Author's response to reviews

Title: Principles of brain plasticity in improving sensorimotor function of the knee and leg in healthy subjects: A double-blind randomized exploratory trial

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Version: 2 Date: 1 July 2009

Author's response to reviews: see over
Dear Editor,

Thank you for your response and for the reviewers’ valuable comments and constructive criticism. Changes have been made in order to meet the criticism raised, and in cases where we have not made the suggested changes, our reasons for not doing so are given.

We hereby submit the revised version of the paper for consideration regarding publication along with response to each of the specific comments provided by the reviewers.

Sincerely,
Eva Ageberg
Authors’ response to Reviewer George Peat (comments for the authors are included)

Thank you for your valuable comments, suggestions and constructive criticism. Changes have been made in order to meet the criticism raised, and in cases where we have not made the suggested changes, our reasons for not doing so are given. Response to each of the specific comments is provided below (each comment is included, followed by our response).

Reviewer's report:
Summary
This article describes an exploratory RCT of the effects on knee sensorimotor function of applying topical analgesia to adjacent skin in 28 subjects without history of major orthopaedic lesions. The authors report no effect and conclude that although negative, the selection of ostensibly healthy controls and differences in the somatotropic organisation of the knee compared with hand and foot suggest that further investigations in injured subjects are warranted.

I enjoyed reviewing this piece of innovative experimental clinical research. The need to explore and test underlying hypothesised mechanisms of injury/disease and recovery is fundamental to developing new therapies. This particular experiment is original but clearly the latest in a series by these investigators, having previously conducted similar studies with positive findings in the hand and foot. It was clearly written throughout.

Discretionary revisions
My understanding is that the receptor fields of nociceptors and wide dynamic range neurons in the dorsal horn expand under conditions of local inflammation or other peripheral nociceptive stimuli. This is one of the mechanisms producing hyperalgesia and allodynia at sites adjacent to actual tissue damage. By removing sensory input from adjacent areas, aren’t you creating a condition that is more conducive to expansion of nociceptive receptor field in clinical subjects by essentially ‘clearing the way’ for their expansion? In other words, this experiment may produce findings that are completely different in pain-free healthy controls compared with painful clinical subjects? A rebuttal of this argument in your Response or brief inclusion in the manuscript itself would be welcome.

AUTHORS’ RESPONSE AND ACTION
A comment on this topic is now addressed in the discussion (page 16): “Previous studies on the upper and lower extremity [22] [23] [24] as well as the present study have been done on subjects without pain. A peripheral nociceptive stimulus, e.g., a painful knee, is known to induce plasticity changes in the spinal cord and at subcortical and cortical levels. Thus, treating patients with a painful joint using cutaneous deafferentation may give a different result compared to that for individuals without pain. This needs to be addressed in future studies.”
Major compulsory revisions
1. Have the authors correctly identified the reasons for the negative findings in the knee in contrast to the positive studies in the hand and foot?

The authors should provide information or Discuss alternatives. Namely,
- random error in the assessment of outcomes. Unlike self-report outcomes, reliability demonstrated in previous studies may not be sufficient for observer-dependent measures which some of the sensorimotor outcomes were. Please state who conducted the assessments, what experience they had of each measure, and whether or not any training, pre-piloting or formal pilot studies were conducted with those assessors.

AUTHORS’ RESPONSE AND ACTION
This information is now added (page 8): “An experienced assessor, who was well trained in all outcome measures from previous studies and pilot-testing preceding the present study, performed the measurements.”

- lack of specificity in the outcome measures, single-leg hop clearly tests the integrity of sensorimotor function beyond just the knee joint.

AUTHORS’ RESPONSE AND ACTION
We are well aware that the one-leg hop test is a measure of extremity function, and that it is not a measure of knee function only. However, this test is widely used when examining patients with knee injury, which is the reason for including this test in our study. Previous studies show that the one-leg hop test can be used as a predictor of functional stability. It has also been shown to be related to self-reported knee function, such as trusting the knee. That is, patients with functional instability will be less confident in taking off and landing on the injured leg than on the non-injured one, and also less confident than patients with good compensatory functional stability. The one-leg hop test is reliable, valid, and sensitive in detecting impairment and it is related to self-reported knee function. For clarification, the following has been added in the method section regarding the one-leg hop test for distance (page 10): “The one-leg hop test is widely used for predicting functional knee stability [1] [3]. Muscle strength, balance and confidence in the knee are contributing factors to the performance of this test.

- the dermatomal area of local cutaneous analgesia (proximal thigh) is innervated by the same spinal levels as the knee joint itself (L2-L4) and overlies the primary agonists of the knee (quadriceps). Is this the same situation as for the hand and the foot?

AUTHORS’ RESPONSE AND ACTION
In this study the EMLA was applied circumferentially above and below the knee. This information is added in the method section (page 7):
“Fifty grams of EMLA, or placebo [24], was applied circumferentially on the leg 10 cm above and 10 cm below the center of patella, leaving the area around the knee without cream (Figure 1).”
In the upper extremity, the EMLA was applied to the volar aspect of the forearm, an area innervated by the same spinal levels as the hand. Previously, we have deafferentated an area proximal to the area in which we want to improve function and also adjacent in the cerebral cortex where the largest potential for plasticity changes are. In the present study, we wanted to improve knee function which is more difficult than improving hand and foot function since the knee is anatomically located in the middle of an extremity. Based on the homunculus where the upper and lower leg are situated next to the knee and also the fact that it is almost impossible to deafferentate the foot by using EMLA we decided to deafferentate the adjacent skin areas both proximal and distal to the knee. These aspects are added in the discussion (page 16-17):

“In previous studies on the upper extremity [22] [23], the anesthetic cream was applied to the volar aspect of the forearm and in the previous study on the lower extremity the anesthetic cream was applied circumferentially on the lower leg [24]. Based on these previous studies, it would be logical to deafferentate the foot and lower leg in the current study. However, it is extremely difficult to anesthetize the entire foot using EMLA due to problems with absorption of the EMLA in the sole of the foot and applying an occlusive bandage. Therefore, we decided to anesthetize the skin area adjacent to the knee knowing that following deafferentation, the adjacent cortical areas rapidly occupy the anesthetized area. We also decided to deafferentate circumferentially on the lower extremity because the cortical area devoted to the lower extremity is small compared to the hand and we, therefore, hypothesized that a larger deafferentated skin area was needed (compared to the upper extremity) in order to allow the knee to expand in the primary somatosensory and motor cortex.”

2. In what way has this study altered the design of planned future studies in clinical, injured subjects? Did this piece of work have any important consequences?

AUTHORS’ RESPONSE

We believe that the major reason for the lack of effect from temporary cutaneous anesthesia is that sensorimotor function may not be impaired in uninjured subjects. Thus, the chance of achieving an improvement in sensorimotor function in these subjects by the short-term intervention that we used is most likely limited. For example, a ceiling effect was noted in some of the measures. The possibility of improving kinesthesia by temporary cutaneous anesthesia may be greater in subjects with knee injury than in uninjured subjects, since our previous studies show that patients with knee injury have higher values (poorer kinesthesia) than uninjured subjects. In an effort to reduce the ceiling effect in perception of touch, several sites around the knee could be tested in further studies. However, the perception of touch of the knee is not as delicate and discriminative as in the hand or the foot sole. Thus, large effects from temporary cutaneous anesthesia may be needed to detect a change in perception of touch of the knee. Also, larger effects of treatment are needed in order to detect an increase in the cortical area of the knee than in that of the hand. We did not find an effect of temporary cutaneous anesthesia in the measures of sensory or motor functions. This could be due to lack of cortical re-organization following the cutaneous anesthesia or that the re-organization was too small to result in a detectable improvement. This is included in the discussion section on pages 15-17. We also included a discussion on the sample size (page 15). In our planned future study on subjects with knee injury, a larger group of subjects will be included.

Thus, we believe that we have included a discussion on important consequences for planned future studies. We also believe that we need to repeat the study on patients with knee injury
and functional limitations. This has now been clarified in the discussion and conclusion: “…patients with knee injury and functional limitations.” After such study, further changes may be made in the design of future studies.

There is an air of inevitability about the conclusion. Was the selection of healthy controls uninformative in the sense that they could not alter the decision about whether to proceed to a trial in clinical subjects? Or did this exploratory study serve other purposes, e.g. to pilot the study procedures, to provide assessors with practical experience in the assessment techniques. Please address in the Discussion.

**AUTHORS’ RESPONSE AND ACTION**

As for the previous studies on temporary cutaneous anesthesia of the hand and foot, we started out a series of experiments by including subjects without injury. The following is read in the introduction: “In this first study of a series of experiments, we included subjects without injury.” To further clarify this, the following has been added in the discussion section on page 17: “…However, the cortical area of the knee is smaller than the cortical area of the hand [12] [13]. Thus, larger effects of treatment are needed in order to detect an increase in the cortical area of the knee than in that of the hand. In line with this reasoning, we found no effect of temporary cutaneous anesthesia of adjacent body parts in the measures of sensory or motor functions of the knee in healthy subjects, whereas previous studies reported improvement in sensory function of the hand and foot in healthy subjects after such treatment [22] [24]….”

3. Was local anaesthesia successfully achieved?

Did the authors check whether local anaesthesia of the areas above and below the knee was achieved and how successful this was? If so, please state in manuscript. If not, please Discuss.

**AUTHORS’ RESPONSE AND ACTION**

Since this was an RCT with a blinded design, that is, both the assessor and the subject were blinded to group allocation, this was not checked for. Moreover, it has not been checked for in previous studies on the hand or foot. In the method section regarding “protocol and masking” the following is added (page 7): “…Therefore, the presence or absence of anesthesia was not verified by the assessor or the subject.” However, we believe you have a good point here, and we may, therefore, include verifying the presence or absence of anesthesia in our planned future study.

**Minor essential revisions**

i. The manuscript would benefit from being written to conform with CONSORT guidelines. Specifically:
- source of subjects, total number invited to obtain 24 eligible consenting participants

**AUTHORS’ RESPONSE**
Since our study had an exploratory character, although it was an RCT, we did not follow all details according to CONSORT guidelines. We have now written all details that we used to conform with CONSORT guidelines, according to your suggestion. However, we are aware that our study will not get the highest rating, since CONOSRT guidelines weren’t followed in detail.

- provide setting and location for data collection

**AUTHORS’ RESPONSE AND ACTION**
This information is now added in the method section (page 8): “The assessment took place at the Department of Orthopedics, Malmö University Hospital.”

- confirm that the sequence of allocation was concealed until treatment was assigned

**AUTHORS’ RESPONSE AND ACTION**
The assessor used computer-randomization lists to assign subjects to treatment/placebo, but the sequence was not concealed until treatment was assigned. The following has now been added in the text (page 7): “To ensure an equal number of men and women in each group two computer-generated randomization lists, one for women and one for men, were drawn up by the statistician. The assessor allocated the next available number on entry into the trial, assigning the subjects to treatment/placebo.”

- clarify who did the allocation sequence, who enrolled participants, who assigned them to treatment/placebo, who assessed outcomes, who administered treatment, who analysed the data and the extent to which allocation was concealed from each and whether or not the success of blinding was evaluated

**AUTHORS’ RESPONSE AND ACTION**
These issues have now been clarified in the subjects and method section:

**“Subjects and randomization**
Twenty-eight (14 women) physically active subjects aged 18-35 years were included in this exploratory double-blind RCT. The subjects were students at the Faculty of Medicine, Lund University or staff members at Malmö University Hospital. They were enrolled by one of the researchers. Subject characteristics, including self-reported outcomes assessed by the Knee injury and Osteoarthritis Outcome Score (KOOS) [30] [31], are given in Table 1. Exclusion criteria were a history of major orthopedic lesions, such as knee injury or fracture, and allergic reactions to anesthetic agents. The physical activity and age distribution of the subjects in this study were chosen in order to match patients with ligament injuries in the knee. Individuals with ligament injuries to the knee are usually young, aged 18 to 35 years, and physically active at a moderate to high level [1] [3]. The subjects were randomly allocated, using a random number generator, to temporary anesthesia using a local anesthetic cream (EMLA®) (EMLA group) or a placebo cream (oil and water emulsion) (placebo group). To ensure an equal number of men and women in each group two computer-generated randomization lists,
one for women and one for men, were drawn up by a biostatistician and given to the assessor. The assessor allocated the next available number on entry into the trial, assigning the subjects to treatment/placebo. The Research Ethics committee of Lund University approved the study, and all subjects gave their written informed consent.

Protocol and masking
Fourteen subjects received a local anesthetic cream containing 2.5% lidocaine and 2.5% prilocaine (EMLA®, AstraZeneca, Södertälje, Sweden) and 14 subjects received a placebo cream of an oil and water emulsion (DAX, Opus Health Care Inc., Malmö, Sweden). The two creams were identical in color, consistency and packaging. A staff member not participating as an assessor or subject in the study distributed the packages with cream to the assessor. Fifty grams of EMLA, or placebo [29], was applied circumferentially on the leg 10 cm above and 10 cm below the center of patella, leaving the area around the knee without cream (Figure 1). The skin areas where the EMLA/placebo was applied were covered with film wrap and a Tubigrip® stocking (MEDLOCK Medical, Oldham, UK). After 90 minutes, during which time the subject was seated, the EMLA/placebo was carefully washed off. The test leader and the subjects were blinded to group allocation, and the subjects were told not to reveal any possible anesthetic sensation. Therefore, the presence or absence of anesthesia was not verified by the assessor or the subject. The success of blinding was not evaluated.

Outcome measures
Measures of sensory and motor functions were assessed before and after 90 minutes of treatment with EMLA or placebo. The tests were performed in the order that they are described below. EMLA/placebo and all tests were performed on the right leg only. The assessment took place at the Department of Orthopedics, Malmö University Hospital. An experienced assessor, who was well trained in all outcome measures from previous studies and pilot-testing preceding the present study, performed the measurements.”

Statistical analysis
“…Group allocation was concealed to the person analyzing the data, until the results were completed.”

ii. p3. Rephrase “…any treatment leading to improved sensorimotor function would be of value for patients…”.” Treatments that carry substantial adverse effects may well not be of (net) value to patients.

AUTHORS’ RESPONSE AND ACTION
The word “any” is omitted. The sentence now reads: “In this perspective, treatment leading to improved sensorimotor function would be of value for patients with knee injury or OA in the short and long term.”

iii. p3. Typo “.larger areas are assigned to sensitive parts…” (Isn’t this argument circular?)

AUTHORS’ RESPONSE AND ACTION
The following is read in the Introduction on page 5: “The somatotopic map does not represent the body in its actual proportions [12] [13]. Instead, larger cortical areas are being assigned to sensitive parts or parts with complex motor demands such as the hands and face [14] [15].” Unfortunately, we do not understand the reviewer’s comment that this statement is circular. Therefore, we have not made any changes here. In case we have misinterpreted your comment, we appreciate clarification so that we can make any required changes.

iv. A biostatistician would be able to specifically advise on whether the correct power calculation was used but a sample requirement of 2 subjects based on the single-leg hop seems incredibly low to me. Can you provide your actual calculations in your Response (not necessarily in the main text of the paper)?

AUTHORS’ RESPONSE
The sample size calculations for improvements within groups were based on results from previous studies on uninjured subjects of the same age and activity level as those included in the current study. The sample size calculations for changes within groups were performed together with a biostatistician and are provided below. The formula we used for sample size calculations within groups was: \(7.9 \times SD_{diff}^2 / \delta^2\)

- TDPM, 30\% change
  - Women: delta 0.4, SD\(_{diff}\) 0.49 → n=12
  - Men: delta 0.5, SD\(_{diff}\) 0.64 → n=13
- Vibrations sense, 20\% change
  - Women: delta 2.6, SD\(_{diff}\) 3.3 → n=13
  - Men: delta 2.6, SD\(_{diff}\) 4.6 → n=25
- Muscle strength, 10\% change
  - Women: delta 16.4, SD\(_{diff}\) 14.5 → n=6
  - Men: delta 25.8, SD\(_{diff}\) 15 → n=3
- One-leg hop test, 10\% change
  - Women: delta 18, SD\(_{diff}\) 8.2 → n=2
  - Men: delta 21, SD\(_{diff}\) 6.5 → n=1

The post-hoc sample size calculations for between groups are given in the discussion section on page 13.

v. Clarify whether all sensorimotor outcomes were measured only on the ipsilateral knee. If not, then justify why not and what effect this might have on the results.

AUTHORS’ RESPONSE AND ACTION
This is now clarified in the method section, page 8: “EMLA/placebo was applied and all tests were performed on the right leg only.”

vi. Not sure if Figure 2 is necessary. Remove and report figure in Table 2. If significantly skewed then either transform or report medians and IQRs.

AUTHORS’ RESPONSE AND ACTION
Figure 2 is omitted and the data is now included in Table 2, according to your suggestion.
vii. It would be helpful to identify in the Title that the study was conducted in ostensibly ‘healthy’ individuals

AUTHORS’ RESPONSE AND ACTION
This has now been added in the title: “Principles of brain plasticity in improving sensorimotor function of the knee and leg in healthy subjects: A double-blind randomized exploratory trial”
Authors’ response to Reviewer Patrick O McKeon (comments for the authors are included)

Thank you for your valuable comments, suggestions and constructive criticism. Changes have been made in order to meet the criticism raised, and in cases where we have not made the suggested changes, our reasons for not doing so are given. Response to each of the specific comments is provided below (each comment is included, followed by our response).

Reviewer's report:
The authors have prepared a manuscript describing the experiment in which cutaneous afferent information was eliminated in the adjacent areas of the knee in healthy subjects. The authors conducted sensory and motor tests to determine whether there was enhanced sensory and motor performance in the knee in the presence of anesthesia of adjacent areas on the limb.

- Major Compulsory Revisions
The authors have prepared a well-written manuscript. There are no major compulsory revisions. The Abstract, Introduction, Methods, Results, and Discussion sections are well developed. There are only a few suggestions for improvement. Please see comments in the Minor Essential Revisions section below.

- Minor Essential Revisions

Abstract:
The first sentence in the manuscript does not support the argument built in the experiment. I recommend introducing the principles of neuroplasticity in the first sentence as they relate to enhanced sensorimotor control associated with anesthesia of adjacent structures.

AUTHORS’ RESPONSE AND ACTION
A sentence introducing brain plasticity is added, according to you suggestion: “Principles of brain plasticity is used in the treatment of patients with functional limitations to improve sensorimotor function.”

Introduction
Throughout the introduction, the authors identify “training” as a means of rehabilitating a person with a knee injury. Please operationally defined the type/types of training (i.e. proprioceptive, balance, plyometric, etc.)

AUTHORS’ RESPONSE AND ACTION
The type of training, that is, neuromuscular training or strength training, is now added, where appropriate, in the introduction and discussion sections, to clarify which type of training is used in the treatment of patients with knee injury and knee OA. For example, the first sentence in the introduction now reads: “Neuromuscular and/or strength training is included in the treatment of knee injury and knee osteoarthritis (OA) to improve both patient-reported...”
function and objective function, such as joint range of motion and sensorimotor (neuromuscular) function.” In the discussion, the type of training is clarified for example in this sentence: “In current neuromuscular training programs for patients with knee injury, principles of brain plasticity such as training of the contralateral extremity are included [1] [3].”

Page 3, paragraph 2: Please clarify the statement, “In order to improve sensory and motor function, more nerve cells are needed.” It may be beneficial to remove the statement entirely.

**AUTHORS’ RESPONSE AND ACTION**
The statement has been removed

*Methods*
*Subjects and Randomization*
Page 5, Lines 5-7: “The physical activity and age distribution…” Please clarify whether these subjects were matched to patients with ligament injuries from another study.

**AUTHORS’ RESPONSE AND ACTION**
Patients with ligament injuries of the knee are usually physically active and aged 18-35 years. This has now been clarified by adding the following sentence: “Individuals with ligament injuries to the knee are usually young, aged 18 to 35 years, and physically active at a moderate to high level [1] [3].”

Page 5, Lines 7-8: “The subjects were randomly assigned…stratified by gender, to temporary anesthesia…” Please revise this sentence for clarity.

**AUTHORS’ RESPONSE AND ACTION**
This has now been clarified according to your suggestion (page 7): “To ensure an equal number of men and women in each group two computer-generated randomization lists, one for women and one for men, were drawn up by a biostatistician and given to the assessor. The assessor allocated the next available number on entry into the trial, assigning the subjects to treatment/placebo.”

Page 10, 1st paragraph: Please provide your interpretation of effect sizes (i.e. <0.4 = small, .41-.7 = moderate, <.7 large).

**AUTHORS’ RESPONSE AND ACTION**
This has now been added and the reference is given (Kazis et al): “An effect size of <0.50 was considered small, 0.50 to 0.79 moderate, and ≥0.80 large [35].”
**Statistical Analysis**
Page 9: Please clarify sample size calculations. Knee kinesthesia 24 (or 12). Was this for pre post measures or between group measures?

**AUTHORS’ RESPONSE AND ACTION**
The sample size calculations were within groups. We agree that it may be confusing to the reader that we provided sample size calculations for both a 20% and a 30% improvement for knee kinesthesia. Therefore, we have removed the values for the 20% improvement. For clarification, the sentence now reads: “For knee kinesthesia, sample size calculations revealed that at least 12 subjects were needed to detect an improvement by treatment of 30% within groups … “ “…within groups” is also added in the following sentences on sample size calculations for the other outcome measures: “For vibration sense, 13 subjects were needed to detect an improvement of 20% (SD\text{diff} 3.3) within groups. For the one-leg hop test, and knee extension peak torque, 2 and 5 subjects, respectively, were needed to detect an improvement by treatment of 10% within groups, with 80% power at the 5% significance level.”

**Results**
The results are presented clearly.

**Discussion**
Throughout the discussion, the word “training” is used. Again, please clarify what is meant by “training.”

**AUTHORS’ RESPONSE AND ACTION**
This has now added in the discussion according to you suggestion (see also response to comment above).

The authors present relevant and valid arguments based on the results of this study. The limitations are clearly addressed. In addition, the authors present logical rationale for the study of those with pathology using these research methods.

The last paragraph of the discussion contains a large amount of speculation, which is appropriate for this type of study.

- **Discretionary Revisions**
  **Statistical Analysis:**
  Why did the authors choose to use paired and independent t-tests? Would an ANOVA with repeated measures be more appropriate? Would comparisons using change scores be more appropriate than pre-post comparisons for all measures?

**AUTHORS’ RESPONSE AND ACTION**
Using t-tests will yield the same results as if we use ANOVA. However, with an ANOVA analysis, within group changes are not given. We choose to use t-test in order to provide data for both within group comparisons (before vs. after in the EMLA and placebo groups, respectively) and between group comparisons (EMLA vs. placebo). These results are provided in Table 2, were we show data for within group changes with mean and 95% CI and between group changes with mean and 95% CI (the column to the right). We have discussed this with our statistical advisor, who recommends us to not make any changes regarding the statistical tests or in how data is presented.
Authors’ response to Reviewer Eleni Kapreli (comments for the authors are included)

Thank you for your valuable comments, suggestions and constructive criticism. Changes have been made in order to meet the criticism raised, and in cases where we have not made the suggested changes, our reasons for not doing so are given. Response to each of the specific comments is provided below (each comment is included, followed by our response).

Reviewer’s report:
General Comments
This paper is very different and interesting as it deals with new aspects of rehabilitation taking advantage of brain reorganization. However, there are a few methodological considerations that authors did not take under account and probably that could have been crucial for their results. The manuscript adheres to the relevant standards for reporting and data deposition but some additional information is needed. Discussion and conclusions are both well balanced and adequately supported by the data, although limitations are not clearly stated and this section needs review. Finally, title and abstract accurately convey what has been found.

Specific comments

Minor Essential Revisions
Abstract

Background
• “Training is included in the treatment of knee injury to improve patient-reported function and sensorimotor function.” Please replace with “….to improve both patient-reported and objective sensorimotor function”.

AUTHORS’ RESPONSE AND ACTION
Changes are made in the abstract and in the background according to your suggestion.

• “The aim was to investigate…..”. Please add “The aim of the current study was to investigate…."

AUTHORS’ RESPONSE AND ACTION
Changes are made in the abstract and in the background according to your suggestion.

• “In a first double-blind exploratory..” Please replace “In this first double-blind exploratory..”

AUTHORS’ RESPONSE AND ACTION
Changes are made in the abstract and in the background according to your suggestion.
**Results**

- Please replace “There were no differences between the groups in effects of treatment” with “There were no differences between the groups due to treatment effect.”

**AUTHORS’ RESPONSE AND ACTION**

Changes are made in the abstract and in the background according to your suggestion.

**Methods**

- Authors use the verb “lay” more than once in the section of outcome measures. Please replace “lay” with “laid” (simple past form of the verb) and if possible replace with synonymous to avoid repetition.

**AUTHORS’ RESPONSE**

The methods have all been used in previous studies, and the text describing these methods have been previously reviewed several times and improved for methodological clarity and language clarity. The word “lay” is past tense in American English (lie is the present tense). To our knowledge, “lay” is a correct word to use here. Therefore, we have not made any changes.

- I think that this extensive referral onto the sample size calculation in statistical analysis section of methods is rather confusing… Furthermore, taking under consideration the fact that no primary outcome measure was determined, this extended section could be avoided. Anyway, the effect sizes in Results section are more reliable and give the same information.

**AUTHORS’ RESPONSE**

No primary outcome measure was determined, since the study has an exploratory character. We expected to find an improvement in more than one of the variables to interpret the results as an effect from treatment. For this reason, we provided sample size calculations for all outcome measures, in order to get an idea of how many subjects we needed to detect changes. We believe that this information is of value for the reader. The sample size calculations were based on results from previous studies on uninjured subjects of the same age and activity level as those included in the current study. We have discussed this matter with our statistical advisor, who recommends us to keep the statistical method section in its present form. Regarding the effect sizes, they give us with information on the effect of the intervention in healthy subjects in the present study.

**Results**
• Table 2. Authors should add t-test values, p values and effect size values in the table for each comparison (t(degrees of freedom) =..., p=..., r=....) replacing mean difference values as it is more appropriate.

AUTHORS’ RESPONSE
It is nowadays recommended that mean and 95% CIs are presented, instead of p-values, since confidence intervals provide us with more information than p-values do. The reader can see how close or far from zero the confidence intervals are and, thus, the reader can interpret how effective, for example, a treatment is. Confidence intervals also give us information on the power of the study. To our knowledge, and also according to the recommendations of our statistical advisor, t-values are not given, since this information is not of crucial importance to the reader. The reviewer suggested that we add r-values, but since we have not made any correlation analysis in our study, r-values are not included. In Table 2, we show data for within group changes with mean and 95% CI and between group changes with mean and 95% CI (the column to the right). According to our statistical advisor this is the appropriate way to present our data.

Discussion
• Please rephrase as it is not clear what you mean: “Since good sensorimotor function is of importance for the overall outcome after injury [6] and in preventing OA [2] [8], it can be argued that training programs need to be more effective in order to improve or restore sensorimotor function after knee injury and knee OA.”

AUTHORS’ RESPONSE AND ACTION
We have rephrased, and it now reads (page 14): “Good sensorimotor function is of importance for the overall outcome after injury [6] and in preventing OA [2] [8]. Although improvements are achieved by neuromuscular and/or strength training, impairment in sensorimotor function often persists [1] [2] [3]. Thus, it can be argued that training programs need to be more effective in order to improve or restore sensorimotor function after knee injury and knee OA.”

Major Compulsory Revisions
Background
• The whole second paragraph that describes brain plasticity is poor. For example, plastic changes of the brain do not only include differences in somatotopic representations (expansion). Differences (neuroplasticity) could be found concerning signal amplitude or activation of additional cortical areas. Furthermore, the different types of neuroplasticity should be discussed (long term and short term mechanisms). According to the study design it is more possible that you refer to short term mechanisms of neuroplasticity. I could suggest for example Boroojerdi et al, (2001), titled “Mechanisms underlying human motor system plasticity”.

AUTHORS’ RESPONSE AND ACTION
The section describing brain plasticity has been rewritten and a discussion of plasticity mechanisms has also been added to the background section:

Introduction (pages 4-5): “One of the most interesting questions in neuroscience concerns the manner in which the nervous system can modify its organization and ultimately its function
throughout an individual’s lifetime based on sensory input, experience, learning and injury [12] [13]. This phenomenon is often referred to as brain plasticity [14] [15]. Plasticity changes can be divided into rapid and long term plasticity. Rapid changes are typically seen minutes after injury or intervention, and are often based on decreased inhibition. Decreased inhibition increases the receptive field size and enables more neurons to be activated by a specific stimulus. This is sometimes referred to as unmasking of synapses or neural structures. Long-term changes are typically seen weeks or months after an injury or intervention and are based on increase or decrease in synaptic transmission or axonal and dendritic sprouting. Synaptic transmission becomes facilitated in a pathway that is frequently used, while those that lay dormant atrophy. Sprouting can be seen in response to injury or to increased functional demand [16]. Axons at the edges of a lesion send new axonal branches into the damaged area and re-innervate dendrites that have lost their synaptic input. Plasticity changes also include changes in nerve signal amplitude and activation of additional cortical areas [14] [15].”

Discussion (page 16): “Neurophysiologic mechanisms in the lower extremity may also differ from those in the upper extremity. Large overlaps in the sensorimotor activation have been shown following movement of the knee, ankle and toes as opposed to the fingers [45]. However, the same plasticity mechanisms likely occur in both the upper and lower extremity, thus making it possible to manipulate plasticity mechanisms also in the lower extremity in order to improve sensorimotor function.”

• Authors should include a brief description of the neurophysiological mechanisms explaining the effect of temporary cutaneous anesthesia on the sensorimotor function of a referred joint rather than simply refer that 3 previous studies have found a positive effect. It is not clear your hypothesis. Please explain why someone could expect both sensory and motor function (especially this) improvement.

AUTHORS’ RESPONSE AND ACTION
A description of the neurophysiologic mechanisms has been added in the background section (see also response to previous comment). Previous studies in a different setting though as discussed below, in the upper extremity and also in the lower extremity have clearly showed an improved sensibility following cutaneous deafferentation of the forearm or lower leg. These studies are focused on sensory function. No previous studies have focused on an improved motor function following cutaneous deafferentation. However, Muellbacher et al showed that following a peripheral nerve block of proximal muscle and skin in patients with impaired hand function following stroke an improved muscle function was seen in the ipsilateral hand. Thus, it is interesting to study whether these effects also can be seen following a cutaneous deafferentation.

• In the last paragraph authors write: “The aim was to investigate if the principle of brain plasticity that has been successfully used on the hand to improve sensory and motor functions, and on the foot to improve sensory function, can be applied on the knee.” I assume that “brain plasticity that has been successfully used on the hand to improve sensory and motor functions” is referred to the study of Bjo¨rkman et al, (2004) as it is the only one that measured motor function. However, it is not true that there were any improvements in motor function according to that study. Please
rephrase and add references in order reader to clarify the sources of your statements.

**AUTHORS’ RESPONSE AND ACTION**
The reviewer is correct on this point and the sentence has been changed and appropriate references are provided (page 6): “The aim of the current study was to investigate if the principle of brain plasticity that has been successfully used on the hand [22] [23] and foot [24] to improve sensory function, can be applied on the knee.”

*Methods*

- Why did you use right leg??? Did you check for footedness?? How did you ensure that all your subjects were right or left foot dominant?? Handedness and footedness influence brain activation during movement of upper or lower limb respectively. Additionally, this factor also influences function outcomes. Please explain and include in methods. In case you did not take this factor under consideration please discuss it as a study limitation at the end of your discussion section.

**AUTHORS’ RESPONSE AND ACTION**
We agree that hand dominance has an influence on brain activation. However, the inconvenience by using “dominant” leg in healthy subjects is that it is determined in different ways: by right- and left-hand preference (e.g., Barber et al, 1990), regarding which leg the subjects prefer to kick a ball with (e.g., Greenberger & Paterno, 1995), by jump preference (e.g., Nyland et al, 1994), or by stance preference when kicking a ball (e.g., Nyland et al, 1997). For this reason we did not determine leg dominance. Moreover, in our previous studies on healthy subjects, we have found no differences between the right and left legs for knee kinesthesia, muscle strength or the one-leg hop test for distance. In addition, it was not possible to assess both legs in the current study since the amount of EMLA that can be used is limited to 60 grams (we used 50 grams). Therefore, only one leg was tested. Based on our results from previous studies showing no differences between the right and the left legs, we believe that the results most likely would have been the same irrespective of whether the right leg or the left leg was used. The right leg was tested in all subjects. Possible bias from choosing the right leg should be minimized due to our study design (RCT). In our study, we hypothesized that temporary anesthesia of the skin area above and below the knee would improve sensorimotor function of the ipsilateral knee and leg. To clarify that all assessments were done on one leg only, the following has been included in the method section: “EMLA/placebo was applied and all tests were performed on the right leg only.”

- Why did you select to anesthetize these areas above and below knee joint?? If your aim was to elicit brain reorganization in a similar way with those three previous studies then it would be more logically to anesthetize foot and ankle areas. I could suggest Kapreli et al, (2007), titled “Lower limb sensorimotor network: issues of somatotopy and overlap.” According to that area lower limb activation pattern differs a lot with upper limb. They found a large overlap in primary sensorimotor cortex (SM1) and cerebellum representations of the three lower limb joints (knee, ankle and foot). I am wondering if a strong limitation of your study is the application of the
anesthetic cream. Please discuss it as a study limitation at the end of your discussion section.

AUTHORS’ RESPONSE AND ACTION
We agree that based on previous results in the upper extremity and foot it would be more consistent to deafferentate the foot and lower leg. However, it is extremely difficult to anesthetize the entire foot using EMLA due to problems with absorption of the EMLA in the sole of the foot and applying an occlusive bandage around the foot. Due to this fact we decided to anesthetize the skin area adjacent to the knee knowing that following deafferentation, the adjacent cortical areas rapidly occupy the deafferented area. This is now addressed in the discussion section (page 16-17):
“In previous studies on the upper extremity [22] [23], the anesthetic cream was applied to the volar aspect of the forearm and in the previous study on the lower extremity the anesthetic cream was applied circumferentially on the lower leg [24]. Based on these previous studies, it would be logical to deafferentate the foot and lower leg in the current study. However, it is very difficult to anesthetize the entire foot using EMLA due to problems with absorption of the EMLA in the sole of the foot and applying an occlusive bandage. Therefore, we decided to anesthetize the skin area adjacent to the knee knowing that following deafferentation, the adjacent cortical areas rapidly occupy the anesthetized area. We also decided to deafferentate circumferentially on the lower extremity because the cortical area devoted to the lower extremity is small compared to the hand and we, therefore, expected that a larger deafferented skin area was needed (compared to the upper extremity) in order to allow the knee to expand in the primary somatosensory and motor cortex.”

We are well aware of the fact that the neurophysiologic mechanisms in the lower extremity may differ from what exists in the upper extremity. However, the same plasticity mechanisms likely occur in both the upper and lower extremity, supported by the results following deafferentation of the lower leg in healthy controls. This information has now been added in the discussion:
“Neurophysiologic mechanisms in the lower extremity may also differ from those in the upper extremity. Large overlaps in the sensorimotor activation have been shown following movement of the knee, ankle and toes as opposed to the fingers [45]. However, the same plasticity mechanisms likely occur in both the upper and lower extremity, thus making it possible to manipulate plasticity mechanisms also in the lower extremity in order to improve sensorimotor function.”

Discussion
• Authors write: “The only difference that we found was a lower value for TDPM, indicating better knee kinesthesia, after compared with before treatment in the placebo group. However, since the 95% CI is close to zero (Table 2), and there may be a learning effect in TDPM [32], the clinical relevance of this improvement of 0.40 degrees can be questioned.” Authors should explain more efficiently this kind of result.

AUTHORS’ RESPONSE AND ACTION
This paragraph has now been rewritten (page 14): “The only difference that we found was a lower value for TDPM, indicating better knee kinesthesia, after compared with before
treatment in the placebo group. However, the 95% CI was close to zero (Table 2), indicating a small change. Moreover, in a previous study on test-retest reliability, we found that there may be a learning effect in TDPM, shown as a significantly lower value in TDPM on the second test session than on the first test session. In that study, the 95% CI was also quite close to zero and, therefore, we questioned the clinical relevance of this learning effect [32]. Since the 95% CI in the current study was close to zero and our previous study show that there may be a learning effect in TDPM, the clinical relevance of the improvement of 0.40 degrees (95% CI -0.05, -0.73), can be questioned.“

• Authors write: “There may be several reasons for the lack of effect from temporary cutaneous anesthesia of the skin area above and below the knee on sensorimotor function of the ipsilateral knee and leg in the uninjured subjects in our study.”
However, authors refer only one reason for the lack of effect (the uninjured subjects). Authors should rephrase or give more reasons.

AUTHORS’ RESPONSE
The several reasons stated in this paragraph (page 15) include: the possible ceiling effect in uninjured subjects, that larger effects from temporary cutaneous anesthesia may be needed to detect a change in perception of touch in the knee compared with the hand and foot, and a possible type II error.

• Authors give as reason of lack of effect the uninjured subjects that they include in their study. However, the two previous studies that they mentioned had found positive results included similarly uninjured subjects in their study…..Could authors explain why??? Please add a comment in discussion section.

AUTHORS’ RESPONSE
This has been clarified in the discussion (page 17): “…However, the cortical area of the knee is smaller than the cortical area of the hand [12] [13]. Thus, larger effects of treatment are needed in order to detect an increase in the cortical area of the knee than in that of the hand. In line with this reasoning, we found no effect of temporary cutaneous anesthesia of adjacent body parts in the measures of sensory or motor functions of the knee in healthy subjects, whereas previous studies reported improvement in sensory function of the hand and foot in healthy subjects after such treatment [22] [24]….“