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Psychoneuroimmunology and Chronic Pain

Sarah E. Giron
University of San Diego

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UNIVERSITY OF SAN DIEGO

Hahn School of Nursing and Health Science

DOCTOR OF PHILOSOPHY IN NURSING

PSYCHONEUROIMMUNOLOGY AND CHRONIC PAIN

by

Sarah E. Giron, PhD(c), CRNA

A dissertation presented to the

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DOCTOR OF PHILOSOPHY IN NURSING

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Dissertation Committee:

Joseph F. Burkard, DNSc, CRNA, Chair

Jane Georges, PhD, RN

Charles A. Griffis, PhD, CRNA

UNIVERSITY OF SAN DIEGO

Hahn School of Nursing and Health Science

DOCTOR OF PHILOSOPHY IN NURSING

CANDIDATE'S
NAME:

Sarah E. Giron

TITLE OF
DISSERTATION:

Psychoneuroimmunology and Chronic Pain

DISSERTATION
COMMITTEE:

Joseph F. Burkard, DNSc, CRNA

Jane Georges, PhD, RN

Charles A. Griffis, PhD, CRNA

Abstract

There is increasing evidence linking chronic pain to altered levels of central nervous system (CNS) inflammation and increased levels of perceived pain, anxiety, depression and sleep disturbance. However the inflammatory molecules responsible for physiologic and psychological components of chronic pain still warrant identification and exploration. Using central inflammation as a paramount factor in the creation and maintenance of chronic pain, this study aimed to investigate and describe the physical and psychological aspects of chronic pain associated with CNS inflammation.

Using a cross-sectional correlational design, cerebrospinal fluid (CSF) inflammatory cytokine patterns present in 8 chronic pain participants were compared to inflammatory cytokine patterns present in 30 control CSF samples using multivariate analysis of variance (MANOVA), with analysis of variance (ANOVA) analysis as a follow-up. Levels of depression, anxiety, sleep disturbance and pain were measured in approximately 8 chronic pain patients and correlated to CSF levels of inflammatory cytokines using Pearson's r , or Spearman's Rho correlations. Demographic information was also explored for relationships to central inflammation and descriptive statistics were examined for responses.

To our knowledge, this is the first study to describe increased CSF levels of Interleukin-8 (IL-8) in a population of majority Failed Back Surgery Syndrome chronic pain patients ($F(1, 36) = 14.89, p < 0.001, \text{partial } \eta^2 = 0.293$). Gender ($F(1,6) = 7.782, p = 0.032, \eta^2 = 0.565$), socioeconomic status ($r = -0.823, p = 0.012$) and educational level ($r = 0.727, p = 0.041$) were also correlated with central levels of inflammation, indicating that central physiologic changes may be related to host sex and psychosocial factors. All

participants reported poor sleep quality and took at least one opioid medication, indicating that sleep and opioids may scaffold a portion of the chronic pain paradigm.

This study richly describes the dynamic experience of chronic pain through the lens of Psychoneuroimmunology. Incorporating both physiologic and psychological aspects of the disease, this study describes an association between chronic pain, central inflammation, gender, socioeconomic status, opioid medications and poor sleep quality. Thus, the future of pain treatment must consider these aspects when treating patients and look to future studies for possible new treatment options which target these factors.

Keywords: inflammation, cytokines, chronic pain, sleep quality, gender, socioeconomic status

Dedication

Magic

Sandra's seen a leprechaun,
Eddie touched a troll,
Laurie danced with witches once,
Charlie found some goblins' gold.
Donald heard a mermaid sing,
Susy spied an elf,
But all the magic I have known
I've had to make myself.

--Shel Silverstein in

Where the Sidewalk Ends: The Poems and Drawings of Shel Silverstein

To those who have taught me how to make magic and have shown me what true magic is. To Dr. Dagny Bloland who taught me that in stillness, I did receive. To Dr. Charles Griffis who has pulled more rabbits out of a hat for me than humanly possible. To Philip for putting me back together again after I had been sawed in half, and for making everyday together true magic. And to the most-supreme magic maker in my life, my Mother: You readied me with the most amazing cape and wand, thank you for teaching me your brand of magic.

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Chapter 1: PROBLEM

Significance

Chronic pain is a health concern of tremendous scope. An estimated 116 million Americans suffer from, and are disabled by this chronic illness (Tsang et al., 2008) at a cost of \$630 billion in lost work revenue, sick time and health care costs (Gaskin & Richard, 2011). Most important, is the cost in quality of life that the patient pays. Patients report not only a wide spectrum of painful symptoms, but also suffer the negative psychological effects of constant pain. Current modalities utilized to treat these patients both physically and psychologically are limited, and are often ineffective for a large portion of the chronic pain population. Recent investigations reported in chronic pain literature have revealed a new direction for therapeutic advancement: central nervous system (CNS) inflammatory mechanisms that are believed to exacerbate and prolong neuropathic pain states (Kreutzberg, 1996; Reeve, Patel, Fox, Walker, & Urban, 2000; Samad, Wang, Broom, & Woolf, 2004; Watkins & Maier, 2003; Watkins, Milligan, & Maier, 2001) also influence anxiety (Baker et al., 2001; Camara et al. 2015), depression (Bonne et al., 2011; Levine et al., 1999; Lindqvist et al., 2009) and sleep disturbances (Dauvilliers et al., 2014). Though preclinical data is accumulating, there remains a dearth of human data in this rapidly developing area of investigation which links central inflammation, chronic pain and the psychology of pain.

This study investigated the emerging relationships between chronic pain syndromes and the role of central inflammation, through examination of cerebrospinal fluid (CSF) patterns of inflammatory mediator molecules. More specifically, this study

investigated whether human CNS levels of pro- and anti-inflammatory cytokines were similar to those described in chronic pain-induced animal studies (Inoue et al., 1999; Morioka et al., 2002; Raghavendra, Tanga, & DeLeo 2004; Song & Zhao, 2001). This study also explored the relationships between central inflammation and the psychological responses to the chronic pain experience (pain perception, anxiety, depression and sleep quality). This was accomplished using the Short-Form McGill Pain Questionnaire (Melzack, 1975; Melzack, 1987), by scoring on the Hamilton Anxiety Rating Scale (Hamilton, 1959), Beck Depression Inventory-II (Beck, Ward, Mendeson, Mock & Arbough, 1961) and the Pittsburgh Sleep Quality Index (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). Keeping central inflammation paramount to the chronic pain experience, this study examined the relationship of central inflammation to chronic pain and the psychology that accompanies chronic pain.

This exploration of psychological and physiologic interaction was supported by a discipline that links the mind and body known as Psychoneuroimmunology. As a field of study, psychoneuroimmunology explores the interactions between behavioral, neural, endocrine and immune processes. Psychoneuroimmunology is a rapidly growing specialty within psychology tasked with exploring the adaptive processes of physiological and behavioral responses to physical and psychological environmental stressors (Ader, 2000). Because there is a scarcity of human evidence supporting the link between physical and psychological pain, and central levels of inflammatory mediator molecules associated with these constructs, this study provided evidence of this multimodal relationship utilizing psychoneuroimmunology as a foundational research

paradigm.

Increased knowledge of the relationships between the psychoneuroimmune responses to prolonged pain states propagated by central inflammatory mechanisms, will lead to information pertinent to more effective analgesic and psychological treatment of these patients. It was the goal of this study to explore and describe the psychoneuroimmune relationship of central inflammation, chronic pain, anxiety, depression and sleep disturbance in the chronic pain population.

Purpose and Specific Aims

The purpose of this study was to examine and characterize the relationships between the physical and psychological components of chronic pain and inflammatory mediator molecules, more specifically cytokines called interleukins (ILs) and tumor necrosis factor-alpha (TNF- α). Specific aims of this study include the following:

- 1) Investigation of altered CSF levels of pro-inflammatory cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF- α) in patients with chronic pain as compared to control samples;
- 2) Investigation of alterations in levels of anti-inflammatory interleukin-10 (IL-10) in chronic pain patients as compared to control samples of CSF.

This study also aimed to explore the relationship between central inflammation and the psychological responses to chronic pain as evidenced by results gathered from the following psychological tests:

- 1) The experience of chronic pain as measured by the Short Form McGill Pain

Questionnaire (SF-MPQ);

- 2) Pain-related anxiety levels as measured by scores on the Hamilton Anxiety Rating Scale (HAM-A);
- 3) The presence or absence of depression as measured by the Beck Depression Inventory-II (BDI-II);
- 4) Sleep disturbances in chronic pain patients as measured by the Pittsburgh Sleep Quality Index (PSQI).

The secondary aims of this study included assessing for relationships between the participant demographics, type of chronic pain diagnosis, length of diagnosis, type(s) of medication(s) used to treat chronic pain and length of time taking those medication(s) to levels of central inflammation.

Hypotheses

1. Patients with chronic pain syndromes will have elevated CSF levels of pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF- α as detected with Multiplex assay analysis when compared to control samples of CSF.
2. Patients with chronic pain syndromes will have decreased CSF levels of anti-inflammatory cytokine IL-10 as detected with Multiplex assay analysis when compared to control samples of CSF.
3. There will be a positive correlation between the severity of perceived pain as measured by the Short Form-McGill Pain Questionnaire (SF-MPQ), anxiety as measured by scores on the Hamilton Anxiety Rating Scale (HAM-A), extent of depression as measured by the Beck Depression Inventory-II (BDI-

II), and sleep disturbance as measured by the Pittsburgh Sleep Quality Index (PSQI), and levels of pro-inflammatory cytokines in the CSF.

4. There will be an inverse correlation between the severity of perceived pain as measured by the Short Form-McGill Pain Questionnaire (SF-MPQ), anxiety as measured by scores on the Hamilton Anxiety Rating Scale (HAM-A), degrees of depression as measured by the Beck Depression Inventory-II (BDI-II), and sleep disturbance as measured by the Pittsburgh Sleep Quality Index (PSQI), and levels of anti-inflammatory IL-10 in CSF.
5. Prolonged length of diagnosis, opioid medications used to treat chronic pain and prolonged length of time taking medication(s) will positively correlate with increased levels of pro-inflammatory cytokines in the CSF of chronic pain patients.
6. There will be an inverse relationship between central levels of anti-inflammatory IL-10 and prolonged length of diagnosis, opioid medications used to treat chronic pain and prolonged length of time taking medication(s).

Theoretical Perspective

For the purpose of this study, the presence of chronic pain was held as the paramount stressor that was integrated into an amalgam of biological and behavioral factors influencing the overall health and disease status of the patient. Pain is defined by the International Association of the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey & Bogduk, 1994). Given that the definition of pain by

IASP incorporates sensory, emotional and biologic components, the Psychoneuroimmunology (PNI) theoretical framework was adopted as a basis for this study because of its multifaceted approach to the health outcome of a patient. More specifically, McCain and colleagues' generic theoretical framework of PNI was adapted and utilized to help guide this study (Figure 1) (McCain, Gray, Walter, & Robins, 2005).

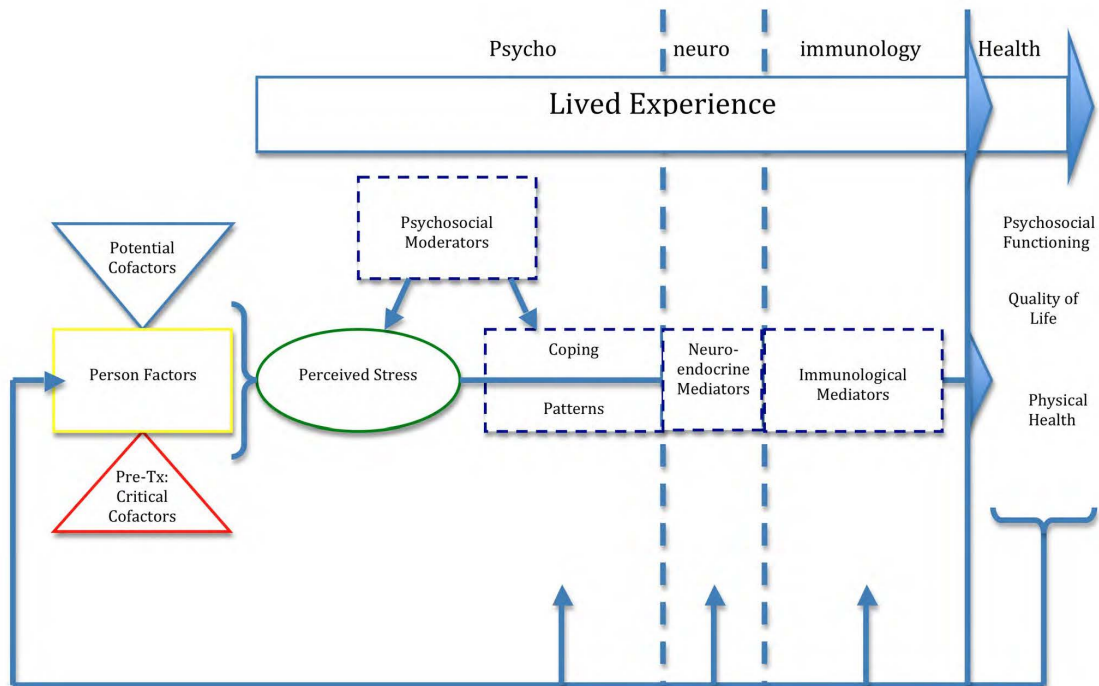


Figure 1. The Psychoneuroimmunology Theoretical Framework adapted from McCain, Gray, Walter, & Robins, 2005, p. 13.

Their framework identifies the patterns, processes and consequences of stress and coping as they relate to the multidimensional psychobehavioral-neuroendocrine-immune system interactions that relate to health dynamics (McCain et al., 2005). The PNI

framework scaffolds this investigation by defining the association between the mind and body. As a framework, it merges psychological variables such as anxiety and depression with the perceived stress of pain and relates the neuroendocrine mediators that influence sleep quality and pain levels as it applies to the chronic pain condition. As a framework, it also correlates the measurable immunological inflammatory markers in the chronic pain study population. Applied to this study, pain and symptoms of depression, anxiety and sleep deprivation can thus be compared to the central biological markers of inflammation associated with chronic pain in a cross-sectional study process (see Figure 2).

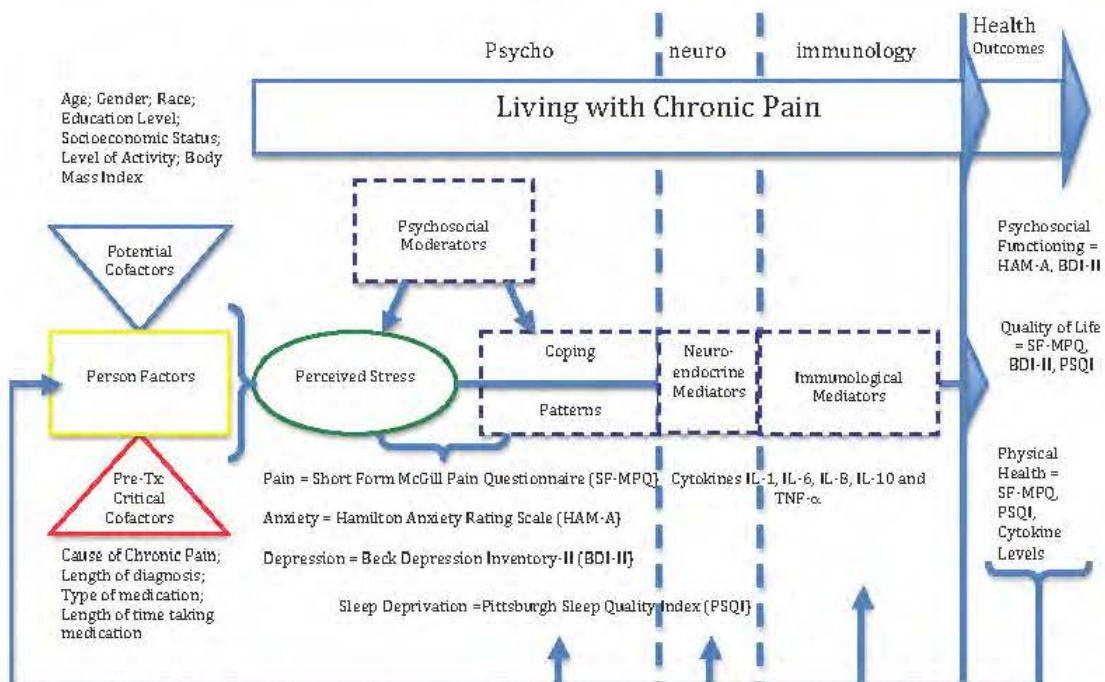


Figure 2. The Psychoneuroimmunology Theoretical Framework applied to the study *Psychoneuroimmunology and Chronic Pain*. Adapted from McCain, Gray, Walter, & Robins, 2005, p. 13.

Within the PNI theoretical perspective, chronic pain is approached as a multifaceted dynamic condition with many involved factors including psychological, neuroendocrine and immunologic components. When considering the factors of pain, anxiety, depression, sleep deprivation, and altered levels of inflammatory biomarkers in the presence of chronic pain, causal pathways are unclear, but these factors have been found to contribute to, or be associated with the chronic pain process in both humans and animals (Baker et al., 2001; Dworkin & Gitlin, 1991; Heffner, France, Trost, Ng, & Pigeon, 2011; Meller, Dykstra, Grzybycki, Murphy, & Gebhart, 1994). The PNI theoretical framework by McCain et al. (2005) acknowledges these relationships and makes them explicit in an iterative, fluid continuum. Use of the PNI theoretical framework thus provides this study a mechanism to bring these multidisciplinary components together in a more inclusive view of the chronic pain process rather than detail the complex physiological and pathophysiological interactions as independent underlying processes.

Psychoneuroimmunology as a paradigm in nursing research supports the premise that there is bidirectional neuroendocrine-immune system interaction (Rabin, Choen, Ganguli, Lysle, & Cunnick, 1989) that justifies psychobehavioral nursing interventions designed to address stress and coping, considering an associated physiological basis (McCain et al., 2005). Psychoneuroimmunology combines the Lazarus and Folkman (1984) cognitive-transactional model of stress with psychology, behavioral, and pathophysiologic processes of the patient's health status. The mind-body interaction (Zeller, McCain & Swanson, 1996) inferred in PNI has been shown in animal and human

models in a myriad of multidisciplinary studies: From the inception of the concept of PNI (Solomon & Moos, 1964) to the work that demonstrated behavioral conditioning's influence on the immune system (Ader & Cohen, 1975), PNI is still a relatively new theory in nursing research. However, it is one that incorporates a more holistic view of specific stressors and the effect on the patient's health spectrum.

Within the PNI framework described by McCain and colleagues (2005), the identified cofactors are those components that have the potential to predispose an individual to certain stress, coping and health patterns (potential cofactors) or the health related features of one's life (pretreatment critical cofactors). The potential demographic cofactors identified in this study were: the patients' age, gender, race, education level, socioeconomic status (SES), level of activity as measured by metabolic equivalents (METS), and body mass index (BMI). The pretreatment critical cofactors identified included: the cause or diagnosis of chronic pain, the length of diagnosis, type(s) of medication(s) utilized to treat the chronic pain symptoms, and the length of time taking medication(s) for chronic pain relief. The psychological --"psycho"-- component of the psychoneuroimmunology model includes sociobehavioral components such as negative affect and distress (McCain et al., 2005). The perceived stress of chronic pain and the effectiveness of the patients coping patterns will be measured by their experience of pain through the Short Form-McGill Pain Questionnaire, their level of anxiety via the Hamilton Anxiety Rating Scale, the extent of depression related to the chronic pain symptoms by the Beck Depression Inventory-II, and their sleep quality via the Pittsburgh Sleep Quality Index. The neurological --"neuro"-- and "immunology" components of the

McCain PNI theoretical framework focus on the physiological responses to psychosocial stressors, the effect of stress on the immune system, and the interrelationship between the neuroendocrine and immune system (McCain et al., 2005). The physiologic responses to the stressor of chronic pain (which amalgamates the effect of stress on the immune system and the interrelationship between the neuroendocrine and immune system) will be measured through patterns of CNS inflammatory cytokines.

Seminal literature demonstrates there is a central neurological component related to the biomarkers measured in this study with the initiation, maintenance and quality of sleep (Fontana, Kristensen, Dubs, Gemsa, & Weber, 1982; Ishimori, 1909; Pappenheimer, Miller, & Goodrich, 1967). Within the PNI framework, and because of the fluid, interrelated interaction of the components within this model, sleep (as measured by the Pittsburgh Sleep Quality Index), will also be regarded within the neuroendocrine function of this framework.

The biological markers investigated by this study will be patterns of biomarkers called cytokines, more specifically IL-1, IL-6, IL-8, IL-10 and TNF- α . These biomarkers are released by both peripheral and central immune cells in response to a wide array of physiologic insults, however nociceptive central sensitization common in chronic pain models has been associated with the release of these biomarkers specific *only* to CNS cells called glia (Zhang et al., 2008). Thus, the PNI framework correctly identifies these CNS cytokines in the neurological --“neuro”-- categorical point of reference within the PNI framework for this specific study. Once glia release inflammatory biomarkers, it is known that these molecules contribute a wide array of immunologic functions. The PNI

framework also correctly maintains these biomarkers as neuroimmune mediators properly categorized under the “neuroimmunology” component of the theoretical framework. The neuroimmune mediators will be measured in this study using Multiplex assay analysis to assess levels of cytokines within the CSF of patients, presumably reflecting inter-related central neuronal and immunological functions. Through triangulation of the various results, this study yields novel information about relationships between chronic pain, the perception of pain, anxiety, depression, sleep deprivation and central biomarker mediators all under the architecture of the PNI framework.

Chapter 2: REVIEW OF THE LITERATURE

Background

Chronic Pain in America

Chronic pain is the loose definition of devastating symptomatology that affects an estimated 116 million Americans (Tsang et al., 2008) and spans across all socioeconomic boundaries, educational levels, cultural, gender and age demographics (Pleis, Ward, & Lucas, 2010). Its causes are indiscriminate, its effects on sufferers can be incapacitating and intolerable. Currently there is no cure for the pain these patients suffer, no remedy or vaccination to restore an enjoyable quality of life.

A developing body of neuroimmune research demonstrates mounting evidence of alternative pain pathways that perpetuate the chronic pain state via physiologic self-propagating, or positive feedback mechanisms. Much of this new evidence points to the inflammatory process involving the signaling molecules secreted by neuronal cells called glia. Investigative work on glial cells has identified a selection of various molecules involved in positive feedback loops responsible for chronic pain, and has established that the underlying mechanisms involved render our current methods of fighting pain ineffective. As previously discussed, some of the proposed responsible molecules secreted by glial cells are called pro-inflammatory mediator molecules, specifically interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF- α). Multiple preclinical investigations have demonstrated that if the effects of these pro-inflammatory mediator molecules are blocked using various approaches, symptoms of chronic pain can be reversed and the analgesic functions of opioid

treatments return.

In addition to widespread and indiscriminate effects, chronic pain also holds an astonishingly high price tag for our health care system. The Institute of Medicine has reported that chronic pain is estimated to cost society an estimated \$630 billion annually in health care related costs and loss of productivity (Gaskin & Richard, 2011). In the United States, chronic pain accounts for millions of sufferers; more than all diabetic, coronary heart disease, stroke and cancer patients *combined* (Tsang et al., 2008). A myriad of anatomical areas are affected by chronic pain (National Centers for Health Statistics, 2006) with a spectrum of severity and quality of pain, making this disease difficult to treat with current practices. The psychological impact of unrelenting pain is also devastatingly prohibitive to a favorable quality of life. A 2006 survey completed by the American Pain Foundation, described more than half of the respondents felt they had little or no control over their pain and reported breakthrough pain at least once a day that severely impacted their quality of life and overall wellbeing (David Michaelson & Company, LLC, 2006). This potentially can lead to the overall feeling of loss of control, helplessness and apprehension, which can compound the negative impact pain holds over the sufferer's life. The survey also disclosed that over half of those surveyed reported that their pain impacted on their overall enjoyment of life, with over 75% reported feeling depressed because of their pain and 74% relaying their pain impacted their energy levels (David Michaelson & Company, LLC, 2006). Clearly chronic pain symptoms are not just incapacitating to the physical being, but spreads through the entire existence of the sufferer and impact all aspects of life.

Physiologic Pain Verses Chronic Pain

Pain is an elegant mechanism developed as a protective measure to preserve cellular function and life. When pain pathways work as they were intended, pain signals transmit from the periphery through the body to the central nervous system (CNS) for processing. Through a complex series of cellular interactions, pain perception (nociception) is the body's way of removing itself from a dangerous situation. In this way, pain, the proper perception of pain, and thus the proper withdrawal reaction to painful stimuli has aided in our species' survival and propagation. However, there are many instances in which individuals' pain perception and reaction to painful stimuli have, through no fault of their own, developed into chronic states of pain that serve no survival or protective benefit. Many individuals with chronic pain can exhibit hyperalgesia, a state in which there is an increased sensitivity to painful stimuli and can, additionally, report allodynia, the sensation of pain in response to a stimuli not normally responsible for pain.

A barrage of aberrant pathways contributes to the initiation and maintenance of nociception in chronic pain states and one of the mechanisms responsible for, and described in the literature is through glial cell activity. Immune cells known as glia are located in the CNS in close proximity to neurons. Until recently, glia were thought to exclusively contribute to the structure and nourishment of neurons (Barres, 1991; Kandel, 1991). Current literature has shown that glia do more than just nourish and provide structure to neurons; glial cells are immunologic cells that are activated following tissue injury, illness or inflammation (Tsuda, Inoue, & Salter, 2005; Watkins & Maier, 2000),

and have been shown to be a fundamental component for enhanced pain perception (Watkins & Maier, 2003). Once activated, glial cells release a cascade of molecules, more prominently pro-inflammatory cytokines, which have been shown to excite pain transmission and experience (Kreutzberg, 1996; Watkins, Milligan, & Maier, 2001). The same pro-inflammatory cytokines can ramp up dorsal horn neurons in pain transmission (Reeve, Patel, Fox, Walker, & Urban, 2000; Samad, Wang, Broom, & Woolf, 2004), they increase the release of neurotransmitters from sensory afferent nerves responsible for pain response (Inoue et al., 1999; Morioka et al., 2002) and may enhance pain via upregulation of AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid) and NMDA (N-Methyl-D-aspartate) receptor expression and activity (Beattie et al., 2002; Samad et al., 2004, Viviani et al., 2003; Wang, et al., 2005).

Cytokines and Chronic Pain

Cytokines are polypeptide, nonstructural proteins that are small (5-140 kDa) and effective at minute concentrations (pM) (Clark, Old, & Malcangio, 2013). They span a spectrum of physiologic functions that are limited in function only by the types of cells that express their receptors (Dinarello, 2007). Because of the vast physiologic effectiveness of cytokines, these soluble messenger proteins have extreme duality: They can be immensely protective (as in the case of immune defense of a host against infection) or highly lethal (as in the cases of cellular apoptosis, sepsis and specific cancers). Cytokines are further denoted by their propensity to be pro-inflammatory, or anti-inflammatory, and subdivided into larger protein families (such as interferons, interleukins, chemokines, mesenchymal growth factors, adipokines or the tumor necrosis

factor family) (Dinarello, 2007).

The interleukin (IL) family is perhaps one of the largest cytokine families that includes over 30 cytokines, both pro- and anti-inflammatory (Dinarello, 2007). Interleukin-1 (IL-1) is a relatively small pro-inflammatory cytokine (17.5 kDa) that also has the ability to initiate the expression and secretion of other pro-inflammatory cytokines. It belongs to a larger IL-1 gene family that is responsible for a myriad of functions including physiologic temperature regulation, T cell immunity against infection and hepatic protein synthesis (Dinarello, 2007). This pro-inflammatory cytokine is primarily secreted by glial cells in the CNS (Felts et al., 2005; Zhang et al., 2008), although there is data to show neuronal expression as well (DeLeo, Colburn, & Rickman, 1997; Sweitzer, Colburn, Rutkowski, & DeLeo, 1999). Additionally, neurons, glia and astrocytes all contain IL-1 receptors (Gruber-Schoffnegger et al., 2013; Zhang et al., 2008), making these CNS cells prime locations for IL-1 synthesis, secretion and cellular action. In order for neurons and astrocytes to express IL-1, an additional enzyme class called matrix metalloproteases has to cleave the inactive precursor (pro-IL-1) to produce the physiologically active form of the cytokine (Kawasaki et al., 2008). Not surprisingly, preclinical trials have demonstrated that rodents that genetically lack IL-1 β , IL-1 α , the IL-1 receptor, or overexpress IL-1 receptor antagonist, all fail to develop either mechanical hypersensitivity, or mechanical and thermal pain behaviors when exposed to an experimentally induced nerve injury (Honore et al., 2006; Wolf, Gabay, Tal, Yirmiya, & Shavit, 2006). Thus, IL-1 plays an unequivocal role in central processes of the pain experience.

Tumor necrosis factor-alpha (TNF- α) is a pro-inflammatory cytokine that is part of a group of over 20 proteins called the tumor necrosis factor superfamily (Dinarello, 2007). It is a relatively small cytokine (17 kDa) and is produced peripherally by macrophages (Aggarwal, Gupta, & Kim, 2012), and centrally by neurons and glial cells (Ohtori, Takahashi, Moriya, & Myers, 2004; Xu, Xin, Zang, Wu, & Liu, 2006). Tumor necrosis factor-alpha also has extreme duality in its function under normal physiology: It aids host immunity, but also has been implicated in sepsis (Eichacker et al., 2002), cancers, cardiovascular, neurologic, pulmonary, autoimmune and metabolic disorders (Aggarwal et al., 2012). Under normative conditions, CNS expression of TNF- α is minimal, however in response to nerve injury, TNF- α induces dorsal horn glia to release additional pro-inflammatory cytokines and increase neuronal sensitivity (Andrade et al., 2011; Clark et al., 2013; Inoue, 2006).

In contrast to the other cytokines discussed above, Interleukin-10 (IL-10) is an anti-inflammatory cytokine. Originally classified as a T-helper-2 (Th2) cytokine, it is now known that the functions of IL-10 encompass more than T cells. The actions of this cytokine are principally derived from the type of cell that produces it, the type of cell that responds to it and the inflammatory cytokine milieu in which it is released (Mosser & Zhang, 2008). This anti-inflammatory cytokine is expressed by mast cells, macrophages, natural killer (NK) cells, eosinophils, neutrophils and glial cells (Saraiva & O'Garra, 2010). It is a potent regulator of immune responses; IL-10 attends to conditions ranging from parasites to allergens (Saraiva & O'Garra, 2010), and is in many ways the physiologic antidote to pro-inflammatory cytokines and overactive immune responses, as

it suppresses Interferon Gamma (IFN-g), IL-1, TNF- α and IL-6 (Dinarello, 2007). Dysregulation of IL-10 thus leads to autoimmune and inflammatory conditions and diseases (Clark et al., 2013; Mosser & Zhang, 2008; Saraiva & O'Garra, 2010). In response to CNS pathology and certain chronic pain states, levels of IL-10 are increased via activation of glial cells (Ledeboer et al., 2003) most likely to combat the transient rise in pro-inflammatory cytokines. However in other chronic pain conditions, IL-10 concentrations have been noted to be lower when compared to healthy controls (Alexander, Perreault, Reichenberger, & Schwartzman, 2007; Backonja, Coe, Muller, & Schell, 2008). Thus, the literature illustrates that the intrinsic anti-inflammatory regulatory mechanism of IL-10 is corrupted or altered in some processes of chronic pain.

Chronic Pain Treatment & Glial Cells

Interestingly, preclinical studies have also shown that glial cells contribute not only to nociception upon insult (Meller, Dykstra, Grzybycki, Murphy, & Gebhart, 1994), but also perpetuate the maintenance of pain when it is treated with opioids. Multiple studies have shown that upon administration of chronic opioids to pain-induced rodents, there was an increase in glial cell activity in the CNS (Raghavendra, Rutkowski, & DeLeo, 2002; Song & Zhao, 2001; Tai et al., 2006). Glia have also been shown to upregulate pro-inflammatory cytokines production and release in the CNS when the organism is treated with longstanding systemic or intrathecal opioids; this increase of cytokine production and release in the CNS has been shown to contribute to opioid tolerance and withdrawal induced pain enhancement (Johnston et al., 2004; Raghavendra, Tanga, & DeLeo 2004; Song & Zhao, 2001). These investigations suggest that not only

are glia partially responsible for the maintenance of chronic pain symptoms, but they are also responsible for decreasing the efficacy of one of the most prominent treatments of pain—opioid drugs. Thus, when a chronic pain-induced rodent is treated for pain, over time the very treatment that relieves the pain will be part of a self propagating cascade of events that will be responsible for perpetuating the pain, increasing the level of pain, decreasing the effectiveness of treatment (through opioid tolerance) and creating painful responses to stressors that normally should have no painful reaction (allodynia).

Glial Cell Inhibition in Treatment of Chronic Pain

Inhibiting glia cells is not an option in the treatment of chronic pain because the full physiologic function of these cells is not well understood. Animal model studies show that arresting glial cell function *in vivo* using fluorocitrate, a glial metabolic inhibitor, suppresses glial cells from amino acid uptake and can result in host seizures. Minocycline, a microglia inhibitor and antibiotic, may prevent glial hyperactivation but it cannot be utilized in cases where there is established neuropathic pain (Milligan et al., 2006). Consequently, a body of research has shifted its approach from directly inhibiting glial cells to investigating what effects could be rendered by targeting the pro-inflammatory mediators released by glia. The results of animal model studies show phenomenal promise for future treatment of chronic pain: Several studies have proposed that morphine tolerance can be slowed or reversed by knocking out the signaling of the glia-produced pro-inflammatory cytokine IL-1, or by blocking IL-1 with a receptor antagonist (IL-1ra) (Hutchinson et al., 2008; Shavit, Wolf, Goshen, Livshits, & Yirmiya, 2005). Thus, animal model studies illustrate a cellular relationship that could be explored

for future treatment of chronic pain, but human research is still lacking.

Preclinical Verses Human Chronic Pain Studies

Unlike the animal model of pain research, where a chronic pain state can be surgically created in a precise manner, such as a chronic constriction injury of the sciatic nerve performed commonly in rodents, human studies have to account for the wide spectrum of pain states and symptoms, the cause(s) of pain and the location(s) of pain. Likewise, rodent studies are also able to meticulously treat a cohort of rodents with the same treatment, medication and dose in a measurable protocol; human studies have to adjust for the individual treatment regimens that utilize many types of medications, at varying doses or holistic treatments that manipulate varying physiologic processes. Additionally, rodent studies are performed in a homogenous population of genetically similar organisms; human studies incorporate varying physiologic and psychological factors associated with pain. Unlike the rodent models in chronic pain research, humans cannot be sacrificed for direct measurement of pro-inflammatory cytokines, and thus the two methods available for cytokine data collection in human models is limited to serum and cerebrospinal fluid (CSF) analysis. The wide variance in types of pain states, medications used for treating pain, co-existing physiologic and psychological variables and the indirect methods of gathering data limit human studies. Preclinical studies, however, offer limited value to understanding the relationship of chronic pain and levels of cytokines in *humans*. Rodent studies offer no insight into the relationship between the psychology of pain and the neuroimmune responses of chronic pain. Anxiety, depression and sleep disturbances are ascertained in rodent models with a certain degree of

interpretation. Rodent models also do not clearly describe what will occur in human physiology. While animal studies may provide a basic understanding of a phenomenon, human studies are nevertheless paramount in progressing towards translation into treatment modalities.

Human Chronic Pain Studies & Inflammation

The human research on pro-inflammatory cytokines is very limited and developing now. Prior human studies focused on measuring pro-inflammatory cytokines through serum analysis secondary to the decreased invasiveness and ease of collection. However, several of these studies discovered confounding levels of cytokines that were measured in the serum versus CSF of the study participants: levels of cytokines found to be high in the CSF were found to be low or undetectable in the serum and vice versa (Backonja et al., 2008; Brisby, Olmarker, Larsson, Nutu, & Rydevik, 2002; Kadetoff, Lampa, Westman, Andersson, & Kosek, 2012; Ludwig, Binder, Steinmann, Wasner, & Baron, 2008; Lundborg, Hahn-Zoric, Biber, & Hansson, 2010; Rozen & Swidan, 2007; Zin, Nissen, Moore, & Smith, 2010). Possible reasons include cytokine production via peripheral physiologic processes, which may or may not be related to nociception, versus cytokine production via glia in the CNS in response to pain signaling. This study will focus on CSF analysis because of the role of CNS processing in chronic pain, and the accompanying central location of glia, cytokines, and neurons. This approach proves more logical than examining serum levels of mediator molecules—subject to multiple competing physiological stimulins of peripheral immune cells—as a means of supportive analysis for developing pain literature.

Currently CSF has been examined in patients with complex regional pain syndrome (Alexander et al., 2007; Alexander, van Rijn, van Hilten, Perreault, & Schwartzman, 2005; Backonja et al., 2008; Munts et al., 2008), fibromyalgia (Bjersing, Dehlin, Erlandsson, Bokarewa, & Mannerkorpi, 2012; Kadetoff et al., 2012), those receiving intrathecal opioid infusions for pain management (Zin et al., 2010), persistent headache (Rozen & Swidan, 2007), neuropathic pain related to previous spinal surgery (McCarthy, Connor, & McCrory, 2013; Capelle, Weigel, Schmelz, & Krauss, 2009), sciatica (Brisby et al., 2002), lumbar radiculopathy (Nagashima, Morio, Yamane, Nanjo, & Teshima, 2009; Ohtori et al., 2011), polyneuropathy (Ludwig et al., 2008), post herpetic neuralgia (Kikuchi, Kotani, Sato, Takamura, Sakai, & Matsuki, 1999; Kotani et al., 2000; Rijdsdijk et al., 2013) and chronic osteoarthritis pain (Buvanendran et al., 2006 ; Lundborg et al., 2010; Yeager et al., 1999). Additional studies analyzing human CSF can be found summarized by Bjurstrom et al. (2014). Grouping of study participants based on diagnosis may control for some error by standardizing the type of pain, quality, location, causes and triggers as well as the treatment for the specific pain. This study included all forms of chronic pain patients with the intention of grouping the types and causes of pain before determining the most meaningful analysis methodology. In general, the preceding studies demonstrated that IL-1, IL-6, IL-8 and TNF- α were found to be elevated in the CSF of human study participants, but more evidence is still needed to corroborate the behavior of these molecules in pain syndromes. Other research has demonstrated a decrease in CSF levels of anti-inflammatory IL-10 (Alexander et al., 2007). This current study targeted IL-1, IL-6, IL-8, TNF- α and IL-10 for analysis and

focused on providing supportive human data for the chronic pain population. By measuring CSF rather than serum, grouping patients based on their diagnosis of pain and basing the cytokine analysis on previous investigations, this study provides a greater understanding of the developing role cytokines play in chronic pain.

Evidence Supporting Inflammatory Contributions to Anxiety and Depression

As previously mentioned, preclinical studies provide little insight into how psychological states (i.e., anxiety and depression) relate to the neuroimmune response of chronic pain in humans. All types of anxiety and depression cannot be directly ascertained in any rodent model, and is an integral component in the pain process and definitive treatment plan of chronic pain. Part of the psychoneuroimmunologic component to pain is the interaction between inflammation, cytokines and the behavioral complex known as sickness behavior or sickness response. Feelings of sickness can be characterized by fatigue, decrease appetite, fever, lack of mobility, sleep disturbances, depression and an inability to concentrate (Dantzer, 2001; Hart, 1988; Watkins & Maier, 2000). Though the neuroimmune-mediated sickness behavior/response may promote unsavory feelings, this natural response to illness is ultimately protective as it aids in healing and is usually short-lived with resolution of the offending illness. However, like chronic pain, when the protective mechanism of the sickness behavior/response goes awry, feelings of depression and anxiety can be perpetuated longer than the illness or stress. If the psychological effects of anxiety and depression are related to the illness or stress of pain, the mechanism is still unclear. Inflammatory cytokines offer one possible explanatory link between all of the psychoneuroimmune responses and pain, but central

levels of cytokines in humans have not been extensively studied thus far. In addition to the relationship of chronic pain to altered levels of centrally-produced cytokines, anxiety and depression must be examined in humans to determine their relationship to chronic pain and central inflammation.

Inflammation & Anxiety

While a majority of research and attention has focused on the relationship between chronic pain and depression (Dersh, Polatin, & Gatchel, 2002), there is literature that supports associations with chronic pain and anxiety disorders. In a large, retrospective analysis of over five thousand participants, McWilliams and colleagues found there was a stronger association of chronic pain and panic disorder (PD) and post-traumatic stress disorder (PTSD) than depression (McWilliams, Cox, & Enns, 2003). Thus, this study and other literature suggest the possibility that efforts to treat chronic pain should be combined with anxiolytic treatment.

Peripheral levels of inflammatory cytokines have been examined with conflicting results in human studies related to anxiety disorders. While some investigations examining relationships between anxiety and systemic cytokines levels have shown increases in serum levels of IL-1 β and IL-6 (Maes et al., 1999; Spivak et al., 1997), results of other studies are contrary to those findings (Koh & Lee, 2004; van Duinen et al., 2008). Possible explanations for the discrepancies between these studies include the type of anxiety (acutely experiment-produced or a chronic underlying diagnosis), whether the cytokines measured were from induced lymphocyte production or systemically circulating levels, and the sensitivity of the assay used to detect the cytokines. Using

Multiplex assay analysis with the Luminex® system (Austin, TX), Hoge and colleagues were able to demonstrate elevations in serum levels of eighteen cytokines including pro-inflammatory IL-1 β , IL-6, IL-8 and TNF- α as well as anti-inflammatory IL-10 in PD and PTSD study participants (Hoge et al., 2009). However, these peripheral results do not clarify if there is a central mechanism that links anxiety, inflammation and pain.

The data on central inflammation and anxiety is very limited. Similar to chronic pain studies that examine peripheral and central cytokine patterns, anxiety research exemplifies the same theme: Peripheral and central levels of cytokines do not appear to agree in magnitude or direction in most investigations. According to Baker et al. (2001), levels of IL-6 were found to be increased in the CSF but not increased in the serum of PTSD participants. Clearly, there is opportunity to examine the relationship between anxiety, commonly found with pain-related conditions, and the centrally mediated response of inflammation.

Inflammation & Depression

The direct correlative relationship between depression and pain has been thoroughly documented in the literature showing that both depressed patients exhibit at least one painful symptom (Vaccarino, Sills, Evans, & Kalai, 2009) and chronic pain patients frequently exhibit depression (Dworkin & Gitlin, 1991). In a study by the American Pain Foundation, over half of the chronic pain sufferers surveyed reported that pain impacted their overall enjoyment of life and over 75% reported feeling depressed because of their pain (David Michaelson & Company, LLC, 2006). Thus pain and depression warrant mutual inspection.

Similar to the anxiety literature, the relationship between levels of pro- and anti-inflammatory cytokines and their neuroimmune relationship in the pathophysiology of depression are not fully understood. Through a meta-analysis of 136 studies on serum levels of cytokines in depressive patients, Dowlati and colleagues found both supportive and contradictory results supporting the role of peripherally measured inflammatory cytokines and depression. They were only able to report a significant increase in IL-6 and TNF- α in depressed subjects when compared to control subjects (Dowlati et al., 2010), and it is unclear to what degree peripheral levels of cytokines relate to depression itself versus the chronic generalized stress depression causes.

Because some antidepressants work centrally and have been shown to reduce levels of inflammatory cytokines (Basterzi et al., 2005; Janssen, Caniato, Verster, & Baune, 2010), the patterns of CNS inflammatory cytokines in the depressed patient begs for more extensive investigation. The evidence of central levels of pro- and anti-inflammatory cytokines and depression in humans is very limited but presently underway. Cerebrospinal fluid levels of cytokines have been measured in unmedicated depressed patients (Levine et al., 1999), suicidal populations (Lindqvist et al., 2009), and chronic PTSD sufferers (Bonne et al., 2011) all with conflicting results. Clearly chronic pain and depressive symptoms are usually experienced together, but there is no data currently available exploring the role of central inflammation and the pathophysiology of these co-existing symptoms. More data must be collected to ascertain the relationship of central inflammation and depression, especially in the chronic pain population. This study adds valuable data in its examination of the relationship of central inflammation

and the depressive psychological component of chronic pain.

Evidence Supporting Inflammatory Contributions to Sleep Deprivation

The complex relationship between pain and poor sleep quality is one that has been extensively documented in the literature, but its causality not fully understood. Both processes of pain causing poor sleep, and poor sleep causing pain are primary paradigms in the bidirectional relationship between pain and sleep. From the protective sickness behavior/sickness response theory, as previously described, sleep has been suggested as a critical component in the restorative process of healing (Dantzer, 2001; Hart, 1988; Watkins & Maier, 2000). Counterproductively, in the presence of chronic pain pathophysiology, the restorative process is impaired by a lack of sleep: In a complex, detrimental dynamic, pain itself causes sleep deprivation and sleep deprivation has been shown to increase pain perception (Chiu et al., 2005; Lautenbacher, Kundermann, & Krieg, 2006; Moldofsky, 2001; Smith & Haythornthwaite, 2004). Up to 53% of chronic back pain patients and 88% of neuropathic pain patients report sleep disturbances (Meyer-Rosberg et al., 2001; Tang, Wright, & Salkovskis, 2007). Furthermore, patients with chronic lower back pain are 18 times more likely than normal control subjects to experience clinical insomnia (Tang et al., 2007). Thus, literature supports that sleep and pain do interact with one another, the abnormalities of sleep are frequently reported by the chronic pain population, and the effects are not benign: The effects of sleep disturbances have been linked with poor physical and psychological outcomes, absence from work, decreased performance, difficulty with personal relationships, accidents and cognitive decline (Fulda & Schulz, 2001). Even in healthy individuals, sleep deprivation

has been noted to cause spontaneous pain (Smith, Edwards, McCann, & Haythornthwaite, 2007). Clearly a holistic approach to treating these patients must involve attention of both the sleep and pain symptomatology. The causes or common mechanisms between poor sleep and pain are just starting to develop and warrant investigation for treatment of these patients. Interestingly, one of the foremost emerging theories on the link between pain and sleep deprivation include circulating inflammatory cytokines.

Inflammation & Sleep

Historic, seminal studies on sleep have demonstrated repeatedly that there is a centrally produced mechanism or substrate for sleep. At the turn of the century, Ishimori demonstrated that upon injecting rested animals with the CSF of sleep deprived animals, the rested animals fell into a deep sleep (Ishimori, 1909). Pappenheimer and colleagues reported similar findings, demonstrating that when the CSF of sleep-deprived animals was injected into rested rodents, a decrease in activity and an increase in sleep was observed (Pappenheimer, Miller, & Goodrich, 1967). Later when it was found that muramyl peptide induced synthesis and secretion of IL-1 by astrocytes, a biological basis for sleep alteration by a centrally mediated mechanism was confirmed (Fontana, Kristensen, Dubs, Gemsa, & Weber, 1982). Repeated animal studies have shown that upon central administration of IL-1 to both rabbits and rats, the result is an increase in various aspects of sleep (Krueger, Walter, Dinarello, Wolff, & Chedid, 1984; Tobler, Borbely, Schwyzer, & Fontana, 1984) independent of, but similar to the somnogenic effects of TNF- α (Shoham, Davenne, Cady, Dinarello, & Krueger, 1987; Kapás et al.,

1992). The concentrations of inflammatory cytokine required to influence sleep, either positively or negatively, is variable in the literature. In one study, Opp and colleagues found that low doses of intracerebroventricular doses of a pro-inflammatory cytokine did not affect rapid eye movement sleep (REMS) while higher doses did impair both non-rapid eye movement sleep (NREMS) and REMS (Opp, Obál, & Krueger, 1991). Thus, there is not only evidence that central inflammatory cytokines influence sleep, but the amount of the inflammatory cytokine circulating influences the quality and type of sleep as well.

Unfortunately, monitoring of central inflammatory cytokines in human sleep studies has been very lacking thus far, and those studies that have monitored peripheral concentrations of inflammation have yielded conflicting results. Peripherally monitored cytokines have been measured in humans, but on a limited basis and have focused on IL-1, IL-6 and TNF- α . Prior human studies have found that peripherally circulating levels of IL-6 have been correlated with decreases in slow wave sleep (Hong, Mills, Lored, Adler, & Dimsdale, 2005) and increased REMS (Irwin et al., 2004) (both of which are associated with poor sleep quality). However, Vgontzas and colleagues found that levels of IL-6 were associated with decreased REMS (Vgontzas et al., 2003) (a result that points to more restful sleep). Patients with excessive daytime sleepiness disorder have been found to express increased plasma IL-6 levels (Motivala, Sarfatti, Olmos, & Irwin, 2005) and TNF- α levels (Vgontzas et al., 1997). Thus it seems that an increase in peripherally circulating inflammatory cytokines is present when there is poor sleep or in conditions of chronic sleep deprivation, but there is conflicting experimental data. Further studies must

describe the means to scaffold concrete treatment options for these patients.

Similar to animal studies that illustrate the pertinent role of inflammatory cytokine concentration on either sleep promotion or sleep disturbance, human plasma studies have also yielded similar results: Peripheral pro-inflammatory cytokine concentrations seem to peak after sleep onset and have higher circulating levels at night (Bauer et al., 1994; Redwine, Hauger, Gillin, & Irwin, 2000). However, when there is poor sleep quality and/or sleep deprivation, higher peripheral levels of pro-inflammatory cytokines are common (Haack, Sanchez, & Mullington, 2007; Moldofsky, Lue, Davidson, & Gorczynski, 1989). These studies beg the question that at what concentration do inflammatory cytokines stop being sleep promoting and begin to be part of the cycle of sleep deprivation? Perhaps the measured peripheral levels of cytokines are not sensitive enough to detect the intricacies of the inflammatory cytokine sleep cycle. Perhaps the central cytokine levels are predominantly the cause of sleep deprivation. Unfortunately none of these studies examined central levels of inflammatory cytokines related to sleep quality, regardless of the seminal sleep literature that suggests central levels of cytokines are somnogenic.

Pain, Inflammation & Sleep

In human pain populations there exists almost a complete deficiency of data comparing inflammatory markers, sleep *and* pain. A study by Heffner and colleagues, substantiates that patients with chronic lower back pain were found to have higher levels of plasma IL-6 than healthy control subjects. In the chronic pain population studied, poor sleep quality was found to be associated with greater pain on the following morning

(Heffner, France, Trost, Ng, & Pigeon, 2011). Thus there is scant data to support the theory that inflammatory cytokines may contribute to the poor sleep quality endured by the chronic pain population, and central levels have not been examined even with the knowledge that there is a CNS component to sleep. Human data is requisite and developing presently. With a better understanding of central inflammatory cytokines associated with chronic pain and poor sleep quality, further discussion can be instigated to encompass a more holistic approach to treating the entire experience of the pain patient. This study offers a novel and exciting exploration of previously unexamined interactions between three critical physiological phenomena: pain, sleep, and the central inflammatory cytokine responses.

Implications for Future Knowledge Development

Because there is a scarcity of human evidence supporting the link between chronic pain, anxiety, depression and sleep deprivation and central inflammation, this study hopes to provide supportive evidence of this relationship. If this study, and future human studies illustrate the same association that preclinical models show, the implications for chronic pain treatment in our patient population are tremendous. Hutchinson and colleagues' preclinical study demonstrated that by blocking the pro-inflammatory cytokine IL-1, the length of time of intrathecal opioid analgesia was doubled and the time of systemic opioid analgesia increased by over one-and-a-half times. The study also demonstrated an approximate seven-and-a-half -fold increase in analgesic efficacy with morphine was possible by co-administrating the opioid with a receptor blocker of IL-1. These results were not just limited to morphine, Hutchinson's

study also produced similar results with methadone as well (Hutchinson et al., 2008). In another preclinical study, Johnston and colleagues indicated that by blocking IL-1 in the CNS, the analgesia of an intrathecally administered opioid was enhanced, opioid tolerance was blunted and both newly developed and established allodynia and hyperalgesia were reduced (Johnston et al., 2004). Because newly marketed antidepressants have elicited central responses through changes in inflammatory cytokine levels (Basterzi et al., 2005; Janssen et al., 2010), the central patterns of these cytokines must be examined in the depressed pain patient.

With supportive evidence that there is a relationship between chronic pain, anxiety, depression and sleep deprivation and central inflammation in humans, the responsible cytokines can be isolated and targeted. With further human evidence that inflammatory cytokines are part of the physiologic and psychological chronic pain cycle, there is a potential to treat chronic pain patients with opioids that would have a longer duration of action and increased efficacy. With further human evidence there is a potential to blunt opioid tolerance that develops over time, and reduce the allodynia and hyperalgesia chronic pain populations experience. With more advanced neurologics that pharmacologically target central inflammation, perhaps anxious and depressive symptoms can be better mitigated. Finally, with further human understanding of the psychoneuroimmunologic aspect of chronic pain, the complete patient will find relief in therapy that addresses all facets of the disease.

Conclusion

Animal models show in repeated studies that chronic pain states, opioid tolerance,

hyperalgesia, allodynia, anxiety, depression and sleep deprivation can be linked to aberrant levels of inflammation. By blocking pro-inflammatory cytokines, animal studies have shown that analgesia time and efficacy can be dramatically increased, opioid tolerance can be decreased and hyperalgesia and allodynia can be resolved. Similar advancements in inhibition of inflammation in the treatment of anxiety and depression are just now showing similar results. Because our current treatment techniques for chronic pain are restrictive and ineffective for certain sufferers, corroborative human evidence needs to be accumulated to illustrate the link between inflammation and chronic pain. If the same relationship that exists in rodent models can be shown in humans, new and innovative treatment modalities can be developed to treat intractable chronic pain and the psychological attributes that accompany this disease. One cannot continue to treat patients with medications and procedures that provide minimal relief for a short duration of time. With new avenues and pathways to target chronic pain, anxiety, depression and sleep deprivation, inflammatory cytokines certainly provide an exciting possibility in the future of chronic pain therapy.

Chapter 3: METHODOLOGY

Design

This was a cross-sectional, correlational study with a convenience sample of approximately eight chronic pain patients recruited from the Pain Management Center at the University of California, Los Angeles (UCLA). One-time collections of cerebrospinal fluid (CSF) samples were analyzed and compared to 30 control CSF samples to allow for testing of the first and second hypothesis. Psychological tool analysis of study participants allowed for testing of the third and fourth hypothesis. Demographic analysis allowed for testing of the fifth and sixth hypothesis.

Sample and Sample Size Calculation

Study Participants

Approximately eight patients with the diagnosis and symptomatology of chronic pain who were eligible for, and medically recommended treatment with an intrathecal opioid infusion pain pump (the Medtronic SynchroMed® II Pain Pump) were enrolled in this study after informed consent (Appendix A) was obtained. Potential participants were recruited from the Pain Management Center at UCLA. Inclusion criteria for this study melded both surgical and experimental criteria; examples of such criteria include the participant's age (the surgical eligibility for an implant of an intrathecal opioid infusion pain pump is 18 years of age) and duration of diagnosis (this study required subjects have a chronic pain diagnosis greater than 6 months duration to be considered "chronic"). Diagnosis of chronic pain was made on standard clinical criteria, as suggested by the International Association for the Study of Pain (IASP). Exclusion

criteria also incorporated a combination of surgical and experimental criteria; surgical criteria that would exclude a patient from obtaining an implanted intrathecal opioid infusion pain pump included meningitis, active upper respiratory infection/flu or febrile nature, acute medical or psychiatric disorders, acute psychological or physiologic danger/instability, altered mental status, pregnancy and the inability to communicate in English. Experimental exclusion criteria included a diagnosis of cancer, human immunodeficiency virus (HIV), recent epidural injections for treatment of chronic pain (within six months), drug abuse, meningitis or history of meningitis, history of blood transfusion(s) and palliative pain treatment. The inclusion and exclusion criteria were confirmed using standard clinical and laboratory methods of analysis, not above or beyond the standard of care, or at any additional burden to study participants. All study participants were instructed to continue all of their medications and therapies without making any changes prior to, or during study involvement.

Control Samples

Control samples of CSF were obtained from the California NeuroAIDS Tissue Network. Thirty random, individual control samples of 1-2 milliliters (mL) of CSF, collected through the HIV Neurobehavioral Research Center between the years 2005-2012, were utilized from this tissue bank as a means to compare levels of central inflammatory cytokines. The CSF samples obtained from this tissue bank were supported by the HIV Neurobehavioral Research Center (HNRC) P30 MH62512 (Principle Investigator: Robert K. Heaton, PhD) and supplied by Scott Letendre, MD through a collaboration with the University of California, San Diego (UCSD). The

control samples of CSF were selected randomly to include seronegative HIV and hepatitis C virus (HCV) donors, with no history of recreational drug use, no diagnosis of cancer and an approximate equal distribution of men and women donors. No other information pertinent to the control sample donors was provided to the research team except the date of collection.

Sample Size Calculation

In order to estimate the sample size needed to yield statistically significant results in the testing of the difference between two means (the CSF cytokine levels between healthy and chronic pain participants), the effect size was estimated using Cohen's *d*. Using this power analysis to estimate the sample size and reduce the risk of a Type II statistical error, aided in estimating the sample size when the population effect size was not known. Human chronic pain CSF studies are just beginning to be explored and as such, this novel new research is not advanced enough to lend proven effect sizes. This could be because the average chronic pain CSF study incorporates small sample sizes (usually 10-40 participants) (Bjurstrom, Giron, & Griffis, 2014) due in part to the invasiveness of collecting CSF, participant burden and risk, and the expense of analyzing cytokines. There are approximately 19 studies to date that have been published on the analysis of CSF cytokines in chronic pain populations. Only two of these studies utilize Multiplex assay analysis (McCarthy et al., 2013; Rijdsdijk et al, 2013). Thus, a sample size calculation for this study was necessary.

Given the significance level was set at 0.05 (to reduced the risk of a Type I statistical error) and the power was set at 0.80 for this study, the effect size was estimated

using results from similar studies that explored CSF levels of cytokines in chronic pain and healthy controls. Rather than estimate the effect size based on the expectations of a small, medium or large effect, Cohen's *d* was calculated for each cytokine analyzed by several prior studies that analyzed chronic pain patients' levels of cytokines (see Table 1).

Table 1

Summary of Cohen's d Score for Similar Chronic Pain Studies

| Author | IL-1 Cohen's <i>d</i> | IL-6 Cohen's <i>d</i> | IL-8 Cohen's <i>d</i> | IL-10 Cohen's <i>d</i> | TNF- α Cohen's <i>d</i> |
|------------------------|--------------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|--|
| Kadetoff et al., 2012 | 0.84 | N/M | 2.37 | N/M | N/M |
| Lundborg et al., 2010 | 0.69 | 0.27 | 0.85 | N/D | 0.52 |
| Backonja et al., 2008 | 1.06 | 0.68 | 0.33 | 0.97 | N/D |
| Ohtori et al., 2011 | N/M | 1.35 | N/M | N/M | N/M |
| Alexander et al., 2007 | N/M | 0.67 | 0.47 | 0.02 | N/M |
| Nagashima et al., 2009 | 1.69 | 1.16 | N/M | N/M | N/D |
| Rozen & Swidan, 2007 | N/M | N/M | N/M | N/M | 0.36 |
| Ludwig et al., 2008 | N/M | 0.52 | N/M | N/M | 0.55 |
| Alexander et al., 2005 | 0.73 | 1.08 | N/M | N/M | 0.18 |
| Average | 1.00 | 0.82 | 1.01 | 0.50 | 0.40 |

Note. N/M denotes that the study did not measure this variable. N/D denotes that the variable was not detectable.

Referring to Polit and Beck’s sample size estimates (Polit & Beck, 2012, p. 424), given the α of 0.05 and a power of 0.8, the approximate sample sizes were obtained from the average Cohen’s d (see Table 2).

Table 2

Estimated Sample Sizes Based on Effect Size Averages of Prior Studies

| Cytokine | Average Cohen’s d from Table 1 (Effect Size) | Estimated Sample Size from Polit and Beck ^a |
|-----------------|--|---|
| IL-1 | 0.96 | <25 |
| IL-6 | 0.76 | 25-33 |
| IL-8 | 1.49 | <25 |
| IL-10 | 0.92 | <25 |
| TNF- α | 0.43 | 40-62 |

Note. ^a Values taken from Table 17.6 in Polit & Beck, 2012, p.424.

The planned number of study participants was set at approximately 30, given the amount of time and the budget for the study, based on this power analysis. The amount of healthy CSF samples obtained from the California NeuroAIDS Tissue Network was 30, allowing for a total $n=60$. According to the Cohen’s d calculation, this sample size would be large enough to detect significance in the majority of inflammatory cytokines tested. Approximately 36 study recruits would be solicited to account for up to 20% attrition.

The actual number of study participants recruited from April 2013 to December

2014 was eight. Potential factors that contributed to the lower than anticipated study participant recruitment included changes in the personnel of the UCLA Pain Management Office, a decrease in the number of physicians performing the intrathecal opioid infusion pain pump procedures, an increase in the number of intrathecal neurostimulator placement procedures and hence, lower than anticipated intrathecal opioid infusion pain pump placement procedures. Only one patient who was solicited to participate in this study refused, making the actual attrition rate approximately 11%.

Setting

The UCLA Pain Management Center was the primary location of subject recruitment, consent and psychological testing. Subjects recruited from the UCLA Pain Management Center had CSF collected in an operative procedure performed at UCLA Santa Monica Orthopedic Hospital Outpatient Surgery Center. The UCLA Cousins Center for Psychoneuroimmunology Inflammatory Biology Laboratory served as the specimen processing, storage and analysis site of this study.

Instrumentation

Multiplexing Assays

To analyze the patterns of inflammatory markers in both chronic pain and control samples of CSF, Luminex Performance Human High Sensitivity Cytokine Panel (R&D Systems, Minneapolis, MN) and the Bio-Plex 200 instrument (Luminex®) with Bio-Plex Manager software version 5.0 were utilized for the precise and highly sensitive measurement of inflammatory cytokines. Multiplex assays were chosen over high-sensitivity enzyme-linked immunosorbant assays (ELISAs) to analyze the CSF samples

because of the minimal amount of sample needed to perform the analysis, the decreased time to analyze the samples for inflammatory cytokines (analytes), the decreased cost of analyzing multiple analytes, and because of its novel new laboratory analysis technique. Multiplex assays utilize fluorescence and spectral analysis as a reporter system instead of the enzyme amplification of antibody-antigen “sandwiches” utilized by ELISAs. Specifically, the Luminex® multi-analyte profiling system uses digital processing to analyze polystyrene beads for up to 500 different analytes at one time (Andreasson, Protelius, Pannee, Zetterberg & Blennow, 2012). It implements a two-laser instrument that detects three-color fluorescence; two colors are used for analyte classification and the third color is used to detect the intensity or amount of analyte. Because of the spherical medium and the use of spectral identification with fluorescence, multiple analytes can be analyzed simultaneously (Elshal & McCoy, 2006).

Precision, accuracy and error. When assessing measurement tools of biophysical samples, such as a new laboratory technique like Multiplex assays, the precision and accuracy of the tool must be reviewed, and the potential error surrounding the tool identified. According to Ryan-Wenger, precision is similar to the concept of reliability; it is not a characteristic of the tool but rather how the tool is utilized by the researcher. Precision reflects the tool’s sensitivity, or tool’s ability to reflect a change in value if there is a corresponding change in the value being measured (Ryan-Wenger, 2010, p. 373). Accuracy is similar to the concept of validity; it is the extent to which the measured values are in agreement with true scores elicited from a gold standard (Ryan-Wenger, 2010, p. 374). Thus, for the purpose of critiquing Multiplex assays, the

precision of the tool reflects the consistency across the measurements, and the accuracy reflects the agreement with the gold standard.

Precision. To assess the consistency across measurements, or precision of Multiplexing in measuring human biological samples, studies were examined that reported on greater than two Multiplex assay analysis on the same biologic sample or control sample. To measure the repeatability or precision of the Luminex® Multiplex tool, the coefficient of variation (%CV) was inspected in several studies. The %CV was examined for Multiplexing as a statistic to relay the error in precision given that tools that have high %CVs are considered to have a high error in their precision, and therefore are not consistent in their measurement.

In a 2013 study examining the validation of three Multiplex kits utilizing the Luminex® platform, Belabani et al. reports that the intra-assay %CV between three Multiplex kits inspected was less than 25% with the exception of one cytokine in one of the Multiplex kits. For the lower limit of quantification, the %CV was less than 30% for all cytokines in all kits with the exception of two cytokines in two kits (Belabani, Rajasekharan, Poupon, Johnson, & Bar-Oh, 2013). DuPont's study shows the inter-assay %CV for two Luminex® Multiplex kits as less than 25%, except for one cytokine in one of the kits. The intra-assay %CV for both kits was less than 25%, except for one cytokine in the aforementioned kit (duPont, Wang, Wadhwa, Culhane, & Nelson, 2005). These studies illustrate the specific nature of this testing modality: Not all kits are appropriate for testing all biomarkers. Manufacturer recommendations should be followed explicitly when selecting a kit for the specific biomarkers to be measured.

Chowdhury's 2009 piece illustrates that the Luminex® Multiplex intra-assay %CV was less than 25%. The mean inter-assay %CV when measuring high concentrations of cytokines was 25%; for low concentrations of cytokines %CV was 30% (Chowdhury, Williams & Johnson, 2009). This exemplifies again the importance of proper kit selection given the expected concentration of analyte. Not all Multiplex kits can measure all ranges of biomarker values.

Kang and colleagues demonstrated intra-laboratory precision values (reported as %CV) for Luminex® Multiplexing between 5.3%-10.8% for the three cytokines analyzed. The inter-laboratory %CV for Multiplexing ranged from 13.1%- 17.9%, demonstrating the importance of developing protocol standards in laboratory techniques for maximizing precision in Multiplexing (Kang, Vanderstichele, Trojanowski & Shaw, 2012).

If precision of a measurement is not a characteristic of the tool, but how the tool is utilized by the researcher, the aforementioned studies illustrate this definition of precision for Multiplexing: Proper kit selection, strict adherence to manufacturer recommendations and standardization of protocols all influence the %CV or precision of this specific tool. Given that the %CV is consistently less than 30% across all studies, precision for the Luminex® Multiplexing assay seems adequate for the analysis of human biologic samples. It is stressed however, that continued validation of the tool must be ongoing and collaborative between laboratories is essential to develop standards for this new assay.

Accuracy. To assess the accuracy of Multiplexing in measuring human biologics,

studies were examined that reported the values of the Luminex® Multiplex tool compared to the gold standard of ELISA analysis. This was done by analyzing the correlations of Multiplexing to ELISA either reported as r or r^2 (correlation or regression coefficients of various statistical analysis). Table 3 (adapted from Elshal & McCoy, 2006) summarizes the correlations of various Multiplex assays to the gold standard ELISA from a variety of studies.

Table 3

Correlations Between Multiplexing and ELISAs

| Cytokines | Keller & Douglass, 2003 | de Jager et al., 2003 | Chen et al., 1999 | Prabhakar et al., 2002 | Richens et al., 2010 | DuPont et al., 2005 |
|-------------------------|-------------------------|-----------------------|-------------------|------------------------|----------------------|---------------------|
| IL-1 β | | 0.964 | | 0.96-0.98 | | 0.838 |
| IL-6 | | 0.847 | | 0.98 | | 0.903 |
| IL-8 | 0.999 | 0.946 | | 0.92-0.96 | 0.9816-0.9859 | |
| IL-10 | | 0.926 | 1.0 | | | 0.820 |
| TNF- α | 0.998 | 0.911 | 0.97 | 0.96 | | 0.938 |
| Correlation Calculation | r | r^2 | r | r^2 | r^2 | r |

Note. Table 3 is adapted from Elshal & McCoy, 2006, p.321 with data from Richens, Urbanowicz, Metcalf, Corne, O’Shea, & Fairclough, 2010.

Unfortunately, limitations of these correlations across the different studies include different methodological approaches, poorly described statistical analysis, utilization of

different Multiplex kits and variation in kit manufacturers. However, from the high degree of correlation detailed in the literature it can be deduced that Multiplexing is appropriate in the analysis of biologic analytes measured by ELISAs. However, throughout the literature, it is stressed that the values elicited from ELISAs and Multiplexing are not interchangeable. Trends or patterns in biomarkers correlate well between ELISAs and Multiplexing, but future comparisons of specific values should note that ELISAs and Multiplex assays do not yield the same values.

Error or bias. Using classic test theory for the evaluation of Multiplexing as a tool to measure cytokines in biologics, it is postulated that the observed value of biomarkers (O) is equal to the true value of biomarkers (T) plus error.

$$O = T + \text{error}$$

Error diminishes the extent to which the measured biomarker values serve as adequate representations of the values trying to be measured. Because error in precision and accuracy is cumulative, the extent of the error or bias must be identified and prevented a priori, controlled for, and accounted for in the research design (Ryan-Wenger, 2010, p.371).

Multiplexing was also assessed for its capacity to analyze biological samples for the presence and amount of biomarkers. However, the error or bias that can occur and convolute the final values involves all parts of the experimental process: from the collection of the sample, through the storage, to the handling of the specimens, through the Multiplex assay process and analysis, and documentation of the results, error can occur. Some of the potential errors with analyzing biological specimens (specifically

CSF) using Multiplexing has been identified by Kang and colleagues. When the error can temporally occur in the experiment, the source of the error and suggestions for correcting the error is presented in Table 4 (Kang et al., 2012). These sources of error cited by Kang and colleagues helped scaffold the methodological approach of this study, as all precautions were taken to avoid these potential sources of error.

Table 4

Potential Error in Multiplexing

| Phase of Experiment | Source of Error in Procedure | Means to Correct Error |
|----------------------------|-------------------------------------|---|
| Preanalytical Phase | Lumbar Puncture | Training and Standardization |
| | | Use of Polypropylene Collection Tubes |
| | | Avoid Blood Contamination of CSF |
| | | CSF Collection in Morning |
| | | Fasting |
| | CSF Handling and Storage | Immediate Freezing of CSF Samples |
| | | Use of Polypropylene Storage Tubes |
| | | Limited Dead Volume in Tubes |
| | | Limit Freeze-Thaw Cycles |
| | | Reagent Handling |
| Analytical Phase | | Follow Manufacturers Instructions |
| | Quality Control Procedures | Predefined Run-Acceptance Criteria |
| | | Use of Aqueous QC Samples and CSF Pools to Assess Within-Run and Between-Run Analytical Performance |
| | | Use of Samples for Lot-to-Lot Consistency |
| | | SOP for Calibration Curve Performance of Analysts |

| | | |
|------------------------------|-----------------------------|--|
| | | Proficiency Panel to Qualify Performance of Analysts |
| | Sample Thawing | Standardization of Sample Thawing Procedure (Temp and Time) Before Testing |
| | Microbead Preparation | Follow Manufacturer's Instructions |
| | Vacuum Pressure | Standardization of Vacuum Pressure and Equipment |
| | Shaking of Reagents | Use Orbital-Type Shaker |
| | Centrifugation of CSF | Standardization of Centrifugation or No Centrifugation Before Testing |
| | Pipetting | Standardized Approach |
| | | Use of Polypropylene Tips |
| | | Avoid Up and Down Pipetting |
| | Equipment | Standardized Regular Validation and Maintenance |
| | Assay | SOP for Testing |
| Post-Analytical Phase | Type of Curve-Fit Algorithm | Use of Nonlinear Logistic Curve-Fit |
| | Run Validation | Predefined Run Acceptance Criteria |
| Inherent to the Kit | Lot-to-Lot Variability | Efforts to Minimize the Variation by Kit Manufacturer |
| | | Lab Establishes Criteria for Acceptable Variability Limits |
| | | Commit to Collaborative Efforts Between Kit Vendors |

| | | |
|--|---------------|---|
| | | and Labs |
| | Documentation | Detailed Standard Operating Procedure |
| | | Identify Possible Sources of Error and Provide Troubleshooting Guidelines |

Note. QC denotes quality control. SOP denotes standard operating procedures. Table 4 is adapted from Kang, Vanderstichele, Trojanowski, & Shaw, 2012, p.487.

With a review of validation studies of Multiplex assays, the precision and accuracy of this measurement tool was explored. From the literature, the precision and reproducibility of Multiplex values were found to be good both intra- and inter-assay. The correlations of various biomarkers were assessed between Multiplexing and the gold standard ELISA, and found to be relevant and acceptable. Multiplexing therefore was chosen to measure biomarkers in this study's biological specimens with the understanding that further validation reports should be reported with this research. The individual analytes, or cytokines, that the Multiplex analysis can detect are summarized in Table 5 along with the sensitivity of each assay.

Table 5

Summary of the Luminex® Performance Human High Sensitivity Cytokine Panel for Laboratory Analysis

| Analytes | Sensitivity (pg/mL) | High Standard Value (pg/mL) |
|---|---------------------|-----------------------------|
| IL-1β | 0.18 | 1,500 |
| IL-2 | 0.28 | 2,450 |
| IL-4 | 2.54 | 7,000 |
| IL-5 | 0.12 | 1,600 |
| IL-6 | 0.31 | 4,050 |
| IL-8 | 0.07 | 3,200 |
| IL-10 | 0.24 | 2,100 |
| IL-12 | 2.96 | 24,500 |
| GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor) | 0.13 | 1,575 |
| IFN-g (Interferon Gamma) | 0.08 | 1,350 |
| TNF-alpha | 0.54 | 3,350 |
| VEGF (Vascular Endothelial Growth Factor) | 1.35 | 2,000 |

Note. Adapted from manufacturer’s website,

<http://www.rndsystems.com/Products/FCST09>, R & D Systems, Inc..

Psychological Instruments

To measure the psychological variables within chronic pain, several tools were selected for this study and are discussed in detail. These psychological tools were

selected for their internal consistency and reliability (reflected in Table 6 as the Cronbach's α), their ease of completion and their use in previous chronic pain inflammatory studies. They were used to measure the study participants' level of pain perception, anxiety, depression, and sleep quality.

Table 6

Summary of Psychological Tools Utilized

| Behavioral Measures | Measurement | Scale | Cronbach's α |
|----------------------------|---|---|---|
| Pain | The Short Form-McGill Pain Questionnaire (SF-MPQ) | Scores for the questionnaire can range from 0 to 45 on the Pain Rating Index, from 0 to 5 on the Present Pain Intensity, and from 0 to 10 centimeters on the Visual Analog Scale. No established scoring noted by authors | ^a 0.73-0.89 ^b 0.96 |
| Anxiety | The Hamilton Anxiety Rating Scale (HAM-A) | A total anxiety score of <17 indicates mild anxiety levels; 18 to 24 mild-moderate anxiety levels; and 25 to 30 indicates moderate-severe anxiety levels | ^c 0.89 |
| Depression | The Beck Depression Inventory-II (BDI-II) | 0–13: minimal depression; 14–19: mild depression; 20–28: moderate depression; and 29–63: severe depression | ^d 0.91 |
| Sleep Quality | The Pittsburgh Sleep Quality Index (PSQI) | Nineteen components that yield a global score of 0-21, with a total of ≤ 5 associated with good sleep quality and > 5 associated with poor sleep quality | ^e 0.83 |

Note. ^a Taken from Burckhardt & Jones, 2003. ^b Reported as an Intraclass Correlation

Coefficient for total pain score by Grafton, Foster, & Wright, 2005. ^c Taken from

Kummer, Cardoso, & Teixeira, 2010. ^d Reported as a Coefficient Alpha by Beck, Steer,

Ball, & Ranieri, 1996. ^e Reported by Buysse, Reynolds, Monk, Berman, & Kupfer, 1989.

Measure of pain perception. The Short Form McGill Pain Questionnaire (SF-MPQ) is a self-reported survey that consists of fifteen descriptors known as the Pain Rating Index

(PRI); eleven sensory descriptors and four that are affective in nature. Each descriptor is rated on a scale of 0-3: 0 (none) to 3 (severe). It also includes a Present Pain Intensity (PPI) index and a Visual Analogue Scale (VAS). Scores for the questionnaire can range from 0 to 45 on the PRI, from 0 to 5 on the PPI, and from 0 to 10 centimeters on the VAS. (Melzack, 1975; Melzack, 1987). This tool can be found in Appendix B.

Measure of anxiety. The Hamilton Anxiety Rating Scale (HAM-A) is a survey completed by the researcher and measures the severity of a patient's anxiety. Based on 14 parameters, each survey item is given a 5-point score - 0 (not present) to 4 (severe) and includes anxious mood, tension, fears, insomnia, intellectual impairment, depression, somatic complaints, autonomic symptoms, physiologic symptoms and behavior at the interview. A total anxiety score of <17 indicates mild anxiety levels; 18 to 24 mild-moderate anxiety levels; and 25 to 30 indicates moderate-severe anxiety levels (Hamilton, 1959). This tool can be found in Appendix C.

Measure of depression. The Beck Depression Inventory-II (BDI-II) is a self-reported survey that assesses and score levels of depression. The BDI-II contains 21 questions, each answer being scored on a scale value of 0 to 3. The cutoffs values used will be: 0–13: minimal depression; 14–19: mild depression; 20–28: moderate depression; and 29–63: severe depression. Higher total scores indicate more severe depressive symptoms (Beck, Ward, Mendeson, Mock, & Arbough, 1961). This tool can be found in Appendix D.

Measure of sleep quality. The Pittsburgh Sleep Quality Index (PSQI) is a self-reported survey that is composed of nineteen components that yield scores on subjective sleep

quality, sleep latency, sleep duration, habitual sleep efficacy, sleep disturbance, use of sleeping medication and daytime dysfunction. These seven components are scored from 0-3 and yield a total score of 0-21. Higher scores indicate worse sleep quality, with a total of ≤ 5 associated with good sleep quality and > 5 associated with poor sleep quality (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). This tool can be found in Appendix E.

Data Collection Protocol

Approximately eight patients with the diagnosis and symptomatology of chronic pain who were eligible for, and medically recommended treatment with an intrathecal opioid infusion pain pump (the Medtronic SynchroMed® II Pain Pump) were enrolled in this study. Potential participants were recruited from the Pain Management Center at UCLA. After informed consent was obtained using Institutional Review Board (IRB)-approved forms (Appendix A), subjects completed the psychological instruments to measure their pain perception, depression and sleep deprivation (Appendices B, D, E). The anxiety tool (Appendix C) was completed by the practitioner collecting the demographic clinical data (Appendix G) and consent for study involvement. Participants underwent a structured interview to compile their demographic information (Appendix F) and results were number coded to ensure participant anonymity. All psychological study results and demographic information were number coded for anonymous participant identification and entered into a password-protected spreadsheet for analysis. Upon completion of the questionnaires, study participants were given a \$25 Visa gift certificate

for their participation in the study. Participants were also reimbursed for their parking fees upon enrollment of the study for the date for the trial operation.

Approximately one month after the preclinical office visit, the study participant underwent a surgical outpatient operation called an intrathecal opioid infusion pain pump trial. This operative surgical procedure and study procedure of collecting intraoperative CSF samples was performed in the operating suite at UCLA Santa Monica Orthopedic Hospital Outpatient Surgery Center. Three mL of CSF were collected from the eight study participants undergoing intrathecal opioid infusion pain pump trials for the treatment of their pain. Standard preoperative preparation and evaluation took place in the preoperative suite of UCLA Santa Monica Orthopedic Hospital Outpatient Surgery Center. A qualified member of the preoperative evaluation team started peripheral intravenous fluids in the preoperative suite and preoperative vital signs were recorded. The patient participant was then brought to the operating room and positioned per surgeon preference, and standard monitors (electrocardiogram, noninvasive blood pressure cuff, pulse oximeter) were applied. Patient participants were given 2-4 L/min nasal cannula oxygen and a combination of intravenous versed and fentanyl was titrated for comfort by a qualified operating room nurse at the surgeon's direction. Strict, standard sterile surgical protocol was followed per the regulations and policies of UCLA Santa Monica Orthopedic Hospital guidelines. Intrathecal access was performed with or without the use of fluoroscopy per the surgeon's discretion. All samples of CSF were collected intraoperatively before 1300 in appropriate polypropylene specimen collection tubes, properly labeled with a number code for anonymous participant identification and

placed on ice in UCLA approved specimen transportation boxes. A member of the research team then transported the specimens from UCLA Santa Monica Orthopedic Hospital to the UCLA Cousins Center for Psychoneuroimmunology Inflammatory Biology Laboratory in the 300 Medical Plaza building of the UCLA Westwood campus via car, with an estimated transportation time of 30 minutes. Upon receipt of the specimens, the UCLA Cousins Center for Psychoneuroimmunology Inflammatory Biology Laboratory centrifuged the samples at 2000G for 10 minutes at 4°C, and partitioned the 3 mL samples into 0.25 mL aliquots with appropriately labeled anonymous participant identification. The aliquots were then stored at -80°C until batch analysis was performed.

After collection of 3 mL of CSF in a specimen collection tube, the surgeon administered a dose of opioid through an intrathecal trial catheter. The catheter was secured and dressed in a sterile fashion and the patient participant was taken to the postoperative suite for recovery. The patient participant was then monitored for side effects and complications arising from the administration of the intrathecal opioid. During the postoperative stay, the participants' hemodynamic stability and vital signs were continually assessed by a member of the pain management team and/or postoperative nursing staff. Once a member of the pain management team determined that the patient participant had not incurred any serious side effects from the administration of an intrathecal opioid, the participant was then eligible to have a permanent opioid infusion pain pump. The catheter was removed, a sterile dressing placed over the operative site and the participant was discharged home with instructions

and emergency contact information.

Control samples of CSF were analyzed from the California NeuroAIDS Tissue Network obtained from Dr. Scott Letendre at the University of California, San Diego (UCSD). Thirty samples of 1-2 mL of CSF were utilized from this tissue bank as a means to compare levels of central inflammatory cytokines. Samples of 1-2 mL were obtained in specimen collection tubes and placed on ice in the appropriate biological hazard transport container. The specimens were shipped from UCSD to the UCLA Cousins Center for Psychoneuroimmunology Inflammatory Biology Laboratory in Los Angeles. Upon receipt of the specimens, the UCLA Cousins Center for Psychoneuroimmunology Inflammatory Biology Laboratory centrifuged at 2000G for 10 minutes at 4°C, and partitioned the 1-2 mL individual samples into 0.25 mL aliquots with appropriately labeled identification. The aliquots were then be stored at -80°C until batch analysis could be performed. Figure 3 depicts the complete cross-sectional study process integrated into the intrathecal opioid pain catheter trial experience.

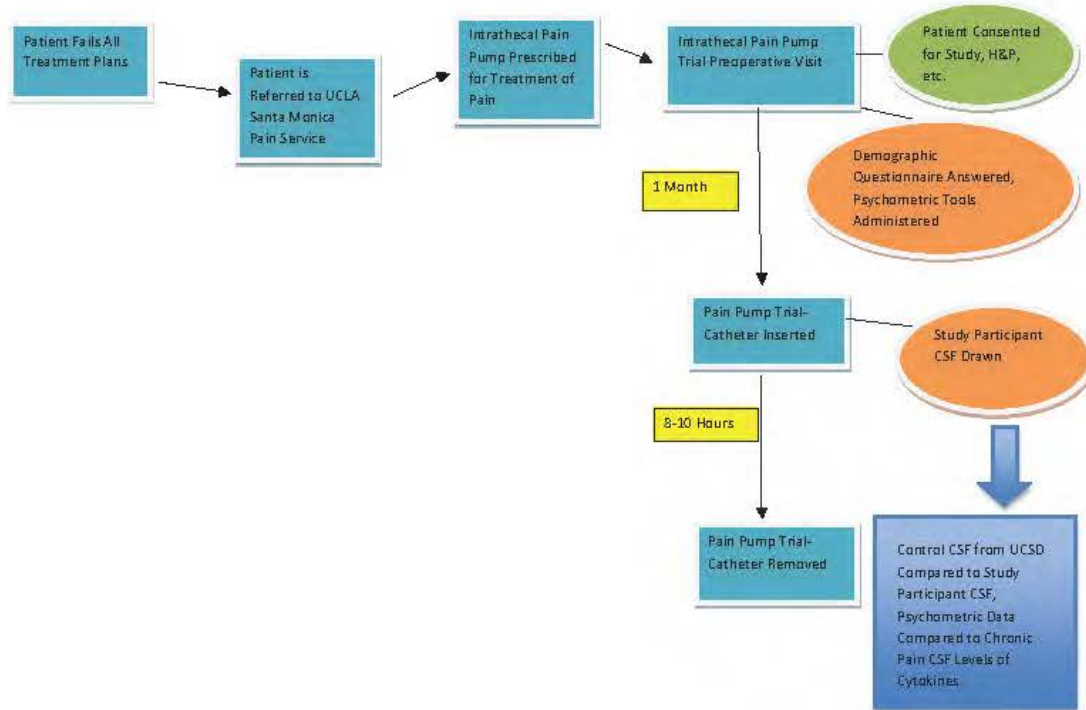


Figure 3. Data collection for cross-sectional study of Psychoneuroimmunology and Chronic Pain.

Cerebrospinal fluid levels of IL-1, IL-6, IL-8, TNF- α and IL-10 were determined using the Luminex® Performance Human High Sensitivity Cytokine Panel (R&D Systems, Minneapolis, MN). All assays were performed in duplicate per manufacturers recommendations. Cerebrospinal fluid samples were diluted 1:2, following the protocol recommended for serum and plasma. All analyses were run in duplicate per the manufacturer’s recommendation. Samples with %CV > 20 % between duplicates were

evaluated on a case-by-case basis for repeat assays and/or modified data analysis. The cytokine levels for all of the samples obtained, both from UCLA and UCSD are reported as picograms/milliliter (pg/ml). The data analysis plan and potential dissemination of study findings are outlined in Figure 4.

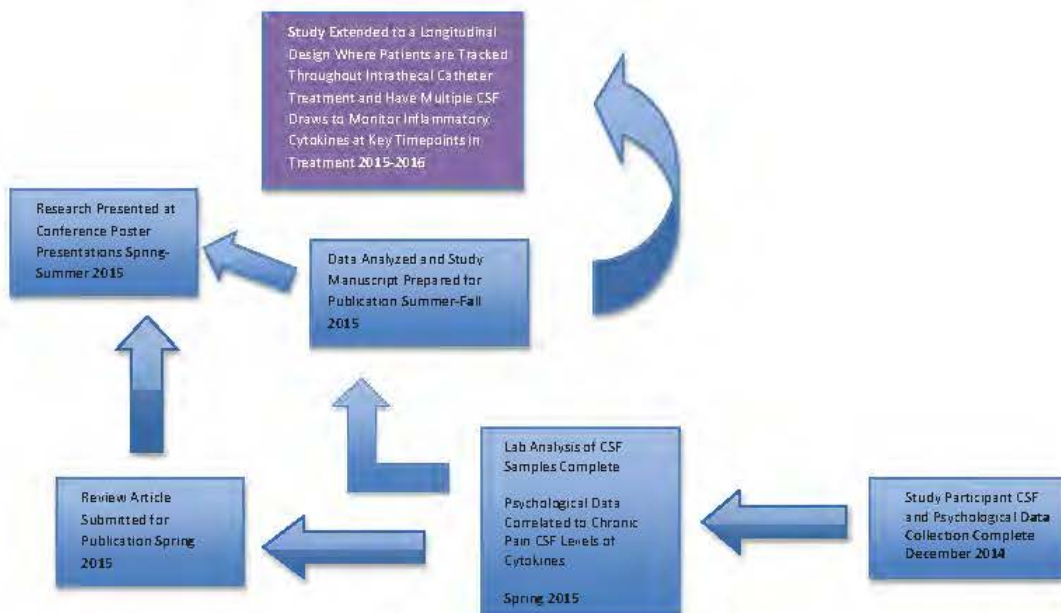


Figure 4. Data analysis plan for cross-sectional study of *Psychoneuroimmunology and Chronic Pain*.

Statistical Analysis

Descriptive statistics were calculated for all variables in the study and examined for responses. Multiple approaches were used to analyze the data and the level of

significance was set at $p < 0.05$ for all statistical testing. To test the first and second hypothesis, CSF levels of inflammatory cytokines in study participants were compared to controls using parametric statistics for normally distributed variables to include multivariate analysis of variance (MANOVA) followed up with analysis of variance (ANOVA) (non-parametric testing using Mann-Whitney U analysis would be implemented for non-normally-distributed variables). To test the third through the sixth hypothesis, correlations were calculated to explore relationships between psychological instrument measures, potential cofactors (demographics), pretreatment critical cofactors and biomarkers using Pearson's r , Spearman's Rho or non-parametric correlations for non-normally distributed data. Multivariate analysis of variance and ANOVA was used to examine differences between gender, diagnosis and types of medications. Calculations were accomplished with SPSS software version 22 (SPSS, IBM Inc., Armonk, NY).

The planned analysis was primarily exploratory given the small study population size, and designed to answer the question: Are there significant relationships between such psychological variables as pain perception, anxiety, depression or sleep deprivation and levels of inflammatory mediators in the CSF of the chronic pain patient?

Compliance Plans

Data and Safety Monitoring Plan

Data and safety monitoring for this study proceeded in an exacting manner and met all UCLA and University of San Diego (USD) IRB requirements. The data collected from paperwork, including but not limited to, the psychological tests administered, were entered into a spreadsheet database that was password-protected, and all paper copies

stored in a locked cabinet at the UCLA Pain Management Center. No electronic data was anticipated to be stored on servers, but in the event that electronic data storage was necessary, UCLA protected servers that are in compliance with Good Clinical Practice (GCP) and Health Insurance Portability and Accountability Act (HIPPA) regulations would have been utilized.

Throughout the study there was a review of data to carefully ensure that there were no adverse effects to study participants. The study was reviewed and all adverse effects would have been reported to the UCLA and USD IRBs by the Principal Investigator (PI) of the study. The Co-Investigators provided a second level of review as the study proceeded. Data was entered on an ongoing basis and weekly reports detailing health events, study progress, data accuracy and completeness was generated for review by the PI and Co-Investigators. Quality assurance activities were supervised by the PI.

Statement of Assumptions and Protection of Human Subjects

Informed Consent

This study protocol was reviewed and approved by the IRBs (Human Subjects Protection Committee) of UCLA and USD. All participants received a California Experimental Subject's Bill of Rights and voluntarily signed an informed consent, specific to the study protocol, prior to study participation in a private setting. All study participants were asked to read and ask questions, and complete consent forms prior to study participation. Risks of study participation were carefully detailed in the consent form. Participants had the option to withdraw consent at any time without prejudice to their future medical care at UCLA Medical Center.

Risks of Study Participation

This study analyzed CSF collected during the surgical procedure necessary for determining chronic pain patients' eligibility to receive a surgically implanted, Food and Drug Administration-approved device for the treatment of their chronic pain symptoms. Patients received this device regardless of their participation in this study, this device did not sponsor this study, the device was not paid for by the study and in no way was being tested by this study. The surgical risks of performing the trial catheter insertion were known risks to the surgical procedure and were consented for by the patient, but did not represent additional patient burden for those participating in this study. Participants were assured via the consent process that they could discontinue their study participation at any time if they decided not to undergo the intrathecal opioid infusion pain pump trial. Minimal adverse effects have been reported with intrathecal opioid infusion pain pump trials. The UCLA Pain Management Center medical doctors that performed the intrathecal opioid infusion pain pump trials in this study have done so in hundreds of past patients with no adverse events related to surgical placement of the intrathecal catheter or pain pump. Additional risks posed to the patient study group above and beyond the surgical risks are listed here.

Cerebrospinal fluid sampling. Study participants were scheduled for a surgical procedure called an intrathecal opioid infusion pain pump trial and CSF drainage is a known and accepted component of performing this surgical technique. Cerebrospinal fluid was available for collection with the same risks and benefits as the surgical procedure for which the participants were consented for with no expected increase in

participant burden. Potential additional risks to the study participants (above the surgical risks) were minimal; a spinal headache is the most common side effect of CSF volume depletion. Because the majority of CSF volume collected was minimized and not anticipated to be greater than what would normally be lost intraoperatively, the risk of the study participant developing a spinal headache was no greater than the surgical risk of developing a spinal headache. The CSF sample represents a minimal amount of CSF present in the adult central nervous system (CNS): Approximately 150 mL of CSF is present in the CNS of the average adult. Cerebrospinal fluid is manufactured and replaced every eight hours, hence the adult body produces around 500 mL of CSF per day (Marieb, 2001). Given the amount of CSF in the CNS and the rapid production of this fluid, the 3 mL sample of CSF necessary for study analysis represented a small but noteworthy participant burden. The risk of developing a spinal headache from 3 mL of CSF sampled was a small, but serious risk to the study participant. The literature supports that spinal headaches occur after approximately 20 mL of CSF is drained from the CNS (Wolff, 1948). While the risk is low, a spinal headache is a serious side effect to monitor for. Throughout the intrathecal opioid pain pump trial, and for approximately 8-10 hours postoperatively, vital signs were continuously monitored and staff was available to talk to participants about any discomforts or side effects they experienced. In accordance with UCLA IRB requirements, and operating room protocols, an Advanced Cardiovascular Life Support (ACLS)-certified study physician was immediately available during all procedures. This was fully explained in the consent form.

Infection. During the surgical procedure where study samples of CSF were

collected, there was a risk of infection to the participant. This risk of infection was the same as the surgical risk of infection and did not represent any additional subject burden above and beyond the surgical risk. An infection after a surgical intrathecal needle stick is rare, but represented a potential risk to the participant. Should an infection occur, treatment including antibiotics or additional surgery may have been necessary.

Bleeding. It is possible, though unusual, to experience an excessive bleeding episode through an intrathecal needle stick. The risk of developing a bleeding episode where an intervention would be necessary was no greater than the surgical risk to study participants. Should bleeding occur, it may require emergency treatment to drain accumulated blood called a hematoma. This was fully disclosed to study participants upon enrollment in the study and fully explained within the consent.

Scarring. In rare cases, scarring from an intrathecal needle stick may result. This represented a small risk to study participants, but not above and beyond the surgical risk of scarring. This was fully disclosed to study participants upon enrollment in the study and fully explained within the consent.

Confidentiality

All records will be kept strictly confidential. No person except the research team will know the subjects were in a research study. Data forms for the collection of health and study data was coded with an anonymous identification number. No data form identified the participants by name. Data forms were kept in a locked cabinet at the UCLA Pain Management Center. No presentation or publication of the results of this study refer to the individual participants or present information that would identify any

participant.

Potential Benefits

The potential benefits for both the participants and broader segments of society include improved understanding of the psychoneuroimmunologic consequences of pain, anxiety, depression and sleep disturbances leading to more effective therapies and prevention. However, participants were informed that no change in their chronic pain treatment or direct benefit was expected to accrue to them from participation in the study.

Subject Reimbursement

Study participants were reimbursed for the parking visit needed for the trial operative day visit. Upon completion of the psychometric tools and after study enrollment, patients were given a \$25 Visa gift certificate.

Inclusion of Women

The chronic pain population of the UCLA Pain Management Center is approximately evenly divided with respect to gender, and women were recruited as part of the study sample. Pregnant women were excluded from this study as it is surgically contraindicated in the intrathecal pain pump trial and permanent pump insertion.

Inclusion of Minorities

The chronic pain population of the UCLA Pain Management Center is extremely ethnically diverse. Recruitment efforts were equally directed toward members of all racial and ethnic groups.

Inclusion of Children

Subjects who are 18-20 years of age (defined as “children” by the National

Institutes of Health) were eligible for recruitment from the UCLA Pain Management Center. Children below the age of 18 would have been excluded as the experience of pain in the pediatric population differs from that of adults (McGrath & Brigham, 1992). Further, the imposition of surgical intrathecal pain pump trials and permanent pump insertions on children below the age of 18 is usually surgically contraindicated.

Safety Precautions and Emergency Protocols

Trained medical and nursing personnel were in attendance during all surgical procedures. Any adverse event related to CSF sampling was immediately evaluated by the study physician. If injury occurred and deemed minor, treatment would have been administered on-site and the participant followed on an outpatient basis. In the extremely unlikely event of a significant injury, the subject would have been referred to the appropriate UCLA service for treatment and follow-up care. The UCLA and USD IRB would have been informed by telephone within 24 hours by the investigators of any serious adverse event, and a written report would follow within three days.

Description of the System for Maintenance of Records

Upon consent and enrollment in the study, the subject's name, address, email, phone number(s), medical record number, enrollment date, intrathecal catheter trail date and demographic data (including the patient's age) was all recorded on a single spreadsheet representing that specific study subject. All identifying information was removed from all subsequent research paperwork, psychological tools and CSF samples, and alphanumerically coded to maintain anonymity. The study anticipated approximately thirty study subjects to participate in this study and alphanumerically coded each study

participant in random assignment of study enrollment. Study subjects enrolled were alphanumerically assigned a code from SGP001 to SGP030. Additional numbers could have been added if there were additional study recruits. The paper copies of the aforementioned information and copies of psychological surveys completed by the study subjects (identifiable only by the subjects alphanumeric code) were stored in a locked cabinet at the UCLA Pain Management Center. A key or legend linking the study subject's information to their alphanumeric identifier was kept in a locked file cabinet at the UCLA Pain Management Center. The key or legend included the assigned alphanumeric code (i.e. SGP001) and the patient's last name and first initial. The laptop computer used to create the study subjects spreadsheet and code list was password protected, utilized only secure Internet connections and was maintained only for study purposes. Sarah Giron was the sole user of this laptop computer.

Cerebrospinal fluid samples from patients were stored in deep freeze storage at the UCLA Cousins Center for Psychoneuroimmunology Inflammatory Biology Laboratory with only the alphanumeric code identifier. Samples were stored with anonymous identification in deep freeze storage until batch analysis was performed per manufacturers recommendations. Throughout the laboratory analysis of the samples, only the alphanumeric code was available to lab personnel for identification of samples.

Limitations

Because of the cross-sectional, correlational nature of this study's design, causality of chronic pain and the causal relationships between levels of pro- or anti-inflammatory cytokines, chronic pain, anxiety, depression and sleep deprivation were not

determined. The study design itself limits the findings of this study to simply providing support for prior studies and serving as a basis for developing future studies. Given the specific criteria and study population of this cross-sectional study, this study design also limited the ability to generalize the results.

The small sample size also limited study findings: Even though a power analysis was performed and determined that the sample size was adequately powerful enough to determine significant changes between three of the five cytokines analyzed, the sample size would have been possibly too small to detect changes with IL-10 and TNF- α . The sample size necessary to detect changes in IL-10 and TNF- α would have been 64-99 participants, but given the budget and time available for this study, this sample size was unrealistic. Nevertheless, because IL-10 and TNF- α are cytokines analyzed on the same Multiplex assay as the other three cytokines, they were still explored with minimal increase in cost or time.

Obtaining the control samples from an outside source was also a limitation of this study. While obtaining these samples confirmed that 30 healthy CSF samples could be obtained and it decreased the time to acquire the CSF samples, lessened the participant burden and cost of recruiting participants to obtain these samples, these control CSF samples only came with a limited description and external assurance. The control samples may not adequately represent the CSF of healthy participants in the general public.

The unequal number of participants between the chronic pain and control CSF samples, as well as the small sample size, may limit the reliability of statistical analysis

of this study. Likewise, because psychological testing was not performed on the control subjects from whom the control CSF came, statistical analysis between inflammatory cytokines and psychological instrument data will only be possible in chronic pain participants.

Three of the four psychometric tests utilized by this study were from direct participant report, while the Hamilton Anxiety Scale was completed by the practitioner consenting and obtaining demographic information from study participants. While this study tried to limit the number of practitioners completing the scales on the participants, to limit inter-reporter variability, a total of four practitioners ultimately completed the Hamilton Anxiety Scale for this study. This number of practitioners may have skewed the results for this psychological variable.

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Chapter 4: MANUSCRIPTS

Manuscript I

UNIVERSITY OF SAN DIEGO

Hahn School of Nursing and Health Science

Chronic Pain and Decreased Opioid Efficacy: An Inflammatory Link

Sarah E. Giron, PhD(c), CRNA

Charles A. Griffis, PhD, CRNA

Joseph F. Burkard, DNSc, CRNA

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Abstract

Chronic pain is a devastating amalgam of symptoms that affects millions of Americans at a tremendous cost to our healthcare system and, more importantly, to patients' quality of life. Literature and research demonstrate that neuroimmune cells called glia are not only responsible for initiating and maintaining part of the chronic pain disease process, but also release inflammatory molecules responsible for decreasing the efficacy of one of the most prominent treatments for pain, opioid analgesia. This article describes chronic pain as a disease process that has ineffective treatment modalities; explores the mechanisms of glial cell activation and inflammatory responses that lead to chronic pain and decreased opioid treatment efficacy; and hypothesizes novel chronic pain treatment modalities based on the glial cell inactivation and anti-inflammatory pathways.

Keywords: Inflammation, Chronic Pain, Cytokines, Opioids, Glia

Chronic pain is a serious health concern of tremendous scope that spans socioeconomic boundaries, educational levels, and cultural, gender, and age demographics (Pleis, Ward & Lucas, 2009). An estimated 116 million Americans suffer and are disabled by this chronic condition (Tsang et al., 2008), with a cost of \$630 billion in lost work revenue, sick time, health care costs (Gaskin & Richard, 2011), and, most importantly, at the cost of patients' quality of life. Patients report not only a wide spectrum of painful symptoms and psychological effects, but also suffer from different types of pain in various anatomical locations. Current modalities utilized to treat these patients are limited in scope, and often ineffective for a large portion of the chronic pain population with a host of dangerous or self-limiting side effects. This raises the question of what more can be done for a patient population larger than all diabetic, coronary heart disease, stroke and cancer patients *combined* (Tsang et al., 2008)?

For decades, pain research focused on neuronal opioid receptors as the mainstays of pain physiology and management. Isolated from opium in the 1800's, morphine has become a mainstay of pain therapy (Ahlbeck, 2011; Mehendale, Goldman, Mehendale, & Rana, 2013), and clinicians eventually developed an array of powerful synthetic opioids for pain relief. Yet, despite the useful addition of variably efficacious anti-depressive and anti-convulsant drugs, which decrease neuropathic pain symptomatology (Vranken, 2009), consistently effective treatment of chronic pain eludes the medical community. Fortunately, recent scientific discoveries have started to unmask various chronic pain neuroimmune pathways, which could lead to more effective treatment of this epidemic. One of the new directions for inquiry highlights central nervous system (CNS)

inflammatory mechanisms that are believed to exacerbate and prolong neuropathic pain states via positive feedback mechanisms (Griffis, 2011). Recent pain literature suggests that the inflammatory mechanisms responsible for the initiation and prolongation of chronic pain are perpetuated by opioid therapy (Raghavendra, Rutkowski, & DeLeo, 2002; Tai et al., 2006). Given the widespread use of opioids in the anesthetic or pain management of patients with chronic pain, it is imperative for nursing and medical practitioners to understand the underlying mechanism of neuroimmune-mediated CNS inflammation, its hypothesized role in chronic pain, and the possibilities for novel pain management modalities if these inflammatory mechanisms are targeted.

Acute Pain Verses Chronic Pain

Pain is an elegant physiologic mechanism that functions as a protective measure to preserve cellular function and life. The International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey & Bogduk, 1994, p. 213). However there are instances when pain ceases to be protective and begins to be destructive. The neuroimmune-mediated CNS inflammatory mechanisms described here offer one possible way to differentiate between these two very different pain states.

Acute Pain

When pain pathways work as intended to detect tissue damage or trauma, pain-sensing receptors known as nociceptors detect chemical, mechanical, or thermal stimuli. Nociceptive detection is then converted and transduced as neural electrical impulses. A δ

and C primary afferent nerve fibers transmit these nociceptive signals to synapse at Rexed's Laminae II (located in the substantial gelatinosa) of the dorsal horn of the spinal cord (Todd, 2012). Via ascending spinothalamic tracts in the spinal cord, these nociceptive neural signals are transmitted to the thalamus where the pain sensation is relayed to higher cortical regions for processing. The basal ganglia, anterior cingulate cortex, amygdala, periaqueductal grey region, hypothalamus, prefrontal cortex and rostral ventromedial medulla are all areas of the higher cortex that process ascending nociception. As nociception proceeds, descending pain modulatory systems may be activated, resulting in increases and decreases in pain perception by the host. All of the higher cortical regions except the basal ganglia participate in descending pain modulation. Figure 1 depicts the acute pain process as described (Garland, 2012). Through a complex series of peripherally and centrally mediated neuro-cellular interactions, and reflex and cognitive actions, sensing and responding to acute nociceptive pain helps the host remove itself from danger. Thus, pain, the proper perception of pain, and the proper withdrawal reaction to painful stimuli has aided in our species' survival.

Chronic Pain

Unfortunately, there are many conditions in which individuals' nociception and reaction to painful stimuli have developed into chronic pain states that provide no survival or protective benefit. Chronic pain, a term loosely and inconsistently defined in the literature, can be classified as any pain that endures beyond the expected healing phase following an injury or in the absence of injury, or pain that lasts longer than three

months (Ruetsch et al., 2013). Common attributes of chronic pain, include hyperalgesia, an increased sensitivity to painful stimuli, and allodynia, a sensation of pain in response to stimuli not normally responsible for pain. A barrage of sustained pain signaling via afferent pathways contributes to the maintenance of the chronic pain state and has been described in the literature as central sensitization (Mifflin & Kerr, 2013; Woolf, 2011), however this paper will focus on the contributions of neuroinflammation to chronic pain syndromes as well as resistance to opioid analgesic therapy.

Glial activation. Glia are neuroimmune cells located in the brain and spinal cord and comprise approximately 70% of all cells in the CNS. Though there are multiple glial cells responsible for the proposed inflammation-driven chronic pain pathways; in accordance with most of the experimental investigations of the phenomenon, this discussion will focus on two innate immune cell types known as microglia and astrocytes.

Historically, glia were thought to contribute exclusively to the structure and nourishment of neurons, until research using a rodent model of sciatic nerve injury identified a positive correlation between astrocyte activity and the level of hyperalgesia observed after an experimentally-induced nerve injury (Garrison, Dougherty, Kajander, & Carlton, 1991). Over a decade later, multiple pre-clinical investigations consistently support what Garrison et al.'s study initially suggested: glial functions comprise much more than nourishment, protection, and structural support of neurons. Glial cells are involved in the development and formation of the blood brain barrier and myelin sheath (Watkins, Hutchinson, Johnston, & Maier, 2005; Watkins et al., 2007) and are activated following tissue injury, illness, or inflammation (Tsuda, Inoue, & Salter, 2005; Watkins

& Maier, 2000). Once activated by peripheral or central stimuli, glial cells in the brain and spinal cord have been shown to release a cascade of molecules, including pro-inflammatory cytokines, contributing to CNS plasticity in pain responses (Mika, Rojewska, Makuch, & Przewlocka, 2010; Wei, Guo, Zou, Ren, & Dubner, 2008). Studies have also suggested that glial cells may represent some of the fundamental components for nociception, and neural detection of pain (Watkins & Maier, 2003; Wieseler-Frank, Maier, & Watkins, 2004). Figure 2 depicts a possible mechanism by which activated glia contribute to increased pain perception through increased neuronal firing via the ascending spinothalamic tract.

Subsequent research among glia cells has delineated a specific order of cellular activation that is fundamental to the genesis of chronic pain states. These data suggest that microglia may *initiate* the chronic pain cycle, as they are the first glial cells activated by excessive sensory afferent neural signaling, and may play a role in astrocyte activation. Astrocytes may be implicated in the *persistence* of chronic pain, remaining activated long after microglial activation has ceased (Fleming et al., 2006; Ledebøer, Hutchinson, Watkins, & Johnson, 2007; Romero-Sandoval, Chai, Nutile-McMenemy, & DeLeo, 2008). Published reports propose that in chronic pain scenarios microglia cells undergo a transformation from a resting, homeostatic state, to an activated state in the dorsal horn minutes to hours after nerve damage or injury, followed by astrocyte cellular activation 24 hours to several days later. The activated microglia remain activated for several months, and astrocyte activation persists after microglial activation has subsided, sometimes for months to years (Fleming et al., 2006; Ledebøer et al., 2005; Romero-

Sandoval et al., 2008). Figure 3 illustrates the temporal nature of glial cell activation in chronic pain. The exact characteristics of neural signaling stimuli that initially cause microglia to activate and begin the chronic pain cascade, as well as the molecular signals that transmit the message from activated microglia to initiate astrocyte activation remain unclear, though fractalkine (a neuron-bound chemoattractant molecule) may play a role in neuron-to-glia signaling (Chapman et al., 2000; Milligan et al., 2004; Milligan, Sloane, & Watkins, 2008). What is more certain from research is that there may be a specific order of cellular activation among these glial cells that is necessary for chronic pain to be initiated and then maintained (Fleming et al., 2006; Ledebor et al., 2007; Ledebor et al., 2005; Romero-Sandoval et al., 2008).

Acute pain models demonstrate, and may be associated with a lack of microglial cellular activation. With stimuli that results in acute pain, microglia are not activated, astrocyte activation occurs immediately (rather than the delayed response in chronic pathways) and then subsides after several days (rather than months to years in the chronic pain process) (Romero-Sandoval et al., 2008). Figure 4 illustrates the temporal nature of acute pain glial activation. Thus, without the activation of microglia to initiate the chronic pain process, acute pain models currently demonstrate that microglia and astrocyte activation is a specific and necessary component to chronic pain physiology. However, because there is evidence indicating glial activation can occur within one week of injury (Winkelstein, Rutkowski, Sweitzer, Pahl, & DeLeo, 2001), the possibility that microglial activation contributes to acute pain responses cannot be ruled out.

Inflammatory response from glia. Once activated in the chronic pain model, glial cells release inflammatory mediator molecules including cytokines; more specifically these cells release pro-inflammatory interleukins (ILs) and tumor necrosis factor-alpha (TNF- α). Research suggests that pro-inflammatory cytokines can increase pain transmission in dorsal horn neurons (Reeve, Patel, Fox, Walker, & Urban, 2000), and increase the release of neurotransmitters from peripheral nociceptive sensory afferent nerves (Inoue et al., 1999; Morioka et al., 2002). These pro-inflammatory cytokines may also enhance pain via upregulation of AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid) and NMDA (N-Methyl-D-aspartate) receptor expression and activity (Beattie et al., 2002; Viviani et al., 2003; Wang et al., 2005). The pain-enhancing effects of pro-inflammatory ILs and TNF- α have been implicated in both pre-clinical (Dubovy, Jancalek, Klusakova, Svizenska, & Pejchalova, 2006; Sacerdote et al., 2008; Sung et al., 2004; Zelenka, Schafers, & Sommer, 2005) and human studies (Backonja, Coe, Muller, & Schell, 2008; Kadetoff, Lampa, Westman, Andersson, & Kosek, 2012; Zin, Nissen, O'Callaghan, Moore, & Smith, 2010) of chronic pain syndromes. All of these investigations indicate that where there is chronic pain, there are excessive levels of CNS pro-inflammatory cytokines. Thus, the literature supports the theory that through the intricate activation sequence of neuroglial cells, followed by the production and release of inflammatory molecules, chronic pain is initiated and maintained.

Chronic Pain Treatment

To date, the most common form of treatment for the chronic pain patient is opioid analgesics. Approximately 3% of adults in the U.S. receive long-term opioid therapy for

treatment in chronic non-cancer pain, with close to six million hospital visits per year directed at obtaining opioid prescriptions (Boudreau et al., 2009; Caudill-Slosberg, Schwartz, & Woloshin, 2004). A study of over 70,000 chronic pain patients' healthcare utilization patterns found that prescriptive costs to treat the pain increase dramatically after a second year of treatment. Remarkably all of the chronic pain patients in this study realized a rise in their prescriptive costs, often exceeding \$5,100 per year (Ruetsch et al., 2013). The rising costs indicate what many practitioners treating chronic pain already know: As the pain continues, so does the use of increasing doses of medications to control the pain. As these drugs are used over time, tolerance (the need for increasing dosages to control pain) develops. Interestingly—glial activation and pro-inflammatory cytokines, known to be present in and contribute to chronic pain syndromes, may offer an explanation for the decrease in opioid efficacy.

Opioid-Glia Interactions

As previously described, studies have repeatedly shown that glial cell activation contributes to increased nociception following injury, and in the initiation and maintenance of chronic pain states. Recently, multiple preclinical studies have shown that the administration of long-term opioids also results in an increase in CNS glial activity (Eidson & Murphy, 2013; Hutchinson et al., 2011). This phenomenon could be mediated in part, by microglial kappa- and mu-opioid receptors (Chao et al., 1996), as well as toll-like receptors on glial cells (TLRs) that bind opioid molecules (Chao, Hu, Sheng, Gekker, & Peterson, 1997). Opioid molecules binding to both types of receptors initiate microglial activation with subsequent production and release of pro-inflammatory

cytokines (Woller & Hook, 2013). Astrocytes have also been found to have mu-, delta- and kappa-opioid receptors, and respond similarly to opioid agonist effects (Dobrenis, Makman, & Stefano, 1995; Eriksson, Hansson, & Rönnbäck, 1990). Figure 5 depicts how opioid administration increases nociception via activated glia and the pro-inflammatory response.

Thus, based upon this, theories hypothesizing glia as neuroimmune cells that contribute solely to structure and function of neuronal cells are incorrect. Pre-clinical studies of chronic opioid administration in the absence of chronic pain syndromes demonstrate that this glial-induced increase in CNS cytokine activity contributes to both opioid tolerance and pain enhancement in opioid withdrawal (Eidson & Murphy, 2013; Johnston et al., 2004; Raghavendra, Tanga, & DeLeo, 2004). These investigations suggest that not only are glia partially responsible for the maintenance of chronic pain, but they are also responsible for decreasing the efficacy of opioid drugs, and thus, one of the most common treatments of pain. It is hypothesized that tolerance results when opioid-activated immune cell pro-inflammatory cytokine production leads to up-regulation of CNS nociceptive neurons, increasing pain perception and severity (Raghavendra et al., 2002; Raghavendra et al., 2004; Song & Zhao, 2001). Thus, when treating chronic pain with opioids, over time the very treatment prescribed to relieve the pain will become part of a self propagating cascade of events responsible for perpetuating the pain, increasing the level of pain, decreasing the effectiveness of treatment (through opioid tolerance), and contributing to allodynia.

Glial Inhibition in Chronic Pain Treatment

Inhibiting glial cells is not currently an option in treatment of chronic pain patients because the health implications to the host are still being explored. Multiple investigations using pre-clinical neuropathic pain models have shown that arresting glial cell function *in vivo* using fluorocitrate, a glial metabolic inhibitor, attenuates neuropathic pain in these animals (Lan et al., 2007; Nakagawa et al., 2007). Fluorocitrate has also been shown to enhance morphine analgesia (Song & Zhao, 2001). Minocycline, a microglia inhibitor and tetracycline derivative, may prevent glial hyperactivation, but it cannot be used in cases of established neuropathic pain unless it is given prior to the injury and is continued after the injury (Mika et al., 2010). This may be due, in part, to the fact that minocycline suppresses microglia but not astrocytes (Yoon, Patel, & Dougherty, 2012) which are believed responsible for perpetuating chronic pain once established. Intrathecal or peripheral administration of minocycline has, however, demonstrated the ability to reduce the symptoms of allodynia and hyperalgesia (Ledeboer et al., 2005; Mika, Osikowicz, Makuch, & Przewlocka, 2007), and has also demonstrated neuroprotective properties in neurodegenerative disease associated with glial activation (Tikka, Fiebich, Goldsteins, Keinanen, & Koistinaho, 2001). Not surprisingly minocycline has also been shown to reduce tolerance to morphine in a dose-dependent manner when administered intrathecally (Cui et al., 2008).

Inflammatory Mediator Inhibition in Chronic Pain Treatment

A new direction for research in chronic pain treatment involves targeting the pro-inflammatory mediators released by glia. Two methods to alter the activity of pro-inflammatory cytokines are commonly cited in pain studies: inhibiting the production of

the cytokine (Genevay, Stingelin, & Gabay, 2004; Iwatsuki et al., 2013; Ohtori et al., 2012b) or blocking the cytokine's receptor binding site (Hutchinson et al., 2008; Shavit, Wolf, Goshen, Livshits, & Yirmiya, 2005; Wolf, Gabay, Tal, Yirmiya, & Shavit, 2006). The results of animal model studies targeting cytokines are compelling: Several studies have suggested that morphine tolerance can be slowed or reversed by genetically inactivating the pro-inflammatory cytokine interleukin-1 (IL-1) or by blocking IL-1 with a receptor antagonist (Hutchinson et al., 2008; Shavit et al., 2005). As predicted from the theoretical discussion above, the analgesic time and efficacy of both morphine and methadone were found to increase when IL-1 was blocked with a receptor antagonist (Hutchinson et al., 2008). Hence, receptor antagonists may offer one approach to improve opioid pharmacology in the chronic pain population where opioid tolerance and analgesic efficacy must be ameliorated.

Ibudilast, a phosphodiesterase inhibitor, selectively targets the synthesis of pro-inflammatory cytokines by microglia and has been globally used as a treatment for asthma, a disease often associated with inflammation of the respiratory system (Rolan, Hutchinson, & Johnson, 2009). Given that pro-inflammatory cytokines have also been implicated in the initiation and maintenance of chronic pain, it is encouraging to see this drug has been effective in blocking neuropathic pain symptoms in animal models (Ledeboer et al., 2007). In addition, ibudilast has also been shown to enhance morphine and oxycodone analgesia, further supporting the possibility that opioid management of chronic pain patients could benefit by using this type of pharmacotherapy (Hutchinson et al., 2009).

The possible effects of using these approaches to treat chronic pain, delay opioid tolerance in humans, and their potential side effects remain unknown. It should be emphasized, however, that inhibition of pro-inflammatory cytokines does not appear to interfere with the neural basis of the acute pain process (Milligan et al., 2001; Sweitzer, Martin, & DeLeo, 2001). This treatment could potentially offer a possible treatment for chronic pain without interfering with acute, protective pain physiology. Thus, the literature suggests that there are several modalities that could be explored for future treatment of chronic pain involving direct manipulation of glial cells or the inflammatory markers they release, but additional studies are needed. Figure 6 provides a synopsis of glial-mediated inflammation inhibition and the agents used to block the neuroimmune effects (Thomas & Hutchinson, 2012).

Implications for Future Treatment of Chronic Pain

If future human-based studies can illustrate the same link that preclinical studies show between glial activation, inflammatory mediator release, chronic pain persistence and decreased opioid efficacy, the implications for treatment in the chronic pain patient population are far-reaching. Research demonstrates that by blocking a pro-inflammatory cytokine in rodents, the effective analgesic duration of an intrathecally-administered opioid can be doubled, and the analgesic duration of systemically administered opioids can be increased more than one-and-a-half times. The same study also demonstrated an approximately seven-and-a-half-fold increase in analgesic efficacy with morphine by administering the opioid in combination with a receptor blocker of IL-1. These results were found with both morphine and methadone (Hutchinson et al., 2008).

Similar investigations demonstrate that drugs which block a pro-inflammatory cytokine in the CNS enhance intrathecally-administered opioid analgesia, blunt opioid tolerance, and reduce both newly developed and established allodynia and hyperalgesia (Johnston et al., 2004). Ibudilast has been shown to increase the analgesic potency of morphine and oxycodone by three to five times the average potency when administered concurrently with the opioid (Hutchinson et al., 2009). The same class of drugs targeting TNF- α has been used successfully in autoimmune disease therapy for rheumatoid arthritis, psoriasis, and enteritis (all of which have inflammatory etiologies) (Sfikakis, 2010), and has also been shown to decrease neuropathic pain when administered intrathecally in a pre-clinical trial (Marchand et al., 2009). In addition, drugs in this class are being investigated for use in the treatment of sciatica (a painful nerve compression condition) (Genevay et al., 2004).

Similarly, anti-interleukin receptor monoclonal antibody therapy has been shown to reduce pain in patients with lumbar spinal stenosis (Ohtori et al., 2012a) and in rheumatoid arthritis (a painful inflammatory disorder of the joints) (Burmester et al., 2011). In many of these studies, therapy targeting interleukins improved symptoms for weeks with little to no side effects noted except serious infection in one study (Burmester et al., 2011). Pre-clinical investigators noted that anti-interleukin treatment was only effective against mechanical allodynia if given at the time of injury; delayed administration of the TNF- α blocker demonstrated no clinical improvement in mechanical allodynia (Marchand et al., 2009).

Thus, further progress and understanding of safe interruption of glial cell and cytokine participation is necessary for future chronic pain treatment. Research suggests that chronic pain could be treated with opioids that would have a longer duration of action and increased efficacy. With additional understanding, opioid tolerance could be curbed, and the allodynia and hyperalgesia chronic pain patients experience may be reduced.

Conclusion

Animal models show that chronic pain states, opioid tolerance, hyperalgesia, and allodynia can be linked to glial activation and increased levels of pro-inflammatory cytokines. By blocking the pro-inflammatory cytokines or the glial cells which produce them, studies have shown that analgesia time and efficacy can be dramatically increased, opioid tolerance can be improved, and hyperalgesia and allodynia can be diminished. Advancements in inflammatory inhibition of other diseases are showing similar results. Because our current treatment techniques for chronic pain are restrictive and ineffective for many chronic pain patients, additional human evidence needs to be accumulated to illustrate the link between glia, cytokines, and chronic pain. An in depth understanding of these complex relationships will hopefully lead to innovative therapies that can safely interrupt the devastating consequences of the self-perpetuated cycle of neuroinflammation underlying chronic pain syndromes and scaffold highly effective opioid therapy.

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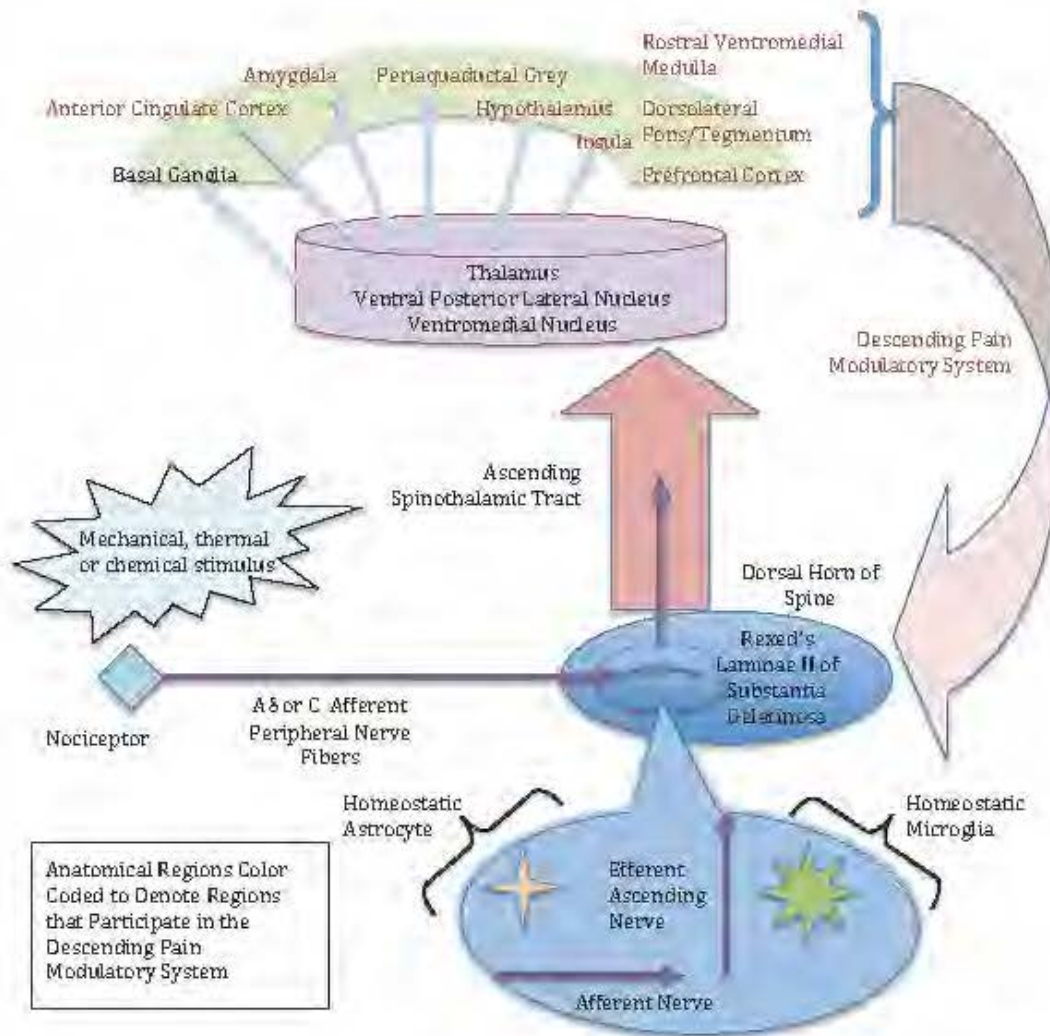


Figure 1. Acute pain process. In the acute pain physiology, nociception is regulated by an ascending and descending modulating system. Microglia and Astrocytes in the central nervous system are not activated and do not increase nociceptive relay of action potentials.

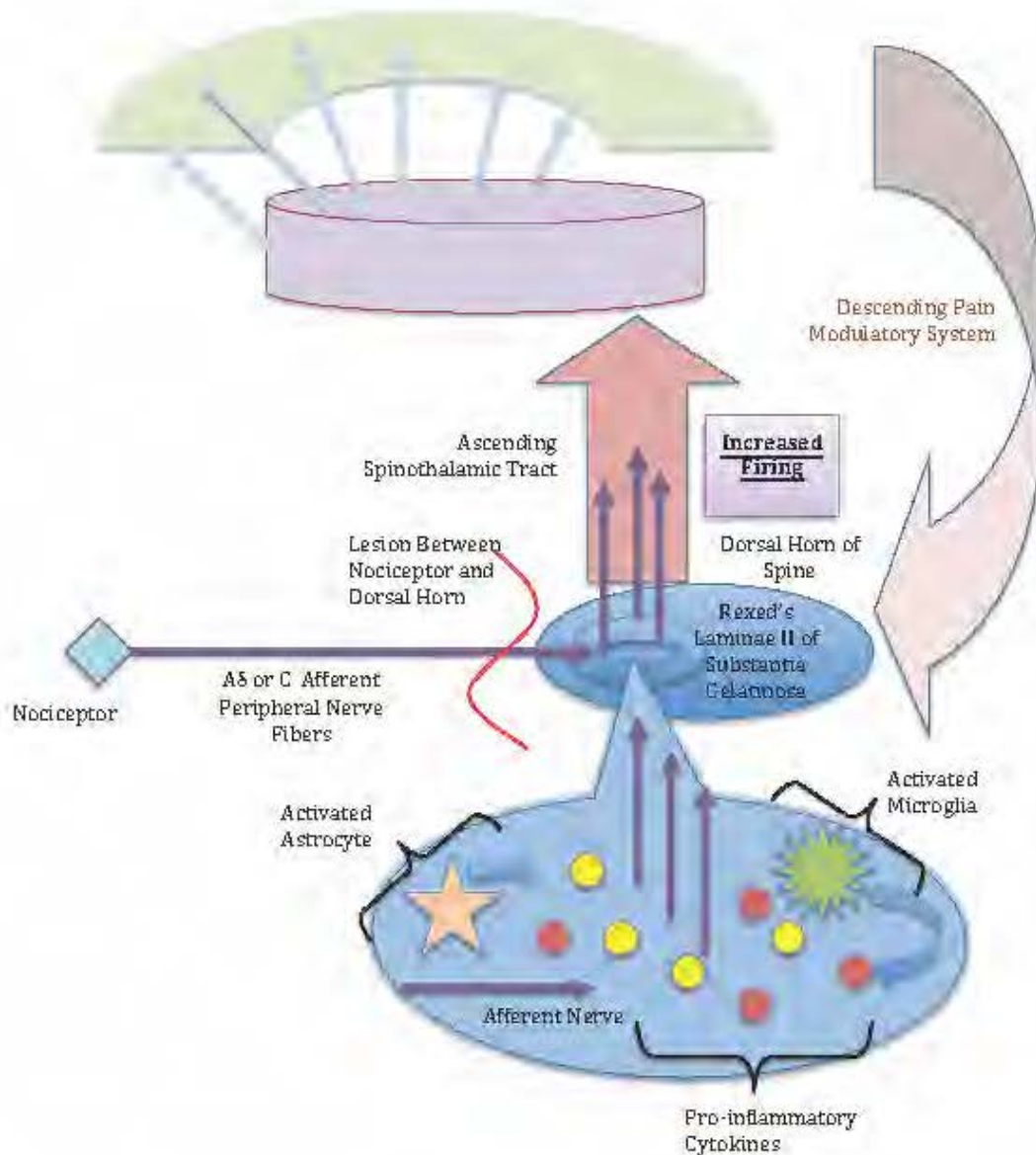


Figure 2. Glial mediated increased nociception. Glial cells increase nociceptive relay of action potentials through release of pro-inflammatory cytokines in the central nervous system after peripheral nerve injury.

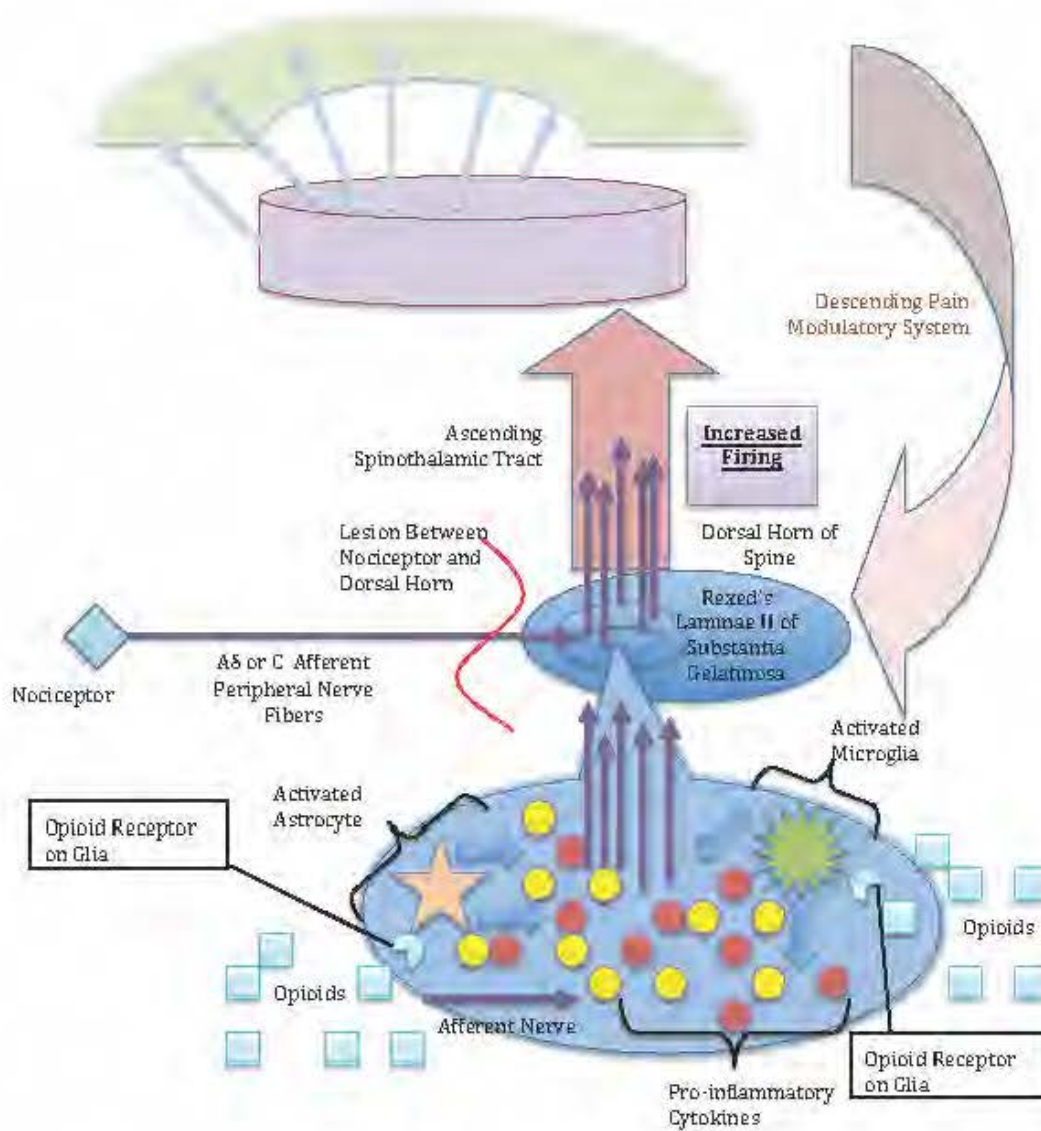


Figure 5. Opioid mediated increased nociception. When chronic pain is treated with opioid analgesia, both a peripheral nerve injury and treatment with opioid analgesia will increase nociceptive relay of action potentials through increased Glial activation in the central nervous system.

Manuscript II

UNIVERSITY OF SAN DIEGO

Hahn School of Nursing and Health Science

American Association of Nurse Anesthetists Foundation General Research Grant

Sarah E. Giron, PhD(c), CRNA

I. Title

Psychoneuroimmunology and Chronic Pain

II. Specific Aims

Chronic pain is a serious health concern of tremendous scope. An estimated 116 million Americans suffer and are disabled by this diagnosis (Tsang et al., 2008) at a cost of \$630 billion in lost work revenue, sick time, health care costs (Gaskin & Richard, 2011) and most importantly, at the cost of the patient's quality of life. Patients report not only a wide spectrum of painful symptoms, but also suffer from the psychological devastation pain can accompany. Current modalities utilized to treat these patients both physically and psychologically are limited, and often ineffective for a large portion of the chronic pain population. Recent investigations of the underlying causes of chronic pain have revealed a new direction for advancement: Central inflammatory mechanisms that are believed to exacerbate and prolong neuropathic pain states (Kreutzberg, 1996; Reeve, Patel, Fox, Walker, & Urban, 2000; Samad, Wang, Broom, & Woolf, 2004; Watkins & Maier, 2003; Watkins, Milligan, & Maier, 2001) as well as sleep deprivation (Dauvilliers et al., 2014), anxiety (Baker et al., 2001) and depression (Bonne et al., 2011; Levine et al., 1999; Lindqvist et al., 2009). Though preclinical data is accumulating, there remains a dearth of human data in this rapidly developing correlation between central inflammation, chronic pain and the psychology of pain.

This study will investigate the emerging relationships between chronic pain syndromes and the role of central inflammation, as measured by cerebrospinal fluid (CSF) patterns of inflammatory mediator molecules. More specifically, this study will

investigate if the same central pro- and anti-inflammatory cytokines found in chronic pain-induced animal models (Inoue et al., 1999; Morioka et al., 2002; Raghavendra, Tanga, & DeLeo 2004; Song & Zhao, 2001) are found in human chronic pain patients. This study will also explore the psychological responses to chronic pain as evidenced by scores on the Pittsburgh Sleep Quality Index, Beck Depression Inventory and the Hamilton Anxiety Scale, as well as chronic pain patients' perception and experience of pain as measured by the McGill Pain Questionnaire. Relationships between the psychological responses to pain and the pain experience will be made and then correlated to CSF levels of inflammatory markers. Because there is a scarcity of human evidence supporting the link between physical and psychological pain and central levels of inflammatory mediator molecules associated with these constructs, this study hopes to provide supportive evidence of this multimodal relationship.

Specific Aim #1: This study will examine and characterize the relationships between chronic pain and pro- and anti-inflammatory mediator molecules.

Hypothesis: Patients with chronic pain syndromes will have elevated CSF levels of proinflammatory cytokines IL-1, IL-6, IL-8 and TNF- α and decreased levels of anti-inflammatory IL-10 as detected on high sensitivity enzyme-linked immunosorbent assay (ELISA) when compared to control samples of CSF.

Specific Aim #2: To explore the relationship between the psychological responses to chronic pain and the concentration of pro- and anti-inflammatory cytokines in the CSF.

Hypothesis: There will be a positive correlation between the severity of chronic pain as measured by the Short Form McGill Pain Questionnaire (SF-MPQ), degrees of depression as measured by the Beck Depression Index (BDI-II), sleep disturbance as measured by the Pittsburgh Sleep Quality Index, and anxiety as measured by scores on the Hamilton Anxiety Scale (HAM-A), and the levels of proinflammatory cytokines in the CSF. There will be an inverse correlation between the severity of symptoms and the anti-inflammatory IL-10.

By gaining knowledge of the relationship between the psychoneuroimmune responses to prolonged pain states propagated by central inflammatory mechanisms, we can acquire the information pertinent to more effective analgesic and psychological treatment of these patients. It is the goal of this study to explore the psychoneuroimmune relationship of central inflammation, chronic pain, sleep deprivation, anxiety and depression in the chronic pain population.

III. Research Strategy

A. Significance

Evidence Supporting Central Inflammatory Contributions to Chronic Pain

A myriad of mechanisms have been hypothesized to contribute to the maintenance of chronic pain states, but one of the most promising new constructs described in the literature is via glial cell activity. Recent literature has shown that glia are neuroimmune cells that are activated following tissue injury, illness or inflammation (Tsuda, Inoue, & Salter, 2005; Watkins & Maier, 2000). Once activated, they release a cascade of molecules, predominantly inflammatory cytokines, which have been shown to

upregulate pain transmission and experience centrally (Kreutzberg, 1996; Watkins, Milligan, & Maier, 2001), thus they are a fundamental component for enhanced pain perception (Watkins & Maier, 2003). These proinflammatory cytokines sensitize dorsal horn neurons in pain transmission (Reeve, Patel, Fox, Walker, & Urban, 2000; Samad, Wang, Broom, & Woolf, 2004), stimulate the release of neurotransmitters from sensory afferent nerves responsible for pain perception (Inoue et al., 1999; Morioka et al., 2002) and may enhance pain via upregulation of excitatory amino acids systems (i.e., AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid) and NMDA (N-Methyl-D-aspartate) receptor expression and activity) (Beattie et al., 2002; Samad, Wang, Broom, & Woolf, 2004; Viviani et al., 2003; Wang, et al., 2005).

Clinical research on central inflammatory cytokines in humans with chronic pain is very limited and developing now. Prior human studies have focused on measuring inflammatory cytokines through serum analysis secondary to the decreased invasiveness and ease of collection. However, there is evidence that these peripheral markers of inflammation may not reflect levels in the central nervous system (CNS), which are those most relevant to the proposed work. Levels of cytokines found to be high in CSF were found to be low or undetectable in the serum (Backonja, Coe, Muller, & Schell, 2008; Lundborg, Hahn-Zoric, Biber, & Hansson, 2010; Zin, Nissen, Moore, & Smith, 2010). While glia are uniformly distributed throughout the CNS, it has been reflected in the literature that *only* glia in the spinal cord are activated following nerve injury (Zhang et al., 2008). Therefore to assess the inflammatory markers related to neuropathic pain, specimens drawn closest to the glia that release the markers would be most accurate in

assessing the types and amounts of markers responsible for this type of inflammatory nociception. This study will focus on CSF analysis because of the central location of glia and cytokines, rather than serum, as a means of supportive analysis for developing literature on the role of central neuroimmune responses and chronic pain.

Prior evidence supporting the use of CSF in the examination of cytokine patterns in chronic pain subjects has focused on patients with Complex Regional Pain Syndrome (Alexander, Perreault, Reichenberger, & Schwartzman, 2005; Alexander et al., 2007; Backonja et al., 2008; Munts et al., 2008); lumbar radiculopathy (Ohtori et al., 2011); chronic pain patients receiving intrathecal opioid infusions for pain management (Zin et al., 2010); persistent headache (Rozen & Swidan, 2007); chronic migraine (Peres et al., 2004); neuropathic pain related to previous spinal surgery (Capelle et al., 2009) and chronic osteoarthritis pain (Lundborg et al., 2010). Various inflammatory cytokines have been found to be elevated in the CSF of human pain study participants including interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and soluble TNF α receptor (TNF α). Other studies have shown a decrease in anti-inflammatory cytokine interleukin-10 (IL-10).

Evidence Supporting Inflammatory Contributions to Anxiety, Depression and Sleep

Deprivation

Part of the psychoneuroimmunologic component to pain is the interaction between cytokines and the behavioral complex known as sickness behavior. Feelings of sickness can be characterized by fatigue, decrease appetite, fever, lack of mobility, sleep disturbances, depression and inability to concentrate (Dantzer, 2001; Hart, 1988; Watkins

& Maier, 2000). Though the neuroimmune-mediated sickness behavior may promote unsavory feelings, this natural response to illness is paramount in healing and is usually short-lived with resolution of the offending illness. However, like chronic pain, when the mechanism of the sickness behavior goes awry, feelings of depression and anxiety, and sleep disturbances can be perpetuated longer than the illness or stress. If the effects of anxiety, depression and sleep deprivation are related to the illness or stress of pain, the mechanism is still unclear. Inflammatory cytokines offer one possible explanatory link between all of the psychoneuroimmune responses and pain, but central levels of cytokines in humans have been relatively unstudied thus far.

Peripheral levels of inflammatory cytokines have been examined with conflicting results in human studies related to anxiety disorders (Hoge et al., 2009; Koh & Lee, 2004; van Duinen et al., 2008). However, these peripheral results do not clarify if there is a central mechanism that links anxiety, inflammation and pain. The data on central inflammation and anxiety is very limited. Similar to chronic pain studies that examine peripheral and central cytokine patterns, anxiety research exemplifies the same theme: Peripheral levels of cytokines do not always exemplify what is found centrally. Baker and colleagues discovered increased IL-6 levels in CSF but not in serum when they analyzed PTSD patients (Baker et al., 2001). Clearly, more studies are necessary to examine the relationship between anxiety, commonly found with pain-related conditions, and the centrally mediated response of inflammation.

The direct correlative relationship between depression and pain has been thoroughly documented in the literature showing that both depressed patients exhibit at

least one painful symptom (Vaccarino, Sills, Evans, & Kalai, 2009) and chronic pain patients frequently exhibit depression (Dworkin & Gitlin, 1991). Similar to the anxiety literature, the relationship between levels of pro- and anti-inflammatory cytokines and their contribution to the pathophysiology of depression are not fully understood. Because antidepressants work centrally and have been shown to reduce levels of inflammatory cytokines (Basterzi et al., 2005; Janssen, Caniato, Verster, & Baune, 2010), the central pro- and anti-inflammatory patterns of these cytokines must be examined in the depressed patient. Cerebrospinal fluid levels of cytokines have been measured in unmedicated depressed patients (Levine et al., 1999), attempted suicide patients (Lindqvist et al., 2009), and chronic PTSD sufferers (Bonne et al., 2011) all with conflicting results. Clearly chronic pain and depressive symptoms are usually experienced together, but there is no data currently available exploring the role of central inflammation and the pathophysiology of these co-existing symptoms. More data is necessary to ascertain the relationship of central inflammation and depression, especially in the chronic pain population.

The complex relationship between pain and poor sleep quality is one that has been extensively documented in the literature, but its causality not fully understood. Up to 53% of chronic back pain patients (Tang, Wright, & Salkovskis, 2007) and 88% of neuropathic pain patients (Meyer-Rosberg et al., 2001) report sleep disturbances. Thus the literature supports that sleep and pain do interact with one another, sleep abnormalities are frequently reported by the chronic pain population, and the effects are not benign (Fulda & Schulz, 2001). Unfortunately, monitoring of central inflammatory

cytokines in human sleep studies has been very lacking thus far, and those studies that have monitored peripheral concentrations of inflammation have yielded conflicting results. Peripherally monitored cytokines have been measured in humans, but on a limited basis and have focused on IL-1, IL-6 and TNF α with conflicting results (Heffner et al., 2011; Hong, Mills, Lored, Adler, & Dimsdale, 2005; Irwin et al., 2004; Vgontzas et al., 2003). There is scant data to support that proinflammatory cytokines may contribute to the poor sleep quality endured by chronic pain patients, but central levels have not been examined even with the knowledge from prior animal models that central levels of cytokines do scaffold involvement with sleep (Kapás et al., 1992; Obál, & Krueger, 1991; Shoham, Davenne, Cady, Dinarello, & Krueger, 1987; Tobler, Borbely, Schwyzer, & Fontana, 1984). More data is crucial to discovering the role of central inflammation on sleep deprivation in the chronic pain patient.

B. Innovation

Review of the current literature on central inflammatory cytokine patterns reveals new relationships among chronic pain, sleep deprivation, anxiety and depression. Though the evidence indicates chronic pain, sleep deprivation, anxiety and depression are associated with central inflammatory changes, the mechanisms responsible require further description. Confirmatory studies are needed to clarify the pathways that have been tentatively identified as new relationships emerge. Thus, the proposed study is justified, in that it will further describe the pathophysiology of pain pathways, as well as explore novel relationships between the psychological responses to chronic pain and inflammatory mediator release.

If this study and future human-based studies can replicate prior findings, the implications for physical and psychological chronic pain treatment in our patient population are highly significant: Prior animal studies have demonstrated that by blocking the release of the proinflammatory IL-1, the length of analgesic effect of an opioid can double, there is an approximate seven and a half-fold increase in analgesic efficacy, opioid tolerance is blunted and both newly developed and established allodynia and hyperalgesia, both of which are common in chronic pain states, can be reduced (Hutchinson et al., 2008; Johnston et al., 2004). Through antagonism of the proinflammatory molecule $TNF\alpha$, the same agent that was shown to decrease both pain and hyperalgesia in animals has also preliminarily been shown to decrease depression, pain and inflammation in human subjects (Aldred, 2001; Sommer, Schäfers, Marziniak, & Toyka, 2001; Tying et al., 2006). Clearly human studies are paramount in progressing towards integrating comprehension of chronic pain pathways with treatment of the entire patient pain experience.

The proposed project will investigate the role of inflammation in the CNS and the psychological impact of chronic pain. This study utilizes a rare opportunity to collect CSF from patients prior to and while undergoing a trial of an intrathecal opiate drug delivery system for treatment of chronic pain. An interdisciplinary team of nurse anesthetist researchers, pain management clinicians and psychoneuroimmunology (PNI) researchers will collaborate to measure CSF levels of inflammatory mediator molecules and evaluate the psychological status as each patient prepares for and then undergoes an intrathecal opioid catheter trial.

This study is a novel and unique approach to bridge chronic pain and PNI research. Additionally, collaboration among nurse anesthetist researchers, the physicians at the University of California, Los Angeles (UCLA) Pain Management Clinic and PNI researchers in the area of chronic pain would be instrumental in developing future clinical studies in the field of chronic pain. This study clearly has translational potential, as it may lead to targeted neuroimmunologic therapies for chronic pain, identification of treatment disparities within this patient population, and scaffold a better understanding of the psychological components of chronic pain. Given the impact chronic pain has on society, the healthcare system and the economy, any discoveries that would allow for improved treatments for chronic pain would be of vital importance.

C. Approach

Design

This will be a cross-sectional, descriptive study with a convenience sample of approximately 30 chronic pain patients recruited from the Pain Management Clinic at UCLA. One time CSF draws will be analyzed and compared to control samples of CSF to allow for testing of the first hypothesis. Psychometric analysis of study participants will allow for testing of the second hypothesis.

Sample

Approximately thirty patients with the diagnosis and symptomatology of chronic pain who are eligible for and medically recommended treatment with a Food and Drug Administration (FDA)-approved intrathecal opioid infusion pain pump (the Medtronic SynchronMed® II Pain Pump) will be enrolled in this study after informed consent is

obtained. Potential participants will be recruited from the Pain Management Clinic at UCLA. Inclusion criteria include the patient being older than 18 years of age and have a chronic pain diagnosis greater than 6 months duration. Diagnosis will be made on standard clinical criteria, as suggested by the International Association for the Study of Pain (IASP). Exclusion criteria will include acute medical or psychiatric disorders, cancer, HIV, prior epidural injections for treatment of chronic pain, drug abuse, non-English speaking, meningitis or history of meningitis, active upper respiratory infection/flu or febrile nature, history of blood transfusion, acute psychological or physiologic danger/instability, altered mental status, palliative treatment, and pregnancy. The inclusion and exclusion criteria will be confirmed using standard clinical and laboratory methods of analysis and all patients will be instructed to continue all of their medications and therapies without making any changes prior to study involvement.

Measures

Beck Depression Inventory II. The Beck Depression Inventory II (BDI-II) will be obtained from each patient before collection of CSF to assess and score levels of depression. The BDI-II contains 21 questions, each answer being scored on a scale value of 0 to 3. The cutoffs used will be: 0–13: minimal depression; 14–19: mild depression; 20–28: moderate depression; and 29–63: severe depression. Higher total scores indicate more severe depressive symptoms (Beck, Ward, Mendeson, Mock & Arbough, 1961).

Hamilton Anxiety Scale. The Hamilton Anxiety Scale (HAM-A) measures the severity of a patient's anxiety, based on 14 parameters, each item given a 5-point score - 0 (not present) to 4 (severe) and includes anxious mood, tension, fears, insomnia,

intellectual impairment, depression, somatic complaints, autonomic symptoms, physiologic symptoms and behavior at the interview. A total anxiety score of <17 indicates mild anxiety levels; 18 to 24 moderate anxiety levels; and 25 to 30 indicates severe anxiety levels (Hamilton, 1959).

Short Form McGill Pain Questionnaire. The Short Form McGill Pain Questionnaire (SF-MPQ) consists of fifteen descriptors, eleven sensory descriptors and four that are affective in nature. Each descriptor is rated on a scale of 0-3: 0 (none) to 3 (severe). It also includes a Present Pain Intensity (PPI) index and a Visual Analogue Scale (VAS) (Melzack, 1975; Melzack, 1987).

The Pittsburgh Sleep Quality Index. The Pittsburgh Sleep Quality Index is composed of nineteen components that yield scores on subjective sleep quality, sleep latency, sleep duration, habitual sleep efficacy, sleep disturbance, use of sleeping medication and daytime dysfunction. These seven components are scored from 0-3 and yield a total score of 0-21. Higher scores indicate worse sleep quality (Buysse et al., 1989).

Procedures

Approximately thirty patients with the diagnosis and symptomatology of chronic pain who are eligible for and medically recommended treatment with an FDA-approved intrathecal opioid infusion pain pump (the Medtronic SynchroMed® II Pain Pump) will be enrolled in this study. Potential participants will be recruited from the Pain Management Clinic at UCLA. After informed consent is obtained using Institutional Review Board (IRB)-approved forms, subjects will complete the psychometric

instruments to measure sleep deprivation, depression, anxiety and pain experience. Patients will undergo a structured interview to compile their demographic information and results will be number coded to ensure anonymity. Upon completion of the psychological questionnaires, study participants will be given a twenty-five dollar Visa gift certificate for their participation in the study. Participants will also be reimbursed for their parking fees upon enrollment of the study for the preclinical office visit date and the date for the trial operation (a total of two parking fees per study participant).

Approximately one month after the preclinical office visit, the study participant will be scheduled to undergo a surgical outpatient operation called an intrathecal opioid infusion pain pump trial. This operative surgical procedure and study procedure of collecting intraoperative CSF samples will be performed in the operating suite at UCLA Santa Monica Orthopedic Hospital. Three milliliters (mL) of CSF will be collected from the approximate 30 patient study participants undergoing intrathecal opioid infusion pain pump trials for the treatment of their pain. Standard preoperative preparation and evaluation will take place in the preoperative suite of UCLA Santa Monica Orthopedic Hospital. A qualified member of the preoperative evaluation team will start peripheral intravenous fluids in the preoperative suite and preoperative vital signs will be recorded. The patient will be brought to the operating room and positioned per surgeon preference, and standard monitors (electrocardiogram, noninvasive blood pressure cuff, pulse oximeter) will be applied. Patients will be given 2-4 liters/minute nasal cannula oxygen and a combination of intravenous versed and fentanyl will be titrated for patient comfort by a qualified operating room nurse at the surgeon's direction. Strict, standard sterile

surgical protocol will be followed per the regulations and policies of UCLA Santa Monica Orthopedic Hospital guidelines. Intrathecal access will be accessed with or without the use of fluoroscopy per the surgeon's discretion. All samples of CSF will be collected intraoperatively during daylight hours in appropriate polypropylene specimen collection tubes, properly labeled with a number code for anonymous identification and placed on ice in UCLA approved specimen transportation boxes. A member of the research team will then transport specimens from UCLA Santa Monica Orthopedic Hospital to the UCLA Cousin's Institute Inflammatory Biology Laboratory in the 300 Medical Plaza building of the UCLA Westwood campus via car, with an estimated transportation time of thirty minutes. Upon receipt of the specimens, the UCLA Cousin's Institute Inflammatory Biology Laboratory will centrifuge and partition the 3 mL samples into 0.5-1 mL aliquots with appropriately labeled anonymous identification. The aliquots will then be stored at -80°C until batch analysis can be performed.

After collection of 3 mL of CSF in a specimen collection tube, the surgeon will place an intrathecal opioid infusion pain catheter and administer of a dose of intrathecal opioid. The catheter will be secured and dressed in a sterile fashion and the patient will be taken to the postoperative suite for recovery. The patient is then monitored for eight to ten hours for side effects and complications arising from the administration of an intrathecal opioid. The patients' hemodynamic stability and vital signs are also continually assessed during this eight to ten hour postoperative stay by a member of the pain management team and/or postoperative nursing staff. Once a member of the pain management team has determined that the patient has not incurred any serious side

effects from the administration of an intrathecal opioid, the patient is then eligible to have a permanent opioid infusion pain pump. The catheter is removed, a sterile dressing is placed over the operative site and the patient is discharged home with instructions and emergency contact information.

Control samples of CSF will be analyzed from the California NeuroAIDS Tissue Network obtained from Dr. Scott Letendre at the University of California, San Diego (UCSD). Thirty random, individual samples of 1-2 mL of CSF, collected from 2005-2012, will be utilized from this tissue bank as a means to compare levels of central inflammatory cytokines. Samples of 1-2 mL will be obtained in specimen collection tubes and placed on ice in the appropriate biological hazard container. The specimens will be shipped from UCSD to the UCLA Cousin's Institute Inflammatory Biology Laboratory in Los Angeles with an estimated travel time of two and half hours. Upon receipt of the specimens, the UCLA Cousin's Institute Inflammatory Biology Laboratory will centrifuge and partition the 1-2 mL individual samples into 0.5-1 mL aliquots with appropriately labeled identification. The aliquots will then be stored at -80°C until batch analysis can be performed.

Control and study participant CSF levels of IL-1, IL-6, IL-8, TNF α and IL-10 will be determined using high sensitivity enzyme linked immunosorbant assay (ELISA) (R&D Systems, Minneapolis, MN) or similar. All assays will be performed in duplicate per manufacturers recommendations. The cytokine levels for all of the samples obtained, both from UCLA and UCSD will be reported as picograms/milliliter (pg/ml).

Variables

Independent Variable: Diagnosis of Chronic Pain (as defined by the International Association for the Study of Pain) greater than 6 months in duration.

Dependent Variables: Proinflammatory Cytokines (IL-1, IL-6, IL-8, TNF α); Anti-inflammatory Cytokine (IL-10); Depression (as measured by Beck Depression Inventory II (BDI-II)); Anxiety (as measured by the Hamilton Anxiety Scale (HAM-A)); Pain (as measured by the McGill Pain Questionnaire).

Categorical Covariate Variables: Age; Gender; Cause of Chronic Pain; Length of time from initial diagnosis; Type of medication used to treat chronic pain; Length of time taking medication; Race; Education Level; Income/Socioeconomic Status; Level of Activity; Body Mass Index.

Possible Exclusion Criteria: Cancer; HIV; Prior epidural injections for treatment of chronic pain; Drug Abuse; Non-English Speaking; Meningitis or history of meningitis; Active URI/flu or febrile nature; History of blood transfusion; Acute psychological or physiologic danger/instability; Altered mental status; Palliative treatment; Pregnancy.

Analysis Plan

Descriptive statistics will be utilized to evaluate study CSF cytokine patterns and participant demographics. Statistically significant differences between patient and control group cytokine levels will be evaluated using multivariate statistical analysis; *t*-tests and chi-square tests for parametric data. To determine the relationship of cytokine

patterns on sleep deprivation, anxiety, depression and self-reported pain in the study participants, analysis of variance (ANOVA) at one/two-tailed $p < 0.05$ for parametric data will be performed. For non-parametric data Mann-Whitney U statistical analysis may be selected as the appropriate statistical tool to test the second hypothesis. Calculations will be accomplished with the aid of the statistical software SPSS version v.19 or newer (SPSS, IBM Inc., Armonk, NY).

The planned analysis at this time is primarily exploratory, and designed to answer the questions: Are there significant relationships between such psychological variables as sleep deprivation, depression, anxiety, or pain intensity and levels of inflammatory mediators in the CSF of the chronic pain patient? Is there a significant difference in the central levels of inflammatory cytokines of chronic pain patients and control samples of CSF? Following literature review, other analyses may be devised using a triangulation approach with the qualitative data obtained during the demographic interview.

D. Investigators

The principal investigator, Sarah E. Giron, CRNA, MS, is currently a Doctoral Student at University of San Diego (USD) Hahn School of Nursing and Health Science's PhD Program. Her prior research experience includes work at the San Diego Veterans Affairs Hospital for which she received the UCSD Undergraduate Excellence in Research Award. Ms. Giron has participated in data collection at UCLA's Department of Integrative Biology and Physiology, as well as a private gastroenterology office, La Jolla Gastroenterology. She has participated in Phase Three clinical trials of Sugammadex at

UCLA's Jules Stein Eye Institute and was awarded the 2012 American Association of Nurse Anesthetists (AANA) Baxter Research Doctoral Fellowship. Ms. Giron will be responsible for managing study operations, coordinating communication amongst team members and patients, and facilitating data collection logistical arrangements.

Co-Investigator Charles Griffis CRNA, PhD, Associate Professor, UCLA Department of Anesthesiology and School of Nursing, is a well-accomplished nurse anesthesia expert in inflammation/chronic pain research. As a highly respected member of the nurse anesthesia academic and UCLA research community, Dr. Griffis was awarded the AANA Researcher of the Year Award in 2010. His numerous publications in a wide array of multi-disciplinary peer-reviewed journals also affirm his expertise in academic research writing. Dr. Griffis will provide faculty support at UCLA, project oversight pertaining to the protocol development and project details at UCLA and provide mentorship to Ms. Giron in academic writing and manuscript publication.

Co-Investigator Joseph Burkard, CRNA, DNSc, Associate Professor, USD Hahn School of Nursing and Health Science, is also a well-accomplished nurse anesthesia educator and researcher. Dr. Burkard has been awarded Instructor of the Year by both the Navy and Kaiser Permanente Programs of Nurse Anesthesia and has been funded by USD and Military Grants for his ongoing research in Simulation and Emergence Delirium. Dr. Burkard will be providing faculty support at USD, oversight for procedures at USD, grant subcontract coordination between UCLA and USD, guidance with statistical analysis and manuscript writing.

E. Environment

The UCLA Santa Monica Orthopedic Hospital and UCLA Cousin's Inflammatory Biology Laboratory will serve as the data collection and analysis site of this study, which will be primarily based at the USD Hahn School of Nursing and Health Science, PhD Program. The study will serve as the dissertation project for USD Doctoral Student, Sarah Giron CRNA, MS. Charles Griffis, CRNA, PhD and Joseph Burkard, CRNA, DNSc will provide academic oversight of the dissertation process. Ms. Giron will be the principal investigator for the project, responsible for developing, managing and collaborating with team members to ensure an ethical and comprehensive research strategy.

The UCLA Department of Anesthesiology, Pain Management Clinic would seem to be an ideal setting for the participant recruitment necessary for this study. Andrea Nicol, MD, Assistant Clinical Professor and Director of Research for UCLA Pain Management Center and Michael Ferrante, MD, Director of UCLA Pain Management Center and Team, UCLA Department of Anesthesiology, have confirmed that once IRB approval is obtained, the patients could be recruited into the study in the clinic setting. The psychological instruments can be administered in the preoperative timeframe. The CSF sample collection can be accomplished at the time of an intrathecal catheter trial, and would not seem to require any escalation in care, nor present any increased risk to the patient. The UCLA Norman Cousins PNI Institute will provide support for the PNI theory aspects of the study through collaboration with Michael Irwin, MD, Professor and Director, an internationally recognized expert in this area. Additionally, Elizabeth Breen, PhD, a research immunologist with extensive experience in cytokine and inflammatory

molecule analysis will provide oversight of the immune analyses. Dr. Breen is the Director of the Inflammatory Biology Laboratory, which is a fully equipped research laboratory, located within the Cousins Center, and will be used for study sample analysis.

IV. Biographical Sketch of Key Researchers

Sarah Giron, CRNA, MS—USD Doctoral Student, Study Principal Investigator, AANA Baxter Research Doctoral Fellowship Grant Recipient

J. Burkard, CRNA, DNSc—Associate Professor, USD Faculty Support and Oversight

C. Griffis CRNA, PhD—Associate Professor, UCLA Faculty Support and Oversight

V. References

See Word Attachment

VI. Timeline

See Word Attachment

VII. Budget

See Attachment

VII. Appendices

Please see the following attachments:

Sarah Giron's CV

Picture of Sarah Giron

Biographical Sketch of Sarah Giron

Dr. Charles Griffis' CV

Dr. Joseph Burkard's CV

Letter to Foundation Addressing IRB Application and Study Progress

High Sensitivity ELISA Instructions for

- a. ELISA HS100C
- b. ELISA hs600b
- c. ELISA hslb00c
- d. ELISA HSTA00D
- e. ELISA MEA001

Psychological Instruments

- a. The Short Form McGill Pain Questionnaire (SF-MPQ)
- b. Beck Depression Inventory II (BDI-II)
- c. The Hamilton Anxiety Scale (HAM-A)
- d. The Pittsburgh Sleep Quality Index

Letters of Support

- a. Dr. Andrea Nicol
- b. Dr. Elizabeth Breen
- c. Dr. Charles Griffis
- d. Dr. Joseph Burkard

IV. Biographical Sketch of Key Researchers

Sarah Giron, CRNA, PhD(c), is a Clinical Instructor of Anesthesiology at the University of Southern California (USC) where she is a full-time Faculty Nurse Anesthetist at the Keck Hospital of USC. She is currently enrolled as a PhD Doctoral Candidate at the University of San Diego's Hahn School of Nursing and Health Science, and is an active Committee Chair and Editor for her state nurse anesthetist association, the California Association of Nurse Anesthetists. She has authored several chapters in nurse anesthesia textbooks and will be featured as the guest speaker for the 2013 graduating USC Program of Nurse Anesthesia Commencement Ceremony. Her most recent funded research endeavors focus on the role of inflammation in the psychology and physiology of chronic pain.

Joseph Burkard, CRNA, DNSc, Associate Professor, USD Hahn School of Nursing and Health Science, is a well-accomplished nurse anesthesia educator and researcher. Dr. Burkard has been awarded Instructor of the Year by both the Navy and Kaiser Permanente Programs of Nurse Anesthesia and has been funded by USD and Military Grants for his ongoing research in Simulation and Emergence Delirium.

Charles Griffis CRNA, PhD, Associate Professor, UCLA Department of Anesthesiology and School of Nursing, is a well-accomplished nurse anesthesia expert in inflammation/chronic pain research. As a highly respected member of the nurse anesthesia academic and UCLA research community, Dr. Griffis was awarded the AANA Researcher of the Year Award in 2010. His numerous publications in a wide array of multi-disciplinary peer-reviewed journals affirm his expertise in academic research writing.

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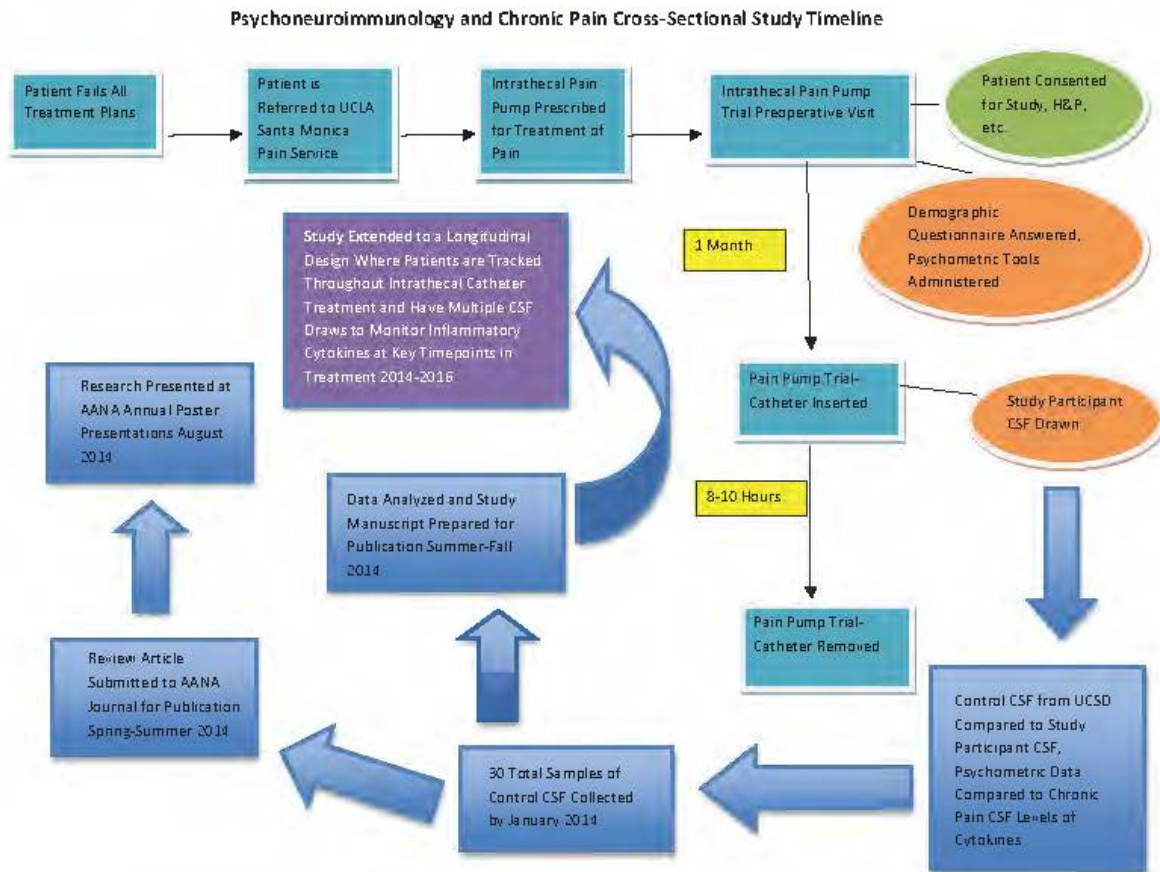
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VI. Timeline



Submitted October 2012 for AANA General Research Grant by S. Giran

VII. Budget

Appendix A – AANA Foundation Grants Budget Template

| DETAILED BUDGET | | Proposal Title: Psychoneuroimmunology and Chronic Pain | | | | | |
|--|---|--|----------|--|------------------|-----------|--------------|
| | | Date: September 26, 2012 | | | | | |
| PERSONNEL | | | | | | | |
| | PROJECT ROLE | | % EFFORT | | SALARY REQUESTED | | |
| Elizabeth Breen, PhD | Co- Investigator | | 81 | | 10582 | | |
| C. Perez | Lab Assistant, Processing Cytokine ELISAs | | 19 | | 2467 | | |
| | | | | | | Subtotal: | 13049 |
| CONSULTANT COSTS | | | | | | | |
| Name | Rate | Amount | | | | | |
| | | | | | | | |
| | | | | | | Subtotal: | |
| EQUIPMENT | | | | | | | |
| Item | Vendor | Quoted Cost | | | | | |
| Included in Personnel & Supply Lined Items | | | | | | | |
| | | | | | | Subtotal: | |
| SUPPLIES | | | | | | | |
| Item | Amount | Item | Amount | | | | |
| Lab Supplies, Processing, CSF High Sensitivity ELISA x 2 2012-13 | 1095 | | | | | | |
| Lab Supplies, Processing, CSF High Sensitivity ELISA x 2 2013-14 | 2738 | | | | | | |
| | | | | | | Subtotal: | 3833 |
| TRAVEL | | | | | | | |
| Name | Relation to Project | Destination | Amount | | | | |
| | | | | | | | |
| | | | | | | Subtotal: | |
| OTHER EXPENSES | | | | | | | |
| Item | Amount | Item | Amount | | | | |
| | | | | | | | |
| | | | | | | Subtotal: | |
| TOTAL DIRECT COSTS: | | | | | | | 16882 |

Budget Justification for Psychoneuroimmunology and Chronic Pain Cross-Sectional Study

Dr. Elizabeth Breen, PhD is an active Co-Investigator in this study and Director for the UCLA Cousins Inflammatory Biology Laboratory where all CSF samples will be analyzed. Her expertise in cytokine analysis and methodological experience with these studies is critical for successful development and completion of this study. Because of her academic appointment and position at the UCLA Cousins Institute of Psychoneuroimmunology Dr. Breen will need 5% salary and benefits to justify her participation in this study. One of her lab assistants, C. Perez, is skilled in handling and performing ELISAs on CSF samples and will be utilized from the Cousins Inflammatory Biology Laboratory as part of this study. This budget reflects the salary and benefits of both Dr. Breen and C. Perez.

All CSF samples will be analyzed with a high-sensitivity ELISA assay to assess for cytokines IL-1, IL-6, IL-8, IL-10 and TNF α . From previous literature, CSF levels of these cytokines are not detectable using a regular ELISA, thus a high-sensitivity is necessary to detect levels. The rates quoted in this budget represent consumables, assay plates, storage test tubes, pipettes, and other lab equipment necessary for analysis.

Upon receiving the CSF samples, the Cousins laboratory will be centrifuging the specimens to separate any precipitate, and making 0.5-1mL aliquots for deep freeze storage. This budget covers the labor of centrifugation and aliquots, and the test tube labeling for correct identification of study samples.

Once centrifuged and the aliquots of CSF samples are made, the specimens need to be preserved at -80°C until all specimens are available for duplicate analysis. This form of batch analysis decreases the rate of discrepancies if assays were performed as CSF samples arrived. Freezer space and handling are included in this budget.

CSF will need to be collected from the patients and transported to the lab in an appropriate container. Per previous studies, polypropylene specimen tubes are appropriate and will not alter the specimens for analysis. Because 2-3 mL of CSF will be collected, 4 mL polypropylene specimens tubes were selected from Fisher Scientific www.fishersci.com. The cost of the polypropylene specimen tubes are included in this budget.

VII. Appendices

Please see the following attachments:

Sarah Giron's CV

Picture of Sarah Giron

Biographical Sketch of Sarah Giron

Dr. Charles Griffis' CV

Dr. Joseph Burkard's CV

Letter to Foundation Addressing IRB Application and Study Progress

High Sensitivity ELISA Instructions

http://www.rndsystems.com/product_detail_objectname_elisa_assay_product.aspx

Psychological Instruments

Found in Appendices of Dissertation

Letters of Support

- Dr. Andrea Nicol
- Dr. Elizabeth Breen
- Dr. Charles Griffis
- Dr. Joseph Burkard

Sarah Elizabeth Giron, CRNA, MS



Home Phone

E-Mail



Education:

- High School Whitney M. Young, Chicago, Illinois, Illinois State Scholar, Honor Roll, 1997
- University University of California San Diego, La Jolla, California, BS (Biochemistry and Cellular Biology), 2001
- University Columbia University, New York, New York, BS (Nursing), Dean's List, 2002
- Graduate School Columbia University, New York, New York, MS (Nurse Anesthesia), Dean's List, 2005
University of San Diego, San Diego, California, PhD (Nursing), Anticipated Commencement 2014
- Honors and Awards
- The AANA Doctoral Research Baxter Fellowship Award, San Francisco, 2012
 - Eight and Forty Scholarship, The American Legion, New York, 2005, 2011, 2012
 - Award for Excellence, Sigma Theta Tau, Graduate Commencement, New York, 2005
 - Excellence in Research, UCSD Faculty Mentorship Program, Undergraduate Symposium, La Jolla, 2000
- Board Certification Registered Nurse, 2002-present
- Other Certified Registered Nurse Anesthetist, 2005-present
BLS and ACLS 2005-present

Professional Background:

Clinical Administrative Appointments

- Clinical Instructor of Anesthesiology, University of Southern California, Los Angeles, California, 2009-present
- Faculty Nurse Anesthetist, University of Southern California, Los Angeles, California, 2009-present
- Senior Certified Registered Nurse Anesthetist, University of California Los Angeles, Los Angeles, California, 2009-present

Angeles, California, 2006-2009

- Certified Registered Nurse Anesthetist, University of California Los Angeles, Los Angeles,

California, 2005-2006

- Burn Intensive Care Unit Registered Nurse, New York-Presbyterian Cornell Weill Medical

Center, New York, New York, 2002-2004

Specific Teaching Responsibilities

- Clinical Preceptor and Didactic Faculty Member, Keck School of Medicine Program of Nurse

Anesthesia, 2009-present

- Clinical Preceptor, Kaiser Permanente and USC Student Nurse Anesthetist out-patient clinical

rotations at UCLA, 2006-2009

- Undergraduate Clinical Coordinator, Fresno State University student Registered Nurse clinical

observation and volunteer rotation at UCLA, 2007-2008

Other

Editor in Chief, California Association of Nurse Anesthetists E-Journal, 2010-present

Editor, California Association of Nurse Anesthetists Newsletter, 2009-present

Society Memberships:

Distinguished

Sigma Theta Tau International, 2002–present

Professional

American Association of Nurse Anesthetists, 2003–present

California Association of Nurse Anesthetists, 2005-present

Service:

Professional Organizations

Editorial Committee Member, California Association of Nurse Anesthetists, 2011-present

Membership Engagement/Information Technology Committee Chairperson, California Association of Nurse Anesthetists, 2009-present

Committee Member, Information Technology Committee, California Association of Nurse Anesthetists, 2008–2009

University/Other Committees

University of Southern California/Keck School of Medicine Program of Nurse

Anesthesia Webmaster, University of Southern California, Los Angeles, California, 2010- present

Faculty Development Chairperson, University of Southern California, Los Angeles, California, 2009-present

LEAP Scholarship Foundation Vice President, Keck School of Medicine Program of Nurse Anesthesia, Los Angeles, California, 2011-present
LEAP Scholarship Foundation Secretary, Keck School of Medicine Program of Nurse Anesthesia, Los Angeles, California, 2010-2011
Quality Assurance Committee, University of California Los Angeles, Los Angeles, California, 2007-2009

Research Activities:

Major Areas of Research Interest

Psychoneuroimmunology, Chronic Pain, Pharmacology

Past Clinical Research

Sugammadex phase 3 clinical trials, University of California, Los Angeles, 2008-2009

Invited Lectures:

Distinguished

"Pain, Opioids and Inflammation: Update on Physiology." CANA Spring 2009 Conference, California Association of Nurse Anesthetists, Rancho Mirage, California, February 2009.

"Sugammadex and a Return to the Land of Neuromuscular Blockade." Winter 2008 Educational Conference, Kaiser Permanente Nurse Anesthetist Association, Anaheim, California, January 2008.

Other

"Sugammadex (Organon 25969) Update." Faculty Development Meeting, University of Southern California, Los Angeles, California, February 2011.

"Ethical Decision Making in Anesthesia." Co-Lecturer, USC School of Nurse Anesthesia, Los Angeles, California, November 2010, 2011.

"Anesthesia for Out-Patient Surgery." Lecturer, USC School of Nurse Anesthesia, Los Angeles, California, September 2010, 2011.

"Opioid Physiology and Pharmacology." Guest Lecturer, Samuel Merritt University, Oakland, California, August 2009, July 2012.

"Opioid and Neuroglial Interactions--What Happens When Opioids Aren't Enough?" Guest Lecturer, Samuel Merritt University, Oakland, California, August 2009, July 2012.

"Opioid Physiology and Pharmacology." Lecturer, USC School of Nurse Anesthesia, Los Angeles, California, June 2009, 2010, 2011, 2012.

"Opioid and Neuroglial Interactions--What Happens When Opioids Aren't Enough?" Lecturer, USC School of Nurse Anesthesia, Los Angeles, California, June 2009, 2010, 2011, 2012.

"Opioids and Benzodiazepines: A Physiology and Pharmacology Review." Continuing Education Series, UCLA Healthcare Registered Nurses, Los Angeles, California, April 2009.

"Sugammadex (Organon 25969) Update." Journal Club Continuing Education, UCLA Healthcare Nurse Anesthetists, Los Angeles, California, September 2006.

Bibliography:

Non Peer Reviewed

1. Sarah Giron: The Next Big Adventure. CANA Newsletter 65 (2) 11-12, 2011
2. Ann Keyes, Sarah Rozycki: Sugammadex (Org 25969) Update. CANA Newsletter 62 (2) 20-21, 2008.

Chapters in Press

1. Sarah Giron: Retina Surgery. In: Case Studies in Nurse Anesthesia 1st Ed; Sass Elisha, CRNA, Ed.D. Jones and Bartlett, Sudbury, Massachusetts, 2011.
2. Sarah Giron, Sass Elisha, Mark Gabot: Steroids. In: Pharmacology for Nurse Anesthesiology 1st Ed; Richard G. Ouellette, M.Ed., CRNA and Joseph Anthony Joyce, BS, CRNA. Jones and Bartlett, Sudbury, Massachusetts, 2011.



BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| | |
|--|--|
| NAME Giron, Sarah Elizabeth | POSITION TITLE Clinical Instructor of Anesthesiology |
| eRA COMMONS USER NAME (credential, e.g., agency login) | |

| EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.) | | | |
|---|---------------------------|-----------------------|--------------------------|
| INSTITUTION AND LOCATION | DEGREE (if applicable) | MM/YY | FIELD OF STUDY |
| University of California, San Diego (La Jolla, CA) | B.S. | 05/2001 | Biochem. & Cellular Bio. |
| Columbia University (New York, New York) | B.S. | 08/2002 | Nursing |
| Columbia University (New York, New York) | M.S. | 08/2005 | Nurse Anesthesia |
| University of San Diego (San Diego, CA) | PhD | Currently Enrolled | Nursing |

NOTE: The Biographical Sketch may not exceed four pages. Follow the formats and instructions below.

A. Personal Statement

Within the medical and nursing community it is imperative that advanced practice nurses take a more prominent role in research. Attaining a previous bachelor's degree in Biochemistry & Cellular Biology and experience with several undergraduate and postgraduate research projects, science has been a staple of my past and will continue to be a necessity for my future. I look forward to continuing research in the field of psychoneuroimmunology with accurate, clinically significant studies that focus on the central inflammatory process and psychological implications of chronic pain. Such studies should help define pain pathways and clarify more effective pain modality treatments both analgesically and psychologically.

B. Positions and Honors

Positions and Employment

| | |
|--------------|--|
| 1999 | Undergraduate Research Assistant, Neurology Dept., <u>University of California, Los Angeles</u> , Los Angeles, CA |
| 2000 | Hepatitis C Lookback Coordinator/Undergraduate Research Assistant, <u>Veterans Affairs</u> , La Jolla, CA |
| 1998-2001 | Lab Technician, La Jolla Gastroenterology, La Jolla, CA |
| 2002-2004 | Burn Intensive Care Unit Staff Nurse, <u>New York-Presbyterian Cornell Weill Medical Center</u> , New York, New York |
| 2005-2006 | Certified Registered Nurse Anesthetist, <u>University of California, Los Angeles</u> , Los Angeles, CA |
| 2006-2009 | Senior Certified Registered Nurse Anesthetist, <u>University of California, Los Angeles</u> , Los Angeles, CA |
| 2009-present | Faculty Nurse Anesthetist, <u>University of Southern California</u> , Los Angeles, CA Clinical Instructor of Anesthesiology, <u>University of Southern California</u> , Los Angeles, CA |

Other Experience and Professional Memberships

| | |
|--------------|---|
| 2002-Present | Sigma Theta Tau International Member |
| 2003-Present | American Association of Nurse Anesthetists Member |
| 2005-Present | California Association of Nurse Anesthetists Member |

CURRICULUM VITAE (04/2011)
CHARLES ALAN GRIFFIS, CRNA, Ph.D.



Date of Birth August 25, 1951

Education

Fall 2005 Doctor of Philosophy

School of Nursing, Graduate Division
University of California at Los Angeles
Los Angeles, California

Fall 1985 - 1987 Ed.Psych, Doctoral Student (36 hrs course work)
College of Education
Learning and Instruction
University of California at Los Angeles
Los Angeles, California

1979 - 1981 Master of Science in Nurse Anesthesia
Program of Nurse Anesthesia
College of Medicine
UCLA
Los Angeles, CA

1976 Bachelor of Science in Nursing (Magna cum
Laude)
College of Nursing
University of South Florida
Tampa, Florida

1974 Associate of Science in Nursing
(Magna cum Laude)
Hillsborough Community College
Tampa, Florida

Academic Appointments

9/11 – present Associate Professor
Research and Didactic Instruction
UCLA School of Nursing

7/10 – present Associate Clinical Professor

Department of Anesthesiology
UCLA School of Medicine
Los Angeles, CA

8/89 – 7/2010

Assistant Clinical Professor
Department of Anesthesiology
UCLA School of Medicine
UCLA School of Nursing (secondary)
Los Angeles, CA

9/84 - 3/87

Adjunct Assistant Professor
Department of Anesthesiology
UCLA School of Medicine
Los Angeles, CA

1995-Present

Anesthesia

Visiting Faculty
University of Southern California Program of Nurse
Anesthesia
Kaiser Permanente Program of Nurse Anesthesia

Professional Employment

Sept. 2011-present

Per Diem Staff Nurse Anesthetist and Clinical Instructor
Greater Los Angeles VA Healthcare System
Department of Anesthesia
Los Angeles, CA

Sept. 2009
Education

Professional Lecturer, Institute for Post-Graduate

2006-Present

Principal Nurse Anesthetist
Assistant Administrative Director, Nurse Anesthesia
Clinical Educator, Educational Director
UCLA Department of Anesthesiology

5/87 - 2006

Staff Senior Nurse Anesthetist and Instructor
UCLA Department of Anesthesiology
Los Angeles, CA

3/92 – 12/98

Staff Nurse Anesthetist
Clinical Instructor
West Los Angeles Veteran's Administration

| | |
|-------------|--|
| | <p>Medical Center Department of Anesthesia Los Angeles, CA</p> |
| 6/89 - 8/89 | <p>Staff Nurse Anesthetist (Part-time) Sepulveda Veterans Admin Medical Center Sepulveda, CA</p> |
| 1/85 - 5/87 | <p>Director UCLA Program of Nurse Anesthesia UCLA Department of Anesthesiology School of Medicine Los Angeles, CA</p> |
| 6/81 - 1/85 | <p>Staff Nurse Anesthetist Clinical Instructor UCLA Department of Anesthesiology Los Angeles, CA</p> |
| 6/81 - 1/85 | <p>Private Anesthesia Practice (Part-time) Plastic Surgery Offices Los Angeles, CA</p> |
| 1974 - 1979 | <p>Registered Professional Critical Care Nurse Tampa, Florida Emergency Department Staff Nurse St. Joseph Hospital</p> <p>Intensive Care Unit Staff and Charge Nurse Tampa General Hospital</p> <p>Coronary Care Unit Staff and Charge Nurse St. Joseph Hospital</p> <p>Cardiac Surgery Intensive Care Unit Staff and Charge Nurse Tampa, Florida</p> <p>Surgical Intensive Care unit Veteran's Administration Hospital</p> |

Tampa, Florida

Societies

California Association of Nurse Anesthetists
American Association of Nurse Anesthetists
International Association for the Study of Pain
International Anesthesia Research Society

CPR Certification

American Heart Association
Basic Life Support, biannual recertification
1974 - present
Advanced Cardiac Life Support, bi.an.recert
1978 – present
ACLS Instructor, volunteer with UCLA Dept of Pre-

Hospital Care

2008-2009

Educational Certification

Approved Continuing Education Provider #CEP 14381
California Board of Registered Nursing

Conference Presentations

UCLA Department of Anesthesiology Clinical Case Conference
“Syncope During Local Anesthesia”, Feb 1983
“Cardiac Depression Following Vancomycin”, Oct 1983
“Anaphylaxis Following Ancef”, May 1984
“Tension Pneumothorax in the Recovery Room”, June 1984
“The Effects of Naloxone on the Exercise Hyperpnea”, August 1984
“HIV Chemoprophylaxis”, May 2001

West Los Angeles VA Medical Center, Department of Anesthesia
Grand Rounds

“Propofol and Alfentanil for Sedation”, August 1993
“HIV Disease and Anesthesia”, April 1996
“Update on HIV and Anesthesia”, November 1997
“HIV/AIDS Drugs: Anesthetic Implications”, July 1997

Federation of American Societies for Experimental Biology, Annual Meeting April 1984.
“The Influence of the Endogenous Opiate System on the Control of the Exercise Hyperpnea in Man”

Harbor-UCLA Medical Center, Division of Respiratory Physiology and Medicine Research Conference, June 1984. "The Effects of Naloxone on the Exercise Hyperpnea"

American Association of Nurse Anesthetists Annual Meeting, August 1984. "The Effect of Naloxone on the Exercise Hyperpnea"

Malignant Hyperthermia Association of the United States Annual Meeting, September, 1984. "Monitoring Techniques in the Operating Room"

American Association of Nurse Anesthetists Annual Meeting, August 1985. "End-Tidal CO2 Monitoring"

California Association of Nurse Anesthetists, May, 1986. "Cardiovascular Implications of PAR Nursing Care"

American Society of Plastic and Reconstructive Nurses, October, 1986. "Malignant Hyperthermia"

California Association of Nurse Anesthetists, February, 1987. "The Future of Nurse Anesthesia Education"

California Association of Nurse Anesthetists, October, 1988. "Clinical Implications of the Harvard Standards for Monitoring"

California Association of Nurse Anesthetists, October, 1989. "The Use of Alfentanil for Sedation in Ophthalmologic Outpatient Surgery"

California Association of Nurse Anesthetists, February, 1992. "Propofol and Alfentanil in Outpatient Anesthesia"

American Association of Nurse Anesthetists Annual Meeting, August, 1993. "Use of Propofol/Alfentanil Bolus and Infusion for Monitored Anesthesia"

California Association of Nurse Anesthetists, May 1996 Annual Meeting. "HIV and AIDS - An Update for the Anesthesia Provider"

American Association of Nurse Anesthetists Annual Meeting, August, 1997. "Perioperative Management: Anesthesia for the Ambulatory Patient" and "Risk Management in the Ambulatory Setting"

California Association of Nurse Anesthetists, Fall Meeting, October, 1997 - "Update on the AIDS Epidemic and Chemoprophylaxis for Occupational Exposure to HIV"

American Association of Nurse Anesthetists Annual Meeting, August, 1998. "HIV/AIDS Drugs: Anesthesia Implications"

California Association of Nurse Anesthetists, May 1999 Annual Meeting, "Anesthetic Implications of HIV/AIDS Drugs"

24th Annual Gastrointestinal Nurses Seminar, March, 2000 - "Conscious Sedation: Do's and Don'ts"

California Association of Nurse Anesthetists, May 2000 Annual Meeting, "Risk Management in Anesthesia Practice"

American Association of Nurse Anesthetists, August 2006 Annual Meeting, Research Forum, "The Effect of Pain on Proinflammatory Cytokines and Leukocyte Cellular Adhesion Molecules"

UCLA Department of Anesthesiology, Mortality and Morbidity Conference, September 2006, "Anesthesia, Pain, Stress, and Immune System Responses"

UCLA Cousins's Center for Psychoneuroimmunology, Psychoneuroimmunology Seminar, November, 2006, "Review of Stephen Maier and Linda Watkins Papers: Pain and Cytokine Responses"

West Los Angeles Veteran's Administration Medical Center, Dept. of Anesthesia Morbidity and Mortality Conference, March, 07, 2007, "Implications of Perioperative Stress to Immune Function"

California Association of Nurse Anesthetists, April 2007, Annual Meeting, "Implications of Anesthesia and Surgical Stress to Perioperative Immune Function"

Kaiser Permanente Nurse Anesthetists Association, January, 2008, "Outpatient Anesthesia: Issues and Update"

UCLA Ronald Reagan Medical Center, Emergency Department Pediatric Conference, Los Angeles County Emergency Department Approved for Pediatrics (EDAP) requirement, November 2008, "Pediatric Sedation in the Emergency Room; Acute Pediatric Pain Management"

California Association of Nurse Anesthetists, February 2009, Spring Meeting, "Pain, Opioids and Neuroinflammation" (2 hours); "Risk Management: Error Prevention" (1 hour)

UCLA Nursing Practice Research Council, April 2009, "Pain and Inflammatory Mediators: A Research Review"

UCLA Department of Anesthesiology, Weekly Morbidity and Mortality Conference, June 2009, "The Contribution of Neuroimmune Activation to Chronic Pain"

American Association of Nurse Anesthetists, Annual Meeting, San Diego, California, Research Forum, Oral Poster Presentation, "Preliminary Data: Pain and Neuroinflammatory Mediators"

Nebraska Association of Nurse Anesthetists, October, 2009, "Chronic Pain and Neuroinflammation; Outpatient Anesthesia-Update on Issues; Anesthesia for Out of the OR Settings"

California Association of Nurse Anesthetists, February 2010, Spring Meeting, "OPSU— Issues and Update" (1 hour)

American Association of Nurse Anesthetists, Assembly of School Faculty, February, 2011, Research Report of the John F. Garde Researcher of the Year, "Pain, Cellular Adhesion Molecules, and Inflammatory Mediator Production"

California Association of Nurse Anesthetists, March, 2012, Spring Meeting, "Policy Governance and CANA"

American Association of Nurse Anesthetists, August 2012, Annual Meeting, "Anesthetic Management of the Patient with Implanted Cardiac Devices"; "Anesthesia for Out of OR Cases"

Invited Lectures

Medical Media Associated, Inc., Continuing Education Lecture Series for Professional Nurses, October 1984. "Airway Management in the Anesthetized Patient"

UCLA Allied Health Program, Center for the Health Sciences. December 1984 - "Anesthesia, A Nursing and Medical Specialty"; June 1985 - "UCLA Nurse Anesthesia Program"; October 1985 - "Nurse Anesthesia at UCLA Medical Center"

Post Anesthesia Recovery Nurses, UCLA, Continuing Education Lectures. July 1985 - "Anesthetic Agents"; January 1986 - "The Choice of General Vs. Regional Anesthesia; August 1986 - "Post Anesthesia Care of the Trauma Patient"

UCLA Operating Room Postgraduate Nursing Program: “The Role of the OR Nurse in Anesthesia Care” and “Anesthesia and Emergency Drugs” - 1985, 1986, 1987, 1988, 1989, 1990

Professional Symposiums, Inc. Continuing Education Lecture Series for Professional Nurses, February, 1986. “Anesthesia and Perioperative Nursing Care”

CRNA Staff and Faculty, UCLA, May, 1986. “Motivation Theory and Nurse Anesthesia Education”

UCLA Operating Room Nurses Continuing Education Conference, August 1986. “Anesthesia and Perioperative Nursing Care”

CRNA Staff and Faculty, UCLA December, 1986. “Cognitive Psychological Approaches to Nurse Anesthesia Education”

UCLA Operating Room Nurses Weekly Teaching Conference, UCLA, August, 1988. “Malignant Hyperthermia”

UCLA Department of Anesthesiology, Educational Lecture Series for Stuart Pharmaceutical Sales Representatives. August - October 1988 - “Monitoring in Anesthesia”; “Preoperative and Postoperative Assessment and the Ambulatory Surgery Unit”

UCLA Post Anesthesia Care Unit, UCLA, August, 1988. “Malignant Hyperthermia”

Jules Stein Operating Room Nurses, December, 1989. “The Pharmacology of Alfentanil and Its Applications to Ophthalmologic Anesthesia”

UCLA Surgery Center Perioperative Nurses, June - September, 1990. Lecture Series entitled: “Continuing Education on Anesthesia Care for the OR Nurse”, composed of 10 two to four hour lecture-discussion classes.

UCLA Surgery Center Operating Room Nurses, February, 1991. “Review of Commonly Used Anesthetic Techniques and Drugs”

September, 1995; August 1996; February 1997 UCLA Perioperative Nurses, “Preoperative Anesthesia Evaluation for the Perioperative Nurse”(24 hours lecture)

UCLA Surgery Center Operating Room Nurses, April 1996. “Monitoring and Sedating Patients in the OR”

UCLA Surgery Center Operating Room Nurses, Perioperative Evaluation Suite Nurses, and Pheresis Unit Nurses, Fall, 1996. "Chemoprophylaxis for Occupational HIV Exposure"

Association of Operating Room Nurses, San Fernando Valley Chapter, March 1997. "HIV and AIDS - Implications for Perioperative Nursing Care"

UCLA Surgery Center Operating Room Nurses, March, 1997; April, 1997, May, 1997 - "Anesthesia Equipment, Cart, Drugs, and Machine - A Hands-on Workshop"

September, 1997 UCLA Perioperative Nurses, "Review of Preoperative Evaluation and Conscious Sedation"

UCLA Surgery Center Operating Room Nurses, October, 1997 - "Review of Cardiac Arrest Procedures and Crash Cart in the OR"

UCLA Surgery Center Operating Room Nurses, November, 1997 - "Malignant Hyperthermia"

November, 1997 West Los Angeles Veteran's Administration Medical Center Perioperative Nurses, "Update on HIV Epidemic and Chemoprophylaxis for Occupational Exposure"; Educational Workshop Organizer and Coordinator.

UCLA Surgery Center Operating Room Nurses, March, 1998 - "Sedation and Airway Management for OR Nurses"

March, 1998 UCLA Perioperative Nurses, "Conscious Sedation"

March, 1998 UCLA Perioperative Nurses, "Perioperative Assessment for Anesthesia Care At UCLA Surgery Center"

Jules Stein Eye Institute Perioperative Nurses, Fall, 1998 - "Discharge Criteria for General Anesthesia and Monitored Anesthetic Care Patients"

October, 1998 UCLA Perioperative Evaluation Suite Nurses, "Perioperative Preanesthesia Evaluation for the Perioperative Nurse" (24hrs of lecture; proctored and tutored Angela Jackson, RN, BSN in assisting with class instruction)

UCLA Surgery Center Operating Room Nurses, January, 1999 - "Local Anesthetics: Clinical Use and Pharmacology; Treatment of Adverse Reactions"

UCLA Surgery Center Operating Room Nurses, March, 1999 - "Review and Demonstration of Defibrillator/Pacemaker and Crash Cart"

UCLA Surgery Center Operating Room Nurses, September, 1999 -"Review of Anesthesia and Sedation Equipment and Drugs"

November, 1999 UCLA Ambulatory Surgery Center Anesthesia Technicians, "Part 1 - Introduction to Theory and Practice of Anesthesia - An Overview"; continuing through Nov 2000, 15 one-hour lectures

Dept of Anesthesiology, Kaiser Harbor City Medical Center, March, 2000 - "Implications of HIV Disease to Perioperative Care"

Medical Procedures Unit Nurses, UCLA Medical Plaza, May, 2000 - "Pre-Sedation Assessment"

Procedure and Treatment Unit Nurses, UCLA Operating Room, May, 2000 - "Review of Pre- and Post-Anesthesia Assessment and Care"

UCLA Ambulatory Surgery Center OR Nurses, May, 2000 - "Review of Crash Cart and Cardiac Arrest in OR"

PACU Nurses, UCLA Operating Room, July 2000 - "Review of Anesthesia Techniques and Drugs"

UCLA Ambulatory Surgery Center PACU Nurses, November 2000 – "Airway Management"

PACU Nurses, UCLA Operating Room, November 2000 - "Preoperative Assessment - Implications for Postoperative Care"

Procedure and Treatment Unit Nurses, UCLA Medical Center, November 2000 - "Implications of Pain Drugs and Antiemetic Drugs to Outpatient Treatment and Discharge"

Jules Stein Eye Institute OR Nurses, May 2001, "HIV Chemoprophylaxis"

August, 2001 UCLA Perioperative Nurses, "Pre-anesthesia Evaluation"

Procedure and Treatment Unit Nurses, UCLA Medical Center, September 2001 – "Perioperative Geriatric Care"

June, 2002 UCLA Perioperative Nurses, "Preanesthesia Assessment"

September 2005 UCLA Healthcare Enterprise Santa Monica Perianesthesia Nurses, "Coagulation Pathways and Implications for PACU Care"

October 2005 UCLA Healthcare Enterprise Santa Monica Perianesthesia Nurses, "Principles of Outpatient Surgery Nursing Care"

November 2005 UCLA Healthcare Enterprise Santa Monica Perianesthesia Nurses, "Propofol: Pharmacology and Anesthesia Use"

Jules Stein Perioperative Nurses, January 2006, "Intravenous Therapy"

February 2006 UCLA Main OR Nurses, "Conscious Sedation Part 1"

Journal Club Leader, UCLA CRNA Group, March, 2006, "Complications of Ophthalmic Regional Anesthesia"

April 2006 UCLA Procedure and Treatment Unit and Post-Anesthesia Care Unit Nurses, "Ophthalmic Surgery Postop Care"

UCLA Healthcare Enterprise Westwood Perianesthesia Nurses, April 2006, "Phase 1 Care, Post Anesthesia Care Unit"

UCLA Healthcare Enterprise Santa Monica Perianesthesia Nurses, May, 2006, "Outpatient PACU Care and Discharge Criteria"

May 2006, UCLA Westwood MOR Nurses, "Conscious Sedation Part 2"

June, 2006, UCLA Westwood MOR Nurses, "Conscious Sedation Part 3:"

UCLA Jules Stein Eye Institute Perianesthesia Nurses, July, 2006, "Jules Stein PACU Care"

UCLA Santa Monica Perianesthesia Nurses, August, 2006, "Hemodynamic Monitoring Principles"

UCLA Westwood MOR Nurses, August, 2006, "Conscious Sedation, Part 4"

UCLA Medical Procedures Unit Nurses, September, 2006, "Sedation Patient Assessment and Preparation"

UCLA Medical Procedures Unit Nurses, October 4, 2006, "Combining Sedatives"

UCLA Radiology Services Nurses, October 20, 2006, “Pharmacology and Physiology of Sedation”

UCLA Medical Procedures Unit Nurses, October 25, 2006, “Difficult Sedation”

UCLA Radiology Services Nurses, October 27, 2006, “NPO Status, Propofol, and Emergency Sedation”

UCLA Medical Procedures Unit Nurses, November 1, 2006, “NPO Status, Propofol, Assessment and Emergency Sedation”

UCLA Medical Procedures Unit Nurses, November 22, 2006, , “Obstructive Sleep Apnea and Sedation”

UCLA Medical Procedures Unit Nurses, December 6, 2006, “Vasopressors, Reversal Drugs and Sedation”

UCLA Emergency Department Nurses, April, 2007, “Sedation in the ER and ETCO₂ Monitoring”

Santa Monica UCLA Medical Center, ICU Nurses, May 21, May 27, July 3, August 28, 2007, “PACU Care for the ICU RN”

Jules Stein Operating Room Nurses, June 2007, July 2007, “Pre-Anesthesia Evaluation”

UCLA Radiology Nurses, August, 2007, “Vasopressor Infusions”

Santa Monica UCLA Medical Center, OR/Procedure Unit Nurses, September 29, 2007, “Moderate Sedation”

Jules Stein Perioperative Nurses, October, 2007, “Malignant Hyperthermia”

UCLA Radiology Nurses, November, 2007, “Avoiding Oversedation”

UCLA Radiology Nurses, February, 2008, “Treating Delirium During Sedation”

Western University, Pomona California, Nurse Practitioner Program, March 2008, “Perioperative Stressors and Preoperative Preparation of the Patient by the Primary Care Nurse Practitioner”

Santa Monica UCLA Medical Center, OR Unit Nurses, May, July 2008, “Basic Conscious Sedation—Introduction; Part 2, Part 3”

Jules Stein Perioperative Nurses, June, 2008, “Perioperative Cardiac Arrest and Resuscitation”

Santa Monica UCLA Medical Center, OR Unit Nurses, August/September 2008, “Arrhythmia Interpretation”, Parts 1 – 4, one-hour lectures

UCLA Ronald Reagan Medical Center, Emergency Department Nursing, October 2008, December 2008, “Sedation in the Emergency Room”

Santa Monica UCLA Medical Center, OR Unit Nurses, Medical Procedures Unit Nurses, October 2008, “Arrhythmia Interpretation”

Santa Monica UCLA Medical Center, Medical Procedures Unit Nurses, December 2008, “ECG Interpretation”

UCLA Ronald Reagan Medical Center, Ambulatory Nursing, March, 2009, “Pediatric Sedation”

UCLA Ronald Reagan Medical Center, Ambulatory Nursing, March 2009, “Adult Sedation”

Western University, Pomona California, Nurse Practitioner Program, March 2009, “Perioperative Stressors and Preoperative Preparation of the Patient by the Primary Care Nurse Practitioner”

UCLA Ronald Reagan Medical Center, Emergency Department Nursing, May, 2009, “Pediatric Sedation”

UCLA Ronald Reagan Medical Center, Radiology-Cardiac Catheterization Nurse Group, July, August, 2009, “Adult Sedation”

Jules Stein Eye Institute, OR Nursing Group, September 2009, “Malignant Hyperthermia”

Santa Monica/UCLA PACU Nurses, January, 2010 “PACU Phase 1 and 2”

UCLA Ambulatory Surgery Center, OR Nurse Group, July, 2010, “Update on Anesthesia Drugs, Techniques, and SCIP Requirements”

California Student Nurse Association, “Nurse Anesthesia as an Advanced Nursing Career Choice”, October, 2010

UCLA Medical Procedures Unit RNs, “Adult Sedation”, January, 2011

Research Activities

1. Thesis Research Project: “The Effects of Naloxone on the Exercise Hyperpnea”. Human laboratory study.
2. Independent Research: “The Use of Alfentanil in Outpatient Ophthalmologic Surgery”: a clinical investigation of the use of the short-acting narcotic alfentanil in 26 patients for monitored anesthetic care at the Jules Stein Eye Institute, UCLA Medical Center, May-August, 1989.
3. Doctoral Dissertation Research Project: “The Effect of Pain on Leukocyte Cellular Adhesion Molecules” (human subjects). UCLA IRB Approval January 05, 2004. Dissertation Defense 12-02-05.

Grant Support:

UCLA School of Nursing, Intramural Grant Award, \$10,000, January, 2004

UCLA General Clinical Research Center, NIH/NCRR Grant #M01RR00865, \$10,000, January 2004-August 2005

American Association of Nurse Anesthetists Research Foundation General Research Grant, \$13,625, March 2004 – August 2005

4. Post-doctoral Research Project, begins 08-04-06, UCLA General Clinical Research Center

Grant Support: National Institute of Nursing Research, “The Effect of Pain on Leukocyte CAMs”, Grant #1 RO-3 NR009106-01A1, August 2005

UCLA General Clinical Research Center, NIH/NCRR Grant, #M01RR00865, April 2006

American Association of Nurse Anesthetists Research Foundation, Post-doctoral Fellowship Grant, May, 2006

5. Research Rotation for USC Program of Nurse Anesthesia students, practicum portion of ANST 512, Research Integration: Capstone Experience. Students attended 3 hours of theory lecture on research methodology and project theoretical framework; assisted with data collection and entry; chose a topic related to project and wrote a review paper, Sept 2007-May 2008.

6. Intramural Grant Award, UCLA Claude D. Pepper Older Americans Independence Center, \$10,000. December 22, 2008 – June 30, 2009. Project Title: “Nuclear Factor Kappa-B Activation and Pain.

7. American Association of Nurse Anesthetists “State of the Science” Poster and Oral Session August 2009, “The effect of pain on leukocyte cellular adhesion molecules and inflammatory mediators”.

8. National Institutes of Health, Grant # 1 R21 DA027558-01, “Pain Opioids and Pro-Inflammatory Immune Responses”, September, 2010. Co-Investigator.

9. General Research Grant, American Association of Nurse Anesthetists Foundation, "Pain Opioids and Pro-Inflammatory Immune Responses", January, 2011. Co-Investigator.
10. American Association of Nurse Anesthetists "State of the Science" Poster and Oral Session August 2012, "Acute painful stress and inflammatory mediator production."

Publications

RESEARCH PAPERS

1. Thesis - "The Effects of Naloxone on the Exercise Hyperpnea"
Program of Anesthesiology for Registered Nurses
Department of Anesthesiology, School of Medicine
University of California at Los Angeles
Los Angeles, CA
2. Griffis, C.A., S.A. Ward and R.D. Kaufman. "Naloxone and the ventilatory response to exercise in man." *European Journal of Applied Physiology* 55: 624-629, 1986.
3. Griffis, C.A. "Monitored anesthetic care for outpatient cataract surgery with alfentanil" *Nurse Anesthesia* 1(2): 71-78, 1990.
4. Dissertation. Griffis, C. "The effect of pain on proinflammatory cytokines and leukocyte cellular adhesion molecules"
School of Nursing, Graduate Division
University of California at Los Angeles
Los Angeles, California
December 05, 2005
5. Griffis, C., Irwin, M.R., Martinez, O., Doering, L., Nyamathi, A., Kaufman, R.D., Elashoff, D., Aziz, N., Compton, P. "Pain-related activation of leukocyte cellular adhesion molecules: preliminary findings". *Neuroimmunomodulation* 14: 224-228, 2007.
6. Griffis, C., et al., "Acute painful stress and inflammatory mediator production". *Submitted 9/5/2012.*

BOOKS

BOOK CHAPTERS

Elisha, S.M. (ed.), *Case Studies in Anesthesia*, "Ruptured Globe", author C.Griffis & E. McCall, Jones & Bartlett: Sudbury, MA, 2010, pp. 373-382.

Joyce, J. & Oullette, R. (ed.'s), *Pharmacology for the Nurse Anesthetist*, "Implications of the Immune System to Pain", author C. Griffis, Jones & Bartlett: Sudbury, MA, 2011, pp. 437-444

REVIEW ARTICLES

Griffis, C.A. "Anesthetic implications of vasovagal syncope", *CANA Newsbulletin* 40(1): 5-10, October, 1985.

Griffis, C.A. "Anesthetic implications of essential hypertension", *CANA Newsbulletin* 41(1): 10 - 17, October, 1986.

Griffis, C.A. "The future of nurse anesthesia education: curricular design, purpose and evaluation" *CRNA Forum* 2(4): 11-12, 1986.

Griffis, C.A. "End-tidal CO2 Monitoring", *AANA Journal* 54(4): 312 - 318, 1986.

Griffis, C.A., Gerbasi, R., Samph, T., Stoll, D. "The impact of present day trends in testing on the certification process for nurse anesthetists" *AANA Journal* 55(3): 198-201, 1987.

Griffis, C.A., Kelley, J. "The future of nurse anesthesia education", *CANA Newsbulletin* 41(2): 20-21, 1987.

Griffis, C.A. "Nurse Anesthesia: An Awakening Specialty" *California Nursing Review* 10(2): 32 - 35, 1988.

Griffis, C.A. "Implications of recent antiviral pharmacology to prevention of occupational HIV transmission in anesthesia clinical practice" *AANA Journal* 64(6): 525-527, 1996.

Griffis, C.A. "Anesthetic Implications of Human Immunodeficiency Virus" *Nurse Anesthetist Forum* 1(3): 3,11-12, 1998.

Griffis, C.A. "Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome-related drug therapy: anesthetic implications. *CRNA The Clinical Forum for Nurse Anesthetists* 10(3):107-116, 1999.

Griffis, C.A. "Prevention of occupational transmission of bloodborne diseases in nurse anesthesia practice" *CRNA The Clinical Forum for Nurse Anesthetists* 11(1): 2-7, 2000.

Griffis, C., Compton, M., Doering, L. "The effect of pain on leukocyte cellular adhesion molecules". *Biological Research for Nursing*, 7(4): 297-312, 2006.

Griffis, C., Page, G., Kremer, M., Yermal, S. "Implications of immune function to anesthesia care". *American Association of Nurse Anesthetists Journal*, 76(6): 449-454, 2008.

Griffis, C. "Neuroimmune activation and chronic pain". *American Association of Nurse Anesthetists Journal*, 79 (1):31-37, 2011.

CASE REPORTS

Griffis, C.A. "Case Report--Management of examination under anesthesia and electroretinogram in a child with degenerative neurological disease", *CANA Newsbulletin Flowmeter Column* 43(3), 1989

Griffis, C.A. "Case Report--Use of retrobulbar block during general anesthesia", *CANA Newsbulletin Flowmeter Column* 44(2), 1990.

Rochin, M., Griffis, C., Kaufman, R. "The development of Mobitz 1 heart block under general anesthesia following chronic beta-adrenergic receptor antagonism", *CANA Newsbulletin Flowmeter Column* 44(3): 18-21, 1990.

Griffis, C., Austin, J., Ritter, J. "The use of propofol and nitrous oxide for inguinal hernia repair" *CANA Newsbulletin Flowmeter Column* 45(1), 1990.

Griffis, C.A. "The effect of intraoperative retrobulbar block on anesthetic management of enucleation under general anesthesia" *Nurse Anesthesia* 2(1): 28-32, 1991.

Griffis, C. "Acute postoperative pain management and malfunctioning epidural catheter" *Oncology Nursing Forum* 30(2): 217-218, 2003.

LETTERS TO THE EDITOR

Griffis, C. "Expert witness testimony and CRNA practice". *AANA Journal* 72(5): 334, 2004.

EDITORIALS

Griffis, C. "The Flowmeter", an educational column originated and written by C. Griffis in the *California Association of Nurse Anesthetists Newsbulletin*: "Review of research methods and statistical analysis for the mystified and/or totally disinterested" *CANA Newsbulletin Flowmeter Column* 41(4), 1987

“Isoflurane: an emerging controversy” *CANA Newsbulletin Flowmeter Column* 42(1), 1988

“Review of the Harvard Standards for Patient Monitoring: implications for practice” *CANA Newsbulletin Flowmeter Column* 42(3), 1988.

“The uses of lidocaine in anesthesia” *CANA Newsbulletin Flowmeter Column* 42(4), 1988

“Clinical implications of respiratory physiology” *CANA Newsbulletin Flowmeter Column* 43(2), 1989

“Cranial Nerve anatomy and physiology—clinical implications for the nurse anesthetist” *CANA Newsbulletin Flowmeter Column* 44(1), 1989.

“Review of recent anesthesia-related incidents with medico-legal implications” *CANA Newsbulletin Flowmeter Column* 45(1), 1990.

“HIV and AIDS--An update on the pandemic and some implications for anesthesia providers” *CANA Newsbulletin Flowmeter Column* 50(2): 24-29, February, 1996.

“HIV and AIDS Part 2--New Drugs and Exposure Prophylaxis” *CANA Newsbulletin Flowmeter Column* 51(1): 25-25, October, 1996.

“Pain and Leukocyte Cellular Adhesion Molecules: My Research Journey” *AANA Newsbulletin, Foundation and Research News* 63(4): 36, April 2009.

BOOK REVIEWS

Griffis, C.A. Book review of *Regional Anesthesia: An Illustrated Procedural Guide* by Michael Mulroy, M.D. Boston: Little, Brown and Co., 1989, pp 1-289; appearing in *Nurse Anesthesia* , 1(4): 231-236, 1990.

ABSTRACTS

1. Griffis, C.A., S.A. Ward and R.D. Kaufman “The influence of the endogenous opiate system on the control of the exercise hyperpnea in man” *Fed Proc* 43(3): 634, 1984.
2. Griffis, C.A. “The exercise hyperpnea and the endorphin system: *Abstracts of the 51st Annual Meeting of the American Association of Nurse Anesthetists*, August, 1984.
3. Griffis, C.A. “End-tidal CO₂ monitoring” *Abstracts of the 52nd Annual Meeting of the American Association of Nurse Anesthetists*, 1985.
4. Griffis, C.A. “Monitored anesthesia care for surgery with propofol and alfentanil” *Poster Session Abstracts of the AANA Education and Research Foundation, 57th Annual Meeting of the American Association of Nurse Anesthetists*, August, 1990.

5. Griffis, C.A. "Use of propofol/alfentanil bolus and infusion for anesthesia care" *Summaries of Professional Sessions, 60th Annual Meeting of the American Association of Nurse Anesthetists*, August, 1993.
6. Griffis, C.A. "Perioperative management: anesthesia for the ambulatory patient" *Summaries of Professional Sessions, 64th Annual Meeting of the American Association of Nurse Anesthetists*, August, 1997.
7. Griffis, C.A. "Risk management in the ambulatory setting" *Summaries of Professional Sessions, 64th Annual Meeting of the American Association of Nurse Anesthetists*, August, 1997.
8. Griffis, C.A. "HIV/AIDS drugs: anesthesia implications" *Summaries of Professional Sessions. 65th Annual Meeting of the American Association of Nurse Anesthetists*, August, 1998.
9. Griffis, C. & Compton, P. "The effect of pain on cellular adhesion molecules" *Communicating Nursing Research Conference Proceedings. Western Institute of Nursing Assembly, April, 2004*.
10. Griffis, C., Compton, M., Irwin, M.R., Kaufman, R. "The effect of pain on leukocyte cellular adhesion molecule expression" *AANA Journal*, 74(6): 468, 2006.
11. Griffis, C., Compton, M., Irwin, M. "The effect of pain on leukocyte cellular adhesion molecules and inflammatory mediators" *AANA Journal*, 77(5):384, 2009.
12. Griffis, C., Breen, E., Irwin, M., Compton, M. "Acute painful stress and inflammatory mediator production." *AANA Annual Meeting 2012 Research Abstracts*, 2012.

C. Other Publications

1. Griffis, C.A. Book review of *Regional Anesthesia: An Illustrated Procedural Guide* by Michael Mulroy, M.D. Boston: Little, Brown and Co., 1989, pp 1-289; appearing in *Nurse Anesthesia* , 1(4): 231-236, 1990.

Teaching

A. Classroom Instruction

March 1983; 1984 UCLA Program of Nurse Anesthesia, "Anesthesia for Vascular and Thoracic Surgery"

March 1983, 1984 UCLA Program of Nurse Anesthesia, "Anesthesia for Cardiac Surgery"

October, 1983 UCLA Program of Nurse Anesthesia, "Anesthesia for the Asthmatic Patient"

Fall, Spring, 1984, 85, 87 UCLA Program of Nurse Anesthesia, Lecture Series, Anes 220A, B, C

"Clinical Applications of Respiratory Physiology: (1) Gas Transport (2) Mechanics of Ventilation (3) Pulmonary Gas Exchange (4) Control of Ventilation", Course Coordinator

Fall, 1984 UCLA Program of Nurse Anesthesia, Anes 221 "Overview of Cardiovascular and Respiratory Physiology", Course Coordinator

Fall, Spring, 1985 UCLA Program of Nurse Anesthesia, Anes 221 "Clinical Applications of Cardiovascular Physiology: Anesthesia for the Patient (1) With Hypertension (2) With Coronary Artery Disease (3) Anesthesia for the Patient in Shock"; Course Coordinator

Fall 1985-87 UCLA Program of Nurse Anesthesia, "Orientation and Overview of Nurse Anesthesia"

Fall 1986-87 UCLA Program of Nurse Anesthesia, "Review of Research Methods: (1) Experimental Design (2) Statistics Review

Fall 1987-91 UCLA Program of Nurse Anesthesia, "Chemistry and Physics Review: (1) States of Matter (2) Gas Laws (3) Fluids and Flows (4) Vaporization" (Course coordinator for this lecture series)

Winter, 1989 UCLA Program of Nurse Anesthesia, "Overview of Pharmacology"

Winter, 1989 UCLA Program of Nurse Anesthesia, "Clinical Applications of Respiratory Mechanics"

Spring, 1989-96 UCLA Program of Nurse Anesthesia, "Anesthesia for Ophthalmologic Surgery"

Fall 1989-91 UCLA Program of Nurse Anesthesia, "Clinical Applications of Chemistry to Nurse Anesthesia Practice: (1) General Chemistry (2) Pharmacology and Chemistry (3) Organic Chemistry (4) Biochemistry" (Course coordinator for this lecture series)

Winter, 1991-96 UCLA Program of Nurse Anesthesia, “Anesthesia for Outpatient Surgery”

Fall, 1996 University of Southern California Program of Nurse Anesthesia, “Anesthesia for Ophthalmologic Surgery”

July, 1997 Kaiser Permanente School of Nurse Anesthesia, Los Angeles, “Anesthesia for the Patient With HIV Disease and Chemoprophylaxis for Occupational Exposure to HIV”

September, 1997 University of Southern California Program of Nurse Anesthesia, “Anesthesia, Universal Precautions, and Infectious Diseases”

March 1998 Kaiser School of Nurse Anesthesia, Los Angeles, “Care of the Patient with HIV/AIDS”

May, 1998 Kaiser School of Nurse Anesthesia, Los Angeles, “Anesthesia for Off-site Cases”

September, 1998 USC Program of Nurse Anesthesia, Los Angeles, “Implications of Asepsis and Infectious Disease Control to Anesthesia Practice”

November, 1999 USC Program of Nurse Anesthesia and Kaiser School of Nurse Anesthesia, Los Angeles. “Implications of Asepsis and Infectious Disease Control to Anesthesia Practice”

November, 2000-2005 University of Southern California and Kaiser Permanente Programs of Nurse Anesthesia (combined), “Asepsis and Infectious Diseases - Implications for Anesthesia Care”

January, 2003, 2004, 2005, 2006, 2007, 2008, 2009 UCLA School of Nursing, Nursing 230, Pathophysiology—“Introduction to the Immune System”

January 2004, UCLA School of Nursing, Nursing 264-Roles of the Advanced Practice Nurse, “Issues in Nurse Anesthesia Practice”

April, 2004, Kaiser Permanente Program of Nurse Anesthesia, “Outpatient Anesthesia”

January 2005, UCLA School of Nursing, Nursing 264-Roles of the Advanced Practice Nurse, “Issues in Nurse Anesthesia Practice”

UCLA School of Nursing, Nursing 239- Nurse Practitioner Professional Practice, Prescribing Schedule II Medications, January 2005, “Pain and Addiction Neurophysiology, Mechanism of Opioid Effects ”

April, 2005; April, 2006; June 2007; April 2008; April 2009 UCLA School of Nursing, N206 Theory Development, “Development of My Theory into My Research Project”

January 2006, UCLA School of Nursing, Nursing 264-Roles of the Advanced Practice Nurse, “Issues in Nurse Anesthesia Practice”

January 2006, 2007 UCLA School of Nursing, Nursing 239- Nurse Practitioner Professional Practice, Prescribing Schedule II Medications, “Pain and Addiction Neurophysiology, Mechanism of Opioid Effects ”

September 2006, 2007, 2008, 2009, 2010 Kaiser Permanente School of Nurse Anesthesia, “Principles of Asepsis and Prevention of Occupational Exposure to Infectious Diseases”

UCLA School of Nursing, Nursing 242-Nurse Practitioner Professional Practice, Prescribing Schedule II Medications, January, 2007, “Pain and Addiction Neurophysiology, Mechanism of Opioid Effects ”

Kaiser Permanente School of Nurse Anesthesia, February 2007, February 2008, February 2009, March 2010 “Anesthesia for Off-Site Cases”

UCLA School of Nursing, Nursing 230, Pathophysiology, March, 2007, “Immune System, Stress, and Psychoneuroimmunology”

USC Program of Nurse Anesthesia, Anst 503-Advanced Pharmacology, April 2007, April 2008, April 2009; April 2010 “Anesthesia, Stress and Immune System Responses”

UCLA School of Nursing, Nursing 242-Nurse Practitioner Program, July 2007, “Introduction to Psychoneuroimmunology”

UCLA School of Nursing, Nursing 262-MECN Program, August, 2007, “Overview of Perioperative Nursing Care of the Patient for Surgery”

UCLA School of Nursing, Nursing 262-MECN Program, August, 2008, “Overview of Perioperative Nursing Care of the Patient for Surgery”

Kaiser Permanente School of Nurse Anesthesia, Junior Class, November, 2008, “Principles of Asepsis and Occupational Disease Prevention in Nurse Anesthesia Practice”

Kaiser Permanente School of Nurse Anesthesia, Junior Class, January 2009, March, 2010
“Anesthesia for Ophthalmology”

Kaiser Permanente School of Nurse Anesthesia, Junior Class, January 2009, “Anesthesia
for Bariatric Surgery”

Kaiser Permanente School of Nurse Anesthesia, Junior Class, March 2009, March, 2010
“Off-site Out of OR Anesthesia”

UCLA School of Nursing, N206, Doctoral Students, March 2009, “Theory Development
and the Dissertation Process”

UCLA School of Nursing, N162, Bachelor of Science Students April 2009, “The
Perioperative Nursing Process”

USC Program of Nurse Anesthesia, Junior Class, June 2009 , “Nociceptive and Chronic
Pain, Opioid Physiology, Tolerance and Neuroimmune Activation”

Samuel Merritt Certified Registered Nurse Anesthetist Program, Junior Class, August
2009, “Nociceptive and Chronic Pain, Opioid Physiology, Tolerance and Neuroimmune
Activation”

David Geffen School of Medicine at UCLA, Doctoring 3, Lecturing Faculty, September
2009 – May 2010

UCLA School of Nursing, N206, Doctoral Students, March 2010, “Theory Development
and the Dissertation Process”

UCLA School of Nursing, N270, MECN Students, January, 2011, “Ventilator
Management”

B. Clinical Instruction

1. 1981-1989: supervision and teaching of nurse anesthetist students for the UCLA Program of Nurse Anesthesia at UCLA Medical Center Operating Rooms.
2. 1992 - 97: supervision and teaching of nurse anesthetist students (UCLA Program of Nurse Anesthesia, now known as the University of Southern California Program of Nurse Anesthesia) and medical students (UCLA School of Medicine) rotating through Anesthesia Department, West Los Angeles Veterans Administration Medical Center, Los Angeles.

3. Simulation Laboratory: May 2004; May 2005; May 2006, May 2007; May 2008; May 2009; May 2010. UCLA School of Nursing, N439A Clinical Laboratory Series Acute Care Nurse Practitioner students “Lumbar Puncture Workshop”
4. February 2005-October 2005: Clinical Coordinator of UCLA nurse anesthetist student rotation. Supervision of junior and senior nurse anesthetist students from Kaiser School of Nurse Anesthesia during clinical anesthesia administration.
5. October 2005-Present: Senior consultant to clinical coordinators. Supervised and instructed medical students and nurse anesthetist students from the Kaiser and USC Programs of Nurse Anesthesia in the UCLA Operating Rooms.
6. April, 2006: supervised and instructed Jules Stein perioperative nurses in insertion of intravenous catheters
7. Fall, 2007-2008: arranged and supervised Master’s Entry-Level Clinical Nurse UCLA School of Nursing students in OR visits for observational experiences (Dahianna Lopez [dahianna@ucla.edu]; Evan Jacobson; Susan Chapdelain; Miriam Wesley[mirmir37@ucla.edu]; April Bautista [april185@aol.com])
8. Simulation Laboratory: April & May 2009; March 2010. Supervision and instruction of nurse anesthetist students in the Kaiser Program of Nurse Anesthesia Simulation Training Facility

C. Mentoring

Certified Registered Nurse Anesthetists

1. Sarah Rozycki, CRNA, MSN: mentored for first conference presentation, January, 2008; mentored to supervise nursing student on observational rotation in the Department of Anesthesiology, April 2008; mentored participation in second conference presentation February 2009.
2. Erica Zima, CRNA, MSN: mentored for curriculum vitae preparation (2008); co-authorship of book chapter (2008); leadership guidance (2007-2009)

Registered Nurses

1. Bert B. Mekpongsatom (bmekpongsatom@mednet.ucla.edu), RN, BSN: 4/08 arranged supervised OR visit, counseled about nurse anesthetist school

Research Assistants

1. Fall 2007: trained 4 research assistants (AJ Freeman [d.free.06@gmail.com], DM Verkade[dmverkade@mac.com], L Guerrero [lance.guerrero@gmail.com], and A Keyes [annkeyesm@yahoo.com]) to participate in post-doctoral project; also mentored these RNs in their consideration of and application to schools of nurse anesthesia.
2. Summer, 2008: trained 2 research assistants (John Scott [jascott@mednet.ucla.edu]; Gregory Zweigle [gvbz@yahoo.com]) to participate in post-doctoral project; also mentored for preparation to apply and attend school of nurse anesthesia.

Nursing Doctorate Students

1. Samira Moughrabi (smoughrabi@gmailcom), UCLA School of Nursing: consulted and counseled on operationalization of doctoral study 4/22/08

2. Julia Lassegard (roadrun1@sbcglobal.net) UCLA School of Nursing: consulted and counseled on degree options, progress, and preparation for written qualifying examination 3/08-6/08.

February 2009: Ms. Lassegard will participate in my research project “NFKappa-B and Pain” as a research assistant, and as part of Nursing 299 in the UCLA School of Nursing Doctoral Program; I will supervise, train, and mentor her.

Committee Service (UCLA)

Department of Anesthesiology Committees

1. Program of Nurse Anesthesia, Curriculum Committee (Member) 1982-86.
2. Program of Nurse Anesthesia, Student Evaluation Committee (Member) 1981-1993.
3. Program of Nurse Anesthesia Program Advisory Committee (Chair) 1985-87
Program Evaluation Committee (Chair) 1985 - 87
4. Quality Assurance Committee (Visiting Member) 1997, 1998, 1999; Full Member 2000-2001
5. Task Force Perianesthesia Nursing Cross-training Project, UCLA Medical Enterprise, Chairman, March 2006-Present

UCLA Medical Center

UCLA Committee on Interdisciplinary Practice (Member) 1985-87
Sedation Committee (Member) 2007-Present; (designated as Sedation Committee Educator)

Research Thesis Committees, UCLA Graduate Division

1. Passani, C. Topic: Characteristics of Best Clinical Instructors, Thesis approved, 1985.
2. Brown, D. Topic: The Effect of Lidocaine on Reflex Bronchoconstriction, (Member August 1985-March 1987)
3. Grogan, D. Topic: Cardiovascular Interactions of Lidocaine and Diltiazem Under Isoflurane Anesthesia, (Member) Thesis approved, 1986.

Committee Service Professional Societies

California Association of Nurse Anesthetists (CANA)

- Program Committee (Member) 1984-85
- Nominating Committee (Chairman) 1985-86
- Public Relations Committee (Member) 1985-86
- Government Relations Committee (Member) 1985-86; 90-92
- Co-chairman, CANA Annual Meeting, May, 1986
- CANA - CSA Liaison Committee (Member), 1986-87

Founder, editor, and contributor continuing education column, “The Flowmeter”, CANA Newsbulletin 1987-97
Member, Peer Review Committee, 1988-89-90
Representative to California Board of Registered Nursing, 2008-2009
Board of Directors, Trustee (elected), 2009-Present
Bylaws Committee Chairman 2009-Present

Council on Certification of Nurse Anesthetists (National)
Member, 1986-87
Examination Committee (Chairman) 1987
Examination Committee (Member) 2007-2008-2009
National Board of Certification and Recertification of Nurse Anesthetists
Staff, Item Writer’s Workshop, San Antonio, February, 2010
Examination Committee (Chairman) March, 2010-Feb, 2012

American Association of Nurse Anesthetists (AANA)
AANA Journal, Editorial Advisory Board, 1992-94; 2009-2012
Infection Control Taskforce, Chairman, 2009-present
AANA Foundation
Research Committee, 2009-present

Kaiser Permanente School of Nurse Anesthesia
Admissions Committee, February 2008
Admissions Committee, February 2009
Admissions Committee, February 2011
University of Southern California, Program of Nurse Anesthesia
Admissions Committee, March, 2009; March 2011

Honors and Awards

Phi Theta Kappa National Honor Fraternity (April 1970)

“Who’s Who Among Students in American Junior Colleges”,
Hillsborough Community College, Tampa, Florida (1973-74)

Phi Kappa Phi Honor Society, University of South Florida (April 1973)

**Pi Lambda Theta National Honor and Professional Association in Education, UCLA
Graduate School of Education, 1986**

“Who’s Who in Professional Nursing” (1985-86)

Best Didactic Instructor Award, UCLA Program of Nurse Anesthesia, 1988

Distinguished Faculty Award for academic and clinical excellence in nurse anesthesia education, UCLA/LAC/Oliveview Program of Nurse Anesthesia, 1994

UCLA Department of Anesthesiology, UCLA School of Medicine, Incentive Award Program, Team Award "For his many contributions to the Department July 1, 1996, to June 30, 1997.

UCLA Department of Anesthesiology, UCLA School of Medicine, Certificate of Achievement "For outstanding achievement in Department of Anesthesiology" for the year of 1998, 1999.

UCLA Graduate Division Unrestricted Fellowship Award, 2001-2002 academic year

Sigma Theta Tau International Honor Society of Nursing, Gamma Tau Chapter, 2003

Outstanding Research Presentation Award, "The Effect of Pain on Cellular Adhesion Molecules", Nursing Research Symposium, UCLA School of Nursing, March 2005

American Association of Nurse Anesthetists Research Foundation, Post-doctoral Fellowship Award, August 2006

UCLA Medical Enterprise Employee Incentive Award, June, 2008

University of Southern California, Program of Nurse Anesthesia, Keynote Commencement Speech, August, 2009

AANA Foundation John F. Garde Researcher of the Year Award, 2010

Community Service

UCLA Health Fair Expo, volunteer in producing, 1985-86

AIDS Project Los Angeles: Completing of 52-hour training course for telephone Hotline service in biology and pharmacology of HIV infection and treatment, and in therapeutic communication techniques. Served as Hotline phone volunteer from 1991-97

Assistant volunteer instructor for AIDS Hotline training course, Fall, 1992.

California AIDS Ride (one week long bicycle non-profit event): volunteer
registered nurse in camp, 1995-96-97

Curriculum Vitae

Dr. Joseph F. Burkard, DNSc, CRNA
CDR(ret), NC, USN

Updated: 4 June 2012

Business Address: Hahn School of Nursing
University of San Diego
5998 Alacala Park
San Diego, CA 92110-2492

Home Address:

[REDACTED]

Telephone:

Mobile: [REDACTED]
Office: [REDACTED]
Home: [REDACTED]
e-mail: [REDACTED]
e-Mail: [REDACTED]
e-mail: [REDACTED]

Present Position:

Associate Professor, University of San Diego DNP / PhD Program,
Clinical Researcher / Clinician University of California, San Diego

Immediate Past:

Clinical Coordinator / Department Head, NNCAP, San Diego
Clinical Research Director Naval School of Health Sciences
Vice-Chairman, Institutional Review Board
Program Director, Advanced Cardiac Life Support
Naval School of Health Sciences
34101 Farrenholdt Drive.
San Diego, CA 92134

Didactic Instructor,
Kaiser Permanente School of Nurse Anesthesia
100 South Los Robles Street
Pasadena, CA 91188

Assistant Professor, School of Nursing, Georgetown University
Medical Center: November 2005-2009

Assistant Professor in Nursing, Nurse Anesthesia Program,
Uniformed Services University of the Health Sciences: November
2005- 2010

Nurse Anesthesia Scope / Responsibility / Function: Anesthetic management of patients to be rendered unconscious or insensitive to pain and emotional stress during surgical,

obstetrical, dental and certain medical procedures, including preoperative, intraoperative, and postoperative monitoring, evaluation and treatment.

Professional Experience

| | |
|---|---|
| October 2011-Present February 2010-Present | Jonas Foundation Military Merit Fellow Visiting Health Policy Professor Samuel Merritt University, Oakland, CA |
| August 2010-2011 | Fellow AACN “Leadership for Academic Nursing Program” |
| January 2006-2008 | Member, Council on Accreditation, American Association of Nurse Anesthetists |
| October 2005-2007 | Member, Public Relations Committee, American Association of Nurse Anesthetists |
| October 2005-2008 | Vice-Chair, Institutional Review Board, Naval Medical Center, San Diego, CA |
| October 2004-2008 | Chairman, Advanced Life Support / Simulation Systems, Naval Medical Center, San Diego, CA |
| August 2000-October 2004 | President, Board of Directors California Association of Nurse Anesthetists Practice Chair, Public Relations Committee |
| August 2000- May 2003 | Clinical Instructor Navy Nurse Corp Anesthesia Program Naval School of Health Sciences San Diego, CA 92134 |
| January 1999- August 2000 | Staff Nurse Anesthetist Adjunct Clinical Instructor Naval Medical Center San Diego, CA |
| January 1997- 1999 | Staff Nurse Anesthetist Fleet Surgical Team Nine USS BOXER LHD-4 Phibron Seven, USS BOXER FPO AP 96601-5806 |
| October 1994 - January 1997 | Staff Nurse Anesthetist |

| | |
|-------------------------------------|--|
| | <p>U.S. Naval Hospital USS INDEPENDENCE CV-62 Yokosuka, Japan</p> |
| <p>August 1992 - September 1994</p> | <p>U.S. Navy Nurse Anesthesia Program NSHS Bethesda, MD George Washington University NH San Diego, CA</p> |
| <p>January 1991 - July 1992</p> | <p>Staff Nurse, ICU NH Camp Pendleton, CA Clinical Instructor, ICU, PACU</p> |
| <p>August 1989 - December 1990</p> | <p>Staff Nurse, Med / Surg unit NH Camp Pendleton, CA</p> |
| <p>August 1986 - May 1989</p> | <p>BSN Program Fairfax, Virginia George Mason University</p> |
| <p>January 1986 - July 1986</p> | <p>Supervisor / Head, Lab Department CVN-71 USS THEODORE ROOSEVELT Norfolk, Virginia</p> |
| <p>October 1983 - December 1985</p> | <p>Medical Lab Tech, Research Assistant Point Loma, CA Naval Health Research Center</p> |
| <p>February 1977 - October 1983</p> | <p>Hospital Corpsman, EMT, Paramedic, Work Center Supervisor Various commands</p> |

Educational Experience

| | |
|--|--|
| <p>August 2010-2011</p> | <p>Fellow AACN “Leadership for Academic Nursing Program”</p> |
| <p>July 2001- December 2003</p> | <p>DNSc Program- Acute Care/ Critical Care Nursing, University of Tennessee</p> |
| <p>Dissertation: Bispectral Analysis and Motor Activity Assessment Scores in Mechanically Ventilated Patients in the Intensive Care Unit.</p> | |
| <p>August 1992 - September 1994</p> | <p>MS in Health Sciences (Nurse Anesthesia) George Washington University</p> |

August 1986 - May 1989

**BSN
George Mason University
Fairfax, Virginia**

January 1984 - January 1986

**AA Medical Laboratory
Technology
George Washington University**

Certifications

**Basic Cardiac Life Support Provider (BCLS)
Basic Cardiac Life Support Instructor (BCLS-I)
Advanced Cardiac Life Support Provider (ACLS-P)
Advanced Cardiac Life Support Instructor (ACLS-I)
Director, Advanced Cardiac Life Support
Advanced Cardiac Life Support Affiliate Faculty / Program Director
Pediatric Advanced Life Support Provider (PALS-P)
Pediatric Advanced Life Support Instructor (PALS-I)
Neonatal Advanced Life Support Provider (NALS-I)
Certified Registered Nurse Anesthetist (CRNA)
Critical Care Registered Nurse (CCRN) Inactive
Medical Laboratory Technician (MLT) Inactive
Cooper Institute Health Promotion Director**

Professional Membership

**American Association of Nurse Anesthetists (AANA)
California Association of Nurse Anesthetists (CANA)
American Association of Critical Care Nurses (AACN)
Member Sigma Theta Tau 1989-Present**

Professional Honors / Committees

**October 2011 Faculty Appointment Jonas Foundation Military Merit Fellow
August 2011 Faculty Appointment Uniformed Services University of the Health Sciences
2011-Present
July 2011 Western Institute of Nursing Abstract Reviewer
2011 JOPAN Mary Hana Memorial Journalism Award
USD Senate 2009-Present
USD School of Nursing IRB Representative 2010-Present
Western Institute of Nursing Poster Committee 2011-Present
Fellow, AACN 2010-2011
2009 RADM Mary F. Hall Nursing Publication Award
Council on Accreditation, AANA 2006-2008
Public Relations Committee, AANA 2005-2008**

President, California Association of Nurse Anesthetists, 2002-4
Board of Directors, California Association of Nurse Anesthetists 2000-2004
Golden Key National Honor Society
Surface Warfare Medical Department Officer
Class of 2000 Instructor of the Year, Navy Anesthesia Program
Class of 2002 Instructor of the Year, Kaiser Permanente School of Nurse Anesthesia

Professional Grants

- Burkard, J.F., Skerrett, K, Terry, M.J. (2012, June). *Resiliency Education Program for Physicians, Advanced Practice Nurses and Therapists: Treating the Wounded Warrior and their Families*. The Sence Foundation Grant submitted 1 June 2012 for the amount of \$62,044
- Bopp, E.J, Burkard, J.F., Spence, D., Hazen, A.L. (2012, May). *Is Combat Exposure Predictive of Higher Preoperative Stress in Military Members*. TSNRP Graduate Level grant submitted 30 May 2012 for the amount of \$14,004.
- Burkard, J.F., Nolan, S. (2012, March). *NIH Pain Consortium Centers of Excellence in Pain Education (CoEPS) Grant*. NIH Grant proposed 3 March 2012 for a 3-year plan for the amount of 275,000.
- Burkard, J.F., (2011, December). *USD iPad Classroom Project Proposal*. Grant proposal presented to incorporate iPad technology into the classroom. Faculty Grant proposed 15 December 2011 for 45 iPads and faculty support for the amount of 20,000.
- Burkard, J.F., (2010, February). *Will the inclusion of a PONV-PDNP risk assessment tool improve PONV measureable outcomes?* Grant proposal presented at the ASU Research Intensive Workshop. Arizona.
- Burkard, J.F., (2010, November). Perioperative Outcomes? Faculty grant funded 17 November 2010 for the amount of 4,000.
- Burkard, J.F., (2010, April). Will the inclusion of a PONV-PDNP risk assessment tool improve PONV measureable outcomes? Grant proposal submitted to UCSD "Innovations in Patient Safety & Quality Improvement". San Diego, California. (Ongoing) 20,000.
- Burkard, J.F., (2009, December). Emergence Delirium in the Combat Veteran. Faculty grant funded 1 December 2009 for the amount of 3,000.

Professional Papers/Abstracts/Poster/Book Chapters

- Gohl, M., Heil, J., & Burkard, J.F. (2012, January). Ultrasound guided paravertebral analgesia and multimodal pathway for acute pain management after breast cancer surgery. Accepted for poster presentation, Western Institute of Nursing Meeting, 18-21 April 2012.
- Jones, S., Burkard, J.F. (2012, January). Effects of cofactors on postoperative recovery times of patients with obstructive sleep apnea. Accepted for poster presentation, Western Institute of Nursing Meeting, 18-21 April 2012.
- Kim, Y., Howell, J., & Burkard, J.F. (2012, January). Effective geriatric pain education

- program for nursing home staffs: Evidence based practice model. Accepted for poster presentation, Western Institute of Nursing Meeting, 18-21 April 2012.
- Liaguno, M., Burkard, J.F. (2012, January). Priming the pump: Early hand expression of breast milk in mother's with NICU babies. Accepted for poster presentation, Western Institute of Nursing Meeting, 18-21 April 2012.
- Maclang, F., Burkard, J.F. (2012, January). Implementation of patient educational intervention to decrease the occurrence of type II diabetes in the gestational diabetic mother. Accepted for poster presentation, Western Institute of Nursing Meeting, 18-21 April 2012.
- Nenno, L., Burkard, J.F. (2012, January). Venous thromboembolism assessment and prophylaxis: A process improvement initiative. Accepted for poster presentation, Western Institute of Nursing Meeting, 18-21 April 2012.
- Savage, A.I., Burkard, J.F. (2012, January). Impact of the patient-centered medical home model (PCMH) on selected patient outcomes and staff satisfaction in a primary care clinic in a military treatment facility. Accepted for poster presentation, Western Institute of Nursing Meeting, 18-21 April 2012.
- Todorova, E., Clark, M.J., & Burkard, J.F. (2012, January). Effects of glycemic management in cardiac surgery: Evidence based project. Accepted for poster presentation, Western Institute of Nursing Meeting, 18-21 April 2012.
- Savage, A.I., Burkard, J.F. (2011, September). Impact of the patient-centered medical home model (PCMH) on selected patient outcomes and staff satisfaction in a primary care clinic in a military treatment facility. Poster presentation, Doctors of Nursing practice Conference, 28-31 September 2011, New Orleans, LA.
- Zamora, Z., Clark, M.J., & Burkard, J.F. (2011). Orthotopic neobladder irrigation: Competency Assessment through simulation. *Urologic Nursing*. April 2011.
- Burkard, J.F. (2011, April). Perioperative Outcomes Analysis Phase One Analysis Project. Poster presentation, Western Institute of Nursing Meeting, 13-16 April 2011.
- Eckhardt, J., Burkard, J.F., & Sawrey, P. (2011, April). Observation unit efficacy in decompensated heart failure patients. Poster presentation, Western Institute of Nursing Meeting, 13-16 April 2011.
- Garner, C. & Burkard, J.F. (2011, April). Applying the evidence: Using research based guidelines in treating patients with diabetes. Poster presentation, Western Institute of Nursing Meeting, 13-16 April 2011.
- Ginn, K., Williams, H., Sarter, B. & Burkard, J.F. (2011, April). The relationship of a diabetes flow sheet on the adherence to national standards in an internal medicine office. Poster presentation, Western Institute of Nursing Meeting, 13-16 April 2011.
- Hyacinth, O., Sarter, B. & Burkard, J.F. (2011, April). Weight management during pregnancy utilizing the IOM guidelines. Poster presentation, Western Institute of Nursing Meeting, 13-16 April 2011.
- McPherson, J. & Burkard, J.F. (2011, April). Implementation of high fidelity simulation to enhance critical thinking skills. Poster presentation, Western Institute of Nursing Meeting, 13-16 April 2011.
- Wolfe, T., Burkard, J.F., & Skerrett, K. (2011, April). Long acting injectables to improve medication adherence in psychotic disorders. Poster presentation, Western Institute of Nursing Meeting, 13-16 April 2011.
- Nolan, S. & Burkard, J.F. (2010). Effect of morbidity and mortality peer review on nurse

- accountability and ventilator-associated pneumonia. *JONA*. 40:9, 374-382.
- McGuire, J. & Burkard, J.F. (2010). Risk factors for emergence delirium in U.S. military members. *Jour of PeriAnesthesia Nur.* 25:6, 392-401.
- Burkard, J.F. (2010, October). Evidence Based Practice: Fostering Nursing Scholarship through Nursing Scholarship. Poster presentation, National DNP Conference Meeting, 29-30 October 2010.
- Burkard, J.F. (2010). Liver failure and nonalcoholic steatohepatitis. In S. Elisha, (Ed), *Case Studies in Nurse Anesthesia*. (1st ed., pp. 105-115). Boston: Jones and Bartlett.
- Burkard, J.F., & Vacchiano, C.A. (2010). Regional anesthesia: Upper and lower extremity blocks. In J.J. Nagelhout & K.L. Plaus (Eds.), *Nurse Anesthesia*. (4th ed., pp. 1077-1102). St. Louis: Saunders.
- Burkard, J.F. (2009). 2009 PONV evidence based consensus guideline update. *Anesthesia Abstracts*, 3:9, 1-44.
- McGuire, J., & Burkard, J.F. (2010). Risk factors for emergence delirium in U.S. Active Duty military members. Manuscript submitted for publication.
- Arruda, T., Burkard, J. (2010). Predictors of critical care determinant factors: Safest patient flow for emergency department admissions. Poster presentation: Western Institute of Nursing Meeting, 13-16 April 2010.
- Arellano, C., Izuchukwu, I. & Burkard, J. (2010). The use of simulation in improving the readiness skills of airforce squadron medical technicians in the care of patients with chest tube drainage systems. Poster presentation: Western Institute of Nursing Meeting, 13-16 April 2010.
- Fernandez, A., Burkard, J. (2010). Screening strategies to improve early diagnosis of diabetes mellitus in the adult population. Poster presentation: Western Institute of Nursing Meeting, 13-16 April 2010.
- Macauley, K., Clark, M.J., & Burkard, J. (2010). Warfarin therapy management in primary care. Poster presentation: Western Institute of Nursing Meeting, 13-16 April 2010.
- Nolan, S., Davidson, J., & Burkard, J. (2010). The effect of nursing morbidity and mortality peer review on nursing accountability and ventilator associated pneumonia rates in the intensive care unit. Poster presentation: Western Institute of Nursing Meeting, 13-16 April 2010.
- Zamora, Z., Clark, M.J., & Burkard, J. (2010). Orthotopic neobladder irrigation: Competency assessment through simulation. Poster presentation: Western Institute of Nursing Meeting, 13-16 April 2010.
- Burkard, J.F. & McGuire, J. (2009). Survey of Certified Registered Nurse Anesthetists regarding the concept of emergence delirium. Unpublished manuscript, School of Nursing, University of San Diego, San Diego, California.
- Burkard, J.F., & Vacchiano, C.A. (2010). Regional anesthesia: Upper and lower extremity blocks. In J.J. Nagelhout & K.L. Plaus (Eds.), *Nurse Anesthesia*. (4th ed., pp. 1077-1102). St. Louis: Saunders.
- Williamson, W., Burks, D., Pipkin, J., Burkard, J.F., Osborne, L.A., Pellegrini, J.E. (2009). Effect of timing of fluid bolus on reduction of spinal-induced hypotension in patients undergoing elective cesarean delivery. *AANA Journal*, 77:2, 130-136.
- Rivera, J.J., Vilecco, D.J., Dehner, B.K., Burkard, J.F., Osborne, L.A., Pellegrini, J.E. (2008). The efficacy of ketorolac as an adjunct to the bier block for controlling post

- operative pain following nontraumatic hand and wrist surgery. *AANA Journal*, 76:5, 341-345.
- Burkard, J.F. (2008). Care of the pregnant patient. In C.B. Drain & J. Odom-Forren (Ed.), *Perianesthesia in nursing: A critical care approach*. (pp. 729-737). St. Louis: Sanders-Elsevier.
- The efficacy of ketorolac as an adjunct to the bier block for controlling post-operative pain following non-traumatic hand and wrist surgery. LT Jesse Rivera, CRNA, LT Dante Villecco, CRNA, LT Bryan Dehner, CRNA, CDR Joseph Burkard, DNSc, CRNA, CDR Lisa Osborne, PhD, CRNA, CAPT Joseph Pellegrini, PhD, CRNA Accepted for publication in the October AANA journal.
- Effect of timing of fluid bolus on reduction of spinal induced hypotension in patients undergoing elective cesarean delivery. LCDR Walter Williamson, CRNA, LT David Burks, CRNA, LT Jessica Pipkin, CRNA, CDR Joseph Burkard, DNSc, CRNA, CDR Lisa Osborne, PhD, CRNA, CAPT Joseph Pellegrini, PhD, CRNA. Accepted for publication in the AANA Journal.
- Prevention and Management of Postoperative Nausea and Vomiting: A Look at Complementary Techniques. Myrna E. Mamaril. MS, RN, CPAN, CAPA, Pamela E. Windle, MS, RN, CAN, CPAN, CAPA, Joseph F. Burkard, DNSc, CRNA
- Vocal Cord Dysfunction: A Case Report Erik C. Cline, LCDR, NC, USN, MSN, CRNA Roger Davis, LCDR, NC, USN, MSN, CRNA Joseph F. Burkard, CDR, NC, USN, CRNA, DNSc, AANA 2006 October journal.
- Burkard, J.F. (2006, August). Can Anesthesia Providers Accurately Predict Their Patient's Preoperative Anxiety? Oral and Poster Presentation, AANA Annual Meeting Washington, D.C.
- Will The Addition of a Sciatic Nerve Block to a Femoral Nerve Block Provide Better Pain Control Following Anterior Cruciate Ligament Repair (ACLR) Surgery? Oral and Poster Presentation, AANA Annual Meeting Washington, D.C.
- Burkard, J.F. (2005). Kearns Sayre Syndrome: A Case Report. Accepted for publication in the GTJNA 2005 Journal.
- Burkard, J.F. (2005). A Modification of the Traditional Bier Block: A Case Report. Accepted for publication in the GTJNA 2005 Journal.
- Burkard, J.F. (2005). Propofol-Ketamine Infusion in an Umbilical Hernia Repair: A Case Report. Accepted for publication in the GTJNA 2005 Journal.
- Burkard, J.F. (2005). Vocal Cord Dysfunction: A Case Report. Accepted for publication in the AANA 2005 Journal.
- A comparison of ondansetron and transdermal scopolamine patches for patients identified to be high risk for the development of post operative nausea and vomiting. Accepted for publication in the AANA 2005 Journal.
- Author chapter 42, Regional Anesthesia, Third edition of Nurse Anesthesia by Nagelhout and Zaglaniczny.
- The effects of fatigue on the performance of anesthesia providers in a simulator setting. Poster presented at the AANA annual meeting, Seattle, WA 4-11 August 2004
- Effects of fiberoptic versus laryngoscopic intubation as measured by the bispectral index (BIS). Poster presented at the AANA annual meeting, Seattle, WA 4-11 August 2004
- Burkard, J.F. (2003, November). Effect of Air versus Saline in the Loss of Resistance Technique in Identifying the Epidural Space when placing a Continuous Epidural

- Catheter among the Parturient. Poster presented at the 15th annual Karen Rieder Poster Session, AMSUS, San Antonio, TX 15-19 November 2003
- Burkard, J.F. (2003 November). A comparison of ondansetron and transdermal scopolamine patches for patients identified to be high risk for the development of post operative nausea and vomiting. Poster presented at the 15th annual Karen Rieder Poster Session, AMSUS, San Antonio, TX 15-19 November 2003
- Bispectral Analysis and Motor Activity Assessment Scores in Mechanically Ventilated Patients in the Intensive Care Unit. Poster presented at the 15th annual Karen Rieder Poster Session, AMSUS, San Antonio, TX 15-19 November 2003
- The effects of clonidine added to bupivacaine on analgesia and post-operative outcomes to femoral / sciatic nerve blocks. Accepted for publication in the AANA 2004 journal. Published July-August 2004
- A comparison of levobupivacaine and ropivacaine in patients undergoing extremity surgery with brachial plexus block. Accepted for publication in the AANA 2004 journal.
- Comparison of axillary Brachial Plexus Block success using a 26 Gauge 1/2 Inch Needle versus a Standard Gauge 1 1/2 Inch Needle. Published in the AANA Journal January 2004
- The effects of clonidine added to bupivacaine on analgesia and post-operative outcomes to femoral / sciatic nerve blocks. Poster presented at the AANA annual meeting, Orlando, FA 1-8 August 2002
- Comparison of axillary Brachial Plexus Block success using a 26 Gauge 1/2 Inch Needle versus a Standard Gauge 1 1/2 Inch Needle. Poster presented at the AANA annual meeting, Orlando, FA 1-8 August 2002
- A comparison of levobupivacaine and ropivacaine in patients undergoing extremity surgery with brachial plexus block. Accepted for publication in the AANA 2004 journal. Poster presented at the AANA annual meeting, Orlando, FA 1-8 August 2002

Professional Presentations

- Burkard, J.F. (2012, May). Joining Forces through Creative Curriculum Approaches: A Panel Discussion. *AACN Leadership Collaboration Webinar*. 14 May 2012.
- Burkard, J.F. (2012, March). Evidence-Based Practice: I know what it is, but how do I do it. *Scripps 9th Annual Advanced Practice Oncology Providers Symposium: Innovation Through Practice*. San Diego, Ca.
- Burkard, J.F. (2012, February). Health Policy for the Advanced Practice Nurse Anesthetist. Visiting Professor Workshop presentation, Samuel Merritt University 25-27 February 2012.
- Burkard, J.F., Skerrett, K., Stanley, L., Suarez, M.J., & Terry, M.J. (2011, December). Readjustment Strategies for the Wounded Warrior. *Military Healthcare Convention & Conference*. Workshop conducted at the Institute for Defense and Government Advancement, Paradise Point, San Diego, Ca.
- Burkard, J.F. (2011, September). APRN's Returning for Doctoral Degrees: Identifying Health Policy Needs. Podium presentation, Doctors of Nursing practice Conference, 28-31 September 2011, New Orleans, LA.
- Burkard, J.F. (2011, September). Research Ethics: Issues Concerning Human Subjects. Research methods training symposium conducted at Naval Medical Center, San Diego California.

- Burkard, J.F. (2011, August). CRNA's Returning for Doctoral Degrees: Identifying Health Policy Needs. Podium presentation, American Association of Nurse Anesthetists National Meeting 6 August 2011.
- Burkard, J.F. (2011, March). Innovation in Anesthesia Practice. Podium presentation, California Association of Nurse Anesthetists Spring State Meeting 25-27 March 2011.
- Burkard, J.F. (2011, February). Health Policy for the Advanced Practice Nurse Anesthetist. Visiting Professor Workshop presentation, Samuel Merritt University 25-27 February 2011.
- McGuire, J. & Burkard, J.F. (2011, March). Post Traumatic Stress in the Combat Surgical Patient. First Place Award, Academic Research Competition. San Diego California
- Burkard, J.F. (2011, January). Preventing and treating PONV: An evidence approach. Podium presentation, University of California, San Diego 15 January 2011.
- Burkard, J.F. (2010, July). Pain and sedation management: state of the art 2010. Podium presentation, University of California, San Diego 22 July 2010.
- Burkard, J.F. (2011, January). Preventing and treating PONV: An evidence approach. Podium presentation, University of California, San Diego 15 January 2011.
- Burkard, J.F. (2011, March). Innovation in Anesthesia Practice. Podium presentation, California Association of Nurse Anesthetists Spring State Meeting 25-27 March 2011.
- McGuire, J. & Burkard, J.F. (2011, March). Post Traumatic Stress in the Combat Surgical Patient. First Place Award, Academic Research Competition. San Diego California
- Burkard, J.F. (2009, December). A Multidisciplinary Expert Forum on Perioperative Nausea and Vomiting. Panel presentation done at the Institution of Medical Education and Research 15 December, 2009. Miami Florida
- Burkard, J.F. (2010, February). Analysis of Health Policy for the Advanced Practice Nurse. Health Policy Visiting Professor Series. Visiting professor symposium conducted at Samuel Merritt School of Nurse Anesthesia, Oakland California.
- Burkard, J.F. (2010, January). Evaluating the evidence in evidence based practice. Symposium conducted at the 23rd annual University of California San Diego anesthesia update, San Diego California.
- Burkard, J.F. (2010, March). Fostering Nursing Scholarship Through Academic and Service Partnership. In S. Hardin panel presentation given during the Janet A. Rodgers Nursing Leadership workshop. San Diego California
- Burkard, J.F. (2010, March). Trauma and Healing Health Policy Issues. . In K. Skerrett and S. Hardin, Trauma and Healing Workshop. Symposium conducted during the Hahn School of Nursing Trauma and Healing Workshop March 5th, 2010. San Diego California
- Burkard, J.F. (2009, August). Responsible conduct of research. Research methods training symposium conducted at Naval Medical Center, San Diego California.
- Burkard, J.F. (2009, April). *Trauma & ARDS an EBP clinical case study review*. Symposium conducted at the 2009 Critical Care Code Trauma Seminar, San Diego California.
- Burkard, J.F. (2009, April). *Responsible conduct of research*. Research methods training symposium conducted at Naval Medical Center, San Diego California.

- Burkard, J.F. (2009, January). *Evidence based practice- a model to change practice*. Workshop symposium conducted at the University of San Diego Hahn School of Nursing, San Diego California.
- Burkard, J.F. (2009, January). *Post operative nausea & vomiting: Evidence based consensus guidelines*. Symposium conducted at the 22nd annual University of California San Diego anesthesia update, San Diego California.
- Burkard, J.F. (2008, December). Reviewer Chair, Congressionally Directed Medical Research Programs program for deployment related medical research programs held December 15th, 2008 at Fort Detrick, MD.
- Burkard, J.F. (2008, August). *Responsible conduct of research*. Research methods training symposium conducted at Naval Medical Center, San Diego California
- Burkard, J.F. (2007, August). Regional Anesthesia for the Upper and Lower Extremity: New Trends in Local Anesthetics and Block Techniques. IDAHO Spring 2007 Conference for Nurse Anesthetists
- Burkard, J.F. (2007, August). USC Regional Anesthesia for the Upper and Lower Extremity: New Trends in Local Anesthetics and Block Techniques. USC Spring 2007 Workshop for Nurse Anesthesia Students
- Burkard, J.F. (2006, March). USC Regional Anesthesia for the Upper and Lower Extremity: New Trends in Local Anesthetics and Block Techniques. USC Spring 2006 Workshop for Nurse Anesthesia Students
- Burkard, J.F. (2006, August). Funderberg Lecture Series, Samford University, Birmingham, Alabama, Brachial Plexus and Upper extremity Blocks for the Anesthesia Provider, Anesthesia Awareness Monitoring: A controversial Issue in Anesthesia Practice.
- Burkard, J.F. (2005, October). Presentation on Anesthesia Awareness Monitoring: A Controversial Issue in Anesthesia Practice. My Patient Has a Bleeding Disorder: Transfusion Guidelines. CANA Anesthesia Update Fall 2005 Monterey, CA
- Burkard, J.F. (2005, May). Responsible Conduct / Ethics of Research Presentation. Naval Medical Center CID Research Series, May 2005
- Burkard, J.F. (2005, February). Presented: The incidence of Awareness During Anesthesia: A Multicenter United States Study. Journal Club NMCSO, February 2005
- Burkard, J.F. (2004, October). Presented: Effects on the progress of labor: Do labor epidurals affect labor outcome. Fall CANA state meeting. Berkeley, Ca, October 2004
- Burkard, J.F. (2004, October). Presented: Non-Obstetric surgery during pregnancy: Does anesthesia increase the risk to the parturient. Fall CANA state meeting. Berkeley, Ca, October 2004
- Burkard, J.F. (2004, August). Presented Two Day Advanced Airway Workshop at the AANA Annual meeting Seattle, WA, August 2004
- Burkard, J.F. (2004, May). Responsible Conduct of Research Presentation. Naval Medical Center CID Research Series, May 2004
- Burkard, J.F. (2004, May). Presented AANA Public Relations Topics at National Rural Health Association Annual Conference, San Diego, Ca, May 2004
- Burkard, J.F. (2004, April). Presented Bispectral Analysis Dissertation Manuscript at NMCSO Academic Research Competition, April 2004

- Burkard, J.F. (2003, October). **Outcomes Research in Regional Anesthesia and Analgesia, CANA La Jolla, CA, October 10, 2003**
- Burkard, J.F. (2003, August). **AANA National Conference, Boston, Ma Aug 4-5 2003. Spinal / Epidural Workshop Instructor**
- Burkard, J.F. (2003, June). **Trauma Fluid Resuscitation: An Anesthesia Provider's Perspective. CANA Las Vegas State meeting 8 June 2003**
- Burkard, J.F. (2002, May). **National Speaker ASPAN Conference San Diego, CA Regional Anesthesia in the Post Anesthesia Care Unit, April-May 2002**
- Burkard, J.F. (2002, April). **Training for the Future: Technology and the Anesthesia Team, Kaiser Permanente Anesthesia Symposium, April 2002**
- Burkard, J.F. (2002, March). **Comparison of axillary Brachial Plexus Block success using a 26 Gauge 1/2 Inch Needle versus a Standard Gauge 11/2 Inch Needle. Naval Medical Center San Diego Annual Research Competition, March 2002**
- Burkard, J.F. (2001, October). **CANA Speaker, Napa CA on trauma anesthesia, Napa California, CANA Fall meeting, October 2001**
- Burkard, J.F. (2001, January). **Ethical Decision Making in a Changing Environment. Surface Warfare Medical Institute Presentation. Point Loma, CA, January 2001**
- Burkard, J.F. (2000, August). **AANA National Conference, Chicago, IL Aug 5-10 2000. Spinal / Epidural Workshop Instructor**
- Burkard, J.F. (2000, June). **Video Teleconference Pain Management for the PACU Nurse, Kaiser Permanente, AANA Upper Extremity Workshop Presentation, Chicago, IL, June 2000**
- Burkard, J.F. (2000, May). **Regional Anesthesia for the Millennium. Regional Anesthesia of the Lower Extremity Presentation and Workshop. CANA Spring Conference, Palm Springs, CA, May 2000**

September 20, 2012

AANA Foundation
222 S. Prospect Ave.
Park Ridge, IL 60068

Dear Dr. Lorraine Jordan and the AANA Foundation Grant Selection Committee,

It is with sincere gratitude that I write you this letter extending my appreciation for awarding me the AANA Baxter Research Doctoral Fellowship Grant. I assure you that our current project, Psychoneuroimmunology and Chronic Pain, for which I received your award has developed into a stellar project that is progressing rapidly. The purpose of this letter is to present you with the plan for utilizing the AANA Baxter Research Doctoral Fellowship funds, as well as the funds applied for in this AANA General Research Grant. I would also like to appraise you of the progress we have made since my proposal submission in April 2012 when I submitted my Fellowship application. Since April, we have added an additional psychometric component to the study to reflect another highly pertinent component of chronic pain symptomatology. After a thorough literature search and through our collaboration with Dr. Michael Irwin, UCLA Professor and Director of the Cousins Institute of Psychoneuroimmunology, it was decided that a measurement of sleep deprivation would be added to the study to provide a more robust and complete picture of the symptoms experienced by chronic pain patients. Through my readings of the literature I found that not only is sleep deprivation and pain highly interrelated, but I have found that inflammation has been evolving throughout this body of literature as well. This is a very pertinent measurement to add to this study, there is relatively little known about the central inflammation levels related to sleep deprivation in chronic pain patients and the research is developing now. This is yet another cutting-edge component of this project that not only increases the overall elegance, but also

makes it one of the most progressive studies on chronic pain, inflammation and the psychological components associated pain.

I am elated to report that the UCLA Institutional Review Board application is being submitted and we will have pending status shortly.

I can report that our budget has been comprehensively created to be both cost-conscious and practical for the volume and scope of laboratory and psychometric testing we are proposing to accomplish. Through collaboration with our Co-Investigator Dr. Elizabeth Breen, PhD, Director of the Cousins Inflammatory Biology Laboratory, as well as our Co-Investigator Dr. Andrea Nicol, MD, MS, Director of Research at the UCLA Pain Management Clinic, we have formulated a budget that incorporates the AANA Fellowship Grant monies. The \$10,000 AANA Fellowship Grant funds will cover the total laboratory expenses for approximately half of our desired study population with three of the five desired cytokines being analyzed. With the funds sought from this General Research Grant, we would be able to analyze five cytokines on our full study population of thirty chronic pain patients. This further funding would allow for a population size that could yield significant results and provide us with the complete five cytokine analysis necessary to illustrate a comprehensive picture of the inflammation set forth in the study design. While our budget reflects that the amount still unfunded to run these laboratory tests is roughly \$17,000, we do hope the Grant Selection Committee will award us considerably more to cover the \$7,000 in indirect costs associated with performing this study at UCLA through USD. If this funding is not currently available from the AANA Foundation, I humbly request you consider me next spring for another Fellowship Grant award upon my application.

Of note, the data collected in this cross-sectional study, will serve as both my dissertation research, as well as a preliminary study for a much more comprehensive longitudinal study designed to investigate inflammation, chronic pain and the psychological components of pain. I look forward to presenting the cross-sectional study findings to the AANA and continuing with Dr. Charles Griffis, CRNA, PhD on expanding this study into a longitudinal study with compelling results. I cannot express how ecstatic I am to

be a part of this revolutionary research and sincerely thank you for your assistance in making this study a reality. Proof is power!

Sincerely,

Sarah Giron, CRNA, MS





Andrea Nicol, M.D., M.S.
Director of Research, UCLA Pain Management Center
Assistant Clinical Professor, Department of Anesthesiology
David Geffen School of Medicine at UCLA
Office Phone: (310) 319-1253
Office Fax: (310) 319-2263

UCLA Pain Management Center

1245 16th Street, Suite 225
Santa Monica, CA 90404
Phone: 310-794-1841
Fax: 310-319-2263

2001 Santa Monica Blvd, Suite 860W
Santa Monica, CA 90404
Phone: 310-828-3209
Fax: 310-828-5165

Dr. Lorraine Jordan, CRNA, PhD
222 South Prospect Avenue
Park Ridge, Illinois 60068-4001
phone: 847-655-1170
fax: 847-692-6968

September 24, 2012

Dear Dr. Jordan,

It is with great pleasure and enthusiasm that I write you this letter to support the AANA Foundation General Research Grant application for Sarah E. Giron, CRNA, MS. I have known Sarah for many years as we were colleagues in the Department of Anesthesiology at UCLA. We both moved on, I to a Pain Management fellowship and Masters in Clinical Research at UCLA and Sarah to USC and now the University of San Diego for a PhD.

I believe Sarah has what it takes to become a leader in the field of anesthesiology and pain research. I have worked with many people during my time at UCLA on research projects and there are many attributes about her that make her unique and special and separate her in a positive way, from the rest of the pack. She takes the initiative in organizing email discussions, in-person meetings, and phone conferences and has the ability to unite the entire study team so that forward progress on projects happens at a very fast pace. She has an affable personality and is extremely pleasant and engaging to work with.

She is well-regarded by her peers, colleagues, and mentors as evidenced by her many awards and honors dating back to 2000. Her academic merit and presence is clearly substantiated as evidenced by the amount of invited lectures and presentations she has given throughout her career. Her dedication to the advancement of current clinical and basic science evidence based medicine in our field is without question, given her return to the classroom to complete coursework and a thesis towards a PhD in Nursing. I have no doubt that she will complete this

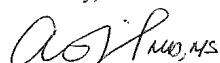
additional educational training with the highest accolades and will have a very bright future in both the clinical and academic/research realms.

Besides highlighting Sarah's strengths, this letter serves to support her application for a research grant through the AANA for the project she plans to complete for her PhD dissertation. I was approached about one year ago by Sarah regarding this project and from the very beginning, I was extremely excited to take part. I will be serving as a co-investigator on this project, which is a cross-sectional study that will investigate the emerging relationships between chronic pain syndromes and the role of central inflammation, as measured by cerebrospinal fluid patterns of inflammatory mediator molecules. In addition to the CSF patterns, the study will be exploring the relationships between the psychological responses to pain and then correlated to cerebrospinal fluid levels of inflammatory markers. This study hopes to gain knowledge of the relationship between the psychoneuroimmune responses to prolonged pain states propagated by central inflammatory mechanisms, with the ultimate goal of acquiring the information pertinent to more effective analgesic and psychological treatment of these patients.

This study is unique, exciting, and unlike any study that has been done before on this topic. There is a great deal of possible knowledge to be gained from the cross-sectional comparison of the CSF between chronic pain and non-chronic pain subjects. Sarah and the rest of our team are extremely hopeful that the data we get from this cross-sectional analysis will provide the justification to perform a longitudinal analysis on the CSF of chronic pain patients as they undergo treatment with intrathecal opiates.

It is with great honor and no reservations that I write this letter in support of Sarah Giron's application for a research grant from the AANA. Please do not hesitate to contact me if you have any questions or need further information.

Sincerely,



Andrea L. Nicol, MD, MS
Director of Research, UCLA Pain Management Center
Assistant Clinical Professor, Department of Anesthesiology
David Geffen School of Medicine at UCLA



September 24, 2012

Lorraine Jordan, CRNA, PhD
Executive Director, AANA Foundation
222 South Prospect Avenue
Park Ridge, Illinois 60068-4001
Re: Giron Proposal "Psychoneuroimmunology and Chronic Pain"

Michael Irwin, M.D.
Director

voice (310) 825-8281
fax (310) 794-9247
www.CousinsPNI.org

Dear Dr. Jordan,

I am pleased to write in support of the research proposal being submitted by Ms. Sarah E. Giron, CRNA, MS, for consideration by the AANA Foundation. Ms. Giron's proposed study examining psychological and immunological measures in chronic pain patients represents both a new area of research for our laboratory, and an extension of previous collaborations on psychoneuroimmunology studies supported by the AANA Foundation (Dr. Charles Griffis, CRNA, PhD, 2011).

Our Inflammatory Biology Core (IBC) Laboratory of the UCLA Cousins Center for Psychoneuroimmunology serves as a research resource for investigators wishing to include assessments of the immune system in their studies. We are able to perform evaluations of cellular signaling, gene expression, and biomarkers of immune system activation and/or inflammation. As the Co-Director of the IBC and an immunology researcher who has investigated immune activation and cytokine biology for more than twenty-five years, I have extensive experience in the collection, processing, and testing of immunological samples in clinical and epidemiologic studies. I oversee the day-to-day operations of the IBC Laboratory, review all results for quality control, and work closely with co-investigators in the interpretation of immunologic data.

I have been providing technical advice and mentoring to Ms. Giron as she has developed her study design and research plan, and I express my enthusiastic support for her application to the AANA Foundation. I hope that we will have the opportunity to pursue the innovative study of chronic pain and the interaction with the mind and the immune system that she has outlined in her proposal, and I look forward to a productive collaboration.

Sincerely,

Elizabeth Crabb Breen, MT(ASCP), PhD
Associate Professor, Department of Psychiatry and Biobehavioral Sciences
Co-Director, Inflammatory Biology Core
UCLA Cousins Center for Psychoneuroimmunology
Office: (310) 206-5738
ebreen@mednet.ucla.edu

September 26, 2012

Lorraine Jordan, CRNA, PhD
Research Director
AANA Foundation

Re: General Research Grant Application
"Psychoneuroimmunology and Chronic Pain"

Dear Dr. Jordan:

I am writing this letter to recommend the study referenced above for General Research Grant funding. As I have indicated in my own writing for the AANA Journal¹, chronic pain is a problem of tremendous scope, causing terrible suffering for millions of Americans and costing our beleaguered health care system billions of dollars a year. Basic and translational research to advance treatment of chronic pain syndromes is desperately needed, and I have found a graduate student who proposes to do just that as a dissertation project. Funding this research is in perfect alignment with the goals and mission of the AANA Foundation.

Sarah E. Giron, CRNA, MS, has proposed a research study examining the cerebrospinal fluid (CSF) of chronic pain patients who are having the implantation of catheters and pumps to allow intrathecal opioid infusion pain treatment.

The study has two specific aims:

Specific Aim #1: This study will examine and characterize the relationships between chronic pain and pro- and anti-inflammatory mediator molecules.

The study will bring together several talented groups to accomplish this aim. Ms. Giron is currently a doctoral student at the Hahn School of Nursing, University of San Diego and has assembled a committee of capable experts to assist her. In the fields of pain physiology and clinical pain intervention, one of the faculty of the UCLA Department of Anesthesiology, Santa Monica Pain Group, Andrea Nicol, M.D. will provide guidance and clinical access. In the area of psychoneuroimmunology, the UCLA Cousins Center of Psychoneuroimmunology is represented, including Dr.

Michael Irwin, an internationally known researcher in body-mind phenomena. Dr. Irwin will focus his efforts on investigating any correlations between the behavioral manifestations of pain and the levels and type of inflammatory mediator molecules found in the cerebrospinal fluid of these patients. Elizabeth Breen, PhD, the Director of the Inflammatory Biology Laboratory, will use her extensive background in immunological laboratory science to oversee and assist in specimen collection and analysis.

The CSF samples from patients will be compared to control samples from a bank established by researchers from the University of California, San Diego in previous investigations, and which are already stored in the Inflammatory Biology Laboratory where the analysis will be conducted. Samples will be examined using ELISA for the presence and levels of immune inflammatory mediator molecules such as cytokines. The data comparing patients and controls will be analyzed using parametric and non-parametric inferential statistics.

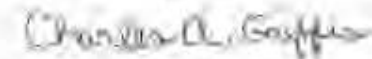
Specific Aim #2: To investigate relationships between pain levels, anxiety, depression, sleep deprivation and the expression of inflammatory molecules in the cerebrospinal fluid of chronic pain patients.

To accomplish this specific aim, Ms. Giron has established a relationship with the UCLA Cousins Center of Psychoneuroimmunology. Dr. Michael Irwin, mentioned above, has joined the dissertation team as a committee member. As a consultant on the grant, he is eager to explore the mind-body connections within the human response to pain. To that end, Dr. Irwin is prepared to lend his expertise to the selection and use of appropriate instruments to assess the behavioral impact of chronic pain, such as anxiety, fear, depression, and sleep deprivation. Dr. Irwin will then assist in analyzing and interpreting the data, which will be examined for correlations between the behavioral manifestations of pain, pain severity, and the expression of inflammatory molecules in the CSF of chronic pain patients.

To date, and to this team's knowledge, there have been less than 10 studies of chronic pain and CSF inflammatory response in humans, though a large body of pre-clinical data, obtained mostly from rodent models of spinal cord or sciatic nerve injury, exists to guide the concepts and interventions. The data so far have been promising, revealing a propensity for the increase of inflammatory molecule production in CSF of chronic pain patients, yet the data is also contradictory in places, and sample sizes have been small. Thus our team firmly believes there is room for further investigation of this phenomenon, especially in view of the prevalence of chronic pain and the high stakes involved in treatment advances. This study will add validity, and hopefully provide some more direction in the clinical search for novel approaches to chronic pain treatment. Hopefully, our results will further guide new approaches to targeting central inflammation in pain patients, which as yet have not seen Phase 1 trials.

In conclusion, the proposed study is supported by a unique combination of disciplines and resources to include psychiatry, psychoneuroimmunology, clinical pain treatment services, and immunology laboratory science. The experts representing these resources in several southern California academic institutions have been confirmed as available and eager to proceed in this novel and cutting edge investigation of chronic pain. This study illustrates that nurse anesthesia basic science research includes the area of human chronic pain, and is congruent with the Foundation Mission Statement: "Advancing the science of anesthesia through education and research". The results of this study will serve to illustrate to the world of pain care and anesthesia services that nurse anesthetists are capable of forging important interdisciplinary academic relationships committed to not only providing clinical pain care, but to producing new knowledge that will contribute to the effective treatment of debilitating chronic pain and further help alleviate suffering. It is our sincere hope that the Foundation Research Committee will decide to fund this important work.

Yours sincerely,



Charles A. Griffiths, CRNA PhD
Associate Professor
UCLA Nursing and Anesthesiology
Cgriffi55@gmail.com

1. Griffiths, C et al. (2011). Neuroimmune activation and chronic pain. *AAANA J*, 79(1), 31-37.



September 26, 2012

Dr. Lorraine Jordan, CRNA, PhD
Executive Director of the AANA Foundation and AANA Senior Director of Research
222 South Prospect Avenue
Park Ridge, Illinois 60068-4001
phone: 847-655-1170, fax: 847-692-6968

AANA General Research Grant: Letter of Support ICO Sarah Elizabeth Giron, CRNA, MS, PhD
Student, USD / Charles A. Griffis, CRNA, PhD

I am writing to express my support for the planned AANA General Research Grant in support of "Psychoneuroimmunology and Chronic Pain" at the University of San Diego and University of California Los Angeles. The University of San Diego and University of California Los Angeles believes the prompt, thorough assessment and treatment of chronic pain is a subject of paramount importance. This study will investigate the emerging relationships between chronic pain syndromes and the role of central inflammation, as measured by cerebrospinal fluid patterns of inflammatory mediator molecules. More specifically, this study will investigate if the same central pro- and anti-inflammatory cytokines found in chronic pain-induced animal models are found in human chronic pain patients.

As a doctoral student at the University of San Diego Sarah has established herself as an outstanding student with a strong academic and research focus. Ms. Giron has received the AANA Palmer Carrier Baxter Scholarship, which has funded the development of her research proposal, facility costs and study preparation costs. The AANA General Research Grant will cover laboratory costs and sample analysis.

I enthusiastically look forward to future collaboration and research in this area between the University of San Diego, UCLA and the AANA. University of San Diego embraces the interdisciplinary model and the collaboration that will proactively move to the prompt and comprehensive treatment of chronic pain at not only this facility but also region wide.

I wholeheartedly support this grant application and support Sarah's team in the review process. I chair her dissertation committee and pain team as she prepares for her PhD proposal defense and institutional review boards.

Joseph Burkard

Manuscript III

UNIVERSITY OF SAN DIEGO

Hahn School of Nursing and Health Science

The Role of Central Inflammation in the Chronic Pain Paradigm

Sarah E. Giron, PhD(c), CRNA

Abstract

There is increasing evidence linking chronic pain to altered levels of central nervous system (CNS) inflammation and increased levels of perceived pain, anxiety, depression and sleep disturbance. However the inflammatory molecules responsible for physiologic and psychological components of chronic pain still warrant identification and exploration. Using central inflammation as a paramount factor in the creation and maintenance of chronic pain, this study aimed to investigate and describe the physical and psychological aspects of chronic pain associated with CNS inflammation.

Using a cross-sectional correlational design, cerebrospinal fluid (CSF) inflammatory cytokine patterns present in 8 chronic pain participants were compared to inflammatory cytokine patterns present in 30 control CSF samples using multivariate analysis of variance (MANOVA), with analysis of variance (ANOVA) analysis as a follow-up. Levels of depression, anxiety, sleep disturbance and pain were measured in approximately 8 chronic pain patients and correlated to CSF levels of inflammatory cytokines using Pearson's r , or Spearman's Rho correlations. Demographic information was also explored for relationships to central inflammation and descriptive statistics were examined for responses.

To our knowledge, this is the first study to describe increased CSF levels of Interleukin-8 (IL-8) in a population of majority Failed Back Surgery Syndrome chronic pain patients ($F(1, 36) = 14.89, p < 0.001, \text{partial } \eta^2 = 0.293$). Gender ($F(1,6) = 7.782, p = 0.032, \eta^2 = 0.565$), socioeconomic status ($r = -0.823, p = 0.012$) and educational level ($r = 0.727, p = 0.041$) were also correlated with central levels of inflammation, indicating

that central physiologic changes may be related to host sex and psychosocial factors. All participants reported poor sleep quality and took at least one opioid medication, indicating that sleep and opioids may scaffold a portion of the chronic pain paradigm.

This study richly describes the dynamic experience of chronic pain through the lens of Psychoneuroimmunology. Incorporating both physiologic and psychological aspects of the disease, this study describes an association between chronic pain, central inflammation, gender, socioeconomic status, opioid medications and poor sleep quality. Thus, the future of pain treatment must consider these aspects when treating patients and look to future studies for possible new treatment options which target these factors.

Keywords: inflammation, cytokines, chronic pain, sleep quality, gender, socioeconomic status

Chronic pain continues to grow in patient numbers and healthcare costs as a condition that has relatively few successful treatment options and dismal recovery. Tolerance to medications, invasive treatment options and debilitating symptoms create a milieu devastating to the physiologic and psychological wellbeing of the patient. Multiple processes have been identified as agencies responsible for the creation and maintenance of chronic pain, one of them being central inflammatory changes brought upon by glial-mediated activation (Alfonso Romero-Sandoval, & Sweitzer, 2015; Taves, Berta, Chen, & Ji, 2013; Wang, Couture, & Hong, 2014).

Prior studies have shown through glial-mediated activation, inflammatory cytokines contribute to the maintenance of chronic pain, as well as tolerance to analgesic treatment (Dubovy, Jancalek, Klusakova, Svizenska, & Pejchalova, 2006; Raghavendra, Rutkowski, & DeLeo, 2002; Sacerdote et al., 2008; Sung et al., 2004; Tai et al., 2006; Zelenka, Schafers, & Sommer, 2005). Interestingly, studies in anxiety, depression and sleep disturbances have also demonstrated altered levels of central inflammatory cytokines (Baker et al., 2001; Bonne et al., 2011; Camara et al., 2015; Dauvilliers et al., 2014; Levine et al., 1999; Lindqvist et al., 2009). However, many of the previous studies in inflammation have failed to monitor the patterns of central inflammatory cytokines in relation to the psychological aspects specific to chronic pain. A high perception of pain, anxiety, depression and sleep disturbances are common within the chronic pain population (Burke, Mathias, & Denson, 2015; Chuang, Weng, Hsu, Huang, & Wu, 2015; Irwin et al., 2012) but yet how these psychological effects manifest physiologically is relatively not well understood. Central inflammation offers one possible mechanism that

links the physiologic and psychological aspects of the chronic pain paradigm. Thus, the purpose of this study was to investigate and describe the physical and psychological aspects of chronic pain associated with central inflammation.

Materials and Methods

Design

A cross-sectional, correlational study design was utilized with a convenience sample of approximately eight chronic pain patients recruited from the Pain Management Center at the University of California, Los Angeles (UCLA). One-time collections of cerebrospinal fluid (CSF) samples were analyzed for 10 inflammatory cytokines and compared to 30 control CSF samples to discern patterns of inflammation present in chronic pain. Psychological analysis of pain perception, anxiety, depression and sleep disturbances in study participants allowed for correlational testing between psychological and physiologic inflammatory patterns. Demographic data analysis allowed for further inspection.

Subjects

Study participants. Approximately eight patients with a chronic pain diagnosis and who were eligible for medical treatment with an intrathecal analgesic infusion pump were enrolled in this study after informed consent was obtained. All study procedures were approved by the University of California, Los Angeles (UCLA) Institutional Review Board (protocol ID: IRB #12-001588). Recruitment and participation in this study commenced between April 2013 and December 2014. Inclusion criteria for this study required that participants be 18 years or older and carry a chronic pain diagnosis of > 6

months. Diagnosis of chronic pain was made on standard clinical criteria, as suggested by the International Association for the Study of Pain (IASP). Exclusion criteria incorporated a combination of surgical and experimental criteria; surgical criteria that would exclude a patient from obtaining an implanted intrathecal analgesic infusion pain pump included meningitis, active upper respiratory infection/flu or febrile nature, acute medical or psychiatric disorders, acute psychological or physiologic danger/instability, altered mental status, pregnancy and the inability to communicate in English.

Experimental exclusion criteria included a diagnosis of cancer, human immunodeficiency virus (HIV), recent epidural injections for treatment of chronic pain (within six months), drug abuse, meningitis or history of meningitis, history of blood transfusion(s) and palliative pain treatment. The inclusion and exclusion criteria were confirmed using standard clinical and laboratory methods of analysis, not above or beyond the standard of care, or at any additional burden to study participants. Only one patient who was approached to participate in the study declined, making the attrition rate for this study 11%. All study participants were instructed to continue their medications and therapies without making any changes prior to, or during study involvement.

Control samples. Control samples of CSF were obtained from the California NeuroAIDS Tissue Network. Thirty random, individual control samples of 1-2 milliliters (mL) of CSF, collected through the HIV Neurobehavioral Research Center between the years 2005-2012, were utilized from this tissue bank as a means to compare levels of central inflammatory cytokines. The control samples of CSF were selected randomly to include seronegative HIV and hepatitis C virus (HCV) donors, with no history of

recreational drug use, and an approximate equal distribution of men and women donors. No other identifying information of the control sample donors was provided to the research team except the date of collection.

Procedures

Data collection protocol. Approximately eight patients with the diagnosis of chronic pain were enrolled in this study. After informed consent was obtained using Institutional Review Board (IRB)-approved forms, participants completed the psychological instruments to measure their pain perception, depression and sleep deprivation. The anxiety tool was completed by the practitioner collecting demographic clinical data and consent for study involvement. Participants underwent a structured interview to compile their demographic information and all results were number coded to ensure participant anonymity. Upon completion of the psychological questionnaires, study participants were given a \$25 Visa gift certificate and parking reimbursement for their participation in the study.

Approximately one month after recruitment, the study participant underwent a surgical outpatient procedure called an intrathecal analgesic infusion pain pump trial. The surgical procedure and study procedure of collecting intraoperative CSF samples was performed in the operating suite at the UCLA Santa Monica Orthopedic Hospital Outpatient Surgery Center. Strict, standard sterile surgical protocol was followed per the regulations and policies of the hospital. Intrathecal access was performed with or without the use of fluoroscopy per surgeons' discretion. Three mL of CSF were collected from each participant intraoperatively in appropriate polypropylene specimen collection tubes

before 1300. All specimens were processed, stored and analyzed at the UCLA Cousins Center for Psychoneuroimmunology Inflammatory Biology Laboratory.

Thirty samples of 1-2 mL of CSF were utilized from the California NeuroAIDS Tissue Network as a means to compare levels of central inflammatory cytokines. All samples were processed, stored and analyzed at the UCLA Cousins Center for Psychoneuroimmunology Inflammatory Biology Laboratory.

Psychological instruments. To measure the psychological variables associated with chronic pain, various tools were selected for this study. These questionnaires were selected for their internal consistency and reliability (reflected in Table 1 as the Cronbach's α s), their ease of completion and their use in previous studies. They were used to measure the study participants' level of pain perception, anxiety, depression, and sleep quality.

Assays. To quantify patterns of inflammatory markers in both chronic pain and control samples of CSF, the Luminex Performance Human High Sensitivity Cytokine Panel (R&D Systems, Minneapolis, MN) and the Bio-Plex 200 instrument (Luminex®) with Bio-Plex Manager software version 5.0 were utilized. All CSF samples were received and centrifuged at 2000G for 10 minutes at 4°C. Samples were then partitioned into 0.25 mL aliquots, labeled for anonymous identification and stored at -80°C until batch analysis could be performed. Before Multiplex analyses, CSF samples were diluted 1:2, following the protocol recommended for serum and plasma. All analyses were run in duplicate per the manufacturer's recommendation. Samples with a coefficient of variation (CV%) > 20 % between duplicates were evaluated on a case-by-case basis for repeat

assays and/or modified data analysis. Table 2 indicates the range and mean detection limits for the cytokines studied.

Data Organization/Statistical Analysis

This study was designed to be a cross-sectional, correlational study. First, a sample size was calculated using Cohen's *d* analysis. Cohen's *d* was selected to estimate the sample size needed to yield statistically significant results in the testing of the difference between two means (the CSF cytokine levels between healthy and chronic pain participants).

Using this analysis to estimate the sample size and reduce the risk of a Type II statistical error aided in estimating the sample size when the population effect size was not known.

The calculated sample size to generate adequate power was far above the actual recruited participants, limiting the interpretation of our results.

Descriptive statistics were then calculated for all variables in the study and examined for significant responses. Data were tested for normal distribution and the level of significance was set at $p < 0.05$ for all statistical testing. All data are reported as mean \pm standard deviation unless otherwise noted. Cerebrospinal fluid levels of inflammatory cytokines in study participants were compared to controls using parametric statistics for normally distributed variables to include multivariate analysis of variance (MANOVA) for related dependent variables and one-way analysis of variance (ANOVA) for additional follow-up. Differences in gender, race, diagnosis and medications between CSF cytokines were also analyzed using MANOVA and ANOVA. Correlations were calculated to explore relationships between psychological instrument measures, demographics, and CSF levels of cytokines using Pearson's *r* or Spearman's Rho

Correlations. Calculations were accomplished with SPSS software version 22 (SPSS, IBM Inc., Armonk, NY).

Results

Demographics

Of the eight chronic pain patients who participated in the study, five participants were female, three participants were male. The age range for the study participants was 41-74 years of age, with a mean age of 53.50 ± 10.53 years. The mean body mass index (BMI) of study participants was 32.35 ± 4.21 kg/m², indicating that the majority of participants in this study were obese. The mean metabolic equivalents (METs) reported by participants was 3.38 ± 1.51 indicating participants could complete moderate intensity activities. The majority of the patients suffered from Failed Back Surgery Syndrome (FBSS) with a mean length of diagnosis of 9.31 ± 7.75 years. The most frequently reported medications for treatment of chronic pain were gabapentin, oxycodone and a fentanyl patch, respectively, with the mean usage of medications 3.50 ± 4.59 years. Table 3 summarizes the demographics of the study participants.

Psychological Results

The eight study participants self-reported a mean pain rating index (PRI) of 22.88 ± 7.1 out of a total score of 45. The mean present pain intensity (PPI) was 3.57 ± 1.13 out of 5, and visual analog scale (VAS) score was $7.38\text{cm} \pm 1.32\text{cm}$ out of 10 cm. The latter two of these variables had one missing data point that was omitted in analysis and not replaced secondary to the small population size. Participants self reported a mean depression score of 19.75 ± 10.51 indicating an overall group level of mild-moderate

depression. The Hamilton Anxiety Rating Scale was administered by a total of four researchers and yielded a mean score of 20.75 ± 11.31 indicating mild-moderate anxiety levels. Table 4 summarizes the psychological data. Of note, the self-reported mean sleep quality was found to be 15.50 ± 3.16 , indicating poor overall sleep quality in the chronic pain study population; every participant reported poor sleep quality.

Cytokine Results

A total of 10 cytokines were assessed using Multiplex assay analyses, including Interleukin-1 β (IL-1 β), IL-2, IL-4, IL-6, IL-8, IL-10, Tumor Necrosis Factor-alpha (TNF- α), Interferon Gamma (IFN-g), Vascular Endothelial Growth Factor (VEGF) and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF). Three cytokines yielded measurable quantities; IL-6, IL-8 and GM-CSF. Interleukin-1 β , IL-2, IL-4, IL-10, TNF- α , IFN-g and VEGF were undetectable in the majority of samples. While CSF from control samples yielded some within-range values for IL-2 and IL-10, they were found at the lower level of detection and were therefore excluded from analysis. Missing values for GM-CSF were replaced with the mean of the data series. Table 5 summarizes CSF cytokine levels detected via Multiplex assay. Figures 1, 2 and 3 display box plots for those cytokines that were detected with Multiplex assay analysis.

Analysis of IL-6, IL-8 and GM-CSF in Chronic Pain

Data was screened and monitored for normality, linearity and homoscedasticity, and descriptive data was inspected. Both IL-6 and IL-8 were found to be non-normally-distributed and transformed using a log transformation. Granulocyte-Macrophage Colony-Stimulating Factor data were found to be normally distributed between

population samples and required no transformation. The dependent variables (IL-6, IL-8 and GM-CSF) were then monitored for association through correlation. With a low correlation between dependent variables, MANOVA was nonetheless conducted to investigate significant differences between study and control samples of CSF cytokines to decrease the rate of a type I statistical error of multiple ANOVA tests.

A one-way MANOVA was conducted to determine IL-6, IL-8 and GM-CSF differences in pain categories. Multivariate analysis of variance results revealed significant differences among the pain categories on the cytokines, Wilks' $\lambda = 0.676$, $F(2,34) = 5.436$, $p = 0.004$, multivariate $\eta^2 = 0.324$. Analysis of variance was then conducted on each cytokine as a follow-up test to MANOVA. Pain differences were found to be significant for IL-8, $F(1, 36) = 14.89$, $p < 0.001$, partial $\eta^2 = 0.293$. The calculated effect size indicated a decent proportion of IL-8 variance was accounted for by the presence or absence of chronic pain. Differences in IL-6 and GM-CSF were not significant, $p = 0.759$ and $p = 0.581$ respectively.

Analysis of Psychological and Demographic Data

Correlations were analyzed to determine significance between demographic data, psychological data and CSF levels of IL-6, IL-8 and GM-CSF. Pearson's r correlations were utilized to assess significance of interval/ratio-level independent variables. Spearman's Rho correlations were utilized to determine significance of ordinal-level independent variables. Tables 6 and 7 summarize correlation analysis and indicate significance.

No significance was found between psychological data and levels of IL-6, IL-8 or GM-CSF, but all participants did report poor sleep quality. Factors that were found to have significant or near-significant positive correlations to cytokines included age ($p = 0.069$), length of diagnosis ($p = 0.082$) and educational level ($p = 0.041$). Socioeconomic status (SES) was found to have a significant negative correlation ($p = 0.012$).

Multivariate analysis of variance and ANOVA were used to assess significance of gender, race, diagnosis and medications to cytokines. Analysis of variance results displayed significant gender differences for IL-6, $F(1,6) = 7.782$, $p = 0.032$, $\eta^2 = 0.565$. The calculated effect size for gender indicated a moderate proportion of IL-6 variance was accounted for by sex. Figures 4, 5 and 6 are box plots that illustrate differences of cytokines and sex. No significant differences were found between race, diagnosis and medications with respect to inflammation, but all participants did report taking at least one opioid medication.

Discussion

The Relationship Between Chronic Pain and Inflammation

Developing research demonstrates mounting evidence of alternative pain pathways that perpetuate the chronic pain state via physiologic self-propagating mechanisms. A barrage of aberrant pathways contributes to the maintenance of nociception in chronic pain states and one of the mechanisms responsible for, and described in the literature is through glial cell activity (Fleming et al., 2006; Loggia et al., 2015). Investigative work on glial cells has identified a selection of various molecules involved in positive feedback loops responsible for chronic pain, and has established that

the underlying mechanisms involved render our current methods of fighting pain ineffective. Some of the proposed responsible molecules secreted by glial cells are inflammatory mediator molecules, specifically interleukins and tumor necrosis factor-alpha (TNF- α). All of these inflammatory cytokines have been shown to excite pain transmission and experience (Kreutzberg, 1996; Watkins, Milligan, & Maier, 2001); inflammatory cytokines can ramp up dorsal horn neurons in pain transmission (Reeve, Patel, Fox, Walker, & Urban, 2000; Samad, Wang, Broom, & Woolf, 2004), they increase the release of neurotransmitters from sensory afferent nerves responsible for pain response (Inoue et al., 1999; Morioka et al., 2002) and may enhance pain via upregulation of AMPA (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid) and NMDA (N-Methyl-D-aspartate) receptor expression and activity (Beattie et al., 2002; Samad et al., 2004, Viviani et al., 2003; Wang, et al., 2005).

To adequately assess the level of inflammatory cytokines released from glial cell activation in response to established chronic pain syndromes, CSF was gathered from eight chronic pain patients and analyzed in this study. As a means for comparison, study participant CSF was compared to the CSF of 30 healthy controls. Interleukin-6, IL-8 and GM-CSF was detectable in CSF through Multiplex assay analyses. Only IL-8 was found to be significantly higher in chronic pain patients. Thus, this study details the significance between chronic pain and increased central IL-8 levels.

While the majority of study participants for this study were FBSS patients, other studies have documented increased CSF levels of IL-8 in osteoarthritis (Lundborg, Hahn-Zoric, Biber, & Hansson, 2010), postherpetic neuralgia (Kikuchi et al., 1999; Kotani et

al., 2000; Rijdsdijk et al., 2013), sciatica (Brisby, Olmarker, Larsson, Nutu, & Rydevik, 2002) and fibromyalgia (Kadetoff, Lampa, Westman, Andersson, & Kosek, 2012). Other studies that utilized FBSS participants indicated increases in IL-6 (Zin, Nissen, O'Callaghan, Moore, & Smith, 2010) and increased levels of the neurotrophic factors Brain-Derived Neurotrophic Factor (BDNF) and VEGF (McCarthy, Connor, & McCrory, 2013). A complete review of these studies was summarized by Bjurstrom et al. in 2014 (Bjurstrom, Giron, & Griffis, 2014). The current study is the first to document increased CSF levels of IL-8 in a majority of FBSS patients.

The Relationship Between Chronic Pain and Sleep

The complex relationship between pain and poor sleep quality is one that has been extensively documented in the literature, but its causality not fully understood. Both processes of pain causing poor sleep, and poor sleep causing pain illustrate the bidirectional relationship between pain and sleep. From the protective sickness behavior/sickness response theory, sleep has been suggested as a critical component in the restorative process of healing (Dantzer, 2001; Hart, 1988; Watkins & Maier, 2000). Counterproductively, in the presence of chronic pain pathophysiology, the restorative process is impaired by a lack of sleep: In a complex, detrimental dynamic, pain itself causes sleep deprivation and sleep deprivation has been shown to increase pain perception (Chiu et al., 2005; Lautenbacher, Kundermann, & Krieg, 2006; Moldofsky, 2001; Smith & Haythornthwaite, 2004). Up to 53% of chronic back pain patients and 88% of neuropathic pain patients report sleep disturbances (Meyer-Rosberg et al., 2001; Tang, Wright, & Salkovskis, 2007). Furthermore, patients with chronic lower back pain

are 18 times more likely than normal control subjects to experience clinical insomnia (Tang et al., 2007). Thus, literature supports that sleep and pain do interact with one another, the abnormalities of sleep are frequently reported by the chronic pain population, and the effects are not benign: The effects of sleep disturbances have been linked with poor physical and psychological outcomes, absence from work, decreased performance, difficulty with personal relationships, accidents and cognitive decline (Fulda & Schulz, 2001). Even in healthy individuals, sleep deprivation has been noted to cause spontaneous pain (Smith, Edwards, McCann, & Haythornthwaite, 2007).

The data linking central inflammation and sleep disturbances has been primarily found in preclinical reports. Repeated animal studies have shown that upon central administration of IL-1 to both rabbits and rats, the result is an increase in various aspects of sleep (Krueger, Walter, Dinarello, Wolff, & Chedid, 1984; Tobler, Borbely, Schwyzer, & Fontana, 1984) independent of, but similar to the somnogenic effects of TNF- α (Shoham, Davenne, Cady, Dinarello, & Krueger, 1987; Kapás et al., 1992). The data on central inflammation, sleep and pain however is very lacking. In human pain populations there exists almost a complete deficiency of data comparing inflammatory markers, sleep *and* pain. A study by Heffner and colleagues, substantiates that patients with chronic lower back pain were found to have higher levels of plasma IL-6 than healthy control subjects. In the chronic pain population studied, poor sleep quality was found to be associated with greater pain on the following morning (Heffner, France, Trost, Ng, & Pigeon, 2011). The current study is one of the first to inspect central cytokine levels for associations with psychological factors known to contribute to the chronic pain

experience. While data collected from the Pittsburgh Sleep Quality Index (PSQI) did not provide statistically significant correlations to levels of the central cytokines measured, it should be noted that all participants scored poor on the sleep measurement tool. Further inspection on the physiologic link between chronic pain and sleep must be explored.

Socioeconomic Differences in Chronic Pain

Socioeconomic status is well described in the literature as a prominent factor affecting health trajectories and outcomes, and studies in pain literature echo the same sentiment. Of those living in lower SES areas, worse pain severity and outcomes are frequently reported (Brekke, Hjortdahl, & Kvien, 2002; Davies et al., 2009; Fuentes, Hart-Johnson, & Green, 2007; Green & Hart-Johnson, 2012). Studies on SES have indicated there is a difference in treatment patterns between the rich and the poor (Haider, Johnell, Weitoft, Thorslund, & Fastbom, 2009; Thorell, Skoog, Zielinski, Borgquist, & Halling, 2012; van Doorslaer, Koolman, & Jones, 2004; Vikum, Bjørngaard, Westin, & Krokstad, 2013), there is an increased association between work disability related to chronic pain in the poor (Bergman et al., 2001; Kronborg, Handberg, & Axelsen, 2009; Patel et al., 2012), and ultimately chronic pain is associated with poorer SES (Jöud et al., 2014). Some of the causal factors associating pain to SES in the literature are income, employment status, social status and education (Bergman et al., 2001; Doran, Drever, & Whitehead, 2004; Saastamoinen, Leino-Arjas, Laaksonen, & Lahelma, 2005). In this study, socioeconomic status was found to have a strong negative correlation ($r = -0.823$, $p = 0.012$) to inflammation indicating that as socioeconomic status goes down, inflammation goes up. This could be related to treatment modalities; higher SES

participants have been found to utilize specialized services, versus the poor who routinely utilize pharmaceuticals (Haider et al., 2009; van Doorslaer et al., 2004; Vikum et al., 2013; Thorell et al., 2012). This could be secondary to the type of strenuous manual labor that is frequented by lower SES persons (Hagen, Holte, Tambs, & Bjerkedal, 2000; Hagen, Zwart, Svebak, Bovim, & Jacob Stovner, 2005). What is not clear is that contrary to previous studies that link a lower educational level to chronic pain, the current study describes educational level to be positively correlated with inflammation ($r = 0.727$, $p = 0.041$). This could be due to an unanticipated bias in study recruitment; patients were recruited from a specialized pain service, and were undergoing a specialized and costly procedure in an affluent part of Los Angeles. This result could also reflect a lack in participant variability with respect to educational levels: The majority of participants in this study were highly educated. Further inspection of pain clinics from a large multi-institutional health system, in multiple languages would aid assessing the relationship between pain, inflammation, SES and educational level.

Gender Differences in Chronic Pain

Gender differences with respect to pain are well documented, but a relatively new research topic. Owing to a 1990 National Institute of Health policy suggesting participation of women in medical and behavioral research, the relationship of gender and pain are just now starting to be included. Women report pain more frequently (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009), the prevalence of several common chronic pain syndromes are found more often in women than men (Mogil, 2012), and literature demonstrates that women consistently report higher pain ratings

compared to men across different diagnoses (Ruau, Liu, Clark, Angst, & Butte, 2012). Likewise their response to pain interventions are also different; women exhibit different responses to opioid analgesics than men (Niesters et al., 2010) and have been reported to take 3 months longer than men to show improvement of painful symptoms after pain management (Keogh, McCracken, & Eccleston, 2005). Postulated mechanisms that influence the difference in pain sensation and treatment include sex hormones (Craft, 2007), differences in cerebral processing of pain (Berman et al., 2006; Derbyshire, Nichols, Firestone, Townsend, & Jones, 2002; Paulson, Minoshima, Morrow, & Casey, 1998), differing endogenous opioid systems (Smith et al., 2006; Zubieta et al., 2002), endogenous dopaminergic differences (McEwen, 2001), and a multitude of psychosocial mechanisms including gender roles (Defrin, Shramm, & Eli, 2009; Robinson, Gagnon, Riley, & Price, 2003; Wise, Price, Myers, Heft, & Robinson, 2002).

In the current study, females were shown to have significantly lower CSF IL-6 levels, and nearly significant lower levels of CSF IL-8 ($p = 0.111$) when compared to men. If women are reporting more pain and experiencing more chronic pain diagnoses than men, this study's findings do not support that the inflammatory cytokines are responsible pain in females. However, it should be noted that inflammatory cytokines do offer one possible physiologic mechanism for the difference in pain experiences between the sexes. Perhaps the decreased levels of inflammation are responsible for the varied responses to treatment. Nevertheless, further research is needed to examine the gender differences with respect to central inflammation and chronic pain.

Opioid Medication in Chronic Pain

It cannot be overlooked that all participants in this study took at least one opioid medication. This is consistent with the general chronic pain population and current national treatment averages (Ahlbeck, 2011; Ballantyne & Mao, 2003; Mehendale, Goldman, Mehendale, & Rana, 2013). Because data from this study describe an increase in at least one inflammatory cytokine, one must consider the relationship between opioids and inflammatory cytokines. Multiple studies have shown that upon administration of chronic opioids to pain-induced rodents, there was an increase in glial cell activity in the CNS (Raghavendra et al., 2002; Song & Zhao, 2001; Tai et al., 2006). Glia have also been shown to upregulate pro-inflammatory cytokine production and release in the CNS when the organism is treated with longstanding systemic or intrathecal opioids; this increase of cytokine production and release in the CNS has been shown to contribute to opioid tolerance and withdrawal induced pain enhancement (Johnston et al., 2004; Raghavendra, Tanga, & DeLeo 2004; Song & Zhao, 2001). Literature has repeatedly championed a relationship between central cytokines and opioid medications, and perhaps it is this relationship that could be exploited for new treatments.

Hutchinson and colleagues' preclinical study demonstrated that by blocking IL-1, the length of time of opioid analgesia was significantly increased. The study also demonstrated that an approximate seven-and-a-half-fold increase in analgesic efficacy with morphine was possible by co-administrating the opioid with a receptor blocker of IL-1 (Hutchinson et al., 2008). In another preclinical study, Johnston and colleagues indicated that by blocking IL-1 in the CNS, the analgesia of an intrathecally administered

opioid was enhanced, opioid tolerance was blunted and both newly developed and established allodynia and hyperalgesia were reduced (Johnston et al., 2004).

Study Limitations

Because of the cross-sectional, correlational nature of this study's design, causality of chronic pain and the causal relationships between levels of pro- or anti-inflammatory cytokines, chronic pain, anxiety, depression and sleep deprivation were not determined. The study design itself limited the findings to providing support for prior studies and serving as a basis for developing future studies. Given the specific criteria and study population of this cross-sectional study, this study design also limited the ability to generalize the results.

The small sample size also limited study findings: While a power analysis was performed and a target of 30 study participants was determined, this study was only able to include eight study participants. This was due to changes in study and pain center personnel, and a trend towards clinically prescribing a different implantable pain device for treatment of chronic pain. While the small sample size does decrease the interpretation of the study results, it should be noted that due to the difficulty in obtaining CSF, this study describes rich results. Other studies with smaller samples sizes have been common in the pain literature that analyzes human CSF, highlighting the incredibly valuable data CSF contains (Alexander, Perreault, Reichenberger, & Schwartzman, 2007; Alexander, van Rijn, van Hilten, Perreault, & Schwartzman, 2005; Backonja, Coe, Muller, & Schell, 2008; Kadetoff et al., 2012; McCarthy et al., 2013; Nagashima, Morio,

Yamane, Nanjo, & Teshima, 2009; Rijdsdijk et al., 2013; Yeager, Lunt, Arruda, Whalen, Rose, & DeLeo, 1999).

Obtaining the control samples from an outside source was also a limitation of this study. While obtaining these samples confirmed that 30 healthy CSF samples could be obtained, it decreased the time to acquire the CSF samples, lessened the participant burden and cost of recruiting participants to obtain these samples, these control CSF samples only came with a limited description and external assurance. The control samples may not adequately represent the CSF of healthy participants in the general public.

Three of the four psychological questionnaires utilized by this study were from direct participant report, while the Hamilton Anxiety Rating Scale was completed by a research practitioner that consented and obtained demographic information from study participants. While this study tried to limit the number of practitioners completing the scales on the participants, to limit inter-reporter variability, a total of four practitioners ultimately completed the Hamilton Anxiety Rating Scale for this study. This number of practitioners may have skewed the results for this psychological variable.

Conclusion

This is the first study to describe increased CSF levels of IL-8 in a majority FBSS chronic pain population. The study provided supportive evidence that there may be a relationship between chronic pain and central inflammation in humans, but clearly the responsible cytokines need further inspection with a larger study population. This data also provides a rich description of the relationship between gender and central

inflammation in chronic pain. Psychosocial variables such as educational level and socioeconomic status were also described in relationship to central inflammation, which illustrates the complex paradigm chronic pain is. All of the study participants took at least one opioid medication for treatment of their pain and reported poor sleep quality, illustrating the need to investigate possible mechanisms of association. This study clearly demonstrates the need for further inspection of both physiologic and psychological attributes of chronic pain. Thus, with a greater understanding of the complete chronic pain picture, the entire patient may find relief in therapy that addresses all facets of the disease.

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Table 1

Summary of Psychological Tools Utilized

| Behavioral Measures | Measurement | Scale | Cronbach's α |
|----------------------------|---|---|---|
| Pain | The Short Form-McGill Pain Questionnaire (SF-MPQ) | Scores for the questionnaire can range from 0 to 45 on the Pain Rating Index, from 0 to 5 on the Present Pain Intensity, and from 0 to 10 centimeters on the Visual Analog Scale. No established scoring noted by authors | ^a 0.73-0.89 ^b 0.96 |
| Anxiety | The Hamilton Anxiety Rating Scale (HAM-A) | A total anxiety score of <17 indicates mild anxiety levels; 18 to 24 mild-moderate anxiety levels; and 25 to 30 indicates moderate-severe anxiety levels | ^c 0.89 |
| Depression | The Beck Depression Inventory-II (BDI-II) | 0-13: minimal depression; 14-19: mild depression; 20-28: moderate depression; and 29-63: severe depression | ^d 0.91 |
| Sleep Quality | The Pittsburgh Sleep Quality Index (PSQI) | Nineteen components that yield a global score of 0-21, with a total of ≤ 5 associated with good sleep quality and > 5 associated with poor sleep quality | ^e 0.83 |

Note. ^a Taken from Burekhardt & Jones, 2003; ^b Reported as an Intraclass Correlation

Coefficient for total pain score by Grafton, Foster, & Wright, 2005; ^c Taken from

Kummer, Cardoso, & Teixeira, 2010; ^d Reported as a Coefficient Alpha by Beck, Steer,

Ball, & Ranieri, 1996; ^e Reported by Buysse, Reynolds, Monk, Berman, & Kupfer, 1989.

Table 2

Summary of the Luminex® Performance Human High Sensitivity Cytokine Panel for Laboratory Analysis

| Analytes | Sensitivity (pg/mL) | High Standard Value (pg/mL) |
|---|----------------------------|------------------------------------|
| IL-1beta | 0.18 | 1,500 |
| IL-2 | 0.28 | 2,450 |
| IL-4 | 2.54 | 7,000 |
| IL-5 | 0.12 | 1,600 |
| IL-6 | 0.31 | 4,050 |
| IL-8 | 0.07 | 3,200 |
| IL-10 | 0.24 | 2,100 |
| IL-12 | 2.96 | 24,500 |
| GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor) | 0.13 | 1,575 |
| IFN-g (Interferon Gamma) | 0.08 | 1,350 |
| TNF-alpha | 0.54 | 3,350 |
| VEGF (Vascular Endothelial Growth Factor) | 1.35 | 2,000 |

Note. Accessed from manufacturer's website on March 7, 2015

<http://rndsystems.com/Products/FCST09>

Table 3

Characteristics of Chronic Pain Population

| Gender | Age | Race | Education Level | SES | METs | BMI (kg/m²) | Diagnosis | Length of Diagnosis | Medication | Length of Medication |
|---------------|------------|-------------|------------------------|--------------|-------------|-------------------------------|------------------|----------------------------|--------------------------|-----------------------------|
| F | 74 | AA | Doctorate | N/R | 1 | 25.3 | FBSS | >10 years | FP, G, O | >2 years |
| M | 54 | W | College | \$100-\$149K | 6 | 35 | DPN | >5 years | O, G | >1 year |
| F | 43 | Asian | Master's | \$100-\$149K | 3 | 28.9 | FBSS | 8 years | FP, M, OA | 6 years |
| F | 52 | W | Part College | \$100-\$149K | 3 | 30 | FBSS | >10 years | Mor, Pre, HA, Mel | 2 years |
| F | 41 | Hispanic | Part College | \$80-\$89K | 2 | 33.8 | S &AS | >6 months | Flex, Mor | >6 months |
| F | 52 | AA | < High School | <\$10K | 4 | 35.1 | FBSS | 2 years | O, Dil, Rob, G | 2 years |
| M | 50 | W | Master's | \$100-\$149K | 4 | 38.7 | FBSS | 25 years | OA, Ibu, Tap, Dic | > 6 months |
| M | 62 | Hispanic | College | \$60-69K | 4 | 32 | FBSS | 14 years | Cym, Lex, FP, G, HA, Rob | 14 years |

Note. SES= Socioeconomic Status, METs= Metabolic Equivalent of Tasks, BMI= Body Mass Index, AA= African-America, W=White, N/R=not reported, FBSS=Failed Back Surgery Syndrome, DPN=Diabetic Polyneuropathy, S=Sacroiliitis, AS=Ankylosing Spondylitis, FP=Fentanyl Patch, G=Gabapentin, O=Oxycodone, M=Methadone, OA=Oxycodone/Acetaminophen, Mor=Morphine, Pre=Pregabalin, HA=Hydrocodone/Acetaminophen, Mel=Meloxicam, Flex=Flexeril, Dil=Dilaudid, Rob=Robaxin, Ibu=Ibuprofen, Tap=Tapentadol, Dic=Diclofenac, Cym=Cymbalta, Lex=Lexapro

Table 4

Psychological Data

| ID | Pain Rating Index | PPI | VAS (cm/10 cm) | HAM-A | BDI-II | PSQI |
|-----------|--------------------------|------------|-----------------------|------------------|---------------|-------------|
| 1 | 27 | 4 | 8.4 | 17 Mild | 28 Moderate | 19 Poor |
| 2 | 22 | 4 | 6.8 | 19 Mild-Moderate | 10 Minimal | 16 Poor |
| 3 | 28 | 4 | 7.0 | 39 Severe | 38 Severe | 13 Poor |
| 4 | 33 | 4 | 7.9 | 7 Mild | 21 Moderate | 17 Poor |
| 5 | 27 | N/R | N/R | 10 Mild | 7 Minimal | 18 Poor |
| 6 | 18 | 5 | 9.6 | 36 Severe | 20 Moderate | 16 Poor |
| 7 | 12 | 2 | 5.9 | 20 Mild-Moderate | 10 Minimal | 9 Poor |
| 8 | 16 | 2 | 6.1 | 18 Mild-Moderate | 24 Moderate | 16 Poor |

Note. PPI=Present Pain Intensity; VAS=Visual Analog Scale; HAM-A=Hamilton Anxiety Rating Scale; BDI-II=Beck Depression Inventory-II; PSQI=Pittsburgh Sleep Quality Index; N/R=Not Reported.

Table 5

Cerebrospinal Fluid Cytokine Levels

| Cytokine | Healthy Controls (pg/mL) | Chronic Pain (pg/mL) | <i>p</i> value |
|-----------------|-------------------------------------|---------------------------------|-----------------------|
| IL-1 | OOR | OOR | |
| IL-2 | OOR | OOR | |
| IL-4 | OOR | OOR | |
| IL-6 | 1.33 ± 0.58 | 1.19 ± 0.26 | <i>p</i> = 0.50 |
| IL-8 | 49.49 ± 12.81 | 63.04 ± 12.16 | <i>p</i> < 0.001 |
| IL-10 | OOR | OOR | |
| TNF- α | OOR | OOR | |
| IFN-g | OOR | OOR | |
| VEGF | OOR | OOR | |
| GM-CSF | 0.24 ± 0.07 | 0.26 ± 0.07 | <i>p</i> = 0.58 |

Note. OOR denotes values were out of range of Multiplex Assay Analyses.

Table 6

Pearson's r Correlations for Interval/Ratio Variables

| Psychological Tools | IL-6^a | IL-8^a | GM-CSF |
|-------------------------------------|--------------------------|-------------------------|---------------|
| Pain Rating Index | -0.458 | -0.289 | -0.517 |
| Present Pain Intensity ^b | -0.515 | -0.393 | 0.153 |
| Visual Analog Scale ^c | -0.411 | -0.609 | 0.529 |
| Hamilton Anxiety Rating Scale | -0.271 | 0.109 | 0.409 |
| Beck Depression Inventory-II | -0.089 | 0.113 | 0.139 |
| Pittsburgh Sleep Quality Index | -0.179 | -0.370 | 0.080 |
| Demographics | | | |
| Age | 0.670^d | 0.427 | 0.447 |
| Body Mass Index | 0.095 | -0.040 | 0.08 |
| Length of Diagnosis | 0.648^e | 0.524 | -0.007 |
| Length of Medication | 0.288 | 0.046 | 0.183 |

Note. ^a Values analyzed using log transformation; ^b One value omitted from analysis; ^c

One value omitted from analysis; ^d $p = 0.069$; ^e $p = 0.082$.

Table 7

Spearman's Rho Correlations for Ordinal Variables

| Demographics | IL-6^a | IL-8^a | GM-CSF |
|-----------------------------------|-------------------------|--------------------------|---------------------------|
| Educational Level | 0.341 | 0.727^c | -0.007 |
| Socioeconomic Status ^b | -0.013 | 0.650 | -0.823^d |
| Metabolic Equivalents | 0.568 | 0.393 | 0.105 |

Note.^a Values analyzed using log transformation; ^b One value omitted from analysis; ^c

Statistical significance at $p = 0.041$; ^d Statistical significance at $p = 0.012$.

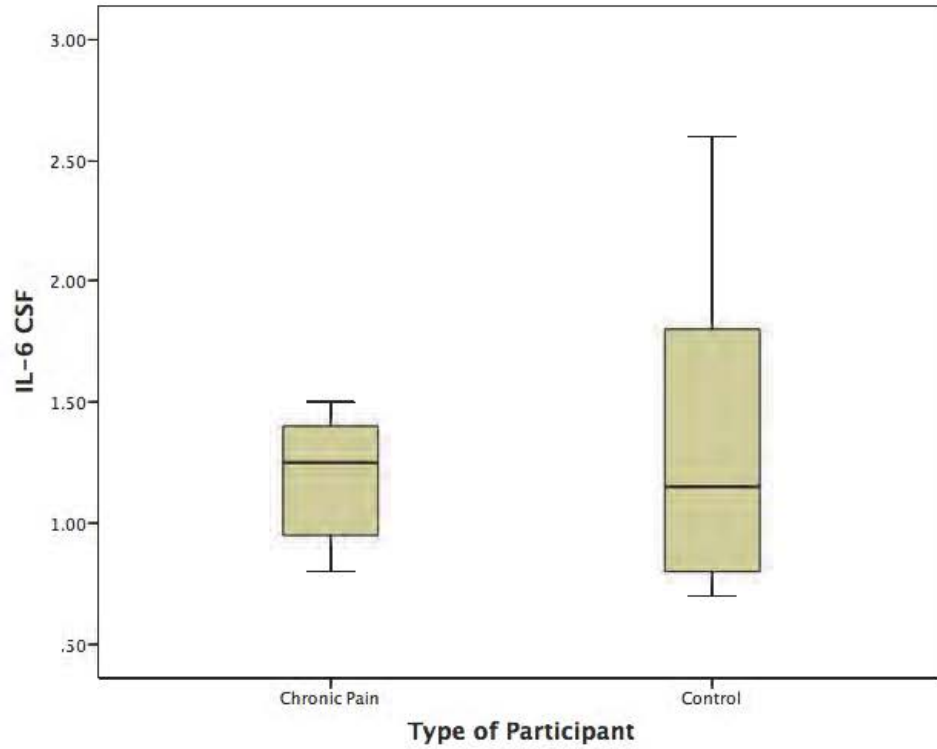


Figure 1. Cerebrospinal fluid levels of IL-6 in chronic pain verses controls.

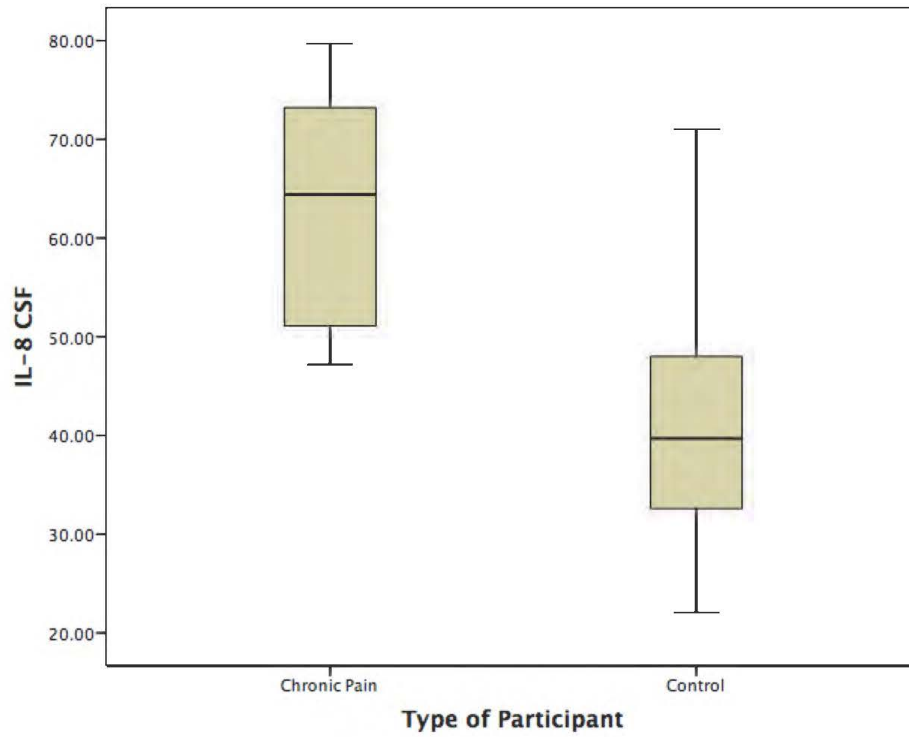


Figure 2. Cerebrospinal fluid levels of IL-8 in chronic pain verses controls.

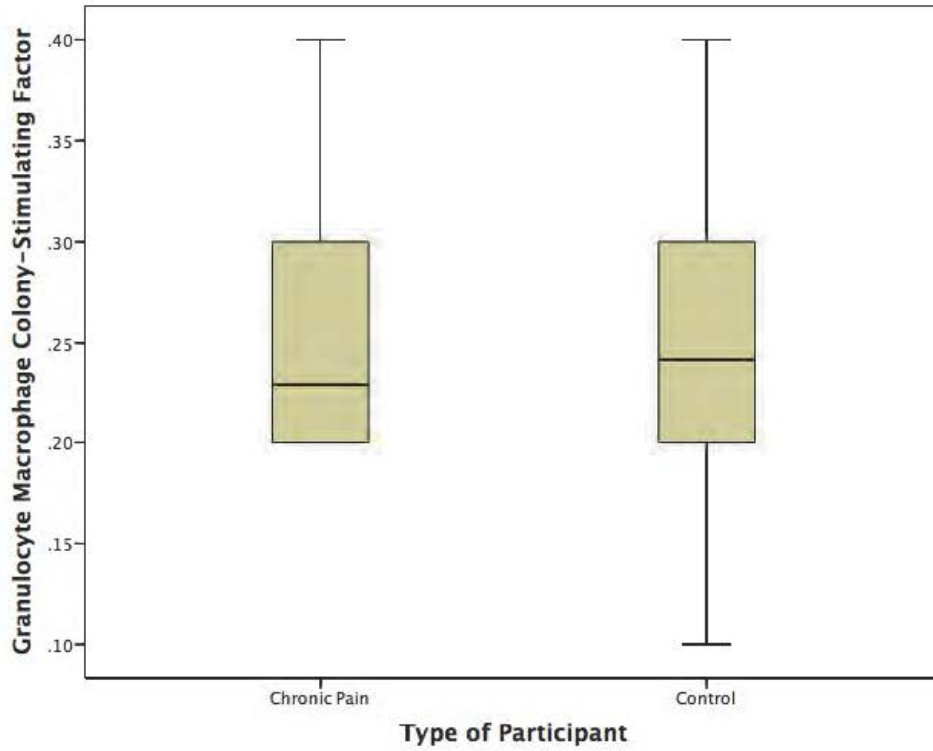


Figure 3. Cerebrospinal fluid levels of GM-CSF in chronic pain verses controls.

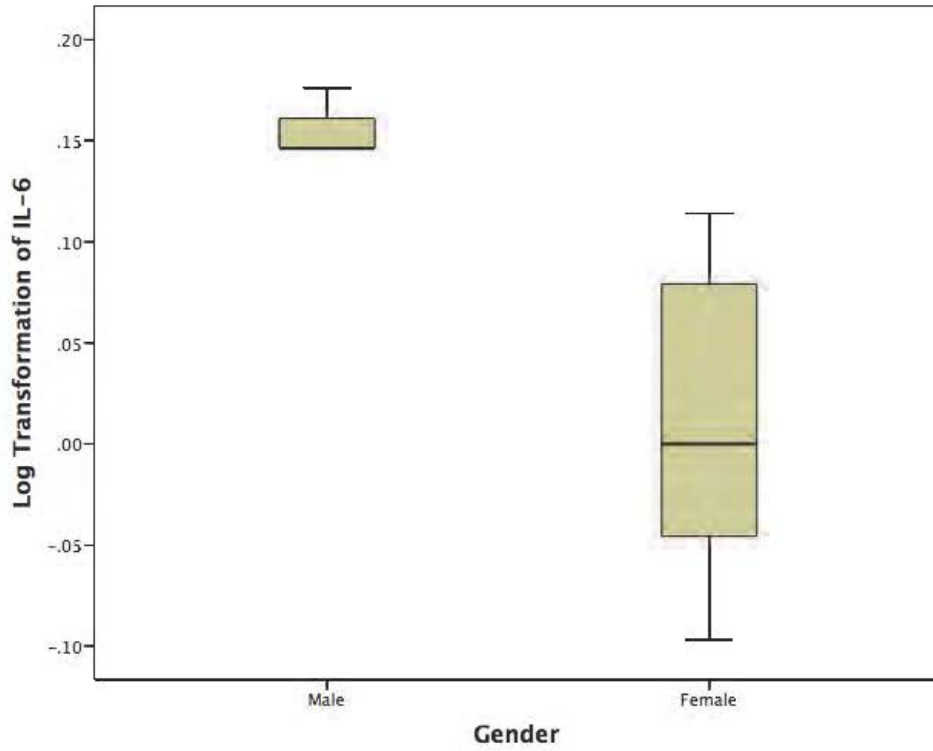


Figure 4. Cerebrospinal fluid levels of IL-6 by gender.

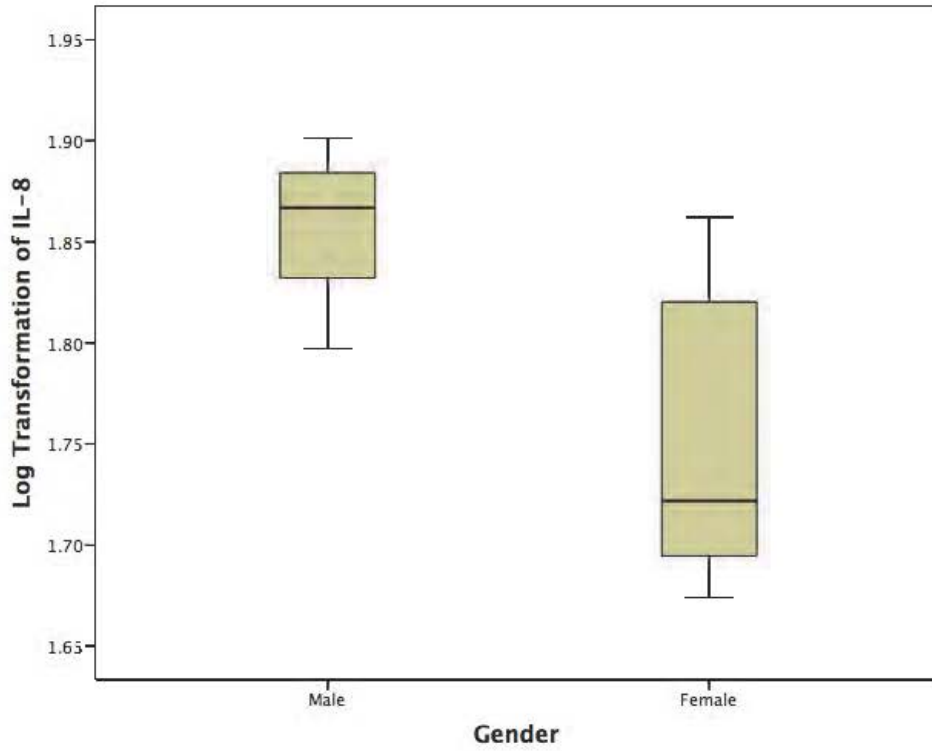


Figure 5. Cerebrospinal fluid levels of IL-8 by gender.

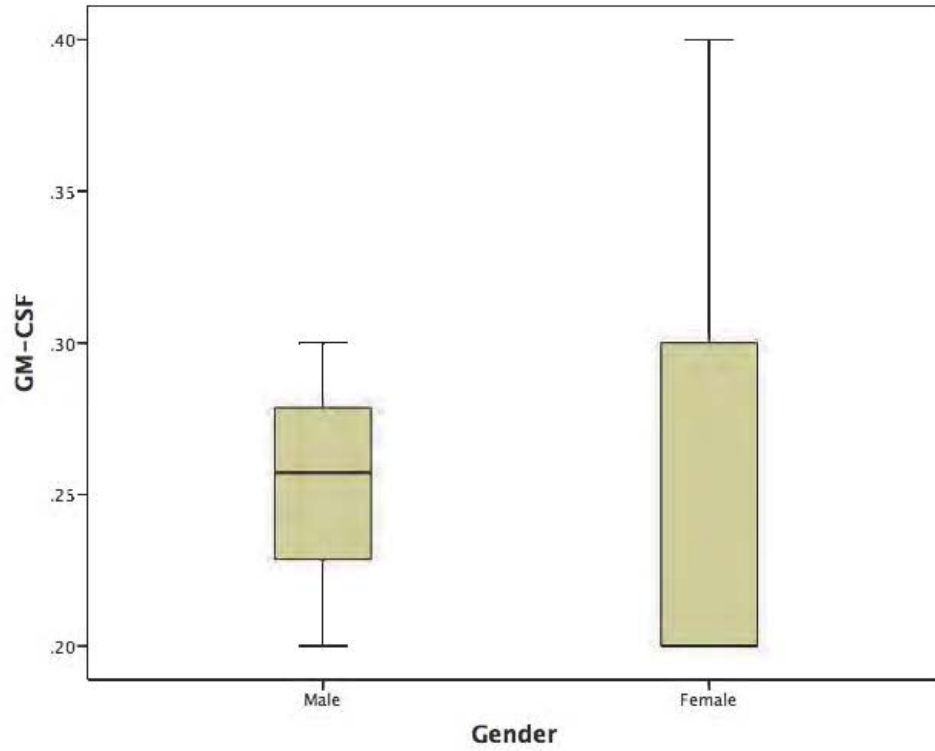


Figure 6. Cerebrospinal fluid levels of GM-CSF by gender.

Appendix A

University of California, Los Angeles Institutional Review Board-Approved Consent Form

UNIVERSITY OF CALIFORNIA LOS ANGELES CONSENT TO PARTICIPATE IN RESEARCH

Psychoneuroimmunology and Chronic Pain

INTRODUCTION

Dr. Irene Wu, and associates from the Department of Anesthesiology/Pain Management Clinic and Cousin's Center for Psychoneuroimmunology at the University of California, Los Angeles are conducting a research study.

The researchers will explain this study to you. **Research studies are voluntary and include only people who choose to take part.** Please take your time about deciding whether to participate in this study. Before deciding:

- You can discuss this study with friends and family.
- You can also discuss it with your health care doctor or request a second opinion.
- If you have any questions, you can ask the researchers for more information before deciding to participate.

The research team is asking you to be in this study because you have been diagnosed with a chronic pain syndrome and are scheduled to have a trial of opioid pain medication administered to you through a tube (catheter) that is placed in your cerebrospinal fluid (CSF). The CSF is located in an area of your body called the thecal sac. Since the catheter is placed inside the thecal sac, this procedure is called an intrathecal opioid catheter trial. Should you respond favorably to the trial, you will be considered to be a candidate for permanent implantation of a pain pump. This pain pump is called an intrathecal pain pump. An intrathecal pain pump is a surgically implanted device that continuously infuses and delivers opioid pain medications into your CSF for the treatment of chronic pain.

WHY IS THIS STUDY BEING DONE?

The purpose of this research study is to investigate the relationships between pain, inflammation, anxiety, depression and sleep patterns.

Changes in inflammation particles found in the fluid surrounding the brain and spine (cerebrospinal fluid or CSF) and blood, and psychological responses related to chronic pain will be analyzed over the treatment period that encompasses 1) the intrathecal opioid catheter trial, 2) the permanent insertion of an intrathecal opioid infusion pain pump, and 3) at three to six months post insertion of an intrathecal opioid infusion pain pump.

This study is being funded by the American Association of Nurse Anesthetists and a CTSI grant funded through the UCLA Cousins Center of Psychoneuroimmunology.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

Approximately 30 people will take part in this study at UCLA.

WHAT WILL HAPPEN IF I TAKE PART IN THIS STUDY?

Before you begin the study:

Before you begin the study, you will need to be diagnosed with a chronic pain condition and as part of your standard clinical care be scheduled to have an intrathecal opioid catheter trial and possible insertion of intrathecal pain pump for the treatment of your pain.

During the study:

If you take part in this study, you allow the researchers to collect a small amount of the fluid that surrounds the brain and spine (cerebrospinal fluid or CSF) which would otherwise not be collected during the intrathecal opioid catheter trial and the permanent placement of the intrathecal pain pump. Specifically, less than a teaspoon of your CSF will be collected for inflammation analysis on three separate occasions, during your 1) intrathecal opioid catheter trial, 2) permanent pump insertion and, 3) at your pump refill visit.

As part of this research, no additional needles sticks are necessary for the collection of this fluid except on the pump refill visit, which will take place 3-6 months after the permanent insertion. However, should it be determined that you are not a candidate for permanent insertion of the pain pump, you will not proceed with the study after the intrathecal catheter trial.

During the pump refill visit, which is part of your clinical care, there will be an extra procedure for research purposes only. Researchers will use fluoroscopy (a live x-ray machine) to guide a very small needle to draw less than a teaspoon of CSF from your implanted pump. This should not take more than five minutes of additional time.

During all three visits where your CSF is collected, approximately 2-3 teaspoons of blood will be collected from the IV started for the procedure or through venopuncture (a procedure where a small needle is used to withdraw the blood from your vein).

Additionally, the researcher(s) will ask you to fill out three questionnaires at or during three doctor visits: 1) prior to the intrathecal catheter trial either on the day you are seen in clinic or the day of your intrathecal opioid catheter trial, 2) preoperatively the day of your permanent pump insertion and, 3) the day you return to have your pump refill. These questionnaires will evaluate your mood, anxiety, sleep patterns and level of pain. You will also be asked to complete forms or answer questions related to your demographic information and diagnosis of chronic pain. Your responses to these questions will be kept strictly confidential and will not become part of your UCLA medical record. Also, the way you answer the questions on the questionnaires will not

influence the medical care you receive. Answering all of the questionnaires should take approximately 20-30 minutes during each visit.

Should a participant be determined to not be a candidate for permanent implantation of intrathecal pump based on clinical information gathered at the time of trial (no improvement in pain response to the intrathecal pain medications or significant side effects to the pain medication), the participant will not proceed with the treatment course of a permanent intrathecal pump and any further study procedures. Should the participant not respond to the trial, and the permanent pump is not implanted, the patient's cerebrospinal fluid will be analyzed for the presence of the inflammation particles and only the initial three questionnaires on levels of anxiety, depression and sleep patterns will be used for research purposes.

HOW LONG WILL I BE IN THIS STUDY?

This study will last approximately one year.

WHAT KINDS OF RISKS OR DISCOMFORTS COULD I EXPECT?

Known risks and discomforts:

The possible risks and/or discomforts associated with the procedures described in this consent form include:

Cerebrospinal Fluid (CSF) Sampling: Your treatment involves undergoing a surgical procedure called an intrathecal opioid catheter trial with possible permanent intrathecal pain pump insertion. During these procedures, the sac containing CSF is punctured and a catheter is inserted in order for opioid pain medications to be infused. Prior to and during the placement of the catheter, a small amount of CSF is normally lost or drained through the needle. This drainage is a known and accepted component of performing this surgical technique.

Your participation in the study involves allowing us to collect three milliliters (less than a teaspoon) of CSF from the needle that is placed. Collection of the CSF will not significantly increase the risks or reduce the benefits of your already scheduled surgical procedure to place the trial catheter or permanently implant the pump. However, you should be aware that there are risks during the collection of the last CSF sample once the pump is permanently in place.

During the final CSF draw at the pump refill visit, a 25-gauge needle will be inserted precisely (using an x-ray called fluoroscopy) through the study participant's surgically prepped and draped abdomen into the catheter access port on the Medtronic SynchroMed® II Pain Pump. Two milliliters (less than a teaspoon) of CSF will be drawn out and this specimen collection procedure is anticipated to take less than five minutes of surgical time. This CSF sample represents the only amount of CSF that is above and

beyond the surgically anticipated surgical CSF loss and represents a minimal amount of CSF present in the adult central nervous system (CNS).

The 2 milliliters (less than a teaspoon) sample of CSF necessary for study analysis and the five minutes of surgical time to collect this sample represent a small but noteworthy participant burden. The risk of developing a spinal headache from the 2 milliliters of CSF sampled is a small, but a serious risk to the study participant. A spinal headache is a severe headache that can increase in severity when one sits up or changes positions abruptly. Usually persons with spinal headaches report that the headache is less severe when lying down. Most of the time spinal headaches resolve on their own but occasionally a spinal headache requires treatment. Treatments for spinal headaches include a procedure called an epidural blood patch. This treatment involves taking a sample of blood and injecting it into the epidural space to stop the leakage of CSF and thus, the spinal headache.

Infection: During the final CSF collection from the implanted pump, participants will receive an additional needle stick. The needle stick will be performed utilizing the same surgical sterile preparation and draping as the pump refill procedure but this collection of CSF does represent an additional risk of infection. An infection after a 25-gauge needle stick is rare, but does represent a potential additional risk to the participant. Should an infection occur, treatment including antibiotics or additional surgery may be necessary.

Radiation: During the pump refill visit in which CSF will be withdrawn from the catheter access port of the Medtronic SynchroMed® II Pain Pump, fluoroscopy will be utilized to locate the catheter access port of the device. Fluoroscopy will be utilized to minimize the number of needle sticks necessary to access and precisely locate the catheter access port of the pain pump. This is both for your comfort and safety.

You are exposed to radiation on a daily basis, both from natural (sun and earth) and manmade sources. The estimated radiation dose that you will receive as a volunteer for this type of research has been compared to the limits allowed for a radiation worker. This limit is low and is not expected to be harmful. The person obtaining your consent can answer any questions you have, and provide detailed written information about the amount of radiation resulting from this study.

Pain Pump Reprogramming Malfunction: During the final visit for the pump refill of pain medication, CSF will be collected from a port on the Medtronic SynchroMed® II Pain Pump. Once the CSF has been collected, the pump will need to be refilled and electronically reset with the approved Medtronic N'Vision® 8840 Programmer to ensure the proper rate of pain medication infusion via the pain pump and intrathecal catheter.

Although unlikely, malfunctioning of the repriming and reprogramming of the Medtronic SynchroMed® II Pain Pump can result in a wide spectrum of side effects expectant from an overdose of narcotic medications ranging from pruritis (itching) to ventilatory depression (difficulty breathing) to death if incorrectly programmed and left untreated. The UCLA Pain Management medical doctors that perform intrathecal opioid infusion pain pump maintenance, including pump refills, have done so in hundreds of past

patients in chronic pain treatment endeavors with no adverse events related to repriming and reprogramming of the intrathecal catheter or pain pump.

Bleeding: It is possible, though unusual, to experience a bleeding episode through a 25 gauge needle stick. Should bleeding occur, it may require emergency treatment to drain accumulated blood called a hematoma.

Scarring: In rare cases, scarring from a 25-gauge needle stick may result. This represents a small but additional risk to study participants. There is no treatment for this risk.

Blood Sampling:

Infection. Before the surgical procedure where study samples of CSF will be collected, there is a risk of infection upon insertion of an intravenous catheter or upon venopuncture. An infection after an intravenous catheter insertion or venopuncture is rare, but does represent a potential risk. Should an infection occur, treatment including antibiotics or additional surgery may be necessary.

Bleeding. It is possible, though unusual, to experience an excessive bleeding through an intravenous needle stick or venopuncture site. The smallest appropriate catheter or venopuncture needle will be used in every effort to minimize bleeding and discomfort for study participants. Should bleeding occur, it may require emergency treatment to drain accumulated blood called a hematoma.

Scarring. In rare cases, scarring from an intravenous needle stick or venopuncture may result. This represents a small risk to study participants.

Unknown risks and discomforts:

The experimental procedure may have side effects that no one knows about yet. The researchers will let you know if they learn anything that might make you change your mind about participating in the study.

ARE THERE ANY BENEFITS IF I PARTICIPATE?

Possible benefits to me:

You will not benefit directly from participating in this study.

Possible benefits to others or society:

This study will help the researchers learn more about inflammation and chronic pain, depression, anxiety and sleep deprivation. Hopefully this information will help in the treatment of future patients with chronic pain like yours.

WHAT OTHER CHOICES DO I HAVE IF I DON'T WANT TO PARTICIPATE?

If you decide not to take part in this study, or if you withdraw from this study before it is completed, the following alternative procedures or courses of treatment are available: Your alternative is not to participate in the research. You can proceed with the same procedures and treatments for your pain without participating in the research.

CAN THE RESEARCHERS REMOVE ME FROM THIS STUDY?

The researchers may end your participation in this study for a number of reasons, such as if your safety and welfare are at risk, if you do not follow instructions or if you miss scheduled visits. The researchers or the study sponsor might also decide to stop the study at any time.

If you decide to stop being in the study, or are removed from the study, or the study is stopped the researcher will ask you to submit in writing your intent to discontinue your involvement in this study.

HOW WILL INFORMATION ABOUT ME AND MY PARTICIPATION BE KEPT CONFIDENTIAL?

The researchers will do their best to make sure that your private information is kept confidential. Information about you will be handled as confidentially as possible, but participating in research may involve a loss of privacy and the potential for a breach in confidentiality. Study data will be physically and electronically secured. As with any use of electronic means to store data, there is a risk of breach of data security.

Use of personal information that can identify you:

- The researchers will make every attempt to protect your confidentiality and to make sure that your personal identity does not become known. This signed consent form will be stored in a locked file that will be accessible only to a very small number of authorized people involved in this project. The research team will carefully follow the coding, storage, and data sharing plan explained below.

How information about you will be stored:

- No identifiable information about you will be kept with the research data.
- All identifiable information about you will be replaced with a code. A list linking the identifiable information and associated code will be locked in two secure locations.
- Some research data and records will be maintained in a secure location at UCLA. Only authorized individuals will have access to it.
- Some research data and records will be stored on a laptop computer that is passcode protected and your identifiable information will be kept separate from the research data.

People and agencies that will have access to your information:

The research team and authorized UCLA personnel may have access to study data and records to monitor the study. Research records provided to authorized, non-UCLA

personnel will not contain identifiable information about you. Publications and/or presentations that result from this study will not identify you by name.

How long information from the study will be kept:

- The researchers intend to keep the research data and records until the research is published and/or presented or until the analysis of the records are complete, whichever is longer.
- In the future, data collected for this study may be shared with other researchers for other studies that are unknown at this time. Any data shared with other researchers, will not include your name or other personal identifying information.

ARE THERE ANY COSTS FOR TAKING PART IN THIS STUDY?

You or your insurer will be billed for the costs of any standard medical care you receive during your participation in the study and you will be responsible for any associated co-payments and deductibles. Above the standard medical care costs, there are no additional cost burdens to you. You will not be charged for the laboratory tests that will be run on the CSF samples we collect from you.

WILL I BE PAID FOR MY PARTICIPATION?

You will be given a \$25 VISA pre-paid gift card for your participation in this study at the time of enrollment. Should it be determined that you are not a candidate for permanent pain pump insertion, you will not proceed with the remainder of the study and will not receive any further compensation. Should you proceed to the remaining steps of the study and complete the study, you will receive an additional \$25 VISA pre-paid gift card at the time of your last CSF draw visit.

You will be reimbursed for the following out of pocket expenses that you might have:
Parking fees for each doctor visit that is research related.

WHAT OTHER THINGS SHOULD I CONSIDER BEFORE PARTICIPATION?

Use of My Specimens:

Any specimens (e.g., CSF or blood) obtained for the purposes of this study will become the property of the University of California. Once you provide the specimens you will not have access to them. The University may share your specimens in the future with other researchers or outside institutions. Information that identifies you will not be shared with anyone outside of UCLA. The specimens will be used for research and such use may result in inventions or discoveries that could become the basis for new products or diagnostic or therapeutic agents. In some instances, these inventions and discoveries may be of potential commercial value and may be patented and licensed by the University. You will not receive any money or other benefits derived from any commercial or other products that may be developed from use of the specimens.

All of the specimens of cerebrospinal fluid will be labeled with a numeric code rather than any personal identifiers to maintain your anonymity. Only limited research personnel will have access to the list that links your personal information to your numeric code and this list will be destroyed once the research is published and/or presented or until the analysis of the records are complete, whichever is longer.

WHO CAN I CONTACT IF I HAVE QUESTIONS ABOUT THIS STUDY?

The Research Team:

You may contact Dr. Irene Wu and Dr. Michael Ferrante at (310) 794-1841 with any questions or concerns about the research or your participation in this study. You can also call the UCLA Page Operator at (310) 825-6301 to reach Dr. Irene Wu 24 hours a day, 7 days week.

UCLA Office of the Human Research Protection Program (OHRPP):

If you have questions about your rights while taking part in this study, or you have concerns or suggestions and you want to talk to someone other than the researchers about the study, you may contact the UCLA OHRPP by phone: (310) 825-5344; by email: mirb@research.ucla.edu or U.S. mail: UCLA OHRPP, 11000 Kinross Ave., Suite 211, Box 951694, Los Angeles, CA 90095-1694.

WHAT HAPPENS IF I BELIEVE I AM INJURED BECAUSE I TOOK PART IN THIS STUDY?

It is important that you promptly tell the researchers if you believe that you have been injured because of taking part in this study. You can tell the researcher in person or call him/her at the number(s) listed above.

If you are injured as a result of being in this study, UCLA will provide necessary medical treatment. The costs of the treatment may be covered by the University of California, or billed to you or your insurer just like other medical costs, depending on a number of factors. The University and the study sponsor do not normally provide any other form of compensation for injury. For more information about this, you may call the UCLA Office of the Human Research Protection Program at 310-825-5344 or send an email to mirb@research.ucla.edu.

WHAT ARE MY RIGHTS IF I TAKE PART IN THIS STUDY?

Taking part in this study is your choice. You can choose whether or not you want to participate. Whatever decision you make, there will be no penalty to you and you will not lose any of your regular benefits.

- You have a right to have all of your questions answered before deciding whether to take part.
- Your decision will not affect the medical care you receive from UCLA.
- If you decide to take part, you can leave the study at anytime.

- If you decide to stop being in this study you should notify the research team right away. The researchers may ask you to complete some procedures in order to protect your safety.
- If you decide not to take part, you can still get medical care from UCLA.

HOW DO I INDICATE MY AGREEMENT TO PARTICIPATE?

If you agree to participate in this study you should sign and date below. You have been given a copy of this consent form and the Research Participant's Bill of Rights to keep. You will be asked to sign a separate form authorizing access, use, creation, or disclosure of health information about you.

SIGNATURE OF THE PARTICIPANT

Name of Participant

Signature of Participant

Date

SIGNATURE OF PERSON OBTAINING CONSENT

Name of Person Obtaining Consent

Contact Number

Signature of Person Obtaining Consent

Date

Appendix B

Measurement of Pain Perception

SHORT-FORM MCGILL PAIN QUESTIONNAIRE
RONALD MELZACK

PATIENT'S NAME: _____ DATE: _____

| | NONE | MILD | MODERATE | SEVERE |
|-------------------|----------|----------|----------|----------|
| THROBBING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| SHOOTING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| STABBING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| SHARP | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| CRAMPING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| GNAWING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| HOT-BURNING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| ACHING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| HEAVY | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| TENDER | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| SPLITTING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| TIRING-EXHAUSTING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| SICKENING | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| FEARFUL | 0) _____ | 1) _____ | 2) _____ | 3) _____ |
| PUNISHING-CRUEL | 0) _____ | 1) _____ | 2) _____ | 3) _____ |



P P I

- 0 NO PAIN _____
- 1 MILD _____
- 2 DISCOMFORTING _____
- 3 DISTRESSING _____
- 4 HORRIBLE _____
- 5 EXCRUCIATING _____

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Appendix C

Measurement of Anxiety

Hamilton Anxiety Rating Scale (HAM-A)

Below is a list of phrases that describe certain feeling that people have. Rate the patients by finding the answer which best describes the extent to which he/she has these conditions. Select one of the five responses for each of the fourteen questions.

0 = Not present, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Very severe.

1 Anxious mood 0 1 2 3 4

Worries, anticipation of the worst, fearful anticipation, irritability.

2 Tension 0 1 2 3 4

Feelings of tension, fatigability, startle response, moved to tears easily, trembling, feelings of restlessness, inability to relax.

3 Fears 0 1 2 3 4

Of dark, of strangers, of being left alone, of animals, of traffic, of crowds.

4 Insomnia 0 1 2 3 4

Difficulty in falling asleep, broken sleep, unsatisfying sleep and fatigue on waking, dreams, nightmares, night terrors.

5 Intellectual 0 1 2 3 4

Difficulty in concentration, poor memory.

6 Depressed mood 0 1 2 3 4

Loss of interest, lack of pleasure in hobbies, depression, early waking, diurnal swing.

7 Somatic (muscular) 0 1 2 3 4

Pains and aches, twitching, stiffness, myoclonic jerks, grinding of teeth, unsteady voice, increased muscular tone.

8 Somatic (sensory) 0 1 2 3 4

Tinnitus, blurring of vision, hot and cold flushes, feelings of weakness, pricking sensation.

9 Cardiovascular symptoms 0 1 2 3 4

Tachycardia, palpitations, pain in chest, throbbing of vessels, fainting feelings, missing beat.

10 Respiratory symptoms 0 1 2 3 4

Pressure or constriction in chest, choking feelings, sighing, dyspnea.

11 Gastrointestinal symptoms 0 1 2 3 4

Difficulty in swallowing, wind abdominal pain, burning sensations, abdominal fullness, nausea, vomiting, borborygmi, looseness of bowels, loss of weight, constipation.

12 Genitourinary symptoms 0 1 2 3 4

Frequency of micturition, urgency of micturition, amenorrhea, menorrhagia, development of frigidity, premature ejaculation, loss of libido, impotence.

13 Autonomic symptoms 0 1 2 3 4


Dry mouth, flushing, pallor, tendency to sweat, giddiness, tension headache, raising of hair.

14 Behavior at interview 0 1 2 3 4

Fidgeting, restlessness or pacing, tremor of hands, furrowed brow, strained face, sighing or rapid respiration, facial pallor, swallowing, etc.

Appendix D

Measurement of Depression

| | | |
|---|----------------------------------|---------------------------------|
|  | Beck Depression Inventory | Baseline |
| V 0477 | CRTN: _____ CRF number: _____ | Page 14 patient initials: _____ |
| BDI-II | | Date: _____ |

Name: _____ Marital Status: _____ Age: _____ Sex: _____
 Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including item 16 (Changes in Sleeping Pattern) or item 18 (Changes in Appetite).

| | |
|--|--|
| <p>1. Sadness</p> <p>0 I do not feel sad.</p> <p>1 I feel sad much of the time.</p> <p>2 I am sad all the time.</p> <p>3 I am so sad or unhappy that I can't stand it.</p> <p>2. Pessimism</p> <p>0 I am not discouraged about my future.</p> <p>1 I feel more discouraged about my future than I used to be.</p> <p>2 I do not expect things to work out for me.</p> <p>3 I feel my future is hopeless and will only get worse.</p> <p>3. Past Failure</p> <p>0 I do not feel like a failure.</p> <p>1 I have failed more than I should have.</p> <p>2 As I look back, I see a lot of failures.</p> <p>3 I feel I am a total failure as a person.</p> <p>4. Loss of Pleasure</p> <p>0 I get as much pleasure as I ever did from the things I enjoy.</p> <p>1 I don't enjoy things as much as I used to.</p> <p>2 I get very little pleasure from the things I used to enjoy.</p> <p>3 I can't get any pleasure from the things I used to enjoy.</p> <p>5. Guilty Feelings</p> <p>0 I don't feel particularly guilty.</p> <p>1 I feel guilty over many things I have done or should have done.</p> <p>2 I feel quite guilty most of the time.</p> <p>3 I feel guilty all of the time.</p> | <p>6. Punishment Feelings</p> <p>0 I don't feel I am being punished.</p> <p>1 I feel I may be punished.</p> <p>2 I expect to be punished.</p> <p>3 I feel I am being punished.</p> <p>7. Self-Dislike</p> <p>0 I feel the same about myself as ever.</p> <p>1 I have lost confidence in myself.</p> <p>2 I am disappointed in myself.</p> <p>3 I dislike myself.</p> <p>8. Self-Criticalness</p> <p>0 I don't criticize or blame myself more than usual.</p> <p>1 I am more critical of myself than I used to be.</p> <p>2 I criticize myself for all of my faults.</p> <p>3 I blame myself for everything bad that happens.</p> <p>9. Suicidal Thoughts or Wishes</p> <p>0 I don't have any thoughts of killing myself.</p> <p>1 I have thoughts of killing myself, but I would not carry them out.</p> <p>2 I would like to kill myself.</p> <p>3 I would kill myself if I had the chance.</p> <p>10. Crying</p> <p>0 I don't cry anymore than I used to.</p> <p>1 I cry more than I used to.</p> <p>2 I cry over every little thing.</p> <p>3 I feel like crying, but I can't.</p> |
|--|--|

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Subtotal Page 1

Continued on Back

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Appendix E

Measurement of Sleep Quality

Subject's Initials _____ ID# _____ Date _____ Time _____ AM
PM

PITTSBURGH SLEEP QUALITY INDEX

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?
BED TIME _____
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?
NUMBER OF MINUTES _____
3. During the past month, what time have you usually gotten up in the morning?
GETTING UP TIME _____
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)
HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you . . .
 - a) Cannot get to sleep within 30 minutes

| | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
 - b) Wake up in the middle of the night or early morning

| | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
 - c) Have to get up to use the bathroom

| | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|

- d) Cannot breathe comfortably
- | | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
- e) Cough or snore loudly
- | | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
- f) Feel too cold
- | | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
- g) Feel too hot
- | | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
- h) Had bad dreams
- | | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
- i) Have pain
- | | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
- j) Other reason(s), please describe _____
-

How often during the past month have you had trouble sleeping because of this?

| | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|

6. During the past month, how would you rate your sleep quality overall?

Very good _____

Fairly good _____

Fairly bad _____

Very bad _____

7. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?

Not during the past month _____ Less than once a week _____ Once or twice a week _____ Three or more times a week _____

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month _____ Less than once a week _____ Once or twice a week _____ Three or more times a week _____

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all _____
 Only a very slight problem _____
 Somewhat of a problem _____
 A very big problem _____

10. Do you have a bed partner or room mate?

No bed partner or room mate _____
 Partner/room mate in other room _____
 Partner in same room, but not same bed _____
 Partner in same bed _____

If you have a room mate or bed partner, ask him/her how often in the past month you have had . . .

a) Loud snoring

Not during the past month _____ Less than once a week _____ Once or twice a week _____ Three or more times a week _____

b) Long pauses between breaths while asleep

Not during the past month _____ Less than once a week _____ Once or twice a week _____ Three or more times a week _____

c) Legs twitching or jerking while you sleep

Not during the past month _____ Less than once a week _____ Once or twice a week _____ Three or more times a week _____

d) Episodes of disorientation or confusion during sleep

| | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|

e) Other restlessness while you sleep; please describe _____

| | | | |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|
| Not during the past month _____ | Less than once a week _____ | Once or twice a week _____ | Three or more times a week _____ |
|------------------------------------|--------------------------------|-------------------------------|-------------------------------------|

Appendix F

Demographic Clinical Data Questionnaire

Code: _____

To Be Filled Out by Research Practitioner:

Cause/Diagnosis of Chronic Pain (i.e. Failed Back): _____

Length of Chronic Pain Diagnosis: _____

Types of Medications Taken by Patient for Treatment of Chronic Pain:

| | | | |
|--|--|--|--|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

Length of Time Patient Has Been Taking Medications:

| | | | |
|--|--|--|--|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

Appendix G

Demographic Data Questionnaire

Code: _____

Please Do Not Fill in Above

Age: _____

Height: _____ Weight: _____

Gender: M F (Please Circle One)

Race: White White, Non-Hispanic African-American Hispanic
Asian-Pacific Islander Native American (Please Circle One)

Education Level: (Please Circle One)

Less than High School
High School/GED
Some College
2-year College Degree (Associates)
4-year College Degree (BA, BS)
Master's Degree
Doctoral Degree
Professional Degree (MD, JD)

Total Household Income: (Please Circle One)

Less than \$10,000
\$10,001-\$19,999
\$20,000-\$29,999
\$30,000-\$39,999
\$40,000-\$49,999
\$50,000-\$59,999
\$60,000-\$69,999
\$70,000-\$79,999
\$80,000-\$89,999
\$90,000-\$99,999
\$100,000-\$149,999
>\$150,000

Physical Activity Level (Please Circle Highest Level of Current Activity You Can Complete Without Stopping)

- 1 Sit Upright
- 2 Eat, dress, use toilet, make bed

| | |
|-----|---|
| 3 | Walk around house, shower |
| 4 | One flight stairs, walk up hill, walk flat – 2 block, 2mph Light house work, dust, wash dishes, golf, bowl |
| 5 | Two flights stairs, walk on flat at 4mph, Sex |
| 6 | Scrubbing floors, move furniture |
| 7 | Weight lifting |
| 8 | Shovel Snow |
| 9 | Doubles tennis, swing dancing |
| 10 | Singles tennis, soccer, basketball, skiing, running |
| 12+ | Competitive Sports |

Appendix H

University of California, Los Angeles Institutional Review Board Approval

3/17/13 11:12 AM



University of California Los Angeles
11000 Kinross Avenue, Suite 211
Los Angeles, CA 90095-1694

<http://ohrpp.research.ucla.edu>
GC-IRB: (310) 825-7122
M-IRB: (310) 825-5344

APPROVAL NOTICE New Study

| | |
|--------------|---|
| DATE: | 12/20/2012 |
| TO: | ANDREA NICOL ANESTHESIOLOGY |
| FROM: | JAMES MC GOUGH, MD Chair, MIRB3 |
| RE: | IRB#12-001588 Psychoneuroimmunology and Chronic Pain Version: 11/23/2012 v2.0 |

The UCLA Institutional Review Board (UCLA IRB) has approved the above-referenced study. The UCLA IRB's Federalwide Assurance (FWA) with Department of Health and Human Services is FWA00004642 (IRB00004473).

Submission and Review Information

| | |
|------------------------------|--|
| Type of Review | Full Board Review |
| Approval Date | 12/20/2012 |
| Expiration Date of the Study | 12/12/2013 |
| Funding Source(s) | 1) AMERICAN ASSOCIATION OF NURSE ANESTHETISTS FOUNDATION <i>Grant Title:</i> Psychoneuroimmune Responses and Chronic Pain |

Specific Conditions for Approval

-- **Research Participants Bill of Rights** - By California law, a copy of the Research Participants Bill of Rights in a language in which the participant is fluent must be given to all research participants in this study as there is a real or foreseeable risk of biomedical harm. Numerous translations are available for download on the HRPP website at <http://www.ohrpp.research.ucla.edu/pages/bill-of-rights>.

Documents Reviewed included, but were not limited to:

| Document Name | Document Version # |
|--|--------------------|
| 12-001588_Consent v3.0 CSF Study.pdf.pdf | 0.01 |

Important Note: Approval by the Institutional Review Board does not, in and of itself, constitute approval for the implementation of this research. Other UCLA clearances and approvals or other external agency or collaborating institutional approvals may be required before study activities are initiated. Research undertaken in conjunction with outside entities, such as drug or device companies, are typically contractual in nature and require an agreement between the University and the entity.

General Conditions of Approval

As indicated in the PI Assurances as part of the IRB requirements for approval, the PI has ultimate responsibility for the conduct of the study, the ethical performance of the project, the protection of the rights and welfare of human subjects, and strict adherence to any stipulations imposed by the IRB.

The PI and study team will comply with all UCLA policies and procedures, as well as with all applicable Federal, State, and local laws regarding the protection of human subjects in research, including, but not limited to, the following:

- Ensuring that the personnel performing the project are qualified, appropriately trained, and will adhere to the provisions of the approved protocol,
- Implementing no changes in the approved protocol or consent process or documents without prior IRB approval (except in an emergency, if necessary to safeguard the well-being of human subjects and then notifying the IRB as soon as possible afterwards),
- Obtaining the legally effective informed consent from human subjects of their legally responsible representative, and using only the currently approved consent process and stamped consent documents, as appropriate, with human subjects,
- Reporting serious or unexpected adverse events as well as protocol violations or other incidents related to the protocol to the IRB according to the OHRPP reporting requirements.
- Assuring that adequate resources to protect research participants (i.e., personnel, funding, time, equipment and space) are in place before implementing the research project, and that the research will stop if adequate resources become unavailable.
- Arranging for a co-investigator to assume direct responsibility of the study if the PI will be unavailable to direct this research personally, for example, when on sabbatical leave or vacation or other absences. Either this person is named as co-investigator in this application, or advising IRB via webIRB in advance of such arrangements.

Appendix I

University of California, Los Angeles Institutional Review Board Continuation

Approval 2013

2/18/16, 11:26 AM



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11000 Kinross Avenue, Suite 211
Los Angeles, CA 90095-1694

<http://ohrpp.research.ucla.edu>
GC-IRB: (310) 825-7122
M-IRB: (310) 825-5344

APPROVAL NOTICE

| | |
|--------------|---|
| DATE: | 12/11/2013 |
| TO: | IRENE WU ANESTHESIOLOGY |
| FROM: | JAMES MC GOUGH, MD Chair, MIREB |
| RE: | IRB#12-001588-CR-00001 2013 Review for IRB#12-001588 Psychoneuroimmunology and Chronic Pain Version: 11/23/2012 v2.0 |

The UCLA Institutional Review Board (UCLA IRB) has approved the above-referenced study. The UCLA IRB's Federalwide Assurance (FWA) with Department of Health and Human Services is FWA00004642 (IRB00004473).

Submission and Review Information

| | |
|------------------------------|--|
| Type of Submission | Continuing Review |
| Type of Review | Full Board Review |
| Approval Date | 12/11/2013 |
| Expiration Date of the Study | 10/9/2014 |
| Funding Source(s) | 1) AMERICAN ASSOCIATION OF NURSE ANESTHETISTS FOUNDATION <i>Grant Title:</i> Psychoneuroimmune Responses and Chronic Pain 2) Other: UCLA Cousins Center for Psychoneuroimmunology / UCLA CTSI <i>Grant Title:</i> Interdisciplinary Seed Grant Program of the Cousins Center for Psychoneuroimmunology <i>Grant Number:</i> UCLA CTSI Grant Number UL1TR000124 |

Specific Conditions for Approval

<https://web.irb.research.ucla.edu/Web/IRB/0cc/0/0v4H0C8EMBP4H0eGA3KNLN3N2E/#fromString.html>

Page 1 of 2

Research Participants Bill of Rights - By California law, a copy of the Research Participants Bill of Rights in a language in which the participant is fluent must be given to all research participants in this study as there is a real or foreseeable risk of biomedical harm. Numerous translations are available for download on the HRPP website at <http://www.ohrpp.research.ucla.edu/pages/bill-of-rights>.

Documents Reviewed included, but were not limited to:

| Document Name | Document Version # |
|--|--------------------|
| 12-001588_Final_Consent_v5_Clean.pdf.pdf | 0.02 |

Important Note: Approval by the Institutional Review Board does not, in and of itself, constitute approval for the implementation of this research. Other UCLA clearances and approvals or other external agency or collaborating institutional approvals may be required before study activities are initiated. Research undertaken in conjunction with outside entities, such as drug or device companies, are typically contractual in nature and require an agreement between the University and the entity.

General Conditions of Approval

As indicated in the PI Assurances as part of the IRB requirements for approval, the PI has ultimate responsibility for the conduct of the study, the ethical performance of the project, the protection of the rights and welfare of human subjects, and strict adherence to any stipulations imposed by the IRB.

The PI and study team will comply with all UCLA policies and procedures, as well as with all applicable Federal, State, and local laws regarding the protection of human subjects in research, including, but not limited to, the following:

- Ensuring that the personnel performing the project are qualified, appropriately trained, and will adhere to the provisions of the approved protocol,
- Implementing no changes in the approved protocol or consent process or documents without prior IRB approval (except in an emergency, if necessary to safeguard the well-being of human subjects and then notifying the IRB as soon as possible afterwards),
- Obtaining the legally effective informed consent from human subjects of their legally responsible representative, and using only the currently approved consent process and stamped consent documents, as appropriate, with human subjects,
- Reporting serious or unexpected adverse events as well as protocol violations or other incidents related to the protocol to the IRB according to the OHRPP reporting requirements.
- Assuring that adequate resources to protect research participants (i.e., personnel, funding, time, equipment and space) are in place before implementing the research project, and that the research will stop if adequate resources become unavailable.
- Arranging for a co-investigator to assume direct responsibility of the study if the PI will be unavailable to direct this research personally, for example, when on sabbatical leave or vacation or other absences. Either this person is named as co-investigator in this application, or advising IRB via webIRB in advance of such arrangements.

Appendix J

University of California, Los Angeles Institutional Review Board Continuation

Approval 2014

2/18/16, 11:24 AM



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APPROVAL NOTICE

| | |
|--------------|---|
| DATE: | 8/7/2014 |
| TO: | IRENE WU ANESTHESIOLOGY & PERIOPERATIVE MEDICINE |
| FROM: | JAMES MC GOUGH, MD Chair, MIREB |
| RE: | IRB#12-001588-CR-00002 2014 Review for IRB#12-001588 Psychoneuroimmunology and Chronic Pain Version: 11/23/2012 v2.0 |

The UCLA Institutional Review Board (UCLAIRB) has approved the above-referenced study. UCLA's Federawide Assurance (FWA) with Department of Health and Human Services is FWA00004642 (IRB00004473).

Submission and Review Information

| | |
|------------------------------|--|
| Type of Submission | Continuing Review |
| Type of Review | Full Board Review |
| Approval Date | 8/7/2014 |
| Expiration Date of the Study | 7/23/2015 |
| Funding Source(s) | 1) AMERICAN ASSOCIATION OF NURSE ANESTHETISTS FOUNDATION <i>Grant PI:</i> SARAH GIRON <i>Grant Title:</i> Psychoneuroimmune Responses and Chronic Pain 2) Other: UCLA Cousins Center for Psychoneuroimmunology / UCLA CTSI <i>Grant PI:</i> IRENE WU <i>Grant Title:</i> Interdisciplinary Seed Grant Program of the Cousins Center for |

<https://webirb.research.ucla.edu/WebIRB/00c0/0/KF6FPP6NK0K7E87Q218FD6411/fromString.html>

Page 1 of 3

| | |
|--|--|
| | Psychoneuroimmunology Grant Number: UCLA CTSI Grant Number UL1TR000124 |
|--|--|

Specific Conditions for Approval

| |
|---|
| <p>– UCLA Serving as Reviewing IRB - The UCLA IRB has agreed to serve as the reviewing IRB for University of California San Diego's involvement in this research according to the provisions of the UC MOU.</p> <p>– Research Participants Bill of Rights - By California law, a copy of the Research Participants Bill of Rights in a language in which the participant is fluent must be given to all research participants in this study as there is a real or foreseeable risk of biomedical harm. Numerous translations are available for download on the HRPP website at http://www.ohrpp.research.ucla.edu/pages/bill-of-rights.</p> <p>– UCLA HIPAA Authorization is required for this research. Copies in English and various translations are available at http://ora.research.ucla.edu/OHRPP/Pages/PHIPermissionForms.aspx</p> |
|---|

Documents Reviewed included, but were not limited to:

| Document Name | Document Version # |
|--|--------------------|
| 12-001588_UCSD_Study_Consent_v2.pdf.pdf | 0.02 |
| 12-001588_Final_Consent_v5_Clean.pdf.pdf | 0.03 |

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General Conditions of Approval

As indicated in the PI Assurances as part of the IRB requirements for approval, the PI has ultimate responsibility for the conduct of the study, the ethical performance of the project, the protection of the rights and welfare of human subjects, and strict adherence to any stipulations imposed by the IRB.

The PI and study team will comply with all UCLA policies and procedures, as well as with all applicable Federal, State, and local laws regarding the protection of human subjects in research, including, but not limited to, the following:

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- Implementing no changes in the approved protocol or consent process or documents without prior IRB approval (except in an emergency, if necessary to safeguard the well-being of human subjects and then notifying the IRB as soon as possible afterwards),
- Obtaining the legally effective informed consent from human subjects of their legally responsible representative, and using only the currently approved consent process and stamped consent documents, as appropriate, with human subjects,

- Reporting serious or unexpected adverse events as well as protocol violations or other incidents related to the protocol to the IRB according to the OHRPP reporting requirements.
- Assuring that adequate resources to protect research participants (i.e., personnel, funding, time, equipment and space) are in place before implementing the research project, and that the research will stop if adequate resources become unavailable.
- Arranging for a co-investigator to assume direct responsibility of the study if the PI will be unavailable to direct this research personally, for example, when on sabbatical leave or vacation or other absences. Either this person is named as co-investigator in this application, or advising IRB via webIRB in advance of such arrangements.

Appendix K

University of San Diego Institutional Review Board Approval



**Institutional Review Board
Project Action Summary**

Action Date: April 8, 2013

Note: Approval expires one year after this date.

Type: New Full Review New Expedited Review Continuation Review Exempt Review
 Modification

Action: Approved Approved Pending Modification Not Approved

Project Number: 2013-04-154
Researcher(s): Sarah Giron Doc SON
Dr. Joseph Burkard Fac SON
Project Title: Psychoneuroimmunology and Chronic Pain

Note: We send IRB correspondence regarding student research to the faculty advisor, who bears the ultimate responsibility for the conduct of the research. We request that the faculty advisor share this correspondence with the student researcher.

Modifications Required or Reasons for Non-Approval

None

The next deadline for submitting project proposals to the Provost's Office for full review is N/A. You may submit a project proposal for expedited review at any time.

Dr. Thomas R. Herrinton
Administrator, Institutional Review Board
University of San Diego
herrinton@san Diego.edu
5998 Alcalá Park
San Diego, California 92110-2492

Office of the Executive Vice President and Provost
Hughes Administration Center, Room 214
5998 Alcalá Park, San Diego, CA 92110-2492
Phone (619) 260-4553 • Fax (619) 260-2210 • www.sandiego.edu

Appendix L

University of San Diego Institutional Review Board Continuation Approval 2014



**Institutional Review Board
Project Action Summary**

Action Date: **March 31, 2014** *Note: Approval expires one year after this date.*
Type: New Full Review New Expedited Review Continuation Review Exempt Review
 Modification
Action: Approved Approved Pending Modification Not Approved
Project Number: **201304-154**
Researcher(s): **Sarah Gloria DoeSON
Dr. Joseph Burkard FacSON**
Project Title: **Psychoneuroimmunology and Chronic Pain**

Note: We send IRB correspondence regarding student research to the faculty advisor, who bears the ultimate responsibility for the conduct of the research. We request that the faculty advisor share this correspondence with the student researcher.

Modifications Required or Reasons for Non-Approval:

None

The next deadline for submitting project proposals to the Project's Office for IRB review is **NA**. You may submit a project proposal to expedited review at any time.

Dr. Thomas R. Herlihy
Administrator, Institutional Review Board
University of San Diego
herlihy@usd.edu
5936 Alcala Park
San Diego, California 92110-2492

Office of the Executive Vice President and Provost
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Appendix M

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