Surgical leg rotation: cortical neuroplasticity assessed through brain mapping using transcranial magnetic stimulation

Luigi Tesio[a,b], Maria Grazia Benedetti[c], Viviana Rota[b], Marco Manfrini[d], Laura Perucca[a,b] and Antonio Caronni[a,b]

Rotationplasty (Borggreve-Van Nes operation) is a rare limb salvage procedure, most often applied to children presenting with sarcoma of the distal femur. In type A1 operation, the distal thigh is removed and the proximal tibia is axially rotated by 180°, remodeled, grafted onto the femoral stump, and then prosthetized. The neurovascular bundle is spared. The rotated ankle then works as a knee. The foot plantar and dorsal flexors act as knee extensors and flexors, respectively. Functional results may be excellent. Cortical neuroplasticity was studied in three men (30–31 years) who were operated on the left lower limb at ages between 7 and 11 years and were fully autonomous with a custom-made prosthesis, as well as in three age–sex matched controls. The scalp stimulation coordinates, matching the patients' brain MRI spots, were digitized through a 'neuronavigation' optoelectronic system, in order to guide the transcranial magnetic stimulation coil, thus ensuring spatial precision during the procedure. Through transcranial magnetic stimulation driven by neuronavigation, the cortical representations of the contralateral soleus and vastus medialis muscles were studied in terms of amplitude of motor evoked potentials (MEPs) and centering and width of the cortical areas from which the potentials could be evoked. Map centering on either hemisphere did not differ substantially across muscles and participants. In the operated patients, MEP amplitudes, the area from which MEPs could be evoked, and their product (volume) were larger for the muscles of the unaffected side compared with both the rotated soleus muscle (average effect size 0.75) and the muscles of healthy controls (average effect size 0.89). In controls, right–left differences showed an effect size of 0.38. In no case did the comparisons reach statistical significance ($P > 0.25$). Nevertheless, the results seem consistent with cortical plasticity reflecting strengthening of the unaffected leg and a combination of cross-education and skill training of the rotated leg. *International Journal of Rehabilitation Research* **37:323–333 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Rotationplasty (RP) is a rare limb salvage procedure (Badhwar and Agarwal, 1998; Gupta *et al.*, 2012) that was first introduced in 1930 by Borgreve for severe shortening of the femur and was then applied in 1950 by Van Nes for femoral sarcoma in children (Fixsen, 1983).

Nowadays it is mostly adopted as a limb-sparing procedure for unilateral bone sarcomas in children (Grimer, 2005), although it is occasionally performed in adults (e.g. after severe traumas; Fixsen, 1983; Klos *et al.*, 2010). According to the Winkelmann classification (Winkelmann, 1996), RP can be applied to the distal femur (A1) and proximal tibia (A2) or femur (B1–B3).

In knee RP (KRP) for distal femur tumors, the distal thigh is removed and the tibia is axially rotated by 180°, remodeled, and grafted onto the femoral stump. The neurovascular bundle is preserved. This limb is fitted with modified below-knee prosthesis and permits the ankle to work as a knee joint. Participants may reach high functional levels, even in sports such as cycling, soccer, swimming, and skiing (Hillmann *et al.*, 2007). The body image is preserved, as shown in tests of mental rotation of the foot (Curtze *et al.*, 2010).

Despite the bizarre appearance of the operated leg (Fig. 1), the patients' overall quality of life is reported to be comparable to that of their healthy peers, although problems may arise in areas of body image and sexuality

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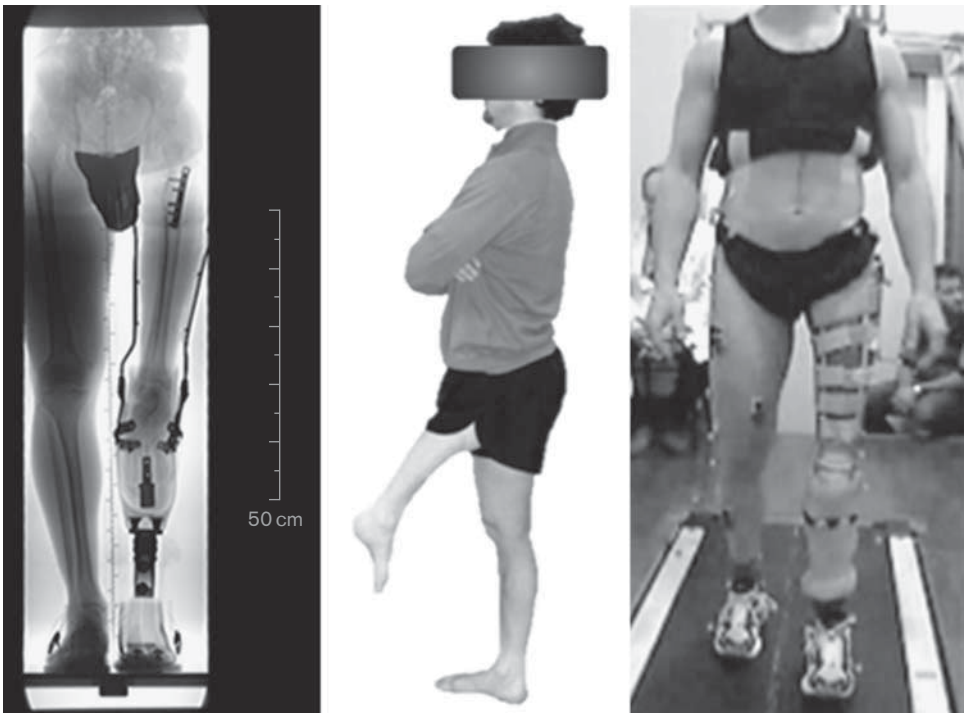
Fig. 1



One of the three KRP patients (patient A, see Table 1). The middle panel gives a full-body picture of the patient (with orthosis). The left panel gives an enlarged radiographic view of the lower limb (orthosis in place). The right panel gives an enlarged view of the lower limbs (same orthosis as shown in the left panel) during walking.

(Veenstra *et al.*, 2000; Hillmann *et al.*, 2007; Forni *et al.*, 2012).

On the operated side, the gastrocnemii are detached at the femoral insertion and are not reinserted. The other muscles are spared. The foot plantar flexors [in particular, the soleus (SOL)] and dorsal flexors (in particular, the tibialis anterior) become functionally homologous to the knee extensors (the quadriceps) and flexors (the hamstrings), respectively. The kinematic and the electromyographic (EMG) patterns at the lower limbs in KRP patients during walking have been thoroughly investigated (Catani *et al.*, 1993; Hillmann *et al.*, 2000). The hip and pseudo-knee joints of the affected side showed nearly normal sagittal rotations, and the timing of recruitment of the rotated muscles was found to be consistent with their new functional role.

Brain plasticity after unilateral KRP has never been investigated. Yet, given the excellent functional recovery it provides, it represents an interesting experimental paradigm for the study of brain neuroplasticity. The aim of the current investigation was to study brain plasticity in response to such a significant anatomical modification. This was done by studying the cortical motor representation of the vastus medialis (VM) and the SOL on the unaffected side (VMun and SOLun, respectively) and of the SOL on the operated side (SOLop) in KRP patients, through transcranial magnetic stimulation (TMS).

Methods

Patients

Recruitment

KRP patients are rare. Since the introduction of KRP in Italy in 1986, a total of 48 patients have undergone this procedure during childhood at the Istituti Ortopedici Rizzoli in Bologna. Thirty of them are long-term (>5 years) survivors (age 12–38 years). For this study, a sample of three adult KRP patients was recruited. These KRP patients were invited to volunteer for the study because of their excellent functional condition.

Every time a volunteer was recruited, a healthy control was sought from among healthcare professionals and students attending the hospital where the study was conducted (age range: 22–32 years). Overall, participant recruitment took 24 months.

Inclusion criteria

Upon cortical TMS, motor evoked potentials (MEPs) had to be recordable from the SOL and the VM muscles on both sides. The participants had to sign a written informed consent form before participation in the study.

The control participants had to be matched with the KRP sample for the following:

(1) sex;
(2) age (by decade);
(3) hand dominance (Edinburgh inventory score ≥ 12 of 15 for side dominance; Oldfield, 1971); and
(4) foot dominance (Waterloo footedness questionnaire –revised; Elias and Bryden, 1998).

Exclusion criteria

(1) History of neurologic or rheumatic diseases (except for transient complications of chemotherapy for KRP patients; see the Results section).
(2) History of major lower-limb trauma.
(3) Unsuitability for brain MRI (e.g. because of implanted devices).
(4) Intake of psychotropic drugs (e.g. benzodiazepines) within 48 h before brain mapping.
(5) Alcohol or coffee intake within 12 h before brain mapping.

Clinical assessment

A physician, specialist in Physical and Rehabilitation Medicine, examined all participants. Clinical examination included the following:

(1) Standard neurologic examination.
(2) Measurement of:
   (a) lower limb length (anterior–superior iliac spine to foot plant under the lateral malleolus);
   (b) calf circumference (at the upper to mid-third transition);
   (c) plantar foot length;
   (d) passive mobility of the hip, unrotated knee, and ankles (using a gravity goniometer); and
   (e) maximal isometric flexion and extension force of the unrotated knee and of both ankles (0–5 grading, Medical Research Council, 1976).
(3) Assessment of hand–foot coordination: after a brief explanation of the test, KRP patients were requested to perform (unrotated side first) full-range flexion–extension cycles of the homolateral hand and foot, to be moved synchronously at the highest possible cadence for at least 10 s (cycles counted by eye, time measured using a chronometer). In normal participants, in-phase coupling (both hand and foot moving upward or downward) can be held at a cadence of around 3.2 Hz, versus no more than 2 Hz for antiphase coupling (Baldissera *et al.*, 1982).

Nerve conduction study

The same physician also carried out a nerve conduction study (see Table 2 for details).

Magnetic resonance imaging

Both KRP and control participants underwent a standard MRI study of the brain [MAGNETOM Avanto I-Class 1.5 T; Siemens AG Medical Solutions (Munich, Germany); 256 slices, matrix size 256×256, voxel size $1 \times 1 \times 1$ mm]. The MRI images were fed into a neuronavigation system (see below).

Transcranial magnetic stimulation testing experimental protocol

MEPs elicited by TMS were used to assess corticospinal excitability and to map cortical representation (Talelli *et al.*, 2006) of the voluntarily activated VM and SOL. The cortical representation of both right and left VM and SOL was mapped in healthy controls, whereas cortical representations of VM and SOL of the unaffected side and SOL of the affected side were mapped in KRP patients.

Each experimental session consisted of multiple recording tests. Each healthy control participated in two experimental sessions: MEPs were sought in the right and left SOL and in the right and left VM in the first and second sessions, respectively. The two sessions were held 1 week apart. KRP patients underwent three MEP testing procedures over the period of a single experimental day, during which knee and ankle muscles were investigated in the following order: SOLop, VMun, and (right) SOLun. For each muscle, the testing procedure took about 1 h. Participants were allowed to rest for 15 min between consecutive MEP tests, and an additional 15-min rest period was allowed on request. During brain mapping, the participants sat comfortably on a chair with a seat height of 55 cm, with backrests and armrests. They wore shorts and a T-shirt and were barefoot. KRP patients did not wear their prosthesis.

To make the MEP amplitude comparable across participants and muscles (see below, data analysis), the peak-to-peak maximum compound motor action potential (cMAP) of the tested muscles was also acquired before the mapping experiments. The MEP amplitude was then standardized to the $cMAP_{max}$ amplitude to control for differences in peripheral origin across participants and experimental sessions (Talelli *et al.*, 2006; Oya *et al.*, 2008).

Stimulating and recording apparatus

TMS was delivered through a figure-of-eight coil (external diameter loop: 9 cm) connected to a Magstim 200^2 stimulator (Magstim, Whitland, UK). The coil was placed tangentially to the scalp with its handle pointing backward, so as to be approximately perpendicular to the expected direction of the central sulcus (lower-limb area).

Conventional self-adhesive surface electrodes (electrodes dimensions: 22×30 mm; recording area 95 mm^2) were used for EMG recording. The EMG signal was amplified

(×1000), filtered (band pass: 50–1000 Hz), A/D converted (sampling frequency: 2000 Hz), and stored on a PC for offline analysis (isolated preamplifier: CED 1902, data acquisition interface: CED Power 1401-3, data acquisition system: CED Signal Version 5; Cambridge Electronic Design Limited, Cambridge, England).

The same EMG electrodes, and filtering and A/D conversion parameters used for the TMS procedure were also used for cMAP recording. The cMAPs were evoked by conventional stimulating electrodes (stimulus intensity: up to 100 mA, pulse duration: 0.2 ms; Synergy NCS EMG EP IOM System; Viasys Healthcare, Old Woking, Surrey, UK). Electrodes were placed in the same position during cMAP and MEP recording.

Corticospinal excitability: motor evoked potential active motor threshold

MEPs were recorded from VM and SOL muscles by TMS of the hemisphere contralateral to the recording site. To keep the TMS stimulus intensity as low as possible, thus minimizing the participants' discomfort, MEPs were elicited in active rather than in resting muscles. It is worth noting that lower-limb muscles, such as the VM, have a high resting motor threshold. Moreover, no MEPs in the resting lower-limb muscles can be evoked in many of the healthy controls (Chen *et al.*, 1998a; Krings *et al.*, 1998). In addition, TMS parameters recorded in the active muscle, such as the active motor threshold, are considered better indicators of the motor cortex excitability, as they have been described to be relatively insensitive to changes in spinal excitability (Talelli *et al.*, 2006).

Defining a background preactivation of the tested muscles

In each recording session, visual feedback on muscle activation was provided through surface EMG recording to help the participants sustain slight contractions of the muscle under investigation. An additional pair of surface electrodes was taped just medially to the electrodes applied for MEP recording. Through these electrodes the EMG signal was filtered and rectified and shown online (acquisition rate: 256 Hz, bandwidth: 0–45 Hz) on a PC screen (ProComp 2 biofeedback device, BioGraph INFINITI software; Thought Technology Ltd, Québec, Canada). The maximum amplitude of the rectified and filtered EMG produced by a 3-s maximal voluntary contraction (MVC) was measured, and a horizontal cursor marking 5% of this level was set on the PC screen. Participants were asked to activate their ankle or knee muscles to keep the rectified EMG trace as close as possible to this 5% threshold. MVC was tested under standardized quasi-isometric conditions: seated participants fully extended their ankle or knee joint, while stabilizing their trunk by grasping both armrests. When testing SOL MVC, the participants were instructed to push with their forefoot against the floor while lifting their heel. When testing MVC of the VM, the participants pushed the dorsum of their foot against a rigid frame with their knee supported by a stool and fully extended. The MVC of SOLop in KRP patients was measured with the patient wearing no prosthesis. KRP patients pushed the sole of their foot upwards so as to keep it at a 90° angle with respect to the leg, against the same rigid frame used for VM MVC evaluation, thus isometrically pushing their pseudo-knee into extension.

The MEP active motor threshold (AMT), defined as the minimum TMS intensity able to elicit MEPs larger than 100 μV in at least three of five trials (Chen *et al.*, 1998b), was measured. AMT was evaluated at the MEP hotspot – that is, the scalp position producing the largest MEP when stimulated at 100% of the maximum stimulator output.

Cortical representation of lower-limb muscles: motor evoked potential mapping

Navigated TMS was used to map cortical representation of VM and SOL. An optoelectronic neuronavigation system (SofTaxic Optic, EMS, Bologna, Italy) was used to digitize the scalp stimulation coordinates, to guide the TMS coil to focus on a stimulation site, and to ensure spatial precision during the procedure. The participant's brain MRI was fed into the system and superimposed, on a PC screen, to outlines of the TMS coil. Matching was ensured by a calibration procedure. At the beginning of the first experimental session, the participants wore a tight-fitting swimming cap with a paper grid taped onto it marking the scalp positions to be stimulated. The position of the grid nodes with respect to participant's nasion and left and right tragus was digitized and stored. The cap was doffed after node digitalization and it was no longer worn during AMT evaluation and the mapping procedure. Scalp positions to be stimulated were spaced 1.5 cm apart and arranged in two 10×10 matrices referred to Cz, one for each hemisphere.

The TMS intensity for the mapping procedure was set at 110% of the AMT. Mapping started at the grid node closest to the MEP hotspot. For each matrix, two or three TMS stimuli (pseudorandom pulse interval: 4–6 s) were delivered at each of the stimulated scalp sites. Their sequence was chosen pseudorandomly. If no MEP larger than 100 μV could be evoked from the first pair of TMS pulses, the third TMS pulse was avoided and that stimulation point was considered outside the map. No scalp positions outside the map boundaries were explored.

Data analysis

Signal processing software (CED Signal Version 5) and custom-made software developed in the MATLAB (version 7; MathWorks Inc., Natick, Massachusetts, USA) environment were used for data analysis. Peak-to-peak amplitudes of each MEP and cMAP amplitude were

measured. The amplitude of the MEPs evoked at the same scalp position was averaged and the mean was subsequently expressed as a percentage of cMAP (Talelli *et al.*, 2006; Oya *et al.*, 2008).

Describing the maps of rotated and unrotated muscles

To compare the maps in KRP patients and controls, various parameters were computed. Left–right (X) and anterior–posterior (Y) coordinates of the center of gravity (cog), that is, the amplitude-weighted center of the map (Talelli *et al.*, 2006), were calculated according to the method of Wassermann *et al.* (1992) and referred to Cz (0, 0 cm, i.e., the vertex). The largest peak-to-peak MEP amplitude (mean of three MEPs), MEP_{max}, across the positions making up the motor map was measured. The map area was defined as the number of scalp positions associated with the mean MEP amplitude larger than 100 μV. The mean amplitudes of the MEPs (standardized to cMAP) evoked from the scalp positions belonging to the map area were summed to obtain the so-called map volume.

Comparing the maps of rotated and unrotated muscles

RP surgery refers to the transplant of the SOL muscle so that it acts as an extensor of the *neo genu* (see the Introduction section). Thus, the SOLop becomes the functional homolog of the (contralateral) VMun. A force contribution from the detached gastrocnemii through nonmyotendinous muscle junctions (see introduction) cannot be excluded. The amplitude, area, and volume of the maps of each side in KRP patients were compared with those in healthy controls. The asymmetry in cortical excitability was analyzed both as a ratio and a difference (expressed in effect size units). Average sample statistics were compared (a) between the SOLun and the SOLop in KRP patients, and right and left SOL in controls; (b) between the SOLun and the VMun in KRP patients, and between the rSOL and the lVM, and the lSOL and the rVM, in healthy controls. Right–left differences between sides were also compared between KRP patients and controls. Ratios close to 1 or effect sizes close to 0 within or between individuals suggest comparable cortical representations. Significance level was set at P less than 0.05 (although statistical significance was not expected, given the tiny sample size). The 95% confidence intervals of the effect sizes were also computed. Statistical computations were performed using STATA version 13.0 software (STATA Corp LP, College Station, Texas, USA). Statistical graphics were produced using STATISTICA Version 11 software (StatSoft Inc., Tulsa, Oklahoma, USA).

Nerve conduction study

Needle EMG was not performed and conduction studies were kept to a minimum to minimize the patients' discomfort. The maximal M-waves of the SOL and VM muscles were recorded bilaterally (see above) to compute the MEP/cMAP amplitude ratio (see above). Signs of peripheral neuropathy or axonal damage on the rotated side were expected given the history of chemotherapy and focal neural surgical stress. A sensory (sural) nerve conduction study was performed on both lower limbs. A motor nerve conduction study was performed on the posterior tibial nerve (cMAP from the abductor hallucis). On the operated leg, posterior tibial nerve and sural nerve stimulating sites were searched for around the groin (i.e. the 'root' of the leg), although the actual pathways of these nerves were unknown.

Ethics

Participants gave their written informed consent. The protocol was approved by the Ethics Committee of the Istituto Auxologico Italiano – IRCCS, Milan, Italy.

Results

Participants

Three KRP patients were recruited. All of them had undergone surgery to the left lower limb 19–24 years before testing, were right-handed and right-footed, and were sports professionals. All had undergone short-course chemotherapy after surgery. Initially, six healthy controls were recruited. In three of them, MEPs from SOL could not be elicited and the experimental session had to be aborted. Table 1 summarizes demographic, anthropometric, and clinical information for the three KRP patients (A, B, C) and the three controls (a, b, c) from whom MEPs could (a, b, c) or could not (x1, x2, x3) be recorded bilaterally from VM and SOL.

Side effects

Neither side effects nor any discomfort were reported by any of the participants examined.

Findings from clinical assessment

Across the KRP sample, the rotated lower limb was 18.5–24 cm shorter than the contralateral lower limb. The maximal circumference of the calf of the rotated leg was 4.5–8 cm shorter compared with the contralateral leg. The plantar surface of the rotated foot was 3–4 cm shorter than the contralateral one. Sensation (touch, pain, vibration) was normal on all limbs. No motor deficits in the upper limbs or the unaffected lower limbs were found. On the rotated limb, passive and active hip extension and abduction could not exceed the neutral position, whereas other movements were normal. Mobility of the rotated foot was normal. Plantar flexion up to 135°–152° was possible: thus, as a pseudo-knee, the ankle lacked 45°–28° to full extension. Isometric force at knee and ankle of the unrotated side was scored as 5+, whereas it was scored 5− for plantar and dorsal flexion of the rotated foot. The patients reported normal perceptions of size, shape, and position of the operated foot (i.e. they perceived it as being rotated). They had no difficulties moving the ankle and the toes in any requested

Table 1 Demographic and clinical information of the three KRP patients (A, B, C), the three matched healthy controls (a, b, c), and the three recruited healthy individuals from whom the requested MEPs could not be elicited (x1, x2, x3)

	ID	Age (years)	Sex	Height (m)	Weight (kg)	Dominant hand	Dominant foot	Sports/level	KRP side	Age at surgery (years)
KRP patients	A	30	Male	1.64	50	Right	Right	Swimming, national paralympic	Left	11
	B	31	Male	1.71	64	Right	Right	Cycling, national paralympic	Left	10
	C	31	Male	1.62	67	Right	Right	Professional body building, fitness trainer	Left	7
Controls	a	25	Male	1.75	72	Right	Right	Soccer, cycling, amateur		
	b	23	Male	1.78	63	Right	Right	Jogging, amateur		
	c	25	Male	1.87	72	Right	Right	Skating, amateur		
	x1	33	Male	1.72	63	Right	Right	Karate, amateur		
	x2	34	Male	1.80	73	Right	Right	Gym, amateur		
	x3	29	Male	1.89	78	Right	Right	Swimming, amateur		

KRP, knee rotationplasty; MEP, motor evoked potential.

directions. Across the KRP sample, synchronous sagittal rotation of the hand and the foot on the unrotated side reached a cadence of 3–3.3 Hz to 2–2.2 Hz during in-phase and antiphase coupling, respectively, like in normal individuals. Unlike that in normal individuals, the higher cadence (3–3.3 Hz) could be reached in both in-phase and antiphase coupling on the rotated side.

Peripheral neurophysiology

Table 2 gives a summary of the neurophysiologic findings. Sural sensory evoked potentials could not be recorded from both sides, the unaffected side, or the rotated side in patients A, B, and C, respectively. Motor conduction speed and cMAP amplitude were suggestive of some form of motor or sensory neuropathy of the rotated side (patient B and C, respectively) or, of bilateral sensory neuropathy (patients A and B). This is consistent with the patients' history of chemotherapy; yet, no specific etiologies could be supported on the basis of these elementary findings. It is worth noting that notwithstanding these subtle signs of neuropathy, all KRP patients' strength and tactile, thermal, and vibratory sensations were intact (details omitted).

Brain MRI

No abnormal findings were found on brain MRI.

Brain mapping

Figure 2 shows a representative tracing of an MEP from the left (rotated) and right (unaffected) SOL of a KRP patient, and from the left SOL of a healthy control, from top to bottom, respectively (see legend for details). In Fig. 3 the panels in each row give the maps of cortical representations of the VM, and the left and right SOL muscles, respectively. In KRP patients, the maps of the rVM and/or rSOL were wider (except for patient B; Fig. 4) and/or showed a higher excitability (black vs. gray spots) compared with the map of the SOLop and with the maps of rVM and rSOL of the healthy controls.

Tables 3–5 summarize the same findings quantitatively. In KRP patients, the mean of all variables from the (unrotated, right side) VMun and SOLun muscles far exceeded those from the (rotated, left) SOLop and the right homologous muscles of controls. By contrast, the values from the SOLop muscles of KRP patients were of higher (MEP_{max} and volume) or slightly lower (area) than those from the lSOL muscles of controls (bottom row in Table 3). Tables 4 and 5 present a statistical comparison based on effect sizes (ESs). The absolute ES values are in general large for SOL and VM of the right side and moderate for the left (operated) SOL. The SOLun and the VMun (the anatomical and functional homologous muscles of SOLop, respectively) are much more excitable than the rotated SOL (note the large effect sizes). However, none of the reported ESs were statistically significant (i.e. all the 95% confidence intervals included zero).

The increased excitability of the cortex on the rotated side is highlighted in Fig. 4, providing a graphic comparison of the results from the unrotated and the rotated sides. The unrotated SOLun and VMun are 'more represented' in the contralateral cortex (same side of the operation) compared with the rotated SOLop in its contralateral cortex. Only the sample average of the ratios between areas of the left and right SOL is slightly lower in KRP patients as compared with controls; all other comparisons show a higher right/left average ratio in KRP patients as compared with controls.

Discussion

In our view, the key findings of this study are the increased cortical excitability (higher peak values and 'volume' of the MEPs) and the spatial over-representation (excitable area) of the unrotated SOLun and VMun, giving rise to hemispheric asymmetries.

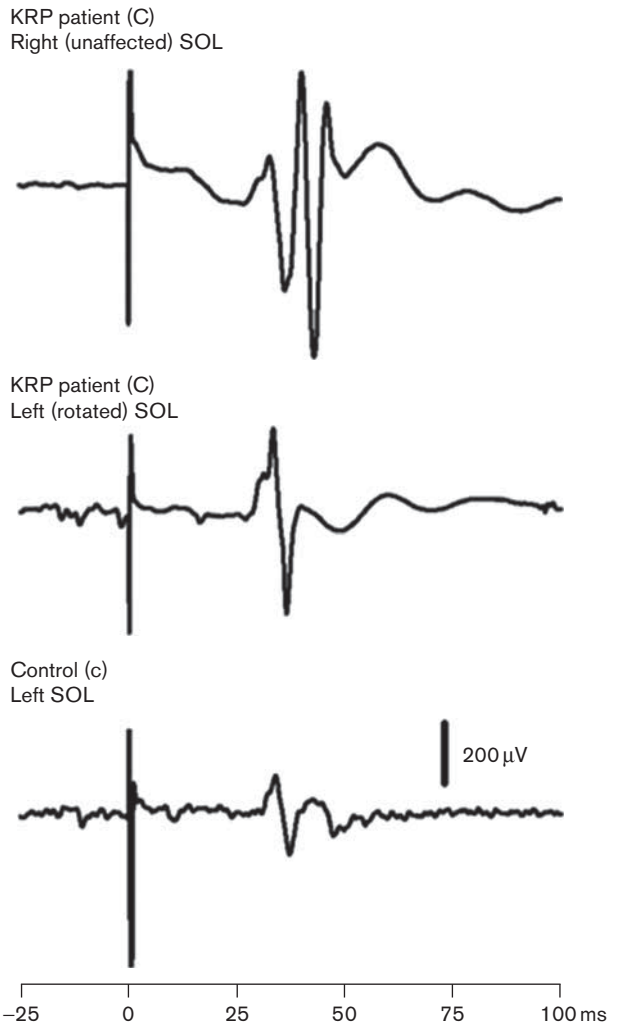
To the authors' knowledge, this is the first study on corticospinal excitability after KRP. Given the peculiarity of the anatomical condition, interpreting the results within the framework of existing knowledge is hazardous. Cortical excitability after lower-limb amputation has been the topic of some studies (e.g. Hordacre and Bradnam, 2013), yet the anatomical analogy between amputation and KRP is doubtful to say the least.

Table 2 Nerve conduction studies and EMG findings from the three KRP patients

	Lower limb – operated			Lower limb – unaffected		
Patient	MAP, peak-to-peak amplitude (soleus) (mV)	Sensory conduction study (SAP, sural nerve), amplitude, speed	Motor conduction study (MAP, tibial nerve, AH muscle), amplitude, speed (knee–ankle)	MAP, peak-to-peak amplitude (soleus)	Sensory conduction study (SAP, sural nerve), amplitude, speed	Motor conduction study (cMAP, tibial nerve, AH muscle), amplitude, speed (knee–ankle)
A	16.8	Absent	9.9 mV; 33.5 m/s	2.6 mV	Absent	11.8 mV; 54.5 m/s
B	4.6	2.7 μV; 40 m/s	0.7 mV; 49.6 m/s	4.0 mV	Absent	8.1 mV; 43.4 m/s
C	8.1	Absent	11.1 mV; 41.7 m/s	17.9 mV	7.9 μV; 53.8 m/s	14.6 mV; 48.5 m/s

AH, abductor hallucis; KRP, knee rotationplasty; MAP, motor action potential.

Fig. 2

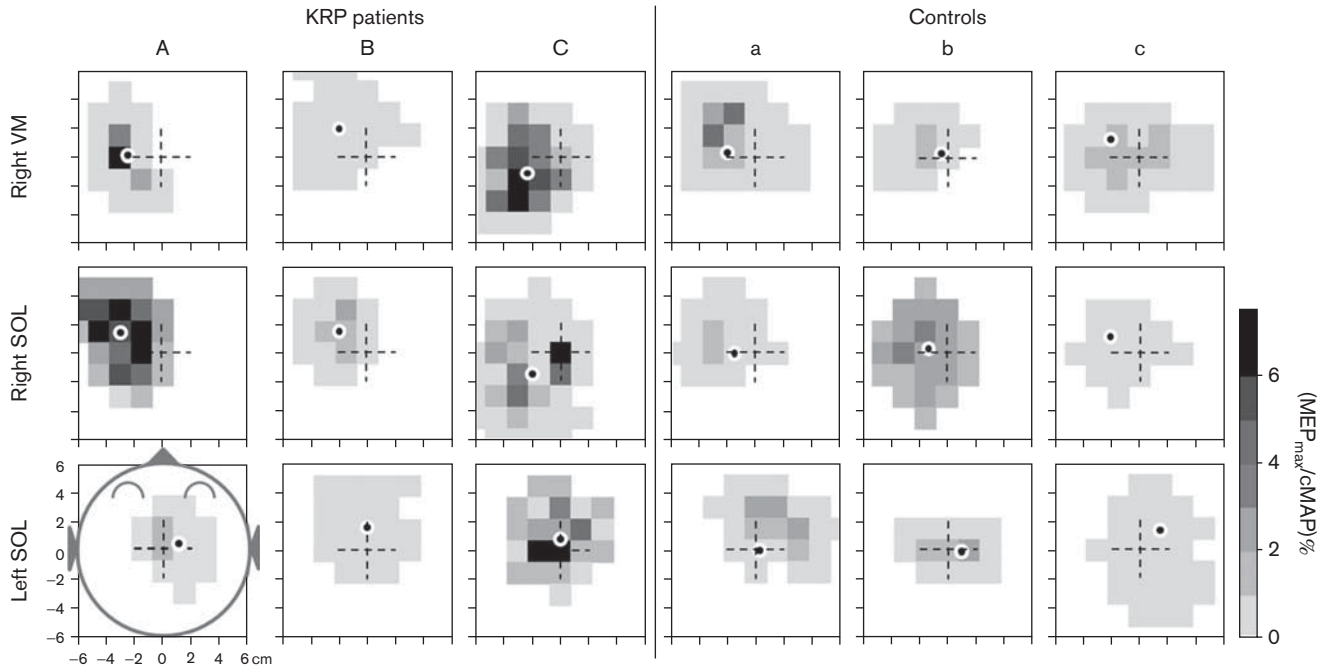


MEPs from the right (unaffected) and left (rotated) soleus muscle in patient C (top and middle trace, respectively), and from a control (c). The horizontal axis is time (ms), with 0 marking the magnetic stimulus on the contralateral cortex. The vertical axis shows the MEP amplitude in μV. The peak-to-peak amplitude of the MEP (clearly recognizable against the background noise) is manually recorded. The traces refer to the largest obtainable MEP (stimulus on the 'hotspot'). KRP, knee rotationplasty; MEP, motor evoked potential.

Functional analogies with KRP can be found in normal individuals. One of these is the unilateral disuse without peripheral nervous damage and/or the unilateral overuse. In fact, in the present KRP patients the rotated leg was hypotrophic. It is also known that under this condition the unaffected limb is overloaded during daily life, in particular during walking (Catani *et al.*, 1993). Finally, the literature provides evidence for enhanced excitability of corticomotor projections targeting muscles proximal to an immobilized, painful, or unstable joint (e.g. after anterior cruciate ligament injury; Héroux and Tremblay, 2006).

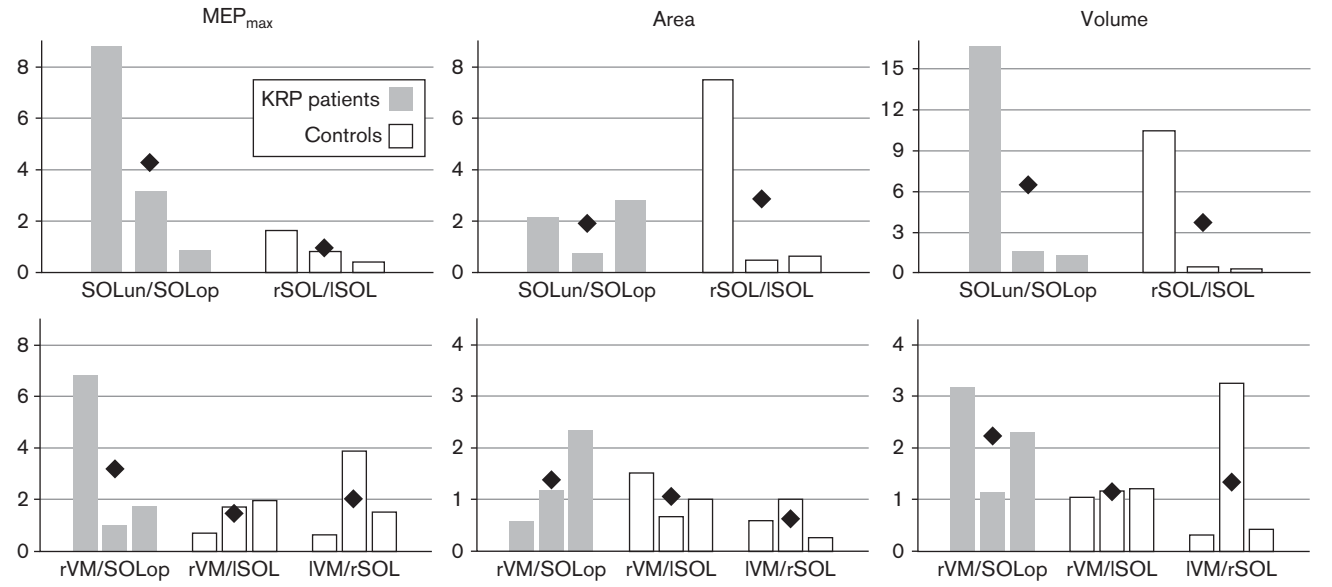
TMS studies on corticospinal excitability after unilateral resistance training point toward an increased amplitude

Fig. 3



TMS motor maps of vastus medialis and soleus muscles in three KRP patients (A, B, C) and three controls (a, b, c). The vertex-to-nose orientation and map scaling (cm) are given in the bottom left panel. The head vertex position (0 : 0 cm) is given in each panel (dashed cross). For each stimulation spot, gray tones represent the peak-to-peak MEP/cMAP amplitude: the higher the amplitude, the darker the spots (white-to-black scaling is presented on the rightmost inset). Dot-circles mark the position of the map center of gravity. cMAP, compound motor action potential; MEP, motor evoked potential.

Fig. 4



Ratios computed on amplitude and spatial parameters of the TMS motor maps. Panels in the left, center, and right columns present ratios of the three map parameters, that is, MEP_{max}, area, and volume, respectively. The ratios are computed between right and left SOL (upper row), and crossed VM and SOL (lower row). In each panel, triplets of gray and white bars refer to KRP patients (A, B, C from left to right, see Table 1) and controls (a, b, c), respectively. The r and l prefix stand for the right and the left side, respectively; the op suffix stands for the operated/rotated (left) side in KRP patients. Diamonds mark the mean across each triplet of bars. KRP, knee rotationplasty; MEP_{max}, maximum motor evoked potential; SOL, soleus; TMS, transcranial magnetic stimulation; VM, vastus medialis.

Table 3 TMS mapping parameters

	MEP_{max}		Area		Volume	
	Controls	KRP patients	Controls	KRP patients	Controls	KRP patients
Right VM	2.6 (1.4–5.0)	7.3 (0.9–12.2)	5.7 (3–8)	7.7 (3–14)	9.6 (3.7–15.6)	27.5 (4.7–63.3)
Right SOL	1.7 (0.7–3.4)	6.7 (2.8–11.1)	8.3 (4–15)	10.7 (4–17)	14.9 (3.8–37.1)	39.7 (6.4–76.9)
Left SOL	1.8 (0.8–2.5)	3.0 (0.9–6.9)	6.7 (2–12)	5.3 (5–6)	8.2 (3.6–12.7)	11.9 (3.9–27.3)

Area is the number of excitable spots on a grid centered on the head vertex with 1.5 cm squared cells (compare with Fig. 3). Volume is the product of MEP_{max} and area. In each cell, means (range) across three KRP patients or three healthy controls are given. Data refer to potentials evoked from the rVM, the rSOL, and the lSOL (the rotated one in KRP patients), respectively (see labels of the corresponding rows).
KRP, knee rotationplasty; l, left; MEP_{max}, highest (peak-to-peak) motor evoked potential evoked, presented as the percentage of the corresponding peripheral compound motor action potential potentials; r, right; SOL, soleus; TMS, transcranial magnetic stimulation; VM, vastus medialis.

Table 4 Differences in excitability (MEP_{max}, area, volume) are expressed as effect size with 95% CIs. Homologous muscles are compared between controls and KRP patients

	Effect size	95% CI
Left (op) SOL, controls–KRP		
MEP_{max}	−0.85	−2.32 (0.97)
Area	0.45	−1.25 (1.97)
Volume	−0.50	−2.01 (1.22)
Mean	−0.30	
Right SOL, controls–KRP		
MEP_{max}	−1.60	−3.03 (0.48)
Area	−0.24	−1.80 (1.41)
Volume	−0.81	−2.29 (1.00)
Mean	−0.88	
Right VM, controls–KRP		
MEP_{max}	−0.90	−2.37 (0.93)
Area	−0.62	−2.12 (1.13)
Volume	−1.04	−2.50 (0.84)
Mean	−0.85	

CI, confidence interval; KRP, knee rotationplasty; MEP_{max}, maximum motor evoked potential; SOL, soleus; VM, vastus medialis.

Table 5 Differences in excitability (MEP_{max}, area, volume) are expressed as effect size with 95% CIs. The differences between right and left sides are shown for both controls and KRP patients

	Controls		KRP patients	
	Effect size	95% CI	Effect size	95% CI
Right (un) SOL–left (op) SOL				
MEP_{max}	0.20	−1.44 (1.76)	0.90	−0.93 (2.37)
Area	0.42	−1.28 (1.94)	1.15	−0.77 (2.60)
Volume	0.84	−0.97 (2.32)	1.18	−0.74 (2.63)
Mean	0.487		1.075	
Right (un) VM–left (op) SOL				
MEP_{max}	0.91	−0.93 (2.37)	0.70	−1.07 (2.19)
Area	−0.34	−0.77 (2.60)	0.75	−1.04 (2.23)
Volume	0.28	−0.74 (2.63)	0.97	−0.88 (2.43)
Mean	0.283		0.808	

The statistics of right SOL and right VM muscles are compared with the statistics of the left SOL. In KRP patients the SOL and the VM of the unaffected (un) side, respectively, represent the anatomical and the functional counterparts of the SOL of the operated (op) side.
CI, confidence interval; KRP, knee rotationplasty; MEP_{max}, maximum motor evoked potential; SOL, soleus; VM, vastus medialis.

of the MEP obtained by stimulating the cortex contralateral to the trained lower limb (e.g. Griffin and Cafarelli, 2007), although the opposite was found by others (Goodwill *et al.*, 2012).

The results obtained from these three KRP patients are consistent with long-term hyperexcitability of the cortex contralateral to the unaffected limb. The MEPs elicited from the SOL and the VM of the unrotated side were higher, and the volume of the excitable areas was invariably larger, compared with both the contralateral side and healthy controls. This increased excitability of the cortex controlling the 'normal' side may seem counterintuitive given that a high level of skill should underlie the excellent motor control of the rotated leg in KRP patients. However, the paradox is only apparent for two reasons. First, if one considers KRP as a condition entailing a sort of unilateral training of the intact lower limb, cross-education has to be expected. Second, strength and skill training may have opposite effects on cortical excitability. For the lower limbs, this was shown by at least two studies. MEPs from the tibialis anterior and the SOL were recorded during 4 weeks of 'strength' (loaded flexion–extension cycles at the ankle) and 'skill' training (balance and postural stabilization while standing on unstable surfaces). The MEP size increased after the former because of decreased interhemispheric inhibition, and it decreased after the latter (Beck *et al.*, 2007).

TMS brain mapping is also affected by the duration of the training. After short-term skill learning (32 min of unilateral ankle motion steering a visual target) an increase, not a decrease, in MAP amplitude from the trained muscles was observed (Perez *et al.*, 2004). It can be said that long-term (in contrast to short-term) skill learning with automation (known as 'overlearning') presumably leads to a decreased cortical 'cost' of movement, due to a migration of the control circuits toward subcortical areas like the basal ganglia and the cerebellum (Puttemans *et al.*, 2005). Consistently enough, it has recently been shown in monkeys that long-term training of an arm-reaching task leads to a decrease in metabolic and overall synaptic activity in the involved primary cortical motor area (Picard *et al.*, 2013).

To sum up, the present findings in KRP patients appear consistent with the effect of chronic strength training of the intact lower limb. A large degree of strength asymmetry had to be expected because of the patients' engagement in professional athletics. The recovery after various unilateral lower-limb impairments, including amputation, is dominated by the preference for an

overload of the intact lower limb during walking, much beyond the requirements of the musculoskeletal constraints (Tesio *et al.*, 1985, 1991, 1998). This is perhaps a special case of the so-called learned non-use affecting the lower limbs (Tesio, 2001).

In contrast, the maintenance of normal excitability of the contralesional cortex, despite weakness and atrophy, appears consistent with a balance between cross-education (increased excitability) and long-term skill learning (decreased excitability). That local skill was increased is corroborated by the capacity of the KRP patients to reach high cadence of antiphase hand–foot coupling.

The many limitations of our study should not be underestimated. It leaves unresolved the question of whether the rotated SOL retains its original cortical representation, whether it is 'adopted' by cortical circuitries originally focused on the VM, or whether it achieves a double representation (i.e. both as an anatomical SOL and as a functional VM). However, the TMS maps of the SOL and VM are known to be largely overlapping (Krings *et al.*, 1998) and not easy to disentangle even in healthy controls. Another limitation stems from the rarity of this anatomical condition. Large study samples and statistical significance are difficult to attain. This makes the present sample of three subjects a relatively relevant one. Multiple comparisons (see Tables 4 and 5 and Fig. 4) consistently pointed toward the conclusion that the ipsilesional hemisphere becomes more excitable after KRP. Two other limitations reflect the need to avoid overstimulation of individual participants (three muscle maps had to be recorded in each of two sessions). First, intrahemispheric and interhemispheric inhibition were not tested. Second, on each spot, only three stimuli with the same intensity (10% above the hotspot threshold) were delivered. This prevented the mapping of motor thresholds and raised an issue of reliability. A series of three stimuli is customary in this research field, also for lower limbs (e.g. Chen *et al.*, 1998a). A recent work provides evidence that a sequence of four stimuli may give reliable results from the stump muscles in above-elbow amputees (Hétu *et al.*, 2011). Hence, reliability in this study might have been only slightly suboptimal.

All things considered, the results suggest that the excellent functional adaptation to KRP seems to correspond the reweighting of the cortical excitability in favor of the ipsilesional cortex. Besides a more in-depth neurophysiologic study, perhaps a study of brain activity through both TMS and functional MRI would be the best way to learn more about this intriguing motor condition (Driver *et al.*, 2009).

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Conflicts of interest

There are no conflicts of interest.

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