**Response to Reviewers**

Large-scale meta-analysis of human medial frontal cortex reveals tripartite functional organization

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Dear Dr. Shaham,

Thank you for the opportunity to revise our manuscript by responding to the reviewers’ insightful comments. Below we outline the major changes to the manuscript to address the concerns by the reviewers, followed a point-by-point response to various specific concerns.

1. We have revised our introduction to more clearly motivate meta-analytic co-activation based clustering. We have emphasized that morphological and connectivity based parcellations infer functional divisions, whereas co-activation based clustering more directly identifies regions with similar co-activation across a wide variety of psychological manipulations.
2. We have more clearly distinguished in our results where our parcellation coincides with previous work, and used the Harvard-Oxford probabilistic atlas to generate more accurate labels.
3. We have simplified the terminology, more liberally cited Figure 1, and revised the methods section to improve clarity.
4. We added post-hoc exploratory tests to determine if certain topics were more strongly associated with particular regions. These post-hoc tests allowed us to address Reviewer 1’s concerns had about differences within the middle zone, and Reviewer 2’s question about function gradients.
5. We have updated Figure 2 to include results from 12 clusters, as well as display the results from 9 clusters in more detail using coronal slices with labeled subcortical regions.
6. The discussion has been substantially revised. In particular, we have been more careful in describing the impact of our work, and not overselling. We have also expanded discussion of future challenges stemming from our work

We believe our manuscript has substantially improved by addressing these comments and hope that you now find it suitable for publication.

Sincerely,

Alejandro de la Vega

**Reviewer 1**

Reviewer’s comments are denoted in italics. All grammatical suggestions were taking into consideration throughout.

We have summarized our changes to the major suggestions by this reviewer. We have also included a point-by-point response to minor comments not encompassed by the major suggestions.

**Major / General**

**Introduction**

*“Could use a little polish. See my specific comments below”*

*“The rationale for partitioning MFC on the basis of meta-analytic co-activation is inadequate, given that this is the central means of identifying the parcels for subsequent profiling*

We have taken into account the reviewer’s specific comments, opening the introduction more broadly, and establishing the problem before jumping into the various fMRI associations with MFC. In the first paragraph, we also more careful outline the limitations of morphological, cytoarchitechtonic and connectivity based parcellation methods. In the final paragraph of the introduction we again highlight the potential advantages of co-activate based clustering:

“In contrast to cytoarchitechtonic and connectivity based parcellations, the present analysis identified clusters with distinct signatures of functional activation across a wide range of psychological manipulations.”

**Method**

*“a. Yes, but at times the complex methodology is challenging to follow. Specifically, the**terminology is unwieldy at points (voxels, features, parcels, zones, sub-regions, and ROI's); be consistent.*

*b. Figure 1 is incredibly helpful, yet, is not cited in the text. It would be helpful to liberally cite each panel as you work your way thru the constituent methods*

*c. Not clear whether co-activation is w/in or b/w studies. If between, why?”*

We have cleaned up the terminology in various places. We avoid the terms “features” and “parcels”, instead referring to “topics” and “clusters”, to be consistent. We more explicitly denote that we will refer to clusters in the 3-clusters solution as “zones” and those in the 9-cluster solution as “sub-regions”. Finally, we consistently use the term “psychological topics” instead of “concepts”, “functions” or other such terms. Additionally, we cite every panel of Figure 1 in the methods.

We have also unpacked the co-activation clustering methodology to elucidate that we are calculating the correlation across studies between MFC voxels and whole-brain PCA components (lines 163-186)

Furthermore, we have noted the version number of the Neurosynth database (line 152) and more clearly stated the address of where to obtain images and code to replicate the present analyses (line 161).

*“How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.? needs to be clarified; see my detailed comments*

*”*

We have more clearly detailed our method for naming and localizing clusters onto anatomy (line 225). We have taken the reviewer’s suggestion to use the Harvard-Oxford anatomical atlas to more precisely localize regions. This revealed that indeed all sub-regions in the middle zone were most likely to occur in either the cingulate or paracingulate sulci. As such, we have renamed these clusters. Moreover, more careful comparison with Picard & Strick (1996) suggested “SMAr” should be re-named to “pre-SMA”. Finally, we have renamed “rACC” to “pgACC”.

See lines 422-442 in the Results for relevant changes. Discussion has been updated accordingly (e.g. lines 684, 691-753).

The reviewer also pointed out that we should clearly state the standardized stereotaxic space we are using, and now discuss this in more detail on line 157:

“As peak activations are populated automatically, the database does not differentiate between Talairach and MNI coordinates; however, all activations are treated as MNI coordinates, and all of the following analyses are in MNI152 coordinate space.”

Results

***“****a second concern is that the authors are in danger of interpreting the null, because (if I understand things correctly) they did not perform contrasts that would license the statements in the discussion about 'more' and 'greater'. “*

We have addressed this concern by calculating 95% confidence intervals for the log odds-ratio loading between topics and regions. Thus, if the 95% CI of log odd-ratio for a specific topic is non-overlapping between two regions, we can argue with some confidence that the association strength between that topic and the two regions differs. In order to reduce the number of post-hoc exploratory comparisons made, we only report 95% CIs for a subset of associations. For example, we report 95% CIS for working memory, pain, reward and fear for all of the sub-regions in the middle zone, as the results from Figure 4 suggests potential differences in these topics between these sub-regions.

We wanted to be careful with these post-hoc exploratory tests, so we ensured to clearly label them as such in the results section.

**Discussion**

*“a. The Discussion needs work. At times, the authors fall prey to over-selling the novelty and significance of their results****.*** *The results themselves are genuinely interesting and exciting, so there is no reason to exaggerate differences with prior research or to over-interpret the theoretical significance. “*

Response: We have scaled back claims of theoretical novelty. For example, in the discussion of the middle zone, the reviewer was convincing that our results are general consistent with the hypothesis that pain, negative affect and cognitive control consistent activate the MCC, and we now point that our clearly (Line 692). However, we also more clearly outline novel implication of our results, such as the finding that reward was consistently associated with ventral MCC, suggesting this region may more generally integrate affect with cognitive control (Line 747).

Moreover, the reviewer was convincing that our results showed less evidence of functional-anatomical specificity. We have removed such claims throughout the manuscript. In addition, we have heeded suggestion to more prominently discuss the finding that “no region is selectively activated by a single psychological concept” by moving expanding and moving that discussion to the second paragraph in the discussion (Line 648).

*“b. At times, the literature cited in the first half of the Discussion seemed dated”*

*“c. The limitations section needs work. Careful scrutiny suggests that these are not necessarily the most important limitations and the most important avenues for future research.*

*d. This study is significant, but the theoretical and translational implications of the work are not clearly outlined in the Discussion. “*

Response: We have taken into account the reviewer’s specific comments. We have more clearly labeled the “future challenges” section (line 825) and expanded this section. We have unpacked the limitations of Neurosynth from more general limitations of fMRI (lines 508-521).

Additionally, we have substantially expanded our future challenges section. We discuss in more detail the possibility that our results suggest a complex many-to-many mapping between regions and functions (lines 478-488) and the dynamic nature of brain region (lines 489-494).

We have suggested that the hypotheses from this study can be tested by 1) the development of novel fMRI studies from the hypotheses proposed by this study and 2) large-scale functional mapping to individual subject anatomy (Lines 495-503).

Moreover, we have attempted to more careful outline the theoretical implications of our work throughout the discussion. For example, in line 459: “Thus, the present results suggest that ventral aspects of MCC may be generally important for incorporating affective information into cognitive control, whereas dorsal MCC may be more important for working-memory dependent aspects of cognitive motor control.”

**Abstract**

We have taken into account the reviewer’s basic comments. See more detail below.

**Figures**

The reviewer suggested we display a coronal slice in Figure 1 to better displays our ROI. Instead, we have added coronal slices to Figure 2. This accomplishes this goal while also showing in more detail the anatomical extent of our clusters. We have also added the silhouette plot to Figure 2 as the last panel.

Figure 5

**Minor / Specific**

*"Since most researchers tend to be intimately familiar with one particular domain of cognition"*

*i strongly agree, but would object to calling it 'cognition' ... maybe 'psychological domain, such as pain'*

We have changed to “psychological domain’. We have also avoiding using the term ‘cognition’ through the manuscript and instead use ‘psychological states’.

*"To determine which voxels across the brain co-activated with each MFC parcel, we performed a meta-analysis resulting in whole-brain maps that indicate which voxels across the brain are active in the studies that activated each parcel."*

*i'm confused; how is this different than the meta-analytic co-activation on page 7?*

*when i 1st read this my comment was -- 'this is not really a meta per se, it seems more like a 'contrast' or a 'meta-analytic contrast' (like a moderator analysis in classic meta)'*

*but then i went and studied figure 1 and realized that (i think; could be wrong) that you are actually describing two steps at once, a meta and a meta contrast; you need to clarify this for the reader*

These are indeed meta-analytic contrasts to determine whole-brain differences between studies that co-activate with one region (e.g. posterior MFC) versus control regions (e.g. middle and anterior MFC). The reason these are meta-analytic contrasts is to highlight the differences between sets of related clusters. Thus, in the 3-cluster solution, we contrast the co-activation of the three clusters to each other, whereas in the 9-cluster solution, we contrast the co-activation of clusters that correspond to the same zone to each other (e.g. vmPFC vs dmPFC & pgACC). We have tried to more clearly explain our methods in lines 231-244, and the caption for Figure 3. We have also avoided using the term “unique”.

*here you insert the additional adj 'specialization,' but given recent critical conversations in the blogosphere, might be better to either drop or use 'func preference profiles'*

We have removed the term “specialization” for the manuscript and now use “functional preference profiles” instead.

**Reviewer 2**

*The authors stress repeatedly the alignment of their findings with previous anatomical MFC studies "to a very substantial degree". Could they be more specific and provide evidence for this assertion. Does the number of clusters align with previous findings? However then I would expect them to find e.g., three distinct cingulate motor areas (as for example Dum & Strick). Or do the authors think that the spatial extent and location of their sub-areas resonates with previous research? Would they be able to demonstrate this? Or does their functional specialization analysis align with previous neurophysiological studies?*

*What does their method of using data-mining fMRI activation peaks to the above mentioned sizeable literature on MFC sub-specialisation? I presume that their method does not allow for finer-grained sub-divisions than cyto-architecture, receptor density or tracer injection based studies? If they wanted for a function-based subdivision could they not have used a "functional localizer" approach as Amiez and Petrides (2014)?*

We have taken multiple steps to address this concern. First, we have more carefully outlined the extent to which our parcellation agrees with previous organizational schemes of MFC (see lines 423-449). In general, we find instances where our parcellation is quite similar to cytoarchtechtonic and connectivity-based approaches, such as the division between SMA and pre-SMA near the VCA. However, we also find several instances of disagreement. For example, as the reviewer notes, we did not identify three distinct cingulate motor areas. In fact, our most posterior cluster spans both SMA and the caudal cingulate zone. As such, we have tempered claims of substantial alignment between the present parcellation and previous studies.

In addition, we have more thoroughly attempted to motivate co-activation based parcellation in the introduction by noting the limitation of previous studies (lines 71-73). In particular, many previous studies indirectly infer functional differences from morphological or connectivity differences, but since they do not directly measure how the MFC responds to various challenges, they cannot directly determine if putative sub-regions are ‘functionally different’. We believe co-activation based parcellation provides a more direct measurement of functional differences.

Moreover, the large-scale meta-analysis in the present work allows us to understand the functional significance of the resulting clusters, across a wide variety of psychological manipulations. Functional localizers are limited in that they can only consider a small subset of possible psychological manipulations. Moreover, the ‘reverse inference’ problem limits the ability of such methods to conclude that a region is preferentially recruited by process in the functional localizer. As we note in the introduction, this is particular problematic for areas with a high rate of activation across studies, like MCC / pre-SMA. We have attempted to make this point more clear in the introduction by unpacking this problem in lines 107-110.

*I am not sure if I follow the assertion: "Although the 12-cluster solution results in a marginally better silhouette score, this comes at the cost of additional complexity." Why would they discard this solution if it fits the criteria that they themselves set better? If they think that MFC organization is indeed more complex why would this be a cost?*

We have address this in two ways. First, we have now included the 12-cluster solution as part of Figure 2. We agree with reviewer that this solution should not be discarded. Moreover, we have attempted to clarify our logic regarding preferring the 9-cluster solution in lines 250-255:

“Although the 12-cluster solution resulted in a marginally better silhouette score, this comes at the cost of additional complexity. The plateauing of silhouette scores suggests that the specific solution that is favored depends on the researcher’s goals, as a greater number of regions results in more accurate, but less reliable clustering solutions (Thirion et al., 2014). Thus, we focused on the 3- and 9- cluster solutions as they provide insight into the functional topography of MFC at two different scales.”

In other words, others have previous argued that the choice of number of clusters largely depends on the researcher’s goals (Thirion et al., 2014). Thus, we use the silhouette score to narrow down on useful solutions—for example, rejecting the 2- and 6- cluster solution. Ultimately, we chose to focus on the 3- and 9- cluster solutions as they are two useful spatial scales for understanding the overall organization of MFC.

*Are there contextual differences in co-activation patterns? E.g., dACC appears to co-activate with DLPFC and amygdala. It also appears to be associated with conflict, decision making and pain. Is it more activated with the amygdala in studies that mention pain and more activated with DLPFC in studies that mention conflict?*

The reviewer brings up a very interesting point. We believe it is the case the results here present an overall picture of the co-activation and function of MFC. As such, certain regions may be dynamically involved with different processes, depending on the context. However, this question is out of the scope of the present report, and would require extensive further research. We have noted this as a potential avenue for future research in a new paragraph in the discussion (lines 489-494).

*Even though the authors state distinct areas with distinct functions and connections there appear to be strong overall similarities in neighboring regions in co-activation and function potentially with gradients of change along different axes. For example "motor" seems to gradually decrease from posterior to anterior. Pain appears to decrease from ventral to dorsal Similarly DLPFC co-activation appears to increase from posterior to anterior. It would be very interesting to see if there is concordance or correlation in these functional - connectivity gradients / changes? E.g., a gradient of decrease in pain is associated with a decrease in amygdala co-activation?*

We have attempted to discuss potential gradients in more detail in the discussion. First, we have tried to formally identify functional gradients by introducing post-hoc exploratory tests of functional differences between sub-regions in the results (lines 381-400). These tests reveal some potential gradients, such as the association with ‘reward’ becoming stronger ventrally in the anterior zone. Although we do not have a test to formally determine if these gradients are accompanied by specific changes in co-activation, we have attempted to discuss such possibilities in the discussion such as:

* “In contrast, pre-SMA (P2) showed a stronger association with cognitive control and co-activated with regions important for goal-directed cognition (e.g. DLPFC, aIns).” Lines 430-431
* “Notably, both dorsal MCC clusters were more strongly associated with WM – and showed great co-activation with fronto-parietal control regions and aIns— while ventral MCC was more strongly associated with affect and co-activated more strongly with subcortical regions, such as amygdala and striatum.” Lines 445-448

*Why does the three-zone subdivision group together regions with vastly different cyto-architecture and separate regions with similar cyto-architecture?*

We think this concern is addressed by the more extensive discussion in our introduction about the limitations of cytoarchitechtonic based parcellations. In short, we don’t believe that there is any reason to expect perfect agreement between these two methods, especially since differences in cytoarchitechtonic properties do not necessarily translate to functional differences. Moreover, as the three-zone solution is so broad, it will necessarily group together regions that differ in morphological and cytoarchitechtonic properties.

We have also tried to address these general concerns in more depth in the discussion. In particular, an important avenue for future work will be to systematically compare parcellation from different modalities (lines 435-437).

*Minor:*

*How well did the Harvard Oxford grey matter match the implicit Neurosynth data-base grey matter? I suppose all Neurosynth foci should lie in the grey matter? What percentage of Neurosynth foci are outside the 30% Harvard-Oxford grey matter atlas?*

We have address this on lines 123-126: “In general, Neurosynth’s activation mask corresponded highly with probabilistic locations of cerebral cortex, with the exception of portions of precentral gyrus, far ventral and frontal medial prefrontal cortex, which showed low activation although they were more than 50% likely to be in cerebral cortex, likely due to signal drop out.”

We could not calculate the exact correspondence between the two masks, as Neurosynth’s masks included sub-cortical regions. In general, Neurosynth’s mask was more liberal due to activations that are often observed in fMRI near, but outside of grey matter. Thus, relatively few voxels were excluded due to low data, as many had already been excluded by the grey matter mask.