# Neural Structures Within the Sheep Temporomandibular Joint

**Abdolghafar Tahmasebi-Sarvestani, MSc**
PhD Student
Department of Anatomy and Histology

**Raymond A. Tedman, PhD**
Senior Lecturer
Department of Anatomy and Histology

**Alastair Goss, DDSc, FRACDS, OMS**
Head
Oral and Maxillofacial Surgery Unit
Department of Dentistry

University of Adelaide
Adelaide, South Australia
Australia

**Correspondence to:**
Dr Raymond A. Tedman
Department of Anatomy and Histology
University of Adelaide
Adelaide, South Australia 5005
Australia

*To better understand pathologic processes associated with arthritis of the temporomandibular joint (TMJ), detailed information on the innervation of TMJ tissues in normal as well as arthritic joints is needed. The aim of this study was to describe the normal innervation of the sheep TMJ in preparation for using this animal as a model for the study of the effects of arthritis on joint innervation. The macroscopic and microscopic appearance plus the distribution of neural structures within the TMJ were examined using fluorescence histochemistry (glyoxylic acid), immunohistochemistry (calcitonin gene–related peptide), silver, and gold chloride techniques. Joints from 10 mature merino sheep were studied. Calcitonin gene–related peptide–immunoreactive nerve fibers were found in the capsule and the synovial membrane, but not in the disc. Nerve bundles and single nerve fibers in the capsule, synovial membrane, and the peripheral 2 to 3 mm of the disc were stained by glyoxylic acid. Ruffini, paciniform-type, and Golgi organ nerve endings plus free nerve endings were located in the capsule, with the highest density of nerve endings occurring at the site of attachment of the disc to the capsule. The highest density of neural structures (using gold chloride) was in the posterior part of the joint. The highest density of autonomic fibers (using glyoxylic acid) was in the anterior capsule. The highest density of sensory fibers (using calcitonin gene–related peptide) was in the synovial and subsynovial tissues of the anterior capsule. These results confirm the existence of autonomic and sensory nerves in the capsule, synovial membrane, and peripheral disc in healthy adult sheep.*
J OROFACIAL PAIN 1996;10:217–231.

**key words:** nerve ending, sheep, temporomandibular joint, immunohistochemistry, calcitonin gene–related peptide, gold chloride

Degenerative changes associated with osteoarthritis or rheumatoid arthritis are common in the temporomandibular joint (TMJ) and often involve pain as well as disruption of normal joint congruity and normal joint movements.[1,2] There is increasing interest in the interaction between disease processes such as arthritis and neural structures, including the mechanisms involved in inflammatory processes. Synovial inflammation, fibrosis, and various cytoskeletal changes are features of these diseases.[3,4] Of the several morphologic and physiologic features of synovium that may provide clues to therapeutic intervention in these diseases, innervation is one aspect that has only recently received some attention. Decreases in the density of fine nerve fibers immunoreactive for substance P (SP), protein gene product 9.5 (PGP 9.5), calcitonin gene–related peptide (CGRP), and neu-

ropeptide Y (NPY) and its C-terminal peptide in synovium from arthritic joints point to some pathologic influence on the innervation of this tissue.[5–8]

To better understand the pathologic processes associated with arthritis of the TMJ, there is a need for detailed information on the innervation of TMJ tissues in normal as well as arthritic joints. The sheep TMJ has been used as a model for the study of TMJ structure and function because the animals are readily available and easily handled, and the sheep TMJ approximates in size and has some features in common with the human TMJ.[9–14] The aim of the present report was to describe the normal innervation of the sheep TMJ. This report is part of a larger study in which the sheep is being used as a model for studying the effect of disease processes such as arthritis on the innervation of the TMJ. Other than the gross anatomic appearance of the nerves supplying the TMJ, nothing is known of the neural structures associated with this joint in the sheep. Consequently, this report describes the appearance and distribution of the neural structures of normal TM joints of healthy sheep through fluorescence histochemistry (glyoxylic acid), immunohistochemistry (CGRP), silver, and gold chloride techniques.

## Materials and Methods

The TM joints of 15 male Australian merino sheep were studied. The sheep were healthy, young adult animals (less than 2 years old). The macroscopic appearance of the branches of the mandibular nerve that innervate the TMJ was studied in six joints from three adult sheep. The joints were dissected under a magnifying lamp. All sheep were sacrificed through an overdose of sodium pentobarbital (Nembutal, Abbott, Sydney, Australia) in accordance with the ethics guidelines of South Australia. All TM joints were removed en bloc with a band saw. The capsule and attached disc were dissected away from the condyle and mandibular fossa and cut into lateral, medial, anterior, and posterior portions for microscopy evaluation. The silver impregnation method described by Linder[15] was used to demonstrate the location of nerve trunks plus the arrangement of major joint structures in sagittal sections of the decalcified TM joints from one adult sheep.

### Gold Chloride Histochemistry

The gold chloride technique was performed to visualize proprioceptors and nociceptors. In five animals, the portions of capsule and disc were immersed in a solution of one part 88% formic acid and three parts fresh lemon juice for 15 minutes in the dark, and they were stained with gold chloride according to the method described by Zimny et al.[16] Frozen sections were cut on a sliding microtome at 70 to 100 µm. Some sections were counterstained with Masson trichome, light green, or van Giesson's. The sections were mounted on slides coated with (3-aminopropyl-triethoxy-silane) (ATS) (Sigma, Aldrich, Castle Hill, Australia) and were allowed to dry. The slides were dehydrated in absolute alcohol, cleared in Histoclear (National Diagnostics, Atlanta, GA), and mounted in pix.

### Fluorescence Histochemistry

Fluorescence histochemistry was performed to visualize autonomic fibers. Six joints were studied using the glyoxylic acid (GA) method described by de la Torre and Surgeon.[17] Each portion of fresh capsule and disc was placed in several drops of distilled water on a metal stub and was frozen in a cryostat at –30°C. Cryostat sections 30 µm thick were mounted on clean glass slides at room temperature. Each slide was quickly dipped (1 dip/s) in a solution of 1.5 g of GA and 115 mL of distilled water, which contained 10.2 g of sucrose and 4.8 g of monobasic potassium phosphate (adjusted to a pH of 7.4 using 35 mL of 1 N NaOH). Slides were air dried using a hair dryer for 5 minutes and then incubated in an oven at 80°C for 5 minutes. The slides were cover slipped with paraffin oil and examined under a fluorescence microscope with a 200-W mercury vapor lamp, 2× BG 12 excitation filters, and a K 515 emission filter. Photomicrographs were recorded on Kodak Tmax 400 film (Kodak, Rochester, NY).

### Immunohistochemistry

Immunohistochemistry was used to visualize sensory nerve fibers. In each of three animals, Zamboni's fixative was injected into the TM joints while the animal was sedated (prior to sacrifice and dissection). Capsule and disc portions of TM joints were immersed in Zamboni's fixative. Pieces of tissue were mounted in Tissue Tek (Miles, Elkhart, IN) on metal stubs and were frozen at –30°C. Serial sections (25 to 35 µm) were cut and mounted on ATS-coated slides and allowed to dry overnight at room temperature. After treatment with 0.3% Triton-X 100 (Rohmand Haas, Philadelphia, PA) in phosphate-buffered saline (PBS)

(PBST) (pH 7.4) for 30 minutes and 0.3% hydrogen peroxide in methanol for 1 hour to inactivate endogenous peroxidase, the sections were incubated for 1 hour with diluted normal blocking serum, which was prepared from the same species in which the secondary antibody was raised. After the excess serum was blotted, the sections were incubated with primary rabbit antiserum to polyclonal rat CGRP (Peninsula Laboratories, Belmont, CA) diluted 1:1,000 in a solution consisting of 1% bovine serum albumin and 0.05% sodium azide in 0.1 mol/L of PBST overnight in a humid chamber at room temperature. After three washings in PBS, the sections were incubated in *(1)* biotintylated goat antirabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:50 in PBST for 2 hours at room temperature and *(2)* avidin-biotin peroxidase complex (Vectastain Elite ABC Reagent, Vector Laboratories) diluted 1:50 in PBST for 1 hour at room temperature. After an additional three washings in PBS, the sections were incubated in peroxidase substrate solution containing diaminobenzidine tetrahydrochloride and nickel (Vector Laboratories) for 6 minutes. Finally, the slides were counterstained with hematoxylin, dehydrated in ethanol, cleared in Histoclear, and mounted in pix. Control samples were made by replacement of the primary antiserum with normal serum.

### Relative Density of Nerve Fibers

Nerve density was examined using a squared graticule eyepiece.[18,19] A qualitative assessment of the density of neural elements (nerve fibers and receptors) was made in relation to the capsule in the following regions of the TMJ: *(1)* anterior; *(2)* posterior; *(3)* medial; and *(4)* lateral. A scale from absence (0) to high (++++) relative density was used. Two fields from each of five sections from each region were examined at 40× magnification for each of the gold chloride, glyoxylic acid, and CGRP immunoreactivity techniques.

## Results

### General Anatomy

No significant differences could be found between left and right joints of the same animal, or between joints from different animals, using the methods described in the present study; consequently, the observations reported are typical of all joints examined.

The sheep TMJ sections consisted of the mandibular condyle separated from the glenoid fossa (temporal or mandibular fossa) of the temporal bone by an articular disc (meniscus). As previously described,[9,10] the condylar head and temporal surface of the joint consist of a relatively acellular fibrous articular surface that overlies cartilage. The disc contained dense, fibrous tissue and scattered chondrocytes. The peripheral part of the disc merged with the capsule, thus separating upper (superior) and lower (inferior) joint compartments (Figs 1a to 1c). Although the posterior attachment of the disc to the capsule consisted of dense fibrous tissue, anteriorly there were loose connective tissue, numerous fat cells, and many blood vessels (Fig 1c). The articular capsule presented an outer fibrous layer and an inner, or synovial, layer. The fibrous layer surrounded the joint, attaching to the temporal bone and mandibular condyle. On the lateral side, the capsule was thickened by additional dense fibrous tissue called the *lateral ligament*. The synovial membrane covered the peripheral parts of the articular surfaces and disc as well as the capsule. It formed villi and folds in the anterior and posterior parts of the joint near the point where the disc joined the capsule.

The auriculotemporal, deep temporal, and masseteric branches of the mandibular division of the trigeminal nerve contributed branches to the TMJ. The masseteric nerve arose from the anterior aspect of the mandibular nerve a few millimeters distal to the foramen ovale. After passing anterolaterally, the masseteric nerve divided into two main branches. The first branch (the deep temporal nerve) passed superiorly deep to the zygomatic process, where it contributed a branch to the medial part of the anterior capsule and entered the deep aspect of the temporal muscle (Figs 1a and 1b). The second branch continued laterally through the mandibular notch after contributing a branch to the lateral part of the anterior capsule of the TMJ. The auriculotemporal nerve branched from the posterior aspect of the mandibular nerve, 2 to 3 mm distal to the origin of the masseteric nerve, and passed medial to the neck of the mandible before sending branches to the posterior part of the capsule of the TMJ. It then passed between the external acoustic meatus and the TMJ (Figs 1a and 1b).

### Silver Impregnation

Silver impregnation was used to show the location of major nerve bundles plus the general arrangement of joint components in sagittal sections of the TMJ (Fig 1c).

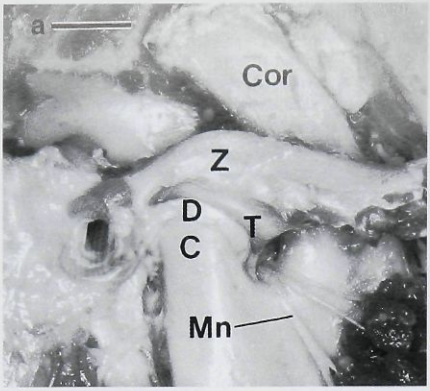


Fig 1a Lateral aspect of the right TMJ of an adult sheep. Zygomatic arch (Z), condylar process (C), and coronoid process (Cor) of the mandible, disc (D), temporal fossa (T), and masseteric branch of the mandibular nerve (Mn) are shown (bar = 10 mm).

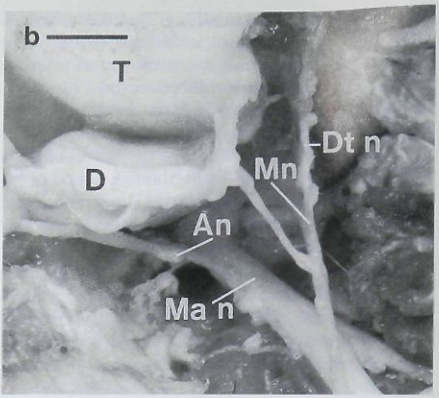


Fig 1b Lateral aspect of the disc (D) and temporal fossa (T) of the right TMJ of an adult sheep showing the mandibular nerve (Ma n), auriculotemporal nerve (An), masseteric nerve (Mn), and deep temporal nerve (Dt n) (bar = 5 mm).

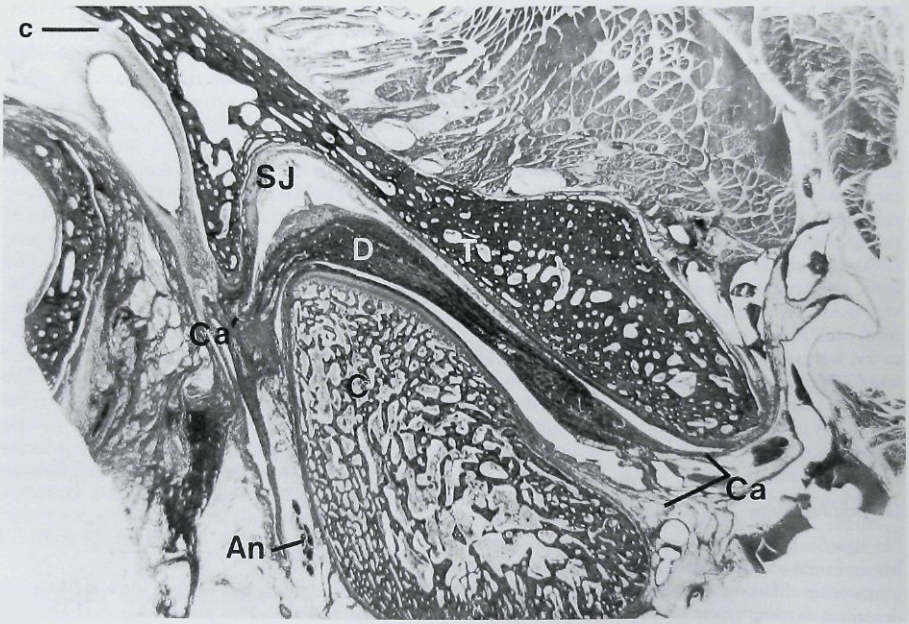


Fig 1c Sagittal section of decalcified, silver-impregnated right TMJ of an adult sheep showing temporal fossa (T), condylar process (C), disc (D), superior joint cavity (SJ), capsule (Ca), and auriculotemporal nerve (An) (bar = 2 mm).

**Table 1** Relative Density of Neural Structures in Four Regions of the Capsule of TM Joints From Adult Male Merino Sheep

| | Regions | | | |
|---|---|---|---|---|
| | Anterior | Posterior | Medial | Lateral |
| Gold chloride technique (4 joints) | ++ | ++++ | + | +++ |
| Glyoxylic acid technique (2 joints) | ++++ | ++ | +++ | + |
| CGRP immunoreactivity (4 joints) | +++ | ++ | + | ++ |

+ indicates low; ++++ indicates high.

### Gold Chloride

Nerve fascicles, single nerve fibers, and nerve endings were clearly demonstrated by the gold chloride technique. Bundles of nerve fibers usually accompanied blood vessels passing through the capsule. Neural structures were found in decreasing density in the capsule, synovium (synovial membrane and adjacent subsynovial tissue), and the disc. Nerve fibers were most dense in the posterior part of the capsule and least dense in the medial part of the capsule (Table 1).

Free nerve endings were seen as either unbranched fibers or simple arborizations from narrow, myelinated and unmyelinated axons (less than 5 μm in diameter). They were found in the capsule, the peripheral disc, and synovium. Networks of these type IV nerve endings were also found in the disc-capsule junction (Figs 2[a] and 3[b]). Free nerve endings extended up to 3 mm into the disc, but no neural structures were found in the thicker, central part of the disc.

More complicated arborizations branching from bundles of myelinated axons (5 to 10 μm in diameter) appeared as simple or complex sprays. The branches within the sprays often contained small swellings or varicosities. These endings were included in the Ruffini category (Fig 2[b]). They did not appear to be encapsulated, and they were present in the capsule and peripheral disc. A simple ending that resembled a ball of threads attached to a single axon or bundle of two axons was called a clew type of spray or Ruffini ending following the description given by Polacek[20] (Figs 2[c and d] and 3[b]). Spray or Ruffini endings were found in all parts of the capsule. Although relatively more endings were found in the lateral capsule, the endings were still sparsely distributed.

All proprioceptive-type sensory endings were more evenly distributed and more dense in the disc-capsule junction than those in the adjacent capsule. The disc-capsule junction in the lateral and posterior regions had more endings than did the medial and anterior regions, respectively. Golgi-type endings were complex spray endings arising from nerve bundles about 15 μm in diameter (Figs 2[e] and 3[c]). The branches had varicose swellings and often ended in expanded tips. These were found in the peripheral disc near the attachment to the capsule.

Encapsulated nerve endings were rare. The paciniform type, in which the myelinated axon terminated in a dense, elongated central core surrounded by a lamellated capsule, was only found in the lateral capsule (Fig 4[a]). The Golgi-Mazzoni type contained a branched central core surrounded by a lamellated capsule and was only found in the lateral disc-capsule junction (Fig 4[b]). An encapsulated spray-type ending was seen in the lateral capsule in only one specimen. It was important to view serial sections when examining structures that looked like receptors, because some blood vessels were also stained by gold chloride.

### Fluorescence Histochemistry

Glyoxylic acid–induced fluorescence of nerve fibers was demonstrated in the capsule, the synovial membrane, and the disc. Numerous varicosities were revealed along the fluorescent axons. Transverse sections of large arteries showed plexiform varicosities embedded in the adventitial sheath. Fluorescent axons with varicosities were present in the inner part of the tunica media. The highest density of fluorescent nerve fibers was in the anterior part of the TMJ (Table 1). Single nerve fibers plus nerve plexuses were localized around blood vessels and in interstitial tissue. Some nerve fibers penetrated the disc to a depth of 3 mm, but no nerve fibers could be seen in the central part of the disc. Large nerve bundles (branches of the masseteric nerve) did not exhibit any fluorescence (Fig 5).

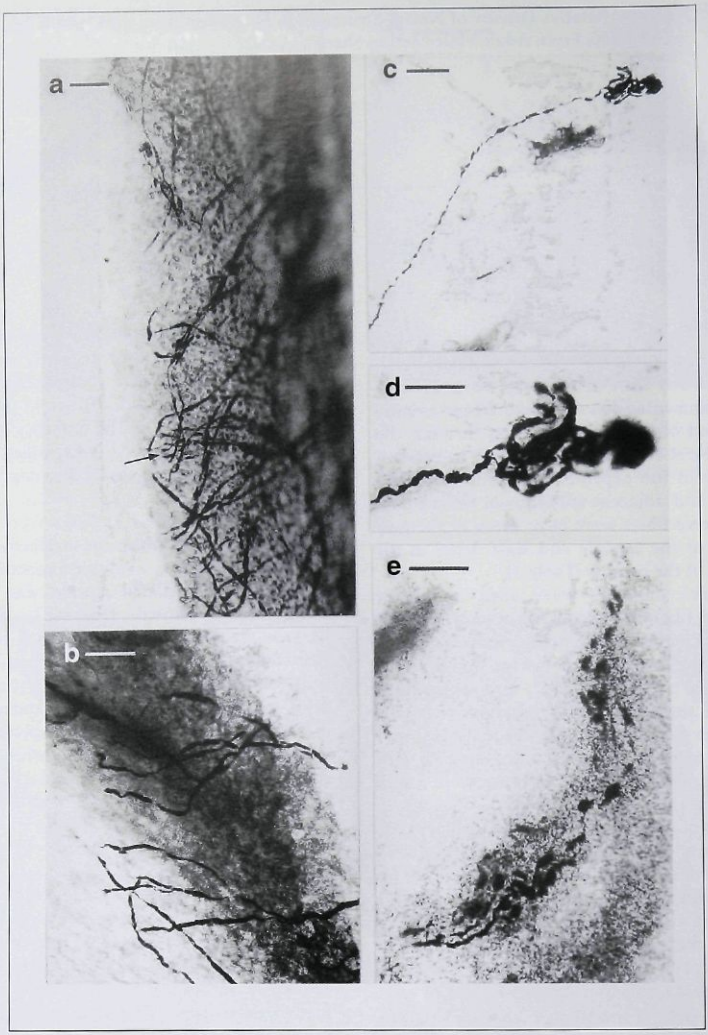


**Fig 2** Light micrographs of gold chloride–stained sections of capsule of TM joints of adult sheep. *(a)* Free nerve endings *(small arrow)* in the synovium (bar = 60 μm). *(b)* Simple, branched Ruffini ending (bar = 10 μm). *(c)* Clew-type Ruffini ending (bar = 50 μm). *(d)* Enlargement of the Ruffini ending shown in Fig 2(c) (bar = 30 μm). *(e)* Part of a Golgi ending (bar = 30 μm).

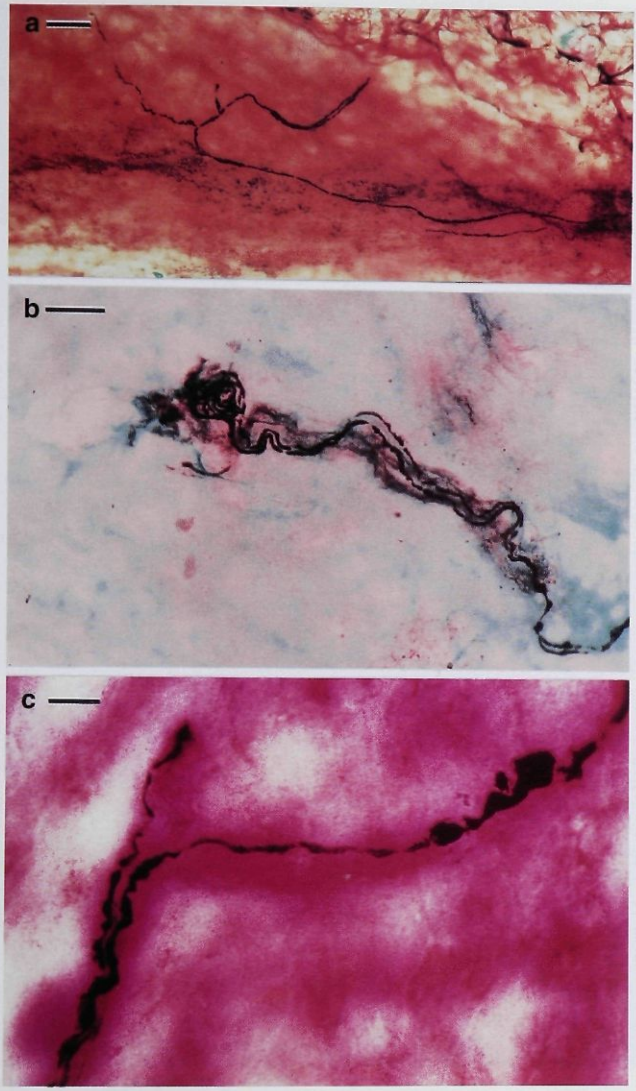


Fig 3 Light micrographs of gold chloride–stained sections of capsule of the TMJ of adult sheep. *(a)* Free nerve endings from a simple arborization (counterstained with Masson trichrome) (bar = 25 μm). *(b)* Clew-type Ruffini ending (counterstained with light green) (bar = 15 μm). *(c)* Part of a Golgi ending (counterstained with van Gieson's) (bar = 6 μm).


Fig 4 Light micrographs of gold chloride–stained sections of capsule of the TMJ of adult sheep. *(a)* Paciniform corpuscle *(arrow indicates central core of corpuscle)* (bar = 40 μm). *(b)* Golgi-Mazzoni corpuscle *(arrowhead indicates axon leading to the corpuscle)* (bar = 10 μm).

### Immunohistochemistry

The majority of CGRP-immunoreactive (CGRP-IR) nerve fibers were located in the synovial membrane and adjacent capsule of the anterior part of the TMJ (Table 1). Free nerve endings were also displayed in the synovium, including synovial villi (Fig 6[a to c]). Small numbers of CGRP-IR nerve fibers penetrated the peripheral part of the disc and terminated in free nerve endings; some more complex spray-type endings resembling Ruffini endings (Fig 6[e]). Neural structures in the synovium were located interstitially; those in the capsule often accompanied blood vessels (Fig 6[c and d]). Immunopositive axons displayed numerous varicosities (Fig 6[a]). Except where a section of capsule contained a large bundle of nerve fibers, the immunoreactive nerve fibers were mostly single axons less than 5 μm in diameter. Many axons were less than 3 μm in diameter. Only small numbers of fascicles containing two to five axons were seen. No immunoreactivity was observed in the control sections.

## Discussion

### Gross Morphology

The TMJ of the sheep is innervated by auriculotemporal (posteriorly) plus masseteric and deep temporal branches (anteriorly) of the mandibular division of the trigeminal nerve. Similar observations have been made for TM joints of the human,[3,21–24] the rhesus monkey,[25] and the cat.[26]

Schmid[22] found that autonomic nerves (a branch of the otic ganglion) entered the medial side of the capsule in the human TMJ. It was presumed that sympathetic fibers came from a sympathetic plexus of the internal maxillary artery that joined the otic ganglion. Several authors[22,27] have found nerve fibers in association with blood vessels in the TMJ. Ishibashi[23] noted that nerve fiber bundles penetrate the capsule accompanied by small arteries in the human TMJ. Although a branch of the otic ganglion to the TMJ was not found in the sheep, and sympathetic fibers could not be detected in major nerves entering the joint, the present study detected sympathetic fibers accompanied by blood vessels, in the capsule, the synovial membrane, and the disc (see discussion of glyoxylic acid). It is most likely that the sympathetic fibers entered the joint accompanying branches of the maxillary artery.

### Gold Chloride Histochemistry

Within joint tissues, articular nerve fibers terminate in four types of endings, three of which are of the encapsulated variety.[28] All three types of encapsulated nerve endings (Ruffini, paciniform, and Golgi organs) are found in the TM joints of rats, rabbits, guinea pigs, cats, monkeys, and humans.[21,25,27,29,30–34] The gold chloride technique

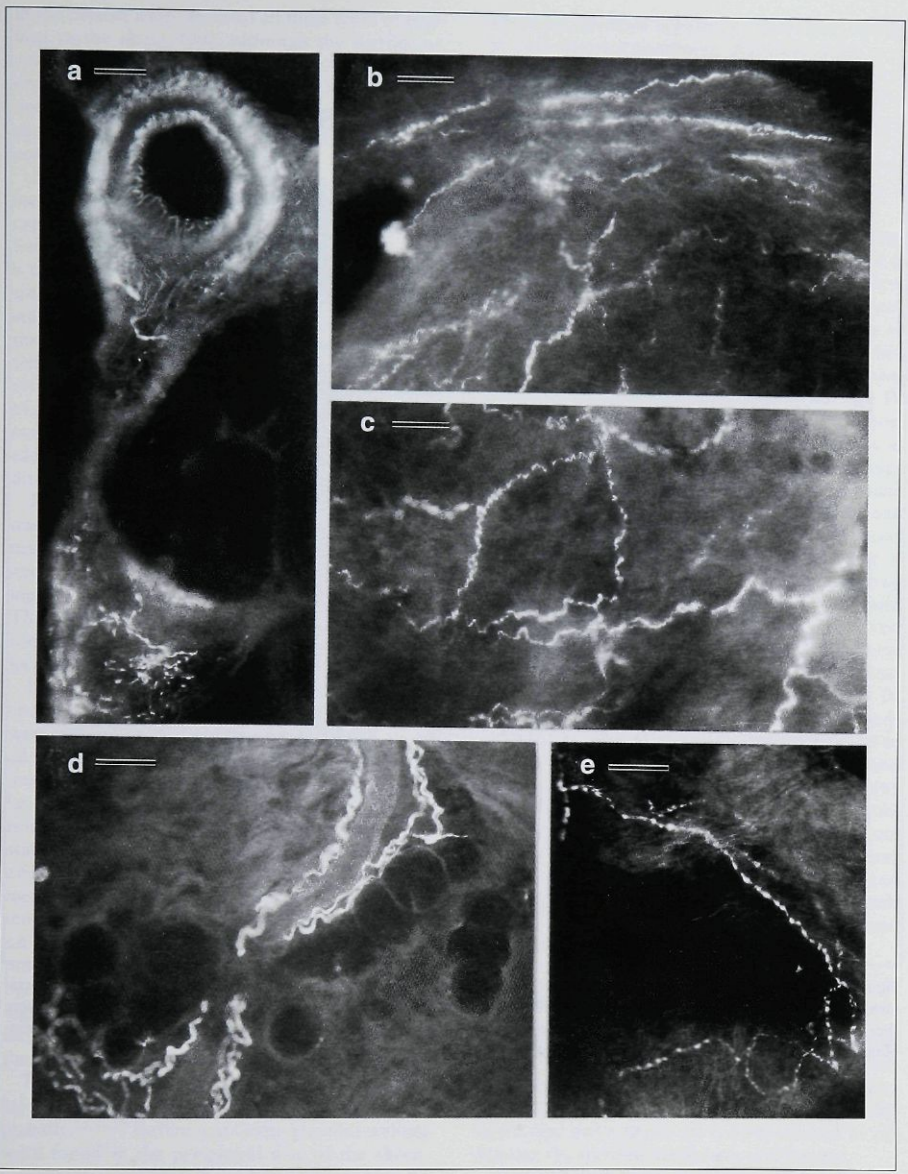


Fig 5 Fluorescence photomicrographs of sections of capsule of the TMJ of adult sheep after treatment with glyoxylic acid. *(a and d)* Adrenergic nerve fibers associated with blood vessels (bars = 35 and 40 μm, respectively). *(b and c)* Adrenergic nerve fibers forming a network in the connective tissue (bars = 50 μm). *(e)* Adrenergic nerve fibers located in the junction between synovial membrane and capsule (bar = 40 μm).

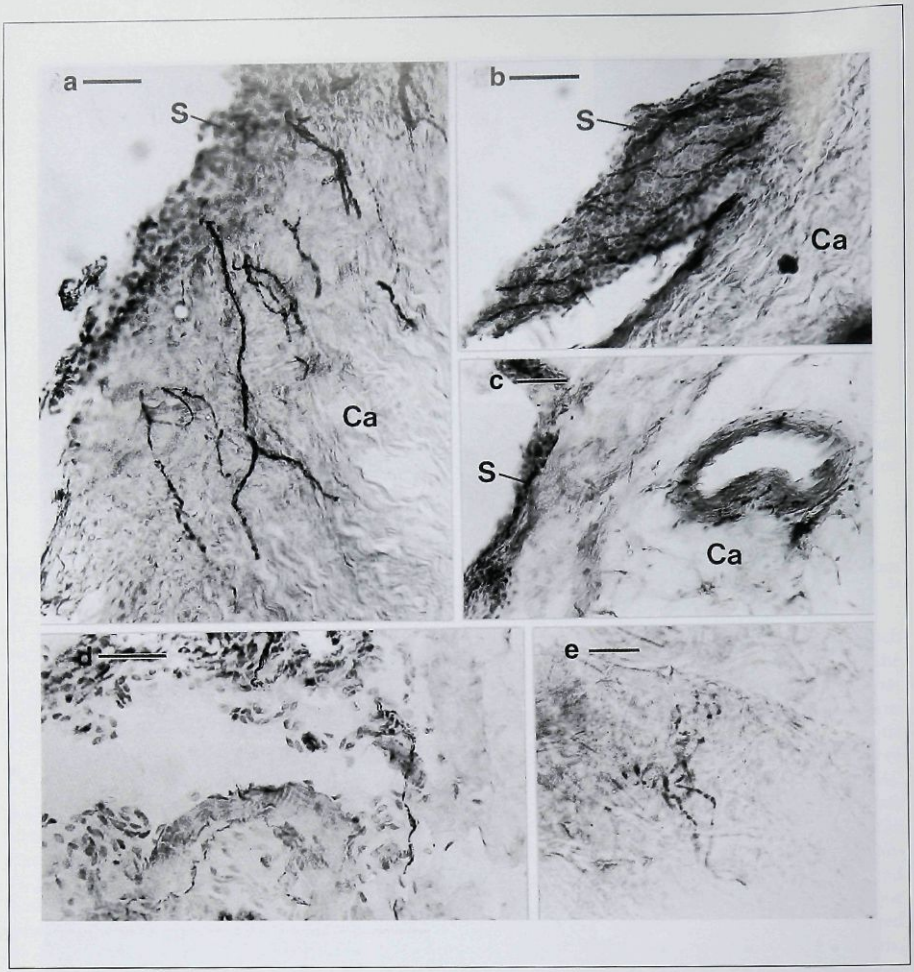


Fig 6 CGRP-immunoreactive nerve fibers in the TMJ of adult sheep. *(a)* Nerve fibers in the capsule (Ca) and synovial membrane (S) (bar = 15 μm). *(b)* Nerve fibers in a synovial fold (bar = 20 μm). *(c)* Nerve fibers in the wall of an artery (bar = 20 μm). *(d)* Nerve fibers entering the synovial membrane lining a lumen (bar = 20 μm). *(e)* Nerve terminal resembling a Ruffini receptor in the peripheral disc (bar = 20 μm).

in the present study revealed all three types of endings in the sheep TMJ, although the Ruffini or spray-type endings were the most frequently encountered type. The criteria used for identification of the receptors are based on the descriptions used by Keller and Moffett,[25] Klineberg,[34] Polacek,[20] and Zimny.[14]

Most of the Ruffini endings in the sheep TMJ lacked a capsule; thus, they resembled the unencapsulated spray endings described by Polacek.[20] Polacek[20] described a series of endings that formed a continuum from simple free nerve endings to simple sprays, and from more complicated encapsulated sprays to the encapsulated corpuscles of the Golgi-Mazzoni type with an inner core. This series of receptors was presumed to represent an evolutionary line with all three well-defined types of receptors occurring in the joint capsules of mammals. Transitional forms occur rarely, and each species of mammal has a particular, clearly distinguishable triad of receptors.

Ruffini or type I articular receptors are numerous in all joints including the TMJ, where they are usually found in superficial parts of the capsule and outnumber the other types of corpuscular endings in cats,[28,32,34] monkeys,[25] and humans.[21] The TMJ usually contains few paciniform-type receptors.[21,25,34] The Golgi-type receptors are mostly located in the deeper parts of the capsule.[28,34]

In the sheep TMJ, the majority of corpuscular endings were located in the lateral capsule and were concentrated at the capsule-disc junction. In all parts of the TMJ, this junction was more richly innervated by receptors than were adjacent capsular areas. Thus, the lateral capsule and all disc-capsule junction areas must play an important role in static and dynamic mechanoreception.[28] Zimny and St Onge[37] found the highest density of mechanoreceptors in areas related to extremes of movement such as the disc-capsule junction of the cat TMJ. Kawamura et al[33] found 85 Pacinian corpuscles in areas of capsule stretched during jaw opening (35) or jaw closure (50). Corpuscular endings are usually much less densely distributed in the medial and lateral parts of the TMJ capsule than in the anterior and posterior parts.[27,31,34,36]

The disc of the TMJ contains all three corpuscular endings, but these are only located peripherally.[25,36,37] Ruffini and Golgi-Mazzoni endings were found in the peripheral disc of the sheep. Corpuscular receptors have been reported in the subsynovial layers of the capsule,[33] as well as in the synovial membrane and villi.[35] However, the methods used by Davies[35] were not described, and the description of innervation was not detailed. There are no other reports of corpuscular endings in TMJ synovial membrane, although several studies have demonstrated free nerve endings and nerve fibers in the membrane and in the disc. The most detailed of these were the immunocytochemical studies, by Ishikawa et al[38] and Kido et al,[39] in which free nerve endings were restricted to the peripheral parts of the adult disc, as was noted in the present study of the sheep TMJ.

Type IV receptors[28] are noncorpuscular endings that belong to small, myelinated (less than 5 μm in diameter) A-type or unmyelinated C-type nerve fibers. They are the most numerous of the four categories of receptors found in the TM joints of humans,[21] cats,[28] rhesus monkeys,[25] mice,[40] rats, guinea pigs, and rabbits.[29] Gold chloride staining revealed numerous free nerve endings (type IV receptors) in the capsule, the disc-capsule junction, the synovial membrane, and the peripheral disc in the sheep TMJ. Freeman and Wyke[41] divide type IV receptors into IVa, or afferent pain fibers found in capsule, ligaments, synovium, menisci, and bone; and IVb, or efferent vasomotor fibers found in walls of small vessels. These receptors exist as networks of nerve endings throughout the fibrous capsule, adjacent periosteum, articular fat pads, and the adventitial sheaths of articular blood vessels.[28] Wyke[28] reported that in the cat, free nerve endings were sparse and largely confined to ligaments. Klineberg[34] described plexuses of type IV nerve endings in the fibrous capsule and fat pads of the cat TMJ. Free nerve endings were found to exist in small numbers in the fibrous capsule and more frequently in ligaments. However, many authors refer to free nerve endings without distinguishing networks of these type IV endings from separate so-called "free" endings. Most free nerve endings are sensitive to chemical, thermal, and mechanical stimuli.[14,42] They have a nociceptive role and may perhaps also serve polymodal mechanoreceptive functions.[40]

### Fluorescence Histochemistry

Glyoxylic acid can be used to demonstrate the localization of noradrenaline-containing fibers (autonomic fibers) using fluorescence histochemistry.[17,43] In the sheep TMJ in the present study, adrenergic nerve fibers were demonstrated in the capsule, the synovial membrane, and the disc.

Schmid[22] found that autonomic nerves (a branch of the otic ganglion) entered the medial side of the capsule in the human TMJ. He presumed that sympathetic fibers came from a sympathetic plexus of the internal maxillary artery that joined the otic

ganglion. Autonomic nerve fibers characteristically form plexuses in the walls of arteries and accompany arterioles and capillaries in joint capsules. Plexuses, which are separate from vessels in the capsule, and synovial membranes are also formed.[44] A retrograde tracing study by Widenfalk and Wiberg[45] revealed a large number of sympathetic efferents to the rat TMJ originating from the superior cervical ganglion. These authors hypothesized that nociceptive input from the TMJ could modulate activity in sympathetic efferents, which normally have a vasomotor role.[35,38,44]

### Immunohistochemistry

Immunohistochemical techniques allow a convenient means to understand the distribution of neural elements in general (PGP 9.5[46,47]), as well as distinction between sensory nerves (SP and CGRP[38,39,47,48]) and autonomic nerves (NPY and vasoactive intestinal peptide [VIP][49,50]) by using specific antibodies raised against these neural epitopes.

In the present study, CGRP-IR nerve fibers were located in the synovial membrane and adjacent capsule of the sheep TMJ. Free nerve endings were also displayed in the synovium. Small numbers of CGRP-IR nerve fibers penetrated the peripheral part of the disc. Neural structures in the synovium were located interstitially, and those in the capsule mostly accompanied blood vessels. Recent immunocytochemical studies have demonstrated sensory nerve fibers and free nerve endings in the TMJ. Substance P–immunoreactive nerve endings have been demonstrated in the capsule, the periosteum, and the adventitia of arteries of the monkey TMJ[27] and the capsule, the synovial membrane, and the disc of rats.[32,39] Substance P and CGRP are neuropeptides characteristically located in unmyelinated and thinly myelinated C-type pain fibers.[51] They may be present interstitially or perivascularly.[19] These peptides may occur separately or together in articular sensory nerves.[48] Ishikawa et al[38] suggested that the CGRP-IR nerves in the rat TMJ might be associated with mediation of pain and/or proprioceptive sensations. Perivascular nerve fibers containing SP and CGRP have been given a putative involvement in the transmission of intracranial pain, and both peptides also influence vascular tone.[51,52] The perivascular nerves, staining for NPY or VIP in the rat TMJ, are believed to be part of the autonomic nervous system and to be involved in the regulation of blood flow in the TMJ.[38]

Neurospecific markers S-100 protein and PGP 9.5 were used by Morani et al[53] to demonstrate the innervation of the capsule and disc of the human TMJ. Perivascular and interstitial fibers were located in the capsule and bilaminar part of the disc. It was suggested that because some fibers were located in avascular parts of the disc, these fibers were most likely proprioceptive or nociceptive in function.

### General Discussion

When the distribution of the four types of nerve endings is considered, the richest innervation occurs in the posterior-posterolateral part of the TMJ capsule in the cat,[34] the mouse,[40] the monkey,[25] and the human.[21] Only Kido et al,[39] using immunocytochemical techniques for the peptides SP and CGRP, have found the highest density of neural elements in the anterolateral part of the capsule of the rat TMJ. Although species differences in the form of the TMJ articulation might account for this apparent discrepancy,[39,40] consideration must be given to the possibility that the techniques used might be highlighting different components or different amounts of the innervation of the TMJ.[39,40,52] Other than the work of Kido et al,[39] all the studies mentioned in the present study in relation to nerve distribution mostly utilized silver, gold chloride, hematoxylin and eosin, or methylene blue staining techniques. These may not have provided accurate analyses of the nerve distribution.[40] Kido et al[39] suggested in their study of the rat that the posterior part of the capsule and disc might be supplied by sensory (SP- or CGRP-containing nerves) and autonomic nerves. Their study did not examine the autonomic nerves (NPY and VIP); and hence, the sensory nerves they stained represented only a part of the total innervation of the joint. Nevertheless, there appears to be an asymmetric arrangement of sensory nerves (and probably autonomic nerves) within the capsule and disc in the rat. Johansson et al[27] found SP-IR nerve fibers to be more sparse in the posterior capsule than in the anterior capsule of the monkey TMJ. Very few nerve endings were seen, but there were regular plexuses in the arterial adventitia in this region. These authors also noted that although SP immunoreactivity and silver impregnation costained some fibers, most SP-IR fibers were not stained by silver impregnation.

Thus, in the sheep TMJ, the distribution of neural elements in the present study using gold chloride resembles that described for a variety of other animals where silver or gold chloride have been used, with the greatest density of neural structures (nerves fibers and nerve terminals) occurring in the

posterior and lateral parts of the capsule. The high density of neural structures in these areas probably reflects the relative importance of these parts of the joint in positional perception, as well as the TMJ reflexes to regulate normal jaw movements and to protect the joint against extreme movements.[20,34,42] These areas are likely to show high sensitivity to pressure changes within the joint, as well as alterations to tension within the capsule during jaw movements.

In addition, the distribution of CGRP-IR nerve fibers in the present study resembles that described for the rat TMJ, in that the greatest density of nerve fibers was found in the anterior part of the capsule. There were more neural elements stained by the gold chloride technique in the posterior and lateral parts of the capsule of the sheep TMJ than by immunolocalization of CGRP-containing fibers. Since the glyoxylic acid revealed low numbers of autonomic fibers in the posterior and lateral capsule, the discrepancy in the numbers of neural elements revealed by the techniques mentioned previously cannot be a result of the autonomic innervation.

The gold chloride technique revealed proprioceptors and free nerve fibers; the immunoreactivity for CGRP revealed nerve fibers and very few free nerve endings, but no spray endings. Thus, it would seem that although immunoreactivity for CGRP most likely demonstrates the distribution of sensory nerve fibers in the sheep TMJ, a more complete picture of the distribution of neural elements, including proprioceptors, can be gained by using gold chloride.

The present study details the appearance and distribution of neural elements in healthy, young adult sheep, and it forms a basis for the next part of the project, in which the sheep is being used as a model for the study of the effects of arthritis (see work by Bosanquet et al,[10] Ishimaru and Goss,[11] and Ishimaru et al[12,13]) on the innervation of the joint. Increasing numbers of studies are showing changes in the distribution and density of neural elements in joint tissue as a result of arthritis.[5–8,19] Future studies will be made to elucidate these changes in the sheep TMJ following surgically induced arthritis.

## Acknowledgments

The skillful technical assistance of Ms G. Hermanis, Mr C. Leigh, and Mr R. Murphy is gratefully acknowledged. The first author would like to thank the Shiraz Medical Science University and Ministry of Health and Medical Education, Tehran, Iran, for providing a scholarship, without which this work would not have been possible. The authors also thank Professor M. Henneberg, Associate Professor G. Townsend, and Drs M. Hukkanen and T. Wilkinson for critically reading the manuscript.

## References

1. Blackwood HJJ. Arthritis of the mandibular joint. Br Dent J 1962;115:317–326.
2. Ogus H. Rheumatoid arthritis of the temporomandibular joint. Br J Oral Surg 1975;12:275–284.
3. Kreutziger KL, Mahan PE. Temporomandibular degenerative joint disease. Part 1. Anatomy, pathophysiology and clinical description. Oral Surg Oral Med Oral Pathol 1975;40:165–182.
4. Meek WD, Raber BT, McClain OM, McCosh JK, Baker BB. Fine structure of the human synovial lining cell in osteoarthritis: Its prominent cytoskeleton. Anat Rec 1991; 231:145–155.
5. Grönblad M, Konttinen YT, Korkala O, Liesi P, Hukkanen M, Polak JM. Neuropeptides in synovium of patients with rheumatoid arthritis and osteoarthritis. J Rheumatol 1988; 15:1807–1810.
6. Konttinen YT, Rees R, Hukkanen M, Grönblad M, Tolvanen E, Gibson S, et al. Nerves in inflammatory synovium: Immunohistochemical observations on the adjuvant arthritic rat model. J Rheumatol 1990;17:1586–1591.
7. Mapp PI, Kidd BL, Terry JM, Revell PA, Ibrahim NBN, Blake DR, Polak JM. Substance P-, calcitonin gene-related peptide- and C-flanking peptide of neuropeptide Y-immunoreactive fibres are present in normal synovium but depleted in patients with rheumatoid arthritis. Neuroscience 1990;37:143–153.
8. Hukkanen M, Grönblad M, Rees R, Konttinen YT, Gibson SJ, Hietanen J, et al. Regional distribution of mast cells and peptide containing nerves in normal and adjuvant arthritic rat synovium. J Rheumatol 1991;18: 177–183.
9. Bosanquet AG, Goss AN. The sheep as a model for temporomandibular joint surgery. Int J Oral Maxillofac Surg 1987;16:600–603.
10. Bosanquet A, Ishimaru J-I, Goss AN. Effect of experimental disc perforation in sheep temporomandibular joints. Int J Oral Maxillofac Surg 1991;20:177–181.
11. Ishimaru J-I, Goss AN. A model for osteoarthritis of the temporomandibular joint. J Oral Maxillofac Surg 1992; 50:1191–1195.
12. Ishimaru J-I, Kurita K, Handa Y, Goss AN. Effect of narrow perforation on the sheep temporomandibular joint. Int J Oral Maxillofac Surg 1992;21:239–242.
13. Ishimaru J-I, Handa Y, Kurita K, Goss AN. The effect of occlusal loss on normal and pathological temporomandibular joints: An animal study. J Craniomaxillofac Surg 1994;22:95–102.
14. Zimny ML. Mechanoreceptors in articular tissues. Am J Anat 1988;182:16–32.
15. Linder JE. A simple and reliable method for silver impregnation of nerves in paraffin sections of soft and mineralized tissues. J Anat 1978;127:543–551.
16. Zimny ML, St Onge M, Schutte M. A modified gold chloride method of the demonstration of nerve endings in frozen sections. Stain Technol 1985;60:305–306.
17. de la Torre JC, Surgeon JW. A methodological approach to rapid and sensitive monomine histofluorescence using a modified glyoxylic acid technique: The SPG method. Histochemistry 1976;49:81–93.
18. Aherne WA, Dunnill MS. Morphometry. London: Edward Arnold, 1982.

19. Pereira da Silva JA, Carmo-Fonseca M. Peptide containing nerves in human synovium: Immunohistochemical evidence for decreased innervation in rheumatoid arthritis. J Rheumatol 1990;17:1592–1599.
20. Polacek P. Receptors of the joints. Acta Fac Med Univ Brun 1966;23:1–107.
21. Thilander B. Innervation of the temporomandibular joint capsule in man. Trans R Sch Dent Stockh Umea 1961;7: 1–67.
22. Schmid F. On the nerve distribution of the temporomandibular joint capsule. Oral Surg 1969;28:63–66.
23. Ishibashi K. Innervation of human temporomandibular joint in adult. Bull Tokyo Med Dent Univ 1974;21(suppl):86–88.
24. Helland MM. Anatomy and function of the temporomandibular joint. In: Grieve GP (ed). Modern Manual Therapy of the Vertebral Column. London: Churchill Livingstone, 1986:64–76.
25. Keller JH, Moffett BC. Nerve endings on the temporomandibular joint of the *Rhesus macaque*. Anat Rec 1990;160:587–594.
26. Romph JH, Capre NF, Gatipon GB. Trigeminal nerve and temporomandibular joint of the cat: A horseradish peroxidase study. Exp Neurol 1979;65:99–106.
27. Johansson A-S, Isacsson G, Isberg A, Granholm A-C. Distribution of substance P-like immunoreactive nerve fibers in temporomandibular joint soft tissues of monkeys. Scand J Dent Res 1986;94:225–230.
28. Wyke B. The neurology of joints. Ann R Coll Surg Engl 1967;41:25–50.
29. Franks AST. Studies on the innervation of the temporomandibular joint and lateral pterygoid muscle in animals [abstract 8]. J Dent Res 1965;43(suppl):947–948.
30. Skoglund S. Anatomical and physiological studies of knee joint innervation in the cat. Acta Physiol Scand 1956; 36(suppl 124):1–101.
31. Kawamura Y, Majima T. Temporomandibular joint's sensory mechanisms controlling activities of the jaw muscles. J Dent Res 1964;43:150.
32. Greenfield BE, Wyke B. The innervation of the cat's temporomandibular joint [abstract]. J Anat 1964;98:300.
33. Kawamura Y, Majima T, Kato I. Physiologic role of deep mechanoreceptor in temporomandibular joint capsule. J Osaka Univ Dent Sch 1990;7:63–76.
34. Klineberg I. Structure and function of temporomandibular joint innervation. Ann R Coll Surg Engl 1971;49:268–288.
35. Davies DV. Synovial membrane and synovial fluid of joint. Lancet 1946 Dec 7;ii(6432):815–819.
36. Wink CS, St Onge M, Zimny ML. Neural elements in the human temporomandibular articular disc. J Oral Maxillofac Surg 1992;50:334–337.
37. Zimny ML, St Onge M. Mechanoreceptors in the temporomandibular disk [abstract 1048]. J Dent Res 1987;66: 237.
38. Ishikawa H, Wakisaka S, Matsuo S, Akai M. Peptidergic innervation of the temporomandibular disk in the rat. Experientia 1989;45:303–304.
39. Kido MA, Kiyoshima T, Kondo T, Ayasaka N, Moroi R, Terada Y, Tanaka T. Distribution of substance P and calcitonin gene-related peptide-like immunoreactive nerve fibers in the rat temporomandibular joint. J Dent Res 1993;72:592–598.
40. Dreessen D, Halata Z, Strasmann T. Sensory innervation of the temporomandibular joint in the mouse. Acta Anat (Basel) 1990;139:154–160.
41. Freeman MAR, Wyke B. The innervation of the knee joint: An anatomical and histological study in the cat. J Anat 1967;101:505–532.
42. Storey A. Temporomandibular joint receptors. In: Anderson DJ, Matthews B (eds). Mastication. Bristol: John Wright and Sons, 1976:50–57.
43. Furness JB, Costa M. The use of glyoxylic acid for the fluorescence histochemical demonstration of peripheral stores of noradrenaline and 5-hydroxytryptamine in whole mounts. Histochemisty 1975;41:335–352.
44. Samuel EP. The autonomic and somatic innervation of the articular capsule. Anat Rec 1952;113:53–70.
45. Widenfalk B, Wiberg M. Origin of sympathetic and sensory innervation of the temporomandibular joint. A retrograde axonal tracing study in the rat. Neurosci Lett 1990; 109:30–35.
46. Ramiera G, Anselmetti GC, Baracchi F, Panzica GC, Viglietti-Panzica C, Modica R, Polak JM. The innervation of human teeth and gingival epithelium as revealed by means of an antiserum for protein-gene product 9.5. Am J Anat 1990;189:146–154.
47. Karanth SS, Springall DR, Kuhn DM, Levene MM, Polak JM. An immunocytochemical study of cutaneous innervation and the distribution of neuropeptide and protein gene product 9.5 in new and commonly employed laboratory animals. Am J Anat 1991;191:369–383.
48. Hukkanen M, Konttinen YT, Rees RG, Santavirta S, Terenghi G, Polak JM. Distribution of nerve endings and sensory neuropeptides in rat synovium, meniscus and bone. Int J Tissue React 1992;14:1–10.
49. Uddmann R, Edvinsson L, Ekman R, Kingman T, McCulloch J. Innervation of the feline cerebral vasculature by nerve fibres containing calcitonin gene-related peptide: Trigeminal origin and co-existence with substance P. Neurosci Lett 1985;62:131–136.
50. Lundberg JM, Terenius L, Hokfelt T, Martling CR, Tatemoto K, Mutt V, et al. Neuropeptide Y(NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. Acta Physiol Scand 1982;116:477–480.
51. Pernow B. Substance P. Pharmacol Rev 1983;35:85–141.
52. Fischer LA, Kikkawa DO, Rivier JE, Amara SG, Evans RM, Rosenfeld MG, et al. Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide. Nature 1983;305:515–521.
53. Morani V, Previgliano V, Schierano GM, Ramieri G. Innervation of the human temporomandibular joint capsule and disc as revealed by immunohistochemistry for neurospecific markers. J Orofacial Pain 1994;8:36–41.

## Resumen

Estructuras Neurales Dentro de la Articulación Temporomandibular de la Oveja

El propósito de este estudio fue el de describir la inervación normal de la articulación temporomandibular (ATM) de la oveja como preparación para el uso de este animal como un modelo para el estudio de los efectos de la artritis sobre la inervación de la articulación. La apariencia macroscópica y microscópica además de la distribución de las estructuras neurales dentro de la ATM fueron examinadas por medio del uso de histoquímica de fluorescencia (ácido glioxílico), inmunohistoquímicos (péptido relacionado al gen de la calcitonina), plata, y técnicas de cloruro de oro. Se encontraron fibras nerviosas inmunoreactivas al péptido relacionado al gen de la calcitonina, en la cápsula y en la membrana sinovial, pero no en el disco. Los fascículos nerviosos y las fibras nerviosas individuales en la cápsula, la membrana sinovial, y los 2–3 mm periféricos del disco fueron teñidos con ácido glioxílico. Se localizaron terminaciones nerviosas del órgano del Golgi, de tipo Paciniforme y de Ruffini, además de terminaciones nerviosas libres, en la cápsula, estando la mayor densidad de éstas en el sitio de la inserción del disco a la cápsula. La mayor densidad de las estructuras neurales (utilizando el cloruro de oro) estaba en la parte posterior de la articulación. Estos resultados confirman la existencia de nervios sensoriales y autónomos en la cápsula, la membrana sinovial y el disco periférico en la oveja adulta sana.



## Zusammenfassung

Neurale Strukturen im Kiefergelenk des Schafs

Das Ziel dieser Studie war die Beschreibung der normalen Innervation beim Schaf, bevor dieses als Tiermodell zur Untersuchung der Effekte von Arthritis auf die Gelenksinnervation verwendet wird. Bei 10 erwachsenen Merinoschafen wurden die Kiefergelenke makroskopisch und mikroskopisch untersucht. Die Verteilung der neuralen Strukturen wurde mittels Fluoreszenz-Histochemie (Glyoxylic acid), Immunhistochemische- (Calcitonin gene–related peptide), Silber und Goldchloridtechniken dargestellt. Calcitonin gene–related peptide–immunoreaktive Nervenfasern wurden in der Kapsel und der Synovialmembran, aber nicht im Diskus gefunden. Nervenbündel und einzelne Nervenfasern in der Kapsel, Synovialmembran und 2–3 mm vom Diskus entfernt wurden mit Glyoxylic acid angefärbt. In der Kapsel wurden Ruffini-, paciniforme und Golgiorgan-Nervenendigungen gefunden, wobei die höchste Nervendichte beim Attachment des Diskus an die Kapsel auftrat. Diese Ergebnisse bestätigen die Existenz autonomer und sensorischer Nerven in der Kapsel, Synovialmembran und am Rande des Diskus im gesunden erwachsenen Schaf.