Your manuscript, JoVE58800 "Real Time Video Projection in an MRI for the Characterization of the Neural Correlates Associated with Mirror Therapy treatment for Phantom Limb Pain," has undergone editorial and peer review and your video has been reviewed by our production department. Note that editorial and production comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.  
  
**Editorial Comments on the manuscript:**  
  
• **Textual Overlap:** Significant portions show significant overlap with previously published work. Please re-write the text indicated in red in the attached document to avoid this overlap.

**Answer**: This is template language for fMRI processing. It is the established FSL pipeline used in the analysis of this protocol, therefore this is what was done using the proper terminology and sequence. We adjusted the text as best as we could to also keep the enough details for the reproduction of the procedure.

• **Protocol Language:** The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief “Note” at the end of the step (please limit notes). Please re-write your entire protocol section accordingly. Descriptive sections of the protocol can be moved to Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study. Any reporting should be moved into the representative results.

**Answer**: The manuscript has been adjusted accordingly as specified by the Journal.

• **Protocol Detail:** Please ensure homogeneity between the written protocol and the video. The manuscript should be a stand alone article, and all specific details mentioned in the video must be present in the protocol.

**Answer**: We have reviewed the homogeneity between the written protocol and the video as stated by the Journal.  
  
• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

**Answer**: The numbering was adjusted in accordance to the Journal requirements  
  
• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs):

1) modifications and troubleshooting 🡪 a modification and troubleshooting were addressed in the manuscript

2) limitations of the technique, 🡪 A limitation paragraph was added

3) significance with respect to existing methods, 🡪 We have stated the significance with respect to other methods.

4) future applications 🡪 We discuss further application of this protocol as a tool for understanding the underlying pathophysiologic mechanism of other neuropsychiatric disorders such as spinal cord injury, stroke among others.

5) critical steps within the protocol. 🡪 The critical steps are detailed in the protocol section.  
  
• **Figure/Table Legends:** Please provide figure legends to adequately describe the figures. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description. This must be placed before the Representative Results section.

**Answer**: We have reviewed all figures and they all have the corresponding legend.

• **References:**  
1) Please use superscripted numbers (e.g. edit line 132).

**Answer**: We have fixed this reference.  
  
• Please define all abbreviations at first use. – Answer: All abbreviations are defined  
  
• Please use standard abbreviations and symbols for SI Units such as µL, mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit. – Answer: This was checked  
  
• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

**Answer**: All our figures were made by the authors and were not published previously.

**Video Comments:**  
  
Audio issues

* • 1:15-1:19 - There are three audible popping sounds during this time. It is unclear until later in the video that these are examples of the audio being played for the patient and not just noise in the narration track. I would recommend removing them here.   
  Editing issues  
  • 2:59 - The edit here is a jump cut, which tends to have a jarring effect on the viewer. It should be smoothed out with crossfades instead.  
  Frame size/proportions issues  
  • 5:10-5:25 - The white background of this figure should be extended to fill the frame.  
    
  Please submit a high resolution version of your video (up to 2 GB) here: <https://www.jove.com/files_upload.php?src=17934048>

**Answer:** All the video issues detailed above were correctly addressed in the new video uploaded

**Comments from Peer-Reviewers:**   
  
  
**Reviewer #1:**

This experiment setup is expected to contribute to research for brain activity in mirror therapy. Also, it has potential to be applied to case studies. There are some comments, please consider.  
  
Major

Introduction  
1. L55-57: You should mention the point of similarity and difference with previous study which was conducted by Diers (Brain Res. 2015) to suggest novelty of your system.

Diers M, Kamping S, Kirsch P, Rance M, Bekrater-Bodmann R, Foell J, Trojan J, Fuchs X, Bach F, Maaß H, Cakmak H, Flor H. Illusion-related brain activations: a new virtual reality mirror box system for use during functional magnetic resonance imaging. Brain Res. 2015 Jan 12;1594:173-82.

**Answer**: Thank you for pointing this out. In fact, we consider the work of Diers et al. as an important influence in our work, and we now have added details outlining similarities and differences of this publication compared to our study. One important issue is that Diers et al. study evaluated brain responses in healthy subjects, while we are evaluating patients with phantom limb pain with associated evidence of impaired neuroplastic reorganization prior to treatment. In addition, Diers et al. evaluated the upper limb while we are exploring neuroplastic reorganization associated with lower limb training. In order to evaluate therapy implicating the lower extremity, we had to develop a novel protocol that included the use of a camera transmitting real time images of the subject’s moving limb. As discussed by Diers et al., there are inherent limitations and challenges in using a mirror-based system alone to make the arm movements seem more natural. This required the use of a complex system of mirrors. However, as stated by our participants, viewing the video transmission made the feeling of movement very real.

Protocol

2. L132-136: Regarding the condition 2), did the experimenter ask the patient to move his phantom leg simultaneously with his intact leg?? Did his muscle around stump contract during seeing mirror visual feedback?? I think that these factors could influence the brain activity.

**Answer**: We did not ask the patient to move their phantom leg simultaneously. The whole point was that the patient was not moving the affected limb in order to avoid signaling from the muscles from the residual limb. In fact, we were clear to specify to the participant to only move the unaffected leg in both conditions (eyes open and eyes closed). Thus, we do not expect any influences on brain activity. A phrase clarifying this instruction has now been included in the protocol.

3. L154-172: I do not think that the actual position of phantom limb spatially matched with mirrored phantom limb. You should describe the detail of method to minimize the discrepancy between actual position of phantom limb and mirrored phantom limb.

**Answer**: There were no methods to assess or match the actual position of the mirror leg to the phantom limb since this was not the goal of this protocol. The goal was to use mirror therapy to give the illusion of having an intact full moving limb. Besides that, most of the amputees do not have phantom limb sensation all the time. The majority of amputees have these sensations a couple of times a day, and sometimes the phantom sensation is in a non-physiological position. In order to match the position, we would have to guarantee that the subject had an ongoing phantom sensation and that the phantom was in a physiological position that could be reproduced. Therefore, we do not recommend adding this step in this protocol.

4. L154-172: Was there time-delay between actual movement and visual feedback?? You should about time-delay because the video transmission could make time-delay among them.

**Answer**: The image was transmitted in real time from the camera directly to a monitor. There is no perceivable time delay between the projection and captured actual movement. The actual movement and the visual feedback are separated by less than a second and did not interfere in the real time feeling as stated by our participants.

Result

5. L238-239: The result of cortical activity in posterior occipital with viewing the projected image of the moving leg was very interesting. You should discussion the brain activity referencing the previous report (Preißler et al. 2013) in a concise description.

Preißler S, Dietrich C, Blume KR, Hofmann GO, Miltner WH, Weiss T. Plasticity in the Visual System is Associated with Prosthesis Use in Phantom Limb Pain. Front Hum Neurosci. 2013 Jun 24;7:311

**Answer:** Thank you for pointing this out. Indeed, the results of Preißler et al. are very interesting and relevant. In our protocol, visual cortex activation is expected and related to the task the participants are performing. The figure presented in the manuscript is comparing the Movement condition with the Mirror condition. During the movement condition (MOV), the participants move their leg keeping their eyes closed. In the mirror condition (MIR), they continue to move the leg but with the eyes open. Therefore, activation of the visual cortex is expected due the differences in the task being carried out (eyes open vs. eye closed).  
  
Discussion

6. You mentioned the technical problems in previous studies which using only video feedback of limb movement. You should have compared the brain activity between condition of mirrored visual feedback and the condition of simple video feedback. You should describe the lack of experimental procedure as limitation and future study.

**Answer:** The limitation of previous studies using video feedback is now discussed. The greatest limitation is that this approach does not provide the feeling of immersion. However, as mentioned, there is a lack of studies comparing the methods of visual illusion with Mirror therapy or mental imagery. Diers et al. showed no differences between visual illusion and mirror box therapy, showing that both techniques may be comparable in providing the illusion we are looking for in this protocol. As the primary goal of this experiment is to better understand the neuroplastic reorganization of participants undergoing mirror therapy, we do not think that a simple video feedback condition is necessary. We have added a comment in the limitations section regarding the lack of experimental procedures as we did not compare brain activity between the condition of mirrored visual feedback and the condition of simple video feedback.

Minor

7. L131: The is typo in reference [56]

**Answer**: We have fixed this typo.  
  
  
**Reviewer #2:  --**   
Manuscript Summary:  
This article present a protocol to study limited aspects of mirror therapy using fMRI.  
  
Major Concerns:  
The authors are not specific enough with what they want to investigate with this set up. I understand their intention is to merely present a protocol and the way they overcame the challenge of having a patient performing mirror therapy in a MRI scan (more on that below). However, every protocol is devised to answer a specific research question which is not clearly stated, nor the protocol is wide enough to answer a range of different questions.

It is unclear to me if the authors want to simply see the activation arising from observing a limb movement from a first-person perspective through a mirror or if they want to answer a question about pain. In the first case they should state how this would advance our understanding and give a better overview of similar research of neuroimaging. In other words, there is not much novelty in showing that with mirror feedback, ipsilateral activation of the sensorimotor cortex is elicited, and there is plenty of evidence for that. If the aim is instead investigating pain, then the authors should give better account of the literature, at least the one on fMRI and PLP (i.e. Jutzeler 2015) and express clearly how this protocol would contribute to improve our understanding. Furthermore, the protocol itself is not sufficient to answer any question about pain as they do not include in their methods any plat to assess that. A fMRI paradigm can be hardly separated from this kind of evaluation, which is also integral part of the experimental protocol.

**Answer**: The goal of this protocol is to describe an approach to perform mirror therapy inside an fMRI scanner. This is entirely in-line and of the scope of this journal by outlining experimental details and methods designed to investigate the structure, function, physiology, and pathophysiology of the nervous system. Here, the technique is described using amputees that have PLP. Mirror therapy is remains commonly used in these patients, even though its underlying neurophysiological mechanisms are not yet completely elucidated.

Furthermore, this experimental procedure can be applied for other conditions that also rely on the use of mirror therapy. This includes conditions such as pain related to stroke and patients with de-afferentation pain.

The novelty of the approach is as follows: previous studies have applied mirror therapy or mental imagery to revel associated brain activation patterns only. Here, we have the opportunity to observe changes in neuroplasticity and how there is functional reorganization after an amputation. Using this experimental protocol, several lines of investigation can be pursued such as comparing cortical reorganization before and after a given treatment, or to correlate changes in brain activation with the degree of phantom pain and other baseline characteristics of the participants.

In our case, this protocol is part of a much larger clinical trial comparing multiple treatment modalities in amputees with PLP (NCT02487966). The goal of this trial is to compare changes in brain activation before and after treatment and correlate findings with pain levels.

In addition, most of the available literature has evaluated upper limb function, which is much simpler to perform technically and the homunculus representation of the hand area is much bigger. This is contrary to that we are investigating in our population (i.e. lower limb amputees) which has been less studied despite the fact that the number of individuals with PLP associated with lower extremity amputation is extremely large. This makes our protocol helpful for general applicability as well as for other disorders such as spinal cord injury and stroke.

R87-94 Authors talked about challenges of mirror therapy in MRI scan but they do not clearly state what they mean with that. It seems that the challenge is to perform the movement inside the limited space of the scan. However this is not much of a challenge for the lower limb (the challenge for the lower limb is to perform movements without moving excessively the head and they do not tackle this problem in any way). Afterwards they vaguely imply that the challenge is the accurate recreation of the visual feedback, even though I do not recall studies of mirror therapy in fMRI scan highlighting such difficulties (i.e. Foel). To show limitation of previous studies they use unacceptable references:

**Answer**: We thank the reviewer for pointing this out. We have now clarified the text with regard to the challenges of performing the mirror therapy within the constrained space inside the scanner. That is, the challenges associated with moving the leg, viewing this movement, and not moving the head. Previous studies, like the one mentioned (Foel), were designed to evaluate changes in cortical mapping using fMRI with upper limb movement and using a mirror placed while subjects performed hand movements observed in real time using a head coil mirror. This was more difficult as they needed extra devices to make the image of the hand appear in the appropriate place on the body. In our protocol, there is no need for additional mirrors or camera systems because for the lower limb, the movements are observed in real time by direct video transmission to a monitor placed at the back of the scanner. However, due to the positioning and space constraints of the MRI machine, a single mirror was used to allow subjects to watch their leg movements. Therefore, the need of this protocol, in which a camera and video system are used to give real time visual feedback is highly relevant and appropriate. This issue is further supported by adding further citations revealing the limitations of previous study protocols.

\*R97 reference 23 is not a proper reference for a peer review article and does not proof how mirror therapy is limited in effectiveness

**Answer**: This reference was deleted.

\*R97 I haven't read reference 24 but from a quick glance at the paper is seems completely out of place. Doesn't seem to provide any evidence that previous attempt at mirror therapy in a MRI scan were limited in effectiveness. What do you mean with effectiveness anyhow?

**Answer**: In this protocol, we discuss why using a real time camera video image is superior to using virtual reality. Our approach provides an accurate and real visual feedback and immersive sensation of the mirror therapy. The reference 24 explains why egocentric distances (i.e. the apparent distance the subject views the object seen in a virtual reality environment is not well perceived) thus further supporting our statement that a real live online live video projection of the subjects’ own leg is better than using a virtual reality imaging of a non-real leg. We have modified the text to make this point clearer.

R102-104 The authors overstated the importance of realistic visual feedback for PLP relief (especially in terms of image resolution), without proper support for this idea

**Answer**: The goal of mirror therapy is to give accurate visual feedback of the missing part of the limb. Only a few studies have compared this technique with other approaches such as virtual reality. Even though, so far, there is no data comparing the efficacy versus image resolution and/or accuracy. The proposed mechanism underlying mirror therapy effects take into consideration the immersive feel provided by real time visual feedback. Therefore, we consider that a realistic visual feedback has a direct effect on the experimental results. We have added more references with regards to the importance of realistic visual feedback and PLP relief.

The authors present ideas on PLP in the introductions as facts, which is not correct, such as "The alleviation of symptoms occurs putatively through the crossmodal re-establishment of afferent inputs, provided by the observation of mirror-reflected images from the non-affected limb". It is not certain that is the reason why alleviation of PLP happens. The whole introduction should be revise to be more clear and what we know is more certain than hypotheses.

**Answer** :This is one of the possible mechanisms and the hypothesis in which we have based our on-going clinical study. We have modified the introduction accordingly to clarify that there are other hypotheses given that the neurophysiological mechanisms are still not well elucidated.

Minor Concerns:

A figure showing the view of the set up from a patient point of view would be helpful.

**Answer:** Unfortunately, taking areal picture or filming of the mirror/monitor inside the scanner is not be feasible due to space constraints and safety issues (i.e. it is not possible to enter with a camera inside the head coil space).

R68 It is unclear what "this reversal" is referring too.

**Answer**: The term “this reversal” refers to the reversal of the maladaptive plasticity theory explained in the same paragraph. We modified the phrase to make this point clearer.

R70-77 Could be a good place to mention limitations of mirror therapy in the treatment of PLP.

**Answer**: We modified the introduction to include more support for the use of mirror therapy and its limitations.

R84-86 Indicate the authors aim to assess correlates of pain relief, and they use the spatial resolution superiority of fMRI as reason to choose that instead of EEG for example. This means however that they are already assuming that the pathophysiology of PLP is completely expressed in the somatosensory and motor cortices, which is reductive.

**Answer**: One of the most accepted models to explain PLP is related to the reorganizational changes and maladaptive plasticity seen in amputees with pain. In addition, there are several studies that show that altering this disorganization is associated with improvement in pain symptoms. Therefore, we are basing our study on available literature. However, we recognize the existence of other theories. In order to address the concern of the reviewer, we have added a statement in which we acknowledge that there are other mechanisms that can be associated with PLP such as Makin et al. Additionally, as stated in the introduction, we state that the exact underlying mechanism of PLP is not well elucidated. Therefore, that is why we believe that this protocol will help to clarify this issue. However, our line of research focus is in investigating these changes in an patient population that has been less studied (i.e. lower limb amputees). We acknowledge that pain causes other neurophysiological alterations that can be better addressed such as EEG or TMS. However, in this case, we are focused on characterizing changes seen in cortical and somatosensory areas.

R68 The authors talk about the reversal of maladaptive plasticity as if it was already mentioned.

**Answer**: The sentence was modified accordingly to clarify what the term reversal is referring to.

R87 The opening sentence is odd

**Answer***:* The sentence was modified accordingly: New sentence now reads as follows: “Phantom Limb Pain (PLP) refers to the sensation of pain perceived within the area corresponding to the missing limb post amputation”.

R131 reference [56] does not exist in the bibliography

**Answer**: This reference was not linked in the reference manager. This is now corrected.

R133 There a more unambiguous way of calling the movement is plantarflexion/dorsalflexion.

**Answer**: This is the most accurate terminology used in the field. However, to avoid any possible misunderstanding, we have added explanations of the movements between parenthesis.

R148-150 are there any instruction given to the patients to rule out the possibility that they are not actually moving the phantom limb during mirror feedback?

**Answer**: There are clear instructions given to the participants, so they do not move the phantom and residual limb in order to avoid any movement of the stump that can alter the signal on fMRI. To clarify, a sentence was included in the instructions section:

*“Give clear instruction to the patient to avoid any movements of the amputated limb as to avoid contraction of stump muscles that can interfere with the brain signal”.*

R226-227 do the subject spontaneously report about the VR immersions or is there an interview? How many patients stated this? How many people out of the total number that underwent the paradigm?

**Answer** :There is no interview or questionnaire carried out. However, in our protocol we do not have any VR, therefore there is no VR immersion. However, if the question is in regards of immersion after our intervention, most of the patients reported immersion, including during protocol setup in which we used healthy controls to test the experiment set up. We have added a sentence in the limitations section that states that we did not have a questionnaire to assess either VR immersion or immersion after our intervention. However, is recommended to use one in further studies.

**Reviewer #3:**

Manuscript Summary:  
This manuscript introduces a protocol to study the neuronal correlates of mirror therapy in the MR scanner environment. For this purpose, a combined mirror-camera-setup is introduced that allows amputees while lying in the scanner to see their moving (intact) limb. In the here introduced protocol, the authors compare a situation where the patient is asked to move the intact limb and to close the eyes, to move the intact limb and to look at the mirror image of the intact limb, or to rest. These three conditions were compared with respect to the BOLD signal change they trigger in sensory cortex. The protocol is of potential interest to researchers studying the neuronal basis of phantom limb pain. However, there are a few serious flaws of the protocol the authors should acknowledge.  
  
Major Concerns:

-The authors write repeatedly in the introduction and discussion that the used set-up is cheap, and easy to construct. This is in fact not true. I have myself worked with a similar online-camera system in the MR-scanner. MR-compatible cameras are very expensive. The model I bought was around 7.000 Euros. I would therefore not consider a setup requiring an MR-compatible camera as cheap or easy at all, because a specific camera has to be bought, or has to be self-constructed, which can be challenging. I would therefore ask the authors to please 1) remove the statements from the manuscript that their setup is cheap, and 2) add details to the exact camera they used, including a link to the website.

**Answer** :We modified the text accordingly, explaining that the camera is not a relatively expensive piece of equipment. The camera used is a Logitech HD Pro C910 with a current cost of approximately $217.00 USD (please see the following link [https://www.amazon.com/Logitech-Webcam-C910-Cameras Frames/dp/B003M2YT96](https://www.amazon.com/Logitech-Webcam-C910-Cameras%20Frames/dp/B003M2YT96)). For safety concerns, we removed all ferrous metal components. This was mostly just the small stand that was attached. Since the camera itself was not placed inside of the MRI bore, there is not a need for the more costly MRI compatible systems. The camera is attached to an MRI safe IV pole via a Loc-Line modular hose to enable positioning changes. The other components are also inexpensive and can be self-built. We also added the information about the camera details as required by the reviewer.

-Because I have used a very similar system myself, I know about the problem using a camera live-image in the MR scanner. Usually, the camera provides a fast, real-time image when the image is directly transferred to the monitor screen. This comes with the cost, however, that the video cannot be implemented into a running program, such as matlab. That is, the camera image is fast, but just a "raw" image, which cannot be switched on or switched off, for example. Because the cues were auditory, and because the patient was asked to close the eyes, I think that the authors chose exactly such a setup here. However, this setup seriously limits the potential applicability of the protocol. For a well-controlled experiment, one would also need a visual condition WITHOUT leg movement, by showing a video. One would also need a leg movement condition with a similar fixation point compared to the visual condition, which would require showing a fixation cross instead of the video image. With this present setup, this cannot be provided. For such a setup, the camera image would have to be imported into e.g. matlab, but then, it would lose its timing accuracy. I therefore do not agree with the authors claims that this setup is ideal, or better than previous ones. I would ask the authors to please comment on all points mentioned above, and to include information about timing accuracy to back up their comments.

**Answer:** We thank the reviewer for pointing this out. This limitation is exactly why we decided to pursue our protocol as stated in the manuscript**.** The goal of this setup was capture the real time experience of moving subject’s own leg. As pointed out, this is only possible by transmitting a high resolution of the leg movement in real time. If the goal of the protocol was not dependent on time accuracy, we would agree that more conditions (e.g. fixation and video) could provide more information. However, as discussed in the introduction, the possible mechanism underlying the effects of mirror therapy that we are evaluating is related to the fact that the mirror image helps to integrate the mismatch between proprioception and visual feedback.

- The authors do not provide any details on the quality of the camera image. A short video sequence has to be included into the manuscript submission, such that the reader can see the quality of the images used here. From my experience, MR-compatible camera images are usually of low quality, because they are very simple system without eg. brightness control. This is one reason why many researchers do not use camera images in the MR scanner. I myself for example have refrained from this option after trying different possibilities for many years. Please provide details.

**Answer:** We also added the information about the camera details as required by the reviewer. We confirm that the camera acquires 1080p image resolution images.

- The authors say in the discussion that their protocol is better than the at present available ones, particularly with respect to immersion. This is pure speculation. It is not clear whether the authors protocol evoked immersion. It is not clear how realistic the images looked. THe protocol was also not compared to previous protocols. I would ask the authors to remove this statement, and to provide a more balanced discussion of their results. It is a serious drawback of the study presented here that participants did not fill out a rubber-hand-like questionnaire where some aspects about the illusory character were assessed. Also this should be acknowledged.

**Answer:** We have modified the text accordingly to avoid the assumption that our protocol is better with respect to immersion. However, we still believe that seeing the participant’s own leg image generates a more immersive feel than seeing a virtual animated representation of a leg. This is due in part to the fact that all participants have morphological differences. Therefore, it is plausible to consider that seeing your own body image will generate a more immersive feel than viewing a generic animation. Still, as mentioned by the reviewer, this is based more on speculation. The text has now been modified to reflect this issue.

Minor Concerns:

- In the introduction, the authors only reference work that provides evidence for the "distortion theory" of phantom pain, according to which distortions in the representation of the missing limb lead to phantom pain. However, there is work by the group of Prof. Makin showing the reverse: preserved topography relates to phantom pain. THis literature should be included.

**Answer:** The most studied theory and the one in which the hypothesis of this study is based on is the theory of cortical reorganization. Therefore, this is the one most discussed in the introduction. However, we agree that it is important to mention all theories involved in the PLP process. We emphasize that this is a procedure paper and thus mechanistic discussions are beyond the purpose and goal of the this journal publication..

- the authors repeatedly say that they monitor the "leg". In fact, participants only move the foot. Please replace "leg" by "foot" throughout the manuscript to avoid confusion

**Answer:**  Although participants are only moving the foot, this was a point we discussed in the beginning of our protocol and we believe that using the word “leg” was more appropriate as we wanted the patient to have the impression that they were moving the whole leg. Therefore, the wording was important after discussing with patients and members of the research staff. We have added a phrase explaining this rational in the revised manuscript.

- the here introduced protocol is different from the usual mirror therapy setup in the sense that here, the patient does not see his/her own leg in the correct perspective. He/she sees the leg from below, not from above. In my view, this is a serious drawback of this protocol. Ideally, the mirror would be mounted in a way that the correct perspective is preserved, as for example done in the design of Keysers et al. 2004 Neuron. I would ask the authors to discuss this issue, and to perhaps try such an arrangement in the MR scanner

**Answer:**  We agree with this potential limitation. Participants usually perform leg mirror therapy in a sitting position, placing the mirror between the legs and looking at the mirror image from the side. This orientation is not possible in the fMRI scanner. In this protocol, patients must lay down to be scanned and the camera is placed on the side of the non-amputated leg and thus observing in real time the leg from that side (in the same way if the participant was sitting). This is in contrast to the study of Keysers et al. 2004 where participants were touched on their legs or observed movies/video images of other people’s limbs or objects being touched.

- Stimulus-associated movements are a serious problem in this design. Could the authors please provide the movement parameters of the MR images in one further figure? Knowing in how far this protocol evokes movements of the head is one important factor for considering / not considering using this protocol

**Answer:** As previously discussed, controlling for head movements is an important limitation and concern as with any fMRI protocol that uses a task requiring movement. For this protocol, we decided not to use head constrains during the images acquisition. However we used foam pads to stabilize the head and avoid movements. From an analysis standpoint, we used dynamic stabilization to minimize motion artifacts, and motion correction was performed as discussed in the analysis section of this protocol:

*“*Volumes with motion  above 0.9mm in any direction should be marked with FSL’s motion outlier detection processing stream and mathematically “scrubbed”  from the final analysis. If more than 25 percent of the volumes are designated for removal, the whole acquisition should be excluded from the total dataset”.

- Could the authors please add more info on the MR images acquired (sequence used, which filter, which segmentation etc.)

**Answer:** All subjects will undergo an MRI scanning protocol using a 3T Philips Achieva system and a thirty-two channel head coil (Center for Biomedical Imaging at Boston University School of Medicine in Boston, MA). The anatomical T1 images have a repetition time (TR) of 3 s and an echo time (TE) of 6.8 ms. Each functional acquisition run provides 182 volumes with a voxel size of 3 mm (isotropic resolution) recorded with TR=2 s, TE=28 ms.

Controlling for potential motion related artifacts is of considerable importance in this study as patient and/or novice individuals unfamiliar with the MRI environment can show greater magnitudes of head motion during image acquisition. This includes prospective motion correction (PMC) which will be used for functional scan acquisitions. PMC measures changes in geometry related to subject motion during the acquisition of a dynamic series and makes real-time adjustments based on the updated measurements. Second, the acquisitions will be processed for automated identification and removal of motion-related artifacts, volumes with motion  above 0.9 mm in any direction should be marked with FSL’s motion outlier detection processing stream and mathematically “scrubbed”  from the final analysis. If more than 25 percent of the volumes are designated for removal, the whole acquisition should be excluded from the total dataset.

**Reviewer #4:**

Manuscript Summary:  
This submission describes a protocol for assessing mirror therapy fMRI correlates with the subject experiencing real-time visualization via video projection inside the scanner. This is an improvement over previous techniques, which may have not matched well in real-time synchronization of movements or felt realistic to a participant. The protocol is aimed at people with leg amputations and phantom limb pain but there would be implications to use this in other contexts like post-stroke pain.  
  
Major Concerns:  
None.  
  
Minor Concerns:

1 - Some justification for the particular choice of leg movements (ie. why just flexion and extension of the foot?) and timing of tasks (why 20 seconds per condition?) during the experiment would be helpful. Was there any dose-finding work? Similarly, the need for a mock scanner beforehand would suggest that there may have been some challenges with subjects beginning with the "real" scanner - any information on how the use of the mock scanner improved the protocol would be helpful.

**Answer:** For the type of the movements, we did several tests in order to get the best signal possible with the least amount of head motion possible, allowing good data quality collection. For fMRI, block design experiments are easy to carry out. The blocks were always of a fixed length, and lasted 20 sec depending on the timing of stimulus. In the case of physiological movements such as the one used here, 20 sec was enough to see clear modulation of the hemodynamic signal.

The mock scanner allows the participant to familiarize themselves with the environment and the protocol leading to improved and more accurate performance at the moment of the real experiment. Therefore, they can be better accommodated and comfortable for the real fMRI experiment.

2 - Authors state this is "real-life immersive sensation provided to the patient" - have subjects actually been asked about their impressions of the protocol and in comparison to others?

**Answer:** There is no interview or questionnaire to address real-life immersive experience. However, several patients reported VR immersion, including during protocol setup in which we used healthy controls. We have added further explanations as part of the protocol limitations in regard to this aspect.

3 - line 111 - change "emersion" to "immersion"

**Answer:** The correction was made.

4 - Since the person with the mutation here is not being treated clinically, would not use the term "patient" - maybe "participant" or "individual"

**Answer:** All the terms were substituted.

5 - Any differences to account for if amputation is above or below the knee?

**Answer:** The level of amputation should not interfere with the protocol execution since all participants use the non-amputated leg to perform the movements and have no visualization of the residual limb or phantom leg.