Neuromuscular recovery pattern after medial collateral ligament disruption in rats

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Laurin J, Dousset E, Mesure S, Decherchi P. Neuromuscular recovery pattern after medial collateral ligament disruption in rats. J Appl Physiol 107: 98–104, 2009. First published May 21, 2009; doi:10.1152/japplphysiol.00317.2009.—The medial collateral ligament (MCL) is one of the most injured ligaments during sport activities. The resulting joint damage effects on neuromuscular system remain unclear. Thus this study was designed to assess the changes in neuromuscular properties of vastus medialis muscle after MCL transection. Complete rupture of MCL was performed on rats, and dynamic functional assessment during locomotion was achieved before and once a week from 1–5 wk postlesion. Twitch properties and metabo- and mechanosensitive afferent fiber responses to specific stimuli were measured 1, 3, and 5 wk after MCL transection. Results indicated that maximum knee angle measured during the stance phase of the gait cycle was decreased during 3 wk after MCL injury and then recovered. Minimum knee angle measured during the stance phase was decreased during 2 wk and showed compensatory effects at week 5. A stepwise decrease in maximum relaxation rate-to-amplitude ratio concomitant with a stepwise increase in half-relaxation time were observed following MCL injury. Variations in metabosensitive afferent response to chemical (KCl and lactic acid) injections were decreased at week 1 and recovered progressively from week 3 to week 5 postlesion. Recovery of the mechanosensitive afferent response to vibrations was not totally complete after 5 wk. Our data indicate that alteration of the sensory pathways from the vastus medialis muscle could be considered as a source of neuromuscular deficits following MCL transection. Our results should be helpful in clinical purpose to improve the knowledge of the influence exerted by ligament rupture on the motor system and permit development of rehabilitation protocols and exercises more appropriate for recovery of functional stability.

mechanosensitivity; metabosensitivity; group III–IV afferent fibers; ligament transection; contractile properties

THE MEDIAL COLLATERAL LIGAMENT (MCL) is considered to be the most commonly damaged ligament occurring during sport injury of the knee. A complete rupture represents almost 50% of the MCL injuries (12, 17), and such disturbance is immediately followed by knee joint damage (joint capsule damages, vascular disruption) and impairment in the neuromuscular system (22, 24). In humans, several investigations have reported that neuromuscular deficits were partially represented by alteration of voluntary muscle activation, prolonged muscle reaction time in response to perturbation, and proprioceptive deficits (23, 29, 41, 48). However, the mechanisms underlying neuromuscular changes are not clear and are still debated.

Among explanations for this, these functional neuromuscular alterations resulting from ligament injury may be attributed to the loss or change in sensory feedback from ligaments and others joint structures (22, 24, 26, 36, 43, 50). Indeed, Hurley et al. showed that patients with knee ligament rupture and associated joint damage had greater reduction in quadriceps femoris activation than those with knee ligament ruptures alone (27). It was suggested that this dysfunction was ascribed to the ankle joint and ligament deafferentation. However, Konradsen et al. showed that suppression of all mechanosensitive afferent informations from ankle joint and ligament did not influence peroneal reaction time (37). They proposed that this disturbance came exclusively from muscle and tendon afferents.

Mechanosensitive afferent fibers (group I) originating from muscle spindle and Golgi tendon organs participated in the detection of joint movement and are involved in α-motoneuron discharge changes (14, 51). Several authors provided evidence that alteration in the “gamma-muscle-spindle system” may be a cause of several neuromuscular deficits such as prolonged peroneal reaction time and decline in muscle activity after ligament injury (35, 36). However, there is no direct evidence of changes in musculotendinous mechanoreceptor activity following MCL injury.

In addition, among the muscle afferent fibers, the thinly myelinated group III and unmyelinated group IV fibers are likely involved in the control of motor unit firing rates (8, 14, 51). Indeed, proprioceptive feedback from spindle Ia afferent could be reduced by group III and IV afferent fiber activity at the spinal level (44). These slowly conducting afferent fibers could also act on the motor cortical output to regulate the α-motoneuron discharge (40, 52). Furthermore, chemically induced discharges in group III and IV muscle afferents can reflexively increase fusimotor discharge (30) and hence increase spindle discharge (38). To accomplish these regulations, group III and IV muscle afferents are activated by increased intramuscular pressure (16), tendon stretch (19), muscle contraction (32, 33), and muscle temperature (21), and by changes in muscle metabolism (33). Thus the first stimulus is mechanical and occurs when contraction distorts the receptive fields of the afferents. The second stimulus is metabolic and occurs when contraction generates chemical by-products. Group III and IV afferents act primarily as mechano- and metabosensitive nerve endings, respectively. However, some group III fibers respond to metabolic stimuli, and some group IV fibers respond to mechanical stimuli. They are selectively activated during and after muscle fatigue (9) or by different agents like...
bradykinin (31), capsaicin (31), lactic acid (LA) (46), arachidonolic acid (46), thromboxane A2 (34), H+ (47), prostaglandin (31), and potassium chloride (KCl) (25). No studies have investigated directly the metabosensitive group III and IV muscle afferent fiber response during MCL transection recovery.

We hypothesize that those sensorimotor changes following ligament injury could be ascribed to alteration of muscle afferent activity (24). This study was designed to examine, during 5 wk following MCL transection, the recovery pattern of the mechanosensitive fiber response to tendo vibrations. We measured as well the recovery pattern of group III and IV metabosensitive afferent fiber response to KCl and LA injections. To be complete, we also assessed changes in locomotion kinetics and contraction properties from quadriceps muscle.

MATERIALS AND METHODS

**Animals.** Forty-seven adults female Wistar rats, weighing 250–300 g (Charles River, Les Oncins, France), were housed in smooth-bottomed plastic cages at 22°C with a 12-h light/dark cycle. Food (Purina, rat chow) and water were available ad libitum. Anesthesia and surgical procedures were performed according to the French law on animal care guidelines, and the Animal Care Committee of University Aix-Marseille II approved our protocols. Furthermore, experiments have been carried out in accordance with the European Community’s council directive of 24 November 1986 (86/609/EEC). No sign of screech, prostration, hyperactivity, anorexia, or paw-eating behavior were observed throughout the experiment.

**Experimental protocol.** Rats were randomized into five groups: control group (C; n = 10) without any surgery, sham group (S; n = 9) in which surgery was made from the left hindlimb without MCL transection, groups with MCL transection in which neuromuscular measurements were made 1 (L1; n = 10), 3 (L3; n = 9), and 5 wk (L5; n = 9) after MCL transection.

The progress of the protocol was the following. In awake animals, 1) measurement of the maximal and minimal knee angles during the stance phase of the gait cycle (dynamic functional assessment) once a week from weeks 1 to 5 after the lesion. In anesthetized animals, 2) analysis of the twitch properties (contraction and relaxation) of the quadriceps muscle, 3) recording of metabosensitive afferent activity in response to chemical injections, and 4) recording of mechanosensitive fiber response to tendon patellar vibrations were assessed.

**Transection of MCL.** The animals were anesthetized with chloral hydrate (Sigma, 60 mg/kg). With the use of an operating microscope (X40, OPM 11 Zeiss, Oberkochen, Germany) and under aseptic conditions, the MCL of the left knee was exposed with a 1-cm medial incision through the skin and fascia. A scalpel curved blade was used to detach the MCL from the surrounding tissues without damaging the ligament. The blade was then passed under the ligament, which is stretched, as close as possible to the femoral bone point insertion because the highest concentration of sensitive receptors was found at the insertion points (13). Finally, the MCL was sectioned with small scissors without damaging the quadriceps muscle. Blood and nerve supplies were also kept intact. Skin incision was then sutured (Flexocin 3-0, B. Braun Medical, Boulogne, France), and the animals were allowed to recover in individual cages. Analgesic agents were administrated immediately and during the first day postsurgery but suppressed in the following days (recovery period) to exclude their potent effect on measured parameters.

**Dynamic functional assessment of hindlimb recovery.** The functional recovery of left knee angle was evaluated in locomotor glass lane once a week using automatic analysis software (SimiMotion software, Simi, Unterschleissheim, Germany) associated to a numerical camcorder (Canon, MV 830i, Courbevoie, France). The size of the glass walking track was 150 cm long, 9 cm wide, and 40 cm high. Heavy lighting was provided with two 500-W spots. Before recording, the lower back and the left hind limb of the rat were shaved. The hip joint (trochanter), the knee joint (condylus femoralis), the ankle joint (lateralis malleolus), and the fifth metatarsal head were marked on the operated leg with a black permanent marker. The camcorder was positioned perpendicular to the vertical plane of the chamber to get a sagittal view of the gait cycles. Rats were placed at one end of the glass lane, and a dark box was placed at the other end. A stimulating noise and a strong light were applied to induce their locomotor activity. Such induced walk was recorded with a 50-Hz acquisition frequency using SimiMotion software (Simi). Three steps were recorded for further analysis. Based on the video analysis, the system computed the different angle joints during the stance phase of the gait cycle. The start of the stance phase was considered to be the point where the foot touches the ground and the end the point where the foot leaves the ground to begin the swing phase.

Maximal and minimal knee angles were averaged on three steps during the stance phase. The two-dimensional positions of the anatomical markers were tracked on each frame. Kinematic data were smoothed using a cubic smoothing spline procedure. The measured gait parameter was the knee joint angle, which was defined as the intersection between the lines extended from the hip to the knee joint and the line from the knee joint to the ankle joint. Based on the position of the toe marker, the gait cycles were divided into a stance and a swing phase. The maximum and minimum angles during the stance phase were computed within the three gait cycles and kept for further analysis. The C group was composed by rats without ligament disruption on which were recorded maximal and minimal knee angles from weeks 1 to 5. Angle values obtained on this group were compared with the angle values obtained with the other groups (S, L1, L3, L5). Processing of the gait parameters was performed using Matlab software (The MatWorks, version 7.5.0 342 R2007b).

**Surgery for neuromuscular measurements.** After MCL transection, rats were re-anesthetized by an intramuscular injection of solution containing a ternary mixture [5 ml of ketamine (Virbac, Carros, France); 2.5 ml of larcagil (Avenis, Paris, France); 2 ml of domitor (Novartis, Mississauga, Canada); 0.1 ml/100 g body wt]. If necessary, anesthesia was prolonged by a supplementary intramuscular injection of ketamine mixture (0.05 ml). Central temperature was maintained constant (~38°C) with a homeothermic blanket (Harvard Apparatus) driven by a rectal thermal probe. Rats were tracheotomized, cannulated, and artificially ventilated (Harvard volumetric pump: rate of 40–60 min⁻¹, tidal volume of 2–4 ml; South Matick, MA). A catheter was inserted into the right femoral artery and pushed up to the fork of the abdominal aorta to transport the chemicals (i.e., KCl and LA) to the contralateral muscle (see below for recording of nerve activity). This catheter was positioned to let the blood flow freely to the muscles of the left lower limb. Finally, the left femoral nerve was dissected free from surrounding tissues on a length of 2–2.5 cm. Animals were positioned in dorsal decubitus during all neuromuscular measurements. The knee and ankle were firmly held by clamps on a horizontal support to avoid disturbing movements and to maintain the 90° knee joint angle during electrical nerve stimulations.

**Twitch measurement.** To measure quadriceps isometric force, a strain gauge (Microdynanometer S60; Ugo Basile Narco Biosystem) was fixed to the muscle and perpendicularly to the ankle to maintain an isometric position at 100°. The contractile response of the quadriceps to femoral nerve stimulation was performed with a neurostimulator (Digitimer D57 A) who delivered single rectangular shocks (duration: 0.1 ms; frequency: 0.2 Hz). The current intensity used to evoke maximal twitch amplitude (from the beginning of the curve to the peak) was determined. Four electrical stimulations were performed, and maximal twitches were averaged. Contractions were recorded with Biopac MP150 system (sampled at 2,000 Hz, filtered with low pass at 150 Hz) and analyzed with Biopac AcqKnowledge 3.9 software (Biopac System). Twitch was analyzed in terms of 1) maximal amplitude (A; in N), 2) contraction time (TPT; time
interval expressed in ms between the beginning of the contraction curve and peak twitch tension), 3) maximum contraction rate (MCR; in N/ms and defined as the slope of a tangent drawn to the steepest portion of the contraction curve from the peak amplitude), 4) half-relaxation time (HRT; time interval expressed in ms between peak twitch tension and the half of the descending portion of the relaxation curve), 5) maximum relaxation rate (MRR; in N/ms and defined as the slope of a tangent drawn to the steepest portion of the relaxation curve from the peak amplitude). MCR and MRR were normalized to A [MCR/A, mean contraction rate constant (ms⁻¹); and MRR/A, mean relaxation rate constant (ms⁻¹)], as suggested by Esau et al. (11), who showed that MRR values are linearly related to A.

Femoral nerve recordings in response to chemical injections and mechanical vibrations. The proximal portion of the femoral nerve was cut to exclude efferent discharges during nerve recordings. To analyze the response of the metabosensitive afferent fibers, femoral nerve was positioned on a pair of bipolar tungsten electrodes and immersed in paraffin oil. The neural signals were referred to a ground electrode implanted in a nearby tissue, amplified (10–100 K), and filtered (30–10 kHz) with a differential amplifier (P2MP SARL, Marseille, France). The afferent discharge was recorded (Biopac MP150 and AcqKnowledge software, Biopac System) and fed into pulse window discriminators (P2MP SARL), which simultaneously analyzed afferent populations. The output of these discriminators provided noise-free tracings (discriminated units) was were displayed on a computer using a data acquisition system (Biopac AcqKnowledge software, Biopac System) at 1-s intervals (in Hz). The discriminated units were counted and recorded on separate tracings.

Before chemical stimuli were injected, a baseline recording was achieved to ensure that the discharge rate was stable. Baseline discharges were also recorded between chemical injections. The impulse activity was recorded without chemical injection during 180 s, and the recording was considered available only if the fluctuation of baseline impulse activity ranged between 100 and 103%. Consequently, variation of firing rate was related only to the stimuli applied and not to environmental conditions.

Once these resting levels of activity were established and stable, varying amounts of KCl (1, 5, 10, and 20 mM) and LA (0.5, 1, 2, and 3 mM) were randomly injected into the artery. It is noteworthy to notice that the injected chemical agents in this investigation did not elicit muscle contraction as indicated by the recorded muscle tension. So considered, no neuromuscular blocking was used for afferent fibers recording. There was a 12-min delay between each injection to let the afferent activity go back to its baseline activity. The poststimulus discharge firing rate was compared with the baseline discharge rate, and variations were expressed in percentage of the corresponding baseline discharge firing rate; i.e., baseline discharges corresponded to 100%. The analysis of the recorded afferent firing rate was performed by a specific Matlab program (The MatWorks, version 7.5.0 342 R2007b). Indeed, the discharge firing rate was measured on 20-s periods all along the recording period. On each 20-s period, the afferent firing rate was calculated on different time intervals lasting from 1 to 20 s and averaged between them. This process excluded any influence of the different time intervals used to calculate the variation in afferent firing rate. Thus the Matlab program calculated the temporal profile of discharge rate variation and extracted the peak discharge rate after each injection. Baseline afferent discharge was analyzed in an 80-s period preceding each injection. Afterward, a post-injection 20-s period of peak discharge rate was compared with the corresponding baseline firing rate.

Muscle tendon vibrations in a range of 10–100 Hz are known to activate muscle mechanosensitive fibers without activating muscle metabosensitive fibers (6, 7). Rectangular mechanical shocks were delivered perpendicularly to the longitudinal muscle axis on patellar tendon by a commercially available vibrator (Ling Dynamic System, LDS group, Herfordshire, UK) driven by a frequency generator (GenTrad Function Generator GF763AF, ELC, Annecy, France).

Vibrations were applied for 5-s periods. The vibration frequency was increased step-by-step from 10 to 100 Hz while the discharge of single afferent units was recorded. The maximal mechanosensitive afferents discharge rate elicited by tendon vibrations was considered as the reference discharge rate (100%). The discharge rate induced by the others frequencies of vibrations were expressed in percentage of the corresponding reference discharge rate.

At the end of the experiment, rats were killed by an intra-arterial pentobarbital overdose.

Statistical analysis. All values are expressed as means ± SE. Data processing was performed using statistical software (Statistica, StatSoft, Tulsa, OK). Concerning kinematic analyses, paired t-tests allowed us to determine changes between pre- and postsurgery conditions. For all other measurements, an analysis of variance completed with a Newman-Keuls’ post hoc test allowed us to assess significant modifications of twitch properties and afferent activity (F_impulse/s) between groups. Differences were considered significant when P < 0.05.

RESULTS

Functional assessment of hind limb recovery. In MCL transection groups, maximum knee angle was decreased from week 1 to week 3 compared with presurgery values (respectively, −15.1°, P < 0.001; −8.56°, P < 0.01; −4.42°, P < 0.01) and returned to control values at week 4 (Fig. 1A). As shown in Fig. 1B, minimum knee angle was decreased at week 1 and week 2 (respectively, −12.28°, P < 0.05 and −5.41°, P < 0.05). It returned to control values at week 3 and week 4.

![Fig. 1](http://jap.physiology.org/)

**Fig. 1.** A: time course of maximal knee angles during the stance phase of the gait cycle during 5 wk. Significantly lower angle in injured ligament groups compared with control (C) group: *P < 0.05; **P < 0.01; ***P < 0.001. B: time course of minimal knee angles during the stance phase of the gait cycle during 5 wk. *Significant differences between injured ligament and C groups (P < 0.05).
However, compared with C group, the minimum knee angle recovery pattern indicates an increase at week 5 (+7.18°, P < 0.05).

**Contractile properties.** As shown in Fig. 2A, no difference was observed between C group and the others groups (S, L1, L3, and L5) in MCR/A ratio. Regarding MRR/A ratio, no difference between C and S groups was observed. However, this ratio exhibited a significant decrease in L1, L3, and L5 compared with C group (respectively, −6.76 ms⁻¹, P < 0.05; −8.31 ms⁻¹, P < 0.05; −9.63 ms⁻¹, P < 0.01).

HRT and TPT followed an equivalent profile (Fig. 2B). Indeed, there was no intergroup difference for TPT. No difference between C and S groups for HRT was observed. HRT increased in L1 compared with C group (+3.46 ms, P < 0.05). Likewise, HRT in L3 and L5 was increased compared with C group (respectively, +4.35 ms, P < 0.05; +5.04 ms, P < 0.01).

**Response of metabosensitive fibers to KCl injections.** In Fig. 3, no difference was observed between C and S groups for all KCl concentrations. A peak discharge for C and S groups was found after KCl (10 mM) (respectively, 108.65 ± 1.17 and 107.06 ± 0.7% of the corresponding baseline values) followed by a slight decrease. The afferent fiber response to KCl 5, 10, 20 mM injections was significantly attenuated in the L1 group (respectively, −3.164%, P < 0.05; −6.809%, P < 0.001; −3.087%, P < 0.05). The dose-dependant profile observed in C group was restored in L3 and L5 groups. It is noteworthy to notice that a significant difference between L3 and L5 was observed, i.e., a higher response to KCl (10 mM) in L5 group compared with L3 group (+7.12%, P < 0.001). Also observed after KCl (10 mM), the response of afferent fibers in the L5 group was higher than the response recorded in the L1 group (+7.07%, P < 0.001).

**Response of metabosensitive fibers to LA injections.** There is no difference between C and S groups for all LA concentrations (Fig. 4). The group III and group IV afferent fiber response to LA injections peaked at LA (1 mM) for C and S groups (respectively, 108.29 ± 1.43 and 107.93 ± 1.71%) and then decreased. In all groups, there is no difference in afferent response to LA injections, except for LA (1 mM). For the L1 group, afferent fiber response to LA (1 mM) was significantly higher response in L3 group compared with L1 group (P < 0.01). #Significantly higher response in L5 group compared with L3 group (P < 0.05).
decreased compared with C group (−5.41%, \( P < 0.01 \)). No difference was observed between C, L3, and L5 groups. However, the afferent fiber response to LA (1 mM) injection was significantly higher in the L3 group compared with the L1 group (+3.57%, \( P < 0.05 \)). Likewise, a higher response was found in the L5 group compared with L1 in LA (1 mM) (+4.32%, \( P < 0.05 \)).

Response of mechanosensitive fibers to patellar tendon vibrations. As shown in Fig. 5, there was no significant difference between C and S groups for all frequency vibrations. Comparing C and L1 groups, significant differences were observed in low-frequency vibrations (at 10 Hz, \( P < 0.05 \); at 20 Hz, \( P < 0.05 \); at 30 Hz, \( P < 0.05 \); at 40 Hz, \( P < 0.05 \); at 50 Hz, \( P < 0.05 \)). No difference was observed between C and L3 groups. When C and L5 groups were compared, significant differences were found exclusively for 10 Hz (\( P < 0.01 \)) and 20 Hz (\( P < 0.05 \)).

Figure 5 also showed that there was a significant difference between L1 and L3 groups for 10 Hz (\( P < 0.05 \)), 20 Hz (\( P < 0.05 \)), and 40 Hz (\( P < 0.05 \)). There was a significant difference between L1 and L5 groups from 10 to 70 Hz (respectively, \( P < 0.001 \), \( P < 0.001 \), \( P < 0.01 \), \( P < 0.05 \), \( P < 0.05 \), \( P < 0.05 \), and \( P < 0.05 \)). Significant differences were observed between L3 and L5 groups for 10 Hz (\( P < 0.001 \)), 20 Hz (\( P < 0.001 \)), 30 Hz (\( P < 0.05 \)), and 70 Hz (\( P < 0.05 \)). All the results indicated that muscle afferent response to vibrations was depressed in the first weeks following MCL transection.

DISCUSSION

In the present study, MCL transection is followed by a significant alteration of the quadriceps muscle metabo- and mechanosensitive fiber response to chemical and mechanical stimuli, respectively. In parallel, we observed a decrease in maximal knee angle during the stance phase of the gait cycle 3 wk following the transection. The recovery of group III and IV muscle afferent response was complete after 5 wk. However, the recovery of mechanosensitive muscle afferent activity as well as the maximal knee angle recovery pattern was not completely achieved after 5 wk. Moreover, the relaxation properties (HRT and MRR/A ratio) of the quadriceps muscle were increasingly impaired during this protocol period.

The S group was studied to check for a possible influence of the surgery itself on measured parameters (contractile properties, afferents fibers response). In all cases, we reported an absence of difference between S and C groups, excluding any influence of MCL transection-induced surgery. Consequently, only the consequence of MCL transection could explain our results.

Alterations of the joint biomechanics are characteristic of ligament rupture (48). In the present study, the maximum and minimum knee angle parameters confirmed that functional instability occurred following MCL transection. Maximal knee angle was referred to the end of the stance phase where the quadriceps muscle was concentrically activated to produce the extension of the leg (just before the swing phase of locomotion). The decreased angle from week 1 to week 3 indicated that concentric activation of the muscle during locomotion was affected by the lesion. In consequence, animals presented difficulty to produce a complete hind limb extension to begin the swing phase. It was not in accordance with Munn et al., who showed that injury did not altered the concentric muscle contraction (42). Methodological differences could explain these opposite results. Indeed, the authors measured eccentric and concentric deficit strength on fibular muscles during ankle perturbation on subjects with unilateral ankle instability instead of kinematic assessment. Minimal knee angle was referred to the first half of stance phase where the quadriceps muscle was eccentrically activated. The decreased angle from week 1 to week 2 indicated that eccentric activation of quadriceps muscle during locomotion was affected by ligament lesion, inducing some difficulty to retain the injured limb during flexion. It was in accordance with a study that showed an ankle strength deficit when fibular muscles were eccentrically activated (42). Maximum knee angle recovered from 4 wk, but it was surprising to note that minimum angle showed an increase at week 5. We can suggest that recovery process could start with compensatory effects. The latter were ascribed to activation of synergic muscles of the quadriceps to protect the affected muscle. Indeed, twitch relaxation properties of the quadriceps showed indirectly, in our study, a failure of contractile machinery (1) induced by MCL transection, which may contribute to the functional instability of the joint. At this stage, the latter muscle and synergic muscles will act together, optimizing the eccentric activation during the stance phase, which could explain the overcompensatory effects at week 5.

Regarding the variation in metabosensitive afferent discharge rate in response to KCl and LA injections, we showed a decrease 1 wk after the lesion for concentrations where afferents are more sensitive (5, 10, and 20 mM for KCl and 1 mM for LA). The response was stepwise recovered to 5 wk. Two possible neural mechanisms are thought to explain decrements in afferents response. First, metabosensitive muscle afferents might be maximally activated following MCL rupture. Actually, MCL injuries lead the development of an inflammatory reaction (10, 13, 49). It is now commonly admitted that inflammatory mediators such as interleukin 6 (IL-6), bradykinin prostaglandins, and an increase of temperature are potent activators of these afferent fibers (8). Moreover, little evidence indicated that some inflammatory agents like cyclooxygenase could sensitize group III and IV afferents in...
response to their mechanical and metabolic stimuli (20, 45). Consequently, their discharge rates could not be increased with additional chemical stimuli. It was demonstrated that blood flow decreased when femoral nerve was transected with MCL transection (28). Moreover, it was shown that complete MCL transection in rabbit led to an increase in blood flow and vascular volume in the injured tissues after 2 wk (3). Metabosensitive afferents are known to respond to vasodilatory agents like papaverine (18). We could speculate, in the present study, that the metabosensitive afferent response could be increased to contribute to the vascular response following MCL transection.

An alternative explanation could be found in metabosensitive threshold rising following MCL lesion. After the MCL rupture, the release of chemicals in interstitial fluid could lead to deconditioning the response of metabosensitive afferents in our study. The latter may be related to cyclooxygenase products, which could be associated with overactivation of group III and IV afferent fibers (20). Consequently, in both of these conditions, metabosensitive afferent fibers could not respond to chemical injections as in animals without ligament injury (C group). To our knowledge, no study shows that metabosensitive fibers are directly affected by ligament injury, but, although this study cannot provide precise information about the mechanisms responsible of metabosensitive fibers alteration, the absence of variation in the responses from muscle afferents surrounding MCL rupture are suggestive. From week 3 to week 5, we observed a progressive recovery of metabosensitive afferent response to chemical stimuli, suggesting a progressive restoration of metabolic messages originating from muscle to the central nervous system. It was well known that these slow conduction muscle afferents contributed to match the motoneuron firing rate discharge and the muscle contractile speed (2, 15). Even if the altered twitch relaxation properties remained from 3 to 5 wk, activation of quadriceps muscle could be optimized after the MCL transection because of the recovery of muscle afferent fiber response.

Previous discussion is based primarily on the hypothesis that recorded afferent fibers originating from quadriceps muscle. It is noteworthy to note that the results of the study could not differentiate the origin of afferents, i.e., from muscle and from the surrounding structures (joint, skin, bone). However, we can exclude skin afferent fibers since they were removed with the skin during electrophysiological surgery, whereas we cannot exclude other types of joint afferents known to be sensitive to inflammatory agents such as bradykinin and serotonin.

The mechanosensitive afferent fibers did not respond to tendon vibrations 1 wk after the lesion, but we observed a progressive recovery from week 3 to week 5 without a complete restoration. Indeed, the altered response of mechanosensitive afferent fibers was maintained for the lowest-frequency vibrations after 5 wk of recovery. The lower response of these afferent fibers could reflect alteration of quadriceps mechanoceptors and may be considered as a source of proprioceptive deficits and prolonged reaction time to joint perturbation induced by ligament rupture. These results reinforce the hypothesis in which the sensorimotor deficits were partially associated with mechanoreceptor damage in the muscle and/or tendon (24, 37). The present study provides direct evidence of the impairment in mechanoreceptors activity from muscle spindles and Golgi tendons organs after MCL transection leading thus to a partial mechanosensitive deafferentation. Moreover, the firing rate of α-motoneurons during voluntary activation was known to depend partially to ια afferent fibers from muscle spindle (39). It was suggested that the altered muscle activation following ligament injury was also ascribed to damage from muscle spindle (36). The lower response may be also attributed to the alteration of joint and ligament mechanoreceptors (24), but response to tendon vibrations was mainly associated with muscle mechanoreceptors. Nevertheless, this study could not differentiate the different group of mechanosensitive afferents. Furthermore, low-frequency vibrations seem preferentially to activate static spindle and perhaps also Golgi tendon organs afferents, whereas high-frequency vibrations are a well known stimulus for dynamic spindle afferents (4, 5). Interestingly, in the present study, the variation in discharge rate was decreased principally for low-frequency vibrations (<50 Hz), suggesting that dynamic spindle afferents were less affected than static spindle and Golgi tendon organs afferents. Our results were in accordance with previous works showing that passive position sense was more greatly impaired than active position sense when the joint receptors are silent (37).

In conclusion, this study confirmed that MCL transection induced a functional instability assessed by dynamic kinematic of locomotion and a progressive alteration of the contractile properties on the quadriceps muscle. Our results also showed changes in metabosensitive afferent fiber response to their stimuli during the first weeks following the injury. The altered sensory pathways from muscle and other joint structures may explain the neuromuscular deficits described after ligament injury. Surprisingly, the relaxation properties were increasingly affected by the ligament rupture, contrary to the others measured parameters. Further investigations are needed to ensure whether rehabilitation exercise may optimize recovery of affected muscle.

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