# Rebuttals

**Reviewer: 1**

This work makes an important contribution to our understanding of the functional connectivity/somatotopic representation of motor brain areas within the medial aspect of the frontal lobe. The paper is well written and the methodology is sound.

The authors might wish to improve their manuscript by considering the following suggestions.

**General comments**

Some sentences are too long and should be broken up for clarity, instead of using colons/semicolons/dashes (e.g. top of p. 10).

**We shortened and reorganized sentences that were too long.**

It could be good for the reader to be provided with a more explicit explanation of their exact hypothesis and what/in what way the present study adds to pre-existing knowledge (bottom of p. 4).

**We made our hypothesis more explicit, underlying what our work adds to previous studies.**

The Discussion needs better structuring (the argument should be more streamlined) and could be more concise (e.g. avoiding repetitions such as on p. 13). The Discussion should also include a final summary of the main findings, plus a suggestion for future research directions

**We re-structured the discussion so that results of the rs-fMRI, those of the BOLD and FC analyses of our previous data, and those of the meta-analysis are first separately commented, and then discussed in terms of their convergences/ divergences. We shortened the discussion avoiding repetitions. We ended the discussion with the main findings, limits of the study and directions for future research.**

**Specific comments**

The title should more scientifically better read "Somatotopic organization…" instead of "Discovering the somatotopic organization".

**We modified the title.**

It would be appropriate to clarify whether the frequency (0.5 Hz) of alternate foot movements was actually measured or estimated (p. 8).

**We clarified this issue both for the hand and for the foot task.**

The headings under 2.3 need to be clearer and more systematic.

**We clarified the headings.**

In the Results section the authors state that the spatial resolution of their data did not permit them to consider each premotor area separately: this point should be addressed in the Discussion section as well.

**We addressed this point in the Discussion.**

There should be more detail and consistency in reporting p values in the Result section.

**Added in the results the p and q value threshold of the analysis.**

The authors are invited to provide more comments on the reviewed literature on the somatotopic representation within the cingulate motor zone (p. 12).

**We added comments on the cited literature.**

In the Methodological considerations, when addressing the influence of age, the authors should specify whether they refer to the resting state study, the task-related study, or both.

**We specified that we referred only to the resting state study.**

**Reviewer: 2**

The purpose of this study is to explore the somatotopic arrangement of the primary motor area using functional connectivity MRI. Segmenting the brain using fcMRI / r-fMRI / rs-fMRI is gaining recent traction in the literature (Margulies et al 2009/ Kim JH et al 2010). (I hope the scientific community will finally come to an agreement on the correct acronym soon.) I applaud the authors for the amount of work performed here and their attention to the inherent issues with fcMRI (physiological noise, etc). However, I do have some concerns about the work that I hope the authors can address. Please respond to my questions both with answers and in revision of the document.

1.) The idea of a predefined arrangement in the motor cortex is one of great debate in the literature. Certainly, even Woolsey and Penfield mention that the motor homunculus was just a simplification scheme in their respective manuscripts. I think this discussion is missing from the paper. The primary sensory somatotopic organization is very well studied and defined but the motor system

seems to be less defined (see work by Donoghue and Sanes). The motor cortex is very plastic. Also the overall structure of the efferent peripheral nervous system wired to the muscles is different than the organized structure of the afferent sensory nervous system. I caution the authors against oversimplification and ask them to revise the document with these concepts in mind.

**We agree with the reviewer on this point; therefore so we approached this problem both in the introduction and in the discussion. We note, however, that the problem of the somatotopic organization of the motor cortex does no vain our method of study of the functional connectivity in resting state. In fact, as suggest by Schieber (Schieber 2001), although the motor representations of contiguous small body parts in the primary motor cortex seem to be distributed, however, it is confirmed that the body regions of head, upper limbs and lower limbs have largely separate topographic representations in the primary motor cortex.**

2.) I am curious if the results are due to the spatial layout of the motor cortex. The author's overall hypothesis may be predefined. Certainly these structures (face/arm/leg) are arranged in a linear order and the center of mass experiment just reinforces that order. The results look like a gradient effect. I would recommend a control outside the linear arrangement or trying a different analysis method instead of the seed voxel / region approach. I worry that the analysis method used might predetermine the results.

**In this paper we performed three additional experiments: ALE Metaanalysis, motor activations and FC during motor task execution in order to obtain a cross-validation of our seed-rsFC results. Only FC during motor task execution use the seed voxel / region approach all the other two approach do not suffer from this potential confound. Furthermore the results of the meta-analysis are generated by a large number of papers which were performed during a motor tasks of hand or face.**

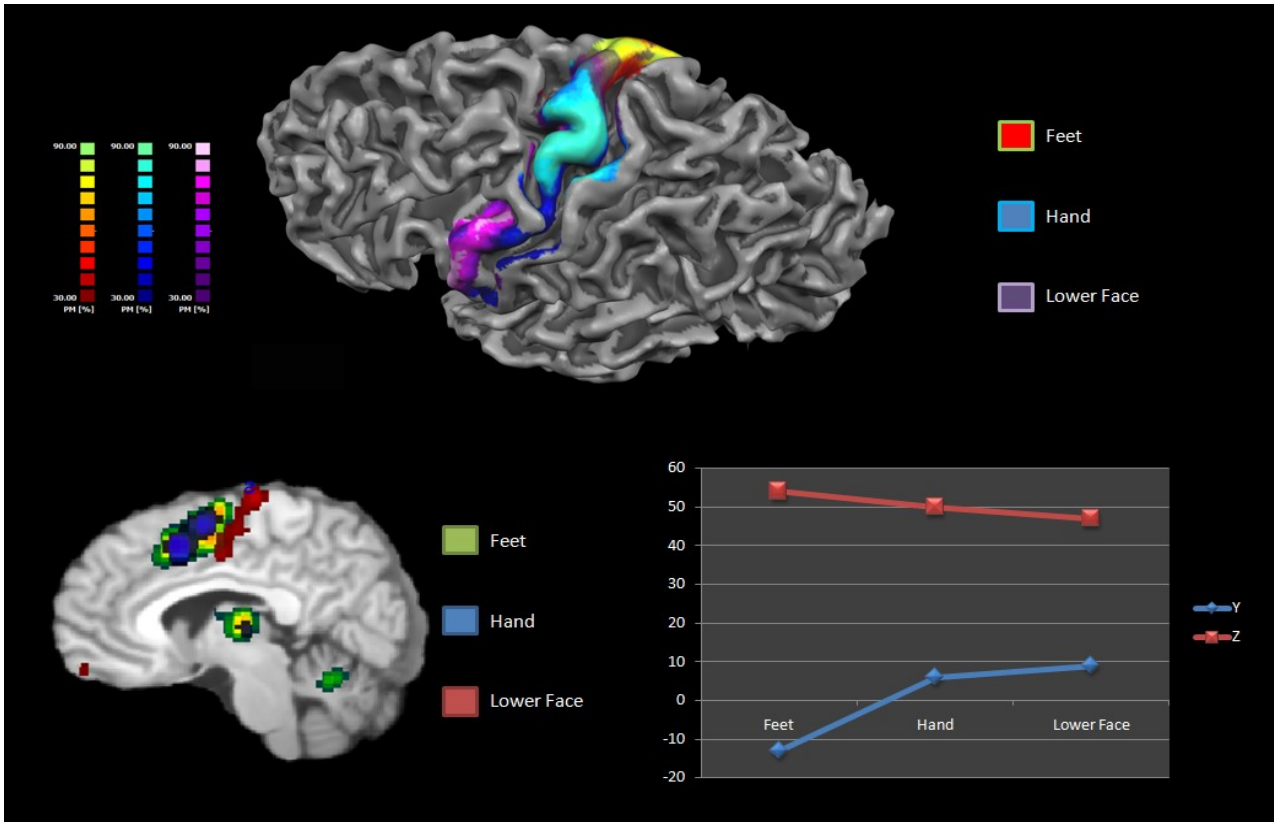
**The recent article by Martijn P. van den Heuvel and Hilleke E. Hulshoff Pol (van den Heuvel and Hulshoff Pol 2009) has shown that there is a strong spatial correspondence between the centers of mass of corresponding areas of the two primary motor cortex mapped by rsFC.**

**To further validate our results as asked by the Referee we performed a supplementary analysis to see if (i) submitted to a unsupervised voxelwise parcellation method the precentral gyrus show a clustered structure and (ii) with a very different method if, even while performing a task, somatotopically homologous areas of M1 (retrived from the parcellation results) show a correspondence on the medial wall with an arrangement similar to that seen with rsFC. To do so we employed the fuzzy clustering of timecourses and the Metaanalytic Connectivity Modeling (MACM, (Robinson, et al. 2009)); MACM focus on specific**

**anatomical regions and address global coactivation patterns across a diverse range of tasks and experimental designs. The consistency of coactivation patterns across experiments is interpreted as a piece of evidence for the functional connection of groups of regions (Robinson, et al. 2009).**

**In fact, two recent meta-analysis studies (Smith, et al. 2009; Toro, et al. 2008) demonstrated that the set of major covarying activation networks identified from large-scale meta-analyses overlaps almost completely with the set of networks that are visualized in the resting brain. These results provide strong evidence that RSNs reflect functional neural networks, and that these dynamic networks are engaged even at rest (Fox and Raichle 2007). Therefore, similarly to previous studies (Koski and Paus 2000; Lancaster, et al. 2005; Postuma and Dagher 2006; Toro, et al. 2008), we used MACM (Laird, et al. 2009) to characterize patterns of functional connectivity in the human brain and compared the emerging patterns with the results of our rsFC analysis to further validate each other (see Rebuttals Figure 1 and Methods for details).**

***Figure 1. Voxelwise parcellation and MACM results***




*Upper panel: Fuzzy clustering of the precentral gyrus of our group.*

*Multi subject summarization with probabilistic maps.*

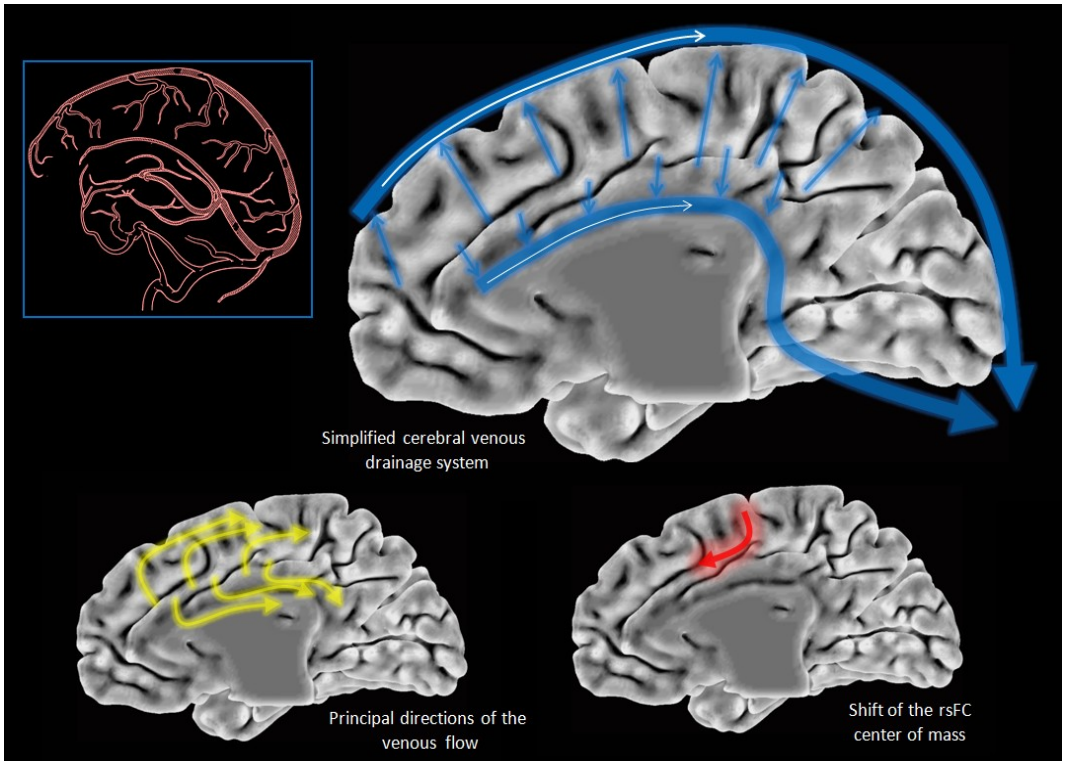
*Lower panels: MACM results confirm the findings of the rsFC as we have seen with other supplementary methods.*

**The fact that: (i) all four additional experiments, made with different techniques and different data, confirm our hypothesis, (ii) submitted to a unsupervised parcellation method the precentral parenchyma show a clusterization that resembles the parcellation outlined by our center of mass-shift results, (iii) in corresponding areas of the contralateral motor cortex there is a correspondence between homologous areas mapped by rsFC as recently demonstrated, (iiii) only two out of five experiments used the seed voxel / region approach, makes us think that it is very unlikely that such evidence may be due to chance or methodological mistake.**

3.) In regards to question 2, could this be just a shared vascular effect? The drainage networks certainly move in similar directions to the somatotopic arrangement. Please comment.

**The subsequent figure show a simplified view of the midline drainage venous system of the brain: it is possible to note that dorsally to the cingulate sulcus the blood flow is moving dorsally and caudally whereas ventrally to the cingulate sulcus the blood flow is moving ventrally and caudally. These directions are almost opposite to the gradient demonstrated by our experiments. We thus consider very unlikely that our results are due or seriously flawed by the venous drainage (see Rebuttals Figure 2).**

***Figure 2. Simplified view of the midline drainage venous system***




***In blue venous system, in yellow venous flow, in red shift effect of hand-leg rsFC***

4.) I am very concerned about the large voxel sizes 5mm x 5mm x 5mm. Is this small enough to detect subtle differences? Penfield used very large recording electrodes as opposed to later peers like Merzenich, Kaas, etc. who used microelectrodes. The later peers were able to find finer somatotopic differences in sensorimotor cortex. Current technology at 3T is about 1.5 mm cubic voxel size. Is the large voxel size confounding your results? How large in terms of cubic tissue is the body/leg or arm representation? Are your methods able to detect differences without partial volume effects?

**The size of the areas identified in the somatotopic representation of the only fMRI high-resolution study (Mayer, et al. 2001) (to our knowledge) exploring the motor areas of the medial wall of the brain ranging from 0.2 up to 4.4 ml, ie largely above our voxel size that is 0.125 ml.**

**In any case the main aims of our work are to confirm the use of seed-rsFC to map the motor somatotopy and to demonstrate of the double gradient of displacement within somatotopically arranged areas of the medial wall. Say that we believe that even with a voxel size of 0.125 ml we can achieve our goals.**

**Nevertheless we still need to take into account that, like most fMRI studies, our data suffer from relatively low spatial resolution and a partial volume effect due to the voxel dimension, the spatial smoothing and the vein drainage effect. It is clear that in order to map the somatotopic distribution of motor areas in a topografically complete and most refined way we need further efforts with high-field scanners and more focused field of view (see for example (Sanchez-Panchuelo, et al.)), but the ambition of this study is not to give a final description of this problem but to explore this issue through an innovative technique and to demonstrate via this novel technique the somatotopical gradient previously suggested by other readers.**

**As mentioned above, given the complexity (already demonstrated by studies on monkey) of the somatotopically motor representation, to have a map as complete as possible is necessary that further studies are carried out with high resolution techniques and methods that can somehow overcome the partial volume problem as multivoxel pattern analysis.**

**In any case the results of our main experiment are strongly confirmed by the results of the four additional experiments. Though it is possible that the voxel size and partial volume effects may have affected the detail of our results we believe, given that the size of our voxel is 50% smaller than the size of a minor somatotopical area in play as mapped with 3T scanner by (Mayer, et al. 2001), that these difficult issues may not subvert our results. This results are also fully confirmed by additional analysis in which datasets are also collected by other groups (see ALE meta-analysis and MACM) and by the findings of the recent paper of**

**Martijn P. van den Heuvel and Hilleke E. Hulshoff Pol (van den Heuvel and Hulshoff Pol 2009).**

Minor Point

1.) The authors mention that subjects that fell asleep were excluded from the study. They later say no subjects were excluded. Please reconcile.

**We corrected this part**

# Rebuttals Methods

## Structure-based meta analysis

We followed the workflow indicated by Laird et al. (Laird, et al. 2009a) for the structure-based meta analyses, also indicated as MACM (Laird, et al. 2009b; Robinson, et al. 2009). We extracted from the BrainMap database (Laird, et al. 2005) all the studies involving only normal subjects that reported an activation in the selected ROI. We used the three ROIs obtained from the voxelwise parcellation, corresponding approximately to the areas of activation of the feet, hands and face. For each group of foci we calculated an ALE probability map using GingerAle 2.0. All the maps are thresholded at $q<0.05$ FDR corrected.

## Voxelwise parcellation

We applied fuzzy clustering on the left unsmoothed precentral gyrus parenchyma to achieve a voxelwise segregation of the underlying motor networks. Precentral gray matter meshes were segmented from each subject morphological image and coregistered using a high-resolution intersubject cortex alignment. Voxels belonging to this region were submitted to an voxelwise unsupervised fuzzy clustering technique. Fuzzy clustering partitions a subset of n voxels in c "clusters" of activation (Smolders, et al. 2007; Zadeh 1977). The z-standardized signal time courses of all voxel are simultaneously considered, compared, and assigned to representative cluster time courses (cluster centroids). This data-driven method thus decomposes the original fMRI time series into a predefined number of spatiotemporal modes, which include a spatial map and an associated cluster centroid time course. The extent to which a voxel belongs to a cluster is defined by the similarity (as measured, e.g., by correlation) of its time course to the cluster centroid. In this method, "fuzziness" relates to the fact that a voxel is generally not uniquely assigned to one cluster, but instead, the similarity of the voxel time course to each cluster centroid is determined. This is expressed by the "membership" of voxel n to cluster c. Cluster time course and membership functions are updated in an iterative procedure (Bezdek, et al. 1984) that terminates when successive iterations do not further change memberships and cluster centers significantly as determined via classical cluster algorithm distance measures. For the current fMRI dataset, the number of clusters was fixed to 2 and the fuzziness coefficient was set to 0.4, as suggested in literature (Fadili, et al. 2000; Fadili, et al. 2001; Golay, et al. 1998; Moller, et al. 2002). We applied principal component analyses to the datasets to reduce dimensionality while capturing at least 90% of the total variance/covariance. Group cluster maps were obtained using probability maps. The

resulting fuzzy clustering maps were reported in the interval [30–90%] and superimposed on a 3D representation of a template brain (average brain).

Voxelwise clustering: optimal number of clusters

The a priori determination of the fuzziness coefficient and the number of clusters are research topics often encountered in the literature (Fadili, et al. 2001). Critically, the "true" number of clusters (i.e. optimal number of classes is usually unknown in Fuzzy clustering. In this perspective, several cluster-validity indices have previously been proposed in the literature to appreciate, in an unsupervised manner, the optimal number of clusters (for a review see (Wang and Zhang 2007)). These indices combined different measures of compactness and separation of the clustering in order to ensure that identified clusters are compact and well-separated. In our paper, we used two different methods: (i) a cross-validation method: the group was split in half, and Jaccard's J (which measures *dis*similarity between sample sets is obtained by dividing the difference of the sizes of the union and the intersection of two sets by the size of the union:

$$J_\delta(A,B) = 1 - J(A,B) = \frac{|A \cup B| - |A \cap B|}{|A \cup B|}.$$

was used to compare clustering solutions across the groups. The minimum number of clusters that minimize the Jaccard dissimilarity index J was choosen. It yielded local maxima of 3-cluster solution. (ii) Using the similarity index generated by the SogIca method (see (Esposito, et al. 2005)) we choose the minimum number of clusters that maximized the combined similarity index for each clustered group. The similarity index here employed is the absolute value of the mutual correlation coefficients, in space for the spatial sources of estimates or in time for the associated basis time-courses; this measure give a combined value of similarity based on spatial and temporal correlation (see (Esposito, et al. 2005)).

**Group components clusterization**

To obtain a unsupervised group components clusterization of all the single subject clusters generated by the Fuzzy clustering technique we employed the Self organizing group ICA. This method (Esposito, et al. 2005), originally developed for single subject ICA results can be successfully employed also for our data, indeed with this method the clusters of single-subject decompositions are grouped according to the combined spatio-temporal information using a self organizing grouping procedure that is based on hierarchical cluster analysis (Himberg, et al. 2004).

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