Université de Montréal

Quantification of Mechanoreceptors in the Canine Anterior Cruciate Ligament

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Tables des matières

Liste des figures

Liste des tableaux

Liste des sigles et abréviations

ACL	Anterior Cruciate Ligament
LCA	Ligament Croissé Antérieur
MRI	Magnetic Resonance Imaging
TKA	Total Knee Arthroplasty

Dédicace

Ce mémoire est dédié à ma famille qui m'a soutenu pendant toutes ces années.

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Sommaire

Purpose:

Although the Anterior Cruciate ligament (ACL) is one of the most studied ligaments in medical science, no one has attempted to quantify the mechanoreceptors of this ligament. These receptors are responsible for the proprioception of the knee. The purpose of this study was to quantify the mechanoreceptors of the normal canine ACL.

Materials and Method:

Ten canine ACLs were harvested preserving their proximal and distal bony attachments. Length was preserved using a special external fixater. The specimens were stained using a modified gold chloride technique. After staining the ligaments they were divided into thirds and then serially sliced at 0.5 microns. The slides were viewed under the microscope at low and high power to identify and count the mechanoreceptors present. Statistical Analysis was performed using an ANOVA and the Sheffé a posteriori tests to determine if a significant difference existed between the number of mechanoreceptors in each third of the ligament as well as to determine where this difference lay. The average number of receptors found were: proximal 67, middle 43, and distal 18. Comparison of these results showed a significant difference existed between the three regions of the ligament ($p<0.001$). The Sheffé test revealed that the proximal third contained a greater mean number of receptors than the middle and distal thirds of the

ligament (S=3.8). There was no significant difference found between the number of receptors in the middle and distal thirds (S=0.85).

Strength and Weakness:

The strengths of this study are that thin sections were used and all mechanoreceptors were counted. The small sections allowed us to have several cuts through each receptor and helped decrease the possibility that artifact was mistaken for a receptor. We did not attempt to classify the types of receptors because we felt that this would be inaccurate. The studies weak points are a small sample and one observer.

Significance:

The number and distribution of mechanoreceptor in the ACL may have clinical consequences after injury or reconstruction. The results of this study cannot be extrapolated to humans, but allow insight into the microscopic structure of the human ACL.

Conclusion:

This study showed a quantitative difference between the number of mechanoreceptors in the proximal third of the canine ACL when compared to the other two thirds of the ligament.

Résumé

Introduction:

Le Ligament Croisé Antérieur (LCA) est un important stabilisateur du genou. Mais son rôle dans la proprioception du genou n'est pas encore completement élucidé, malgré le fait que plusieurs auteurs y aient identifié des mécanorécepteurs de différents types.

Le LCA est important pour la stabilité statique du genou. Une blessure issolé du LCA provoque la translation antérieure excessive du tibia sur le fémur. Chez l'humain cette blessure amène de l'instabilité, la sensation que le genou "lâche". Chez le chien la même blessure est responsable d'une arthrose précoce. Par contre dans le genou humain, une progression de l'arthrose à la suite d'une blessure du LCA n'a pas été démontrée.

La proprioception est l'habileté à percevoir la position d'un membre dans l'espace. Plusieurs recherches sur le rôle du LCA dans la proprioception du genou ont des résultats contradictoires.

Il y a peux de recherche dans la microstructure du LCA. Pour mieux comprendre le rôle proprioceptif du LCA, il est donc important d'approfondir l'étude de l'anatomie microscopique de ce ligament.

But:

Le but de cette étude est d'identifier les mécanorécepteurs dans le LCA du chien. De plus, en comparant le nombre de récepteurs dans les trois tiers du LCA nous voulons identifier l'endroit où se concentrent en plus grand nombre les mécanorécepteurs.

Matériaux et méthodes:

Les ligaments croisés antérieurs de dix chiens ont été prélevés. Les critères d'inclusion étaient l'absence de blessure au genou, utilisation d'animaux ne provenant pas de projet où ceux-ci auraient pu subir des traumatismes du genou. Et le genou a été congelé dans les 2 heures suivant son prélevement. La taille du chien n'influençait pas la décision d'inclusion.

Les LCA ont été prélevés après que l'animal ait été sacrifié. Les genoux ont été ouverts avec une incision parapatellaire médiale et les LCA ont été prélevé avec un bloc d'os proximal et un bloc d'os distale. Un fixateur externe a été utilisé pour garder le ligament à sa longeur de repos. Les ligaments ont ensuite été colorés au chlorure d'or selon la technique de Zimney décrite en détail dans la **Figure 1**. Après coloration les ligaments ont été coupés en tiers proximal, moyen et distal. Chaque section a ensuite été coupée de façon sériée à intervalles de 5 microns et montée sur lame. L'auteur a examiné chaque lame en identifiant chaque mécanorécepteur. Ces données ont été analysées à l'aide d'un ordinateur. Chaque lame a été examiné sous microscope à fort et faible agrandissements pour tenter une identification précise du type de récepteur **(Figure 2)**. Parfois, plusieurs coupes ont été nécessaires pour ce faire.

Une analyse statistique a été faite. Le nombre total de mécanorécepteurs a été calculé pour chaque tiers du ligament. Une moyenne et une analyse de variance ont été faites. La valeur d'alpha a préablement été établie à 0.05 et la valeur de beta à 0.80. Il a également été déterminé qu'un test de Scheffé serait utilisé à posteriori pour déterminer à quel endroit se trouvait, le plus grand nombre de mécanorécepteurs dans le ligament, si ce nombre variait effectivement.

Résultats:

Deux milles lames ont été observées et deux types de mécanorécepteurs ont été identifiés. La **Figure 3** montre un récepteur Pacini, le récepteur le plus souvent identifié. Des récepteurs Ruffini, **(Figure 4)** ont aussi été observés. Des terminaisons nerveuses libres (Free Nerve Endings) ont été identifiées et exclues, car leurs fonctions semblent être la régulation autonomique et la perception de la douleur, et non la proprioception **(Figure 5)**. Le **Tableau 1** (Table 1) montre le nombre de récepteurs retrouvés dans chaque tiers des LCA. On a dénombré 323 récepteurs Pacini dans les tiers proximaux, 100 dans les tiers moyens, et 33 dans les tiers distaux. Le pourcentage de mécanorécepteurs par tiers de ligament est illustré à la **(Figure 6)**. La moyenne de récepteurs par lame est présentée dans le **Tableau 2** (Table 2). Le **Tableau 3** (Table 3) montre le nombre moyen de récepteurs par tiers de ligament. On a constaté une prédominance de récepteurs Pacini dans les tiers proximaux. Par ailleurs, la partie distale du ligament contient moins de récepteurs que les parties proximales.

Analyse statistique:

Les résultats de l'analyse de variance ont démontré une différence entre les nombres moyens de récepteurs de chaque tiers ($p<0.001$). Ces résultats sont exposés en détail dans le **Tableau 4** (Table 4).

Le test statistique de Sheffé a été appliqué aux résultats de l'analyse de varience pour déterminer où la différence significative entre les quantités de récepteurs se trouvait. Les résultats du test de Scheffé ont démontré que la différence se trouvait entre les tiers proximaux du ligament et les tiers moyens et distaux du ligament. Il n'y avait pas de différence significative entre les tiers moyen et distal du LCA canin. Le **Tableau 5** (Table 5) contient les détails de ces calculs.

Discussion:

Le genou est une articulation complexe qui comprend en réalité trois articulations: les articulations tibiofémorale interne, tibiofémorale externe et patellofémorale. Les ligaments du genou sont importants pour donner de la stabilité à l'articulation lors des mouvements du genou. Les ménisques sont des amortisseurs localisés dans les articulations tibiofémorales internes et externes. Ils agissent comme stabilisateurs secondaires de la translation antérieure du tibia sur le fémur mais seulement en cas de rupture du LCA. Les ligaments croisés eux résistent à la translation du tibia sur le fémur. Le LCA plus spécifiquement accomplit cette fonction. Ces ligaments sont extrasynoviaux. Ils sont très forts mais leur position intra-articulaire diminue leur possibilité de guérison. Ceci est secondaire au deplacement des bouts deplacer, presence de liquide synovial après dechirure et destruction de circulation sangine du ligament. La combinaison d'une déchirure du LCA et d'une déchirure du menisque interne s'avérait bien néfaste pour le genou.

Le LCA prévient aussi la rotation du tibia. La fonction de ce ligament est très importante pendant la course à haute vitesse, lors d'un arrêt rapide complet d'un changement soudain de direction, ou à la réception d'un saut.

Le LCA du chien a un rôle plus important dans l'arthrose que celui de l'humain. [3, 30] Le plateau tibial canin a une angulation postérieure plus importante que celle de l'homme.[9] Ceci pousse le tibia antérieur sur le fémur. De plus, le chien marche avec le genou fléchi ce qui augmente cette tendance du plateau tibial à glisser sur le fémur. Une rupture du LCA chez le chien entraine une arthrose précoce. [3]

Des mécanorécepteurs ont été identifiés dans les ligaments de plusieurs articulations chez l'homme et l'animal.[.33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51] Des signaux afférents au cerveau ont été démontrés par Pitman et al.. Dans leur étude, la région qui répondait le mieux au stimulus arthroscopique était dans le tiers moyen.

Un réflexe LCA/ischiojambiers est postulé en réponse à un étirement du croisé antérieur. Quelques auteurs ont démontré la présence de ce réflexe[54, 55] , d'autres non.[57]

Nous avons utilisé les genoux du chien pour plusieurs raisons. Premièrement, ils sont plus faciles à obtenir que des genoux humains. De plus la méthode de coloration avec chlorure d'or a été documentée chez le chien. Et finalement la grandeur du ligament chez le chien facilite sa coloration.

Barrack [25] a démontré une diminution de proprioception après rupture du LCA. Il a aussi démontré le même phénomène chez des patients ayant une laxité ligamentaire généralisée. Cependant Barrett [64], quant a lui, a démontré une amélioration de la proprioception chez les patients qui ont reçu une reconstruction du LCA. Toutes ces études démontrent l'importance des mécanorécepteurs du LCA dans la proprioception du genou.

Notre hypothese est que la rupture du LCA entraîne une petite perte de mécanorécepteurs du genou. Ceci ne devrait pas donner de baisse de proprioception mesurable. En plus l'instabilité qui résulte de la rupture du LCA provient du fait que notre cerveau est incapable d'interpréter les signaux afférents des mécanorécepteurs du genou qui n'ont pas été endommagés. Après reconstruction du LCA, les signaux afférents redeviennent interprétables à la suite du rétablissement de la séquence de décharge reconnaissable par le cerveau.

Il y a plusieurs sources d'erreurs possibles dans cette étude. D'une part, la coloration de chlorure d'or peut ne pas avoir été uniforme. Par contre nous avons tranché nos spécimens très finement, ce qui nous a aidé à identifier les récepteurs en plus d'éliminer l'artefact. De l'autre part, le fait qu'une seule personne ait regardé les lames est un point faible posible. L'identification précise d'un mécanorécepteur peut être difficile. Il nous a été difficile de faire une distinction précise entre les récepteurs de Pacini et ceux de Ruffini. Un autre source d'erreur possible est le petit échantillon étudié.

Néanmoins, malgré les erreurs possibles de notre analyse, nous constatons de façon assez évidente qu'un nombre plus grand de mécanorécepteurs se situent dans le tiers proximal des ligaments étudiés.

Nous sommes intéressés à poursuivre ce travail en appliquant cette technique à des LCA humains. Nous sommes aussi intéressés par le rôle que le LCA joue dans la proprioception du genou.

Conclusion:

Les résultats de cette étude démontrent qu'il y a une différence dans le nombre de mécanorécepteurs dans les diverses parties du LCA du chien. Par contre ces résultats ne peuvent pas être extrapolés à l'humain.

Corps de la thèse

Introduction:

One of the most important stabilizing structures in the knee is the Anterior Cruciate Ligament (ACL). This ligament's stabilizing function is well known but its role in proprioception of the knee is unclear. Mechanoreceptors have been described in human as well as animal ACLs. Despite thier presence the role these receptors play in proprioception is unknown.

ACL injuries in humans are common; indeed the incidence of acute ACL tears is 1 in 3000 Americans. Approximately 95,000 new tears[1] and approximately 50,000 ACL reconstructions are performed annually in the USA[2]. Consequently a lot of research has been performed on the ACL. Most of this research has been on the ACL's mechanical role in stabilizing the knee.

The knee ligaments provide the knee with static stability. These ligaments prevent the knee from moving out of its normal plane of motion. Injury to these structures can result in instability and “giving out” of the knee. In the dog an ACL tear usually results in rapidly progressive osteoarthritis of the knee joint.[3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23] This progression is not necessarily true in humans though many authors consider it to be a risk factor for osteoarthritis.[24,25,26,27,28,2930]

Proprioception is the ability to perceive the position and motion of a joint in space. The mechanism by which the brain performs this task is not completely elucidated. Although some work has been done in this area, our understanding of how much information is transmitted from the ligament mechanoreceptors to the brain is unknown. The process of transmitting this data is also not well understood. The microscopic anatomy of the mechanoreceptors in the ligaments remains unknown. The amount of proprioception that these ligaments provide is debated. There are no studies that correlate increased proprioception with an increased concentration of mechanoreceptors. We feel that the first step in understanding the role of mechanoreceptors in any joint is to delineate their distribution in these ligaments.

Purpose:

The purpose of this study was to attempt to identify the different types of mechanoreptors in the canine ACL and then attempt to quantify these receptors. We wanted to be able to see where the receptors are located and not just how many are present. We wanted to identify the region of the ACL that had the highest concentration of receptors to help us understand the proprioceptive role of this ligament.

Materials and Methods:

Inclusion Criteria:

ACL specimens were obtained from canine knees immediately after sacrifice. To be included in this project the dog had to have been in good overall with no known knee injury. Dogs that had been subjects in musculoskeletal research projects that traumatized the knee were excluded. The leg or knee was frozen within 12 hours of sacrifice. The size of the dogs was not important. The specimens came from several different breeds including mongrels. If the dog was a part of another study then its weight bearing status was recorded.

Exclusion Criteria:

Dogs with evidence of prior injury to their ACL were excluded. Also any animal that had evidence of a prior injury to any other knee ligaments were excluded. The inability to preserve an adequate bone block from the origin or insertion of the ligament was grounds for exclusion of the ligament from the project. The presence of osteoarthritis in the knee meant exclusion of the knee. Specimens sacrificed several hours before removal of the ACL were excluded. The fact that an animal was non-weight bearing on a limb prior to harvest was not considered to be an exclusion criterion. Despite these strict criteria no specimens needed to be excluded.

Specimen Harvesting and Preparation:

Ten canine knees were obtained that met the criteria. Most of these animals were sacrificed as part of another research project. The knees were harvested within 2 hours of sacrifice and the complete knee joint resected. The knees were frozen at –30° Celsius until the author could dissect out the ACL from each knee.

The knees were opened using a medial parapatellar incision. The joint was inspected as were the ligaments and menisci. All muscle and fat attached to the tibia and femur was sharply dissected using a #15 blade. The patella and its tendon were then removed from the tibial tubercle. The following structures; ACL, PCL, and menisci were then removed from each knee. The bony insertion of the ACL and PCL were cut from the tibial plateau and femoral notch. Each animal's PCL and menisci were examined and harvested for other unrelated studies using a similar protocol. The ligaments were then immediately refrozen at –30°C until further processing could be performed.

Slide Preparation:

The ACL with its intact bony origin and insertion were then thawed out over night. The Ligaments were held at their resting length using a special external fixater, specifically designed for this project. The ligaments were then stained using a modified gold chloride stain as described by Zimny[31]. This method comprises several steps and incubation periods where the ligament is left for extended periods of time in different solutions. A detailed description of the staining procedure is found in **Figure 1**.

The ligaments were then sectioned in thirds so that there was a proximal, middle and distal third for each ACL. From these ligaments at least three sections were selected to undergo final processing. Specimens were frozen at -30° Celsius. Each section was serially sliced at 5-micron intervals, while frozen, using a microtome. The individual slices were floated on water and then transferred to the slides. The sections were then fixed to the slides and covered with cover slips.

Slide Examination:

The author examined each slide and the mechanoreceptors present were counted and identified. All slides were visualized using the same technique. The routine was started by inspecting the ligament at the top left corner of the side. The slide was then moved from left to right and receptors noted as they were seen. Once spotted on low power, high power was then used to specify the type of receptor. The slide was then moved toward the right until the end of the specimen was reached. Next the slide was moved down one half the visual field and then moved toward the left until the edge of the specimen was reached again. The process was repeated until the bottom right corner was reached. Approximately five of these passes were necessary per cut **(Figure 2)**.

The quantity of each type of receptor was noted. This data was written on data sheets and then later entered into a spreadsheet. The slides were visualized under low power and then at higher powers to identify each receptor. Position of each receptor was memorized using specific landmarks on the slide (ligament architecture) to prevent accidentally counting the same mechanoreceptor twice. Some times the observer had to examine several different slices in order to identify the receptor and distinguish it from artifact.

Classification of the receptors was performed by comparing the observed receptors to a written description of each type of mechanoreceptor. Pictures from peer reviewed journals were also used to help identify the observed receptor. Unfortunately there are no immunospecific techniques available to specifically identify a type of receptor so that this type of identification was not performed. We feel this would have been the most specific method to identify the type of receptor.

Statistical Analysis:

The total number of mechanoreceptors was calculated for each third of the ligament. The average number of mechanoreceptors per slide was calculated by taking the sum of mechanoreceptors viewed in each section and dividing them by the total number of slides in that section since not al sections had the same number of slides made. This allowed us to calculate the average number of receptors per section of ligament. An analysis of variance was performed on these results. Alpha was set at 0.05 and Beta set at 0.80. P values less than 0.05 were considered significant. Significant values for the ANOVA were further investigated using the Scheffé A Posteriori test to determine where the difference was located.

Results:

Two thousand slides were viewed. Two types of receptors were found. The majority of these receptors were Pacini receptors **(Figure 3)**. They made up the majority of all the receptors that were seen in any third of the ligament. Ruffini receptors **(Figure 4)** were the second, most frequent, type of receptor found in the ACL. Free nerve endings **(Figure 5)** were seen but were not felt to represent mechanoreceptor since their function seems to be pain perception and autonomic nervous system regulation of blood flow.

Slide quality varied. Most slides were very clear and with little artifact. There were some sections that did have some folding of the specimen edges. The thin serial slices helped the identification process, because they allowed the author to examine the section above and below to confirm the presence of a receptor.

Three hundred and twenty three pacini receptors were noted in the proximal third of the ligament **(Table 1)**. There were only 100 noted in the middle third and only 33 were seen in the distal third. There were fewer Ruffini receptors found. Seventy were noted in the proximal portion, which represented the largest number of these receptors in a section. Only 7 receptors were seen in the distal section and 23 in the middle section. A percentage of the total number of mechanoreceptors for each third of the ligament is found in **(Figure 6)**.

The mean number of receptors seen per slide **(Table 2)** varied from 0.65 for pacini receptors in the proximal third to 0.05 Ruffini receptors identified in the distal third. Because of the thinness of the slices the same receptors had to be viewed for at least three consecutive slides, before it was counted. The mean number of receptors per slide for the remaining thirds of the ligaments is shown in detail in **Table 2**.

The mean number of receptors per section also showed a predominance of pacini receptors in the proximal third of the ligament **(Table 3)**. As we move distally in the canine ACL fewer receptors are visualized. This is true not only for the type of receptors but also in the sheer number of receptors seen.

Statistical Analysis:

Results of the analysis of variance (ANOVA) performed on the mean number of mechanoreceptors in each third of the ligament showed that there was a significant difference between the three means ($p<0.001$). These results are given in further details in **Table 4**.

The Sheffé A Posteriori test was then applied to the results to determine where this difference lay. The results showed a significant difference between the number of mechanoreceptors found in the proximal portion of the canine ACL and the number of receptors found in the middle and distal thirds. There was no statistical difference found between the number of mechanoreceptors found in the middle and distal thirds of the ligament **(Table 5)**.

Discussion:

Knee anatomy:

The knee is a complex joint, which in reality is made up of three articulations: the medial tibiofemoral joint, the lateral tibiofemoral joint and the patellofemoral joint. It is composed of three bones and four major ligaments. The bones that make up the knee joint are the femur, tibia and patella. The fibula does not articulate with the femur in humans and dogs but may articulate with the femur in other species. In the human knee the fibula is an important insertion point for the lateral collateral ligament. The tibia and femur actually have two compartments (joint surfaces) that individually bear the weight of the body. Furthermore the femur also articulates with the patella.

Several ligaments give the knee its stability. The collateral ligaments stabilize the joint in the coronal plane. They resist varus and valgus forces applied to the knee during normal walking as well as during running and jumping. Movement in the anteroposterior plane in resisted by the cruciate ligaments. Anterior displacement of the tibia on the femur is resisted by the ACL, while the Posterior Cruciate Ligament (PCL) resists posterior translation of the tibia on the femur. The cruciate ligaments are located inside the knee but are extrasynovial. These ligaments are very strong but because of their position in the knee they heal poorly.

This poor healing potential is due to several factors. When the ACL is torn the ligament ends are no longer in contact and therefore can not heal in an anatomical position. The torn synovium allows contact of the torn ligament with the joint fluid a known inhibitor of bone and ligament healing. The ligaments blood supply is disrupted. Constant motion of the torn ends also diminishes the healing potential of the ligament. All these factors contribute to the poor healing potential of the ligament.

The function of the ACL is to prevent anterior displacement of the tibia on the femur. The ligament also limits rotation of the tibia on the femur. The ACL is subjected to maximum stress when a person moves quickly from full speed to a complete stop, while landing after a jump, and with cutting. Cutting is a rapid change in direction while running. A good example is a wide receiver running an out pattern. In this pattern the player runs full speed approximately 10 meters and then changes directions 90° toward the sideline without decreasing speed.

The slight posterior tilt of the human tibial plateau may explain why humans with ACL deficient knees are less prone to osteoarthritis. The canine tibial plateau has a steeper posterior slope and this may exaggerate the amount of anterior to posterior movement present in the knee of the animals with normal walking. This same mechanism may help explain the premature arthritis seen in the knees of dogs with an ACL tear. The canine knee is held in the flexed position and is never fully extended. This is a major difference between the human and canine knee. The flexed position may functionally increase the posterior tilt of the plateau and encourage anterior translation of the tibial plateau on the femur in the canine knee and result in osteoarthritis after an ACL tear.

ACL strain can vary with knee position. This is well known in the human knee. The ACL seems to give most of its stabilizing effect between 0 and 60 degrees of knee flexion. This is a reason why the Lachman test is more sensitive to instability in the human than the drawer test. The Lachman test stresses the ACL in the position where the ACL has to provide its maximum amount of stability. Increased translation of the tibial plateau in this position is a better indication of the ligament integrity than in a non-functional position.

The ACL is often injured in pivoting sports. In these events a rapid change in direction places a stress on the ACL that can exceed the strength of the ligament. Reconstruction is required in many athletes who wish to continue playing pivoting sports. Studies have shown better function in athletes after reconstruction of the ACL than in athletes treated non-operatively.

The menisci are cartilage shock absorbers that decrease the load placed on the articular cartilage. They also may act as secondary stabilizers of the knee. Menisci diffuse the load borne by the femoral condyles and tibial plateaus by converting compressive forces into hoop stress a form of tension stress. The radial orientation of the collagen in the meniscus absorbs the compressive forces placed on this structure. The menisci are most important when the knee is flexed. Trauma significant enough to rupture the ACL often injures the menisci. Injury to the medial meniscus in an ACL deficient knee may lead to post-traumatic osteoarthritis.[32]

Mechanoreceptors Studies:

Mechanoreceptors are pressure sensitive corpuscles that have been identified in the ligaments of almost all the joints of both humans and animals.[33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51] These receptors have been shown to send impulses to the brain when the knee joint is flexed and extended as well as internally and externally rotated.[52] These studies also document the presence of mechanoreceptors in the ligaments and capsule of the knee of both humans and other mammals.

Mechanoreceptors have been shown to provide afferent stimuli to the cerebral cortex in humans. Pitman et al. stimulated the ACL in three different regions during arthroscopy and measured the response using somatosensory potentials. This showed a sensory potential reaching the cerebral cortex. The greatest stimulation appeared to occur when the mid-portion of the ligament was stimulated. [53]

The ligaments, capsule and menisci of the canine knee have been shown to contain mechanoreceptors. These same receptors have been reported in the canine Anterior Cruciate Ligament. The ACL's unique intra-articular position and orientation give this ligament its function. There are no other structures that are in a position to monitor anterior translation of the tibia on the femur. Consequently, it is felt to be an important source of afferent proprioceptive information in the knee. Tears of the ACL have been shown to decrease a patient's awareness of the position of his knee as well as the patients' ability to detect motion of his knee.

A reflex arc to protect the ACL and the knee has been postulated. This arc results in a reflex contraction of the hamstrings, in response to stretching of the ACL. This is a very controversial subject. Miyatsu et al.'s work suggests that the ACL muscle reflex may play an important role in normal knee kinematics.[54] Beard has described the hamstring reflex that is thought to protect the ACL during physiological activity.[55] He believes that the resulting instability after an ACL tear may be increased by loss of this reflex. Furthermore he was able to show an improvement in response time in this reflex after completion of a rehabilitation program designed to enhance the hamstring reflex.[56] Grabiner et al. on the other hand have shown that the above mentioned reflex may not be functional during activities which strain the ACL.[57]

Gómez-Barrena et al. studied the mechanoreceptors of the feline knee. They were able to demonstrate that the ACL was poorly innervated when compared to the patellar tendon. They also showed that the afferent neurons were segmentaly located in spinal segments whose efferent neurons innervated both the quadriceps and hamstring muscles.[58] Further studies by Gómez-Barrena et al. focused on the electrical activity produced in the knee before and after transection of the ACL. They monitored electrical activity in both the medial articular nerve and the posterior articular nerve. They found an increase in electrical activity in the medial articular nerve after transection of the ACL but no muscular changes in activity. They concluded that the abnormal afferent stimulation in the knee after ACL transection might not be enough to produce muscular changes.[59]

Despite the identification of all these mechanoreceptors there are no studies in which an attempt to quantitate the number of receptors present in the ligaments. This basic fact has been overlooked. Our study was an attempt to try to fill the void.

The Canine Knee:

The canine knee is a good specimen to use to identify mechanoreceptors. Researchers have used gold chloride to identify canine mechanoreceptors in the past. The smaller size of the knees and its ligaments, makes taking serial sections of the ACL easier and also helps stain the ligaments. Since smaller ligaments tend to allow a more uniform dispersion of stain they are less likely to have artifact. This is important when trying to count and identify mechanoreceptors on a slide.

The canine joint has some similarities and differences with the human knee. The same ligaments are found in each and their positions in the knee are similar. Both joints are hinge joints, but while the human knee joint can fully extend the dog is unable to perform this function. The ACL in the canine knee may have a more important role in knee stability than in humans. The flexed knee and steeply inclined tibial plateau both tend to increase the forward thrust of the tibia on the femur. This may explain why dogs have a higher incidence of osteoarthritis after an ACL tear. Increased forward thrust increases the shear forces on the articular cartilage of the tibiofemoral joint that leads to the loss of this tissue.

The human ACL is the best known ligament in the world. This is because it is commonly injured, difficult to treat and has been the subject of a large number of both basic science and clinical research. The increasing popularity of professional sport leagues, the large salaries professional teams pay their athletes, the susceptibility of a star athlete to this career ending injury have all focused our attention on this ligament indirectly.

Studies Dealing with Knee Proprioception:

Several published studies have dealt with proprioception[60],[61] of knee before and after ACL tears. Barrack[62] has shown that patients with ACL deficient knees seem to have decreased proprioception when compared to normal patients. In this study 2 groups of patients were compared using a threshold to detection of passive motion test. The first group had normal knees and served as controls. The second group had a documented isolated injury to their ACL in one knee. In this study patients in the control group had nearly identical proprioception in both knees. Patients in the ACL deficient group had a statistically significant difference between their two knees. The injured knee was less efficient in detecting passive motion of the joint. A multivariate analysis of the data in this study seemed to attribute this loss of proprioception to loss of the ACL rather than to other factors.

This same author has also shown that patients who have ligamentous laxity may also have decreased position sense[63]. In a similar study two groups of patients' proprioceptive ability was compared. The tested group was a group of 12 professional ballet dancers. The control group was a group of active age matched controls. All of the dancers met at least three of the criteria for loose joints. In the control group only one patient met these criteria. The dancers were consistently poorer in reproducing joint angles than the control group. The authors felt that poor position sense might predispose these individuals to musculoskeletal injuries.

Barrett et al.[64] studied the relationship between joint proprioception and osteoarthritis. The authors tested three groups of patients. The first group was the control group and had normal knees. In the second group patients had evidence of osteoarthritis. The third group consisted of 11 patients who had undergone Total Knee Arthroplasty (TKA) using a hinged knee prosthesis and 10 patients who had undergone TKA using a semi-constrained device. Results demonstrated that patients with severe osteoarthritis of the knee seem to have diminished proprioception, at all ages, when compared to normal controls. Age also influenced the results. Increasing age decreased knee position sense in the normal control group. Knee replacement improved the subject's ability to determine the position of the knee especially if the arthroplasty performed was a semi-constrained prosthesis. Finally the use of an elastic bandage greatly increased a patient with poor position sense's performance. The author concluded that proprioception alterations may be the basis of some types of osteoarthritis in the knee. Furthermore the author felt that this decreased proprioception may also allow the arthritis to progress.

A neuropathic hypothesis for osteoarthritis in the canine knee was refuted by O'Connor et al..[6] In their study the dorsal (sensory) ganglia to each knee was destroyed in dogs with normal, ACL deficient, and ACL deficient and denervated knees. The normal knees did not become osteoarthritic. The denervated and unstable knees rapidly became osteoarthritic while the knees that were ACL deficient but had normal sensation became osteoarthritic but at a much slower rate then the ACL deficient denervated knees. They concluded that denervation did not cause osteoarthritis but that it could accelerate OA in the presence of instability. [6]

Skinner et al.[65] has also shown decreased proprioception in the elderly. They studied patient's proprioception using threshold to detection of passive motion and joint position sense. The group studied were 20 patients with normal knees with an age range between 20 and 80 years old. A decrease in proprioception was found to correlate with increasing subject age using both testing methods. The tests were shown to be measuring the same biological parameter. The authors concluded that deterioration of proprioception might be an indicator for sub-clinical degenerative joint disease of the knee. They also stated that these techniques may also be used to determine if a joint's deterioration is neuropathic in origin.

Barrett[66] also studied the effect of ACL reconstruction on knee proprioception. In this study he studied three groups of patients. The first group was normal controls while the second group consisted of patients that had an ACL tear. The third group had undergone a MacIntosh-Jones reconstruction of the ACL. All patients were rated with a standard knee score, their subjective opinion of their reconstruction, and joint position testing. The results showed that there was a difference between the joint position sense of the subject's knees in both the ACL deficient group and the ACL reconstructed group. This difference was more pronounced in the ACL deficient group. The reconstructed group tended to have joint position sense that approached the non-injured knees joint position sense but it was never the same.

Lephart et al.[67] also studied patients after ACL reconstruction. To be included in this study patients had to have had an ACL reconstruction using either an autograft or an allograft and a contralateral normal knee. In their study they were able to demonstrate a significant decrease in threshold to the detection of passive motion in the reconstructed knee at or near the terminal range of motion. The use of a neoprene sleeve improved the patient's ability to detect passive motion at the extremes of motion in both the uninvolved and the involved knee.

MacDonald et al.[68] have not been able to demonstrate a difference in proprioception between an ACL deficient knee and the patients normal knee. Three groups of patients were tested: group 1 controls, Group 2 ACL deficient knees, Group 3 ACL reconstructed knees. The three groups underwent threshold to detection of passive motion testing. The authors failed to find a significant difference between the third groups ability to detect passive motion in the involved knee and group 2's ability to perform the same task. The authors conclude that ACL reconstruction did not improve a patient's ability to detect passive motion. This finding is different than that stated by Barrack in his study. In their discussion the authors state that their measuring technique may not have been sensitive enough to note a difference between the two groups (groups 2 and 3).

Beard[54] has shown that patients with specialized physical therapy regimens can improve their knee proprioception despite the loss of their ACL. He was able to show that a specific training regiment allowed patients to function better. This regiment did not affect their laxity on clinical exam.

The specific functioning of these mechanoreceptors within the ligament is unknown. I think that the ligament is perceived as a whole. The division of the ligament into thirds is artificial and is not a reflection on the physiologic activity of the receptor. It is logical that the mechanoreceptors of the whole ligament act together to effect changes in neural stimulation of the brain.

Further review of the literature shows that there are no studies that compare proprioception before and after ACL reconstruction. In all the above mentioned studies the groups have been composed of patients that had reconstruction in the past. A study comparing a patient's proprioception before and after reconstruction would be interesting to help determine when proprioception returns to the knee. A study such as this may help determine the role of the mechanoreceptors in the ACL.

It is theorized that the mechanoreceptors in the ACL are destroyed after the ligament ruptures. The receptors may continue to exist in the ligament but the neural connection between the receptors and the brain is probably lost. This is most likely true for avulsions of the proximal part of the ligament.

Mechanoreceptors in the ACL represent only a small proportion of the receptors present in the knee joint capsule ligaments and menisci. The isolated loss of such a small number of receptors should probably not result in a measurable loss of proprioception unless the resulting instability causes dysfunction of the remaining receptors. If the brain has a programmed imprint of motion allowed by the knee then the loss of a ligament and its resulting increased abnormal motion may confuse the brain because it cannot comprehend the afferent input of proprioceptive data. The normal sequence of mechanoreceptor firing is lost and the brain can no longer interpret the new pattern that results from the abnormal motion of the ACL deficient knee. Therefore when a patients knee is positioned in space a small amount of anterior or posterior translation of the tibia on the femur may cause the incoming afferent nerve information to be either inaccurate or uninterpretable to the brain. This would decrease patient proprioception. Reconstruction of the ligament would allow the other receptors in the joint to function normally by restoring stability, decreasing abnormal motion, and make the resulting normal pattern of afferent data interpretable to the brain. This would be possible even without any functional mechanoreceptors being present in the graft.

Possible Sources of Error:

There are many possible sources of error in this project. Gold chloride stain has been shown to color tissues containing neural elements. When using gold chloride there is always a possibility of the stain not working uniformly in the ligament. This occurred in some sections of our ligaments. This could have occurred selectively in the middle and distal thirds of the ligament for an unknown reason but this is unlikely. The fact that our ligaments were cut thin allowed us to examine many areas at small intervals and the stain quality seemed in the most part homogenous.

The identification of mechanoreceptors by the author only, could also be considered a weak point in the study. The presence or absence of a receptor is much easier to perform than trying to precisely identify the type of receptor present. Although the qualification of the receptors was performed distinguishing between the different types of receptors is difficult. We did not perform a statistical analysis of this data because it was not felt to be reliable enough to warrant statistical analysis. On the other hand the presence or absence of receptors was accurately measured and therefore feel confident that the results of the counting of mechanoreceptors in general in each section of the ligaments were valid. The quantification portion of this study is reliable, as analyzed statistically, and presented in this paper.

Another source of error is the small sample size. Even without performing statistical analysis on the raw data it was obvious that there were a lot more receptors in the proximal portion of the ligaments. The division of the ligaments into thirds was an arbitrary decision. It was also performed in an arbitrary way. The ligament was sectioned into three equal size sections but this was done by gross estimation instead of after precise measurements because of the difficulty required in measuring and dividing such a small structure. The goal was to try and determine if a difference between the number of receptors present in different sections was real. Since the section was arbitrarily defined as a third, the millimeter of difference that may have existed with a more precise measurement of each section would not have changed the significance of the results and would have added hours of work to the project without providing any real benefit.

Significance of this Study:

The significance of this study is that different parts of the ACL may have a greater afferent source of information or proprioception that the rest of the ligament. This could result in a different perception of what type of ligament tear gives the patient the greatest amount of disability. Proximal injuries would disrupt proprioception more than distal injuries. Therefore, a proximal partial tear of the ACL should result in a greater degree of proprioceptive loss than a similar tear in the distal ligament. This proprioceptive loss could lead to increased instability when the partial tear is in the proximal part of the ligament. In the clinical setting, the diagnosis of a proximal partial tear of the ACL on Magnetic Resonance Imaging (MRI), may require reconstruction because of its greater potential for instability. A partial distal tear may only require observation because of its decreased likelihood to result in an unstable knee.

In complete tears tension through the ligament will be absent. Often times the remaining stump of ligament will scar to the posterior cruciate ligament. After scarring to the PCL the ligament may be able to perceive some translation of the tibia on the femur. In this case a distal injury may provide an intact portion of ligament that perceives translation better than after a proximal tear since a greater number of mechanoreceptors will be intact. Unfortunately most ACL tears occur in the proximal portion of the ligament. Anatomically this part of the ligament has a smaller cross section of fibers.

In the surgical setting, ACL reconstruction may be influenced by these results. Since the proximal ACL contains a higher concentration of mechanoreceptors its preservation may be important. Preservation of the proximal stump during reconstruction may increase the patient's knee proprioception after reconstruction. Reconstruction of the ligament and reattaching the proximal stump of the ligament to the graft would improve the reconstructed ligament 's proprioception and possibly lead to better clinical results.

This study allows us to refine the theory of how proprioception information is transmitted to the brain. In dogs there may be a different amount of proprioceptive information transmitted by the proximal part of the ligament. The canine brain may be organized and programmed to receive more input from the proximal part of this ligament. Further research on this subject is needed.

This study will stimulate us to perform further studies on the human ACL. It will help us try to improve our surgical and bracing techniques to help restore as much proprioception to the injured knee as possible. If human studies show a similar organization of mechanoreceptors in the human knee, bracing of partial distal ACL tears may be studied more closely to see if surgery in this patient population can be avoided. Preservation of the proximal stump of ACL during reconstruction may be studied to see if it will improve proprioception after reconstruction than standard techniques.

Future Studies / Direction:

A similar study using human ACLs would be interesting and help answer the question of whether there are different number of receptors in the ACL of humans. It would allow us to see if the microscopic anatomy of the human ACL has different concentrations of mechanoreceptors in it allow comparison of the concentration of mechanoreceptors in humans and dogs ACLs. This could lead to identification of different concentration of mechanorecptors among different mammalian species.

Another study started by the author (MAA) may also help shed some light on the role of the mechanoreptors of the human ACL. In this study proprioception is being measured before and after ACL reconstruction. This study will attempt to answer when proprioception returns to an ACL deficient knee. Combining this new data with our current study we may be able to determine how the ACL and its mechanoreptors influence knee proprioception. If ACL reconstruction improves knee proprioception immediately after surgery, before receptors can grow into the graft then the stabilizing role of the ligament may affect the brain's ability to determine the position of the limb. This means that all the ligaments of the knee probably help determine position of the limb. Loss of one ligament an its resulting instability would affect the patient by making the afferent information received by the brain uninterpretable. Reconstruction would make the information interpretable again. If proprioception does not return to the knee then it may mean that the mechanoreceptors of the ACL are more important than the other structures in proprioception.

Conclusion:

This study suggests that there are a higher number of mechanoreceptors in the proximal third of the canine ACL than in the rest of the ligament. Although a difference may exist in the number of mechanoreceptors present in each third of the human ligament this study cannot answer this question. A similar study using human ACL would be needed to prove this hypothesis. The role of mechanoreceptors in knee proprioception is still debated. This study provides us with some information that may be helpful in understanding the role of these receptors in the ACL but does not provide any definite conclusions about this subject.

This study suggests that a significant difference exists between the number of mechanoreptors found in the proximal portion of the canine ACL. There was no significant difference found between the middle and distal thirds of the ligament. This is of interest to humans because if there is no difference in the number of mechanoreceptors in the human knee then preservation of the proximal third of the ACL may not be critical.

Figures:

1. Tissue is prepared in a solution of Triton X-100.

   0.3% solution for 20 minutes

   Rinse 3 times for 15 minutes in distilled H_2O

2. Soak specimen in external fixater in following solution:

   1 cc of 88%formic acid

   3 cc Fresh squeezed and filtered lemon juice

   Solution and specimen are placed in the dark to incubate for 15 minutes. Tissue becomes transparent.

3. Remove lemon juice and sponge dry the ligament.

   Cover the specimen with 1% gold chloride solution.

   Incubate in darkness for 20 minutes. Tissue should change into a yellowish color.

4. Remove the gold chloride. Immerse specimen in solution of 25% formic acid

   Incubate in darkness for 20 <u>hours</u>

5. Rinse for 10 minutes X 2 in 70% ethanol

6. Immerse in glycerin in darkness for 24 hours.

7. Dehydrate and dip in paraffin.

8. Section with microtome at 5 microns

Figure 1: Technique used to stain canine ACLs.

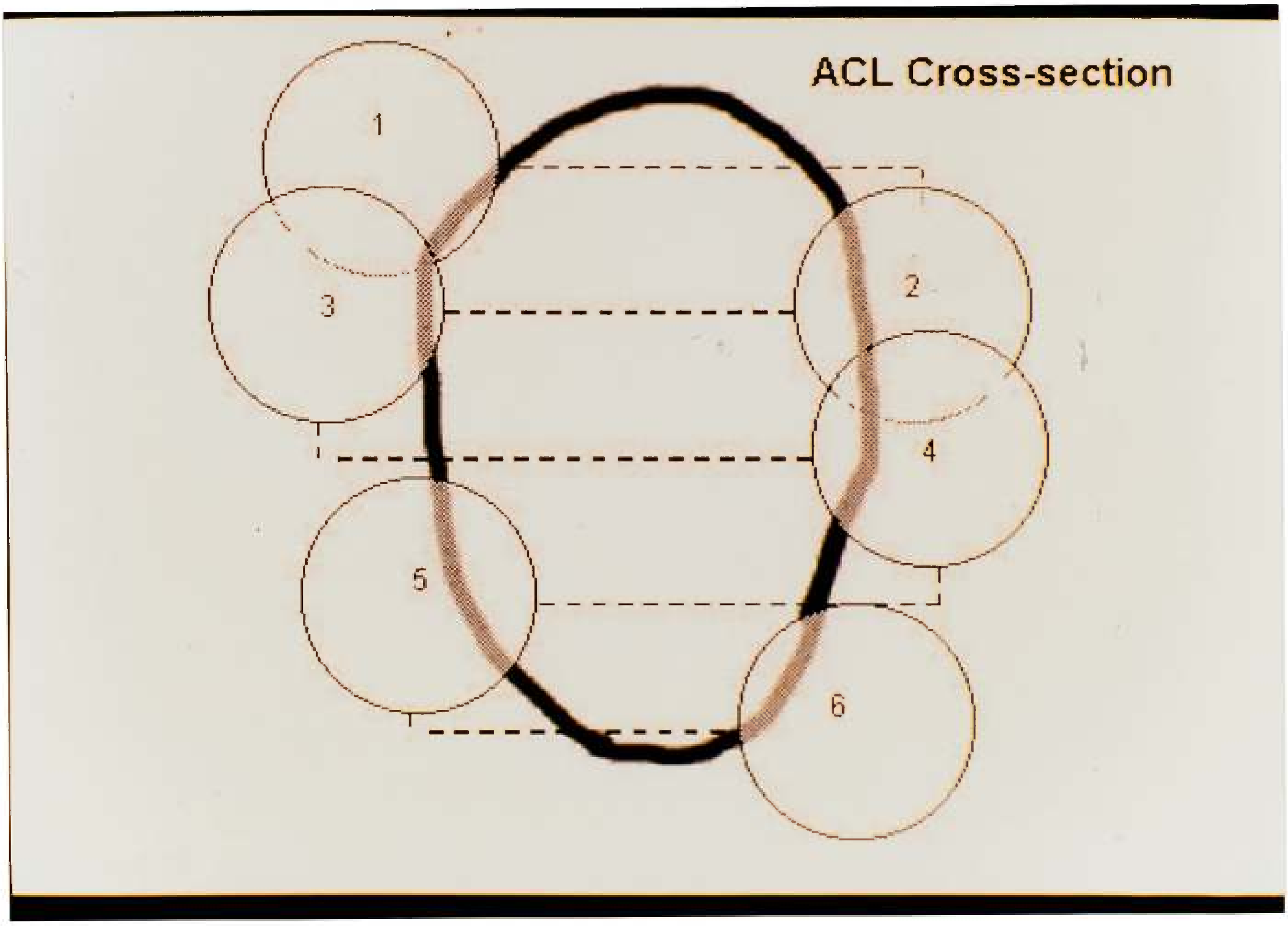

Figure 2: Diagram showing how the slides were examined with microscope. The microscope was started at point labeled 1 and worked down to point 6.

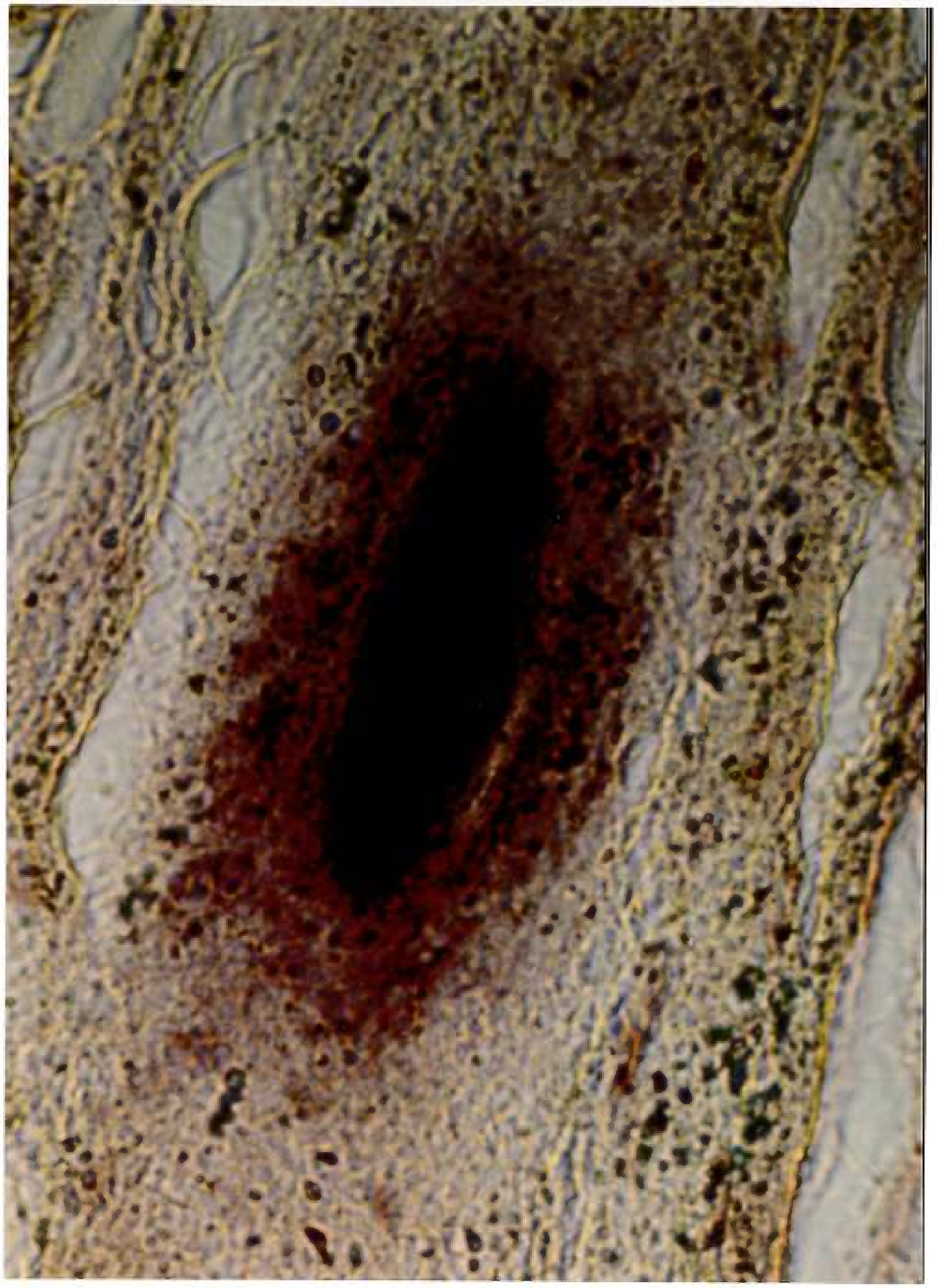

Figure 3: Pacini receptor of canine ACL

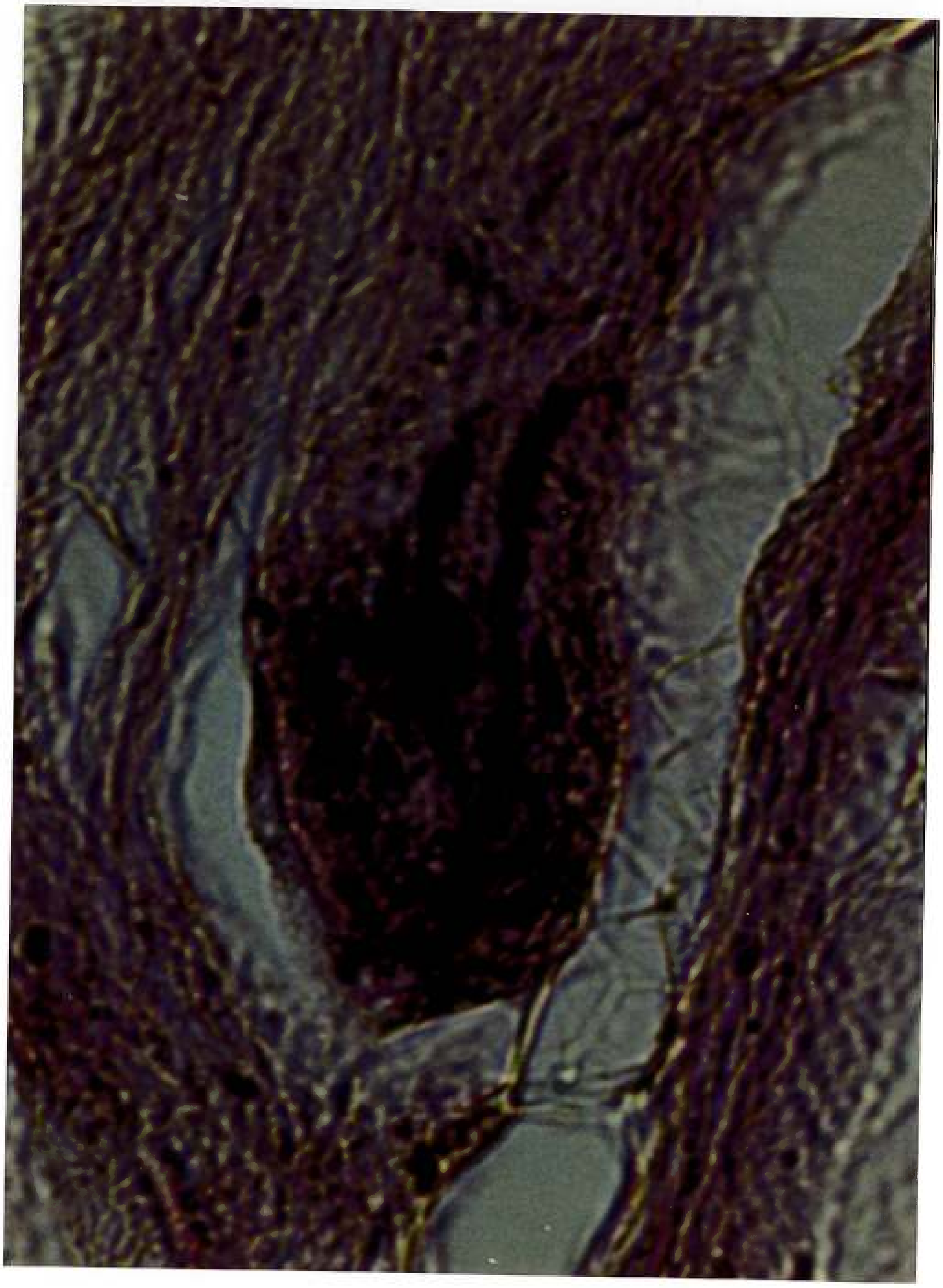

Figure 4: Ruffini receptor of canine ACL

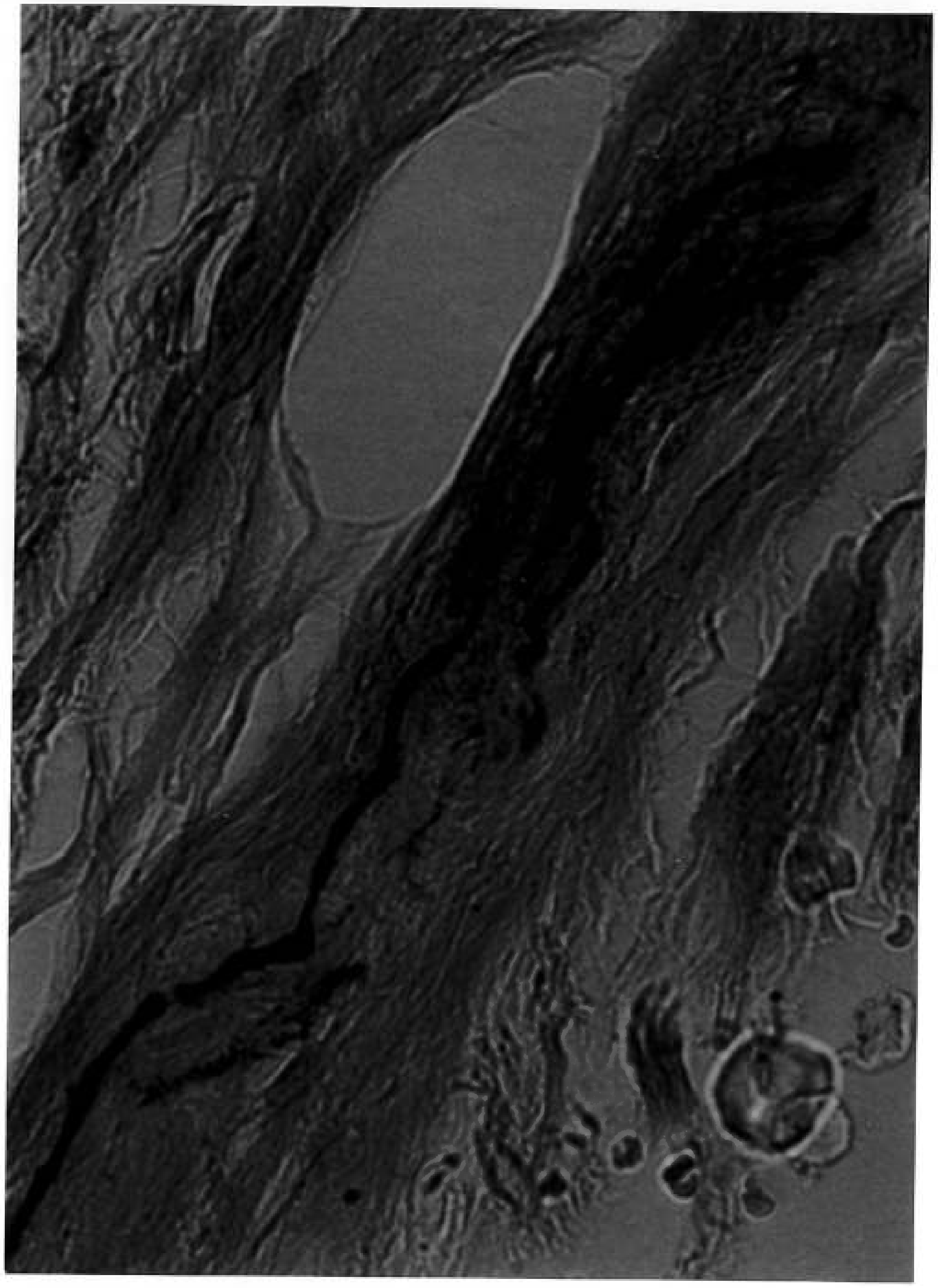

Figure 5: Free Nerve Endings (FNE) in canine ACL

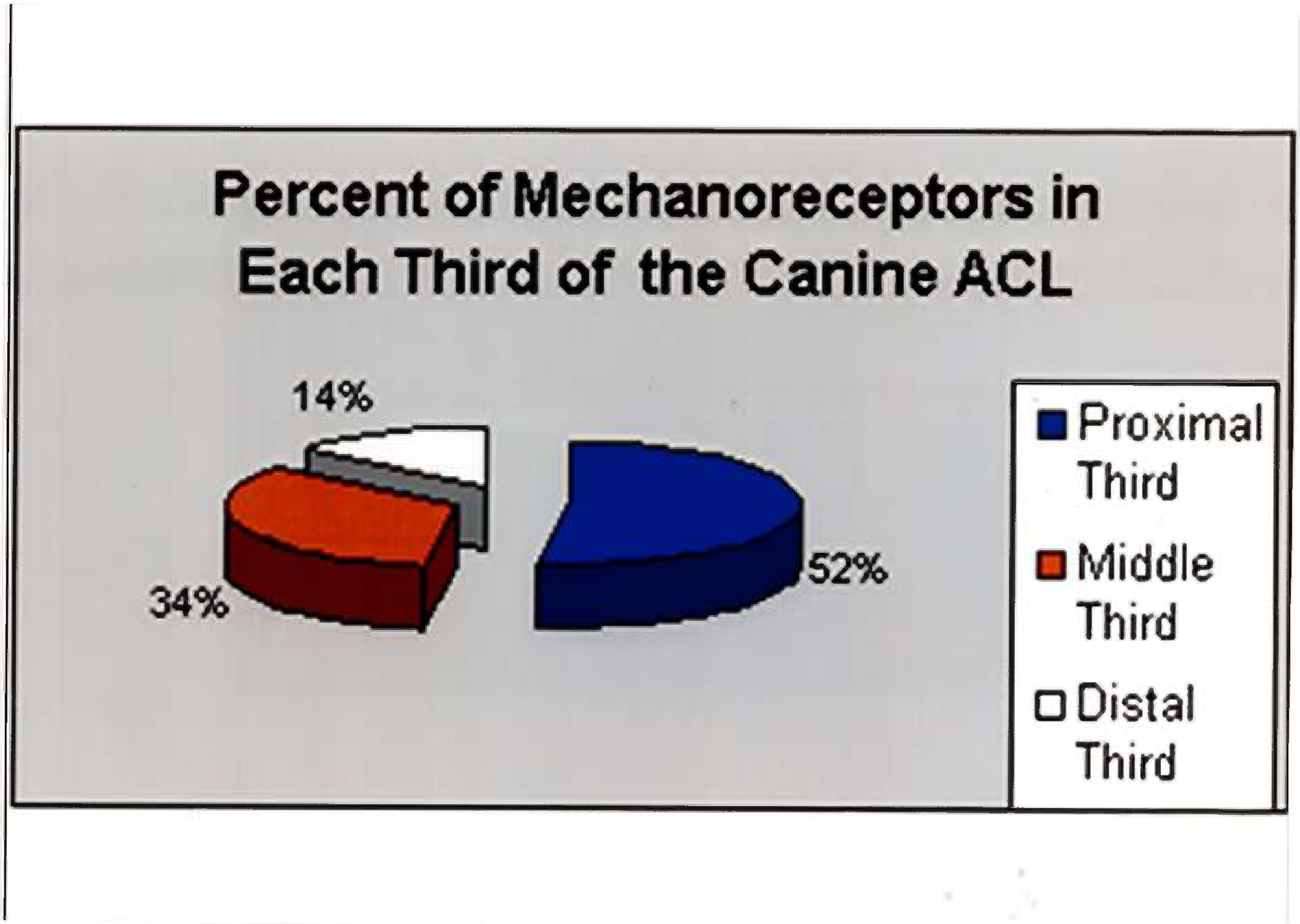

Figure 6: Graph showing the percent of mechanoreptors in each third of the canine anterior cruciate ligament.

Tables:

Number Receptor	Pacini	Ruffini	All
Proximal Third	323	70	393
Middle Third	100	23	123
Distal Third	33	7	40

Table 1: Table showing type of receptor and its location.

Mean Receptors / Slide	Pacini	Ruffini	All
Proximal Third	0.65	0.13	0.78
Middle Third	0.31	0.07	0.38
Distal Third	0.23	0.05	0.28

Table 2: Table showing mean number of receptors found per slide in each third of the ligament.

Mean Receptors / Section	Pacini	Ruffini	All
Proximal Third	48	10	58
Middle Third	25	5.8	30.8
Distal Third	11	2.3	13.3

Table 3: Table showing the mean number of receptors per section of ligament.

ANOVA: Single Factor

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Proximal	522	407	0.77969	2.1145197		
Middle	324	123	0.37963	2.0566735		
Distal	145	40	0.27586	0.506705		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	47.21352	2	23.6068	12.683139	**3.64E-06**	4.6267
Within Groups	1838.936	988	1.86127			
Total	1886.149	990				

Table 4: Table showing the detailed results of the ANOVA on the mechanoreceptor data. These results show a significant result of p=0.00000364.

Scheffé Test

			Calculation										
Contrast	Coefficient	L_1	MSW	sum of C_j/N_j	MSW X C_j/N_j	SR of d	L_1/S_{L1}	K-1	$F_{0.95}$	(K-1)F	$S_{0.01}$	L_1/S_{L1}	
L_1=1-1+0	C_1=1, C_2=-1, C_3=0	0.4	2.765557	0.004989	0.013797	0.117462	3.405362	2	3	6	2.44949	3.405362	ACCEPT
L_2=1+0-1	C_1=1, C_2=0, C_3=-1	0.5					4.256703	2	3	6	2.44949	4.256703	ACCEPT
L_3=0+1-1	C_1=0, C_2=1, C_3=-1	0.1					0.851341	2	3	6	2.44949	0.851341	REJECT
L_4=1-1/2-1/2	C_1=1, C_2=-1/2, C_3=1/2	0.45					3.831033	2	3	6	2.44949	3.831033	ACCEPT
L_5=1/2-1/2+1	C_1=-1/2, C_2=-1/2, C_3=1	0.3					2.554022	2	3	6	2.44949	2.554022	ACCEPT

Table 5: Table showing detailed results of the Sheffé test. These results show significant difference between the proximal and middle thirds as well as between the proximal and distal thirds but no difference between the middle and distal thirds.

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