Analysis of Pain in the Rabbit Temporomandibular Joint After Unilateral Splint Placement

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Information in this paper has been presented at the following conferences: International and American Association for Dental Research General Session, Seattle, Washington, March 2013; Orthopaedic Research Society Annual Meeting, New Orleans, Louisiana, March 2014; and American Association for Dental Research Annual Meeting, Charlotte, North Carolina, March 2014.

Aims: To determine whether behavioral, anatomical, and physiologic endpoints widely used to infer the presence of pain in rodent models of temporomandibular disorders (TMD) were applicable to the rabbit model of TMD associated with altered joint loading. **Methods:** Unilateral molar dental splints were used to alter temporomandibular joint (TMJ) loading. Changes in nociceptive threshold were assessed with a mechanical probing of the TMJ region on nine splinted and three control rabbits. Fos-like immunoreacitvty in the trigeminal subnucleus caudalis was assessed with standard immunohistochemical techniques from three splinted and six control animals. Retrogradely labeled TMJ afferents were studied with patch-clamp electrophysiologic techniques from three splinted and three control animals. Remodeling of TMJ condyles was assessed by histologic investigations of three splinted and three control animals. A Student *t* test or a Mann-Whitney *U* test was used with significance set at $P < .05$ to compare splinted to control samples. **Results:** While variable, there was an increase in mechanical sensitivity in splinted rabbits relative to controls. The increase in Fos+ cells in splinted rabbits was also significant relative to naïve controls (86 ± 8 vs 64 ± 15 cells/section, $P < .05$). The rheobase (364 ± 80 pA) and action potential threshold (–31.2 ± 2.0 mV) were higher in TMJ afferents from splinted rabbits compared to controls (99 ± 22 pA and –38.0 ± 2.0 mV, $P < .05$). There was significant remodeling in the condylar fibrocartilage layers as manifested by a change in glycosaminoglycan distribution and a loss of defined cell layers. **Conclusion:** Behavioral and anatomical results were consistent with an increase in nociceptive signaling in concert with condylar remodeling driven by altered TMJ loading. Changes in excitability and action potential waveform were consistent with possible compensatory changes of TMJ afferents for an overall increase in afferent drive associated with joint degeneration. These compensatory changes may reflect pain-adaption processes that many patients with TMJ disorders experience. *J Oral Facial Pain Headache 2015;29:193–202. doi:10.11607/ofph.1371*

Key words: *altered loading, fibrocartilage degeneration, pain assessments, temporomandibular joint, unilateral splint*

Pain is reported to be the most common reason for seeking treatment for temporomandibular disorders (TMD),[1–3] and up to 4% of the population seeks treatment for TMD.[1] One of the mechanisms that can underlie TMD progression is unbalanced or excessive mechanical loading of the temporomandibular joint (TMJ), which can drive changes in the biochemistry and dysfunctional remodeling of the joint fibrocartilage that lead to joint degeneration.[4] The development of effective interventions for the treatment of TMD has been hindered by the dearth of data linking the changes observed in preclinical models to the development and manifestation of the pathology of the human state. While it is clear that a subpopulation of TMD patients suffer from degeneration of the joint,[4] previous histologic analyses have provided little insight into the nature and extent of the degeneration.[5,6] Furthermore, no study has elucidated whether there is a point at which the degeneration becomes irreversible, or whether there is a relationship between the extent of degeneration and the manifestation of pain.

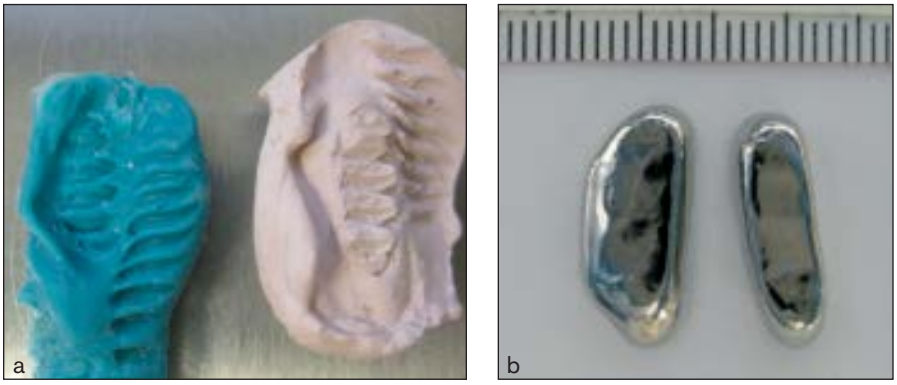

Fig 1 Unilateral molar bite raising splints. **(a)** The splints were made by first taking an impression of the teeth, from which a plaster mold was made. **(b)** The metal splints were cast as crowns; the superior view is shown.

A number of TMD models have been developed in small rodents such as rats and mice in which a variety of behavioral,[7] anatomical,[8] and physiologic[9] endpoints have been used as evidence of pain and/or changes in pain. However, there has been considerably less progress in translating and applying these approaches to larger animal models, such as the rabbit, which may enable the use of analyses not feasible in smaller species. For example, while the main function of the TMJ is mechanical support of jaw movement, it is difficult to determine the mechanical properties of joint tissue from small rodents. Similarly, while parafunctional habits have been linked to the development of TMD, the link between the altered TMJ loading associated with these habits and the manifestation of pain has yet to be determined. To begin to address these issues, a reversible method was developed for inducing TMJ remodeling in the rabbit by using splints; this method enables control over the timing between the onset and resolution of the altered loading. As a first step in the characterization of this model, the aim of this study was to determine whether behavioral, anatomical, and physiologic endpoints widely used to infer the presence of pain in rodent models of TMD were applicable to the rabbit model of TMD associated with altered joint loading.

Materials and Methods

Animal Model

Eighteen skeletally mature, female, New Zealand White rabbits approximately 1 year in age, weighing between 5 and 7 kg, were purchased from Myrtle's Rabbitry, Covance Research Products, and Charles River Laboratories International. All rabbits were examined by a veterinarian prior to use in the study and were found to be in good health. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh and in accordance with the National Institutes of Health guidelines for the use of laboratory animals.

Altered TMJ loading was achieved through the placement of splints, unilaterally over the opposing maxillary and mandibular molar arch of the right side.[5,10,11] Unilateral splints were used as a way to impart sudden abnormal unbalanced occlusion, which could occur in patients who had suffered trauma or a dramatic change in occlusion from a dental procedure such as orthognathic surgery. Splints that were custom made from impressions taken from each rabbit (Fig 1a) were cast as crowns (of nonprecious metal) on molds made from the impressions (Fig 1b). The thickness of each splint was approximately 1 mm, which was about one-third of the height of the molars (approximately 3 mm). The 1-mm thickness was chosen because thinner splints could not be manufactured easily, and much thicker splints did not allow for the rabbit to close its mouth. Splints were subsequently attached to the appropriate arch with dental cement (FujiCEM 2, GC Corp) following cleaning (with water and a cotton swab to remove food debris) and priming teeth (with 34% phosphoric acid tooth conditioner gel, Dentsply). The retention of the splints was checked at 1 week post–splint placement. Both the collection of impressions and the placement of splints were performed with intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg) used for sedation and inhaled 2% isoflurane used to establish and maintain a surgical plane of anesthesia. After 6 weeks, the rabbits were euthanized (100 mg/kg pentobarbital) and the brainstems and trigeminal ganglia were harvested and processed for immunohistochemistry and whole cell patch clamp, respectively. TMJ condyles were also harvested for histology. Of the 18 rabbits used in the study, 12 received splints and the remainder were untreated controls. Some of the tests were performed on the same rabbits, such as behavioral and electrophysiologic recording.

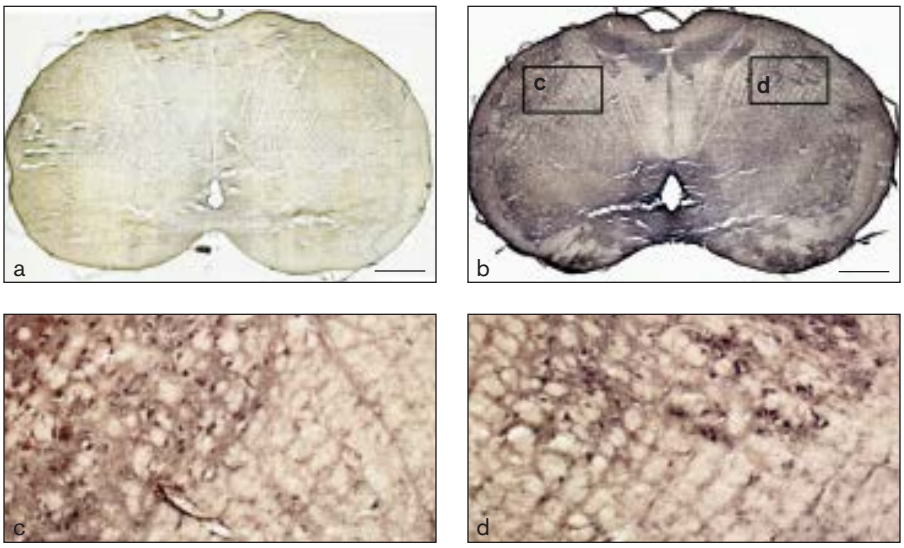

Fig 2 c-Fos immunohistochemistry. **(a)** Negative control showing no staining (scale bar = 1 mm). **(b)** Fos staining on a whole brainstem (scale bar = 1 mm). Regions counted were boxed off. **(c, d)** Regions in which the Fos+ cells were counted, totaled, and averaged for comparison between splinted rabbits and control rabbits.

Behavioral Nociceptive Testing

Previously, joint degeneration has been correlated to pain in small rodent models, such as rats and mice, by using behavioral approaches. The use of calibrated von Frey hairs (VFHs) has been one approach applied in rodent models of TMD pain that assesses the presence of hyperalgesia and allodynia in the skin overlying the TMJ.[7] Mechanical nociceptive threshold was assessed with an electronic VFH device (IITC Life Science Inc) by using a protocol similar to that developed for assessing TMJ sensitivity in the rat.[7] Briefly, the rabbits were habituated to handling for at least 3 days prior to baseline nociceptive testing and were unrestrained for testing. The skin over the TMJ was shaved at least 1 day prior to nociceptive testing. The electronic VFH device fitted with a blunt tip (5-mm diameter) was applied to the skin over the TMJ. The stimulus was applied three times, with a stimulus interval of several seconds. A positive response was noted when the rabbit flinched or pulled its head away from the application of the stimulus. Withdrawal frequency data were compared over time and between rabbits. Testing was performed bilaterally on nine splinted rabbits. Baseline data were collected for at least 3 days prior to taking impressions, and then measurements were collected twice during the first week after the splinting procedure and once a week thereafter for a total of 6 weeks. Data were also collected on three control rabbits without splints over the course of 6 weeks to determine if there was any change over time for healthy rabbits. Behavioral observations were collected by an investigator who was aware of whether rabbits were from control or experimental groups.

c-Fos Immunohistochemistry (IHC)

There is considerable evidence to support the suggestion that the number of neurons demonstrating immediate early gene, c-Fos-like immunoreactivity (Fos-LI) in the superficial spinal dorsal horn[12] and trigeminal[8] subnucleus caudalis can be used as a measure of nociceptive activity, and therefore nociception. Fos-LI has been used previously to monitor ongoing/inflammation hypersensitivity both in somatic joints and the TMJ.[8,13,14] Thus, as a complementary endpoint to changes in mechanical sensitivity, the presence of Fos-LI was assessed in the brainstem. The brainstem and cervical spinal cord (C1-C2) of three splinted and six control rabbits were harvested and postfixed in 4% paraformaldehyde and cryoprotected in 30% sucrose. Sections (50 μm) were taken from the region of brainstem tissues 3 mm rostral to 6 mm caudal to the obex. Floating sections were processed for IHC as previously described.[8,15] Briefly, the sections were first blocked with normal horse serum (Vector Laboratories), then a goat anti-Fos antibody (c-Fos[4]: sc-52-G, Santa Cruz Biotechnology) was applied, followed by a biotinylated horse anti-goat antibody (Vector Laboratories). The sections were processed with avidin-biotinperoxidase complex (ABC) (Vector Laboratories), and a nickel-cobalt diaminobenzidine (DAB) reaction (Vector Laboratories) so that Fos positive (Fos+) neurons could be identified with black staining of the nuclei. Negative controls for the immunohistochemistry were generated by not using any primary antibody; only the secondary antibody was applied (Fig 2a). At least three random sections per rabbit were analyzed, and the number of Fos+ cells were counted in two standardized regions on the left and right sides of the brainstem in ImageJ software (Figs 2b to 2d). The two regions were totaled for each slide and the cell counts from each rabbit averaged. The experimenter counting Fos+ cells was blinded to the group. Brainstems from three splinted rabbits were compared to six control rabbits.

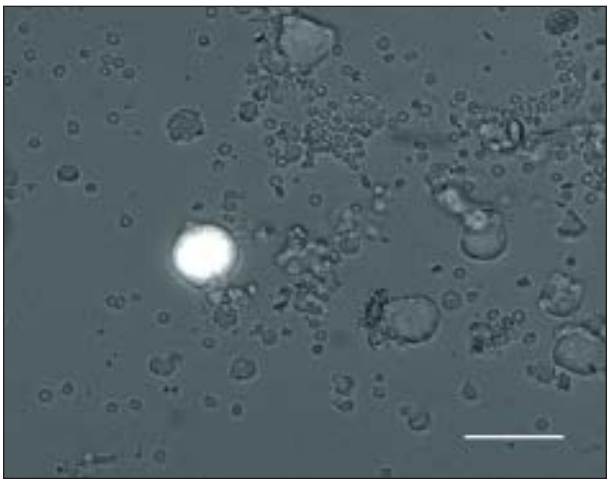

Fig 3 Retrogradely DiI-labeled TMJ afferent. The DiI-labeled neurons were tested with whole cell patch clamp (scale bar = 50 μm).

Data were checked for normality with the Anderson-Darling Test for Normality, and a Student *t* test was used with significance set at $P < .05$ to compare the average number of Fos+ cells in splinted rabbits compared to control rabbits.

Electrophysiology

Electrophysiology has been used previously in rodents to monitor changes in the excitability of afferent neurons innervating the TMJ, under the assumption that sensitization and/or spontaneous activity in putative nociceptive afferents will be associated with ongoing pain and TMJ hypersensitivity.[9] Whole-cell patch-clamp electrophysiologic analysis of acutely dissociated retrogradely labeled TMJ afferents was used as a third measure of altered nociception in the splinted rabbits. Trigeminal ganglia from three control and three splinted rabbits, 6 weeks after splint placement, were harvested and dissociated immediately after the animals were euthanized for study in vitro with protocols developed for rats and mice that were adapted to the rabbit.[9,16] Prior to splint placement on the experimental animals, at the time of impressions while the rabbits were anesthetized, 10 μL of a 17 mg/mL retrograde tracer DiI (1,1×-dioctadecyl-3,3×,3×-tetramethyl indocarbocyanine perchlorate; Molecular Probes) suspension in a saline solution was injected into both the left and right sides of the TMJ.[9,17] The joint capsule was located through palpation, while opening and closing the mouth. The location of the DiI was confirmed at the time of tissue harvest via direct visualization of the joint capsule. A similar procedure was completed on the control rabbits. The assumption was that the ongoing stimulus from the splint should override any effect from the needle. Importantly, however, both control and splinted animals received injections, and comparisons were made between these two groups.

Following dissociation of trigeminal ganglia, DiI-labeled TMJ neurons were readily identifiable under epifluorescence illumination (Fig 3). While it is possible that the rabbit TMJ capsule is innervated by both nociceptive and non-nociceptive afferents, anatomical and electrophysiologic data from rat TMJ afferents in situ suggest that this structure is primarily innervated by C- and Aδ- nociceptive fibers.[18–20] Therefore, all DiI-labeled neurons were studied under the assumption that the majority of recorded neurons are likely to be nociceptive afferents. Whole-cell patch-clamp recording was used to assess the excitability of these neurons. A standardized series of protocols was employed to assess the cell capacitance, action potential threshold, properties of the action potential waveform (overshoot, duration, after hyperpolarization magnitude, and rate of decay of the after hyperpolarization), rheobase, and the response to suprathreshold current injection.[9] Briefly, a single action potential was first evoked with a depolarizing current injection via a 4-ms square pulse to analyze the properties of the waveform. The rheobase, the amount of current injection needed to evoke an action potential, was determined by slowly increasing the current amperage until the pulse evoked an action potential. Then, to assess excitability, a square 750-ms pulse was injected at 1×, 2×, and 3× rheobase, and the number of action potentials evoked at each level was recorded. The slope of the stimulus-response function was compared between control and splinted rabbits. The control data were pooled, as differences between sides were not expected. The experimental data were pooled after no differences were found between the left and right sides. Nociceptive afferents were collected from three splinted rabbits with a total of 20 cells analyzed, and from three control rabbits with a total of 14 cells analyzed. Data were checked for normality with the Anderson-Darling Test for Normality, and Student *t* test or Mann-Whitney *U* test was used with significance set at $P < .05$ to compare the variable values in splinted rabbits compared to control rabbits.

Condylar Histology

Left and right TMJ condyles from control and splinted rabbits (n = 3 per group) were fixed and decalcified in Formical (Decal Chemical Corp) to prepare for standard paraffin embedding and sectioning. Samples were embedded and sectioned by Alizeé Pathology at 6 μm. Slides were stained with Safranin O/Fast Green to visualize the glycosaminoglycans (GAG) and to visualize the shape and distribution of cells in the fibrocartilage.

Table 1 Summary of the Behavioral von Frey Hair Test Results

	Control (n = 3)	Splinted (n = 9)		
Time point	Total no. of rabbits responding	Total no. of rabbits responding	No. of rabbits responding on left side**	No. of rabbits responding on right side
Baseline	0	0	0	0
Week 1	0	2	1	2
Week 2	0	3	2	1
Week 3	0	3	3	1
Week 4	0	3	3	0
Week 5	0	4	4	3
Week 6	0	4	4	2

*Note that adding left and right might not equal the total because some rabbits reacted on both sides.

Results

Behavioral Pain Assessments

The behavioral reactions of the splinted rabbits were varied (Table 1). A positive response was noted when the rabbit flinched or pulled its head away from the application of the stimulus. In the splinted group, three rabbits never responded, one rabbit responded for a time then stopped responding, three rabbits responded after a few weeks and continued to respond through the remainder of the testing period, and two rabbits responded intermittently. The contralateral (left) side appeared to be more affected than the splinted right side, with more rabbits reacting to the stimulus: Of the nine splinted rabbits, four rabbits responded to stimuli applied to the contralateral side and two responded to the ipsilateral/treated side at 6 weeks. The total number of splinted rabbits responding to the applied stimulus increased with time, with two rabbits reacting at 1 week and four rabbits reacting at 6 weeks. Over the course of 6 weeks, six of the nine splinted rabbits reacted to the stimulus at least once. None of the splinted rabbits responded during the first week after splinting. None of the control rabbits reacted to the applied stimulus at baseline or over the course of 6 weeks.

Anatomical Analysis

The Fos-LI was largely restricted to the nucleus of neurons in the subnucleus caudalis, extending from the brainstem into the rostral cervical spinal cord. There were, on average, 64 ± 15 Fos+ cells/section in control rabbits and 86 ± 8 Fos+ cells/section in splinted rabbits. This difference was statistically significant, $P < .05$ (Fig 4). One control brainstem was removed as an outlier from the results because the average number of cells per slide was over three standard deviations away from the average without the data point. There were no differences between the left and right sides of the brainstems for the splinted or control rabbits, so only the total values per section were reported.

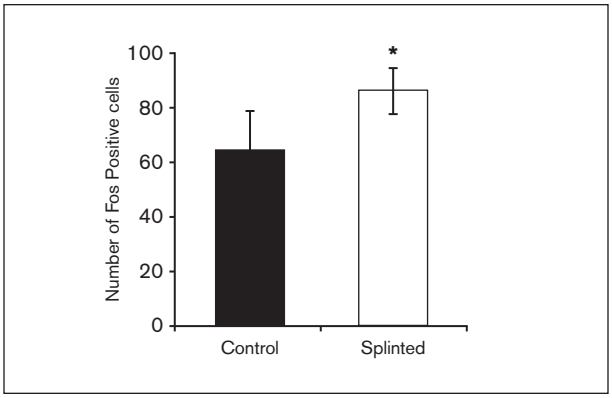

Fig 4 Average number of Fos+ cells were totaled from both regions of splinted and control rabbit brainstems and compared ($P < .05$). Data shown as average ± standard deviation. Samples were collected from three splinted rabbits and six control rabbits.

Electrophysiologic Analysis

For the patch-clamp experiments, the greatest difference between groups was in rheobase or the amount of current required to evoke an action potential. The rheobase (364 ± 80 pA) and action potential threshold (–31.2 ± 2.0 mV) were higher in rabbits with splints compared to rheobase (99 ± 22 pA) and action potential threshold (–38.0 ± 2.0 mV) in the control rabbits ($P < .05$) (Fig 5a). However, there was a trend toward an increase in the number of action potentials evoked in response to suprathreshold stimuli in neurons from splinted rabbits (Figs 5c and 5d). The action potential duration was decreased significantly (from 11.5 ± 1.7 to 6.1 ± 0.9 ms) and the after-hyperpolarization decay time constant was also decreased significantly (from 16.7 ± 1.5 to 13.4 ± 1.2 ms) in neurons from splinted animals when compared to controls ($P < .05$) (Table 2). Action potential waveforms are shown in Fig 5b, and the passive and active electrophysiologic properties of the TMJ neurons are summarized in Table 2.

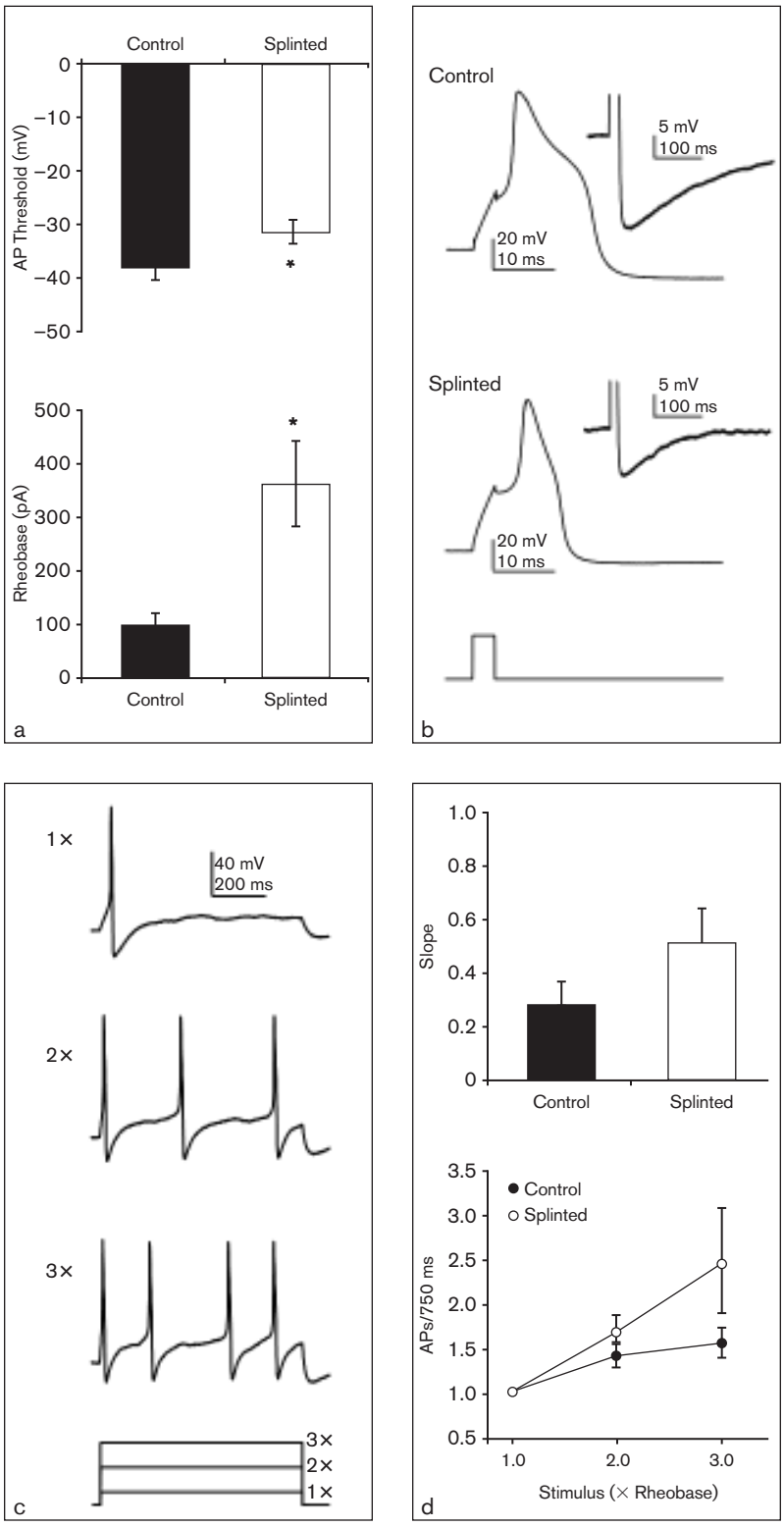

Fig 5 Electrophysiologic results. **(a)** Action potential threshold and rheobase of the TMJ afferent nerves. The action potential threshold was higher than control after 6 weeks of splinting (*P < .05). The rheobase, or the amount of current required to evoke an action potential, was also higher after 6 weeks of splinting (*P < .05). Data presented as average ± standard error of the mean. **(b to d)** Action potential behavior in afferent neurons from control and splinted rabbits. **(b)** Action potentials from a control neuron and a splinted neuron in response to a 4-ms current injection. **(c)** Action potential response to suprathreshold current. 1×, 2×, and 3× rheobase current was injected for 750 ms, and the number of action potentials in response were counted. **(d)** Plot of the number of action potentials (APs) with respect to the stimulus. There was a trend for more action potentials with increasing stimulus for splinted rabbits; however, the slope difference was not statistically significant (P > .05). Putative nociceptive afferents were collected from three treated rabbits, with a total of 20 cells analyzed, and from three control rabbits, with a total of 14 cells analyzed.

Condylar Histology

A change of the GAG distribution in the subchondral layer of the fibrocartilage was observed between the splinted and the control animals (Fig 6). GAG staining was seen throughout the length of the control condyles and the treated side of the splinted rabbits. In the contralateral condyles of the splinted rabbits, the GAG staining ended midway through the anteroposterior

Table 2 Passive and Active Electrophysiologic Properties of Putative TMJ Nociceptive Afferents from Control and Splinted Animals

	Capacitance (pF)	Resting membrane potential (mV)	Amplitude (mV)	Action potential duration (ms)	After-hyperpolarization	
					Amplitude (mV)	Decay time constant (ms)
Control	50.0 ± 3.7	−60.8 ± 1.6	45.4 ± 2.6	11.5 ± 1.7	16.7 ± 1.5	166.4 ± 29.4
Splinted	51.2 ± 5.1	−57.8 ± 1.4	42.6 ± 2.8	6.1 ± 0.9*	13.4 ± 1.2	68.5 ± 7.3*

*$P < .05$.

Putative nociceptive afferents were collected from three treated rabbits with a total of 20 cells analyzed, and from three control rabbits with a total of 14 cells analyzed. Results presented as average ± standard error of the mean.

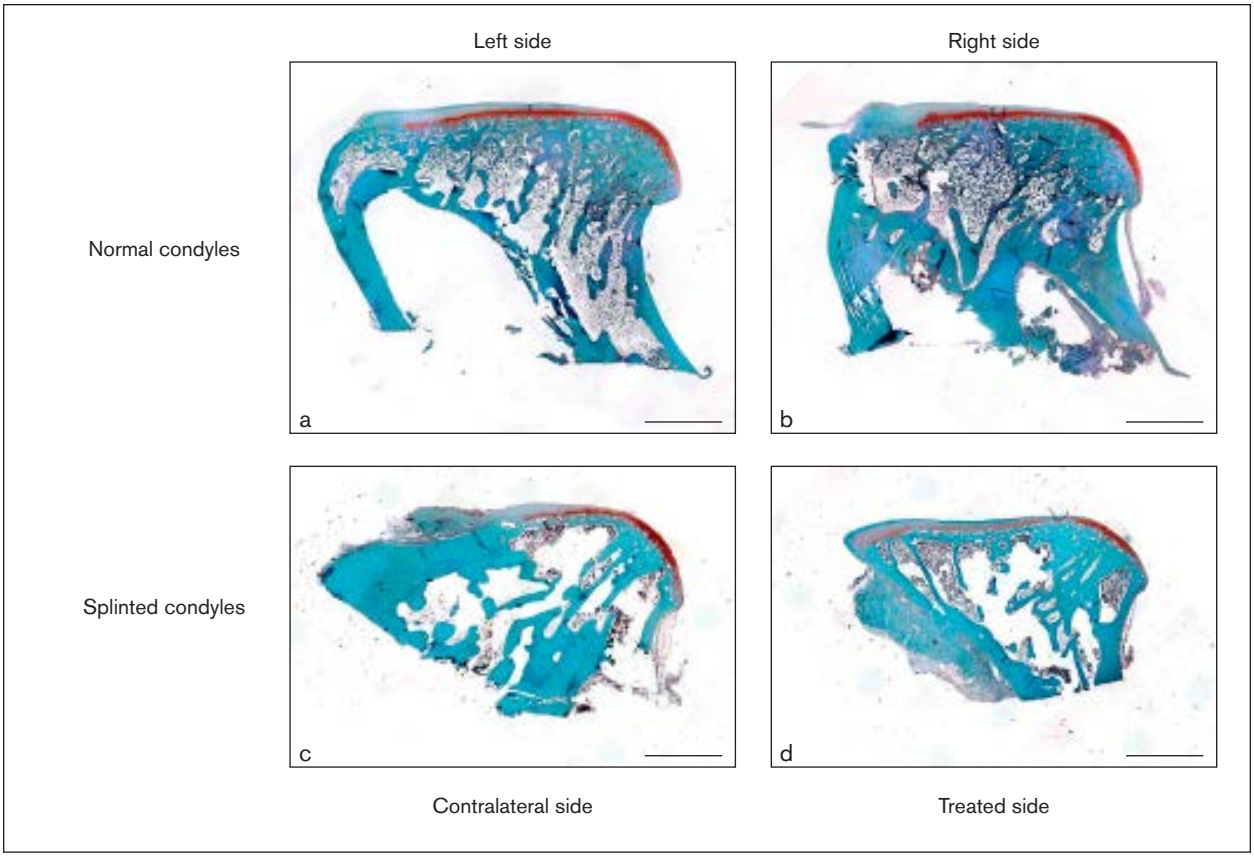

Fig 6 Histology of the TMJ condylar fibrocartilage, showing staining for GAG. The **(a)** left and **(b)** right condyles from normal rabbits and the **(c)** contralateral/left and **(d)** treated/right condyles of the splinted rabbits (scale bar = 2 mm). Images shown are representative samples from n = 3.

length of the condyle, leaving behind a fibrous layer (Fig 6c). The subchondral layer lost definition of the columnar lacunae in both the treated and contralateral condyles from splinted rabbits.

Discussion

The objective of this study was to determine whether endpoints widely used to infer the presence of pain in rodent models of TMD were applicable to a rabbit model of TMD associated with altered joint loading. These endpoints have included increased sensitivity to mechanical probing of the tissue over the TMJ, increases in Fos-LI in the trigeminal subnucleus caudalis, and an increase in the excitability of TMJ afferents. Changes in these endpoints were correlated with changes in joint histology. Six weeks after unilateral splint placement, marked changes in the condylar fibrocartilage were present, which agreed with previous studies.[5,6] These changes were associated with an increase in the response to mechanical probing over the TMJ and Fos-LI. In contrast, a decrease in the excitability of TMJ afferents was observed in splinted animals.

The behavioral changes observed in the rabbit were consistent with results from previous rat studies that showed an increase in the response to mechanical probing over the TMJ following an injection of an inflammatory agent into the joint space.[7] However, differences with previous studies were also observed, where in contrast to rats and mice, there was a limited sensitivity in the rabbit TMJ. The rabbits presented with an all-or-nothing response, and the response was dramatic when observed. This suggests that an alternative behavioral approach may be useful and needed for the rabbit, an approach that lends itself to a more graded assessment of changes in nociception. However, the variability observed in the manifestation of mechanical hypersensitivity may be an important feature of the rabbit model that not only increases its relevance to the clinical picture but may be exploited to help identify the mechanisms underlying the variability in the manifestation of pain in the clinical population of TMD patients.

It is interesting to note that despite the apparently limited sensitivity of the behavioral assay employed, rabbits were more sensitive to mechanical stimulation of the joint contralateral to the splint. This laterality was observed in the absence of evidence for laterality in the changes observed in Fos-LI or electrophysiologic properties of TMJ afferents. These results suggest that the behavioral hypersensitivity is due to changes above the level of the brainstem, perhaps associated with a sensory-motor circuit sensitized in association with the altered pattern of occlusal contact. That said, while most lateral TMD pain appears to be associated with the side associated with occlusal contact,[21] there is evidence of pain at specific sites contralateral to occlusal contact.[22] A more systematic probing of the TMJ and muscles of mastication in the rabbit may have revealed a more nuanced pattern of sensitivity as suggested by the results of this previous analysis of pain in TMD patients.

The Fos-LI data also followed the same pattern previously reported in rat models of TMJ pain,[8,13] where Fos+ cell counts were higher in the experimental animals than the controls. That said, the overall Fos+ cell numbers in the control rabbits were higher than in the control animals in previous small rodent orofacial pain studies. However, it should be noted that Fos+ cell counts comparable to those found here have been reported in studies involving other species, such as cat and guinea pig.[23,24] Nevertheless, the high level of Fos-LI in control rabbits suggests that non-nociceptive inputs drive Fos expression in the rabbit trigeminal subnucleus caudalis, and thus a more specific endpoint may be useful in the rabbit model. And while the splint-induced increase in Fos+ cells observed was significant, there are several likely explanations for the relatively modest increase observed. Differences in the quality and the intensity of the applied stimulus can affect the number of Fos+ cells observed,[25] and most studies in rodents have employed relatively intense stimuli with relatively short intervals between induction of inflammation and analysis of Fos-LI.[8,13,26] Along these lines, while joint degeneration was clearly evident in the rabbit model, the sensitivity to evoked stimuli is particularly problematic in the clinical population and nothing was done to drive additional oromotor activity in the present study prior to tissue harvest. If compensatory changes suggested by the decrease in afferent excitability discussed below were also manifested in the brainstem and/or higher central nervous system centers, the relatively modest increase in Fos-LI may reflect the efficacy of these processes. Additional experiments will be needed to address these possibilities. In the future, it will also be important to determine a detailed time course of the changes in Fos-LI associated with the splint model. The differences that arise between the Fos expression and the VFH test may be due to the relative sensitivity of the VFH test in the rabbit model.

Electrophysiologic results were not only opposite to those expected, but also to those previously reported in rodent models in which an increase in TMJ afferent excitability was observed in association with TMJ inflammation.[9] There are at least four likely explanations for these discrepant results, and these hypotheses will need to be tested in future experiments. The first may simply be due to experimental differences, where increases in excitability were observed after a relatively short period (a few days to a week) of inflammation and a frank inflammatory stimulus was used to drive the changes.[9,16] A very different approach was used in the present study, where the neurons were studied after a considerably longer period of time postinjury, giving the neurons time to compensate for the stimulus. Second, these differences may reflect species differences, such that the increase in nociceptive inputs in the rabbit TMJ model is due to changes in transduction and/or transmitter release, for example, rather than an increase in excitability per se. Even in the rodent literature there is an ongoing debate as to whether the increased mechanical sensitivity observed in models of acute and persistent inflammation are due to an increase in afferent excitability, given that despite robust changes in the response to thermal stimuli, no changes in the response to mechanical stimuli are generally reported in studies involving the skin-nerve preparation.[27–30] Third, while the majority of afferents innervating the TMJ of the rat appear to be nociceptive,[18–20] there is evidence of non-nociceptive afferent innervation in the cat[31] and more relevantly in the rabbit[32] TMJ. This raises the intriguing possibility that TMJ pain may be due to a decrease in non-nociceptive inputs to the

trigeminal subnucleus caudalis, which normally inhibits nociceptive input arising from this structure. Arguing against this possibility, however, was the observation that the electrophysiologic properties of TMJ afferents were relatively homogeneous, while evidence from the rat suggests it is possible to distinguish putative nociceptive from non-nociceptive afferents.[33] Fourth, the changes in excitability and action potential waveform observed are consistent with compensatory changes in TMJ afferents for an overall increase in afferent drive associated with joint degeneration. Given how critical normal TMJ function is to survival, the presence of compensatory inhibitory mechanisms makes intuitive sense. This latter possibility suggests an alternative basis for the manifestation of TMD pain, or at least why joint pathology may not be the best predictor of pain and loss of function, where the failure to engage such compensatory mechanisms may contribute to greater pain and disability observed in subpopulations of TMD patients. Future studies will be needed to determine what is occurring through time to the neurons as they change and compensate for the ongoing pain stimulus.

The electrophysiologic changes are all consistent with an increase in potassium ion (K+) current in TMJ afferents, which include decreased excitability, membrane hyperpolarization, decreased action potential duration, and an increase in after-hyperpolarization amplitude. Currently, treatments for pain are being developed with drugs such as retigabine, which increase K+ channel activity as a means to reduce the nerve activity.[34] In this regard, the present results are particularly striking given that decreases in K+ channel activity have often been identified as mechanisms accounting for the increases in excitability seen in many studies.[34] The rabbit model is therefore one of the first to show evidence of an increase in K+ current in response to a pain-inducing challenge.

The study was sufficient to establish significant changes in joint properties, nociception, Fos-LI, and electrophysiologic properties. While the behavioral changes were variable, suggesting the need for more sensitive assays, the changes with this endpoint were also significant. The potential importance of timing and the ability to recover from the changes in the joint in the absence of another form of intervention suggest that detailed analyses of both onset and recovery will ultimately be important. Furthermore, with a detailed analysis of the timeline of the endpoints employed in the present study, it may be possible to determine whether there is a time point at which changes in the TMJ become irreversible, resulting in the emergence of chronic TMJ pain.

TMD reflect a multifactorial and complex disease process. This study focused on a very particular type of joint degeneration and on discrete parts of the process: altered loading leading to local tissue stress, inflammation, and pain. Taken together, the present data indicate that abnormal TMJ loading can lead to condylar fibrocartilage remodeling. Loss of the subchondral cartilage layer may lead to abnormal fibrocartilage mechanical properties, which if unchecked could lead to further degeneration of the joint. This could be a possible mechanism for the transition from acute to chronic pain in TMD patients. In the acute phase, this fibrocartilage degeneration could also result in the activation of the nerves in the synovium or subchondral bone, and consequently, TMD pain. These findings could help explain why patients in the clinic present with pain but with no apparent damage to their TMJ, as magnetic resonance imaging or panoramic radiography could not detect the changes to the condylar fibrocartilage as observed in the histology of this study. Further development of this rabbit model could lead to a better understanding of TMJ pain and degeneration.

Acknowledgments

The authors would like to acknowledge the help of Dr Kwan Lee in the collection of electrophysiologic data, help of Jesse Lowe with the condyle histology, and funding from the National Science Foundation under grant number 0812348, as well as from the National Institutes of Health under grant number T32 EB003392, the University of Pittsburgh Research Fund, and the University of Pittsburgh School of Dental Medicine. The authors have no conflicts of interest to disclose.

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