# Innervation of the Human Temporomandibular Joint Capsule and Disc as Revealed by Immunohistochemistry for Neurospecific Markers

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*An immunohistochemical investigation for the neurospecific markers S-100 protein and protein gene product 9.5 was carried out on 12 healthy temporomandibular joint discs and capsules that were taken at autopsy from human adults. Large nerve trunks in the joint capsule and posterior disc ligament, as well as small nerve bundles and single nerves in almost all parts of the capsule, were heavily stained by anti-S-100 antiserum. The S-100 immunoreactive chondriocytes were also detected in the disc. Antiprotein gene product 9.5 similarly evidenced nerve fibers in the capsule and posterior disc ligament, and it also labeled a few small nerve bundles and a number of single fibers in the peripheral portions of the disc. No corpuscular or specialized endings were encountered in the specimens. These results give new support to the existence of a rich innervation of the human temporomandibular joint capsule and disc that should be further characterized with regard to type, function, and neuropeptide content.*
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Sensory joint receptors are clearly demonstrated to be involved in the control of movements and in joint protection.[1] There is also growing experimental evidence to show that the central nervous system is involved, not only in sensory reception and motor control, but also in regulating the activities of peripheral tissues in physiologic and pathologic situations.[2] In particular, the nervous system appears to modulate the inflammatory response in various joint pathologies.[3] Many studies suggest that immunoregulatory neuropeptides may be released from the nerve endings into the synovial fluid.[4,5] The various morphologic studies on the innervation of both animal and human joint tissues, however, have failed to produce conclusive results.[6,7]

With regard to temporomandibular joint (TMJ) innervation, studies carried out on human and rat material have produced contradictory results as to the presence of nerve fibers in the TMJ disc and capsule.[6,8–10] However, they were based on gold or silver impregnation, which tends to give inconstant and unspecific results. Recently, immunohistochemical techniques that are able to detect neurospecific structural or neuroactive peptides have made it possible to identify nerve components in many tissues including human specimens. Specifically, general peptidergic markers, such as the glia-specific protein S-100 and the neurospecific marker protein gene product 9.5 (PGP 9.5)[11–13] have been used for this purpose. To the authors' knowledge, few studies concerning the distribution of immunohistochemical markers in primate joint tissue have been published.[14,15]

The present study used antibodies against the glia-associated protein S-100 and the neurospecific marker PGP 9.5, which is believed to be expressed in all parts of the smaller peripheral nerve endings,[13,16–18] with the aim of studying the distribution of nerve fibers in the human TMJ capsule and disc.

## Materials and Methods

The research used 12 joint discs and capsules taken from human adults of both sexes (Table 1), which had no evident local or systemic joint pathologies, 24 to 36 hours after death.

Discs and capsule parts were immediately fixed by immersion in Zamboni fixative (4% w/v paraformaldehyde plus 0.2% picric acid in phosphate buffer; pH 7.2 to 7.4) for 12 hours at 4°C, washed in phosphate buffer saline (PBS) (pH 7.3) plus 15% sucrose for 3 days, then frozen in liquid nitrogen or dehydrated and embedded in paraffin. Sections 15 to 30 μm thick were treated by Sternberger's[19] peroxidase-antiperoxidase (PAP) method or the immunofluorescence method.

Sections treated by the PAP method were pretreated with 0.2% Triton X-100 (Merck, Darmstad, Germany) in PBS (pH 7.3) for 30 minutes and then immersed in 1:1 methanol-PBS containing 0.3% $H_2O_2$ for 30 minutes to inactivate endogenous peroxidases. Primary antibody was incubated for 12 hours at 4°C (anti-PGP 9.5 [Ultraclone, Isle of Wight, UK] was diluted 1:3200 in PBS, and anti S-100 [Dakopatts, Denmark] was diluted 1:400 in PBS). Anti-IgG and PAP complex (Dakopatts) were used diluted 1:50 in PBS for 1 hour at room temperature. The peroxidase reaction was developed in TRIS 0.05 M buffer (pH 7.6) containing 0.02% diamino-benzidine and 0.01% $H_2O_2$ for 10 minutes.

The sections treated by immunofluorescence were incubated in anti-IgG fluorescinate (Dakopatts) diluted 1:50 in PBS for 1 hour at room temperature. Immunohistochemical specificity controls were done *(1)* by substituting the primary antiserum with nonimmune rabbit serum and *(2)* by omitting the secondary anti-IgG serum or the PAP complex.

For the histochemical determination of endogenous peroxidase activity, control sections were incubated in PBS containing 0.025% DAB and 0.003% $H_2O_2$.

## Results

Immunohistochemical controls for the specificity of the immunologic reaction produced no labeling and revealed no endogenous peroxidase activity.

Anti-S-100 immunostaining evidenced many large nerve trunks in almost all parts of the capsule examined; these showed no preferential spatial orientation (Fig 1). Small nerve bundles could also be seen in the peripheral parts of the joint disc, running from the main nerve trunks of the capsule to the most peripheral part of the joint disc (Fig 2). A short stretch of the branches of single fibers, but not their endings, was also observed. In addition, some round or ovoid immunolabeled chondriocytes were visible inside the disc.

Anti-PGP 9.5 antiserum revealed a large number of nerve bundles in the capsular portion (Fig 3). These immunoreactive fibers were visible in all the preparations with both immunoenzymatic and immunofluorescence techniques. These were large trunks, of a similar shade to those evidenced by anti-S-100. Single perivascular fibers were also visible in the capsule and the bilaminar part of the joint

**Table 1** Subjects From Which Joints and Capsules Were Taken

| | Age (y) | Sex* | Dentition | Cause of death |
|---|---|---|---|---|
| 1 | 28 | F | Complete | Aggression |
| 2 | 84 | F | Edentulous | Heart failure |
| 3 | 50 | M | Dentate 1.5–2.5/3.6–4.6 | Heart failure |
| 4 | 50 | M | Dentate 1.5–2.5/3.6–4.6 | Heart failure |
| 5 | 61 | F | Dentate 1.4–2.3/3.5–4.5 | Lung edema |
| 6 | 30 | F | Complete | Trauma |
| 7 | 16 | M | Complete | Trauma |
| 8 | 16 | M | Complete | Trauma |
| 9 | 75 | F | Dentate 1.3–2.3/3.6–4.6 | Heart failure |
| 10 | 78 | F | Edentulous | Heart failure |
| 11 | 66 | M | Edentulous | Brain stroke |
| 12 | 75 | F | Edentulous | Heart failure |

*F = female, M = male.

Fig 1 Large nerve bundle evidenced in areolar pericapsular space (anti-S-100; magnification ×125).

Fig 2 S-100 immunolabeling showing single fibers and a trunk nerve bundle running in the peripheral disc (magnification ×250).

Fig 3 Features comparable to Fig 1 evidenced with anti-PGP 9.5 serum (magnification ×125).

Figs 4a and 4b *(Left)* Nerves and small varicose fibers in the vessel wall evidenced by anti-PGP 9.5 after PAP immunohistochemistry (magnification ×250); *(right)* same features evidenced by anti-PGP 9.5 after fluorescence immunohistochemistry (magnification ×400).

Figs 5a and 5b *(Left)* PGP 9.5 immunoreactive endings observed in the peripheral disc after PAP immunohistochemistry (magnification ×125); *(right)* PGP 9.5 immunoreactive endings observed in the peripheral disc after fluorescence immunohistochemistry (magnification ×125).

disc (Fig 4a), where PGP 9.5 immunoreactive nerves reached the highest concentration. In the disc, some small nerve bundles and numerous single fibers with no preferential orientation were visible (Figs 4b through 5b). These nerves followed tortuous paths and terminated as single fibers without terminal specializations. Examination of serial sections showed that these fibers are apparently limited to the peripheral portions of the disc; they are well represented along the entire disc, but especially in the anterior and posterior areas. Anti-PGP 9.5, unlike anti-S-100, did not reveal any immunoreactive cell element in the capsule nor in the disc.

## Discussion

This study describes the previously unreported distribution of S-100 and PGP 9.5 immunoreactive fibers in the human TMJ. Previous investigations on the innervation of the TMJ capsule and disc in animals and humans using silver or gold impregna-

tion methods have provided conflicting results,[1,6,8] perhaps related to the techniques used. These results are unrepetitive and capricious, especially in articular tissues with a dense network of collagenous fibers. In recent years, immunohistochemistry for neurospecific markers has been shown to be a more reliable method of investigating peripheral nerve fibers.[13,17,18]

The nerve distribution observed in the capsule is similar to that previously described after chromium-silver impregnation.[8,20] However, although these studies only reported capsular and perivascular fibers, assumed to be autonomic in nature, the present study also identified fibers in areas of the joint disc that are certainly avascular, as recently observed by Wink.[6] These nerve fibers should then be suspected of being proprioceptive or nociceptive. They appear as free nerve fibers and are present in regions identified as belonging to the joint disc through contrast staining and observation under the interference microscope. Previous studies have demonstrated nerves containing calcitonin gene-related peptide (CGRP) in rat TMJ discs,[21,22] but other authors failed to detect silver-impregnated or substance P-immunoreactive nerves in monkey TMJ discs.[15] On the other hand, in the cat knee meniscus, it has been shown that over 50% of nerve fibers are afferent rather than autonomic.[17] The neuroactive peptides that were used as neuronal markers are not able to evidence the overall organization of the nerve fibers. The present study used PGP 9.5, which is now believed to be the most ubiquitous marker of the peripheral neurons.[13] The PGP 9.5 is a soluble protein isolated by bidimensional electrophoresis on polyacrylamide gel from extracts of the human brain.[24] It is a major component of neuron cytoplasm, apparently not associated with the cytoskeleton, that may have enzymatic activity. Studies conducted to date have demonstrated its superiority over other neuron markers, such as neuron-specific enolase (NSE) and neurofilaments (NFs), in revealing overall innervation of peripheral organs.[13,17,18]

Nevertheless, it must be considered that the present study utilized material removed from the human body 24 to 36 hours after death. Owing to the speed of molecular decay of the antigens used as markers and the instability of nerve structures, it is probable that a number of nerve fibers, perhaps a considerable number, went undetected. No specialized endings were found in our preparations in the disc or in the capsule. This agrees with observations by Ichikawa et al,[21,22] who used anti-CGRP in rats.

With respect to the functional significance of the nerve fibers, neurophysiologic data indicate that TMJ receptors initiate reflexes that may be involved in the control of jaw movement.[25-27] Efferents from cervical sympathetic ganglia along with afferents from the TMJ to the sensory trigeminal ganglion and to the dorsal root ganglia have been demonstrated in the rat with neuroanatomic tracing techniques.[28] Further neuroanatomic investigations on their central projections are required to clarify whether the nerves observed in the disc are nociceptive or proprioceptive fibers, or are involved in different functions. It should be remembered that some studies show that neuropeptides with a probable trophic function are released in the synovial fluid.[5,29] In vitro, these neuropeptides can modulate the production of immunoglobulins,[24] stimulate T lymphocytes,[31] and induce production of PGE-2 and collagenases by the synovial cells.[32] Many of them are also involved in vasoregulatory and inflammatory mechanisms, eg, in the skin.[33] An increased release of neuropeptides into the synovial fluid in rheumatoid arthritis has, for instance, been suggested.[29] Some of the nerves observed in the TMJ capsule and disc may be responsible for the release of these peptides.

Some S-100 immunolabeled chondriocytes were visible in the disc. This immunoreactivity may be related to the neural crest origin of mesenchymal elements from which the disc fibrocartilage might originate.

In light of the data obtained in this study, it appears that the human TMJ capsule is richly innervated and the disc of the human TMJ has a nervous component, as does other joint cartilage, eg, the cat knee meniscus.

Further investigation is needed to characterize these nerves with regard to type and function, ie, autonomic, proprioceptive, or nociceptive, and to neuroactive peptide content.

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## References

1. Zimny ML. Mechanoreceptors in articular tissues. Am J Anat 1988;182:16–32.
2. Clark RKF, Wyke BD. Contributions of temporomandibular articular mechanoreceptors to the control of mandibular posture: An experimental study. J Dent 1974:2:121–129.

3. Mapp PI, Kidd BL, Merry P, Revell PA, Blake DR. Neuroanatomical features of the synovial membrane. Br J Rheumatol 1988;128:8.
4. Deviller P, Weill B, Renoux M, Menkes C, Pradelles P. Elevated levels of tachykinin-like immunoreactivity in joint fluids from patients with rheumatic diseases. New Eng J Med 1986;314:1323.
5. Larsson J, Ekblom A, Heriksson K, Theodorsson E. Immunoreactive tachikinins, calcitonin gene-related peptide and neuropeptide Y in human synovial fluid from inflamed knee joints. Neurosci Lett 1989;100:326–330.
6. Wink CS, Onge MS, Zimny ML. Neural elements in the human temporomandibular articular disc. J Oral Maxillofac Surg 1992:50:334–337.
7. Ralston HJ, Miller MR, Kasahara M. Nerve endings in human fascias, tendons, ligaments, periosteum and joint synovial membrane. Anat Res 1960:36:137–147.
8. Thilander B. Innervation of temporomandibular joint capsule in man. Trans P Sch Dent Umea 1961;2:1.
9. Dixon AD. Structure and functional significance of the intraarticular disc of the human temporomandibular joint. Oral Surg 1962;15:48–61.
10. Bernick S. The vascular and nerve supply to the temporomandibular joint of the rat. Oral Surg 1962;15:488–498.
11. Thomson RJ, Day INM. Protein gene product 9.5: A new neuronal and neuroendocrine marker. In: Neuronal and Glial Proteins: Structure, Function and Clinical Application. London: Academic Press, 1988.
12. Thompson RJ, Doran JF, Jackson P, Dhillon AP, Rode. PGP 9.5 A new marker for vertebrate neurons and neuroendocrine cells. Brain Res 1983;278:224–228.
13. Gulbenkian S, Wharton J, Polak JM. The visualisation of cardiovascular innervation in the Guinea pig using an antiserum to protein gene product 9.5 (PGP 9.5). J Auton Nerve Syst 1987;18:235–247.
14. Gronblad M, Kontinnen YT, Korkala O, Liesi P, Hukkanen M, Polak J. Neuropeptides in the synovium of patients with rheumatoid arthritis and osteoarthritis. J Rheumatol 1988;15:1807–1810.
15. Johansson AS, Isacsson G, Isberg A, Granholm AC. Distribution of substance P-like immunoreactive nerve fibers in temporomandibular joint soft tissues of monkey. Scand J Dent Res 1986;94:225–232.
16. Bishop AE, Carlei F, Lee V, Trojanowski J, Marangos PJ, Dahl O, et al. Combined immunostaining of neurofilaments, neuron specific enolase, GFAP and S-100. A possible means for assessing the morphological and functional status of the enteric nervous system. Histochem 1985; 82:93–97.
17. Ramieri G, Panzica GC, Viglietti-Panzica C, Spingall D, Polak JM. Uninnervated Merkel cells and Merkel-neurite complexes in human oral mucosa as revealed by an antiserum to protein gene product 9.5. Arch Oral Biol 1992;37:263–267.
18. Ramieri G, Anselmetti GC, Baracchi F, Panzica GC, Viglietti-Panzica C, Modica R, et al. The innervation of human teeth and gingival epithelium as revealed by means of an antiserum to protein gene product 9.5 (PGP g.5). Am J Anat 1990;189:146–154.
19. Sternberger LA. The unlabeled antibody peroxidase-antiperoxidase (PAP) method. In: Sternberger LA (ed). Immunocytochemistry. New York: Wiley, 1979.
20. Lautenbach Von E, Oberbeckmann J. Neurohistologische Untersuchungen an Kapsel und Discus des Kiefergelenkes. Dtsch Zahnärztl Z 1968;23:923–930.
21. Ichikawa H, Matsuo S, Wakisaka S, Akai M. Fine structure of calcitonin gene related peptide immunoreactive nerve fibers in the rat temporomandibular joint. Arch Oral Biol 1990:35:727–730.
22. Ichikawa H, Wakisaha S, Matsuo S, Akai M. Peptidergic innervation of the temporomandibular disc in the rat. Experientia 1989;45:303–304.
23. Langford LA, Schmidt RF. Afferent and efferent axons in the medial and posterior articular nerves of the cat. Anat Res 1983:206:71–78.
24. Jackson P, Thompson RJ. The demonstration of new human brain-specific proteins by high-resolution two-dimensional polyacrylamide gel electrophoresis. J Neurol Sci 1981;49:429–438.
25. Schwaluk S. Initiation of reflex activity from the temporomandibular joint of the cat. J Dent Res 1971;50: 1642–1645.
26. Clark RKF, Wyke BD. Arthrokinetic reflexogenic systems in the temporomandibular joint. J Anat (London) 1974:117:216.
27. Klineberg IJ, Ash MM. Some temporo-mandibular articular reflex effects on jaw muscles [abstract]. J Dent Res 1978;57:130.
28. 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.
29. Mapp PI, Kidd BL, Terry JM, Revell PA, Ibrahim NBN, Blake DR, et al. Substance P-calcitonin gene-related peptide- and C-flanking peptide of neuropeptide Y-immunoreactive fibers are present in normal synovium but depleted in patients with rheumatoid arthritis. Neurosci 1990;37:143–153.
30. Stainisz AJ, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P and somatostatin on immunoglobulin synthesis and proliferation by lymphocytes from Peyers patches, mesenteric lymphonodes and spleen. J Immunol 1986;136:152–156.
31. Payan DG, Brewster DR, Goetzel EJ. Specific stimulation of human T lymphocytes by substance P. J Immunol 1983:131:1613–1615.
32. Lotz M, Carson D, Vaughan JH. Substance P activation of rheumatoid synoviocytes: Neural pathway in the pathogenesis of arthritis. Science 1987:235:893–895.
33. Bloom SR, Polak JM. Regulatory peptides and the skin. Clin Exp Dermatol 1983;8:3–18.

## Resumen

La inervación de la cápsula de la articulación temporomandibular humana y el disco de acuerdo a exámenes immunohistoquímicos para marcadores neuroespecíficos.

Se realizó una investigación immunohistoquímica para los marcadores neuroespecíficos de proteína S-100 y el producto del gen de proteína 9,5, en 12 cápsulas y discos de articulaciones temporomandibulares (ATM) sanas que fueron tomadas de autopsias realizadas en adultos humanos. Los troncos nerviosos mayores en la cápsula de la articulación y el ligamento del disco posterior, así como los paquetes nerviosos menores y los nervios individuales en casi todas las partes de la cápsula, fueron teñidos fuertemente por el anti-suero anti-S-100. También se detectaron en el disco condrocitos inmunoreactivos S-100. El producto del gen de anti-proteína 9,5 puso de manifiesto igualmente, fibras nerviosas en la cápsula y el ligamento del disco posterior, como también marcó unos pocos paquetes nerviosos y un número de fibras aisladas en las porciones periféricas del disco. No se encontraron terminaciones corpusculares o especializadas en los especímenes. Estos resultados proporcionan nuevo soporte a la existencia de una inervación rica en la cápsula de la ATM y el disco, la cual debe ser caracterizada adicionalmente de acuerdo al tipo, función y contenido neuropéptido.



## Zusammenfassung

Die Innervation der menschlichen Kiefergelenkskapsel und des Diskus ermittelt durch Immunhistochemie für neurospezifische Marker.

Eine immunohistochemische Untersuchung für die neurospezifischen Marker "S-100 protein" und "protein gene product 9.5" wurde an 12 gesunden Kiefergelenken (Kapseln und Disci) aus Leichen von gesunden Erwachsenen durchgeführt. Grosse Nervenstämme konnten mit anti-"S-100" Antiserum in der Gelenkkapsel und im posterioren Diskusligament markiert werden, ebenso kleine Nervenbündel und einzelne Nerven in praktisch allen Teilen der Gelenkkapsel. "S-100" immunoreaktive Chondrozyten wurden auch im Diskus gefunden. "protein gene product 9.5" stellte in ähnlicher Weise Nervenfasern der Kapsel und des posterioren Diskusligamentes dar, es markierte auch einzelne kleine Nervenbündel und eine Anzahl einzelner Nervenfasern in den periferen Zonen des Diskus. In den untersuchten Proben konnten keine korpuskulären oder spezialisierten Nervenendigungen gefunden werden. Diese Resultate bekräftigen die Existenz einer ausgeprägten Innervation der menschlichen Kiefergelenkskapsel und des Diskus, die weiter untersucht werden sollte was ihre Art, ihre Funktion und ihren Neuropeptidgehalt betrifft.