A Rat Model of Temporomandibular Joint Pain with Histopathologic Modifications

Steven B. Nicoll, PhD
Assistant Professor
Department of Bioengineering
University of Pennsylvania
Philadelphia, Pennsylvania, and
Associate Professor
Department of Biomedical Engineering
The City College of The City University of New York
New York, New York

Christopher K. Hee, BS
Graduate Student
Department of Bioengineering
University of Pennsylvania
Philadelphia, Pennsylvania

Martin B. Davis, MS
Research Specialist
Department of Neurosurgery
University of Pennsylvania
Philadelphia, Pennsylvania

Beth A. Winkelstein, PhD
Associate Professor
Department of Bioengineering, and
Associate Professor
Department of Neurosurgery
University of Pennsylvania
Philadelphia, Pennsylvania

Correspondence to:
Dr Beth A. Winkelstein
Department of Bioengineering
University of Pennsylvania
210 S 33rd Street
240 Skirkanich Hall
Philadelphia, PA 19104
Fax: 215-573-2071

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Aims: *To develop a rat model of temporomandibular joint (TMJ) pain and to characterize in it the development and temporal response of behavioral hypersensitivity as well as to evaluate if and to what extent a loading protocol is associated with histological changes in the TMJ consistent with osteoarthritic pathology.* ***Methods:*** *A novel rat model of TMJ pain was developed using a noninvasive, mechanical loading protocol. Rats were exposed to steady mouth-opening for 7 days (2 N force, 1 hour/day), and mechanical hyperalgesia (increased pain response) was measured during the loading period and for 14 days thereafter. Histological modifications in the joint cartilage were also evaluated. Outcomes for the mouth-opening exposure were compared to age-matched controls. Thresholds for evoking responses were compared using a ranked ANOVA with repeated measures.* ***Results:*** *Increased mechanical hypersensitivity in the temporomandibular region developed during daily loading and persisted even after the termination of the loading protocol. Histologic characterization revealed thinning of the cartilaginous structures of the joint and irregular zonal cellular arrangements in the condylar cartilage of rats subjected to the daily loading protocol.* ***Conclusion:*** *The injury model presented here is the first to demonstrate mechanically-induced behavioral hypersensitivity accompanied by osteoarthritic pathology in the TMJ.* J OROFAC PAIN 2010;24:298–304

Key words: collagen, condylar cartilage, glycosaminoglycans, osteoarthritis, pain

Studies on the etiology of temporomandibular joint (TMJ) disorders have demonstrated the importance of osteoarthritis (OA) as a major pathology in the joint.[1,2] OA is characterized by degeneration of articular cartilage, with associated morphological changes that include fibrillation, erosion, and eventual loss of condylar cartilage.[1] Such structural alterations may lead to compensatory or pathological alterations in the surrounding tissues (ie, synovium, ligaments, muscles), resulting in symptoms of craniomandibular dysfunction, including pain and stiffness, limited motion, and crepitus.[1,3]

Mechanical overloading of the TMJ has been implicated as a major causative factor in the onset of OA and related orofacial pain disorders.[1] However, the pathophysiologic and cellular mediators that underlie the development of such chronic orofacial pain are not well understood, nor has a relationship to mechanical loading been defined. Several experimental models have been

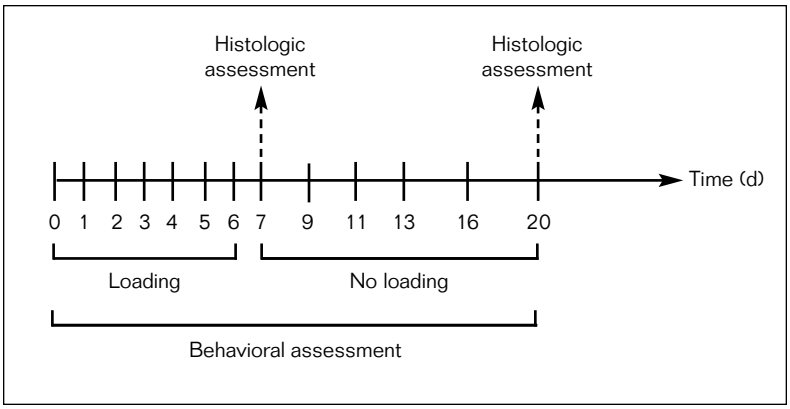

Fig 1 Schematic outline of the study design and outcomes relative to the steady mouth-opening loading protocol.

developed to examine causative factors in TMJ OA progression. Such models often involve intra-articular injections of inflammatory or catabolic agents, such as interleukin-1α or collagenase, to promote the onset of OA, or utilize invasive surgical manipulation of tissue structures in order to alter the kinematics and stability of the joint.[4–8] For example, severing of the discal attachments followed by anterior displacement of the disc has been employed in a rabbit model, while disc perforation and scraping of the condylar surface have been used in sheep models to induce OA symptoms.[6–8] A limitation of the above approaches is that they introduce artificial damage to the joint structures and do not approximate the clinical disorder of mechanically-induced TMJ OA.

Given the role of excessive mechanical loading in the onset of OA, recent investigations have employed forced jaw-opening protocols to establish animal models of mechanically-induced OA that present pathology (ie, formation of cartilaginous lesions) analogous to that observed in humans.[9–11] While these reports suggest OA-associated *histopathology* may result from mechanical loading alone, there has been no examination of such a loading protocol on the onset and/or maintenance of *pain*. Therefore, the purpose of this study was to develop a rat model of TMJ pain and to characterize in it the development and temporal response of behavioral hypersensitivity as well as to evaluate if and to what extent a loading protocol is associated with histological changes in the TMJ consistent with osteoarthritic pathology. It was hypothesized that daily steady-mouth opening is sufficient to induce sustained behavioral sensitivity in the region of the TMJ.

Materials and Methods

Experimental Design

Rats were exposed to steady mouth-opening for 7 days, and mechanical hyperalgesia (increased nociceptive response) was measured during the loading period and following its cessation to evaluate nociceptive behavior (Fig 1). In addition, the TMJs were assessed for modifications in joint cartilage structure at the first day following the cessation of joint loading (day 7) and also at 14 days after loading (day 20) to identify indicators of OA-like disease progression (Fig 1).

Animal Procedures and Loading Protocol

Male Holtzman rats (Harlan Sprague-Dawley), weighing 397 ± 93 g were housed according to the conditions specified by the international and United States regulatory bodies for the care and use of laboratory animals. All experimental procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee and carried out under the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.[12] All rats were housed with a 12–12 hour light-dark cycle and free access to food and water. Mouth-opening was induced to load the TMJ in situ; all joint-loading procedures were performed under halothane inhalation anesthesia (4% for induction and 3% for maintenance during loading procedure). The loading paradigm used in this study was based on that described by Fujisawa et al,[9] for producing OA-like lesions in the TMJ following daily application of steady mouth-opening in the rabbit. For each loading session, rats were placed in a prone position and the mouth was opened via a customized device that

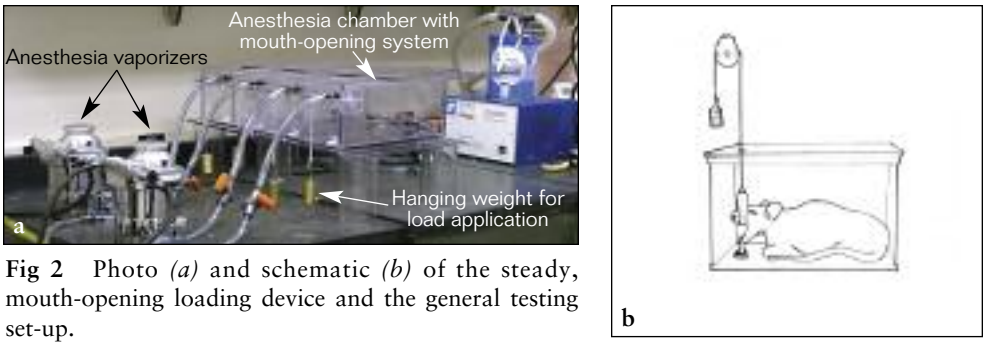

Fig 2 Photo *(a)* and schematic *(b)* of the steady, mouth-opening loading device and the general testing set-up.

held the mandible in a fixed position and opened the maxilla via coupling to a customized sling and pulley system that applied 2 N of force (Fig 2). Each mouth-opening session was applied for 1 hour on each of 7 consecutive days (days 0 to 6) (Fig 1). The maxilla opening load of 2 N was selected to be sufficiently below the load that dislocates the rat TMJ (unpublished findings from pilot studies). Following each loading session, each rat recovered in room air on a heated pad. All rats were monitored continuously during loading and for the period of recovery.

Behavioral Testing

Behavioral testing was performed by a single investigator to assess the induction and maintenance of tactile hypersensitivity in the region of the TMJ. Accordingly, mechanical hyperalgesia was assessed several days before the start of the loading protocol and throughout the study duration by methods used to measure orofacial sensitivity in models of inflammatory TMJ pain.[13–15] Briefly, an ascending series of Semmes-Weinstein von Frey monofilaments (Stoelting) was applied to the skin around the TMJ, starting with a 1.6 mN filament strength. Each filament strength was used to apply mechanical stimulation to the TMJ region five times and the threshold for generating a nociceptive response was defined as the filament producing at least three head withdrawals. Thresholds were confirmed by applying the next higher filament in the series to verify a positive response. Threshold testing was performed separately for each of the right and left joints, with each side undergoing three rounds of testing and the mean value taken as the threshold for that side on each day. Behavioral assessment was performed each morning before the application of loading (on days 0 to 6) and then every other day during the week after the termination of daily loading (days 7 to 13) and on days 16 and 20 (Fig 1). All behavioral assessments were performed without the use of anesthesia. In order to control for variability in the individual responses of each rat, the baseline (day 0) value was taken as the response threshold without loading (control). Because discrete filament strengths were used, thresholds were compared using a ranked ANOVA with repeated measures, with time and loading treatment as the two factors. A Tukey's honestly scientific difference (HSD) post-hoc test was used to determine significance ($P < .05$). Statistical analysis was performed using SYSTAT version 10.2 (Systat Software).

Histological Analysis

The left and right TMJs were harvested for histological analysis at two time points after the cessation of loading as well as from unloaded control rats (Fig 1). The unloaded joints that were harvested for comparison were taken from age- and weight-matched rats for each time point that was assessed (n = 5). In one group of rats, joint samples were harvested at 1 day after the cessation of joint loading (day 7, n = 4); and in a separate group, tissue was harvested at 14 days after the cessation of loading (day 20, n = 4). Rats were randomly assigned to each loading group. Specimens were fixed in acid-formalin/ethanol,[16] decalcified in 10% ethylene-diamine-tetra-acetic acid for 1 week at 4°C, dehydrated in a graded series of alcohol, embedded in paraffin, and serially sectioned (8 μm thickness) along the sagittal plane by using a rotary microtome (Leica model RM 2030). Slides were stained with 1% Alcian Blue (in 3% acetic acid) and 0.1% Picrosirius Red to visualize glycosaminoglycan and collagen localization, respectively.[17] Additional slides were stained with

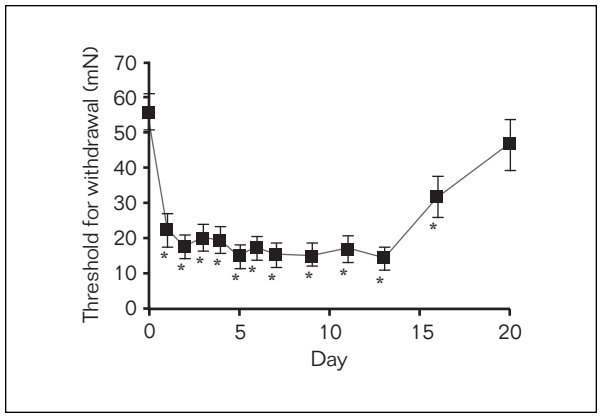

Fig 3 Mechanical hypersensitivity as measured by the average response threshold (mean ± SEM) to von Frey filament stimulation. Increased sensitivity corresponds to a reduced response threshold (n = 8 rats through day 7, n = 4 through day 20). Mouth-opening significantly reduced (*P < .036) thresholds below baseline control responses for all days except on day 20.

hematoxylin Gill number 2 and eosin Y alcoholic (Sigma) to assess cellular organization and cartilaginous tissue structure. Samples were viewed with a Zeiss Axioskop 40 optical microscope, and images were captured using AxioVision software (Carl Zeiss).

Results

All rats (n = 13) exhibited signs of thriving over the study period, with steady weight-gain (approximately 3g per day) during the loading and post-loading periods. Pilot studies assessing the effects of daily exposure to the anesthesia protocol were performed using normal age- and weight-matched rats and demonstrated no effect on weight-gain or behavioral responses in those rats (data not shown). There were no differences in the threshold for eliciting a response between the right and left sides for any rat; as such, the left and right thresholds for withdrawal were averaged for each rat.

The baseline threshold for head withdrawal was 55.6 ± 25.1 mN (Fig 3). This threshold decreased to 18.3 ± 15.7 mN during the period of daily loading (days 1 to 6) (Fig 3) (n = 8), indicating an increased mechanical sensitivity in the temporomandibular region. Sensitivity in the area of the TMJ persisted even after the cessation of the loading protocol (Fig 3). The withdrawal threshold was significantly (P < .036) reduced for each day compared to the baseline control thresholds, with the exception of that measured 14 days after the cessation of loading (day 20) (Fig 3).

Histologic characterization at day 7 (1 day after the termination of loading) (n = 4) showed thinning of the condylar cartilage and articular disc in the TMJs of all rats subjected to the repeated daily mouth-opening loading protocol (Figs 4b and 4e) (n = 4) in comparison to unloaded, normal rats (n = 5) (Figs 4a and 4d). This thinning of the cartilaginous tissues persisted throughout the duration of the study with no evidence of repair at day 20 (n = 4) (14 days following the cessation of loading) (Figs 4c and 4f). In addition, Alcian Blue and Picrosirius Red staining revealed a loss of both glycosaminoglycans and collagen from the extracellular matrix in the loaded joints as indicated by a reduction in staining intensity in the articular cartilage and disc (Figs 4a to 4c). Also, the condyles in the joints of normal animals displayed four distinct regions in the articular cartilage (fibrous, proliferating, mature, and hypertrophic) and characteristic zonal cellular arrangements (Fig 4d). In contrast, the joints from rats undergoing loading exhibited truncated regions in the condylar cartilage and irregular cellular alignment (Figs 4e and 4f). No histological differences were observed between the right and left joints from each animal.

Discussion

Orofacial pain has been studied using a host of well-characterized experimental models,[6,7,14,18–22] the majority of which utilize intra-articular injections in the TMJ.[14,15,20,23] In these studies, the time course of the onset and maintenance of changes in

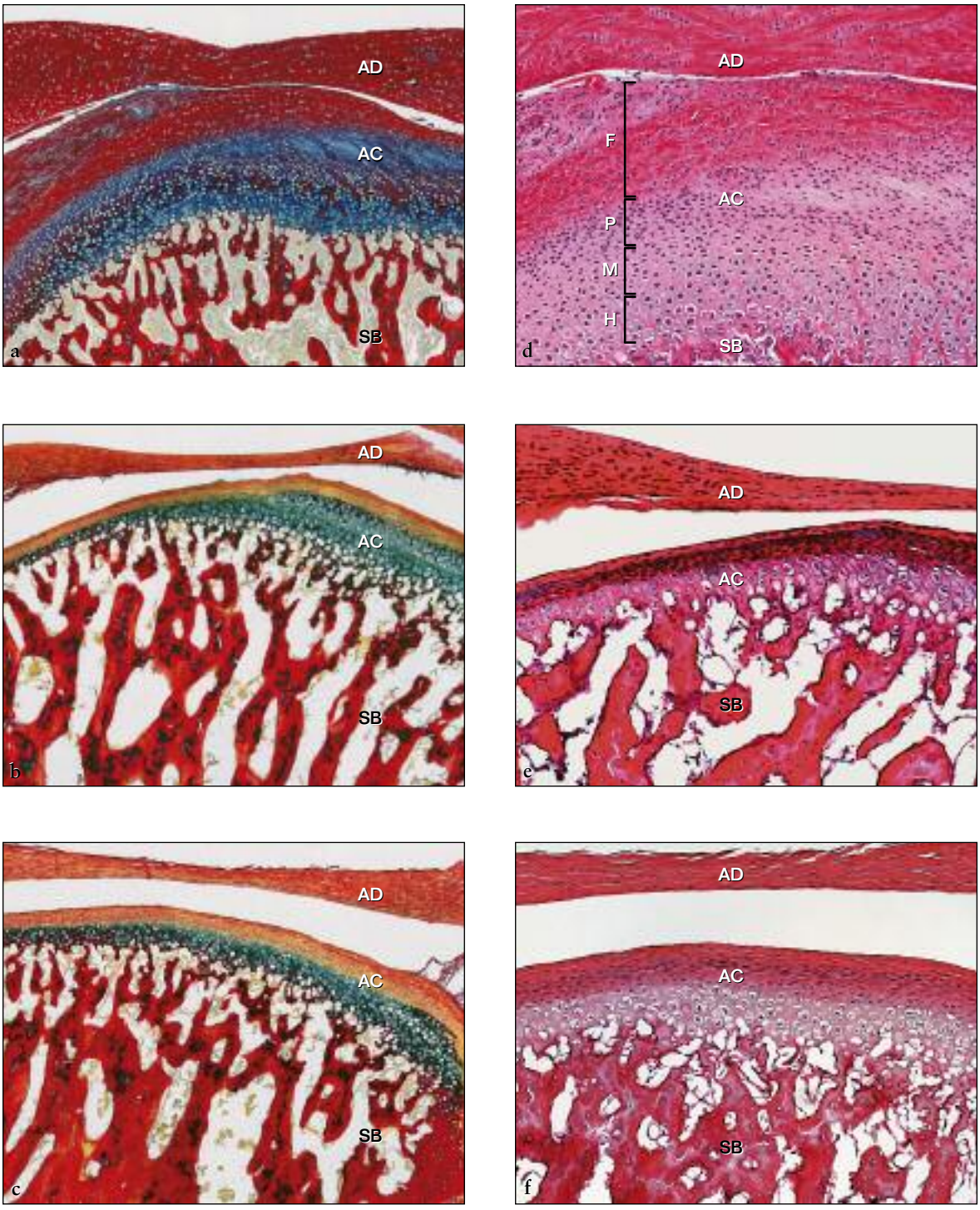

Fig 4 Representative histology of the rat TMJ in normal animals (*a, d*) and those subjected to a steady, mouth-opening loading protocol at day 7 (*b, e*) (1 day after the termination of loading, n = 4) and day 20 (*c, f*) (14 days after the end of loading, n = 4). The articular disc (AD), articular cartilage (AC), and subchondral bone (SB) are indicated in the micrographs, as are the fibrous (F), proliferating (P), mature (M), and hypertrophic (H) cellular zones of the condylar cartilage (panel D). Scale bar = 200 μm (*a through c*) and 100 μm (*d through f*). *Left* column = Alcian blue and picrosirius red; *right* column = hematoxylin/eosin.

the behavioral sensitivity in the orofacial and temporomandibular regions has been well-characterized; increased sensitivity is immediate (as early as 1 day after injection) and can persist for nearly 2 weeks.[14,20,22] The noninvasive TMJ injury model presented here is the first to demonstrate mechanically-induced behavioral hypersensitivity accompanied by osteoarthritic pathology. Mechanical hypersensitivity produced in this model was sustained even after the termination of joint loading, and is consistent with that shown in other models of TMJ pain,[13,14] but without requiring invasive techniques or chemical insults.

Changes in joint tissue, characterized by destruction of condylar cartilage and pathology in the TMJ capsule, have been reported following injection of inflammatory substances such as adjuvants and degradative enzymes.[5,14] For example, Shinoda et al[14] noted severe erosion of the condylar cartilage and increased fibrosis in the synovial membrane, underlying connective tissue, and joint capsule after injection of complete Freund's adjuvant, providing evidence of an association between local changes in the joint tissue and an increased orofacial pressure sensitivity in that TMJ pain model. However, unlike rheumatoid arthritis, OA originates primarily through noninflammatory mechanisms. Therefore, chemically-induced models of TMJ OA are not accurate representations of the disease. Alternative noninvasive models of TMJ disorders based on mechanical overloading served as the basis for the loading paradigm in the current study. Fujisawa et al[9] first reported a mechanical-stress-induced model of TMJ OA due to daily steady mouth-opening in the rabbit. In their model, steady forced jaw-opening (2N for 3 hours/day for 5 days) produced a loss of cartilage and proliferation of chondrocytes at the articular surface of the condyle at 7 days following termination of loading. Proteoglycan expression was greatly reduced at 1 day after the end of loading but returned to naïve levels over time at sites of nested chondrocyte proliferation. Although that study provided support for a nonsurgically-induced model, it did not measure nociceptive behavior associated with OA in the TMJ, and modifications in joint tissue structure were not sustained. In the experimental model used in the present study, behavioral hypersensitivity was coupled with irreversible thinning of the cartilaginous structures of the TMJ and disruption of zonal cellular alignment, consistent with early signs of degeneration and progression to OA.[1] More recently, a forced jaw-opening protocol was applied in rats with a prescribed separation between maxillary/mandibular incisors (3 hours/day) that demonstrated OA-like lesions and altered jaw muscle activity 2 weeks after the end of loading.[11] As such, future studies are necessary to determine if the observed increased behavioral sensitivity in the present steady mouth-opening model is due to injury of the orofacial muscles or ligaments which stabilize the joint rather than to degeneration of the TMJ cartilage. The marked loss of glycosaminoglycans and collagen from the cartilaginous structures of the joint following steady mouth-opening is in agreement with previous investigations employing anterior disc displacement to promote OA in the rabbit TMJ.[24–26] Specifically, these investigators observed a reduction in staining intensity for multiple sulfated glycosaminoglycans (ie, keratan sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate) and collagens (types II, VI, and IX) in the condylar cartilage and articular disc 2 weeks after surgical induction of disc displacement. Altered glycosaminoglycan and collagen profiles in osteoarthritic joints have been attributed to both decreased biosynthesis and increased degradation of the matrix components.[27,28] However, the present study did not explore the mechanisms of extracellular matrix macromolecule depletion or those that gave rise to the observed cartilage thinning.

Taken together, the present results suggest that the forced jaw-opening loading protocol may provide a useful *in vivo* model to define those mechanisms that lead to painful OA of the TMJ and may offer a viable platform to evaluate treatment alternatives for TMJ OA. In particular, the system may be used to assess pharmacologic antagonists to specific cellular and biochemical mediators of TMJ pain and inflammation as well as to investigate tissue-engineering approaches for restoration of joint structures and functions.

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