We have determined whether somatosensory evoked potentials (SEPs) were detectable after direct mechanical stimulation of normal, injured and reconstructed anterior cruciate ligaments (ACLs) during arthroscopy. We investigated the position sense of the knee before and after reconstruction, and correlated the SEP with instability.

Reproducible SEPs were detected in all 19 normal ACLs and in 36 of 38 ACLs reconstructed during a period of 13 months. Of the 45 injured ACLs, reproducible SEPs were detected in 26. The mean difference in anterior displacement in the SEP-positive group of the injured ACL group was significantly lower than that in the SEP-negative group. In the reconstructed group, the postoperative position sense was significantly better than the preoperative position sense. Our results indicate not only that sensory reinnervation occurs in the reconstructed ACL, but also that the response to mechanical loads can be restored, and is strongly related to improvement in position sense.

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Very little information is available regarding the sensory role of the anterior cruciate ligament (ACL). Although the mechanical contribution of the ligament is well documented, recent information suggests that it may have an important sensory role in initiating protective reflexes to control surrounding muscle tone through the 1a-muscle spindle to the afferent-nerve system. Many studies have shown the existence of several types of mechanoreceptor in human and animal ACLs. Whether these mechanoreceptors and their associated functions are restored after reconstruction of the ACL is uncertain.

Our aim therefore was to determine whether regenerated nerve fibres and mechanoreceptors in the reconstructed ACL could respond to mechanical loads applied to the ligament and to test for afferent impulses and somatosensory evoked potentials (SEPs), which would indicate functional restoration of axons and mechanoreceptors.

Patients and Methods

We enrolled 110 patients (110 knees) with intact posterior cruciate ligaments (PCL), but with knee injuries, into the study. Their mean age was 31 years (9 to 66). All gave consent and the protocol was approved by the Ethical Board of our institution. In four knees, mechanical stimulation of the intact PCL produced no cortical SE, because of technical defects and these individuals were excluded leaving 106 knees (106 patients, 49 men and 57 women) for analysis.

The patients were placed into one of three groups. Group I comprised 21 men and 24 women of mean age 29.8 years with an injury to their ACL, who had been diagnosed with anterior instability before arthroscopy. They underwent an arthroscopically-assisted reconstruction of the ligament. In this group the ACL had been ruptured, but remnants were observed bridging the femur and tibia. These attachment sites were not anatomical and the bridging tissue was flimsy. Knees in which the remnant of the ACL was attached to the PCL were excluded from group I, since the mechanical stimulation of the remnant through its attachment could induce cortical SEPs derived from neurones in the PCL. Patients in group II, 21 men and 21 women of mean age 32.9 years, had undergone reconstruction of the ACL in which quadrupled semitendinosus or doubled semitendinosus and gracilis tendon grafts had been used. The time of examination was immediately after reconstruction in two patients, at three and six months after reconstruction in two others, and at more than 13 months after reconstruction in the remaining 38. Finally, group III, the control group, consisted of seven men and 12 women of mean age...
28.4 years. They had normal ACLs but had undergone arthroscopic examination for an internal derangement of the knee (3 knees), meniscal tear (13 knees), or superficial chondral lesions (3 knees). The three groups were similar in regard to age and gender.

**Knee stability and position sense.** The anterior laxity of each reconstructed knee was examined before and after operation, using a KT-2000 knee arthrometer (MED Metric Corporation, San Diego, California) with a force of 133 N applied anteriorly and with the knee flexed at 30°. Both knees were examined; any difference in millimetres was recorded as laxity. No abnormalities were observed in the uninjured knees.

Using the procedure as modified by Skinner et al., the position sense of the joint was defined as the ability to reproduce an angle at which the joint had been placed moments before. The examiner extended one of the legs at a slow, steady rate of approximately 10°/s from a starting angle of 90°. The leg was stopped at a random angle between 5° and 25° and held by the examiner for three seconds. The knee was then returned to the starting angle, and the patient was asked to put it back to the target position. Inaccuracy was recorded as the difference in degrees between the perceived angle and the actual angle of flexion. Each knee was measured ten times, and the mean inaccuracy of the normal knee in degrees was subtracted from that of the injured knee to give a measurement of position sense. For analysis, reconstructed knees were classified as ‘stable’ if the right-to-left difference in anterior displacement between the two knees was less than 3 mm and as ‘unstable’ if the difference was 3 mm or more.

**SEPs.** All patients were examined under general anaesthesia induced with nitrous oxide in oxygen with fentanyl citrate, 0.1 ml/kg. Inhaled anaesthetics such as halothane, isoflurane, and enflurane, were not used to avoid their depressant effects on cortical responses. No local anaesthesia was used. SEPs were measured within 15 minutes of the induction of anaesthesia and after intubation before arthroscopy of the knee for reconstruction of the ACL or during a second-look procedure. The ACL of each patient was stimulated at the midpoint using a mechanical stimulator (DPS-270; Diamedical Inc, Tokyo, Japan). In Group I, remnant tissue was similarly examined. The mechanical stimulator consisted of a plastic tip 3 × 5 mm in size connected to a moveable coil transducer, controlled by a feedback circuit (Fig. 1). The tip of the stimulator was positioned on the surface of the ACL (Fig. 2). To produce an indentation in the ACL, 200 stimuli, each with a force of 3.92 N, were delivered at a rate of two per second. The room temperature was maintained at between 23° and 25°C. Electrode impedance was maintained at between 2 and 10Ω.

Cortical responses were recorded over a bandwidth of 0.5 to 1500 Hz for 100 ms. The 200 stimuli were averaged, and two responses were recorded for each trial and superimposed to ensure consistency. The cortical response was recorded using a system for measuring evoked potentials (Synax 1100; NEC Medical Systems, Tokyo, Japan). The SEPs were synchronously monitored by a surface electrode placed on the scalp at a point 2 cm lateral and 2 cm posterior to the CZ position, according to the international 10 to 20 system, with the reference of the FPz or ear. A peak or trough in each SEP (usually a P1 trough or an N1 peak) was chosen as the feature for programmed automatic detection for a monitoring session.

The SEPs produced by the PCL after mechanical stimulation were recorded under the same conditions. The values of the latency and potentials of P1 and N1 induced by mechanical stimulation in the PCL, were referred to as the control. P1 and N1 latency and the difference between the potentials of P1 and N1 were calculated as the voltage of the SEP (Fig. 3). In addition, the ratio of the voltages of the SEPs from the ACL and the PCL was calculated according to the following formula:
N1
P1
10 msec
1 µV

Ratio = (voltage of SEP induced by ACL stimulation)/
(voltage of SEP induced by PCL stimulation)

In order to assess the reproducibility of SEPs, the recording was repeated at least twice and confirmed by the method of Bland and Altman. The higher voltage was selected in each case.

In the injured group, any remnant of the ACL was electrically stimulated using a bipolar electrode inserted into the mid-portion of the remnant before mechanical stimulation. This electrode has a Teflon-coated tip (AU-1; Inter Medical Co Ltd, Tokyo, Japan) so that the stimulus is produced only at its tip, is localised in the ACL and does not spread to other structures within the knee through synovial fluid or physiological saline. The stimulus was a square wave administered for 0.2 ms with an intensity of 10 mA and at a frequency of three per second. As with the mechanical stimulation, the cortical response was recorded over a bandwidth of 0.5 to 1500 Hz for 100 ms, 200 stimuli were averaged, using Synax 1100, and the SEP was monitored from the surface electrode placed on the scalp.

Statistical analysis. The reproducibility of SEPs was examined by the method of Bland and Altman (MedCalc statistical software, Mariakerke, Belgium) using the latency of P1 and N1 and the SEP voltage in the control group of 19 subjects. All numerical data were expressed as the mean with 95% confidence intervals. Differences in the measurements, such as the latency and potential of P1 and N1 and the voltage ratio between the three groups, were assessed using one-way analysis of variance, followed by Scheffe’s test when a statistical difference was found. The relationships between the potential of the SEP, the degree of instability and diminution of position sense were estimated using Pearson’s correlation coefficient (r).

The unpaired Student t-test was used to compare the voltage or the position sense between the stable and the unstable knees in the reconstructed group. The position sense was analysed by the paired Student t-test to determine whether the values varied significantly between preoperative and postoperative conditions. A p value of less than 0.05 was regarded as statistically significant. All analyses were undertaken with the StatView 4.5 statistical software package (Abacus Concepts Inc, Berkeley, California).

Results

Reproducibility of the SEPs. The mean differences (+95% CI) of the P1 and N1 latencies and SEP voltage were 0.04 (-0.72 to 0.80), 0.02 (-0.48 to 0.52), and 0.002 (-0.0170 to

Table I. Details of mechanically-stimulated SEPs (mean; 95% CI) in all three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of knees</th>
<th>SEP voltage (µV)</th>
<th>P1 (ms)</th>
<th>N1 (ms)</th>
<th>Ratio (ACL/PCL)</th>
<th>Instability (mm)</th>
<th>Position sense (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Injured</td>
<td>45</td>
<td>1.30 (1.06 to 1.55)</td>
<td>31.1 (28.8 to 33.4)</td>
<td>43.6 (40.9 to 46.3)</td>
<td>0.96 (0.78 to 1.13)</td>
<td>4.99 (4.25 to 5.60)</td>
<td>1.14 (0.87 to 1.42)</td>
</tr>
<tr>
<td>SEP+</td>
<td>26</td>
<td>1.27 (1.12 to 1.43)</td>
<td>31.0 (29.2 to 32.9)</td>
<td>42.4 (40.2 to 44.7)</td>
<td>1.00 (0.89 to 1.12)</td>
<td>1.61 (0.83 to 2.40)</td>
<td>0.44 (-0.06 to 0.94)</td>
</tr>
<tr>
<td>SEP−</td>
<td>19</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.00 (0.58 to 1.42)</td>
<td>-1.88 (-7.48 to 3.37)</td>
<td>0.88 (-0.40 to 2.15)</td>
</tr>
<tr>
<td>II. Reconstructed</td>
<td>42</td>
<td>1.27 (1.12 to 1.43)</td>
<td>31.0 (29.2 to 32.9)</td>
<td>42.4 (40.2 to 44.7)</td>
<td>1.00 (0.89 to 1.12)</td>
<td>0.75 (0.09 to 1.41)</td>
<td>0.36 (-0.25 to 0.99)</td>
</tr>
<tr>
<td>SEP+</td>
<td>36</td>
<td>1.27 (1.12 to 1.43)</td>
<td>31.0 (29.2 to 32.9)</td>
<td>42.4 (40.2 to 44.7)</td>
<td>1.00 (0.89 to 1.12)</td>
<td>1.61 (0.83 to 2.40)</td>
<td>0.44 (-0.06 to 0.94)</td>
</tr>
<tr>
<td>Stable</td>
<td>28</td>
<td>1.21 (1.03 to 1.39)</td>
<td>30.9 (28.9 to 32.8)</td>
<td>42.1 (39.5 to 44.8)</td>
<td>1.00 (0.89 to 1.12)</td>
<td>0.75 (0.09 to 1.41)</td>
<td>0.36 (-0.25 to 0.99)</td>
</tr>
<tr>
<td>Unstable</td>
<td>8</td>
<td>1.49 (1.05 to 1.94)</td>
<td>31.6 (25.4 to 37.7)</td>
<td>43.4 (38.2 to 48.7)</td>
<td>1.00 (0.58 to 1.42)</td>
<td>4.63 (3.37 to 5.88)</td>
<td>0.73 (-0.16 to 1.61)</td>
</tr>
<tr>
<td>SEP−</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.00 (0.58 to 1.42)</td>
<td>0.75 (0.09 to 1.41)</td>
<td>0.36 (-0.25 to 0.99)</td>
</tr>
<tr>
<td>III. Control</td>
<td>19</td>
<td>1.42 (1.16 to 1.68)</td>
<td>31.3 (29.1 to 33.5)</td>
<td>43.4 (40.8 to 45.9)</td>
<td>1.10 (0.91 to 1.27)</td>
<td>1.00 (0.13 to 1.87)</td>
<td>0.38 (0.35 to 0.72)</td>
</tr>
<tr>
<td>SEP+</td>
<td>19</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.10 (0.91 to 1.27)</td>
<td>1.00 (0.13 to 1.87)</td>
<td>0.38 (0.35 to 0.72)</td>
</tr>
<tr>
<td>SEP−</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.10 (0.91 to 1.27)</td>
<td>1.00 (0.13 to 1.87)</td>
<td>0.38 (0.35 to 0.72)</td>
</tr>
</tbody>
</table>
Knee stability. The mean side-to-side difference of anterior displacement was 4.99 mm in group I, 1.26 mm in group II and 1.00 mm in the control group (Table I). The difference in the injured group was significantly larger than that of the other two groups (p < 0.0001). The reconstructed and the control groups also differed significantly (p < 0.0001). The ratio in the control group was higher than that of SEP-positive knees in the reconstructed and the injured groups, but there was no significant difference between the three groups.

Position sense. The mean inaccuracy of joint position sense was 1.14° in the injured group I, 0.48° in 40 knees of the reconstructed group II (the remaining two knees were measured immediately after reconstruction), and 0.38° in the control group III. The mean inaccuracy of group I was significantly larger than that of the other two groups (p = 0.008). The postoperative accuracy of joint position sense in group II was significantly improved (p = 0.03) from the preoperative inaccuracy of 1.01° (0.73° to 1.28°). Also, in the SEP-positive knees of group II, the inaccuracy seen in the stable knees (0.36°) was not significantly differ-

Discussion

Histological studies in animals have shown axonal regeneration with mechanoreceptors after reconstruction of the ACL.\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\) It was, however, unclear whether the mechanoreceptors were functional. Aune et al\(^5\) using immunohistochemical methods, found no neuropeptides in biopsies of remodelled human ACLs after reconstruction. By contrast, Ochi et al\(^13\) detected cortical SEPs after direct electrical stimulation of injured and reconstructed ACLs. These results did not necessarily indicate regrowth of mechanoreceptors or the functional restoration of regenerated axons since the SEPs induced by the electrical stimulation only indicated regeneration or retention of axons in the injured ACLs or axonal regeneration in the reconstructed ACLs. It was unclear whether regenerated axons and mechanoreceptors could be activated when a mechanical load such as that induced by the anterior translation of the tibia during daily or sports activity was applied to the reconstructed ACL.

Our results suggest that mechanical stimulation elicited reproducible SEPs in 36 of 38 knees (95%) more than 13 months after reconstruction of the ACL, indicating functional recovery of axons and mechanoreceptors. Since it was also found that mechanical stimulation of the remnants of injured ACLs elicited reproducible SEPs in 26 of 54 knees (58%), we conclude that preserved neurones and mechanoreceptors in the remnants of injured ACLs also retain some function.

The first recordings of ligamentous afferent nerves were made by Andrew and Dodt\(^14\) and Andrew\(^15\) in 1954 using very small loads (below 100 g) applied to the medial collateral ligament. In the same year, Boyd\(^16\) reported recordings from two Golgi tendon organ afferents in the cruciate ligament of a cat. In 1985, Cohen, Starr and Pratt\(^17\) demonstrated SEPs in man in response to natural forms of somatosensory stimulation of the lower limb including stretching of muscles, tapping on muscle bellies and tapping on cutaneous surfaces. More recently, Solomonov et al\(^18\) have shown that direct mechanical stress of up to 116 N applied to the ACL could induce activity in the hamstrings of anaesthetised cats, but Pope, Cole and Brandt\(^19\) detected no reflex activity in the quadriceps or hamstrings muscles of anaesthetised cats after loading of up to 125 N, although they observed output in the posterior articular nerve. Reflex activity in the quadriceps or hamstring muscles of dogs and cats with a precollicular transection was observed by Miyatsu, Atsuta and Watakabe\(^20\) after axial loading of 30 N by traction on a block of bone which has been isolated with the tibial insertion of the ACL. Krauspe et al\(^6\) reported nerve activity in the ACL on passive hyperextension.
It seems clear that mechanical stimulation of the normal ACL in animals can induce output in the peripheral nerve connected to mechanoreceptors within the ACL. It is uncertain, however, whether SEPs are elicited by mechanical stimulation in the normal human ACL, or whether mechanical stimulation of the remnant of the ACL, or the reconstructed ACL, can induce reproducible SEPs.

In the injured group in our study, positive electrical SEPs were observed in 73% (33 of 45), and the incidence of positive mechanical SEPs was 58% (26 of 45). By contrast, Ochi et al\textsuperscript{13} reported positive electrical SEPs in 47% (15 of 32). During our study knees with negative electrical SEPs always had negative mechanical SEPs. We therefore did not monitor the mechanical SEPs in 30 knees because reproducible electrical SEPs could not be obtained. Since these knees were excluded from the analysis, the accuracy of positive electrical SEPs, as well as mechanical SEPs, may appear to be high. On the other hand, 36 of 38 knees (95%) in which the ACL had been reconstructed using autogenous hamstring tendons, showed functional restoration of regenerated sensory neurones and mechanoreceptors more than a year after reconstruction. Ochi et al\textsuperscript{13} also showed that the voltages of the electrical SEPs of the reconstructed ACL and of the normal PCL were 1.14 (0.45) $\mu$V and 1.14 (0.42) $\mu$V, respectively. These values are lower than those of the mechanical SEPs measured here under the same conditions and with the same anaesthesia and measurement system as the previous study. A partial explanation for this difference is that, although electrical stimulation is more effective than mechanical stimulation in activating sensory neurones, it can only elicit discharges of regenerated neurones between the tips of the electrode, a distance of 5 mm, whereas mechanical stimulation can probably excite all regenerated nerve fibres in the reconstructed ACL as well as nerve fibres in the areas where the reconstructed ACL is attached to bone.\textsuperscript{21} However, a higher-voltage SEP may not necessarily indicate a denser sensory neurone.

In regard to our technique for mechanical stimulation of only the structure of the ACL, this may have caused micromovement of the femorotibial joint and activation of the mechanoreceptors in the capsule. If this speculation is correct, the positive mechanical SEPs would have been observed immediately after reconstruction of the ACL and we should have observed some knees with positive mechanical SEPs when negative electrical SEPs were obtained. This was not the case. The positive SEPs from mechanical stimulation appear to be induced by activation of the mechanoreceptors in the ACL grafts or remnants.

Several histological studies in animals report nerve regeneration as well as regrowth of mechanoreceptors in the ACL graft. By contrast, in a histological study of ten human ACLs, Aune et al\textsuperscript{13} found no evidence of regrowth of mechanoreceptors or axonal regeneration in biopsied tissue from patellar tendon autografts between five and 37 months after surgery. They could, however, not fully examine the degree of regeneration of sensory neurones because the amount of biopsied tissue obtained was small.

It is therefore reasonable to examine the reconstructed ACL electrophysiologically by recording SEPs after direct electrical stimulation. In the study of Ochi et al\textsuperscript{13} more than 18 months after operation, relatively high voltages were recorded in the SEPs in 22 knees reconstructed using autogeneic tendons, indicating the presence of regenerated neurones.

The fact that mechanical stimulation of the reconstructed ACL or the ACL remnant can induce SEPs clearly indicates that sensory neurones in the ligament may provide sensory information about its deformation which may contribute to the stability of the joint. Since SEPs were recorded after mechanical stimulation of the ACL remnant in 26 of 45 knees, augmenting the ACL without resecting the entire remnant may help to preserve sensory neurones and mechanoreceptors.

Our study confirms not only that sensory reinnervation occurs in the reconstructed human ACL, but also that electrical output induced by mechanical loads to the grafted ACL can be conveyed to the central nervous system. These findings suggest that sensory function of the ACL can be restored, probably through the regeneration of mechanoreceptors.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References


