The carrageenan-alone low gamma activity was the significant predictor of the mechanical sensitivity in the final model (carrageenan-alone, $\beta = 2.06, p = .033$; poke, $\beta = .40, p = .183$; thermal, $\beta = 1.26, p = .100$). The positive correlation suggests that as the left CeA low gamma activity increased, there was a corresponding decrease in mechanical sensitivity of the injured left paw at 48 – 72 hours (Figure 3-42A-C).

![Figure 3-42: Left CeA low-gamma oscillations correlated with thresholds of the injured left paw at 48 - 72 hours](image)

The left CeA low gamma activity in the carrageenan-alone and thermal conditions (A and C) strongly correlated, while the poke induced activity (B) weakly correlated, with the paw withdrawal thresholds. The low gamma activity in the carrageenan-alone condition was the only significant predictor of mechanical sensitivity ($p < .05$).
3.4.6 **High Gamma Oscillations and Mechanical Sensitivity**

The right CeA high gamma oscillations negatively correlated with the non-injured left paw withdrawal thresholds at hour 3, $r = -.84$, $p = .035$. The results of the hierarchical multiple regression analysis, revealed that taken together the poke and thermal induced high gamma activity accounted for only 7.3% of the variance in the withdrawal thresholds and the effect was not significant, adjusted $R^2 = .07$; $F(2, 3) = .831$, $p = .516$. The addition of carrageenan-alone high gamma activity to the model explained 53.3% of additional variance in the withdrawal thresholds after controlling for the poke and thermal induced high gamma activity but the effect was not significant, $\Delta R^2 = .53$, $\Delta F (1, 2) = 9.53$, $p = .091$. The high-gamma activity associated with all the three stimuli did not significantly predict mechanical sensitivity in the final model (carrageenan-alone, $-1.60$, $p = .091$; poke, $\beta = 1.13$, $p = .290$; thermal, $\beta = -.293$, $p = .722$). The high gamma oscillations were negatively correlated with the paw withdrawal thresholds (Figure 3-43A-C). Thus an increase in the right CeA high-gamma activity was associated with an increase in mechanical sensitivity of left paw.
At hour 3 after the carrageenan injection, the carrageenan-alone (A), poke (B) and thermal (C) induced high gamma oscillations were negatively correlated with the PWTs of the non-injured left paw, but did not significantly predict peripheral mechanical sensitivity ($p > .05$).

3.5 Correlations between CeA Oscillatory Activity and Thermal Sensitivity

3.5.1 Delta Oscillations and Thermal Sensitivity

For the right CeA group with right paw injury, the baseline delta oscillations significantly correlated with the left paw thermal withdrawal latencies, $r = -.87$, $p = .026$. The results of the hierarchical multiple regression analysis, indicated that the poke and thermal-induced right CeA delta oscillations accounted for 98.1% of the variance in the withdrawal latencies of the non-injured left paw and the effect was significant; adjusted $R^2 = .98$; $F (2, 3) = 130.12$, $p = .001$. The addition of baseline delta activity to the model explained less than 0.1% of additional variance in the withdrawal latencies, after controlling for the poke and thermal induced delta activity, $\Delta R^2 < .001$, $\Delta F (1, 2) = .005$, $p = .950$. In the final model, the thermal-
induced delta activity was the only significant predictor of the paw withdrawal latencies (thermal, $\beta = -.99, p = .031$; baseline, $\beta = .02, p = .950$; poke, $\beta = .07, p = .581$). The baseline and thermal-induced delta activity negatively correlated with the paw withdrawal latencies. Thus an increase in delta activity was associated as an increase in thermal sensitivity at baseline (Figure 3-44A-C.)

![Figure 3-44: Right CeA delta oscillations correlated with the paw withdrawal latencies at baseline](image)

The baseline (A) and thermal-induced (C) delta activity negatively correlated with the paw withdrawal latencies. The thermal-induced (C) delta activity was the only significant predictor of peripheral thermal sensitivity ($p < .05$).

For the right CeA group with left paw injury, the carrageenan-alone delta oscillations were significantly correlated with the non-injured right paw withdrawal latencies at hour 5, $r = -.88, p = .009$. The results of hierarchical multiple regression analysis revealed that the poke and thermal-induced delta activity accounted for 57.5% of the variance in the paw withdrawal latencies but the effect was not significant, adjusted $R^2 = .58; F (2, 4) = 5.05, p = .080$. The addition of carrageenan-alone delta activity to the model explained 14.1% of additional variance in the
withdrawal latencies after controlling for the poke and thermal induced delta activity, $\Delta R^2 = .14$, $\Delta F(1, 3) = 2.96, p = .184$. These results suggest that the right CeA delta oscillations associated with all the three stimuli did not significantly predict thermal sensitivity. The negative correlation between the delta oscillations with the withdrawal latencies (Figure 3-45A) indicates that as the right CeA delta activity increased, the peripheral thermal sensitivity also increased.

Figure 3-45: Right CeA delta oscillations correlated with latencies of the non-injured right paw at hour 5

At hour 5, the delta activity in the carrageenan-alone (A), and thermal (C) condition negatively correlated with the paw withdrawal latencies. However, the delta oscillations associated with all the three stimuli did not significantly predict of peripheral thermal sensitivity ($p > .05$).

For the left CeA group with right paw injury, the carrageenan-alone delta oscillations significantly correlated with the injured right paw withdrawal latencies between 48 – 72 hours, $r = -.79, p = .019$. The results of the hierarchical multiple regression analysis, indicated that taken together, the poke and thermal-induced delta activity accounted for only 8.6% of the variance in the withdrawal latencies of
the injured right paw and the effect was not significant, adjusted $R^2 = .90$; $F(2, 5) = 1.33, p = .345$. The addition of delta activity to the model explained 34.5% of additional variance in the withdrawal latencies after controlling for the poke and thermal induced delta activity but the effect was not significant, $\Delta R^2 = .35, \Delta F(1, 4) = 4.48, p = .102$. These results suggest that the delta oscillations associated with all the three stimuli did not significantly predict the paw withdrawal latencies. The negative correlation between the carrageenan-alone delta oscillations and the withdrawal latencies indicates that as the left CeA delta activity increased, the peripheral thermal sensitivity of injured right paw also increased (Figure 3-46A-C).

![Graph showing correlation between delta oscillations and withdrawal latencies](image)

Figure 3-46: Left CeA delta oscillations correlated with the latencies of the injured right paw at 48 – 72 hrs

During the second phase of the inflammatory response the correlation between the delta oscillations and the paw withdrawal latencies, was strongest and more linear in carrageenan-alone condition (A) compared to poke (B), and thermal (C) stimulation conditions. However, in all the three conditions delta activity was not a significant predictor of peripheral thermal sensitivity ($p > .05$).
For the right CeA group with left paw injury, baseline theta oscillations significantly correlated with the left paw thermal withdrawal latencies, $r = .89$, $p = .008$. The hierarchical multiple regression analysis examining the relationship between right CeA theta activity with the left paw withdrawal latencies at baseline, indicated the poke and thermal-induced theta activity accounted for 17.1% of the variance in the withdrawal latencies but the effect was not significant; adjusted $R^2 = .17; F (2, 4) = 1.62, p = .305$. The addition of baseline theta activity to the model explained 49.2% of additional variance in the withdrawal latencies after controlling for the poke and thermal induced theta activity, $\Delta R^2 = .49, \Delta F (1, 3) = 24.17, p = .016$. In the final model, the baseline theta activity ($\beta = .77, p = .016$) was a significant predictor of the withdrawal latencies but not the thermal ($\beta = .31, p = .147$) and poke ($\beta = .43, p = .085$) induced activity. These results indicate that the baseline theta activity was positively correlated with the paw withdrawal latencies; indicating that a decrease in baseline theta activity was associated with an increase in peripheral thermal sensitivity at baseline (Figure 3-47A).
Figure 3-47: Right CeA theta oscillations correlated with baseline left paw latencies at baseline

The correlation between the theta oscillations and the paw withdrawal latencies was strongest and more linear in the baseline (A), compared to poke (B) and thermal (C) stimulation conditions. The baseline theta activity was also a significant predictor of peripheral thermal sensitivity ($p < .05$).

For the left CeA group with right paw injury, the carrageenan-alone theta oscillations significantly correlated with the withdrawal latencies of injured right paw between 48 – 72 hours, $r = .74$, $p = .038$. The hierarchical multiple regression analysis results indicated that the poke and thermal-induced theta oscillations accounted for 17.1% of the variance in the withdrawal latencies and the effect was not significant, adjusted $R^2 = .17$; $F (2, 5) = .52$, $p = .625$. The addition of the carrageenan-alone theta activity to the model explained 67.3% of additional variance in the withdrawal latencies but the effect was not significant, $\Delta R^2 = .67$, $\Delta F (1, 4) = 17.25$, $p = .014$. The carrageenan-alone theta activity was the significant predictor of thermal sensitivity in the final model (carrageenan-alone, $\beta = .85$, $p = .014$; poke, $\beta = -.56$, $p = .059$; thermal, $\beta = .04$, $p = .843$) (Figure 3-48A-C).
Figure 3.48: Left CeA theta oscillations correlated with latencies of the injured right paw at 48-72 Hours

The correlation between the theta oscillations and the paw withdrawal latencies was strongest and more linear in carrageenan-alone condition (A), compared to the poke (B) and thermal (C) stimulation conditions. The theta activity associated with the carrageenan-alone condition was also a significant predictor of peripheral thermal sensitivity.

3.5.3 Beta Oscillations and Thermal sensitivity

For the right CeA group with right paw injury, the beta oscillations significantly correlated with the non-injured left paw withdrawal latencies with at 3 ($r = -.83, p = .041$), and 48 - 72 ($r = .97, p = .002$) hours, and injured right paw at 48 - 72 hours ($r = .90, p = .013$). The results of the hierarchical multiple regression analysis revealed that taken together the poke and thermal-induced right CeA beta activity accounted for 8.3% of the variance in the withdrawal latencies of the non-injured left paw at hour 3, and the effect was not significant; adjusted $R^2 = .08; F (2, 3) = 1.23, p = .408$. The addition of the carrageen-alone beta activity to the model explained 35.0% of additional variance in the withdrawal latencies after controlling
for the poke and thermal induced beta activity but the effect was not significant, $\Delta R^2 = .35$, $\Delta F(1, 2) = 3.52$, $p = .201$. The negative correlations between the right CeA beta oscillations and the paw withdrawal latencies suggest that the beta activity increased with thermal sensitivity (Figure 3-49A-C).

![Figure 3-49: Right CeA beta oscillations correlated with PWLs of the non-injured left paw at 3 hours](image)

The right CeA beta oscillations negatively correlated with the paw withdrawal latencies of the non-injured left paw at 3 hours after the carrageenan injections. Thus, beta activity increased with thermal sensitivity. However, the multiple regression analysis results indicated that beta activity was not a significant predictor of thermal sensitivity ($p > .05$).

The results of the multiple regression analysis examining the relationship between beta activity and thermal sensitivity of the non-injured left paw at 48-72 hours revealed that the poke and thermal-induced right CeA beta activity accounted for 50.8% of the variance in the withdrawal latencies but the effect was not significant, adjusted $R^2 = .51$; $F(2, 3) = 1.55$, $p = .345$. The addition of carrageenan-alone beta activity to the model explained 46.5% of additional variance in the withdrawal latencies and the effect was significant, $\Delta R^2 = .47$, $\Delta F(1, 2) = 34.48$, $p =$
.028. In the final model, the carrageenan-alone beta activity was the only significant predictor of thermal sensitivity (carrageenan-alone, $\beta = .86$, $p = .028$; thermal, $\beta = .12$, $p = .627$; poke, $\beta = .32$, $p = .263$) and positively correlated with the paw withdrawal latencies (Figure 3-50A-C).

![Figure 3-50: Right CeA beta oscillations correlated with PWLs of the non-injured left paw at 48 – 72 hours](image)

The positive correlations (A and B) indicate that the carrageenan-alone and poke-induced beta oscillations decreased with increasing thermal sensitivity, while the negative correlation (C) suggests that thermal-induced beta activity increased with thermal sensitivity. Only the carrageenan-alone beta activity was a significant predictor of thermal sensitivity ($p < .05$).

The results of the multiple regression analysis examining the relationship between beta activity and thermal sensitivity of the injured right paw at 48-72 hours revealed that the poke and thermal-induced right CeA beta activity accounted for 80.0% of the variance in the withdrawal latencies and the effect was significant, adjusted $R^2 = .80$; $F (2, 3) = 11.01$, $p = .042$. The addition of carrageenan-alone beta activity to the model explained only 4.2% of additional variance in the withdrawal
latencies and the effect was not significant, $\Delta R^2 = .04$, $\Delta F(1, 2) = 1.09$, $p = .407$. In the final model, none of the three stimuli-associated beta activity was a significant predictor of thermal sensitivity (carrageenan-alone, $\beta = .96$, $p = .407$; thermal, $\beta = .347$, $p = .576$; poke, $\beta = .257$, $p = .719$) and there was variation in the direction of the correlations (Figure 3-51A-C).

Figure 3-51: Right CeA beta oscillations correlated with the withdrawal latencies of the injured right paw at 48 – 72 hours

The positive correlations (A and B) indicate that the carrageenan-alone and poke-induced beta oscillations decreased with increasing thermal sensitivity, while the negative correlation (C) suggests that the thermal-induced beta activity increased with thermal sensitivity. The beta activity associated with all the three stimuli was not a significant predictor of thermal sensitivity ($p > .05$).

For the left CeA group with right paw injury, the carrageenan-alone beta oscillations significantly correlated with the non-injured left paw withdrawal latencies between 48 – 72 hours, $r = .75$, $p = .031$. The hierarchical multiple regression results indicated that the poke and thermal-induced beta activity accounted for 29.9% of the variance in the paw withdrawal latencies and the effect
was not significant, adjusted $R^2 = .30; F (2, 5) = .20, p = .829$. The addition of carrageenan-alone beta activity to the model explained 53.7% of additional variance in the withdrawal latencies but the effect was not significant, $\Delta R^2 = .54, \Delta F (1, 4) = 5.49, p = .079$, and there was a variation in the direction of the correlations (Figure 3-52A-C).

Figure 3-52: Left CeA beta oscillations correlated with the non-injured left paw withdrawal latencies at 48-72 hrs

The correlation between beta oscillations and the paw withdrawal latencies was strongest and more linear in carrageenan-alone condition (A), compared to the poke (B) and thermal (C) stimulation conditions. The beta oscillations associated with all the three stimuli were not significant predictors of peripheral thermal sensitivity ($p > .05$).

3.5.4 **Low Gamma Oscillations and Thermosensitivity**

For the right CeA group with right paw injury, the low gamma oscillations correlated with the thermal withdrawal latencies of the left paw at 1 ($r = -.85, p = .031$), 3 ($r = -.96, p = .001$), and 48 – 72 ($r = .82, p = .044$) hours; and withdrawal latencies of the injured right paw at 3 ($r = -.84, p = .036$) hours. The hierarchical
multiple regression analysis, did not reveal significant results for the poke and thermal-induced low gamma activity at hour 1, adjusted $R^2 = .23$; $F(2, 3) = .54, p = .632$. The addition of the carrageenan-alone low gamma activity to the model did not have a significant effect, adjusted $\Delta R^2 = .63; \Delta F(1, 2) = 11.74, p = .076$. Thus the right CeA low gamma oscillatory activity was not a significant predictor of thermal sensitivity in all the three conditions at 1 hour after the carrageenan injection (A-C).

![Graph](image)

Figure 3-53: Right CeA low-gamma oscillations correlated with the left paw withdrawal latencies at hour 1

The correlation between low gamma oscillations and the paw withdrawal latencies at 1 hour after the carrageenan injection was strongest before stimulation (A), compared to the poke (B) and thermal (C) stimulation conditions. In all the three conditions, the low gamma activity was not a significant predictor of thermal sensitivity ($p > .05$).

The hierarchical multiple regression analysis results indicated that taken together the poke and thermal-induced low-gamma oscillations at hour 3, accounted for 85.9% of the variance in the withdrawal latencies of the non-injured left paw and the effect was significant, adjusted $R^2 = .86; F (2, 3) = 16.24, p = .025$. The poke-
induced low-gamma activity was the significant predictor of thermal sensitivity in the first model (poke, $\beta = -0.79, p = 0.018$; thermal, $\beta = -0.52, p = 0.053$). The addition of carrageenan-alone low gamma activity to the model only explained 5.9% of additional variance in the withdrawal latencies, $\Delta R^2 = 0.06$, $\Delta F (1, 2) = 4.54$, $p = 0.167$ and the activity associated with all the three conditions did not significantly predict thermal sensitivity in the final model (carrageenan-alone, $\beta = -0.90, p = 0.167$; poke, $\beta = -0.02, p = 0.972$; thermal, $\beta = -0.23, p = 0.329$). Thus the poke-induced low-gamma activity was the only significant predictor of the left paw withdrawal latencies and the effect was only observed in the first model. The correlation was negative, which suggests that the low gamma activity increased with thermal sensitivity (Figure 3-54A-C).

![Figure 3-54: Right CeA low-gamma oscillations correlated with the non-injured left paw withdrawal latencies at hour 3](image)

The right CeA low gamma oscillations were negatively related with the non-injured paw withdrawal latencies. The low gamma activity increased with thermal sensitivity (A-C) but the poke-induced activity was the only significant predictor of peripheral thermal sensitivity ($p < 0.05$).
The hierarchical multiple regression model examining the relationship between the right CeA low gamma oscillations and thermal sensitivity of the injured right paw at hour 3, revealed that taken together the oscillations induced by the poke and thermal stimulation accounted for 19.6% of the variance in the withdrawal latencies and the effect was not significant, adjusted $R^2 = .20$; $F (2, 3) = 1.61$, $p = .335$. The addition of carrageenan-alone low gamma activity to the model explained 46.4% of additional variance in the withdrawal latencies after controlling for the poke and thermal induced low gamma activity, $\Delta R^2 = .46$, $\Delta F (1, 2) = 49.47$, $p = .020$. In the final model the carrageenan-alone and thermal induced low-gamma oscillations significantly predicted the right paw withdrawal latencies (carrageenan-alone, $\beta = -1.27$, $p = .020$; thermal, $\beta = .70$, $p = .039$; poke, $\beta = .30$, $p = .233$). The carrageenan-alone and poke-induced low gamma oscillations positively correlated with the paw withdrawal latencies, indicating that they increased with thermal sensitivity, while the thermal induced activity decreased as the sensitivity increased (Figure 3-55A-C).

Figure 3-55: Right CeA low-gamma oscillations correlated with the injured right paw withdrawal latencies at hour 3
The carrageenan-alone (A) and thermal (C) stimuli-induced low gamma oscillations were the significant predictors of peripheral thermal sensitivity ($p < .05$).

For the left CeA group with left paw injury, the low gamma oscillations significantly correlated with the injured left paw withdrawal latencies at hour 5, $r = .98$, $p < .001$. The hierarchical multiple regression analysis results indicated that the poke and thermal-induced low gamma activity accounted for 50.6% of the variance in the withdrawal latencies of the injured left paw, but the effect was not significant, adjusted $R^2 = .51$; $F(2, 4) = 4.08$, $p = .108$. The addition of carrageenan-alone low gamma oscillations to the model explained 30.9% of additional variance in the withdrawal latencies after controlling for the poke and thermal induced low gamma activity, $\Delta R^2 = .31$, $\Delta F(1, 3) = 47.35$, $p = .006$. The low-gamma activity associated with the carrageenan-alone condition was the significant predictor of thermal sensitivity in the final model (carrageenan-alone, $1.20$, $p = .006$; poke, $\beta = .11$, $p = .514$; thermal, $\beta = .22$, $p = .153$). The positive correlation with the paw withdrawal latencies, suggests that the low gamma activity decreased as the thermal sensitivity increased (Figure 3-56A-C).
Figure 3-56: Left CeA low-gamma oscillations correlated with the injured left paw withdrawal latencies at hour 5

The correlation between the low gamma oscillations and the paw withdrawal latencies was strongest and more linear in carrageenan-alone condition (A), compared to poke (B) and thermal (C) stimulation conditions. The carrageenan-alone activity was also a significant predictor of the thermal sensitivity ($p < .05$).

The left CeA low gamma oscillations significantly correlated with the withdrawal latencies of the injured left paw at the 48 - 72 hours after the carrageenan injection, $r = -.80$, $p = .032$. The results of the hierarchical multiple regression analysis revealed that taken together, the mechanical (poke) and thermal-induced left CeA low gamma activity accounted for 87.0% of the variance in the withdrawal latencies, and the effect was significant, adjusted $R^2 = .87$; $F (2, 4) = 20.99$, $p = .008$. Both the poke ($\beta = .58$, $p = .017$) and thermal ($\beta = -.79$, $p = .006$) induced low gamma oscillations were significant predictors of thermal sensitivity in the first model. The addition of carrageenan-alone low gamma activity to the model explained only 1.6% of additional variance in the withdrawal latencies after controlling for the poke and thermal induced low gamma activity, and the effect was not significant $\Delta R^2 = .02$, $\Delta F (1, 3) = .68$, $p = .470$. The poke-induced low-gamma
activity was the significant predictor of thermal sensitivity in the final model (carrageenan-alone, $\beta = -0.38$, $p = 0.470$; poke, $\beta = 0.68$, $p = 0.040$; thermal, $\beta = -1.14$, $p = 0.084$). The direction of the correlation for the poke-induced activity was also different from that of the carrageenan-alone and thermal-induced activity (Figure 3-56A-C).

![Figure 3-57: Left CeA low-gamma oscillations correlated with the injured left paw withdrawal latencies at 48 - 72 hours](image)

The carrageenan-alone (A) and thermal induced (C) negatively correlated, while the poke-induced oscillations positively correlated, with the paw withdrawal latencies. Both the thermal and poke-induced low gamma activity significantly predicted thermal sensitivity, but the poke-induced activity was the only significant predictor in the final hierarchical multiple regression model ($p < 0.05$).
Chapter 4

Discussion

4.1 *Overall Results*

The main purpose of this study was to determine the role of the left and right CeA in the onset and progression of inflammatory pain. In order to accomplish this goal, the study set out to achieve three specific objectives: 1) to identify the differences in the right and left CeA activity during the onset and progression of inflammatory pain; 2) to determine the effect of unilateral lesion of the left and right CeA on the onset and progression of inflammatory pain; and 3) to examine whether the right, but not the left, CeA neural oscillations are positively correlated with, and significantly predict peripheral mechanical, but not thermal, sensitivity. The study found that: 1) there were variations in the oscillatory activity of the right and left CeA as the pain progressed; 2) the right CeA lesions induced early onset, and enhancement of mechanical hypersensitivity; 3) both the left and right CeA neural oscillations strongly correlated with, and predicted thermal and mechanical sensitivity, but there were some inconsistencies in the direction of the correlations. Since this is the first study involving recording of progressive pain-related changes in the CeA oscillatory activity in freely moving animals, the results are discussed in the context of: 1) the known nociceptive mechanisms of the CeA; and 2) the molecular mechanisms involved in the generation of neural oscillations, and their functional significance. Overall, the results revealed evidence of lateralization of CeA activity in some, but not all oscillatory frequency bands and conditions.
4.2 Differential trend of Increase in Delta Oscillatory Activity

As predicted, there was significant increase in the delta oscillations in both the right and left, but the effect only persisted in the right CeA as the pain progressed. Furthermore, the right CeA delta activity increased in response to both the ipsilateral and contralateral injury, and the effect did not significantly differ on the basis of the side of injury. On the other hand, the left CeA delta activity significantly increased in response to the contralateral, but not the ipsilateral, injury. The overall delta activity of the left CeA was also greater in response to the contralateral, compared to the ipsilateral, injury.

This difference in the patterns of the left and right CeA delta activity is consistent with the pain-related responses and changes in the CeA. There is documented evidence indicating that both the left and right CeA receive, and are activated by nociceptive input (Carrasquillo & Gereau, 2007), but persistent pain-induced changes in synaptic plasticity and sensitization occur in the right, but not the left, CeA neurons (Carrasquillo & Gereau, 2008; G. Ji & Neugebauer, 2009). This lateralization of pain-related changes occurs in a time-based manner, which is characterized by initial activation of both the right and left CeA, and a shift to unilateral activation of the right CeA as the pain progresses (Gonçalves & Dickenson, 2012). The finding that the right CeA delta oscillations were induced by both the ipsilateral and contralateral injuries is also consistent with previous evidence indicating that the CeA has bilateral receptive fields (Bird et al., 2005; Neugebauer & Li, 2002a). The left CeA mainly responds to contralateral, while the right CeA consistently responds to both ipsilateral and contralateral noxious stimuli (G. Ji &
Neugebauer, 2009). The consistency of the changes in delta activity observed in the current study, with the pain-related responses and mechanisms of the CeA reported in previous studies, suggests that these neural oscillations might be a biomarker of nociceptive activity in the CeA.

Delta oscillations are slow and represent an oscillatory cycle that completes at a rate above 250 ms (Cheng et al., 2008). Although the role of these slow neural oscillations in the modulation of pain is still unclear, research evidence indicates that they increase during emotional arousal and persistent pain (Knyazev, 2012). Delta oscillations are also involved in various processes including attention, learning, consolidation memories, and synaptic down scaling (Steenland, Wu, Fukushima, Kida, & Zhuo, 2010). Since the CeA is the main output nucleus of the amygdala, and a substrate for the integration of the sensory and emotional dimensions of pain (Ji et al., 2010; Jiang, Fang, Kong, Jin, et al., 2014b), the changes in delta activity observed in the current study could be an amalgamation of the neuronal mechanisms involved in the processing of pain-related attention, memories, and emotional arousal.

Interestingly however, in almost all the experimental conditions there were no significant time-dependent changes in the sensitivity of delta oscillations to mechanical and thermal stimulation of the injured and non-injured paws. The lack of significant changes in both the left and right CeA delta oscillations during thermal and mechanical stimulation could be attributed to emotion-induced disruption of the CeA neuronal activity. Previous evidence indicates that there is an overlap in the CeA neural circuitry involved in modulation of pain, emotions, and other
processes (Manning & Mayer, 1995). Thus, the emotional arousal induced by thermal and mechanical stimulation can interfere with the pain-related responses of the CeA. Furthermore, noxious stimuli-induced emotional arousal can activate the BLA-mediated inhibition of the CeA activity via the GABAergic ITC neurons (Rea et al., 2009) and subsequently interfere with the mechanisms involved in the generation of neural oscillations. Thus, the absence of significant changes in delta activity during peripheral thermal and mechanical stimulation could be a result of the emotion-induced disruption of the CeA mechanisms involved in the generation of these oscillations.

4.3 Lateralized Decrease in Alpha Oscillations

Contrary to prediction, there was significant decrease in the right, but not left, CeA alpha oscillations as the pain progressed. Furthermore, mechanical and thermal stimulation induced an overall decrease in alpha oscillations in both the left and right CeA, but this effect was also significant in the right, but not left, CeA. The decrease in alpha activity in response to nociceptive stimuli is consistent with previous evidence indicating that in almost every sensory system, alpha oscillations increase during sensory disengagement or in absence of sensory input (Buzsáki, 2006). This finding is further supported by results of previous studies indicating pain-induced decrease in alpha oscillations in different brain regions (Backonja et al., 1991; P. Chang & Arendt-Nielsen, 2003; P. F. Chang, Arendt-Nielsen, & Chen, 2005; A. C. N. Chen, 2001; W. Peng, Hu, Zhang, & Hu, 2014; Schulz, Tiemann, Witkovsky, Schmidt, & Ploner, 2012). Overall, there was a trend of decreased alpha
activity in all the experimental conditions of the current study, which suggests that suppression of these oscillations is the most likely nociceptive response in the CeA.

4.4  **Theta Oscillations Decreased during Thermal Stimulation**

Contrary to prediction, inflammatory pain did not induce significant time-based changes in both the right and left CeA theta oscillations. The significant effects observed in the study, included an increase in the right CeA theta activity of the non-lesion group at 1 hour after the carrageenan injection, and lesion group during mechanical stimulation of the non-injured left paw at 3 hours. There was a consistent trend of decreased theta activity during thermal stimulation, which was significant in the right CeA at 1 and 5 hours, and in the left CeA between 48 – 72 hours after the carrageenan injection. The increase in the right CeA theta activity is consistent with previous evidence indicating that pain-related emotional responses increase theta oscillatory power in the amygdala (Jeon et al., 2010; Paré & Collins, 2000; Sangha et al., 2009). In the amygdala, theta oscillations are involved in retrieval of memories associated with noxious stimuli (Seidenbecher, Laxmi, Stork, & Pape, 2003) and amygdala neurons generate these oscillations during noxious stimuli-induced emotional arousal (Paré & Collins, 2000; Seidenbecher et al., 2003). Furthermore, the pain-induced changes in synaptic plasticity and sensitization in the CeA positively correlate with the emotional dimension of pain (Marcello et al., 2013; Tappe-Theodor et al., 2011). Thus, both the emotional and sensory dimensions of pain would be expected to increase theta oscillations in the CeA, but due to unclear mechanisms there was a significant decrease in activity during thermal stimulation.
One of the possible explanations for the decrease in the CeA theta oscillations could be the activation of the endogenous opioidergic system during emotion arousal. Emotion-induced activation of opioid receptors in the CeA suppresses adenylyl cyclase synthesis (Nakamura et al., 2013), which is required in the generation of theta oscillations (H. C. Pape, Narayanan, Smid, Stork, & Seidenbecher, 2005; H. Pape & Pare, 2010). Thus, whereas emotional arousal increases activity in the amygdala, some of the CeA mechanisms such as the activation of the opioidergic system during pain-related emotional arousal can have a suppressive effect on the generation of theta oscillations. This suppressive effect could also be attributed to the emotional arousal-induced inhibition of the activity of the CeA via the BLA-mediated activation of the GABAergic ITC cells.

4.5 *Lateralized Changes in Beta Oscillations*

Contrary to prediction, the right CeA beta oscillations of both the contralateral and ipsilateral injury groups significantly decreased during the first phase of the inflammatory response. On the other hand, the left CeA beta oscillations of the contralateral, but not ipsilateral, injury group significantly decreased during both the first and second phases of the inflammatory response. Mechanical stimulation induced significant changes in the left, but not right, CeA beta oscillations, and these changes were characterized by an increase, and a decrease, in activity during stimulation of the injured left paw at 1 and 5 hours, respectively. Although, there was an overall decrease in both the right and left CeA activity in response to the injury, there was a significant increase in the beta oscillations of the left CeA during thermal stimulation at 48 – 72 hours after the carrageenan injection.
The overall response in both the left and right CeA as the pain progressed was a decrease in beta oscillations, which is consistent with evidence indicating pain-induced suppression of beta oscillations in brain imaging studies (Mouraux, Guérit, & Plaghki, 2003; Schulz et al., 2012). The pattern of the decrease in the left and right CeA beta activity was also consistent with previous evidence indicating that the pain-related changes in the right CeA occur in response to both ipsilateral and contralateral, while those of the left CeA are consistently induced by contralateral, nociceptive stimuli (G. Ji & Neugebauer, 2009). This consistency in the pattern of responses suggests that the changes in the left and right CeA beta oscillations observed in the current study were due to nociceptive activity.

However, there were significant changes in the left CeA beta activity during stimulation of the injured ipsilateral paw, which is inconsistent with the evidence indicating that the left CeA consistently responds to contralateral, but not ipsilateral, stimuli. A possible explanation could be that the stimulation of the injured ipsilateral paw induced some mechanisms of the left CeA involving beta oscillations, but not necessarily evoked by input via the direct nociceptive pathways. On the other hand, there is evidence suggesting that the left CeA also responds to high-threshold nociceptive input from the ipsilateral side of the body (G. Ji & Neugebauer, 2009).

The finding that the decrease in beta oscillations remained in the left, but not the right, CeA as the pain progressed, suggests possible time-based lateralization of the CeA mechanisms associated with these oscillations. Since previous evidence suggests that the left CeA does not undergo pain-related changes in synaptic
plasticity and sensitization, the persistent significant decrease in beta oscillations might be associated with the anti-nociceptive mechanisms of the CeA. An *in vitro* study found that the generation of beta oscillations in the basolateral amygdala involves activation of NMDA receptors (Randall, Whittington, & Cunningham, 2011). NMDA receptors play a major role in inducing pain-related changes in synaptic plasticity and sensitization of the right CeA neurons (Bird et al., 2005; Fu et al., 2008; Han et al., 2006). Thus, the persistent decrease in beta oscillations of the left CeA could be related to the anti-nociceptive properties of the CeA, involving inactivation of these receptors.

Although it is not clear whether the activity of the NMDA receptors is affected by changes in dopamine levels, there is evidence indicating that beta oscillations are also generated as a consequence of a decrease in the levels of dopamine (Mallet et al., 2008). Pain-induced emotions such as fear and anxiety (Watabe et al., 2013) can increase dopamine levels in the CeA (Smith, Geissler, Schallert, & Lee, 2013) and consequently decrease beta activity. The underlying mechanisms are beyond the scope of the current study, but further research could establish whether the changes in dopamine levels and/or the activation of NMDA receptors, are the underlying mechanisms in the generation of pain-related beta oscillations in the CeA.

The increase in beta activity at some of the time-points during both thermal and mechanical stimulation was contrary to expectation, since evidence from the current and previous studies suggests that beta activity decreases during pain. There is however, evidence indicating that beta oscillatory power increases with GABAergic inhibition (O. Jensen et al., 2005), which can occur in the CeA during
emotional arousal (Day, Nebel, Sasse, & Campeau, 2005). The emotional arousal induced by thermal and mechanical stimulation can result in GABAergic inhibition and its associated increase in beta activity. On the other hand, the GABAergic disinhibition which occurs in the CeA during persistent pain (Jiang, Fang, Kong, & Jin, 2014; Ren & Neugebauer, 2010) is a possible underlying mechanism for the decrease in beta oscillations as the pain progressed. However, this GABAergic disinhibition in the CeA is associated with changes in synaptic plasticity and sensitization which are lateralized in the right CeA. Therefore, since the suppression of beta activity was persistent in the left CeA, there might be other unknown mechanisms involved.

4.6 Lateralized Changes in Gamma Oscillations

Contrary to prediction, the low-gamma oscillations of the right CeA significantly decreased at 5 hours after the carrageenan injection, and there were no other notable changes in both the low- and high-gamma activity during the progression of inflammatory pain. On the other hand, there were significant changes in both the low- and high-gamma oscillations of the left CeA. The high- and low-gamma activity in the left CeA respectively increased immediately and at 48 – 72 hours after an ipsilateral carrageenan injection. The high-gamma oscillations significantly decreased in response to a contralateral injection and the effect persisted to 48 – 72 hours. Furthermore, compared to the oscillations recorded without simulation, both the left and right CeA low- and high-gamma oscillations did not significantly change during mechanical stimulation, but the left CeA high gamma oscillations decreased in response to thermal stimulation.
The significant increase in gamma oscillations is consistent with evidence from previous studies indicating that gamma oscillations increase during emotional arousal (Headley & Paré, 2013). The CeA is a substrate for the integration of the sensory and emotional dimensions of pain (Ikeda et al., 2007; Langevin, 2012; Li & Neugebauer, 2004a, 2004b; Neugebauer et al., 2004; Veinante et al., 2013a) and the pain-related emotions can induce an increase in gamma oscillatory power. Interestingly, the increase in the left CeA low-gamma oscillations only occurred in response to the ipsilateral, but not contralateral, injury. Since the left CeA mainly responds to contralateral stimuli, the increase in gamma activity in response to ipsilateral injury may be due to mechanisms, which do not involve direct nociceptive input. However, on the basis of previous evidence (e.g. Ji & Neugebauer, 2009) it could also be a consequence of a high-threshold nociceptive input in the ipsilateral receptive field.

Overall, both the low- and high-gamma activity decreased as the pain progressed, and the effect was consistent in the left, but not the right, CeA. The underlying mechanisms for the generation of gamma oscillations are similar to those involved in the development of pain-related synaptic plasticity and sensitization in the CeA. Under normal conditions, the CeA neurons are under GABA-mediated tonic inhibition (Diaz & Morton, 2014; Ehrlich et al., 2009; Oka et al., 2008). The development of pain-related synaptic plasticity and sensitization requires a decrease in this GABAergic inhibition (Jiang, Fang, Kong, Jin, et al., 2014; Ren & Neugebauer, 2010) and the activation of ionotropic and metabolic glutamate receptors (Ren & Neugebauer, 2010). Similarly the principle determinant of the
generation of oscillatory activity in the gamma frequency range is a decrease in GABA$_A$ receptor activation (McNally, McCarley, McKenna, Yanagawa, & Brown, 2011) and an increase in the activation of ionotropic or metabolic glutamate receptors (Bartos et al., 2007; McNally et al., 2011; Phillips et al., 2012; Randall et al., 2011). The shared underlying mechanisms for the generation of gamma oscillations and the pain-related changes in synaptic plasticity and sensitization of the right CeA neurons, suggests that the significant decrease in both the low and high gamma oscillations of the left CeA might be linked to anti-nociceptive processes.

This observation is consistent with previous evidence indicating that gamma oscillations facilitate changes in synaptic plasticity during learning (Ole Jensen, Kaiser, & Lachaux, 2007; Lu, Jefferys, Toescu, & Vreugdenhil, 2011; Popescu, Popa, & Paré, 2009), whose underlying molecular mechanisms have some similarities with pain-induced synaptic plasticity (R. R. Ji, Kohno, Moore, & Woolf, 2003). Since the right, but not left, CeA neurons undergo pain-related changes in synaptic plasticity and sensitization (Carrasquillo & Gereau, 2007, 2008; Ji & Neugebauer, 2009) gamma oscillations would be expected to increase in the right, but not left, CeA. Although this may account for the consistent significant decrease in left CeA gamma oscillations in the current study, there was no significant increase in the right CeA gamma activity. A possible explanation for this observation could be the dual pro- and anti-nociceptive mechanisms of the CeA which can counterbalance each other, and have a net effect on the extent of the increase or decrease in the neural oscillations generated in the CeA. However, a conclusion on the basis of the current
study would be speculative and thus the exact underlying mechanisms could be determined by future studies.

4.7 Right CeA Lesion-induced Early and Enhanced Mechanical Sensitivity

In the current study, it was hypothesized that the right CeA lesions would eliminate the processes involved in the development of pain-related sensitization, and hence, prevent the onset of mechanical sensitivity. However, contrary to prediction, the right CeA lesions induced an early onset of mechanical hypersensitivity in the injured ipsilateral and contralateral paws, as well as the non-injured ipsilateral paws. For the left CeA lesion group, mechanical hypersensitivity was confined in the injured paws and was only significant at hour 5 in the ipsilateral paw, and started at hour 3 in the contralateral paw.

For the control group, mechanical hypersensitivity started at 3 hours for the injured paws, and 48 - 72 hours for non-injured right paws. Furthermore, mechanical sensitivity of both the injured and non-injured paws of the right, but not left, CeA lesion group with left paw injury was significantly higher than that of the control group with injury in the same receptive field. However, the mechanical hypersensitivity that occurred after injury on the right side of the body was confined in the injured paws and there were no significant group differences.

These results indicate that the right CeA lesions induced early onset and enhancement of mechanical sensitivity. On the other hand, the left CeA lesions demonstrated that when the right CeA is left intact (spared) the early onset and enhancement of mechanical sensitivity, as well as the contralateral hyperalgesia were eliminated. Previous evidence indicates that the pain-related changes such as
the activation of protein kinases, PKA (G. Ji & Neugebauer, 2009) and ERK (Carrasquillo & Gereau, 2008) which are lateralized in the right CeA, play a major role in the development of mechanical hypersensitivity (G. Ji & Neugebauer, 2009). It would thus be expected that lesions made before inducing inflammatory pain, would eliminate these protein kinases and other pro-nociceptive mechanisms of the right CeA, and thus prevent inflammation-induced mechanical hypersensitivity. The finding that the right CeA lesions increased mechanical sensitivity is thus inconsistent with the hypothesis of the current study.

On the other hand, the evidence from previous studies indicates that the CeA plays dual pain-inhibitory (Nandigama & Borszcz, 2003; Neugebauer et al., 2004; Palazzo et al., 2011) and pain-facilitatory roles (G. Ji & Neugebauer, 2008; Neugebauer et al., 2004; Veinante et al., 2013; Zhang & Hammond, 2009). Thus the early onset and enhancement of mechanical sensitivity induced by the lesions, demonstrate that the anti-nociceptive mechanisms of the right CeA play a major role in suppressing the onset of pain sensitivity. This observation is further supported by the finding that the left lesions, which spared the right CeA, were associated with a late onset and less mechanical sensitivity, as well as an absence of contralateral hyperalgesia.

The finding that contralateral hyperalgesia occurred in the rats with right, but not left, CeA lesions, indicates that its underlying mechanisms might also be lateralized. The evidence for possible lateralization of the modulation of the spinal neural circuitry involved in contralateral hyperalgesia is demonstrated by the observation that blocking of ERK activation in the right CeA completely inhibits
hypersensitivity in the contralateral non-injured paw (Carrasquillo & Gereau, 2007; R.-R. Ji, Gereau, Malcangio, & Strichartz, 2009). In the current study, contralateral hyperalgesia occurred in response to the left, but not right, paw injuries, which might suggest that the contralateral input to the right CeA could be more involved in inducing hypersensitivity in the non-injured paw, but a firm conclusion requires further investigation. Overall, the results of the current study demonstrate that the anti-nociceptive properties are lateralized in the right CeA, and have a significant suppressive effect on the onset and progression of mechanical sensitivity. The lesions resulted in a loss of these anti-nociceptive mechanisms of the right CeA, which induced early onset and enhancement of mechanical sensitivity.

4.8 The CeA Lesions Did Not Have Profound Effects on Thermal Sensitivity

Consistent with prediction, the right CeA lesions did not have a profound effect on thermal sensitivity. In the left paw injury condition, thermal hypersensitivity only occurred in the right CeA lesion group, but in all the study conditions, the right paw injuries were associated with thermal hypersensitivity in both the injured and non-injured paws. Furthermore, the onset of the thermal hypersensitivity was at 3 hours in all the conditions, and there were no significant differences between the thermal sensitivity of the rats in the control and lesion groups, except for higher sensitivity in the non-injured left paw of the control compared to the right CeA lesion group at 48 – 72 hours after the carrageenan injection.

The finding that only the right CeA lesions were associated with thermal hypersensitivity in response to a left paw injury, suggests that the right CeA might
be more involved in the modulation of thermal sensitivity. However, some of the previous studies found that the CeA seems to be involved in the modulation of mechanical, but not thermal, sensitivity (Carrasquillo & Gereau, 2007; Neugebauer & Li, 2003). On the other hand, there is evidence suggesting that the two pain modalities might be modulated by different mechanisms in the CeA. For example, microinjection of opioids and a GABA_\text{A} receptor antagonist (bicuculline) into the CeA reduced thermal sensitivity, while the infusion of a GABA_\text{A} receptor agonist (mucinol) increased thermal sensitivity (Rashvand et al., 2014). Furthermore, bilateral NMDA-induced lesions of the CeA eliminated morphine-induced antinociception among rats that were subjected to a tail-flick test (Manning & Mayer, 1995). Whereas this evidence suggests that the CeA might be involved in the modulation of both thermal and mechanical sensitivity, the results of the current and some of previous studies suggest that the CeA is more prominent in the modulation of mechanical sensitivity.

4.9 Correlation between CeA Activity and Pain Sensitivity

In the current study, it was hypothesized that the inflammatory pain-induced changes in the right, but not left, CeA activity would be positively correlated with and significantly predict mechanical, but not thermal, sensitivity. Contrary to prediction, the results revealed that both the right and left CeA neural oscillations strongly correlated with, and significantly predicted both thermal and mechanical sensitivity. Furthermore, even for the same oscillatory frequency band, there were variations in the direction of correlations.
4.9.1 *Delta Oscillations Correlated with Peripheral Thermal and Mechanical Sensitivity*

Contrary to prediction, the right CeA delta oscillations positively and negatively correlated with mechanical sensitivity of the injured left and right paws, respectively. On the other hand, both the right and left CeA delta oscillations positively correlated with thermal sensitivity. Furthermore, the right CeA delta activity was a significant predictor of both thermal and mechanical sensitivity. The positive correlations are consistent with previous research evidence, indicating that delta oscillatory power increases in situations of paying attention to biologically relevant stimuli and cognitive preoccupation (Cahn, Delorme, & Polich, 2013; Hauck, Metzner, Rohlfis, Lorenz, & Engel, 2013; Ishii et al., 2009; Knyazev, 2012). Since the CeA is involved in the modulation of attention to painful stimuli and emotional processing (Davis & Whalen, 2001; Ji et al., 2010) delta oscillatory power is expected to increase with thermal and mechanical sensitivity. The negative correlation with mechanical sensitivity observed in the current study is contrary to expectation, but may be associated with pain-induced disruption of attention and other processes involving delta activity.

4.9.2 *Theta Oscillations Correlated with Thermal, but not Mechanical Sensitivity*

Contrary to prediction, both the left and right CeA theta oscillations negatively correlated with, and significantly predicted thermal, but not mechanical sensitivity. The lack of significant correlation with mechanical sensitivity is inconsistent with previous evidence indicating that the CeA responds to both stimuli but the pain-related changes occur in response to mechanical, but not thermal,
stimuli (Neugebauer & Li, 2003). The negative correlations suggest that an increase in pain sensitivity was associated with a decrease in theta activity, which is also inconsistent with previous evidence indicating that neurons in the amygdala generate theta oscillations during noxious stimuli-induced emotional arousal (Paré & Collins, 2000; Seidenbecher et al., 2003). The pain-induced emotional responses in the CeA would thus be expected to increase theta oscillations as the pain sensitivity increases.

On the other hand, the negative correlation observed in the current study is consistent with previous evidence indicating that theta oscillatory power can decrease as a result of processes similar to the pain-related mechanisms of the CeA. Persistent pain induces GABAergic disinhibition in the CeA (Jiang, Fang, Kong, & Jin, 2014; Ren & Neugebauer, 2010) and the loss of the fast-acting GABA_A receptors mediated inhibition can disrupt the neural mechanisms involved in the generation of theta oscillations, and reduce their power (Leppä et al., 2011). Thus the loss of GABAergic inhibition in the CeA is a probable explanation for the decrease in theta oscillations as the pain sensitivity increased, but further research is needed to identify the underlying mechanisms which were beyond the scope of the current study.

4.9.3 Alpha Oscillations did not Correlate with Pain Sensitivity

Contrary to prediction, the right CeA alpha oscillations did not correlate with both mechanical and thermal sensitivity. These results are inconsistent with previous research evidence indicating that pain induces a decrease in alpha oscillations in various areas of the brain. For example, a brain imaging study found
that alpha oscillations decreased in response to thermal pain and significantly
correlated with pain sensitivity (W. Peng et al., 2014). Alpha oscillations increase
when there is disengagement from environment stimuli and decrease when there is
sensory input or activity (Buzsáki, 2006). Thus, alpha oscillations would be expected
to decrease with increasing pain sensitivity. Further research with larger sample
sizes, could provide evidence in regard to whether alpha oscillations in the CeA
correlate with, and predict pain sensitivity.

4.9.4 Both the Right and Left CeA Beta Oscillations Correlated with Thermal
Sensitivity

Contrary to prediction, both the right and left CeA beta oscillations strongly
correlated with thermal sensitivity. The right CeA activity also correlated with
mechanical sensitivity. However, even for same CeA side and stimulus modality
there were variations in the direction of the correlations between beta oscillations
and pain sensitivity. For example, the right CeA beta oscillations positively
correlated with mechanical sensitivity in all the three conditions and thermal
sensitivity in the carrageenan-alone and poke (mechanical stimulation) conditions.
However, the thermal-induced beta activity correlated positively with thermal
sensitivity at 3 hours, and negatively at 48 – 72 hours.

The negative correlation between beta oscillations and thermal sensitivity is
consistent with evidence of previous studies indicating that these oscillations are
generated as a consequence of a decrease in the levels of dopamine (Mallet et al.,
2008). Pain-induced emotions can increase dopamine levels in the CeA (Smith et al.,
2013) and consequently result in a decrease in beta activity as the pain severity and
sensitivity increase. This assertion is further demonstrated by the finding that the correlation was negative in the conditions where beta activity was a significant predictor of thermal and mechanical sensitivity. However, with the inconsistency in the direction of the correlations observed in the current study, further research is needed to draw firm conclusions about the nature of the relationship between the CeA beta oscillations and peripheral pain sensitivity.

4.9.5 Low Gamma Oscillations Correlated with, and Predicted Mechanical and Thermal Sensitivity

Contrary to prediction, both the right and left CeA low gamma oscillations strongly correlated with both mechanical and thermal sensitivity. The direction of the correlations of both the right and left CeA oscillations with mechanical and thermal sensitivity varied; that is, the correlations were positive and negative at different time-points. The positive correlations between low gamma oscillations and pain sensitivity are consistent with the results of previous studies which indicate that gamma oscillatory power increases with pain intensity (Hauck, Lorenz, & Engel, 2007; W. Peng et al., 2014; Schulz et al., 2012) as well as emotional arousal (Headley & Paré, 2013) which is a characteristic response of rats to noxious stimuli.

The negative correlations are contrary to expectation since the rats with high pain sensitivity would be expected to have more gamma oscillatory power. Gamma oscillations increase during emotional arousal (Headley & Paré, 2013) and the pain-related responses of the CeA include the emotional dimension of pain. Thus the pain-related emotions would be expected to evoke gamma oscillations, which is also consistent with previous research evidence indicating positive correlations
between gamma oscillations and pain sensitivity (W. Peng et al., 2014). The negative correlations observed in the current study are thus contrary to expectation, but may be indicative of some underlying mechanisms since they were observed at the time points during which the inflammatory response was at the highest peak. Furthermore, in conditions where it negatively correlated, the CeA gamma activity was a significant predictor of pain sensitivity. Correlations do not imply a causal link, but the decrease in gamma activity with increasing pain sensitivity may be an indication of possible pain-related mechanisms of the CeA.

The gamma oscillations have been implicated in pain perception (Hauck et al., 2007; Schulz et al., 2012) and in the initiation and execution of movement (Schulz et al., 2012) which are some of the functions modulated by the CeA (Smith et al., 2013). Therefore, since in the current study the determination of both the thermal and mechanical sensitivity involved withdrawal of the paws from the stimuli, the strong correlations and predictive power of the gamma activity might be related to the CeA’s role in the modulation of pain perception as well as other sensorimotor functions.

4.9.6 **High-Gamma Oscillations Correlated with Mechanical Sensitivity**

Consistent with prediction, the right CeA high gamma oscillations positively correlated with mechanical, but not thermal, sensitivity. The positive correlation between the right CeA high gamma oscillations and mechanical sensitivity is consistent with previous research evidence indicating that gamma oscillations increase during emotional arousal (Headley & Paré, 2013) and may thus be
indicative of an increase in pain-related emotions among the rats with more pain sensitivity.

On the other hand, the positive correlation between gamma oscillations and pain sensitivity may also be associated with the sensory dimension of pain, since the underlying mechanisms for the generation of gamma oscillations are similar to those involved in the development of pain-related synaptic plasticity and sensitization in the CeA. The changes in pain-related synaptic plasticity and sensitization of the CeA neurons require a decrease in this GABAergic inhibition (Jiang, Fang, Kong, Jin, et al., 2014; Ren & Neugebauer, 2010) and activation of ionotropic and metabolic glutamate receptors (Ren & Neugebauer, 2010). The same mechanisms, that is, the decrease in GABAergic inhibition (McNally et al., 2011) and activation of glutamate receptors (Bartos et al., 2007; McNally et al., 2011; Phillips et al., 2012; Randall et al., 2011) are required for the generation of gamma oscillations. Thus the increase in gamma oscillations with pain sensitivity could have been a result of the changes in both the emotional and sensory dimensions of pain.

4.10 Limitations and Future Directions

4.10.1 The Effect of Inflammatory Pain on the CeA Oscillatory Activity

This is the first in vivo study, to investigate pain-related changes in neural oscillations, and their lateralization in CeA. There were thus no previous studies to use as a framework of comparison when discussing the observed pain-related changes in the neural oscillations of the CeA. The results were thus discussed in the context of known pain-related changes in CeA activity, and the mechanisms for
generation of neural oscillations and their functional significance in different brain regions.

Furthermore, the study does not provide direct evidence of whether the observed changes occurred in response to the sensory and/or emotional dimensions of pain. LFP oscillations are a summation of the extracellular neuronal activity (R.-K. Cheng et al., 2008) and can increase or decrease in response in different sensory, emotional, cognitive, and other neuronal events (Buzsáki, 2006; Knyazev, 2012; Magri et al., 2012; Pfurtscheller, Neuper, Brunner, & Lopes Da Silva, 2005; Rubino, Robbins, & Hatsopoulos, 2006). Therefore, further research using specific receptor agonists and antagonists could elucidate the specific circumstances involved in the generation of these oscillations as well as their pain-related roles in the CeA.

4.10.2 CeA Lesions and Pain Sensitivity

The current study used electrolytic lesions which destroyed the entire left or right CeA. When carried out at adequate parameters, electrolytic lesions are more restricted to the CeA compared to the excitotoxic lesions which damage neurons in the adjacent amygdala nuclei (Lázaro-Muñoz et al., 2010) but they are nonspecific and damage passage neural pathways and cell bodies (Blankenship, Huckfeldt, Steinmetz, & Steinmetz, 2005). Being the main output nucleus of the amygdala the CeA has multisynaptic projections to various brain sites involved the modulation of emotions and endogenous pain control (Han et al., 2006; Neugebauer et al., 2004; Phelps & LeDoux, 2005) including the basal forebrain, substantia innominata dorsalis, brainstem, and periaqueductal grey (PAG) (Li & Neugebauer, 2004a) and
rostral ventromedial medulla (RVM) (Gebhart, 2004; Han et al., 2006; Palazzo et al., 2011). Thus due to their nonspecific destructive effect, the electrolytic lesions disrupt several processes mediated by the CeA. Therefore, future research could restrict the disruption of function to only the mechanisms of interest, by using selective chemical lesions which target specific receptors, cell bodies, or enzymes of particular CeA neurons.

Furthermore, as indicated in the introduction and discussion the pain-related processes may involve interactions between the BLA and CeA mechanisms. The BLA modulates the CeA activity through direct projections and indirectly through glutamatergic activation of GABAergic intercalated cell masses (ITC) (Pape & Pare, 2010; Ren & Neugebauer, 2010; Tappe-Theodor et al., 2011). A recent study found that lesions that eliminated the BLA and spared the CeA prevented mechanical hypersensitivity (Veinante et al., 2013) while the results of the current and some of the previous studies indicate that lesion of the right CeA that spares the BLA enhances mechanical sensitivity. Therefore, future studies should investigate the interplay between the CeA and BLA as well as the LA mechanisms and the role of this interaction in the modulation of pain sensitivity.

4.10.3 Circadian rhythm and pain sensitivity

In the current study, all the experiments were carried out during the night period of the circadian cycle (5:00 PM – 7:00 AM). A number of studies have found that the circadian rhythm can influence pain sensitivity in both humans and animals (Bruguerolle & Labrecque, 2007; Chang, Smith, Thorpe, Barratt, & Karim, 2010; Mousa, Schiller, & Zucker, 2014) and can even interfere with the effectiveness of
analgesic medications (Kusunose et al., 2010; Minett, Eijkelkamp, & Wood, 2014). Previous evidence indicates that as a result of melatonin-mediated modulation of neurotransmitters the night period is associated with increased sensitivity to inflammatory pain (Liu, He, & Huang, 2014; Perissin et al., 2004). During the night, there is an increase in the concentration of pro-inflammatory mediators such as substance P, which can result in increased activation of nociceptors in rats (Bruguerolle & Labrecque, 2007). It is believed that the circadian rhythm does not only influence pain sensitivity, but can also result in misinterpretation as well as a failure to detect pain-related behavioral responses (Minett et al., 2014). Since all the data in the current study were collected during the night period, the generalization of the results would require further investigation involving daytime behavioral testing. Future studies could include both phases of the circadian cycle to assess the whether there are diurnal variations in the CeA-mediated pain sensitivity.

4.11 Conclusion

In conclusion, the results of the current study provide the first in vivo evidence of pain-related changes in the neural oscillations of the CeA during the progression of inflammatory pain. The pattern of the right and left CeA oscillatory responses to inflammatory pain was consistent with the known pain-related CeA activity. For example: 1) the right CeA delta and beta oscillations, respectively increased and decreased in response to both ipsilateral and contralateral, while the left CeA oscillations consistently changed in response to contralateral injury; 2) there were initial pain-induced oscillatory responses in both the left and right CeA, followed by lateralized responses as the pain progressed. In addition, the right, but
not left, CeA lesions induced early onset and enhancement of mechanical sensitivity, but had a lesser effect on thermal sensitivity. The right and left CeA neural oscillations strongly correlated with, and were significant predictors of peripheral thermal and mechanical sensitivity, but the observations were more frequent for the right CeA. The consistency of the results of the current in vivo study with the evidence from previous in vitro studies accentuates the central role of the CeA in pain modulation, and lateralization of pain-related processes. Future in vivo studies should investigate the specific underlying mechanisms for generation of pain-related oscillations in the CeA, with special emphasis on the possible variations in response to the sensory and emotional dimensions of pain.
Appendix A

Tables
Table A-1: Right CeA oscillations correlated with mechanical and Thermal sensitivity associated with left paw injury

<table>
<thead>
<tr>
<th>Oscillatory Activity</th>
<th>Mechanical sensitivity (PWT) (N = 7)</th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left Paw (injured)</td>
<td>Right Paw</td>
<td>Baseline</td>
<td>HR 1</td>
<td>HR 3</td>
<td>HR 5</td>
<td>HR 48-72</td>
<td>Baseline</td>
<td>HR 1</td>
<td>HR 3</td>
<td>HR 5</td>
<td>HR 48-72</td>
</tr>
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<td>Delta</td>
<td>Pearson Correlation</td>
<td>Sig. (2-tailed)</td>
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<td>-0.047</td>
<td>-0.863*</td>
<td>0.328</td>
<td>0.670</td>
<td>0.180</td>
<td>0.219</td>
<td>-0.593</td>
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<td>-0.307</td>
<td>-0.560</td>
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<th>HR 1</th>
<th>HR 3</th>
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** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Table A-2: Right CeA oscillations correlated with mechanical and thermal sensitivity associated with right paw injury

<table>
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<tr>
<th>Oscillatory Activity</th>
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<th>Right Paw (injured)</th>
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<tbody>
<tr>
<td></td>
<td>Left Paw</td>
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<td>Sig. (2-tailed)</td>
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<td>.746</td>
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<th>Right Paw (injured)</th>
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<td>Left Paw</td>
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** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Table A-3: Left CeA oscillations correlated with mechanical and thermal sensitivity associated with left paw injury

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<th>Oscillatory Activity</th>
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<td>Right Paw</td>
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<td>Pearson Correlation</td>
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<td>Theta</td>
<td>Pearson Correlation</td>
<td>-.408</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.364</td>
</tr>
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<td>Pearson Correlation</td>
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<td>Pearson Correlation</td>
<td>-.407</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.364</td>
</tr>
<tr>
<td>Low Gamma</td>
<td>Pearson Correlation</td>
<td>.540</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.456</td>
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</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.938</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).**

*Correlation is significant at the 0.05 level (2-tailed).
Table A-4: Left CeA oscillations correlated with mechanical and thermal sensitivity associated with right paw injury

<table>
<thead>
<tr>
<th>Oscillatory Activity</th>
<th>Mechanical sensitivity (PWT) ($N = 8$)</th>
<th>Thermal sensitivity (PWL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Left Paw</strong></td>
<td><strong>Right Paw (injured)</strong></td>
</tr>
<tr>
<td></td>
<td>Baseline HR 1 HR 3 HR 5 HR 48-72</td>
<td>Baseline HR 1 HR 3 HR 5 HR 48-72</td>
</tr>
<tr>
<td>Delta</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
</tr>
<tr>
<td></td>
<td>-.324 -.261 -.308 .208 .171 -.324  -.101 -.323 .171 .337*</td>
<td>-.088 -.233 -.564 .243 .208 -.104 .221 -.156 -.144</td>
</tr>
<tr>
<td>Theta</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
</tr>
<tr>
<td></td>
<td>.260 .079 .172 .222 -.167 .260 .016 .370 -.013 -.129</td>
<td>-.114 .322 .356 -.533 -.370 .439 -.056 .075 -.288 -.331</td>
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<tr>
<td>Alpha</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
</tr>
<tr>
<td></td>
<td>.157 .365 .331 .343 -.091 .157 .097 .033 -.149 -.137</td>
<td>-.417 .342 .266 -.211 .361 -.417 .410 .598 .501 .491</td>
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<tr>
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<td>Pearson Correlation Sig. (2-tailed)</td>
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<tr>
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<td>.439 .272 .356 -.533 -.370 .439 -.056 .075 -.288 -.331</td>
<td>-.114 .322 .356 -.564 .243 .208 -.104 .221 -.156 -.144</td>
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<tr>
<td>Low Gamma</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
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<td>.208 -.088 -.233 -.564 .243 .208 -.104 .221 -.156 -.144</td>
<td>.304 .407 .492 .617 .380 .304 .314 .117 .206 .217</td>
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<td>Pearson Correlation Sig. (2-tailed)</td>
<td>Pearson Correlation Sig. (2-tailed)</td>
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<tr>
<td></td>
<td>-.417 .342 .266 -.211 .361 -.417 .410 .598 .501 .491</td>
<td>-.114 .322 .356 -.564 .243 .208 -.104 .221 -.156 -.144</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).**

**Correlation is significant at the 0.05 level (2-tailed).**


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Biographical Information

Mayanja M. Kajumba earned his Bachelor of Science degree, and a Master of Science in Clinical Psychology degree at Makerere University, Uganda. He was awarded a Fulbright Grant by the United States Department of State to pursue a PhD in Experimental Psychology (Health and Neuroscience, major) at the University of Texas, Arlington. During the first two years of his PhD program he investigated the effect of analgesic medications on pain, symptom distress, and quality of life of lung cancer patients. He also investigated the effect of electrical stimulation of the central nucleus of the amygdala (CeA) on the spinal cord neuronal responses to peripheral pain, as well as the pain-induced changes in the local field potential activity of the CeA. His research was presented at various international conferences, including the Society for Neuroscience annual meetings. His dissertation project was the first in vivo study to investigate the pain-related changes in the CeA neural oscillations, as a basis for demonstrating lateralization of pro- and anti-nociceptive properties. He is a lecturer at Makerere University, and intends to continue investigating the neurophysiology of pain conditions using pharmacological and electrophysiological procedures.