



Topography of sensory receptors within the human glenohumeral joint capsule

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Background and hypotheses: Sensory receptors in the joint capsule are critical for maintaining joint stability. However, the distribution of sensory receptors in the glenohumeral joint of the shoulder, including mechanoreceptors and free nerve endings, has not been described yet. This study aimed to describe the distributions of different sensory receptor subtypes in the glenohumeral joint capsule. Our hypotheses were as follows: (1) Sensory receptor subtypes would differ in density but follow a similar distribution pattern, and (2) the anterior capsule would have the highest density of sensory receptors.

Methods: Six glenohumeral joint capsules were harvested from the glenoid to the humeral attachment. The capsule was divided into 4 regions of interest (anterior, posterior, superior, and inferior) and analyzed using modified gold chloride stain. Sensory receptors as well as free nerve endings were identified and counted under a light microscope from sections of each region of interest. The density of each sensory receptor subtype was calculated relative to capsule volume.

Results: Sensory receptors were distributed in the glenohumeral joint capsule with free nerve endings. The anterior capsule exhibited the highest median density of all 4 sensory receptors examined, followed by the superior, inferior, and posterior capsules. The median densities of these sensory receptor subtypes also significantly differed ($P = .007$), with type I (Ruffini corpuscles) receptors having the highest density (2.97 U/cm^3), followed by type IV (free nerve endings, 2.25 U/cm^3), type II (Pacinian corpuscles, 1.40 U/cm^3), and type III (Golgi corpuscles, 0.24 U/cm^3) receptors.

Conclusion: Sensory receptor subtypes are differentially expressed in the glenohumeral joint capsule, primarily type I and IV sensory receptors. The expression of sensory receptors was dominant in the anterior capsule, stressing the important role of proprioception feedback for joint stability. The surgical procedure for shoulder instability should consider the topography of sensory receptors to preserve or restore the proprioception of the shoulder joint.

Level of evidence: Anatomy Study; Histology

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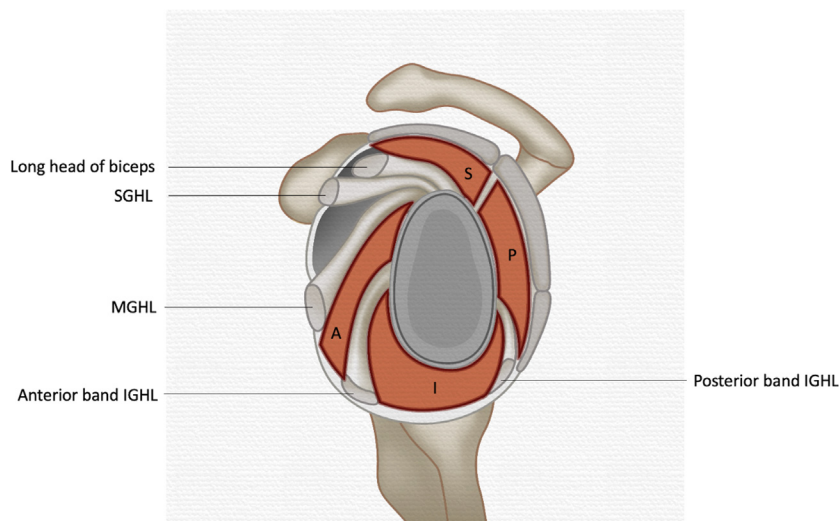


Figure 1 Glenohumeral joint capsule regions of interest (red): anterior (A), posterior (P), superior (S), and inferior (I) capsules. SGHL, superior glenohumeral ligament; MGHL, middle glenohumeral ligament; IGHL, inferior glenohumeral ligament.

Damage to the glenohumeral joint capsule is involved in a variety of shoulder problems, from instability to adhesive capsulitis (frozen shoulder).²⁹ Ligaments and the capsule act as important passive restraints for glenohumeral joint stability and establish physical continuity of the humeral head with the rotator cuff muscles.¹³ Sensory receptors embedded in the ligament and thickened capsule provide proprioceptive and nociceptive feedback that trigger various reflexes to protect the joint.^{6,9,10,14-16,22,23,25,37,39,41} However, studies on sensory receptors in the glenohumeral joint have primarily focused on nociceptors of the sub-acromial bursa and their relationship with joint pain,^{12,28,31,38} whereas functions in protective reflexes have scarcely been investigated. The neuroanatomic distribution of the mechanoreceptors in the glenohumeral joint capsule has been studied based on their histomorphologic characteristics.^{7,13,29,35} However, the differences in the mechanoreceptors' density in each area of interest in the glenohumeral joint capsule remain unknown. The purpose of this study was to describe the distributions of different sensory receptor subtypes in the glenohumeral joint capsule. Our hypotheses were as follows: (1) Sensory receptor subtypes would differ in density but follow a similar distribution pattern, and (2) the anterior capsule would have the highest density of sensory receptors.

Materials and methods

Cadaveric dissection and specimen preparation

Internal review board exemption was obtained for this study (IRB exemption number S2019-1181-0002). The cadavers used in this study were donated to the institution. Bilateral glenohumeral

capsules were harvested from 3 fresh frozen cadavers, 1 male and 2 female cadavers, with a mean age of 59 years (range, 56-69 years). All 6 glenohumeral capsules were harvested within 24 hours of the cadavers being thawed to room temperature. The capsuloligamentous structure was not damaged during dissection or separation from the bone. Dissections were performed by 2 orthopedic surgeons (E.K. and J.-M.K.). After dissection, the specimens were immersed in 4% paraformaldehyde solution at neutral pH. After 24 hours of fixation, each specimen was divided into 4 regions of interest (ROIs). The ROIs included (1) the superior capsule, extending from posterior to the biceps long head to the level of the scapular spine; (2) the posterior capsule, extending from the level of the scapular spine to the posterior band of the inferior glenohumeral ligament; (3) the inferior capsule, extending from the posterior band to the anterior band of the inferior glenohumeral ligament; and (4) the anterior capsule, extending from the anterior band of the inferior glenohumeral ligament to the middle glenohumeral ligament (Fig. 1). The time taken from specimen preparation to the staining process was 48 hours.

Modified gold chloride staining

The modified gold chloride staining method was used to visualize capsular sensory receptors.³⁴ In brief, specimens were transferred to vials containing fresh lemon juice and 98% formic acid in a 3:1 ratio and shaken using a Penetron shaker (Penetron Swirling Shaker Model Mark IV; Sunkay Laboratories, Tokyo, Japan) inside a fume hood (A-MB-1200TYPE; DH Science, Daejeon, Republic of Korea) for 10 minutes at 30°C. Gold chloride solution (200 mg/dL in deionized water, HT1004; Sigma-Aldrich, St Louis, MO, USA) was poured into the vials, and shaking was continued for 90 minutes. This process was repeated for each specimen using recycled gold chloride solution from the previous specimen after filtration. The specimen was then removed from the gold chloride solution and soaked in 25% formic acid solution with shaking for an additional 12 hours (minimum). Thereafter,

Table I Freeman and Wyke sensory receptor classification⁶

Type	Name	Morphologic features	Dimensions, μm	Functional characteristics
I	Ruffini corpuscle	Globular or round shaped, thin myelinated capsule, arborizing nerve terminal	100×40	Mechanoreceptor: low threshold, slowly adapted
II	Pacinian corpuscle	Cylindrical or conical, thick myelinated capsule, single nerve terminal	280×120	Mechanoreceptor: low threshold, rapidly adapted
III	Golgi corpuscle	Fusiform or spindle shaped, thin myelinated capsule, arborizing nerve terminal	600×100	Mechanoreceptor: low threshold, slowly adapted
IV	Free nerve endings	Nonmyelinated, irregular nerve end	Diameter of 0.5-5	Nociceptor: non-adapting

specimens were washed 3 times in running distilled water for 5 minutes, transferred to individual conical tubes filled with 30% sucrose solution, and stored in a refrigerator at 4°C for 1-2 days. After the specimens sank to the bottom of the conical tubes, they were transferred to new vials containing 30% sucrose and optimum-cutting temperature compound and shaken for 2 hours. Stained specimens were then embedded in 30% sucrose and optimum-cutting temperature compound (3:2 ratio) and frozen according to a previously described technique.³⁴ Frozen specimens were sectioned at 30 μm thickness parallel to the longitudinal (horizontal) axis and perpendicular to the vertical axis with a cryosectioning machine (Leica CM3050-S Research Cryostat; Leica Biosystems, Wetzlar, Germany). Sections were then mounted on glass microscope slides for examination.

Microscopic examination

An inverted light microscope was used for determination of receptor subtype and distribution. The Freeman and Wyke classification⁶ was used as a reference for evaluating the morphology of Ruffini, Golgi, and Pacinian corpuscles (Table I). The slides were analyzed by 2 orthopedic surgeons (E.K. and J.-M.K.). Free nerve endings were identified based on a technique used in a previous study.³⁸ The slides were first visualized under low-power magnification (100 \times) and then under higher-power magnification (200 \times) to identify each receptor subtype. Sensory receptor structure was observed on serial slides to assess continuity. If continuity was confirmed, the structure under observation was counted as 1 sensory receptor unit. Discontinuous objects with uncertain morphologies were not counted. We reconfirmed each structure at 400 \times magnification to minimize misidentification and bias.

Density calculation

A customized software program was developed to determine capsule volume.¹⁴⁻¹⁶ The volume of 1 slide was multiplied by 30 μm to obtain the total dimension. The volume of each ROI (in cubic centimeters) was then calculated. The density of the sensory receptors (in units per cubic centimeter) was calculated by dividing the number of sensory receptors (in units) by the total area of the ROI. Densities were then compared among subtypes and ROIs.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normality of each data set distribution. As sensory receptor densities were skewed, values were expressed as median (range).¹⁵ The nonparametric Kruskal-Wallis test was used to evaluate the dominant (highest-density) sensory receptor subtype in each ROI with post hoc tests for pair-wise comparisons. The significance level was set at $P < .05$ for all tests. All descriptive and statistical analyses were conducted using SPSS software (version 15.0; IBM, Armonk, NY, USA). The interobserver reliability of the microscopic examination was expressed as the percentage of absolute agreement.¹

Results

Sensory receptors in glenohumeral joint capsule

The glenohumeral joint capsule sections contained 3 types of encapsulated nerve endings: type I (Ruffini corpuscles), type II (Pacinian corpuscles), and type III (Golgi corpuscles) receptors. Unencapsulated (free) nerve endings, classified as type IV sensory receptors, with no specific morphology were also observed. The Ruffini corpuscles were dendritic in shape and 200-300 μm in largest diameter (Fig. 2, A); the Pacinian corpuscles were lamellar and cylindrical in shape and 100-200 μm in largest diameter (Fig. 2, B); and the Golgi corpuscles were spindle shaped and 450-500 μm in largest diameter (Fig. 2, C). The free nerve endings varied in size from 100 to 450 μm , with inconsistent shape (Fig. 2, D). In total, 15,000 stained sections were examined, encompassing a total volume of 9386.25 mm^3 . The interobserver reliability of the microscopic examination was 81%, which was interpreted as acceptable agreement.²⁷

Density of mechanoreceptors

Table II summarizes the overall density of each receptor subtype within the glenohumeral joint capsule. Ruffini corpuscles were observed at the highest median density, followed by free nerve endings, Pacinian corpuscles, and

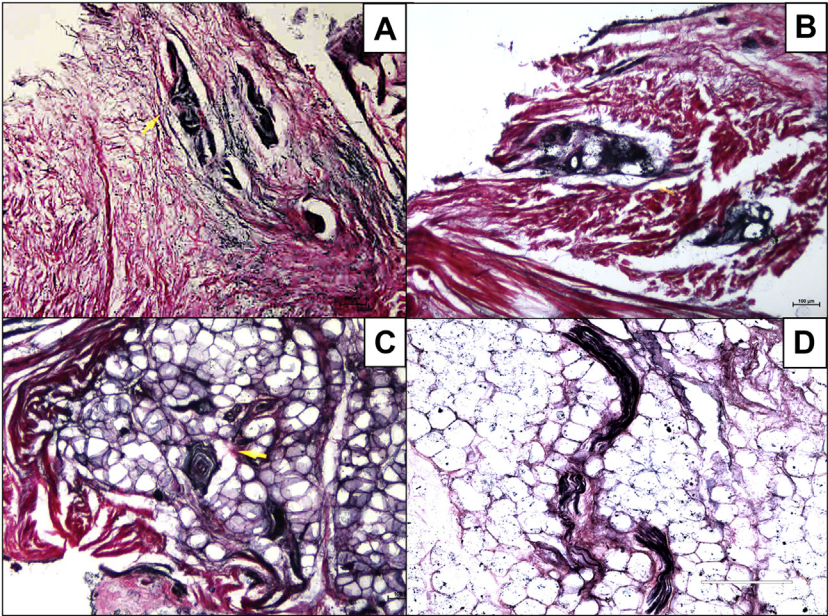


Figure 2 The sensory receptors of the glenohumeral joint capsule include 3 types of encapsulated nerve endings: type I (Ruffini corpuscles, →) (A), type II (Pacinian corpuscles, →) (B), and type III (Golgi corpuscles, →) (C). (D) Type IV (free nerve endings) is also expressed in the glenohumeral joint capsule (modified gold chloride staining, original magnification ×200).

Table II Overall median density of sensory receptors in glenohumeral joint capsule					
	Type I	Type II	Type III	Type IV	P value
Median, U/cm ³	2.97	1.40	0.24	2.25	.007*
Minimum-maximum, U/cm ³	0-3.55	0.41-2.22	0.11-0.35	1.41-3.52	

* Kruskal-Wallis test with significance level set at $P < .05$.

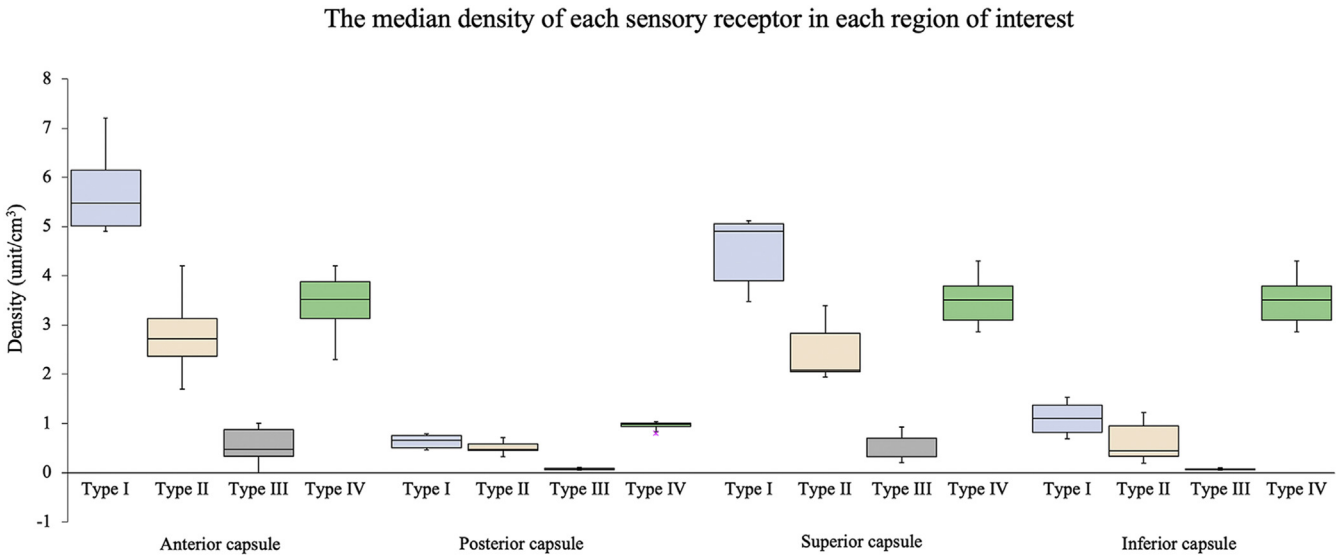


Figure 3 Distribution of each sensory receptor in each region of interest.

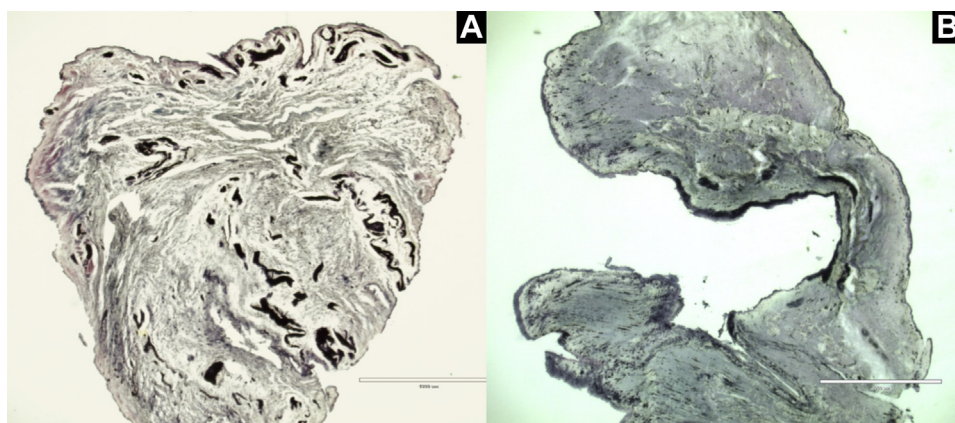


Figure 4 Expression of sensory receptors in anterior capsule (A) vs. posterior capsule (B) (modified gold chloride stain, original magnification x40).

Golgi corpuscles ($P < .001$). However, on pair-wise post hoc comparisons, only the densities of Ruffini and Pacinian corpuscles differed significantly owing to the large variation across samples.

Figure 3 presents the median density of each sensory receptor subtype within each ROI of the glenohumeral joint capsule. Significant differences in density were found among ROIs for all subtypes (Ruffini corpuscles, $P < .001$; Pacinian corpuscles, $P = .003$; Golgi corpuscles, $P = .03$; and free nerve endings, $P = .02$). Figure 4 shows an example of the density of sensory receptors expressed in the anterior capsule compared with that expressed in the posterior capsule.

Ruffini corpuscles were measured at the highest density in the anterior capsule, followed by the superior, inferior, and posterior capsules, although there was no significant difference between the inferior capsule and either the superior ($P = .838$) or anterior ($P = .462$) capsule by pair-wise post hoc analysis. Similarly, Pacinian corpuscle density was the highest in the anterior capsule, followed by the superior, inferior, and posterior capsules, but density did not differ significantly between the posterior and inferior capsules ($P = .648$) or between the anterior and superior capsules ($P = .561$) by pair-wise post hoc analysis. Golgi corpuscle density was also the highest in the anterior capsule, followed by the superior capsule. Golgi corpuscles were equally distributed between the inferior and posterior capsules. However, on post hoc analysis, Golgi corpuscle density in the anterior capsule differed significant only compared with that in the posterior capsule ($P = .010$) and inferior capsule ($P = .010$).

As in the case of encapsulated receptors, free nerve endings were at the highest density in the anterior capsule, followed by the superior, inferior, and posterior capsules. Density did not differ significantly between the posterior and inferior capsules ($P = .902$) or between the anterior and superior capsules ($P = .806$).

Discussion

The primary finding of this study was the existence of sensory receptors in the substance of the glenohumeral joint capsule. The sensory receptors are differentially expressed in the human glenohumeral joint capsule but follow a similar distribution pattern, with the highest median density in the anterior capsule, followed by the superior, inferior, and posterior capsules. The joint capsule is critical for joint structural stability and prevents injury by passively restraining movement.¹⁰ However, the role of proprioception in shoulder stability is debatable. Warner et al³³ evaluated proprioception in individuals with normal shoulders, those with unstable shoulders, and those after surgical stabilization. They reported that the unstable shoulders demonstrated a considerable decrease in proprioceptive control and that surgical stabilization normalized the proprioception. Consistently with this finding, surgeons who advocate stabilization not only address the mechanical instability that results from joint injury but also implement functional rehabilitation to encourage the patient's return to sport.¹⁹ However, the use of proprioception-enhancing physiotherapy without surgical stabilization was opposed by a previous study³² because the neural elements are not injured but rather need to be activated to the normal stage.³⁰ Thus, basic scientific knowledge of mechanoreceptors and their contribution to glenohumeral joint stability is essential to confirm the best course of treatment.

Several histologic studies have described the neural anatomy of the shoulder joint.^{12,13,18,28,29,31,38} However, previous reports have focused on the nociceptors of the subacromial bursa^{12,28,31,38} and the proprioceptors of the ligamentous complex^{13,18,29} compared with those of the glenohumeral joint capsule.^{7,35} The glenohumeral joint capsule has been studied for the distribution of sensory receptors.^{7,35} However, a previous report limited the microscopic analysis to the anteroinferior and

posteroinferior portions of the glenohumeral joint capsule.³⁵ A study by Gohlke et al⁷ analyzed the entire glenohumeral joint capsule for the presence of sensory receptors. However, the density of the reported sensory receptors was not normalized to the dimension of the harvested tissue, which only represented the relative density rather than the true density. For these reasons, previous studies have possibly underestimated the true value of the sensory receptors.

Our study described the mechanoreceptor subtype distribution within the glenohumeral joint based on the morphologic classification of Freeman and Wyke⁶ with modified gold chloride staining.³⁴ The staining technique was modified to improve the tissue transparency and provide uniform staining throughout the depth of the tissue. The gold chloride staining method used was modified from an earlier technique²¹ and has demonstrated consistency in identifying sensory nerve endings.^{14-16,24,34,35} Our observations revealed that type I and IV sensory receptors are the most numerous and densely distributed mechanoreceptors, followed by type II and III sensory receptors, which is similar to findings observed in the elbow and hip joints.^{10,14-16} The type I sensory receptors are activated from their resting phase when the joint is stressed by angular and compression forces, as is shown in the hip joint.¹⁰ Furthermore, the type IV sensory receptors, which are the second most dominant sensory receptors, act as a nociceptive system in the joint that is activated when the joint is subjected to chemical or mechanical stimuli.⁸ The sensory receptors are activated when they detect the amount of strain that is only reached when the joint is subjected to extreme joint rotation (ie, shoulder dislocation).²⁰ Clinically, the response of type I and IV sensory receptors may similarly explain the positive apprehension test results in shoulder instability patients. Therefore, the term “capsuloperception” is perhaps appropriate to describe the strain detection mechanism of a joint capsule. We observed that type II and III sensory receptors were less expressed in the glenohumeral joint capsule. The absence of type II and III sensory receptors was also described in the anterolateral ligament of the knee joint,² which is inseparably considered a capsular structure.⁵ The lesser distribution of type II and III sensory receptors perhaps suggests the less important proprioceptive role of peripheral capsular structures than that of the central pivot ligaments such as the anterior cruciate ligament of the knee joint, which shows the comparable existence of all sensory receptor types.^{25,26,40}

The anterior capsule exhibited the highest overall density of mechanoreceptors. This preferential location of mechanoreceptors is consistent with the essential role of the anterior capsule in maintaining shoulder stability. Mechanoreceptors provide afferent feedback as the capsular tissue is stretched³⁹ to reflexively increase muscle tone and push the humeral head back toward the glenoid fossa, thereby preventing joint dislocation. Patients with anterior shoulder instability demonstrate various soft tissue and osseous

pathologies, which often include detachment and stretching of the capsule and/or labrum.^{11,36} A prospective study of 212 patients with ≥ 1 documented shoulder dislocation revealed that most patients ($n = 168$, 79%) had anterior capsular insufficiency. Thus, patients with capsular detachment and stretching following a traumatic shoulder dislocation are advised to undergo surgery as soon as possible both to regain neuro-physiological feedback mechanisms and to restore anatomic integrity. Indeed, a systematic review concluded that operative treatment (ie, anatomic Bankart repair) is superior to nonoperative treatment and/or arthroscopic lavage for reducing recurrence in young patients with first-time traumatic shoulder dislocation.⁴

Mechanoreceptor density may decrease with age, as evidenced by a study of the human coracoacromial ligament,¹⁸ and the anterior capsule is a major site of pathology associated with anterior shoulder dislocation without rotator cuff tear in older patients.¹⁷ These findings strongly suggest that the high mechanoreceptor density of the anterior shoulder capsule is critical to maintain joint stability and that reduced mechanoreceptor density with a concomitant decrease in proprioception contributes to the enhanced risk of shoulder dislocation in elderly persons.

Limitations

The major limitation of this study is the small number of specimens. Additional specimens from different age groups are required to reach conclusive results. Cadaver age and time from death to fixation are also confounding factors, particularly as age is known to affect mechanoreceptor density.³ Necrosis during the postmortem period before freezing may also influence the shape and number of mechanoreceptors. Another limitation is the lack of control staining methods (hematoxylin-eosin staining and immunostaining) and 3-dimensional reconstruction of the morphology of sensory receptors. In future studies, other staining methods should be applied, along with 3-dimensional reconstruction software, to verify the morphology of sensory receptor subtype. Despite the limited number of samples, all samples were examined thoroughly under high-power magnification and demonstrated similar subtype distribution patterns. The finding of peak receptor density in the anterior capsule has important clinical implications for shoulder stabilization surgery.

Conclusion

Sensory receptor subtypes are differentially expressed but distributed in a similar pattern throughout the human glenohumeral joint capsule, with the anterior region having the highest density. This distribution is consistent with mechanoreceptor functions in maintaining joint

stability via proprioceptive reflexes and suggests that restoration of proprioception is critical for successful shoulder instability treatment.

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