Neural and muscular electric activity in the cat's knee

Changes when the anterior cruciate ligament is transected

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We studied the response of the normal and unstable knee to passive motion and anterior tibial displacement in the cat. 6 cats were anesthetized and the deep level of anesthesia was controlled by electroencephalograms. We recorded electric activity in the articular nerves (posterior PAN and medial MAN) and periarticular muscles (quadriceps and hamstring), while performing passive flexion, extension, internal and external rotation. We then produced anterior displacement of the tibia at 30° and 90° of flexion, as in the Lachman and the anterior drawer maneuvers. The anterior cruciate ligament was surgically sectioned and the same series of passive displacements was performed. We observed statistically significant increased activity in the MAN, the PAN and the quadriceps muscle during knee flexion, in the MAN during extension, and in the PAN and hamstring during external rotation with the knee 90° flexed. Anterior cruciate transection caused anterior displacement of the tibia during stress. This produced a significant increase in the MAN activity and a significant decrease in the hamstring electric activity at 30° and 90° of flexion, as in Lachman and anterior drawer maneuvers. We conclude that electric activity in the articular nerves and periarticular muscles, in response to passive motion and anterior tibial displacement, is altered in the cat's knee after anterior cruciate transection. This suggests that various patterns of periarticular muscle reaction in the anterior cruciate-deficient knee may be related to the unconscious perception of abnormal motion.

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Morphologic studies have confirmed the presence of neural fibers and endings in knee structures (Skoglund 1956, Freeman and Wyke 1967, Schultz et al. 1984, Schutte et al. 1987, Halata and Haus 1989, Sjölander et al. 1989, Haus and Halata 1990). Articular nerves (named MAN—medial articular nerve—and PAN—posterior articular nerve—in the cat) (Freeman and Wyke 1967) lead information from the anterior cruciate and other knee structures to segmental spinal ganglia (Gómez-Barrena et al. 1996).

To clarify how this innervation contributes to knee function, neurophysiologic studies were performed in anesthetized and decerebrate cats. Changes in the activity of gamma-motor neurons related to soleus, gastrocnemius, biceps femoris and semimembranosus muscles were observed after stretching the cruciate ligaments (Johansson et al. 1990). The sensory network in the cruciate ligaments may affect the control of periarticular muscle activity around the knee (Johansson et al. 1991). Electric activity in afferent PAN fibers was obtained after local pressure on the proximal anterior cruciate ligament and during passive knee motion (Krauspe et al. 1992). In EMG studies, Solomonow et al. (1987) showed an anterior cruciate-hamstring reflex arc after axially stretching the ligament with high loads. They concluded that ligament overload may contribute to functional stability of the knee joint by the regulation of periarticular muscle contraction. Further experiments by Miyatsu et al. (1993) showed discharges at the PAN, and muscle motor unit activity, not only in the hamstring but also in the quadriceps muscle, after axially stretching the anterior cruciate ligament with physiologic loads (between 1% and 10% of the ligament's maximum tensile strength). These results suggested that the anterior cruciate-muscle reflex may play a physiologic role during knee function.

At this point, neurophysiologic data show that the anterior cruciate ligament can induce PAN electric activity during experimental axial loading (loads ranging from physiological to high magnitude), local pressure and passive knee motion (within the normal

range and considerably increased near the limits). Periarticular muscle activity around the normal knee is elicited by axial loading of the anterior cruciate ligament at the hamstring, quadriceps and gastrocnemius. The main purpose of our study was to evaluate whether the electric activity in the articular nerves (MAN, PAN) and periarticular muscles (quadriceps and hamstrings) in the anesthetized cat's knee, in response to passive motion and anteroposterior translation, is significantly altered when the anterior cruciate ligament is transected.

Khalsa and Grigg (1996) recently showed that mechanoreceptor neurons in the posterior capsule of the cat's knee joint do not change significantly after mechanical derangement of the knee due to anterior cruciate transection. The confirmed normal behavior of capsule afferents allows a further assessment of the role of propioception in the unstable knee. Proprioception is believed to regulate periarticular muscle contraction for the purpose of stabilizing a joint, whether stable or unstable. With normal afferents in other knee structures, the unstable knee may take advantage of the undamaged structures to obtain proprioceptive information for regulating muscular contraction in response to motion and stress. This different origin of proprioceptive information would explain the adaptive changes observed in the EMG and gait of patients with an unstable knee (Solomonow et al. 1987, Andriacchi and Birac 1993, Tibone and Antich 1993, Ciccotti et al. 1994). The second aim of our study was to assess how the articular nerves and periarticular muscles react to passive motion and anteroposterior translation when the anterior cruciate ligament is transected.

Animals and methods

Experiments were conducted on 6 cats weighing 2–3 kg, under intraperitoneal pentobarbital anesthesia (30 mg/kg). Cortical activity was recorded by electroencephalography (EEG) during the experiment to ascertain the level of anesthesia. Supplementary doses of anesthetic were given when a decrease in the amplitude of the slow wave activity was observed on the EEG. No voluntary activity in response to stimuli was obtained at that time. All the experiments were performed according to the Declaration of Helsinki and following the recommendations of the Spanish Committee of the International Council for Laboratory Animal Science and the European Community Directive 86/809/EC to avoid animal suffering.

In order to record the afferent potentials from the MAN and PAN, these were exposed under a Zeiss OPMI 19 surgical microscope (Jena, Germany) and placed over a nichrome hook electrode. We recorded the EMG of the quadriceps muscle and the hamstring, using a pair of bluntly-cut, insulated, nichrome wire electrodes (120 μm diameter) placed in the middle of each muscle. The afferent potentials and the EMG were filtered (0.3–3 KHz), amplified and recorded on a magnetic tape with the beginning of the movement as a temporal reference for off-line analysis. The start and the end of every maneuver were signaled by the examiner. No precise measurement of the latency was obtained using this protocol, designed to determine the amplitude of the electric activity evoked by the maneuvers. To record the EEG, a macroelectrode (120 μm diameter, bluntly-cut, insulated, nichrome wire) was inserted (1.5 mm deep) in the frontal cortex. The EEG was filtered (0.3–30 Hz), amplified and continuously monitored throughout the experiment with an analogic oscilloscope.

We made our recordings during the passive range of motion, in the anesthetized cat's knee in a supine position, starting with the knee at 90° flexion and the hip at 90°. From the starting position, we performed passive maximum flexion and back, maximum extension, maximum external rotation, and maximum internal rotation. The anterior instability examination included passive anterior tibial displacement, with the knee at 30° of flexion (as is the Lachman maneuver), and at 90° of flexion (anterior drawer). Each movement and maneuver was repeated 10 times, at a rate of 0.2 Hz. No muscular or neural fatigue was observed.

We obtained a first set of recordings in the normal knee. We then obtained a second set of recordings in the same knee after a limited anterolateral arthrotomy (to avoid articular nerve surgical damage) and surgical transection of the anterior cruciate ligament (causing a demonstrated, severe anterior instability of the knee). We compared neural and muscular activity in the same knee of the 6 animals before and after anterior cruciate transection. Each anterior cruciate-deficient knee had its own control in the same knee a few minutes previously, under the same experimental conditions.

We performed the statistical analysis on a PowerPC 7100/80 Macintosh computer (Cupertino, CA, USA). The recorded data were fed into the computer at a sampling frequency of 10 KHz. Afferent multiunit potentials from the nerves and muscle motor units were detected, using a voltage threshold. Summed peristimulus time histograms (PSTHs) were calculated between the afferent potentials of the motor units, using the beginning of the movement as a zero reference (binwidth 50 msec) with *Spike2* software (C.E.D., Cambridge, U.K.). Response was defined as

the mean afferent potential/bin or motor unit/bin during the movement, at least twice as large as the spontaneous activity prior to the beginning of the movement. The amplitude of the response for each movement was calculated as the mean afferent potential/bin or motor unit/bin during the movement, minus the mean spontaneous activity calculated during 200 ms recorded before the movement. All data are given as mean ± standard error. A paired Student's t-test was used for the statistical analysis between the response amplitudes in the control condition and after the anterior cruciate ligament transection, using a significance level of at least 0.05.

Changes in the electric activity recorded at the articular nerves (MAN and PAN) and periarticular muscles (quadriceps QUAD and hamstring HAMS) after anterior cruciate ligament transection during passive motion and anterior tibial displacement

	MAN	PAN	QUAD	HAMS
Passive motion				
Flexion	↑[b]	↑[a]	↑[b]	ns
Extension	↑[b]	ns	ns	ns
External rotation	ns	↑[b]	ns	↑[b]
Internal rotation	ns	ns	ns	ns
Anterior tibial displ.				
at 90° flexion	↑[b]	ns	ns	↓[b]
at 30° flexion	↑[b]	ns	ns	↓[b]

[a] $p < 0.05$, [b] $p < 0.01$, NS not significant

Results (summarized in Table)

Flexion

In the normal knee, the MAN, the PAN, the quadriceps muscle, and the hamstring showed a response to electric activity during passive flexion, from the 90° starting position. While the MAN and the hamstring displayed a vigorous response, the PAN and quadriceps responses were weak (Figure 1A, control). The MAN and PAN activities showed an alternated pattern during flexion, as well as during the other maneuvers under study (Figures 1A and 4A, controls). We found an increase in the spontaneous activity and in the flexion-evoked activity in the MAN, PAN, and quadriceps muscle, after cruciate transection (Figure 1B, unstable). Spontaneous activity was also increased in the hamstring but flexion provoked no response, as indicated by the flat PSTH (Figure 1B, unstable). When the mean area of the PSTH obtained for the 6 cases during flexion was plotted, significant increases in the flexion-evoked activity in the MAN ($p = 0.0002$), PAN ($p = 0.04$), and quadriceps muscle ($p = 0.003$) were observed after anterior cruciate transection (Figure 2A, flexion).

Extension

In the normal knee, the MAN, the quadriceps, and the hamstring showed electric activity in response to passive extension, starting at 90° flexion, while the PAN showed hardly any response. When the ligament was transected, only the MAN electric activity showed a significant increase ($p = 0.0004$) (Figure 2B, extension). Periarticular muscles activity showed a decrease, but this was not statistically significant.

External rotation

The MAN, the quadriceps, and the hamstring showed electric activity during passive external rotation of the knee at 90° flexion. When the ligament was transected, the electric activity at the MAN and the quadriceps remained unchanged. However, a significant increase was found in the extension-evoked electric activity at the PAN ($p = 0.0017$) and the hamstring ($p = 0.001$) (Figure 3A, external rotation).

Internal rotation

All the studied elements showed electric activity during passive knee internal rotation at 90° flexion. When the ligament was transected, no significant variations were detected. The hamstring activity was increased, but considerable variability was noted and the difference was not statistically significant (Figure 3B, internal rotation).

Anterior tibial displacement

When the maneuver was performed at 90° knee flexion (as in the anterior drawer), some electric activity was observed, even in the normal, stable knee in the MAN and the quadriceps, but mostly in the PAN and the hamstring (Figure 4A). When the anterior cruciate ligament was transected, the electric activity was significantly increased in the MAN ($p = 0.0001$) and decreased in the hamstring ($p = 0.0001$), as shown in Figure 5B. When the maneuver was performed at 30° knee flexion (as in the Lachman test), the electric activity observed in the normal, stable knee in the MAN and the PAN was more evident than that in periarticular muscles (Figure 5A). After damaging the ligament, the same changes were found at 30° flexion, as at 90° flexion: a significant increase in the MAN's activity ($p = 0.006$), and a significant decrease in the hamstring ($p = 0.0001$). The MAN and the PAN maintained the alternated activity pattern in response to the maneuvers, and this was best observed after anterior cruciate damage (Figure 4).

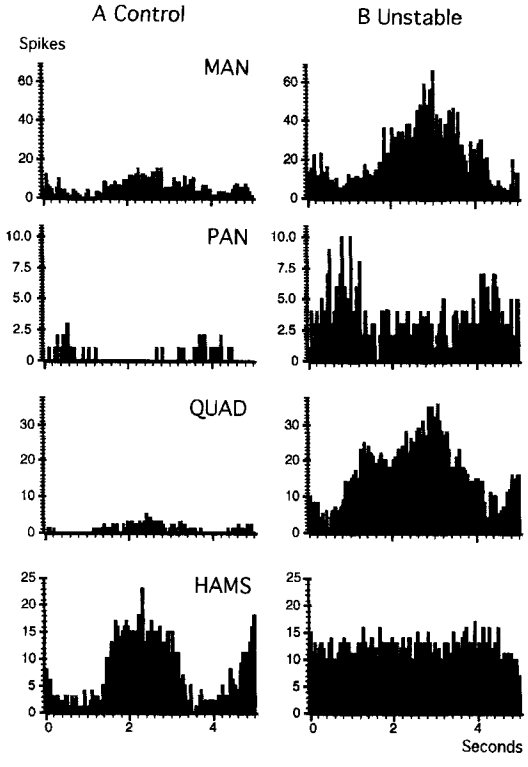
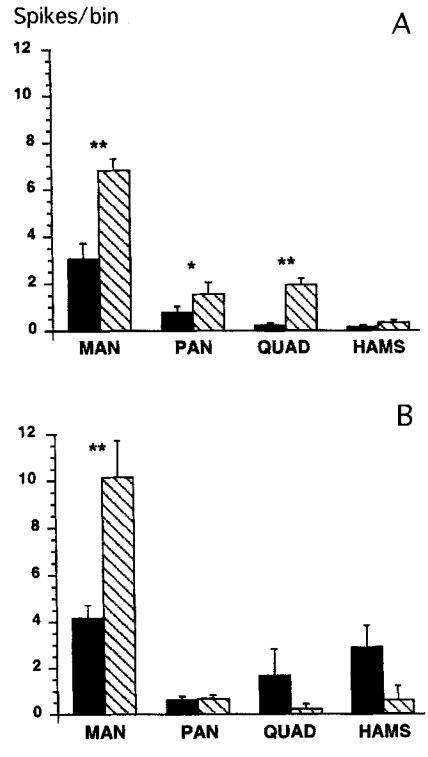


Figure 1. PSTH of afferent potentials in the MAN and the PAN, and motor units in the quadriceps (QUAD) and hamstring (HAMS). Ten repeated maneuvers of passive flexion, from 90° to maximum knee flexion, in a single, representative experiment, before (A) and after anterior cruciate transection (B).



Figures 2. Plots of the response area at the MAN, the PAN, the quadriceps muscle (QUAD), and the hamstring (HAMS), for all the knees (n 6) during passive knee *flexion* (A) and *extension* (B), before (solid bars) and after anterior cruciate transection (hatched bars). Significant differences are shown by * ($p < 0.05$) and ** ($p < 0.01$).

Discussion

Little is known about how the knee reacts to anterior cruciate damage. Most clinical studies stress the altered subjective perception of passive motion and joint position, which are interpreted as altered proprioception (Barrack et al. 1989, Barrett 1991). We do not refer to proprioception as the conscious perception of passive motion or joint position, but rather as the transmission of mechanically-evoked electrical nerve signals (which we detect at the articular nerves) triggering muscular responses (which we detect by the EMG). The precise role of a single structure (such as the anterior cruciate ligament) is important for understanding how the knee reacts when this structure is damaged. Knee function must be considered as a whole, producing proprioceptive information and reacting to it. Our experimental model was designed to study knee proprioception and its reaction to passive motion and passive stress with or without the anterior cruciate ligament, in the anesthetized cat.

Decerebrate animals have been used to determine motor neuron output in various studies (Ekholm et al. 1960, Miyatsu et al. 1993). A better detection of motor unit discharges is the reason for using experimental decerebration. Chloralose anesthesia has been employed (Solomonow et al. 1987), although other authors found no reflex response to anterior tibial displacement using this anesthetic (Pope et al. 1990). These techniques, although avoiding cortical control entirely, cause a peripheral hyperreflexive state that is far from the normal periarticular muscle reaction. In order to obtain electric activity patterns closer to normal function, we used pentobarbital general anesthesia. Pentobarbital deep anesthesia disconnects cortical responses from the peripheral stimuli. Although muscle relaxation is not induced, voluntary motion is inhibited. Thus, muscular contraction in response to passive motion is related to this proprioceptive stimulus, in the absence of another source because of the deep level of anesthesia. In our experiments, the level of anesthesia was controlled using EEG recordings. Changes in the electric activity obtained after ligament damage were not related to awakening, as no EEG changes were observed. In our measurements,

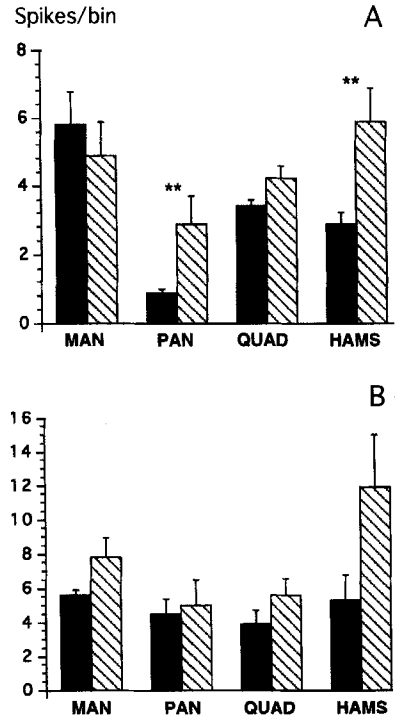


Figure 3. Plots of the response area at the MAN, the PAN, the quadriceps muscle (QUAD), and the hamstring (HAMS) for all the knees (n 6) during passive *external* (A) and *internal* rotation (B), before (solid bars) and after anterior cruciate transection (hatched bars).

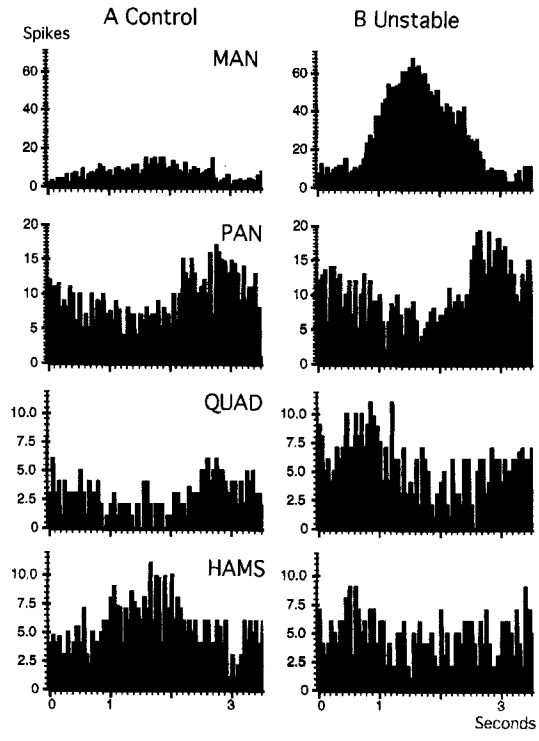


Figure 4. PSTH of afferent potentials in the MAN and the PAN, and motor units in the quadriceps (QUAD) and hamstring (HAMS). 10 repeated maneuvers of anterior tibial displacement with the knee at 90° flexion (anterior drawer) in a single, representative experiment before (A) and after anterior cruciate transection (B).

basal activity before the maneuver was subtracted to calculate the area of the PSTH evoked by the maneuver.

Passive knee hyperextension and external/internal rotation produced increased activation of neural fibers from the anterior cruciate ligament, in the experiments by Krauspe et al. (1992) in the normal knee. Some clinical EMG studies show that quadriceps-hamstring activity is altered in anterior cruciate-deficient knees (Solomonow et al. 1987, Draganich et al. 1989), while enhanced hamstring contraction is seen as a protective pattern. After transection of the anterior cruciate ligament, Khalsa and Grigg (1996) observed that posterior capsule afferents continue to behave normally. We showed that passive knee flexion, extension, external and internal rotation induce electric activation of neural fibers in the MAN and the PAN, as shown in Figures 1, 2 and 3. After anterior cruciate transection, knee kinematics are altered, as also is the neural fiber activation in the MAN and PAN during passive motion. These changes are significant in flexion at the MAN and PAN, in extension at the MAN, and in external rotation at the PAN. These changes reflect different stimuli and different origins of proprioception: medial proprioception (through the MAN) during flexion and extension, and posterior proprioception (through the PAN) during external rotation.

We found that anterior tibial displacement produced some neural fiber activation with the knee at 30° of flexion (as in the Lachman test) at the MAN and PAN, and little activation with the knee at 90° of flexion. However, when the ligament was transected and anterior instability was present, a significant increase in the MAN activation was observed at 30° and 90°, suggesting that the medial meniscus and other medially innervated structures may be overloaded, thus producing more medial information. Although PAN activity is present in our study in the normal knee, as found by Miyatsu et al. (1993), MAN activity is also present and appears to be significantly more important when the ligament is damaged.

When the muscular response was studied by EMG, we observed quadriceps and hamstring activation dur-

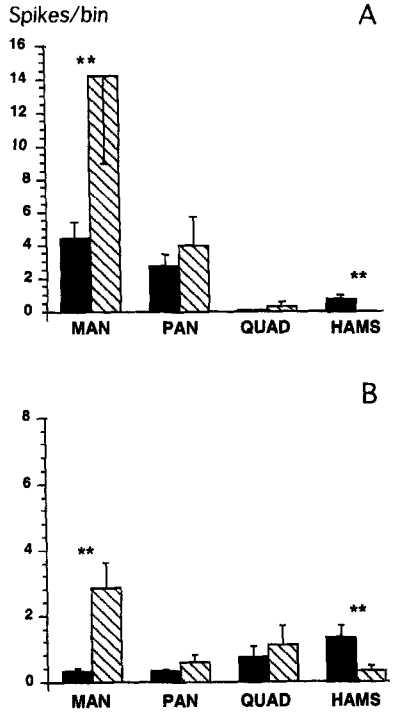


Figure 5. Plots of the response area at the MAN, the PAN, the quadriceps muscle (QUAD), and the hamstring (HAMS) for all the knees (n 6) during *anterior tibial displacement* with the knee at 30° (A) and 90° flexion (B), before (solid bars) and after anterior cruciate transection (hatched bars).

ing extension as well as internal and external rotation, as observed by Krauspe et al. (1992). Transection of the anterior cruciate produced a significant increase in hamstring activity during external rotation. No significant changes were observed in our experiments in extension or internal rotation, or in the quadriceps muscle. Muscle activity was detected when anterior tibial displacement was forced in the stable knee at 90° flexion. Hamstring reflex activity significantly declined in forced anterior tibial displacement, at both 30° and 90° of flexion. This supports the finding of Solomonow et al. (1987) who observed hamstring reflex activity when the normal anterior cruciate was loaded. In our study, if the ligament is damaged, this activity diminishes.

Regarding the relevance of our study, clinical interpretations can be based on the data obtained during extension, anterior tibial displacement, and external rotation. When the knee with the damaged anterior cruciate is extended, increased information flows through the MAN, probably due to the overloading of medial structures (namely, the medial meniscus). However, no muscular change is detected. Thus, the medial information is insufficient to induce a muscular response when the anterior cruciate is absent, even before any muscular atrophy, as in our model. This would explain why the medial meniscus can be damaged in the unstable knee (Hirshman et al. 1990), if the stabilizing efforts of the medial meniscus in the anterior cruciate-deficient knee are not enhanced by the powerful muscular stabilization.

As during extension, anterior displacement data also show that medial structures are overloaded, and that information through MAN produces no muscular response in our model. The hamstring reaction, that would be expected during anterior tibial displacement, is instead significantly inhibited when the anterior cruciate is damaged. It would be difficult to believe that any muscular hypertrophy of the hamstring, after rehabilitation protocols, would compensate for this inhibition, considering that our measurements were obtained in the acute knee, with no muscular atrophy.

When the knee is externally rotated, PAN activity is increased after anterior cruciate damage, probably due to an overloading of the posterolateral corner. This overload provokes a significant, protective hamstring contraction, opposing the unprotected medial meniscus overloading in extension and anterior tibial displacement. When the posterolateral corner is injured, we can hypothesize that the hamstring would be less reactive, and a poorer muscle stabilization would be expected. This would support clinical reports of failed anterior cruciate reconstructions when the posterolateral corner was damaged and not repaired (O'Brien et al. 1991).

Our first hypothesis is confirmed: the electric activity in the articular nerves and periarticular muscles is significantly altered after anterior cruciate ligament transection. Our findings verify our second aim, assessing the reaction of the knee to anterior cruciate damage. The clinical extent of proprioceptive damage is probably significant for the functioning of the anterior cruciate-deficient knee.

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