THE FOURTH SCIENTIFIC MEETING OF THE TMJ ASSOCIATION

A Systems Approach to the Understanding of TMJ as a Complex Disease

Meeting Abstracts

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… Moving TMJ Research into the 21st Century

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Factors Characterizing TMJ Diseases and Disorders: A Survey of Affected Individuals

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What is collectively referred to as temporomandibular joint disorders (TMJ) represents a poorly understood, but often profoundly incapacitating, complex array of symptoms. In an effort to determine the prevalence and distributions of TMJ symptoms, as well as to identify environmental and iatrogenic associations with TMJ, we have conducted a survey of individuals on The TMJ Association Registry. The research project was approved by the Medical College of Wisconsin Institutional Review Board.

Registrants of The TMJ Association Registry were sent an invitation via email to participate in the survey. Respondents were asked to invite a friend to serve as a control subject by completing a similar, but abbreviated, questionnaire. The email directed the recipient to the site where the web-based informed consent document and the questionnaire could be accessed. Ten thousand initial contacts were made with individuals who are registered with The TMJ Association. One thousand five hundred twenty individuals (15.2%) responded to the invitation to participate. Over one hundred individuals who did not respond to the invitation to participate were queried about reasons for not responding. Reasons for non-response included lack of time to complete the survey or because the individual had not been diagnosed with TMJ. A follow-up survey was conducted to determine differences in key variables between respondents and non-respondents.

Key variables in the survey included: demographic information (age, sex, marital status), reproductive history, family history of TMJ disorder, age at which TMJ symptoms first occurred and age of TMJ diagnosis, respondent's concepts about causes of TMJ symptoms, procedures related to TMJ, and selected medications (anti-anxiety, anti-depressant, non-steroidal anti-inflammatory agents, both non-narcotic and narcotic pain relievers, and skeletal muscle relaxants).

The presentation will present findings from the survey. The purpose of the presentation is to identify key issues and ideas related to predisposing factors for TMJ and to describe the distribution of TMJ symptoms.

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Interdisciplinary Approaches for the Study of TMJ Patients: Co-morbid Conditions That Can Arise from Chronic Pain

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Patients with severe and persistent TMJ pain often report sensory, motor, autonomic and/or affective symptoms that are poorly captured by present diagnostic classifications and inadequately addressed by clinical research and preclinical model systems. Both clinical description and the scientific investigation of these complex clinical presentations, often falling outside the topographical domain of the presumed primary focus, are hampered by the need for competent interdisciplinary investigative teams.

Reported symptom aggregates and observable signs are inconsistent from patient to patient, and may vary over time within a given subject. Complications from multiple unsuccessful treatments may further confound the clinical scenario, negatively affecting any systematic clinical research. It is unfortunate that those TMJ patients in most serious need for help are faced with little or no scientific evidence in support care decisions. It is only through systematic research of the pathogenesis of these symptom aggregates, including understanding of the individual response patterns, that management of the subset of TMJ patients in the most desperate situations can expect scientifically justifiable care. Without the use of suitable model systems, validated against clinical material, advancements in this area are highly questionable.

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The C1-C2 Spinal Cord: Nociceptive Processing of Input from the Vagus and the Neck and Jaw

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Temporomandibular joint diseases and disorders are difficult to diagnose, because facial pain can be a symptom that represents many conditions. Most of the sources of the pain are attributed to diseases originating in the head. The purpose of this presentation is to expand the symptoms of facial pain to include angina pectoris, which often results from ischemic heart disease. Angina pectoris is commonly referred to the chest and left arm, and sometimes to the right arm; however, occasionally, the pain radiates to the neck and jaw. Historically, angina pectoris was treated by performing surgical sympathectomies, which relieved the pain in approximately 70% of the patients. However, in many of the unresponsive patients, pain was experienced in the neck and jaw. The clinicians proposed that the vagus nerve was the pathway for producing the pain. These observations led to the hypothesis that spinothalamic tract (STT) cells of C1-C3 segments received convergent input from the heart via the vagus and from somatic structures located in the neck and jaw. The STT was studied because it transmits visceral and somatic nociceptive information from neurons in the gray matter of the spinal cord that ascend to the ventral posterior lateral and medial nuclei of the thalamus. This information is then relayed to the somatosensory area in the postcentral gyrus of the cortex to discriminate the location of the painful stimuli and to areas of the frontal and limbic lobes of the cortex and the insula that contribute to motivational affective behavior and autonomic adjustments associated with painful stimuli. To perform this study, extracellular action potentials of C1-C2 spinothalamic tract cells in anesthetized primates were examined for their responses to algesic chemicals that stimulated the nociceptive afferents of the heart and to mechanical manipulation of somatic structures. Of the neurons responding to noxious cardiac stimulation, 80% of them were excited and these same neurons received input from noxious mechanical stimulation of the somatic receptive fields. Most of the somatic receptive fields included the mandibular region of the jaw, the neck and/ or head regions. Approximately 70% of the STT cells responded to innocuous and noxious somatic stimulation and 30% responded only to noxious stimulation. None of these neurons responded only to innocuous stimulation. Nerve ablations and spinal cord transections were used to determine the pathways by which cardiac afferent information was transmitted to the C1-C2 STT cells. These interventions revealed that the noxious cardiac input was transmitted primarily in vagal afferent fibers directly and indirectly to the C1-C2 STT neurons. These results confirmed the suggestion of clinicians who performed surgical sympathectomies for the relief of angina pectoris that the vagal afferent fibers transmitted the noxious cardiac information to the C1-C2 STT neurons. Recently, we have also shown that noxious esophageal stimulation can also activate C1-C2 spinal neurons. Based on these studies, some patients with asymptomatic jaw pain may be experiencing bouts of myocardial ischemia or esophageal disorders such as gastroesophageal reflux.

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Uncovering the Local Biochemical Milieu of Myofascial Trigger Points: Implications in TMJ-Related Pain

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Myofascial trigger points (MTrPs) are a common cause of non-articular musculoskeletal pain. Myofascial pain arises from discrete hyperirritable palpable nodules (trigger points) in taut bands of muscle and can only be diagnosed by systematic palpation of the soft tissue by an experienced examiner. MTrPs in the masseter, lateral pterygoid, sternocleidomastoid (clavicular division), medial pterygoid and upper trapezius are common causes of pain in the region of the temporomandibular joint. Although data demonstrate a role for endogenous substances such as bradykinin, serotonin, prostaglandins and others in muscle pain, the pathogenesis of myofascial pain remains elusive.

To measure the local biochemical milieu, we developed a novel, 32-gauge microanalytic system capable of the in-vivo collection of small volumes (~ 0.5 μl) and sub-nanogram levels of solutes <75kDa from muscle tissue. This device improves on standard microdialysis probes by combining the size, safety and handling ability of an acupuncture needle with a custom-designed microdialysis probe fabricated to exacting specifications.

We assessed the local muscle biochemical milieu in 9 healthy subjects divided into 3 groups based on the following findings in the trapezius: Active (painful MTrP present; 3 subjects), Latent (non-painful MTrP present; 3 subjects) and Normal (no pain, no MTrP present; 3 subjects). Concentrations of bradykinin, calcitonin gene-related peptide (CGRP), substance P (SP), tumor necrosis factor-α , Interleukin -1β, serotonin, and norepinephrine were significantly higher in the Active group than either of the other two groups ($p<.01$). pH was significantly lower in the Active group than the other two groups ($p<.03$).

We demonstrated 1) that we can collect continuous, near real-time samples from soft tissue without harmful effects on subjects; 2) proof-of-principle of the system's ability to distinguish among subjects who have clinically distinct soft tissue findings; and 3) that subjects with neck pain secondary to a MTrP in the upper trapezius had significantly elevated levels of biochemicals (e.g., cytokines, neuropeptides, catecholamines, etc.) associated with pain and inflammation.

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Translating New Insights in Our Understanding of Chronic Pain from the Bench to the Bedside

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The present Director of NIH has initiated an interdisciplinary and translational road map for NIH research that focuses on collaborative research among scientists in many different disciplines. It emphasizes the need to move basic research from the bench to research approaches that rapidly *translate* into clinical research and improvement in the management of disease. The pain field is a model for multidisciplinary and interdisciplinary research. There is an interaction of neuroscience, specialties of medicine such as neurology, neurosurgery, anesthesiology, psychiatry and others, the fields of experimental and clinical psychology, psychophysics, dentistry, and nursing. This merging of ideas and approaches is seen in the organization of the major pain societies, the International Association for the Study of Pain and the American Pain Society, which are truly multidisciplinary organizations. Pain research is also a model of translational research which I would define as research that 1) takes laboratory findings and applies them to clinical problems to study underlying mechanisms of disease and illness; 2) develops opportunities for better diagnosis based on basic research findings; and 3) utilizes basic research findings to develop new technical approaches leading to better treatment. It is followed closely by the application of this knowledge in clinical trial studies. Translational research also provides the link for the reverse approach: the ability to examine clinical evidence and utilize that knowledge to study underlying mechanisms. In spite of major advances over four decades, basic research findings in pain have not been readily translated into new approaches to pain management which still rely mainly on drugs that have been available in one form or another since the early 20th century. The NIH Roadmap will provide increased funding opportunities for new interdisciplinary approaches to the study of disease and the translation of basic science findings into the clinical arena. How can we take advantage of these new funding opportunities? First, we need to realize that there will be no silver bullet for the treatment of persistent pain. Second, new advances in interdisciplinary research will be particularly important in view of new research in genetics and brain imaging. Large-scale research projects will require collaboration among multiple disciplines in the extramural community, the NIH and industry. The NIH roadmap hopefully can provide the merging of innovative ideas to achieve our goal of new approaches to pain management.

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Pain and the Complex Entity of Reflex Sympathetic Dystrophy

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Complex regional pain syndromes (CRPS) are clinically characterized by pain, abnormal regulation of blood flow and sweating, edema of skin and subcutaneous tissues, trophic changes of skin, appendages of skin and subcutaneous tissues, and active and passive movement disorders. Some patients have sympathetically maintained pain (SMP), indicating that there exists a coupling from sympathetic (noradrenergic) fibers to afferent neurons in the periphery. CRPS is classified into type I (previously reflex sympathetic dystrophy; developing after trauma without nerve lesion) and type II (previously causalgia; developing after trauma with nerve lesion).

(A) Based on multiple evidence from clinical observations, experimentation on humans, and experimentation on animals, the hypothesis will be put forward that CRPS is a disease involving the central nervous system as well as the peripheral nervous system. CRPS patients exhibit changes which occur in somatosensory systems processing noxious, tactile and thermal information, in sympathetic systems regulating various effector systems (blood vessels, sweat glands, possibly inflammatory cells etc.), and in the somatomotor system. These observations argue that the central representations of these systems are changed in CRPS patients (particularly type I).
(B) It is important to emphasize, *(1)* that CRPS I may develop after trivial trauma, trauma remote from the affected extremity or even visceral or central lesions; *(2)* that the clinical symptomatology of CRPS is frequently entirely out of proportion to the trauma; and *(3)* that a few temporary blocks of the sympathetic supply to the affected extremity of CRPS patients with SMP sometimes may lead to a long-lasting (even permanent) pain relief and to resolution of the other changes present in CRPS. Thus, looking at CRPS in this way shifts the attention away from interpreting this syndrome conceptually in a narrow manner and to reduce it to *one* system or to *one* peripheral or central mechanism only (e.g., to sympathetic-afferent coupling, to an adrenoceptor disease, to a peripheral inflammatory disease, to a psychogenic disease).
(C) The directions to be taken in basic and clinical research to unravel this puzzling syndrome should have the following priorities: *(1)* Basic research focusing on the brain in order to find out in which way the brain orchestrates the changes seen in the somatosensory, sympathetic and somatomotor systems. *(2)* Basic research focusing on the peripheral inflammatory and other peripheral processes (e.g., edema, trophic changes, sympathetic-afferent coupling) and on how these peripheral changes are linked to the central changes. *(3)* Basic research focusing on the question in which way pain is related to the development and maintenance of CRPS. *(4)* Studies to validate existing models and to develop new models of CRPS or its components.

Research on CRPS may serve as model for exploration of the pathophysiological mechanisms in clinically related fields, such as neural regulation of rheumatoid disease, fibromyalgia, irritable bowl syndrome, inflammatory bowel disease or TMJ disease.

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Evidence of Nociceptive-Neuroendocrine Control of Pain

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Clinical pain syndromes are generally classified as inflammatory, neuropathic or generalized. The latter category includes a large number of common pain syndromes (e.g., TMJ, FMS and IBS) whose mechanisms remain poorly understood. It has recently been appreciated that there is a large overlap between the Generalized Pain Syndromes (GPS), both with respect to co-occurrence in the same individual and the symptoms experienced (e.g., fatigue, sexual dimorphism in incidence, sleep disturbances, impact of stress, normal routine labs, as well as pain). The pathogenic mechanism of GPS is very poorly understood and currently available analgesic therapies have proven to be only partially, and unpredictably, effective. Given the lack of objective signs and laboratory abnormalities, generalized pain syndromes have traditionally been classified as psychological in origin, or more recently as a CNS disorder. However, a growing body of literature supports an important role of peripheral, nociceptive-neuroendocrine mechanisms.

Progress in this area has been seriously hindered by the lack of an adequate experimental model of chronic generalized pain. We have determined that interruption of vagal-afferent activity from the abdomen in the rat can result in a state of chronic generalized hyperalgesia. Following subdiaphragmatic vagotomy, the nociceptive threshold to mechanical stimulation decreased and bradykinin-induced mechanical hyperalgesia was enhanced. Similar to GPS, this vagotomy-induced hyperalgesia exhibits marked sex-dependence, with the enhancement of bradykinin-induced hyperalgesia significantly greater in females. The vagotomy-induced generalized hyperalgesia depends on activity of the adrenal medulla, a characteristic also relevant to GPS syndrome in which chronic stress-induced activation of the sympathoadrenal and hypothalamic-pituitary-adrenal axes may contribute. Therefore, another important dimension of our model is the demonstration of generalized hyperalgesia induced by chronic intermittent stress.

The purpose of this presentation is to further characterize the role of nociceptive-neuroendocrine mechanisms underlying chronic generalized pain.

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Integration and Multiscale Analysis of Biological Pathways Responsible for Complex Diseases Such as TMJ

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The complex disease of essential hypertension will be presented as a template for the study of TMJ as a complex disease. Genetic mapping of humans and rats has now identified hundreds of quantitative trait loci of blood pressure-related phenotypes and the task of gene identification and validation is progressing rapidly. Rat model systems with homogeneous genetic backgrounds that mimic many elements of the human disease have been developed that enable well-controlled functional studies to be performed to link candidate genes to complex networks of genes/proteins and function. A genomics systems biology map of cardiovascular function established in rat model systems using linkage studies and chromosomal substitution techniques will be presented. Bioinformatic tools and novel computational strategies of comparative genomics enable data obtained from the broadly conserved elements of the genomes to guide our understanding of human function and disease. The tools of physiological genomics and proteomics provide remarkable opportunities to advance our understanding of complex diseases such as hypertension, arthritis, and TMJ diseases with anticipation of identifying the genetic variants that contribute to an individual's vulnerability to these diseases and leading us towards individualized prevention and therapy.

The quantitative understanding of the complex interactions of mammalian organisms remains the most daunting challenge of modern biology and the so-called field of 'systems biology' is emerging to meet this challenge. Towards this end, collaborations of many experts in diverse areas including medicine, basic sciences, and computational biologists at our institution are working to advance our integrative and quantitative understanding of complex function and disease. The utility of such approaches and the application to understanding of other human complex diseases will be discussed. Studies currently under way will be presented that are aimed at developing sets of computational, multi-scale, biophysical models of mitochondria and cell function that respond to inputs from the nuclear genome, proteome, mitochondria, cytoplasm and extracellular signals. Computational models such as these provide the means for translating information between experimental regimes such as those obtained from *in vitro* recordings on isolated cells and *in vivo* measurements in the intact animal. It is hoped that such experimental paradigms and multidisciplinary systems approaches will pave the way for the studies of many other complex diseases such as those of the TMJ.

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Is Menopausal Hormone Therapy a Protective Factor or a Risk Factor? Challenges of Managing a Complex Hormonal State

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The transition to postmenopause is one of the more remarkable physiological changes a woman undergoes. This transition, which typically occurs around age 51, signals the end of ovarian hormone secretion and the appearance of secondary changes in metabolism and multiple endocrine systems. Moreover, postmenopausal women are thought to be at increased risk for several diseases compared to age-matched cycling women such as cardiovascular disease, cerebrovascular stroke, osteoporosis, hip fracture, dementia, and Alzheimer's disease. The hypothesis that ovarian steroids exert protective actions and their absence makes postmenopausal women more vulnerable to these diseases is supported by numerous clinical observational studies and a wealth of data based on animal models. However, disparate results from recent clinical trials have prompted an exceedingly useful re-examination of the actions of ovarian hormones outside of the reproductive axis and a re-evaluation of the broad use of estrogens and progestins during the postmenopausal years. This is leading to consideration of more individualized and mechanism-based hormone therapies that recognize that: estrogen treatment outcomes can be radically altered by the subject's age and duration of hormone deprivation as well as the sub-clinical disease progression; different estrogens (or progestins) do not produce equivalent actions; and hormone delivery regimens have a major impact on outcome measures. An additional and perhaps crucial element is our emergent understanding of estrogen's interface with inflammation and with the adipose/metabolic system. There are direct effects of estrogen on the inflammatory process and on adipose tissue distribution and function, and the question is how does this contribute to the phenotypic shifts in dyslipidemia, diabetes, and cardiovascular and neurodegenerative diseases in women throughout their lifespan. Using the example of the cardiovascular system as context, it will be argued that conclusions regarding harmful, neutral, or beneficial effects of hormone therapy must derive from a mechanism-based evaluation of the complex interactions of estrogen's targets.

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Genetics as the Glue for Translational Research

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Translational Science has become a common buzz-word since Dr. Zherouni initiated the Roadmap Initiative at the National Institutes of Health. The meaning in general implies from laboratory bench to patient bedside. Exactly what that means is further defined by the user who can further define to virtually all forms of biomedical research. From my perspective the key word is translation and how this is accomplished. For many diseases such as TMJ there is a need to integrate data from a variety of sources, patients, animal models, cell culture and tissue engineering studies, to name a few. This type of translational science is essential to enabling new discoveries. Genetics and genomics offer several key aspects to the knowledge-generating aspect of the translational research paradigm. Bioinformatics that was developed from the genetics and genomics teams generated huge datasets requiring large databases, and sophisticated analysis tools is arguably the most immediate platform to help translational research for TMJ. With the high density genotyping platforms and the promise of genomic sequence for all patients in the not so distant future, the management of data becomes even more critical. However, it is the ability to capture medical records electronically and to generate large patient databases for research that is likely to yield the most promise for TMJ over the next few years. Finding patients that meet rigorous study criteria and are willing to participate in the study is arguably the single largest bottleneck for translational research. The TMJ Association and its active members offer a chance to build such a resource, which could be used for a wide number of study types from the genetics to clinical trials. This dataset could then be used for whole genome association studies as a means to try and identify the genetic basis of TMJ. Finally, the ability to use comparative genomics and genetics down to the level of a single nucleotide, due to the large number of species having been sequenced, opens opportunities to integrate data across multiple species and to inform investigators about the disease process and eventually help with the development of new therapeutics. As an example of what could be developed for TMJ, I will discuss how we have deployed genetics and genomics into our studies of common complex diseases, as well as discuss how the genomics information is likely to change the practice of clinical medicine.

Training Multidisciplinary Translational Clinical Scientists

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Progress in the application of basic research findings to clinical conditions has been hampered by many barriers—among them is a paucity of researchers able to translate scientific advances from the bench to the bedside and back again to the bench. Recent attention has focused on overcoming this obstacle through multidisciplinary team research and preparation of researchers to participate in these teams to foster translational and clinical research. This presentation provides practical information of special interest to current and future trainees and mentors regarding training in translational and clinical research. The objective is to inform and empower researchers to enable them to best use available resources to advance towards the ultimate goal of improving prevention and management of pain.

Understanding and Treating TMJ Disorders: A Modest Proposal

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Beginning with the identification of a clinical problem, the ultimate goal of clinical research is to more effectively prevent and treat disease and disability. For a number of years, the TMJ Association has been collecting information to document the protean nature of these disorders and urging both investigators and NIH to invest resources into studies of their pathogenesis and treatment. NIH has recently introduced a new program encouraging academic medical enters to create a "home" for the conduct of clinical research and the training of clinical investigators—the Clinical and Translational Science Award (CTSA). A goal of this award is to provide the infrastructure to facilitate multidisciplinary collaboration among laboratory scientists, clinical investigators, and the community. This would be an opportune time to develop a model interdisciplinary, collaborative research program, with a focus on TMJ disorders. Suggested steps to accomplish this might include the following: a) convene a group of clinicians from the various disciplines involved in treating TMJ disorders to develop criteria for establishing a diagnosis of TMJ disease; b) convene a multidisciplinary group of laboratory scientists and clinical investigators to develop one or more protocols related to the pathogenesis and/or treatment of TMJ disorders; c) provide funding mechanisms for multidisciplinary research programs, including clinical trials.

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Poster Abstracts

Inflammation of Craniofacial Muscle Induces Widespread Mechanical Allodynia Which Is Abolished by CGRP Receptor Antagonist

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Introduction: Recent studies demonstrate the involvement of calcitonin gene-related peptide (CGRP) in deep tissue craniofacial pain. The clinical effectiveness of a potent CGRP antagonist for treatment of migraine headache represents a potential therapeutic venue for other chronic deep tissue pain disorders in which CGRP levels are elevated. We investigated the modulation of behavioral responses evoked by nociceptive stimuli following a local injection of complete Freund's adjuvant (CFA) and the effect of CGRP antagonist on CFA-induced behavioral responses.

Methods: CFA (150µl, 0.5mg/ml) was injected into the masseter muscle of male rats (n=50) to induce inflammation. Rats then received an injection of CGRP (8-37) into the tail vein (n = 12; 0.15 mg/kg in saline, i.v) or saline (n=10,i.v) as vehicle control. Animals were tested for their response to mechanical stimulation.

Results: Inflammation of the masseter muscle evoked mechanical allodynia not only in the region of inflammation ($p<0.001$), but also secondary mechanical allodynia in the contralateral head ($p=0.025$), ipsilateral hindpaw ($p<0.001$) and contralateral hindpaw ($p<0.001$). Intravenous administration of CGRP (8-37) but not saline two minutes prior to the injection of CFA into the masseter muscle completely blocked CFA-evoked reductions in withdrawal thresholds (one-way ANOVA indicated no difference between pre-injection thresholds and post-injection thresholds $p=0.60$).

Conclusion: The widespread modulation of nocifensive behavior evoked by inflammation of deep craniofacial tissue found in this study resembles the widespread deep tissue pain reported in fibromyalgia, whiplash injury and some temporomandibular disorders and thus may provide insight into the mechanisms of these musculoskeletal pathologies. Further, these data implicate CGRP receptors in deep tissue nociceptive mechanisms and suggest that CGRP antagonists may have therapeutic potential for musculoskeletal pain.

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Nociceptive Modulation of Jaw Muscle Spindle Activity Requires Fusimotor Drive

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Introduction: The purpose of this study was to demonstrate that gamma fusimotor activity is required for nociceptor-induced alteration of proprioceptive signaling from jaw muscles.

Methods: Microelectrode recordings were made from jaw muscle spindle afferent neurons in the trigeminal mesencephalic nucleus (Vmes). Ramp and hold openings of the jaw were used to characterize the response of single masseter spindle afferents before injecting hypertonic saline (HS, 100µl, 5%) into the homonymous muscle. The mean firing rate (MFR) of each unit was calculated for the rest, open, and hold phases of repeated ramp and hold movements before (baseline) and up to 5 minutes after injection.

Results: Injections of HS produced highly reproducible changes in MFR in one or more phases of the stretch. Injections with isotonic saline did not affect MFR. Units that were consistently modulated by HS injections (n=20), in one or more phases of the jaw stretch, were retested with HS after infusing gallamine triethiodide (2gm/kg, i.v.) for 20 minutes to effectively block gamma fusimotor influences. Responses to HS were compared before and after gallamine infusion. Gallamine infusion reliably eliminated the HS-induced modulation of Vmes neurons in all phases during the ramp and hold movements.

Conclusion: Consistent with observations of proprioceptive modulation in the hindlimb and neck muscle spindle afferents, nociceptive-induced alterations in proprioceptive responses to jaw opening appear to be mediated through the gamma fusimotor system. The results of this study suggest that eliminating the gamma fusimotor drive prevents alteration in jaw muscle spindle afferent discharge following algesic chemical stimulation.

Analgesia Produced by Epoxyeicosatrienoic Acid in the Ventral Periaqueductal Gray of the Rat

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Opioid analgesics are clearly the most efficacious agents currently available for treatment of moderate-to-severe pain. However, their use in the management of chronic pain is limited due to impairment of Na^+, K^+ -ATPase activity after chronic opiate treatment, a possible mechanism of tolerance/addiction. Furthermore, opioid peptide (enkephalin) interaction with mu and delta opioid receptors increase the levels of IP_3, intracellular $[Ca^{2+}]i$ and PKCα phosphorylation. Based on the data showing that epoxyeicosatrienoic acids (EETs) induce elevation in $[Ca^{2+}]_i$, activation of Ca^{2+} dependent second messengers, phosphatases and kinases, along with our demonstration of a novel cytochrome P450 4X1 epoxygenase that is localized to neurons, we hypothesize that EETs, metabolites of arachidonic acid, may have analgesic effects similar to opioid receptor activation.

Groups of male SD rats were microinjected with different concentrations of EETs (39 - 78 nmol) and vehicle (saline) into the ventral periaqeductal grey area (vPAG). The tail-flick (TF) response, an index of antinociception, was then determined. Microinjection of EETs or morphine produced dose-dependent analgesia (EETs, 39 and 78 nmol: 7.10±1.08 and 8.80±1.20; Morphine: 9.22±0.36), whereas microinjection of vehicle did not alter the baseline of TF latency (control: 3.47±0.18). However, the mechanism through which EETs produce analgesia is not clear at this time. Therefore, further study of the mechanism by which EETs produce analgesia will be important since EETs may offer a potentially novel therapeutic strategy for the management of chronic pain.

Antagonism of Neurokinin-1 Receptors in the Rostral Ventromedial Medulla Attenuate Inflammation-Induced Thermal Hyperalgesia in Rats

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Introduction: The rostral ventromedial medulla (RVM) is involved in the descending modulation of inflammatory hyperalgesia. There is moderate labeling of substance P in the nucleus raphe magnus (NRM), a sub-region of the RVM, and a moderate density of neurokinin-1 (NK-1) receptors in this supraspinal region.

Methods: The present experiments examined the effects of L-733,060 (NK-1 receptor antagonist) or vehicle microinjection (500 nl injected over 90 sec) into the RVM on paw withdrawal thresholds to noxious thermal stimuli before and after complete Freund's adjuvant (CFA)-induced inflammation in the rat. We used Western blots to examine the effect of CFA-induced inflammation on NK-1 receptor protein expression in the RVM.

Results: When the NK-1 receptor antagonist was administered after inflammation (posttreatment), there was an attenuation of thermal hyperalgesia as compared to animals that received a vehicle microinjection. More specifically, the increase in paw withdrawal latencies was seen 2 hr ($p<0.05$) and 5 hr ($p=0.01$) following L-733,060 microinjection and returned to the vehicle control level 24 hr following microinjection. When the NK-1 receptor antagonist was administered before inflammation (pretreatment), there was an attenuation of thermal hyperalgesia at 24 hr ($p<0.05$) following inflammation as compared to vehicle microinjected animals. The hyperalgesia returned to vehicle control levels 3 days following inflammation. Western blots show increased NK-1 receptor protein expression 2 hr, 24 hr, 3 d, and 7 d following inflammation as compared to naïve controls.

Conclusion: These findings indicate that there is increased NK-1 receptor expression in the RVM following CFA-induced inflammation and antagonism of NK-1 receptors in this supraspinal region results in attenuation of thermal hyperalgesia.

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Effect of Interleukin-1Beta on NR1-Serine 896 Phosphorylation in the Rat Spinal Trigeminal Nucleus

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Introduction: Previously studies have shown that glial interleukin-1beta (IL-1beta) in the spinal trigeminal nucleus (Vsp) is activated after masseter inflammation and plays a role in the development of orofacial hyperalgesia. To study the interaction of IL-1 receptor (IL-1R) with N-methyl-D-aspartate receptors (NMDAR) and the biochemical signaling pathways in response to orofacial injury, we examined the effect of IL-1beta on serine 896 phosphorylation of the NMDAR NR1 subunit (P-ser896-NR1) in the Vsp.

Method: Transverse slices including the causal Vsp (0.5 mm thick) were obtained from 8-10-week old male rats. The slices were kept in oxygenated artificial cerebrospinal fluid (aCSF) at room temperature. After treatment, the tissues were frozen, and the caudal subnucleus caudalis (Vc) of Vsp was punched out. The P-ser896-NR1 level was measured by Western blot.

Result: After a 15 min-IL-1beta (10 ng/ml & 50ng/ml) treatment, the P-ser896-NR1 was significantly increased to 215% of the control ($n=4$, $P<0.05$). Pretreatment of slices with IL-1R antagonist blocked the IL-1beta-induced increase in P-ser896-NR1. Pretreatment with chelerythrine (0.01mM), a protein kinase C inhibitor, blocked IL-1beta-induced P-ser896-NR1 ($n=3$, $p<0.05$). Pretreatment with 2APB (0.072 mM), an IP3 receptor inhibitor, also blocked IL-1beta-induced P-ser896-NR1, suggesting that intracellular calcium plays a role in regulation of P-ser896-NR1 in the Vsp. In contrast, the NMDAR channel blocker MK-801 (0.03 mM) did not block IL-1beta-induced P-ser896-NR1.

Conclusion: Our results suggest that IL-1beta modulates NMDAR function through NR1-Ser896 phosphorylation and the IL-1beta-induced P-ser896-NR1 involves a signaling cascade dependent on PKC activation and intracellular calcium release.

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Regulation of CGRP Expression in an in Vivo Model of TMJ Inflammation

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Introduction: Peripheral release of calcitonin gene-related peptide (CGRP) from sensory trigeminal ganglion neurons contributes to neurogenic inflammation within the temporomandibular joint (TMJ) and central release mediates pain and central sensitization. CGRP levels are elevated in TMJ synovial fluid samples in patients suffering from TMJ disorders and correlate with reported pain levels.

Methods: The goal of this study was to determine the effects of inflammatory and anti-inflammatory agents on CGRP levels within trigeminal ganglion by radioimmunoassay and immunostaining using an in vivo model of TMJ inflammation.

Results: Initially, neurons innervating the TMJ were localized within the ganglion by retrograde labeling using True Blue. Next, immunohistochemistry was used to identify CGRP and TRPV1 expressing neurons within the entire ganglion. Almost all CGRP containing neuronal cells (>90%) within the V3 region of the ganglion also expressed TRPV1, a marker of sensory C fibers. The effect of capsaicin injection into the TMJ capsule for 2 h caused an ipsilateral decrease in CGRP levels in the ganglion. This decrease was attenuated by overnight pretreatment with a concentrated cocoa bean extract, enriched in polyphenolic compounds. Injection of the NO donor SNP at pH 5.5 for 24 h caused an ipsilateral increase in CGRP levels that was blocked by pretreatment with the polyphenolic compound catechin. In addition, the effect of the cell permeable p38 MAP kinase inhibitor SB 203580 on basal, unstimulated and NO/pH 5.5-stimulated CGRP levels was investigated. Overnight treatment with SB 203580 decreased basal levels of CGRP and inhibited stimulated levels following a 2 h injection of a NO/pH 5.5 solution.

Conclusion: Data from our study supports the use of this in vivo model to more thoroughly investigate the cellular and molecular mechanisms that control CGRP synthesis and release from trigeminal ganglion neurons under normal, pathological, and therapeutic conditions.

Activation of Trigeminal Nociceptors by Mechanical Stimulation via Integrins

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Introduction: Patients suffering from degenerative temporomandibular joint (TMJ) disorders commonly complain of debilitating pain evoked by jaw movement (i.e., mechanical allodynia). The exact mechanism involved in mechanically evoked nociception is currently unknown. Here, we provide new evidence that RGD-binding integrins regulate mechanoreception of trigeminal ganglion (TG) nociceptors *in vitro*. Integrins are heterodimeric transmembrane receptors that mediate cell-extracellular matrix interactions.

Methods: Ferromagnetic beads (8.0 μm diameter) coated with GRGDSP (RGD) peptide were bound to rat trigeminal neurons cultured (1 day cultures) on a laminin substrate. Trigeminal nociceptors were identified in TG cultures by measurement of a 10 nM capsaicin-evoked free intracellular Ca^{++} (iCa^{++}) flux using a standard fluorescence microscopic method employing the indicator Fura-2 (340/380 nm). Cultures were then subjected to a brief magnetic field (25 G; 1 Hz) to produce twisting movements of the magnetized RGD-coated beads under controlled conditions (i.e., magnetic twisting cytometry) in the presence or absence of an anti-β1 integrin blocking antibody (Chemicon Int). Neuronal responses to mechanical stimulation were monitored by measurement of free iCa^{++}. Data were normalized to peak free iCa^{++} evoked by 50 mM KCl.

Results: Eighty-two percent of capsaicin-sensitive TG neurons responded to mechanical stimulation via RGD-coated beads. Peak free iCa^{++} following capsaicin or mechanical stimulation was 67.8% and 69.1% of maximum KCl response, respectively. Preincubation with an anti-β1 integrin blocking antibody, but not heat denatured antibody, significantly attenuated responses of capsaicin-sensitive TG neurons to mechanical stimulation.

Conclusions: These data are consistent with the hypothesis that RGD-binding integrins of the β1 integrin subfamily mediate mechanically-evoked nociception in the trigeminal system. If this hypothesis is confirmed with future studies, then therapies targeting specific integrins mediating this response could provide relief of movement associated jaw pain experienced by patients with TMJ disorders.

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Gap Junction Proteins in Reactive Astrocytes Contribute to Inflammatory Hyperalgesia

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Introduction: Glia is now recognized as important contributors in pathological pain conditions. It has been shown that the activation of astrocytes involves calcium waves spreading between astrocytes through the gap junction and that spinal gap junctions are potentially involved in pain facilitation. We have examined the role of astrocytic gap junction protein, connexin 43 (Cx43), in masseter hyperalgesia.

Methods: Tissue injury was produced by injecting complete Freund's adjuvant (CFA, 0.05 ml, 1:1 dilution), an inflammatory agent, into the masseter muscle of the Sprague-Dawley rat. The protein levels were examined by immunohistochemistry and Western blot. Mechanical hyperalgesia was assessed with von Frey microfilaments.

Results: The injection of CFA resulted in activation of Cx43 in the spinal trigeminal complex including the trigeminal subnuclei interpolaris/caudalis (Vi/Vc) transition zone and Vc, the two regions involved in trigeminal pain processing. The Cx43-like immunoreactivity colocalized with glial fibrillary acidic protein, a marker of astrocytes, but not CD11b, a marker of microglia, and NeuN, a neuronal marker. In masseter-inflamed rats, the increased Cx43 staining was clearly associated with hypertrophied astrocytes, characteristic of reactive astrocytes. Western blot confirmed the upregulation of Cx43 in the spinal trigeminal complex. The mechanical hypersensitivity of the masseter muscle was demonstrated by enhanced head withdrawal responses to applications of von Frey filaments. Post-treatment with gap junction decouplers octanol (4, 8 nmol) and carbenoxolone (0.08-8 nmol) microinjected into the Vi/Vc or Vc significantly attenuated mechanical hypersensitivity after CFA.

Conclusion: These results suggest that reactive astrocytes with increased gap junction connectivity contribute to the central mechanisms underlying CFA-induced masseter hyperalgesia.

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The Effect of Interleukin-10 and Fluorocitrate on Masseter Inflammatory Hyperalgesia

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Introduction: Studies indicate that CNS cytokines, in association with activated glia, may modulate neuronal function. We have studied the effect of interleukin (IL)-10, an antiinflammatory cytokine, and fluorocitrate, a glial inhibitor, on masseter hyperalgesia.

Methods: Tissue injury was produced by injecting complete Freund's adjuvant (CFA, 0.05 ml, 1:1 dilution), an inflammatory agent, into the masseter muscle of the Sprague-Dawley rat. Mechanical sensitivity of the masseter and orofacial skin 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. A decrease in EF_{50} indicates mechanical hyperalgesia/allodynia.

Results: Injection of CFA into the masseter produced mechanical hyperalgesia/allodynia beginning 30 min and peaking at 1d after CFA. A secondary hyperalgesia at the perioral region also developed. We then injected IL-10 (0.6, 1.0 and 10 ng) intrathecally at the obex level at 1d after inflammation. Both primary masseter ($p<0.01$) and secondary perioral ($p<0.05$) hyperalgesia were attenuated in IL-10-treated rats. The attenuation of hyperalgesia was further confirmed by focal microinjection of IL-10 (0.006-1 ng) into the ventral subnuclei interpolaris/caudalis (Vi/Vc) transition zone, an important site for the processing of trigeminal deep input. Intra-Vi/Vc IL-10 significantly attenuated both masseter and perioral hyperalgesia ($p<0.05$). Further, the reduction of EF_{50}s for the masseter and perioral skin was also significantly attenuated after microinjection of fluorocitrate (1,000 ng) into the Vi/Vc.

Conclusion: These results suggest that glial inhibition and interruption of the central cytokine cascade after inflammation may block or attenuate CFA-induced masseter hyperalgesia.

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Sensory Feedback from Mechanical Nociceptors in the Masseter Muscle Can Be Presynaptically Motivated

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Introduction: Muscle pain, including that which occurs in many temporomandibular disorders (TMD), is a significant clinical problem for which no efficacious therapy is currently available. In spite of its high clinical relevance, little is known about the processing of noxious sensation from muscle. Therefore we explored noxious mechanical feedback from the masseter muscle using an animal model.

Methods: Intra-axonal recordings were made from group III masseter muscle afferents in anesthetized rats (n=10). After physiological characterization, afferents were intracellularly stained with biotinamide. Sections were then immunostained with a monoclonal antibody for glutamic acid decarboxylase (GAD-65) which is present in GABA-containing nerve terminals. Using confocal microscopy, optical sections (0.5 microns) were collected through the region of mechano-nociceptor, primary afferent boutons.

Results: Putative GAD-65 boutons were found juxtapositioned with masseter muscle primary afferent boutons on up to five consecutive optical sections. When three-dimensional reconstructions of masseter muscle axon collaterals were generated and rotated in space, GAD-65 boutons were closely apposed to muscle afferent boutons in all perspectives. These results show that the anatomical substrate for presynaptic modulation of noxious mechanical sensory feedback is present within the brainstem. Additional electrophysiological data indicates that following intramuscular injection of hypertonic saline, masseter muscle afferents exhibit responses comparable to dorsal root responses in spinal afferents which are indicative of presynaptic modulation.

Conclusion: These results provide morphological and electrophysiological evidence that sensory feedback evoked by noxious mechanical stimulation of the masseter muscle may be presynaptically modulated. These results also indicate that GABA receptors should be explored as potential therapeutic targets for the treatment of muscle pain and the craniofacial muscle pain which occurs in TMD.

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Sex Differences in Antinociceptive Circuitry Associated with TMJ Inflammation

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Objectives: The prevalence, severity and duration of pain associated with temporomandibular disorder (TMD) are greater in women than in men. Because TMD pain often resolves in the absence of clinical intervention, it is of interest to determine factors that underlie recovery of TMD in males and females. We have previously observed that TMJ inflammation results in a feeding deficit in rats. In male rats, this feeding deficit recovered within 3 days even though inflammation was still present. This recovery was naloxone reversible, suggesting the involvement of endogenous opioids. The present study was designed to test the hypothesis that the impact of TMJ inflammation will be greater in female rats and that any recovery of feeding deficit will be opioid independent.

Methods: A conditioning paradigm was used to assess feeding behavior in rats where the time between food rewards (inter-feeding-interval (IFI)) was used as the dependent variable. TMJ inflammation was induced with Complete Freud's Adjuvant (CFA). Four groups of rats were studied: intact males, intact females, ovarectimized females (OVx), and OVx females with estrogen replacement (OVx+E).

Results: 24h after CFA injection the median IFI increased in all four groups of rats. While the increase in females was larger than that in males, this difference was not statistically significant. By 72 h, the median IFI returned to baseline in all 4 groups. This recovery was naloxone reversible in males and OVx, but not intact females or OVx+E.

Conclusions: These results suggest that there is a sex difference in the mechanisms underlying antinociceptive circuitry engaged by TMJ inflammation. This sex difference appears to depend on the presence of estrogen. Importantly, these results support the suggestion that the use of opioids may be less effective for pain relief in women.

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Estrogen and Inflammation Induce Facial Secondary Hyperalgesia and Erk Activation in Trigeminal Neurons

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Introduction: Temporomandibular disease is much more common in women than men, and painful episodes are linked to the hormonal fluctuations of the menstrual cycle. Trigeminal ganglion neurons from ovariectomized rats show increased excitability with estrogen replacement and inflammation of the temporomandibular joint (Flake, et. al., 2005). The activated form of extracellular-signal regulated kinase (ERK) a member of the MAPK family, has been shown to mediate inflammatory pain in dorsal root ganglia (Obata et. al., 2004).

Methods: To investigate the effect of estrogen and inflammation on trigeminal hyperalgesia and ERK activation in vivo, we injected complete Freunds adjuvant (CFA) or saline into the masseter muscle in ovariectomized Sprague-Dawley rats treated with estradiol (E2) or vehicle. Three days after treatment, facial hyperalgesia was assessed using von Frey filaments, and trigeminal ganglia were harvested.

Results: CFA-injected and estrogen-replaced rats showed increased withdrawal percentages to von Frey stimulation of the whisker pad compared to saline and vehicle treated controls, with an additive effect occurring in rats receiving both CFA and E2. Immunohistochemical labeling of trigeminal ganglia showed an increased percentage of p-ERK positive neurons in the CFA, E2, and CFA+E2 treated groups. E2 treatment increased ERK activation throughout the ganglia, while CFA effects were concentrated in the V3 division. Experiments are ongoing to determine whether an additive effect of CFA+E2 exists within specific anatomic subregions of the ganglia.

Conclusion: These data show that estrogen and inflammation of the masseter induce secondary hyperalgesia of the whisker pad, and they suggest that ERK activation is the mechanism by which estrogen exacerbates inflammatory pain.

Supported by: NIH DEO1582.

CFA-Induced Plasma Extravasation in the Rat TMJ Is Sexually Dimorphic

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Introduction: We have recently reported that chronic estrogen and persistent inflammation increase the excitability of rat temporomandibular joint (TMJ) afferents. The present study was performed to determine whether the influence of estrogen was specific to neuronal excitability, or whether chronic estrogen had a more generalized pro-inflammatory influence within the TMJ.

Methods: We addressed this question by assessing the impact of sex and gonadal hormones on plasma extravasation (PE) in and around the TMJ at baseline and at various times following induction of inflammation. The Evans Blue (EB) assay was the method used to assess PE. Inflammation was induced by injecting Complete Freund's Adjuvant (CFA) into the TMJ. Groups of rats included: intact females (F), ovariectomized females with vehicle (OvX-V), ovariectomized females with estrogen (OvX-E), intact males (M), gonadectomized males with vehicle (Gx-V) and gonadectomized males with testosterone (Gx-T).

Results: The most striking differences between groups were between M and F. These two groups differed with respect to both baseline PE (M>F) and with respect to the time course of CFA-induced changes. While testosterone and estrogen both appear to be suppressing inflammation –induced TMJ PE, testosterone has a greater suppressing effect than estrogen.

Conclusion: From these results, we can conclude that TMD pain may reflect estrogen-induced suppression of PE. These changes may also contribute to a higher incidence, severity, and duration of TMD pain in females than in males.

Decreased Loading of the TMJ Inhibits the Effects of Ovarioectomy in CD-1 Mice

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Introduction: Ninety percent of those seeking treatment for TMJ disorders are women of childbearing ages suggesting a hormonal component of the disease process. Recently, in bone, estrogen was shown to mediate the anabolic effects of mechanical loading. We believe that estrogen may also mediate the mechanical loading response in the TMJ fibrocartilage.

Methods: Thirty-six, 21-day-old female mice were either sham operated, had their ovaries removed (OVX) or were OVX and received estrogen replacement (10 μg/kg day). The mice were then divided into two sets. In the first set the mice were fed a normal pellet diet. In the second set the mice had decreased loading conditions (the mice were fed a soft diet and had their incisors trimmed out of occlusion). When the mice reached 49-days-old they were sacrificed and processed for histology. Mice were weighed once a week.

Results: There was no statistically significant difference in the weight in any of the groups or sets of the animals at any time point of the experiment. In the normal pellet diet set, the removal of estrogen by OVX caused a decrease in the width of the hypertrophic layer of the mandibular condylar cartilage compared to the sham operated mice, which was rescued by estrogen replacement. In contrast, in the soft diet set, OVX did not cause any change in the height of the hypertrophic layer of the mandibular condylar cartilage compared to the sham operated mice.

Conclusion: Normal physiological loading is required for the estrogen removal effect on the mandibular condylar cartilage. Our current working model is that the increase in estrogen in women during childbearing ages makes the TMJ joint more sensitive to mechanical loading causing an exaggerated response, thus making the TMJ joint more prone to injuries.

Influence of Macroscopic Collagen Fiber Architecture of the Mandibular Condylar Cartilage of Tensile Behavior of the Tissue

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Introduction: Previous microscopic structural investigations provide little evidence of anisotropy of mandibular condylar cartilage. However, a previous tensile evaluation of the porcine condylar cartilage suggested that this tissue may be anisotropic. This study aimed to explore the macroscopic collagen fiber orientation of the condylar cartilage, and for the first time correlate this to regional and directional tensile behavior.

Methods: For visual inspection of collagen fiber orientation, 30μm sections of porcine mandibular condylar cartilage were prepared and mounted on slides for polarized light microscopy at 200X magnification. For tensile testing, uniform rectangular cartilage specimens were prepared from anterior, superior and posterior regions in the mediolateral direction, and from medial, central and lateral regions in the anteroposterior direction. Each specimen was tare loaded to 0.03N in 37°C PBS, subjected to 10 preconditioning cycles to 6% strain, and loaded to 20% strain at 6 mm/min.

Results: Macroscopic collagen fiber arrangement of the condylar cartilage had a peripheral arrangement of the fibers at the boundaries and predominantly anteroposterior arrangement inside the periphery. Under tension, regions in the anteroposterior direction were stiffer than regions in the mediolateral direction. In the anteroposterior direction, the medial, central, and lateral region moduli were 21 ± 13, 32 ± 15, and 25 ± 11 MPa, respectively (mean standard deviation). In the mediolateral direction, the anterior, superior, and posterior region moduli were 19 ± 5, 7.1 ± 0.8, and 9.1 ± 6.4 MPa, respectively.

Conclusions: Polarized light microscopy revealed the presence of fiber arrangement in the condylar cartilage similar to that of the TMJ disc. The tensile moduli confirm the anisotropy and display a heterogeneity of mandibular condylar cartilage not previously reported in the literature. The structure and mechanical behavior elucidated in this study would be consistent with a mechanical environment where surface shear that is predominantly, but not exclusively, anteroposterior.

Mechanical Stimulation of Photopolymerized Hydrogel Scaffolds for TMJ Articular Cartilage Regeneration

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Introduction: Temporomandibular joint (TMJ) disorders are often associated with TMJ disc dislocation, which can damage the articular cartilage of the condylar process. Little attention has focused on therapies to treat damaged TMJ articular cartilage. In this study, photopolymerized poly(ethylene glycol) hydrogels were investigated as cell-carriers for regenerating articular cartilage in the TMJ under the application of mechanical loading.

Methods: Bovine cartilage was explanted from the condylar process of the TMJ. Bovine articular chondrocytes were isolated from the metacarpalphalangeal joint. Chondrocytes were photoencapsulated in poly(ethylene glycol) hydrogels and cultured for 24 hours. The constructs were placed in a custom designed cell-straining apparatus and either subjected to 15% amplitude compressive strains at 0.3 Hz or left unstrained for 48 hours. Cartilage explants and hydrogel constructs were enzymatically digested and total DNA content, proteoglycan content (chondroitin sulfate (ChS)), cell proliferation ([^{3}H]thymidine incorporation) and/or proteoglycan synthesis ($^{35}SO_4$ incorporation) were measured.

Results: Articular cartilage explants from the TMJ condylar process contained 90±3% water, 60±28 ng DNA/mg wet weight (ww) and 6.1±1.4 μg ChS/μg DNA. PEG crosslinking density was varied to produce hydrogels that contained 92±0.7% water. When chondrocytes were encapsulated in these gels and cultured for 3 days (unstrained), the constructs contained 26±6 ngDNA/mg ww and 2.8±0.6 μgChS/μgDNA. When subjected to dynamic loading, cell proliferation and PG synthesis were stimulated by 41±6% and 41±10% compared to unstrained controls. These results demonstrate that hydrogels may be fabricated to match properties of the TMJ articular cartilage (in terms of water content), maintain chondrocyte viability, promote the production of cartilage extracellular matrix (ECM) molecules and upon loading stimulate cell proliferation and PG synthesis.

Conclusions: Photopolymerized hydrogels are promising cell-carriers for regenerating TMJ articular cartilage. Dynamic loading further enhances cell proliferation and ECM production. Future studies will investigate degradable scaffolds on long-term culture for TMJ articular cartilage regeneration.

Temporomandibular Joint Reconstruction in the Minipig Using a Computer-Tomography-Based Scaffold Design Manufactured from Degradable Polycaprolactone

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Introduction: We engineered a biodegradable condylar-ramus-unit (CRU) scaffold for implantation in both an adolescent and a mature-adult animal model for TMJ reconstruction following condylectomy. Our objective was to evaluate the regenerative potential of a biodegradable CRU implant and determine if microstructure of the scaffold design had an influence on bone growth.

Methods: Dilated anatomically shaped CRU scaffolds were designed using representative computed-tomography (CT) datasets to recreate condylar contour. Three separate designs were manufactured using epsilon-polycaprolactone (PCL) powder for the adolescent animals, and two were created for the adult animals. A unilateral condylar ostectomy was performed in eleven adolescent minipigs, and 6 adult minipigs. Autologous iliac crest bone marrow was placed into the PCL scaffolds. The CRU implant sleeve was secured to the posterior border of the ramus. Minipigs were sacrificed at either 30 or 90 days. The CRU scaffolds were recovered with intact articular disc and eminence and were evaluated using micro-computed tomography (microCT) and histological analysis.

Results: All of the animals returned to full masticatory function. Data from microCT for all adolescent minipigs demonstrated significant new bone formation, within and surrounding the condylar head of the scaffold, regardless of CRU scaffold design. Histology in this group demonstrated cartilage formation along the condylar surface. In the mature-adult animal group, microCT data showed 15-30% new condylar bone growth guided by the implant design.

Conclusions: PCL CRU scaffolds did not impede bone growth. A reasonable amount of bone ingrowth was achieved in a mature-adult animal model. This study demonstrates that adolescent minipigs are a poor model for evaluation of TMJ reconstruction due to their innate injury response displaying abundant bone growth, and their ability to regenerate pseudocondyles following condylectomy without replacement. The mature-adult Yucatan minipig appears to be a more appropriate animal model for future human investigation due to its limited growth potential.

TMJ Chondrocytes Isolated from Biglycan/Fibromodulin Double-Deficient Mice Have Altered Proliferation and Differentiation in Vitro

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Introduction: Mice doubly-deficient in two small leucine-rich proteoglycans, biglycan and fibromodulin develop accelerated temporomandibular joint osteoarthritis (TMJ OA). Our goal was to develop an *in vitro* system to investigate the early mechanistic basis of the disease. In this study the proliferation and differentiation potential of TMJ chondrocytes obtained from wildtype (WT) and biglycan/fibromodulin double-deficient (DKO) animals were measured.

Methods: TMJ condyles from 5-week old WT and DKO animals were dissected and single cell suspensions were obtained by digesting with collagenase/dispase II. The cells were cultured in α-modified minimum essential medium, 20% serum, 2mM glutamine, 2-mercaptoenthanol, and antibiotics. When ~80% confluent, the cells were passaged for proliferation and differentiation assays. Cell proliferation was examined using a BrdU ELISA. To examine differentiation, 4 x 10^6 cells/60 mm dish were cultured in chondrogenic media consisting of Dulbecco's modified Eagle's medium, 10% serum, 100 μg/ml ascorbic acid, 2mM glutamine, and antibiotics. Type I collagen, type II collagen, decorin, sox-9 and cartilage oligomeric protein (COMP) mRNA expression were analyzed by RT-PCR and normalized to gapdH expression levels.

Results: The BrdU ELISA revealed that the DKO cells had increased proliferation levels as compared to WT cells. RT-PCR analysis showed that there were no obvious differences in type I collagen, decorin and sox 9 mRNA expression levels between WT and DKO cells. However, the DKO cells had decreased type II collagen mRNA levels and dramatically increased COMP mRNA levels compared to WT cells, indicating differences in cell differentiation. These alterations *in vitro* reflect changes previously observed in the DKO TMJ *in vivo*.

Conclusions: We show, for the first time, the combined importance of biglycan and fibromodulin in regulating the proliferation and differentiation of TMJ chondrocytes. This in vitro system provides a new tool to test deeper mechanistic questions that may reveal early pathological events underlying TMJ OA.

Mechanical Signals Generated by Appropriate Physical Therapies Can Prevent Fibrocartilage Degradation and Induce Repair in TMJ Disorders (TMJDS)

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Introduction: TMJDs, frequently inflammatory in nature, cause pain and degeneration of joints. Although there is no universal treatment, physical therapies are invariably beneficial to inflamed TMJs. However, a lack of understanding of their mechanisms on inflamed joints has impeded the use of such therapies to the full extent. We hypothesize that mechanical signals are antiinflammatory and initiate repair by upregulating wnt signaling involved in TMJ development.

Methods: TMJ fibrochondrocytes grown on flexible bottom plates were either unstretched or subjected to cyclic tensile strain (CTS) (12%; 0.05Hz; Flexcell) with or without interleukin (IL)-1β. PCR, Western blots, and immunofluorescence were used to examine regulation of the NF-kB and Wnt signaling pathways.

Results: The results revealed that CTS: (i) suppresses IL-1β-induced upregulation of proinflammatory mediators including nitric oxide, prostaglandin-E2, and matrix metalloproteases (MMPs); (ii) blocks IL-1β-mediated nuclear translocation of NF-κB by inhibiting IKK activation; (iii) counteracts IL-1β-induced inhibition of constitutive wnt3a, wnt4, and wnt7a mRNA expression within 4h, and proteins by 24h; (iv) further downregulates wnt co-receptor LRP6 over IL-1β inhibition; and (v) represses IL-1β induced β-catenin migration to the cytoplasmic periphery restoring β-catenin to its normal cytoplasmic orientation.

Conclusion: These findings are the first to demonstrate that signals generated by dynamic strain antagonize inflammation as well as upregulate fibrocartilage repair via matrix synthesis. These signals, by inhibiting IL-1β-dependent transcriptional activation of nitric oxide synthase-II, cyclooxygenase, and MMPs, rescue cartilage from degradation. Importantly, during inflammation, these signals may promote cartilage repair by counteracting IL-1β-mediated inhibition of the canonical and non-canonical wnt pathways. CTS restores β-catenin pools through wnt3a and wnt7a stiumulation and β-catenin relocation. Wnt4 signaling through the Wnt/Ca^{2+} pathway can inhibit β-catenin mediated gene transcription as evidenced by further down regulating of LRP6. Mechanistic understanding of these signals is essential for development of proper therapeutic interventions to treat/prevent progression of inflammatory TMJDs.

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Identification of Proteoglycan 4 in the Temporomandibular Joints of Baboons

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Introduction: The temporomandibular joint (TMJ) is a synovial joint whose movements are facilitated by an articular disc. Recent evidence suggests that a defect in the lubricating system of the TMJ may be responsible for the development of TMJ derangement and subsequent degenerative TMJ disease. A secreted molecule, proteoglycan 4 (PRG4), is believed to provide boundary lubrication to articulating joints. At least four splice variants of PRG4 are expressed in articular joints and there is compelling evidence suggesting that one or more of these variants are critical for normal lubrication of joints. However, no studies to date have examined the presence and function of PRG4 or its variants in the TMJ. The purpose of the proposed research is to better understand the lubricating system of the joint in an effort to identify causative factors responsible for common TMJ derangements.

Methods: Baboon TMJ cartilage, articular disc and synovium total RNA was isolated and cDNA synthesized. Quantitative real-time PCR (qRT-PCR) was performed to investigate mRNA expression levels of PRG4 in baboon synovium and disc cells. Standard PCR was performed on baboon cartilage, disc, and synovium to determine the presence of PRG4 alternatively spliced transcripts using primers designed to amplify the spliced regions.

Results: qRT-PCR results indicated PRG4 transcripts are expressed 8,000 fold higher in the synovium than in the disc. Standard PCR suggests the presence of differential alternative splicing of PRG4 among the various cDNAs tested with amplicons corresponding to expected sizes of alternatively spliced products.

Conclusion: These studies show for the first time that one or more splice variants of PRG4 are expressed in the TMJ of baboons. We anticipate that understanding better the functions of this molecule will provide important insights into early molecular mechanisms that may be involved in common degenerative TMJ diseases.

Masseter Muscle Exhibits Impaired Repair Following Eccentric Muscle Contraction

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Introduction: Muscle tension is strongly related to temporomandibular disorders and craniofacial pain. One craniofacial muscle, the masseter, is of particular interest, because recent evidence indicates that it has a reduced regenerative capacity compared to limb muscles. This study investigated whether masseter muscle contraction induces muscle damage, inflammation, and regeneration.

Methods: Eccentric muscle contraction (n=500) was produced using surface electrodes in anesthetized male rats (n=80). Muscle damage and inflammation was quantified using behavioral, histological, immunohistochemical and stereological techniques.

Results: Nocifensive head withdrawal thresholds were significantly decreased ipsilaterally (4 hrs. - 4 days) following eccentric contraction while no changes were found in the contralateral threshold. Muscle edema was analyzed by comparing the wet/dry muscle weight (n=6). These results indicated a 5% increase in the wet weight of the contracted muscle. Plasma extravasation, measured via Evans Blue method, was significantly elevated in the contracted masseter 24 hours following eccentric contraction. No changes were present in the skin overlying the masseter nor in the contralateral masseter or skin. Inflammation and damage were evident in hemotoxylin and eosin (H & E) stained muscle sections, after 96 hours. The experimental muscle exhibited significantly more angular-shaped ($p<0.001$) and compacted ($p<0.01$) myofibers than control myofibers, indicative of myofiber damage. Immunohistochemical analysis showed infiltration of ED1 macrophages within 48-96 hours after muscle contraction. Stimulated muscle showed only minimal signs of muscle repair in both H & E and immunohistochemical sections 45 days after muscle contraction.

Conclusion: This study shows that the muscle contraction model used here produces an inflammatory response. The observed impaired ability for repair and regeneration of the masseter muscle may become an important factor in craniofacial muscle disorders.

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Regulatory Genes Involved in TMJ Formation

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Introduction: Temporomandibular joint disorders (TMJD) represent a significant health problem and a major source of non-dental orofacial pain. The significant heterogeneity in TMJD has not been well characterized and there are no known biomarkers to define TMJD subsets. Thus, we sought to obtain a better understanding of TMJ biology by characterizing genes involved in TMJ development.

Methods: 7μm cryosections of E16 mouse embryos were subjected to Laser Capture Microscopy. RNA from captured articular surfaces of condyle and glenoid fossa was amplified and analyzed in triplicate using Affymetrix GeneChip mouse genome array 430 2.0, with coverage of over 34,000 well characterized transcripts. Microarray analyses were carried out using the GeneSifter program.

Results: 3,035 probe sets were enriched >2-fold in the condyle and 1,465 probe sets were enriched >2-fold in the fossa, when compared to an E16 whole embryo reference sample. Triplicates showed high correlation. 220 condyle candidate genes represented signaling molecules and 190 represented transcription factors. Among these were members of the Notch family, POU domain transcription factors, zinc finger proteins and bone morphogenetic protein family members (BMPs). Similarly, among the genes enriched in the fossa, 170 candidates represented genes encoding signaling molecules and 95 represented transcription factors. Examples of these included catenin, a member of the Wnt signaling pathway, a BTB (POZ) domain containing gene, and a member of the Msx family. Most of these genes are known to play key roles in patterning. Approximately 60% of the genes obtained encoded signaling molecules or transcription factors specific to one tissue compartment.

Conclusions: The identification of differentially expressed genes present in the articular surfaces of the TMJ will provide a starting point for dissecting the signaling and transcriptional pathways controlling TMJ development. In addition, the genes may potentially serve as novel markers for more accurate diagnosis for disorders affecting the TMJ.

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Models of Stress and Allostatic Load as Applied to Complex, Multi-System Disorders

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The past century has seen a profound shift in diseases of humankind. Acute, unifactorial diseases, such as from infection by a single bacterial strain, are being replaced increasingly by chronic, multifactorial disorders that arise from complex interactions among genes, environment, and time. Temperomandibular joint disease, chronic fatigue syndrome, postural tachycardia syndrome, neurocardiogenic syncope, pseudopheochromocytoma, fibromyalgia, and metabolic syndrome may be examples of this phenomenon. These disorders involve dysfunction of multiple body systems, in somewhat overlapping syndromes, although each is typically diagnosed and treated by the most prominent symptoms or signs within a single system. Researchers in cardiology, neurology, endocrinology, rheumatology, and psychiatry must develop and apply fundamentally new concepts to take into account this development, by appreciating the multi-disciplinary, integrative, and often "mind-body" nature of chronic multi-system disorders. According to the concept of allostasis, there is no single, ideal set of steady-state conditions in life. Allostasis reflects active, adaptive processes that maintain apparent steady states, via multiple, interacting effectors regulated by homeostatic comparators—"homeostats." Stress can be defined as a condition or state in which a sensed discrepancy between afferent information and a setpoint for response leads to activation of effectors, reducing the discrepancy. "Allostatic load" refers to the consequences of sustained or repeated activation of mediators of allostasis. From the analogy of a home temperature control system, the temperature can be maintained at any of a variety of levels (allostatic states) by multiple means (effectors), regulated by a comparator thermostat (homeostat). Allostatic load and risks of system breakdown increase when, for example, the front door is left open in the winter. Applying these notions can aid in understanding how acute and chronic stress might exert adverse health consequences via allostatic load. This abstract presents models of homeostatic systems that incorporate negative feedback regulation, multiple effectors, effector sharing, environmental influences, intrinsic obsolescence, and destabilizing positive feedback loops. These models can be used to predict effects of environmental and genetic alterations on allostatic load and therefore on the development of chronic multi-system disorders.

Sex Differences in the Nociceptive Processing of Temporomandibular Disorder (TMD) Patients

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Introduction: Our previous work demonstrated that female TMD patients exhibit enhanced nociceptive processing compared to age matched, pain-free controls. This enhancement, measured as increased temporal summation of pain, was found on the hand – a body site distant from the region of clinical pain. This finding suggested that these patients had a body-wide increase in central nervous system (CNS) nociceptive processing. Our model incorporating these observations was that this central nociceptive hyperexcitability was one component contributing to the pain of TMD, and could explain why minor and sometimes even undetectable pathology of the TMJ region could result in a distressing chronic pain condition. These previous studies only evaluated women, since an overwhelming majority of TMD patients are women. We now describe the results of testing male TMD patients in the same manner.

Methods: Our temporal summation of mechanical pain protocol was used as previously. Sharp probes are repeatedly pressed against the skin of the finger, while the subject reports the perceived pain intensity for the 1st, 5th, and 10th stimulus of the series. While the stimulus force is the same each time, rapid repetition of the stimuli produces a gradually increasing perception of pain, which is attributable to CNS nociceptive signal summation.

Results: In marked contrast to the female TMD patients, the male TMD patients do not show any temporal summation of pain. Additionally, the male TMD patients are different from healthy male subjects, who do show significant temporal summation, but less so than either healthy women or women with TMD.

Conclusion: The central nociceptive processing upregulation thought to be contributing to TMD pain for women is not present in men. These results suggest a distinct sex difference in the role of altered nociceptive processing for TMD pain.

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Impact of Psychosocial Factors on Pain in Adults with Temporomandibular Joint Disorder (TMD)

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Introduction: Pain is the primary presenting complaint in patients with TMD. An extensive literature documents relationships between pain and psychosocial factors (e.g., stress, negative mood, catastrophizing). Catastrophizing, a negative cognitive and affective response to pain that includes elements of magnification, helplessness, pessimism, and rumination, is reliably associated with heightened pain experience and emotional distress across many chronically painful conditions. Some research indicates that individuals high on catastrophizing show more pain behaviors in the presence of another person.

Methods: We examined the relationship between catastrophizing and two important dimensions of pain: self-reported daily life pain and pain reported during a painful examination that included palpation of masticatory muscles (MAST), neck muscles (NECK), and head muscles (HEAD). Individuals (N=111; 76% female) were being screened for participation in a RCT comparing the effects of nortriptyline and cognitive-behavioral therapy in reducing TMD-related pain. Sex differences were observed on MAST, NECK, and overall pain on palpation (POP).

Results: Multivariate analyses examining correlates of pain during daily life revealed lower education and greater catastrophizing to be significant predictors of higher daily life pain. Regression analyses found younger age, greater catastrophizing, and higher daily life pain to be associated with greater MAST. Similarly, more depressive symptomology and higher daily life pain were found to be significant predictors of greater NECK. Lastly, regression analyses found more depressive symptoms, greater catastrophizing, and higher daily life pain to be predictive of greater POP.

Conclusion: Generally, higher levels of pain-related catastrophizing were consistently associated with great self-reported pain during a dental examination as well as pain during daily life. Depressive symptoms and pain during daily life were also associated with pain reported during a dental examination. These findings indirectly suggest that interventions that increase adaptive coping and decrease catastrophizing may help to buffer some of the deleterious functional consequences of TMD.

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