THE FIFTH SCIENTIFIC MEETING OF THE TMJ ASSOCIATION

Can Studies of Comorbidities with TMJDs Reveal Common Mechanisms of Disease?

Meeting Abstracts

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TMJDs: Overcoming the Scientific Challenges of a Complex Phenotype

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Sensory, motor, autonomic and affective systems are shaping - to individually varying degrees - the symptom complex linked to TMJ diseases and disorders, and many other persistent pain-stress conditions. Allodynia, impaired motor function, problems with appetite, sleep, reproduction, cardiovascular systems, and - last but not least - negative emotions represent the many comorbid phenomena that drive the individual disease experience. Understanding the mechanisms in effect is expected to have far reaching impact beyond TMJ diseases and disorders, including many chronic diseases, notably those involving pain-stress. The epidemiologically established risk of women in their reproductive years in terms of exhibiting greater disease prevalence, severity and persistence points to biological mechanisms impacting on the respective symptom generators. Of further mechanistic significance is the fact that the efficacy of available treatments for persistent TMJ diseases and disorders - irrespective of the specific nature of the therapy - does not differ statistically from a credible placebo. Furthermore, negative affect appears to boost the beneficial effect of a credible placebo when compared to the presumably active treatment. Not only does this suggest that the respective treatment targets are in question, together the observations point to the fact that disease processes do not appear to differ from other complex diseases for which gene x environment interactions serve as explanatory models.

Although the characterization of clinical case phenotype is problematic - mostly in terms of scope - there are experimental opportunities to arrive at a first approximation of the mechanisms, including gene x environment interactions in effect in clinic cases.

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Overlaps Between Tension-Type Headaches and TMJDs

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A variety of painful problems can be experienced in the head and face but often seem to affect deep craniofacial tissues. Both temporomandibular joint disorders (TMJDs) and tension-type headaches (TTH) are believed to have a significant contribution from the skeletal muscles and have several clinical features in common. However, it is still unclear to what extent these two prevalent disorders are separate entities or have similar pathophysiological background (Svensson 2007). There is now reasonably good evidence that myofascial TMJD patients are more likely to have a TTH problem and vice versa, however, the overlap is not complete (Ballegaard et al. 2008). Studies have documented similarities regarding sensitization of the nociceptive pathways, dysfunction of the endogenous pain modulatory systems as well as contributing genetic factors, but there are also a number of distinct differences between TMJDs and TTH that need to be considered. Currently, there are two internationally accepted classifications of TMJDs and TTH: The Research Diagnostic Criteria for TMD (Dworkin and LeResche 1992) and the International Headache Society Classification (2004). Both are based on the consensus of world-leading researchers and clinicians and include operationalized diagnostic criteria. Both classifications have also been subject to extensive testing in terms of reproducibility (John et al. 2005), whereas validity testing has been shown to be more difficult because of the lack of a gold standard or distinct neurobiological markers. Thus both TMJDs and TTH are mainly based on a combination of well-defined signs and symptoms rather than a good understanding of the underlying pathophysiology, i.e., it has not been possible to make a mechanism-based classification because essential information is still missing. In fact, it is not known if pains associated with TMJDs are i) transient, nociceptive types of pain ii) inflammatory pain associated with tissue damage, iii) neuropathic pain associated with lesions or diseases in the somatosensory system, or iv) functional types of pain (Woolf 2004). Using the current classification systems, TMJDs and TTH disorders do overlap and appear to share many of the same pathophysiological mechanisms, however, it would be premature to consider them as identical entities, since the importance of, for example, the affected muscles and associated function, as well as systemic risk factors, need to be established. Orofacial pain specialists and headache specialists should collaborate to further develop diagnostic procedures and management strategies of TMJD and TTH.

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TMJDs: A Mosaic of Clinical Phenotypes and Systemic Disorders

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Temporomandibular joint disorders (TMJDs) are a heterogeneous family of musculoskeletal disorders that represent the most common orofacial pain conditions[1,2]. Although there are several forms or subclasses of TMJDs, the most common and debilitating forms are associated with persistent pain in the region of the temporomandibular joint, the periauricular region, and muscles of the head and neck[1,2]. Worldwide epidemiological studies report the prevalence of TMJDs to range from 5 to 50% with most studies reporting a prevalence rate of approximately 10%[2].

TMJDs are associated with several comorbid conditions and symptoms - including, but not limited to, irritable bowel syndrome, fibromyalgia syndrome, vulvar vestibulitis, affective disorders, abnormalities in sleep and autonomic dysfunction[3]. At present, we have a poor understanding of the pathophysiological mechanisms that mediate TMJDs and related comorbidities. However, several recent studies have provided evidence that persistent pain conditions like TMJDs and associated comorbidities are multifactorial and are composed of mosaics of intermediate phenotypes that manifest as enhanced sensitivity to painful events (i.e, a state of pain amplification), autonomic imbalances, and psychological sequel. There is now considerable evidence that these intermediate phenotypes are influenced by multiple polymorphisms in genes that code for proteins which modulate pain processing, affect/mood, and autonomic function[3-6].

Dr. Maixner will present findings from recently completed[5,7] and ongoing (see www.oppera.org) cross-sectional and prospective studies that examine the biopsychosocial and genetic factors contributing to the onset and maintenance TMJDs and related conditions. (Supported by DE07509, NS45685, DE16558, NS41670, and DE017018).

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Patient Round-Table
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Chronic Headache
Teri Robert, Help for Headaches & Migraine, Parkersburg, WV.
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Generalized Pain Conditions
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Disclosure: None

Irritable Bowel Syndrome
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Disclosure: Receives honorarium from Ventrus for an advisory board role

Endometriosis
Mary Lou Ballweg, President and Executive Director, Endometriosis Association, Milwaukee, WI
Disclosure: Receives EA from Organon for expert panel role. Receives EA from KV Pharmaceutical for "hands-off" recruitment mailing role. Receives receives EA from Mary Jo Liseek, M.D. for "hands-off" recruitment mailing role

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Rheumatoid Arthritis
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Disclosure: None
Sandy Canfield, Patient Advocate, Burke, VA
Disclosure: None

Temporomandibular Joint and Muscle Disorders
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Disclosure: None

Chronic Headache and TMJDs

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Over the past 20 years, many studies have examined the epidemiology and comorbidities of chronic headache disorders. The two most common primary headache disorders are episodic tension type headache and migraine. Migraine is traditionally divided into two major types based on symptom profile: migraine without aura (MO) and migraine with aura (MA). It is also divided into two types by attack frequency: episodic migraine with less than 15 headache days per month and chronic migraine (CM) with 15 or more headache days per month. Population-based studies have revealed that episodic migraine is associated with several psychiatric comorbidities (depression, generalized anxiety disorder, panic disorder and bipolar disease among others), epilepsy and stroke. Migraine with aura is particularly associated with stroke, myocardial infarction, angina, myocardial revascularization procedures and claudication. Most comorbidities show a stronger association with CM than episodic migraine. In addition, many pain disorders seem to aggregate within individuals.

Comorbidities may arise through several possible pathways. First, apparent comorbidities might arise due to methodologic artifacts such as selection bias or ascertainment bias. To guard against the identification of spurious comorbidities we recommend systematic ascertainment of the conditions of interest in representative samples. Second, comorbidity might arise through a process of unidirectional causation. That is, migraine and TMJD may be associated because TMJD aggravates migraine, increasing its incidence or duration. A third possibility is that the conditions may share environmental risk factors such as trauma. A forth possibility is that conditions may be linked by genetic risk factors. For example, if certain polymorphisms increase vulnerability to chronic pain, two pain disorders may be linked through that common genetic predisposition. Similiarly, if particular genotypes protect against pain, the same individuals may be less likely to have either migraine or TMJD.

In this talk, I will consider the evidence for the comorbidity of TMJD with both migraine and tension type headache, and then review the evidence for and against each of the possible mechanisms which may link migraine and tension type headache.

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Role of Neuronal-Satellite Glial Cell Interactions in the Underlying Pathology of Migraine and TMJ Disorders

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Activation of trigeminal ganglion nerves is implicated in the pathology of migraine and temporomandibular joint (TMJ) disorders. Cell bodies of trigeminal neurons reside in the ganglion in close association with satellite glial cells [1]. Neuron-glia interactions via gap junctions and paracrine signaling are thought to be involved in all stages of inflammation and pain associated with several CNS diseases[2, 3]. However, the role of neuron-glia communication within the trigeminal ganglion under normal and inflammatory conditions is not known. Based on recent studies, we now have evidence of increased neuron-satellite glial cell signaling via gap junctions within the trigeminal ganglion in response to trigeminal nerve activation [4]. The number of gap junction plaques formed by connexin 26 between neurons and satellite glial cells is transiently increased following capsaicin injection into the TMJ. However, connexin 26 gap junction plaques between neurons and satellite glia are stably expressed in response to injection of complete Freund's adjuvant into the TMJ capsule. Interestingly, stimulation of V3 neurons leads to increased gap junction communication as well as increased expression of the MAP kinases p38 and ERK, not only in neurons and satellite glial cells in the V3 region, but also those cell types located in the V1 and V2 regions of the ganglion. Based on our findings, neuronal-glial communication via gap junctions and paracrine signaling are likely involved in the development of peripheral sensitization within the trigeminal ganglion and thus play an important role in migraine and TMJ pathologies. Furthermore, we propose that propagation of inflammatory signals within the ganglion may help to explain the significant comorbidity associated with migraine and TMJ pathology.

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The Challenges of Targeting Mechanisms of Pain Following Nerve Injury

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It is becoming apparent that neither differing pain symptoms nor differing disease diagnoses can explain reliably the variability in analgesic responses following nerve injury. We may therefore infer that, despite similar causative processes such as in painful diabetic neuropathy, mechanisms of pain are likely to be heterogeneous. Our challenge is to identify within individual patients which specific mechanisms are operating to produce which signs and symptoms, so that we may improve the diagnoses and initiate novel treatment strategies for pain.

Pain mechanisms in patients have been categorized by disease, by symptoms (including responses to quantitative sensory testing), by sensitivity to selective drugs, and by specific tests such as functional imaging. Unfortunately, the treatment of chronic pain is not clearly defined by etiology or anatomical lesion. The history and physical should be focused on the qualities of the spontaneous or ongoing pain, as well as the nature and intensity of specific mechanical, thermal, or chemical stimuli that may evoke pain. Quantitative sensory testing may be a clinically useful tool to determine the relative contribution of sensory loss and the presence of hyperexcitability. A rational treatment program is dependent on an understanding of the relative contribution of each type of pain to the patient's overall pain experience.

We will review the putative pain mechanisms upon which our clinical decisions are based. We will discuss several classes of analgesic and antihyperalgesic agents, including antidepressants, anticonvulsants, sodium channel blockers, glutamate receptor antagonists, and opioids, in specific clinical pain states. We will also discuss the potential sources of variability that challenge the ability to detect treatment effects in clinical trials, such as inadequate dosing, dose-limiting side effects, and other dosing considerations including hepatic metabolism and potential drug interactions.

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Glia as the "Bad Guys" in Dysregulating Pain and Opioid Actions: Implications for Improving Clinical Pain Control

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Work over the past 15 years has challenged classical views of pain & opioid actions. Glia (microglia & astrocytes) in the central nervous system are now recognized as key players in: pain amplification, including pathological pain such as neuropathic pain, compromising the ability of opioids, such as morphine, for suppressing pain, causing chronic morphine to lose effect, contributing to opioid tolerance, driving morphine dependence/withdrawal, driving morphine reward, linked to drug craving & drug abuse, & even driving negative side effects such as respiratory depression. Atop this, what is both fascinating & fundamentally important, is that these opioid effects on glia are via the activation of a non-classical, non-stereoselective opioid receptor distinct from the receptor expressed by neurons that suppresses pain. This implies that the effects of opioids on glia & neurons should be pharmacologically separable.

Our studies, led by Dr. Mark Hutchinson, have revealed that opioids induce an opposing process, namely glial release of proinflammatory cytokines that oppose opioid analgesia. Glial opposition of analgesia occurs in response to a broad range of opioids, including, but not restricted to, morphine & methadone. Upon opioid administration, both pain suppression & proinflammatory cytokine-induced pain enhancement simultaneously occur, as opponent processes. Blocking proinflammatory cytokines markedly enhances the magnitude & duration of analgesia. Indeed, morphine dose response functions performed in the absence vs. presence of cytokine inhibitors reveals a marked leftward shift in the dose response function when proinflammatory cytokine actions are blocked, demonstrating that these endogenous proinflammatory mediators naturally compromise the analgesic efficacy of both intrathecally & systemically delivered opioid analgesics.

Glial proinflammatory cytokines upregulate in response to chronic opioids, importantly contributing to the development of opioid tolerance, dependence/withdrawal, & reward. Of fundamental importance is our discovery that opioids activate glia via a non-stereoselective receptor separate from the classical opioid receptor - toll like receptor (TLR)-4. Given that neuronally inactive (+)-naloxone blocks this glial receptor but not neuronal opioid receptors, this finding predicts that (+)-opioids such as (+)-naloxone should potentiate opioid analgesia by not blocking morphine effects on neurons, yet removing glial activation that opposes analgesia. This is true.

In sum: our data predict that suppressing glial activation will suppress pathological pain of various etiologies, improve opioid analgesia, & suppress opioid (a) tolerance, (b) dependence, (c) reward linked to drug craving/drug seeking & (d) respiratory depression. Further, our data demonstrate that opioid activation of glia is fundamentally different than that for neurons. Glial receptors are not stereoselective, opioid effects on glia must be via different receptors (TLR4) than those for neurons. Effects of glia & neurons should be separable, & to increase the efficacy of opioids one should either modify opioids so they don't bind glia &/or create long-lasting, orally available versions of [+]-naloxone.

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Clinical and Neurobiological Aspects of Brain-Gut Interactions in IBS Patients

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Irritable bowel syndrome (IBS), characterized by chronically recurring abdominal pain or discomfort, associated with altered bowel habits, is one of the most common syndromes seen by gastroenterologists and primary care providers, with a worldwide prevalence of 10 to 15%.[1] In the absence of detectable organic causes, IBS is referred to as a "functional" syndrome and remains defined by symptom criteria.[2] IBS is part of a larger group of common "functional" disorders which frequently overlap in the same patient at the time of presentation, or manifest at different times in a patient's life time,[3] show frequent comorbidity with other somatic pain disorders (such as fibromyalgia, chronic pelvic pain and interstitial cystitis)[4] and have a disproportionate impact on health care utilization[1] and health-related quality of life (HRQoL).[5] Non-GI factors (such as symptoms of vital exhaustion and symptom-related fears) play an important role in HRQoL impairment in IBS patients, and excessive health care utilization and costs are commonly related to non-GI symptoms.

About 10% of patients develop IBS-like symptoms following bacterial or viral enteric infections ("post-infectious IBS"), with female gender, duration of gastroenteritis, and several psychosocial factors (including major life stress at the time of infection, somatization) being established risk factors.[7] Symptoms of IBS or of related functional disorders (such as recurrent abdominal pain and functional constipation) commonly date back to childhood, where the estimated prevalence of IBS is similar to that in adults.[8] The female to male ratio in most population-based samples is 2:1, with generally higher rates in health care seeking samples, suggesting that both biological and psychosocial factors (e.g., threshold for seeking medical care) are likely to contribute to this gender bias.

The fact that all functional disorders share stress-sensitivity of symptoms, show a high degree of comorbidity with psychological symptoms (primarily anxiety and somatization) and psychiatric disorders, and respond to central nervous system (CNS)-directed therapies (both psychological and pharmacological) point towards an important role of the CNS in symptom generation. In the majority of patients, IBS symptoms result from a complex dysregulation of the brain-gut axis, involving variable contributions of peripheral, spinal and supraspinal abnormalities (reviewed in[1, 9, 10]). Vulnerability to develop IBS is thought to be influenced by an interaction between genetic factors and early adverse life events. Alterations in GI motility have been identified in some patients and, together with alterations in intestinal fluid handling, may play an important role underlying IBS-related bowel habit irregularities.[1] Enhanced perception of signals arising from the GI tract ("visceral hypersensitivity") is considered a key factor underlying abdominal pain and discomfort.[10] Considerable preclinical and clinical evidence supports the presence of altered central arousal/stress circuits which may play a key role in central pain amplification, altered autonomic and hypothalamic-pituitary-adrenal axis responses and in associated symptoms of anxiety.[11, 12] Recent evidence implicates a possible alteration in intestinal microflora, host-microbial interactions and mucosal neuroendocrine immune interactions. However, it remains to be determined which of the various reported abnormalities truly contribute to IBS symptoms, to health care seeking and to HRQoL impairment, which targets are relevant for drug development, and which of the growing list of abnormalities represent secondary effects or epiphenomena.

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Neural Mechanisms of the Pains of Endometriosis and Comorbid Disorders

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Endometriosis is a disorder whose signs are growths of endometrial tissue in abnormal locations, and whose symptoms include distressing pelvic visceral and muscle pains. Surprisingly often, endometriosis co-occurs with other painful conditions in widely disparate bodily regions, such as irritable bowel syndrome, interstitial cystitis/painful bladder disorder, temporomandibular joint disorders, migraine headaches, vulvodynia, and others. The enigma of endometriosis is that its signs fail to correlate with its visceral, muscle, somatic, and comorbid pain symptoms. Recent studies in experimental animals and women suggest that the ectopic growths of endometriosis develop their own sensory and sympathetic nerve supply. This innervation, together with the peripheral generation of algogenic agents, likely contributes to engagement of the central nervous system (CNS) in generating individually-different pain symptoms. Mechanisms by which the central nervous system becomes engaged in pain related to endometriosis include central sensitization, remote central sensitization, and central hormonal modulation. These mechanisms likely apply generally to other chronic pain conditions, such as temporomandibular joint disorders, and encourage a deliberate multifactorial approach to assessment and diagnosis, followed by an individualized multifactorial approach to treatment.

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Clinical Features and Neurobiological Aspect of Interstitial Cystitis

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Interstitial cystitis/Painful Bladder Syndrome (IC/PBS) is a common chronic bladder syndrome characterized by bladder pain and discomfort (urgency) and increased frequency of urination. It is associated with significant decrements in HRQOL, productivity and increased health care costs. Patient symptoms in IC/PBS reflect a hypersensitive state with exaggeration of normal genitourinary sensations, whereby bladder sensation and fullness occur at lower volumes than normal and the discomfort of a full bladder is perceived as pain. Bladder biopsy findings of patients with IC/PBS appear to separate the condition into two general groups: (1) patients with Hunner's ulcers, characterized by a chronic pancystitis with mast cell infiltration, submucosal ulcerations, denuded epithelium, inflammatory infiltrate, and lower bladder capacity (10% of patients) and (2) the majority of patients with non-ulcerative IC/PBS, who can present with bladder biopsies showing completely normal bladders or mild inflammation and normal bladder capacity. The pathophysiology of IC/PBS is poorly understood. Numerous theories, including problems with urothelial cell barrier exposing the bladder to urine toxins, allergic response, infectious causes, autoimmune disease, and abnormal nerve responses have been postulated. Current treatment modalities usually are empiric and parallel proposed pathophysiological theories including: pentosan polysulfate sodium, aimed at repairing the urothelial GAG layer, intravesical anti-inflammatory therapies, intravesical pro-inflammatory therapies, H2 blockers, tricyclic antidepressants, etc. Most medications that have been studied in well-designed placebo controlled studies have failed to show efficacy over placebo.

There is growing evidence that IC/PBS shares several features with other chronic functional pain disorders including Irritable Bowel Syndrome (IBS), TMJ, and Fibromyalgia Syndrome (FMS), all pointing to the potential for shared underlying central nervous system (CNS) mechanisms and possible associated genetic polymorphisms. Thus IC/PBS can be viewed as a syndrome resulting from enhanced responsiveness of central stress circuits to exteroceptive (psychosocial) stressors common to many medical and psychiatric disorders, as well as enhanced responsiveness to interoceptive (visceral afferents, neuroendocrine) stressors that are specific to IC/PBS. While secondary peripheral changes in the bladder are likely to play a role in maintaining IC symptoms, alterations in the central stress responsiveness seem to play a key role in the mediation of autonomic and neuroendocrine responses, and of pain modulation.

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Neural-Epithelial Interactions in Interstitial Cystitis

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Symptoms of the chronic painful bladder condition interstitial cystitis (IC) include urinary frequency, urgency, nocturia and pain. A comparable disease in cats, termed feline interstitial cystitis (FIC), exhibits nearly all of the characteristics and symptoms of human IC. There are a number of theories explaining the pathology of IC, including alterations in the epithelial lining of the bladder termed the urothelium. Recent studies also suggest that there may be a number of common neural mechanisms in IC associated with other chronic pain conditions.

Urothelial cells (UTC) lie in close proximity to afferent nerves and data accumulated over the last several years now indicate that these cells express a number of receptors/ion channels similar to those in sensory nerves.[1,2] Examples of "sensor" molecules expressed within the urothelium are receptors for bradykinin, neurotrophins, purines, norepinephrine, acetylcholine, and TRP channels, as well as estrogen receptors. In addition, release of chemical mediators (nitric oxide, NO; ATP; acetylcholine) from UTCs suggests that these cells exhibit specialized sensory and signaling properties that could allow reciprocal communication with neighboring UTCs, as well as nerves or other cells in the bladder wall.

Injury or inflammation may alter the response of both UTCs and sensory afferents to nociceptive/non-nociceptive stimuli. For example, increases in mechanosensitivity (allodynia) could trigger urgency/pain in patients with bladder dysfunction, including those with IC. These findings fit closely with our results in FIC cats, where we have detected abnormalities in detection of chemical or mechanical stimuli in both FIC urothelium and sensory neurons.[3] This type of allodynia/hyperalgesia could be due to a defect in "sensor" or "transducer" mechanisms resulting from changes in expression/sensitivity of ion channels or receptors near the target organ (which could influence both sensory nerves and urothelium). For example, similar to that shown in IC patients, we have reported altered purinergic receptor expression and ATP release from FIC bladder UTCs.[4] Mechanosensitive ATP from the urothelium has a number of consequences, such as activation of P2X or P2Y receptors on bladder nerves, or promotion of autocrine activation of P2Y receptors on urothelial cell surface.

In addition, it has been demonstrated that inflammation or injury-induced increases in endogenous neurotrophic factors, such as nerve growth factor (NGF) in the target organ, may link tissue damage and hyperalgesic responses. Similar to that reported in IC patients, we have evidence that FIC urothelium express significantly larger amount of NGF as compared to urothelium from normal cat bladder. It has been shown that neurotrophic factors have a profound effect on the morphology and capsaicin-sensitivity of sensory neurons. In this regard, we have also found altered capsaicin sensitivity in addition to an increase in the diameter of dorsal root ganglion (DRG) neurons from FIC as compared to normal healthy controls.[5]

Though the urothelium maintains a tight barrier to ion/solute flux, factors such as tissue pH, mechanical or chemical trauma, or bacterial infection can modulate this epithelial barrier function. Alterations in bladder epithelium have been demonstrated both in patients with IC, as well as in FIC cats. For example, we have shown that FIC UTCs are associated with a decreased rate of proliferation as well as heparin-binding epidermal growth factor production.[6] Thus, these factors may adversely influence epithelial integrity, which could result in passage of toxic and irritating urinary constituents through the epithelium, leading to changes in properties of sensory pathways.

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Recent evidence has shown that glial cells are involved in pain enhancement in chronic clinical pain states. Activation of astrocytes and microglia may result in altered cell morphology, changes in surface membrane, cytoplasmic protein expression and release of factors that can contribute to changes in neuronal function. We have evidence of changes in morphology and expression patterns of spinal cord glial cells (astrocytes/microglia) in FIC.[7] These and other findings may indicate an active role for glial cells in the pain-signaling pathway. Taken together, evidence also suggests that urothelial cells can receive and integrate multiple stimuli, thus providing an important "link" in the transfer of information from the urinary bladder to the nervous system.

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Mechanisms in "Central" Pain Syndromes: Lessons Learned from Fibromyalgia

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Objectives. To provide an overview of the findings regarding the underlying pathogenesis of FM, and to compare these findings with the results of recent treatment studies, to determine whether there is concordance between what "should work" and "what does" in this illness.

Findings. A number of different experimental modalities have been employed to demonstrate conclusively that FM is characterized by a "left-shift" in stimulus-response function, i.e. that FM patients display both hyperalgesia and allodynia. The fact that this occurs to several different types of sensory stimuli (pressure: heat or electricity applied to the skin; auditory stimuli) suggests that this augmented pain processing is primarily a central rather than a peripheral process. There is additional evidence suggesting that there is a deficiency of descending analgesic activity in FM, and that this is primarily due to decreased serotinergic/noradrenergic activity, rather than to an inherent defect in opioid-induced analgesic systems. Additional evidence from experimental pain testing studies suggests that there is increased "wind-up" in FM patients, reminiscent of the central sensitization due to increased glutamatergic and/or neurokinin activity noted in animal models. Finally, in addition to these neurobiological aberrations, it is well known that many individuals with FM have or develop behavioral, psychological, and cognitive adaptations to their illness that may perpetuate or worsen symptoms.

These findings have been corroborated by functional MRI showing increased neuronal activity to many of these same stimuli in several brain regions known to be involved in processing the sensory and affective processing of pain and sensory stimuli, particularly the insula. A number of other functional neuroimaging modalities such as PET and H-MRS have also led to pathogenic insights into this condition, which are supported by clinical trials showing that classes of drugs that decrease the activity of excitatory neurotransmitters (e.g., glutamate) or increase activity of pain-inhibiting neurotransmitters (e.g., serotonin/norepinehprine) can improve pain and other symptoms in this condition. Ongoing studies using several types of functional neuroimaging in the context of clinical trials is likely to lead to even more information about subsets of individuals with varying underlying causes for this augmented pain and sensory processing, and might eventually help us tailor therapy for this and related conditions.

Conclusions. Mechanistic studies reveal a number of neurobiological abnormalities in FM that would be expected to lead to pain and/or sensory amplification, and clinical trials of drugs aimed at these targets have yielded the expected positive results. These trials suggest that different sub-groups of patients may respond to different drugs, and that some patients may benefit from the concurrent use of education, cognitive-behavioral, and exercise programs that address comorbid problems often seen in FM patients.

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Comorbidity of TMJDs and Fibromyalgia

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Fibromyalgia and temporomandibular disorders (TMD) are frequently comorbid conditions, have many characteristics in common, and may share important and possibly heritable causal factors. Reported rates of TMD have ranged from 42 to 94% of patients with fibromyalgia. However, only 10 to 18% of patients with TMD have also met criteria for fibromyalgia. In a recent study, fibromyalgia was identified in 82% of TMD patients with masticatory myofascial pain. These results suggest that the presence of masticatory myofascial pain is an important factor in the comorbidity of fibromyalgia and TMD.

Fibromyalgia shares several clinical features with TMD, particularly chronic TMD with myofascial pain of the masticatory muscles. Both disorders are more common in women than men, and have similar associated symptoms including muscle pain, pain sensitivity, fatigue, sleep and concentration difficulties, bowel complaints, headaches, and depressive and anxiety symptoms. They are also affected by similar modulating factors such as stress, weather changes, cold, and warmth. However, there are also important distinguishing characteristics of fibromyalgia and TMD. Notably, patients with fibromyalgia report more functional disability, work difficulty, and greater dissatisfaction with health. In addition, the prevalence of TMD appears to decrease with age in women, while the prevalence of fibromyalgia increases with age. It is possible that the disorders are part of a continuous spectrum of chronic pain with fibromyalgia on the more severe end of the spectrum.

Recent evidence supports the possibility of some shared pathophysiologic mechanisms in fibromyalgia and chronic TMD. Fibromyalgia and reduced pressure pain thresholds aggregate in families, which suggests that genetic factors are involved in the etiology of fibromyalgia and in pain sensitivity. In addition, fibromyalgia coaggregates in families with major mood disorders, as well as mood disorders, anxiety disorders, eating disorders, irritable bowel disorder, and migraine, taken collectively. Genetic studies implicate the involvement of a functional polymorphism of the gene, catechol-O-methyltransferase (COMT), in fibromyalgia and TMD. COMT activity has been found to substantially influence pain sensitivity. Both disorders are associated with augmented temporal summation of pain, suggesting that alterations in central nervous system processes contribute to enhanced pain sensitivity. In addition, high somatization scores and depression are predictive of the development of TMD and fibromyalgia. Finally, fibromyalgia and chronic TMD are responsive to antidepressant treatment, although studies of antidepressant treatment of TMD are limited.

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Generalized Pain Syndromes: Mechanisms

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The primary symptom and most debilitating aspect of TMJD and other generalized pain syndromes (e.g., FMS and IBS) is chronic generalized pain. The pathogenic mechanisms of pain in these syndromes are very poorly understood and currently available analgesic therapies have proven to be only partially, and unpredictably, effective. Progress in this area has been seriously hindered by the lack of an adequate experimental model of chronic generalized pain. We determined that interruption of vagal-afferent activity from the abdomen in the rat can result in a state of enhanced chronic generalized hyperalgesia. The vagotomy-induced generalized hyperalgesia depends on activity of the adrenal medulla, a characteristic also relevant to TMJD and other generalized pain syndromes in which chronic stress-induced activation of the sympathoadrenal and hypothalamic-pituitary-adrenal axes may contribute. Another important dimension of our model is the demonstration of generalized enhanced cytokine-induced hyperalgesia induced by chronic intermittent stress. In this model rats exposed to non-habituating sound stress exhibited no change in mechanical nociceptive threshold, but showed a marked increase in hyperalgesia evoked by cytokines. This enhancement, which developed more than a week after exposure to stress, required concerted action of glucocorticoids and catecholamines at receptors located in the periphery on sensory afferents. The altered response to pronociceptive mediators involved a switch in coupling of their receptors. Thus, an important mechanism in generalized pain syndromes may be stress-induced co-activation of the hypothalmo-pituitary-adrenal and sympatho-adrenal axes, causing a long-lasting alteration in intracellular signaling pathways, enabling normally innocuous levels of immune mediators to produce chronic hyperalgesia. The model provides important new insights into the pathophysiology of generalized pain conditions, including that associated with TMJD.

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Imaging the Cognitive Modulation of Pain in Fibromyalgia

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Introduction: Fibromyalgia (FM) is a disabling disorder characterized by chronic and widespread musculoskeletal pain (5). Our previous brain imaging data demonstrated that, compared to healthy controls, FM patients exhibited exaggerated functional magnetic resonance imaging (fMRI) responses to both non-painful and painful heat stimuli (1). These data, and those reported by Gracely and colleagues (2), have provided objective evidence of augmented central processing of nociceptive stimuli in FM, and consistent with psychophysiological data, support the emerging view that abnormalities in central pain processes act to maintain FM pain. However, cognitive aspects of pain, such as expectancy and attention, can change the sensory and affective experience and have been viewed as crucial in the development of chronic pain (3; 4). Further, it is unclear whether augmented central processing of sensory stimuli is unique to FM or is a general consequence of chronic pain.

Purpose: The overall purpose of this project is to determine the influence of expectancy and attention on brain responses to non-painful and painful stimuli in FM compared to healthy controls (CON) and a pain control group with rheumatoid arthritis (RA).

Methods: We are recruiting three groups of women, FM (n=40), RA (n=40) and CON (n=40). Anticipation is being manipulated by randomly assigning participants to 'pain' and 'no pain' conditions. Attention is being manipulated by using a demanding cognitive task, the Stroop color-word task, and measuring brain responses to painful and non-painful heat stimuli. The first day of testing involves psychophysical pain and Stroop testing in a mock-MRI unit. The second day involves collection of functional neuroimaging data on a 3 Tesla GE SIGNA MRI (GE Medical Systems, WI). During scanning participants receive either moderately painful (pain rating of '13' on 0-20 scale) or non-painful heat stimuli alone and while performing the Stroop color-word task. Data are analyzed with Analysis of Functional NeuroImages (AFNI) software.

Preliminary Results: Augmentation - FM patients showed significantly greater ($p<0.001$) brain responses to moderately painful stimuli in several pain-relevant brain regions, including the anterior insula and the anterior cingulate cortex (ACC) compared to CON. Anticipation - Both FM and RA patients, but not CON, exhibited significantly greater ($p<0.001$) pain-relevant brain responses to warm stimuli in the 'pain' condition compared with the 'no pain' condition. Attention/Distraction – For CON, performing the Stroop color-word task in the presence of a painful stimulus engaged the frontal cortex, ACC and PAG to a significantly ($p<0.001$) greater extent, and the insula to a significantly ($p<0.001$) lesser extent compared to receiving painful stimuli or performing the cognitive task alone. FM patients did not show significant increases in frontal or ACC activity, or decreases in insula activity during pain and distraction, but did show greater PAG activity ($p<0.001$). Further, FM patients exhibited decreased activity ($p<0.001$) in inferior temporal and parietal cortices, suggestive of increased attention to pain. Linear regression of the change scores for pain showed that decreases in pain perception were significantly ($p<0.001$) and positively ($r^2 = 0.65$ to 0.80) related to brain activity in the superior frontal cortex, ACC and the PAG for CON but not for FM.

Discussion: Our preliminary functional brain data replicate and extend our previous finding of augmented neural responses to pain in FM. Our data also show that anticipation of pain alters the neural response to non-painful stimuli in FM and RA. Finally, our preliminary data show that FM patients do not engage the same neural system as controls while receiving pain and performing a distracting cognitive task. Controls show brain responses typical of top-down cognitive pain control, while FM patients demonstrate a neural response suggestive of enhanced attention to pain.

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Understanding the Biology of Chronic Fatigue Syndrome to Improve Objective Diagnosis and Intervention

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Chronic diseases are some of the most common maladies of the 21st century and pose a tremendous public health challenge. But, there is a great deal of optimism that genomic approaches can improve diagnosis, treatment and, ultimately, prevent chronic diseases. Sophisticated technologies exist to scan the genome, detect disease-associated differences and potentially predict who is at risk for developing disease. However, there are still large gaps that need to be closed before this revolution in genomic medicine can be translated into biologic significance and have its anticipated impact.

Chronic fatigue syndrome (CFS) puts the aspirations of genomic medicine to the test. CFS is a complex trait controlled by many genes and whose inheritance does not follow the simple rules of Mendelian genetics. Rather, CFS is a result of the interplay between many genes and an individual's environment over the lifespan. This means that genes and gene products are context dependent and in the case of CFS, potentially affected over time by other diseases and comorbidities, infection, trauma, and behavior.

Genomic information can be used to further our understanding of the biology and pathophysiology to improve diagnosis and treatment of CFS. Genomics have been successfully used to synthesize the molecular profiles that make up the heterogeneous mix of CFS. There are examples of how genomic information has been used to customize therapeutic interventions in a variety of diseases. I will propose a genomic medicine model and explain how adaptation of this model will influence not only the diagnosis and treatment of CFS, but could be applicable to many complex chronic conditions.

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Research Advances in Chronic Fatigue Syndrome

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Chronic Fatigue Syndrome is an illness that has been difficult to study. Case definition issues, overlap with the fibromyalgia population, and lumping vs. splitting debates all have confounded early studies. Population-based studies suggest a US CFS population in excess of 1,000,000, with 85% of the population undiagnosed. With such a high percent of the potential study population undiagnosed, there are also concerns that the population available for study may not fully represent the larger group. The clinic-based population also tends to shift away from the higher risk ethnic subgroups, African American and Hispanic, toward Caucasians with longer duration illness.

Despite these obstacles, there has been progress. In recent years efforts to subgroup populations of patients with CFS into more homogeneous subpopulations has resulted in a better understanding of the pathogenesis of this disabling illness. As described in Dr Vernon's talk, the biologic underpinnings of this complex disorder are becoming more clear, and clinical trials that are pathogenesis-driven are replacing trials oriented towards symptom management alone. Thus there are studies focused on immunomodulators, antivirals, stage 4 sleep induction, and autonomic dysfunction in addition to studies of pain management approaches, stimulants and coping strategies. There is a considerable body of literature on the benefits of cognitive behavioral management and reconditioning: the benefits seen are similar to those seen in other chronic conditions. In this talk the current literature will be reviewed, including studies evaluating potential diagnostic markers and biomarkers, and recent and past clinical trials of CFS.

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Rheumatoid Arthritis and Cardiovascular Risk

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RA is a chronic inflammatory disease that affects 1-2% of the population, and has a 2-fold higher prevalence in women than men. RA is associated with a reduced lifespan (standardized mortality ratio for RA of 1.28-3.0). Cardiovascular (CV) disease has been consistently identified as the leading cause of excess deaths in RA. In addition, a higher incidence of non-fatal CV events [e.g., myocardial infarction (MI), congestive heart failure [CHF], and stroke], and a higher prevalence of subclinical atherosclerosis, in RA patients compared to controls, have been demonstrated. Although some studies have suggested a reduction in CV events in the RA population with the emergence of methotrexate and/or anti-tumor necrosis factor (TNF) therapy, results to date are inconsistent. Interestingly, RA patients with myocardial infarctions (MIs) are also less likely to be symptomatic than non-RA patients with MIs. Adjustment for conventional CV risk factors does not account for the higher rates of CV events in RA populations, *suggesting that rheumatoid inflammation is an independent risk factor for CV disease.* This hypothesis is supported by data in the general population that high normal or modest elevations of circulating inflammatory markers are potent independent risk factors for CV events, and that the most prone-to-rupture atherosclerotic lesions are those that exhibit a robust inflammatory infiltrate.

The mechanism(s) by which RA accelerates atherogenesis remains unclear and is undoubtedly multifactorial and complex. However, two potential pathways, that are ***not*** mutually exclusive, have been suggested. In the first, high levels of circulating and/or or intravascular cytokines, such as TNF-α, directly damage endothelium and promote atherosclerosis. Several lines of evidence suggest that vascular injury is an early and ongoing event in rheumatoid disease. Inflammation of blood vessels ("vasculitis") is well described in RA, both clinically and histopathologically. A major inciting factor is thought to be deposition in blood vessels of circulating immune complexes, containing RA-associated autoantibodies. Enhanced activation of endothelium in RA is supported by elevated levels of soluble adhesion molecules in RA patients, especially those with vasculitis, and by a higher prevalence of endothelial dysfunction in RA patients, compared to controls. In the second pathway, these cytokines act on extra-vascular target organs to promote the development of metabolic risk factors that secondarily accelerate atherosclerosis. In particular, in muscle, TNF-α inhibits insulin signal transduction and impairs glucose metabolism *in vitro* and, when infused in humans, induces insulin resistance. Insulin resistance is a potent risk factor for atherosclerosis and is believed to be at the core of the so-called "metabolic syndrome", a group of metabolic risk factors for CV disease that cluster in individuals. Indeed, the prevalences of insulin resistance and metabolic syndrome are higher in RA patients compared to controls, and insulin sensitivity improved in RA patients, but not diabetics, following anti-TNF treatment. Thus, insulin resistance in RA may be induced, in part, by the effect of disease-driven inflammatory cytokines on insulin receptor function and number in muscle.

Taken together, these data provide strong circumstantial evidence for a critical role for RA specific immunological and inflammatory pathways in promoting atherogenesis.

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Rheumatoid Arthritis Immunopathogenesis

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RA is the most common inflammatory arthritis and affects about 0.5 to 1% of the world's population, and about 1% of the United States population. RA is more common among females, with a ratio of 2:1 to 4:1 females : males. The causes of RA remain unknown to date. What is generally thought is that a genetically susceptible host encounters an unknown etiological agent, which results in an immunopathogenic response culminating in the development of arthritis. Both genetic and environmental causes may play a role. RA is associated with HLA-DR4 in most populations studied. Analysis of the major histocompatibility complex has revealed shared epitopes in the beta chains of DR that predispose to RA development. Environmentally implicated etiological factors include infectious diseases due to *Mycobacteria* species, and viruses. Other potential etiological agents include reactions of the body to "self" constituents, such as collagen. In recent years a cartilage glycoprotein termed gp39 has been implicated as an autoantigen in RA. Though gp39 appears very specific for RA, only a minority of patients have detectable antibodies to gp39. Another potential causative factor is glucose-6-phosphoisomerase. This antigen was first identified in a spontaneous arthritis model which develops in mice bearing the T cell receptor recognizing bovine pancreas ribonuclease, which are cross bred with non-obese diabetic mice. The disease results from antibodies to glucose-6-phosphoisomerase antibodies to this antigen, which are themselves capable of inducing arthritis in recipient mice. However, once again, only a small percentage of patients with RA have antibodies to glucose-6-phosphoisomerase. Antibodies may also arise to nonarticular structures. Rheumatoid factor was one of the initial factors leading to the concept of autoimmunity as causative in RA. Rheumatoid factor, which is present in approximately 75-80% of patients with RA, is an antibody directed against an antibody. High rheumatoid factor titers are associated with the presence of extraarticular features and more severe disease. A recent example of antibodies arising to nonarticular structures are antibodies to citrullinated peptides. These antibodies recognize a host of proteins such as filaggrin and keratin. Antibodies to citrullinated peptides (anti-CCP) appear to be new markers useful in the detection of RA, with a specificity greater than that of rheumatoid factor. One of the strongest arguments for antibodies to CCP being implicated in the pathogenesis of RA, is that antibodies are often detected in the serum of RA patients many years prior to the onset of RA. Another etiological factor that has gained attention, particularly in studies performed on European populations, is smoking. Some studies have suggested that smoking can predict the development of RA and influence disease severity. Many of these factors likely act in concert to initiate the development of RA.

The main site of pathogenesis of RA is in the synovium, which contains a variety of cells that participate in immune reactions, including T lymphocytes, B lymphocytes, macrophages, endothelial cells, and synovial tissue fibroblasts. Numerous cytokines and inflammatory mediators are produced by these cells, contributing to disease. Some of these include interleukin-1, tumor necrosis factor-alpha, and chemokines. Current clinical therapies target some of these mediators. There are a number of future drug targets being considered for RA, including: anti-interleukin-6, anti-interleukin-18, upregulation of "anti-inflammatory" cytokines such as interleukin-4, inhibitors of lymphcytes, adhesion molecule blockers, blockers of osteoclast activation, and angiogenesis inhibitors. Tumor necrosis factor-alpha blockers have made a large impact on disease progression already, and other targeted therapies are on the horizon, offering many therapeutic options for patients with RA.

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Mechanisms of Deep Tissue Pain in the Orofacial Region: Role of Glial-Cytokine-Neuronal Interactions in the Trigeminal Transition Zone

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Previous studies have established the role of the medullary dorsal horn or subnucleus caudalis of the spinal trigeminal complex, a homolog of the dorsal horn of the spinal cord, in trigeminal pain processing. Less attention has been given to other components of trigeminal nociceptive pathways beyond the medullary dorsal horn. Advances in our understanding of trigeminal nociceptive processes have occurred in recent years. In addition to the medullary dorsal horn, studies have pointed out increased excitability and sensitization of the subnuclei interpolaris/caudalis transition zone (Vi/Vc) of the spinal trigeminal nucleus and its role in trigeminal pain processing. Sensitization of Vi/Vc neurons occurs after orofacial inflammation, which involves a cascade of cellular events including activation of neurotransmitter receptors and glial-cytokine-neuronal interactions. In response to orofacial inflammation, glia in the Vi/Vc transition zone are activated and inflammatory cytokines released, contributing to activity-dependent plasticity and hyperalgesia. It is noteworthy that the injury-induced glial activation depends on neuronal input and is coupled to N-methyl-D-aspartate receptor phosphorylation, suggesting reciprocal interactions between central glial cells and neurons in the development of orofacial hyperalgesia. Our findings further establish a role of Vi/Vc transition zone in trigeminal pain processing and provide mechanisms by which the non-neural elements contribute to activity-dependent neuronal plasticity and persistent pain. (Supported by NIDCR DE11964, DE15374, DE018573.)

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Human Brain Neurotransmitter Responses to Temporomandibular Pain and Psychophysical Correlates

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The central nervous system regulation of pain signaling is complex, involving a number of circuits and neurotransmitter systems. Much information has been gathered on the regulation of pain signaling in the nerves conducting the pain signal and at the level of the spinal cord. There is increasing recognition, however, that pain processing also involves higher order circuits (the so-called "pain matrix"). These include brain regions that overlap with those involved in cognitive functions and other forms of sensory processing. Interindividual variations in the function of these circuits are thought to confer vulnerability and resiliency to the development of persistent pain conditions and the complexity of the clinical syndromes.

We have studied the central regulation of temporomandibular pain using molecular neuroimaging techniques (positron emission tomography), targeting neurotransmitter systems traditionally involved in the regulation of pain (endogenous opioid system) and others, such as the dopaminergic, more recently implicated in responses to painful and generally salient stimuli.

In healthy subjects, we observe profound interindividual variations in the function of the endogenous opioid system that are related to the capacity to suppress and regulate sustained painful stimuli. These studies also show that this neurotransmitter responds to pain signal in numerous brain regions, with variations in regional responses being related to the individual characteristics of the pain experience.

We then studied factors that contributed to the variability in endogenous opioid system function. Sex differences were prominent, as were the effects of circulating gonadal steroids. Under low estradiol, low progesterone conditions females did not activate this system at similar pain levels as men did, resulting in hyperalgesic responses. The situation was reversed under high estradiol conditions, where women released endogenous opioids and suppressed the sustained pain experience to levels comparable or superior to those of men. These data suggest that states accompanied by anovulation in women or a reduction in estrogen effects (e.g. ongoing pain, or otherwise stressful states, anti-estrogen medications) may create a situation where pain is not suppressed effectively, promoting chronicity.

Additional influences on the function of this neurotransmitter system included genetic polymorphisms. Two recently identified, which were related to substantial differences in the capacity to suppress pain through these mechanisms, include those affecting the function of the enzyme catechol-o-methyl transferase and neuropeptide Y. Reductions in the function of the endogenous opioid system have been recently described in a sample of patients diagnosed with fibromyalgia syndrome (FM) and related to clinical pain.

These data highlight the multiple influences promoting a dysregulation of pain processing systems in the human brain and direct future research in the area.

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Animal Models of Depression and TMJ Pain Processing

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Aims: Temporomandibular joint/muscle disorders (TMJD) represent a family of persistent pain conditions involving the TMJ and muscles of mastication. The basis for chronic TMJD is uncertain; however, female gender and depression have been identified as risk factors. Animal models for depression have been available for many years, yet none have been applied to studies of TMJD. The goal of this study was to determine if induction of depression-like behavior in female and male rats influences the initial stage of TMJ nociceptive processing, at the level of the spinomedullary (Vc/C_{1-2}) dorsal horn.

Methods: Ovariectomized (OvX) rats were given high (HE, 10 μg/d) or low (LE, 1 μg/d) dose estradiol 3-benzoate 1 h prior to daily sessions of the forced swim test (FST, 10 min/d X 3 d), an animal model that predicts the efficacy of antidepressant drugs or sham swim (SS). Males received daily injections of saline and FST or SS. Immobility time (IT) and forelimb grip force (GF, muscle pain) were measured daily. On day 4, rats were anesthetized with isoflurane and TMJ units recorded in laminae I-II at the Vc/C_{1-2} junction. Unit activity was evoked by injection of ATP (0.001-1mM) into the joint space.

Results: IT increased and GF decreased after FST conditioning in all groups consistent with depression-like behavior. FST enhanced the ATP-evoked unit activity in HE and male, but not LE, rats relative to SS. Compared to SS, FST enhanced the magnitude of evoked responses to low and high dose ATP in HE rats, enhanced the response to high dose ATP in males, and no effect in LE rats. FST did not affect the threshold dose of ATP across groups. FST did not affect spontaneous activity or cutaneous RF areas of TMJ units.

Conclusions: These results indicated that the encoding properties of TMJ units in laminae I-II at the Vc/C_{1-2} junction are modified by estrogen status and the induction of depression -like behavior and support the hypothesis that this region is a critical target for sex hormone and stress-related modulation of neural circuits relevant for TMJ pain processing in females. This model may provide a means to examine TMJ nociceptive processing without overt inflammation of the joint, a common feature in chronic TMJD.

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A Systems Approach to Understanding TMJDs
Workshop Summary Report

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Based on a previous TMJ Association meeting and discussions of the NIDCR National Advisory Council, "A Systems Approach to Understanding TMJDs" Workshop was held in Bethesda, MD. The participants were assembled as a Working Group of the NIDCR Advisory Council, September 16-18, 2007. Most of the speakers were expert in fields other than TMJDs and had experience in systems approaches to studies of biology and disease. These thought-leaders were pleased to be asked to explore a new approach to a set of prevalent disorders that afflicts many patients and frustrates the healthcare community for lack of evidenced-based methods with which to treat them.

A systems approach to research and design is regularly used in engineering but is relatively new to biology. The approach is usually one of a study of a process to determine a desired outcome and the most effective way of obtaining this outcome. In undertaking this approach one does not have an understanding of all (or perhaps any) of the intermediate steps. Systems approaches and analyses are powerful methods to complement the reductionist research that predominates in biological research.

There was a range of opinions on the timing of introducing systems approach initiatives in TMJD research. The workshop participants did develop the following possible research opportunities for consideration by the NIDCR Advisory Council:

1) Support multidisciplinary research on the causes, pathophysiology, prevention, diagnosis and treatment of TMJDs.
    a) Discovery and hypothesis-driven research by co-principal investigators
    b) Resource human and animal databases available to applicants
    c) Annual research planning and investigator meetings
    d) Data network formatted for availability to everyone

2) Establish data-mining studies of clinical, patient advocacy and basic science databases (e.g., OPPERA, other chronic diseases, and TMJ Association Databases).
    a) Genetic, environmental, therapeutic and management differences could be analyzed using bioinformatic systems models for specific outcomes, new associations and variances.
    b) This could produce new hypotheses and new approaches for investigator-initiated research grant applications.

3) Implement TMJD training programs.
    a) Summer program, nationally advertised, for introducing trainees to TMJDs
    b) Post-doc fellowships, research residencies, faculty exposure/retraining (basic and clinical)
    c) Mentoring and development of new faculty (K awards)

Disclosure of Relevant Financial Relationships: None

Poster Abstracts

Poster A

Catechol *O*-Methyltransferase (COMT) Met/Met Genotype Influences Cortisol Response and Pain Symptoms after Minor Motor Vehicle Collision (MVC)

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Catechol *O*-Methyltransferase (COMT) is an enzyme which degrades catecholamines, including epinephrine, norepinephrine, and dopamine. A common functional polymorphism of the *COMT* gene (*Val*^{158}*Met)* decreases COMT thermostability, resulting in a 3-4 fold reduction in COMT enzyme activity. The allelic effects are codominant, so that individuals with the *met/met* genotype have the least COMT enzyme activity. In animal models, it has previously been shown that heightened catecholamine levels increase pain sensitivity. In addition, it was recently reported that individuals with the *COMT met/met* genotype exhibit a lower cortisol response to the Trier Social Stress Test. We assessed the association between *met/met* genotype and cortisol response and pain symptoms after minor MVC. 47 adult Caucasians presenting to the emergency department (ED) for care after minor MVC were recruited. ED evaluation included serial ED cortisol assessment, blood collection for subsequent DNA analysis, and an assessment of neck pain symptom severity (0-10 NRS). Dissociative symptoms were also assessed (Michigan Critical Events Perception Scale). Genotyping was performed using the Sequenom Platform. Associations between *COMT met/met* genotype and cortisol response and pain symptoms were assessed via repeated measures analysis and t-test, respectively. Individuals with COMT *met/met* genotype had a reduced ED cortisol response (AUC .106 vs. 467, p = .05) and increased pain symptoms in the ED (6.9 vs. 3.0, p = .002) vs. other genotypes. Individuals with the *met/met* genotype also had more dissociative symptoms (16.1 vs. 11.4, p .002). In contrast, injury Severity Score, highest Abbreviated Injury Severity score, airbag deployment, and police officer rating of crash severity at the scene were poor predictors of pain outcomes. These data provide the first report of a link between genes influencing the stress response and the physiologic and symptom response to a traumatic event.

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Disclosure of Relevant Financial Relationships: None

Poster B

Effect of Intranasal Delivery of Carbon Dioxide on Trigeminal Ganglion Neurons: Inhibition of Neuron-Glia Gap Junction Communication and SNAP-25 Expression

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Objective: The goal of this study was to determine whether administration of 100% CO_2 to the nasal mucosa inhibits trigeminal ganglion neuron-glia signaling and neuronal cell secretion.

Background: Activation of trigeminal nerves is implicated in the pathology of migraine and allergic rhinitis. Recent clinical evidence supports the use of noninhaled intranasal delivery of 100% CO_2 for treatment of migraine and allergic rhinitis with multiple randomized placebo-controlled studies showing evidence of efficacy in both indications. The mechanism of action is not well understood but is likely to involve repression of trigeminal nerve activation, neuronal-glial cell signaling, and paracrine signaling within the ganglion. We have previously shown that CO_2 suppresses stimulated CGRP secretion from cultured rat trigeminal neurons.

Methods: Sprague Dawley rats were injected in the whisker pad or the eyebrow with capsaicin and the effect on gap junction activity determined by dye coupling and the levels of S100B, CGRP, and SNAP-25 determined by immunohistochemistry. To test the effect of CO_2 administration, animals were left untreated (control) or treated with 100% CO_2 at a flow rate of 10 ml/sec for 40 sec immediately before stimulation with capsaicin. Trigeminal ganglia were removed 2 hrs following capsaicin injection and data acquired using fluorescent microscopy.

Results: Increased signaling via gap junctions between trigeminal neurons and satellite glial cells was seen 2 hrs after capsaicin injection into the whisker pad or eyebrow. This gap junction communication was greatly reduced in animals treated with CO_2 prior to capsaicin stimulation. CO_2 treatment also blocked the stimulatory effect of capsaicin on S100B and CGRP expression. In addition, CO_2 caused a decrease in levels of SNAP-25, a protein involved in controlling CGRP release from trigeminal nerves.

Conclusions: Results from this study provide the first evidence of a unique regulatory mechanism by which CO_2 inhibits sensory nerve activation, neuron-glial cell signaling, and subsequent neuropeptide release.

Key words: trigeminal, migraine, carbon dioxide, CGRP, gap junction, glia

Study supported by: Capnia, Inc.

Disclosure of Relevant Financial Relationships: None

Poster C

Contribution of Primary Afferent Input to Trigeminal Glial Activation, Cytokine Induction and NMDA Receptor Phosphorylation

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Introduction: Studies implicate a role for glia/cytokines in persistent pain. However, the mechanisms by which these non-neural elements are activated and contribute to CNS activity-dependent plasticity and pain are unclear. Here we tested the hypothesis that primary afferent input plays a role in trigeminal glial activation after tissue injury in rats.

Methods: Complete Freund's adjuvant (CFA) was injected into the masseter muscle in rats. This procedure upregulates glial fibrillary acidic protein (GFAP), a marker of astrocytes, interleukin-1beta (IL-1beta), an inflammatory cytokine, and phosphorylation of the NR1 subunit (P-NR1) of the NMDA receptor and induces inflammatory hyperalgesia. To produce local anesthetic block, lidocaine was infiltrated into tissues surrounding the masseter nerve. The electrical stimulation (ES) consisted of trains of 4 square pulses (100 Hz, 3 mA, 0.5 ms pulse width). Western blot and immunohistochemistry were performed on brainstem tissues.

Results: The CFA-induced increase in GFAP, IL-1beta and P-NR1 was significantly reduced ($p<0.05$) in rats receiving local anesthesia. Compared to the SHAM rats, there was an increased immunoreactivity against GFAP, IL-1beta and P-NR1 in the subnuclei interpolaris/caudalis trigeminal transition zone in rats receiving ES of the masseter nerve, which were also attenuated by peripheral local anesthesia. The astrocytic gap junction protein Cx43 was also increased after ES. Double staining showed that IL-1beta was selectively localized in GFAP-positive cells and P-NR1-immunoreactivity was localized to neurons.

Conclusion: These findings indicate that primary afferent inputs associated with peripheral nociceptor activation are necessary and sufficient for the activation of astroglia and upregulation of IL-1beta, as well as neuronal NMDA receptor activation.

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Disclosure of Relevant Financial Relationships: None

Poster D

Sex-Differences in Peripheral Delta Opioid Receptor (Dor) Function and Involvement of G Protein-Coupled Inward Rectifying Potassium Channels (Girk) in Dor-Mediated Attenuation of Masseter Hypersensitivity

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Introduction: While the evidence supporting the role of peripheral opioid receptors (PORs) in mediating analgesic and anti-hyperalgesic effects has been accumulating, potential sex-differences and mechanisms in POR effects are not clearly understood. Pro-nociceptive stimuli such as capsaicin increase peripheral analgesia by increasing cell surface expression of DORs in primary afferent neurons (PANs). Spinal opioid receptors (ORs) produce analgesia by activating GIRK, however little is known about GIRK involvement in OR-mediated analgesia in PANs. Here we investigated whether there are sex-differences in cell surface expression of DORs in trigeminal ganglia (TG) that correlate with DOR efficacy and whether the DOR-mediated analgesia involves GIRK in a craniofacial muscle pain model.

Method: Sex-differences in the effect of a DOR agonist on capsaicin-induced masseter hypersensitivity were assessed. Western blot technique was employed to examine changes in cell surface expression of DORs in TG under the same condition. We then examined whether GIRK1-4 subunits expressed in the CNS are expressed in TG by RT-PCR and Western blot techniques, and whether local application of a GIRK blocker attenuates the anti-hyperalgesic effects of peripheral DORs. GIRK and OR co-expression on masseter afferents was demonstrated with immunohistochemistry.

Results and Conclusion: Local application of DPDPE significantly attenuated capsaicin-induced masseter hypersensitivity in male, but not in female, rats. Capsaicin-induced increase in cell surface expression of DORs in TG was seen only in male rats. GIRK1-4 mRNAs and protein products of GIRK1 and 2 were reliably detected in TG. Tertiapin-Q, a high affinity inhibitor for GIRK, blocked the analgesic effect of DPDPE, suggesting that DOR effect is potentially mediated via GIRK. These data reveal a novel mechanism for sex-differences in DOR-mediated analgesia and provide first evidence for GIRK expression in masseter afferents and their involvement in POR-mediated analgesic and anti-hyperalgesic effects under the acute inflammatory muscle pain condition.

Disclosure of Relevant Financial Relationships: None

Poster E

Prevalence of Comorbid Conditions in Individuals with TMJD Compared to Matched Controls

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Introduction: This study was undertaken to compare the prevalence of comorbidities among individuals with TMJD and matched controls.

Methods: Initially, 3,500 TMJD registry participants were invited to complete a web-based survey of comorbid conditions and symptoms. For comparison purposes, respondents were encouraged to invite a friend of the same sex and similar age, but unaffected by TMJDs, to respond to similar questions. Friends were used as controls in order to decrease the likelihood of major differences in confounding variables between the groups. A total of 1,540 of the TMJD registry participants who were contacted responded by completing the questionnaire and 57 friends of these individuals also completed questionnaires. The current report is limited to comparisons of cases and controls. The 57 controls were matched by age, education and sex to 4 TMJD cases. Conditional logistic regression procedures were used to compare group differences in the prevalence of selected self-reported conditions.

Results: Overall, the TMJD registry respondents were predominantly female (90%), white (97%), college graduates (79%), 41 years old on average and had first experienced TMJD symptoms 16 years earlier. Demographic variables were similar between the cases and controls. Group differences in selected comorbid conditions are summarized below:

Co-morbidity	Odds Ratios	95% CI	P-value
Chronic fatigue	7.0	2.4-19.9	0.0005
Migraine headache	3.8	2.1-10.3	0.0005
Tension headache	4.7	2.5-9.4	0.0005
Depression	2.7	1.4-5.4	0.004
Degenerative arthritis	4.6	1.8-11.5	0.001
Fibromyalgia	5.1	1.2-21.9	0.03
Auto immune problems	3.7	1.1-12.5	0.03
Allergies	1.2	1.1-3.5	0.03
Popping Joints	20.8	9.0-48.5	0.0005
Chronic pain	4.6	2.5-8.5	0.0005

Conclusions: This study found associations between TMJD and many comorbid conditions (some not shown). Study limitations include the small sample size, the use of prevalence data, and potential biases due to self-selection and self-report. Nonetheless, the findings suggest that prospective studies of comorbidities among incident TMJD cases are warranted.

Disclosure of Relevant Financial Relationships: None

Poster F

Modulation of Tactile Responsiveness in Somatosensory Cortex by Noxious Heat: Implications for Female TMD Patients

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Introduction: One putative explanation for pain associated with temporomandibular disorders (TMD) is dysfunction of sensory-regulating mechanisms in the CNS. Understanding the mechanisms through which pain modulates the processing of innocuous tactile information may provide insight into the complexity of chronic pain conditions. Using functional Magnetic Resonance Imaging (fMRI), we investigated the cortical response evoked by innocuous and noxious cutaneous stimuli in females with and without TMD.

Methods: Female TMD patients and age-matched controls experienced three types of stimuli on the right hand: 1) innocuous tactile (26 Hz vibration), 2) noxious heat (49° C, producing moderate pain), and 3) concurrent vibration and heat. Stimulus events occurred every 32-62s and were 4-10s in duration. Subjects rated the average intensity of the tactile stimulus in the presence and absence of noxious heat.

Results: In the presence of heat producing pain, ratings of tactile intensity for controls decreased 14.4%, on average (stdev=6.97%); however, only 6 of 12 TMD subjects rated the tactile stimulation less intensely in the presence of heat pain. In control brain images, noxious heat not only inhibited the response of some cortical areas to tactile stimulation, it caused other areas to respond to tactile stimulation that would not otherwise. TMD subjects differed from controls in the areas activated by tactile stimulation in the absence of noxious heat, suggesting possible activation of sensory-regulating mechanisms by the subjects' on-going clinical pain.

Conclusion: Pain differentially modulates the tactile responsiveness of subregions of somatosensory cortex and the pattern of modulation is different in TMD. These findings should lead to an improved theoretical understanding of patients' pain complaints and to novel means for assessing the efficacy of TMD therapeutic interventions.

Disclosure of Relevant Financial Relationships: None

Poster G

Prevalence of Orofacial Pain among Women with Vulvodynia: Prospective Two-Year Follow-Up Study

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Introduction: We recently demonstrated that orofacial pain (OFP) is a common co-morbidity among patients with vulvodynia and that psychological characteristics of subgroups of women with OFP significantly differed from those without. The primary objective of this study was to assess the stability of our original findings while investigating the change in OFP symptoms over a 2-year period. Our secondary objective was to investigate the common subtype(s) of OFP among women with vulvodynia.

Methods: Seventy-six women from the original cohort of 137 (55%) returned the completed questionnaires assessing dyspareunia (Gracely pain scale), anxiety (State/Trait Anxiety Inventory), and somatization (Pennebaker Inventory of Limbic Languidness). Diagnosis of OFP was rendered based on responses to a standardized questionnaire; participants were categorized into the following three categories: clinical, sub-clinical, and no OFP. Seven women were clinically examined to confirm the questionnaire-based diagnosis of OFP and identify its subtype.

Results: Participants were grouped within OFP categories: subclinical (n=24, 32%), clinical (n=25, 33 %), and no OFP (n=27, 36%). Baseline demographics (e.g. age, race, education) did not differ. Consistent with our initial report, women with OFP had higher degrees of anxiety (p=0.022) and somatization (PILL, p=<0.001) despite similar intensities of dyspareunia. Prevalence of OFP symptoms differed between baseline and 2-yr follow up (chi2= 0.001). Patients were more likely to have changed OFP categories if they had initially belonged to the subclinical OFP group. Less variability in diagnosis status was observed among the OFP (73%) and OFP-free (53%) groups. Of those who were clinically examined (n=7), 5 had temporomandibular disorder (TMD) and 2 were OFP-free.

Conclusion: OFP is a common complaint among women with vulvodynia with TMD being the most common form. Further investigation into the nature of overlap between the two conditions may advance our understanding of the underlying biological processes between these two intuitively disparate conditions.

Disclosure of Relevant Financial Relationships: None

Poster H

Brainstem Neuronal Activation Differs Following Contraction-induced Versus Adjuvant-induced Muscle Inflammation

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Introduction: Muscle pain is a substantial clinical problem whose pathophysiology is poorly understood. Current models of muscle pain are invasive and do not simulate muscle pain pathologies. We compared c-fos expression following muscle inflammation produced by eccentric muscle contraction (EC), a physiologically relevant stimulus, with Complete Freund's adjuvant (CFA).

Method: The masseter muscle was inflammed unilaterally in adult male rats (n=16) either by EC or injection of CFA. After 24 hrs, the skin overlying the masseter was anesthetized and the masseter was probed (2.9N) to activate muscle mechanoreceptors. One hour after probing, animals were sacrificed and processed for Fos immunocytochemistry. The distribution of Fos-positive neurons was analyzed using a non-biased stereological method (optical dissector).

Results: In the trigeminal subnucleus caudalis (Vc) the number of Fos-positive neurons following both EC and CFA was substantially (up to 3X) elevated versus control. In both EC and CFA animals, ipsi- and contralateral dorsal Vc contained approximately 3 times more Fos-positive neurons than control. In contrast to this, three times more Fos-positive neurons were found in the ventral ipsi- and contralateral Vc of EC versus CFA animals. In the principal sensory nucleus (Vp) the number of Fos-positive neurons was greater for EC than either CFA or control. Fos-positive neurons were particularly abundant in the parabrachial region and dorsomedial Vp. In the cervical spinal cord (n=5), significantly more Fos-positive neurons were present following EC than in naive animals or when the masseter was passively stretched.

Conclusion: These results indicate that EC and CFA activate neurons above control levels. Muscle contraction, a physiologically relevant stimulus, evokes a different distribution of Fos-positive neurons than CFA suggests that EC activates different neuronal circuits and may provide unique insight into muscle pain.

Disclosure of Relevant Financial Relationships: None

Poster I

Characterization of a Glial Component in a Novel Model of Neuropathic Facial Pain

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Neuropathic orofacial pain is a complex syndrome often resistant to conventional analgesics. We present a new, straightforward model for the study of chronic neuropathic facial pain and a preliminary characterization of pain from this model.

Methods: A skin incision was made to expose the maxillary branch of the trigeminal nerve. Exposure of this trigeminal branch at this point in its course was far more straightforward than other alternatives in the literature. This branch was constricted using 3 chromic gut ligatures loosely tied around the nerve. Tactile allodynia was measured 3, 7, 10, 14, 21, 28, 35 and 42 days post-surgery. IL-1 receptor antagonist (IL-1ra) and other inhibitors/antagonists of inflammatory mediators were administered intracisternally to characterize the role of immune activation in pain behaviors. Immunohistochemistry will be used to define the anatomical distribution and timecourse of microglial and/or astrocytic activation with this model.

Results: Significant allodynia was present from 7-35 days post-constriction injury. Central (intracisternal) IL-1ra reversed established allodynia, implicating proinflammatory cytokines in this pain enhancement. Pain behavior assessment following blockade of other inflammatory mediators is ongoing, as are immunohistochemical analyses.

Conclusion: Our trigeminal neuropathic pain model produces significant allodynia that is modulated by inflammatory mediators. This model will allow us to better investigate treatments for chronic neuropathic orofacial pain and the interplay between neuropathic orofacial pain and other facial pain disorders.

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Poster J

Microinjection of IL-1beta into the Trigeminal Transition Zone Produces NMDA Receptor-Dependent Orofacial Hyperalgesia in Rats

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Introduction: We have previously shown that IL-1beta, a prototypic inflammatory cytokine, is upregulated in the subnuclei interpolaris/caudalis (Vi/Vc) transition zone of the spinal trigeminal complex, an important structure in processing orofacial deep input, after masseter muscle inflammation. Further, IL-1beta affects neuronal function through signal coupling with N-methyl-D-aspartate (NMDA) receptors. Here we investigated the effect of microinjection of IL-1beta into the Vi/Vc on orofacial hyperalgesia in rats.

Methods: A guide cannula was chronically implanted into the Vi/Vc of the Sprague-Dawley rat under pentobarbital anesthesia and mechanical sensitivity of the orofacial site was assessed with von Frey microfilaments. The EF_{50} values, defined as the von Frey filament force (g) that produces a 50% response frequency, were used as a measure of mechanical sensitivity.

Results: Intra-Vi/Vc IL-1beta dose-dependently produced mechanical hyperalgesia/allodynia, which could be detected at bilateral facial sites. The hyperalgesia started from 30 min (ipsilateral) and 60 min (contralateral) after injection and lasted for about 6 h ($p<0.01$). Intra-Vi/Vc pretreatment with IL-1receptor antagonist (IL-1ra) significantly attenuated the IL-1beta-induced hyperalgesia ($p<0.01$), confirming that the effect was mediated through the IL-1 receptor. Pre-injection of AP-5 and MK-801, two NMDA receptor antagonists, significantly attenuated IL-1beta-induced hyperalgesia ($p<0.05$). However, intra-Vi/Vc pretreatment with fluorocitrate, a gliotoxin, and minocycline, a microglial inhibitor, did not attenuate IL-1beta-induced hyperalgesia, suggesting that the IL-1beta-produced effect was downstream to glial activation.

Conclusion: These results provide behavioral evidence that support a role of inflammatory cytokine IL-1beta and its coupling to NMDA receptors in the central mechanisms of orofacial hyperalgesia.

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Poster K

Propranolol in TMD Treatment: Preliminary Findings

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Introduction: Temporomandibular disorders (TMD), a heterogeneous family of musculoskeletal disorders, represent the most common chronic orofacial pain condition. Considerable evidence now suggests that the painful myalgia seen in TMD patients may result from impairment in central pain processing pathways which are modulated by adrenergic systems. The aim of the research is to test whether low dose propranolol, a non-selective beta-adrenergic receptor blocker, can reduce pain and restore function in TMD patients.

Methods: 40 healthy control female subjects and 40 female TMD patients are enrolled in a 4 week trial. TMD patients complete a double-blind, placebo-controlled, two period case-crossover study of propranolol treatment (40 mg/day) or placebo. All subjects are examined using the Research Diagnostic Criteria (RDC) for TMD. Participants are genotyped and phenotyped using quantitative sensory testing procedures to assess pressure and heat pain sensitivity. Participants also complete a daily symptom calendar and a series of psychological and pain-related questionnaires.

Results: Treatment data are currently available for 26 TMD patients and suggest that propranolol therapy increases mechanical and thermal pain threshold and reduces clinical pain. The effect of propranolol appears to be associated with polymorphisms in genes related to the metabolism of catecholamines – catechol-O-methyltransferase *(COMT)* and beta-2 adrenergic receptor *(ADRB2)*.

Conclusion: Supporting earlier studies, results from the present research show that TMD patients are hypersensitive to noxious stimuli. Preliminary results suggest that propranolol may diminish mechanical and thermal sensitivity and pain symptoms associated with TMD. Moreover, these findings suggest that patients with *COMT* and *ADRB2* genetic haplotypes that contribute to a heightened adrenergic response will benefit most from propranolol treatment.

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Poster L

Chewing Performance of TMD vs. Controls: A New Experimental Model

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Objectives: Limitations in masticatory function are a common complaint by patients with TMD. However, published data, relying mostly on a classic sieve method for assessing particle size, suggest equivalent chewing performance by TMD patients and healthy controls. This study focused on developing a new measurement approach using optical scanning and then testing its utility.

Methods: In Phase I, standard objects of various sizes, shapes, and number were used to develop a valid scanning protocol. For Phases II & III, each sample was based on 2 grams of Optosil® chewed at the rate of one cycle/second for forty seconds, expectorated, rinsed, dried, spread on a flat bed scanner, and scanned. In Phase II, methods reliability was assessed using the ICC statistic for the parameters count, median, upper and lower quartile, and minimum and maximum values. In Phase III, two trials of chewed samples were obtained from 20 asymptomatic controls and 25 TMD patients in order to assess subject reliability and overall utility. Standard scanner software, Image J, and Stata were used for analysis.

Results: Phase I resulted in a valid protocol for count and size estimation of known samples. In Phase II, scanning method reliability for 5 of 6 parameters exceeded ICC of 0.95. In Phase III, the best subject reliability for the two trials was 0.6 among the 6 parameters. Using the statistics from trial 2, the comparison between subject groups was assessed with individual t-tests, and the TMD group exhibited worse performance ($p<0.05$) for 5 of the 6 measurement parameters.

Conclusions: The optical measurement method is valid and reliable for 5 of 6 parameters with respect to chewed Optosil particles. These results demonstrate that individuals with TMD chew less well compared to controls.

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Poster M

Masticatory Function and Pain: Experimental Reliability and Utility

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Objective: A common complaint by individuals with TMD is that masticatory function is impaired due to pain. The pain-adaptation model by Lund, based on acute experimental pain, suggests that function is necessarily limited by pain due to inhibitory influences on motoneurons; pain and its impact on function may differ, however, in individuals with clinical pain. We present preliminary data from a new laboratory model testing chewing efficiency using a range of foods; our goal was to assess reliability of the model, the role of pain in affecting function, and functional parameters based on electromyographic (EMG) activity.

Methods: Individuals with RDC/TMD pain diagnoses (16 community cases, 8 clinic cases) as well as 20 asymptomatic individuals were recruited. Subjects chewed 6 foods and 1 non-food ranging in texture, while EMG was measured from 5 muscles; present pain was reported as well. Each food was repeated twice.

Results: In terms of trial reliability, EMG activity exhibited ICC ratings above 0.9, time to swallow ranged from 0.69-0.81, and number of chewing cycles varied between 0.83-0.92. The EMG magnitudes did not differ among the 3 subject groups for any of the foods (ANOVA, $p>0.05$). In contrast, pain increased in the TMD groups as the texture of the food increased but did not differ between community and clinic cases. There was a trend of fewer chewing cycles in the clinic cases with increased food texture and a shorter time to swallow, compared to the control group.

Conclusions: This experimental chewing model produces reliable data when measuring EMG activity, chewing parameters, and pain across groups, tasks and trials. In contrast to the pain adaptation model, individuals with TMD appear to function at the same level as healthy controls with respect to muscle contraction during function despite increasing pain as the task demand increases.

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