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# Improved Functionnectome by dissociating the contributions of white matter fiber classes to functional activation

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## Running title: Functionnectome with white matter classes

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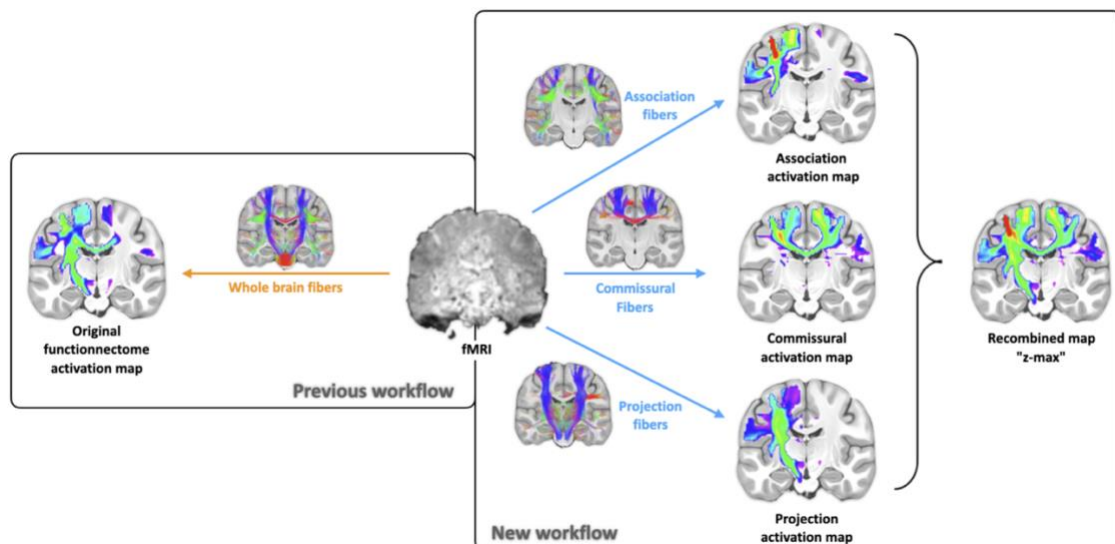
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**Keywords:** task-related fMRI, diffusion MRI, tractography, human brain

## Abstract

Integrating the underlying brain circuit's structural and functional architecture is required to explore the functional organization of cognitive networks properly. In that regard, we recently introduced the Functionnectome. This structural-functional method combines an fMRI acquisition with tractography-derived white matter connectivity data to map cognitive processes onto the white matter. However, this multimodal integration faces three significant challenges: 1) the necessarily limited overlap between tractography streamlines and the grey matter, which may reduce the amount of functional signal associated with the related structural connectivity; 2) the scrambling effect of crossing fibers on functional signal, as a single voxel in such regions can be structurally connected to several cognitive networks with heterogeneous functional signals; and 3) the difficulty of interpretation of the resulting cognitive maps, as crossing and overlapping white matter tracts can obscure the organization of the studied network. In the present study, we tackled these problems by developing a streamline-extension procedure and dividing the white matter anatomical priors between association, commissural, and projection fibers. This approach significantly improved the characterization of the white matter involvement in the studied cognitive processes. The new Functionnectome priors produced are now readily available, and the analysis workflow highlighted here should also be generalizable to other structural-functional approaches.

## Graphical Abstract



We improved the Functionnectome approach to better study the involvement of white matter in brain function by separating the analysis of the three classes of white matter fibers (association, commissural, and projection fibers). This step successfully clarified the activation maps and increased their statistical significance.

## Introduction

Magnetic resonance imaging (MRI) is one of the best tools to study the human brain in vivo (Bandettini 2012; Lerch et al. 2017). It offers a wealth of modalities, leveraging different properties of matter to infer and contrast brain tissues' state and inform us of their structural and functional characteristics. Notably, the blood-oxygen-level-dependent (BOLD) signal of functional MRI (fMRI) (Ogawa et al. 1990) has shown tremendous success in mapping cognitive areas and networks in the grey matter (Yarkoni et al. 2011). Analysis of fMRI data traditionally involves leveraging the link between the BOLD signal and hemodynamic response to physiological processes. It reveals brain areas in which the BOLD signal displays a specific pattern related to cognition or other functional organization of the brain. Likewise, diffusion MRI (dMRI) data, in tandem with tractography algorithms (Conturo et al. 1999; Jeurissen et al. 2019), have been harnessed to tackle the challenge of charting the white matter tracts defining the brain's structural connectivity. Diffusion MRI yields information about the local directions of water diffusion across the brain (Le Bihan et al. 1986). Because water tends to diffuse in the same direction as axonal fibers in the white matter, tractography uses dMRI data to try and reconstruct fiber bundles – or “streamlines” – that can be employed to estimate structural connectivity in the brain (Jeurissen et al. 2019).

Over the past decade, the exploration of the brain's function using MRI has shifted from unimodal fMRI to the combination of functional and structural information (Douaud et al. 2011; Hermundstad et al. 2013). One domain that has particularly benefited from this is the functional study of white matter in the brain. While there is emerging evidence that the white matter holds meaningful BOLD signals (Gore et al. 2019; Li et al. 2019), it is too weak in comparison to the grey matter. Yet, the white matter plays a major role in cognition: it is the structural foundation of the functional networks classically observed in grey matter (for a review see (Thiebaut de Schotten and Forkel 2022)). Assessing the functional involvement of the white matter in cognitive processes is thus crucial to better understand the brain and to shed light on how diseases and damage to the white matter affect it. Traditionally, this aspect has mainly been investigated through lesion-symptom mapping, correlating cognitive deficit with the location of brain lesions. In this framework, and more generally in the study of brain connectivity, the white matter fibers are classified among three groups: association, commissural, and projection fibers (Catani and Thiebaut de Schotten 2012). The association fibers correspond to the axons connecting different parts of the cortex in the same hemisphere; the commissural fibers correspond to the axons connecting the cortex of the two hemispheres (crossing the interhemispheric fissure); and projection fibers correspond to axons connecting the cortex with subcortical structures. This categorization of the brain connections reflects the functional organization of the cognitive networks they support and is very useful in the description and clinical study of these networks (Aralasmak et al. 2006; Catani and Thiebaut de Schotten 2012; Hasan et al. 2010).

Yet, relying solely on the clinical approach to study the function of the white matter greatly limits our insights into the subject as symptoms cannot reflect all the brain's cognitive processes. By undertaking a multimodal approach and complementing the grey matter functional information with structural connectivity derived from tractography, it is possible to map cognitive processes onto the white matter of the healthy human brain. In this regard, we recently introduced the Functionnectome (Nozais et al. 2021), a structural-functional method – and an open-source program – that combines an fMRI acquisition with whole-brain, tractography-derived, white matter connectivity data. It has been successfully applied to task fMRI (t-fMRI) – mapping white matter domains linked to specific cognitive tasks – and to resting-state fMRI (rs-fMRI) – mapping the white matter and grey matter joint contribution to resting-state networks (Nozais et al. 2023).

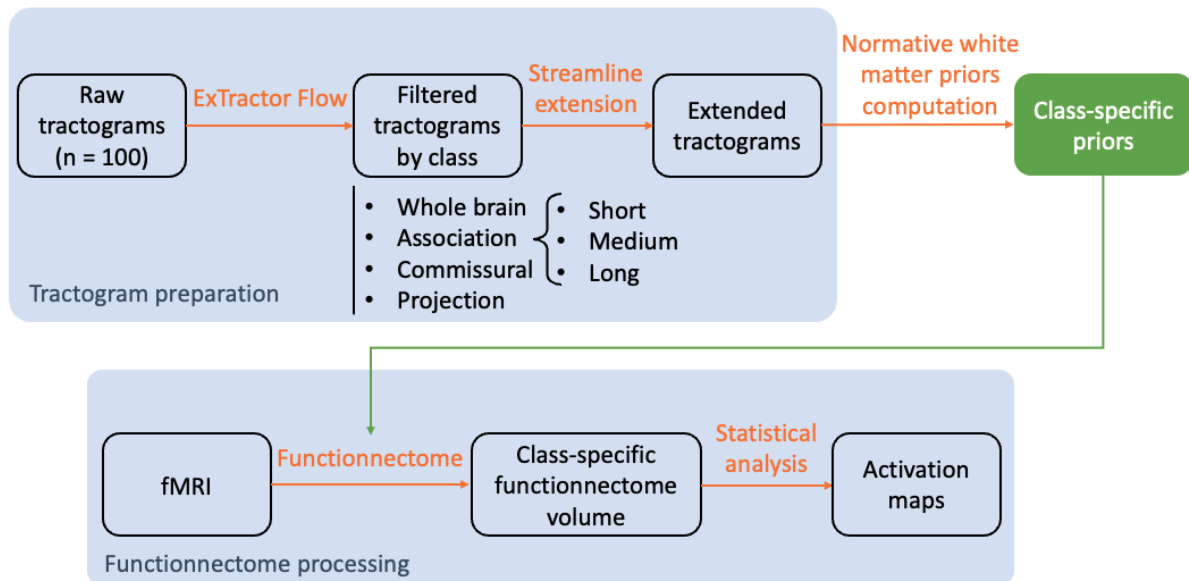
However, integrating fMRI and tractography data is not trivial, and multiple factors may impact the results and their interpretability. Among them, three potential pitfalls stand out: 1) By essence, current tractography algorithms do not reach deep into the grey matter, usually stopping streamlines at the boundary between white and grey matter. However, properly associating streamlines with the grey matter voxel at their ends is crucial to effectively combine the grey matter functional signal with the related white matter connectivity. The resulting structural-functional relationship between the two could thus be underestimated if the streamlines do not fully reach the grey matter. 2) The structural-functional integration in white matter areas with crossing fibers can be problematic. In these regions, a single voxel can be structurally connected to several functional networks with widely different cognitive roles, effectively reducing its apparent involvement with any specific cognitive process (i.e., locally lowering the signal-to-noise ratio in the functionnectome volumes). 3) Probably the most important point lies in the interpretability of the brain maps derived from structural-functional integration. Indeed, using whole-brain tractograms to estimate the structural connectivity necessarily leads to maps involving the whole studied networks without distinctions between which fiber tract is being activated. This is a problem when these tracts are close or even overlapping, as it would lead to ambiguity regarding the architecture of the studied functional network.

In the present study, we explored the effect of these three points on structural-functional analysis using the Functionnectome. More specifically, we first implemented and tested a procedure to modify existing streamlines by adding short segments at their ends to reach deeper into grey matter and help improve the association between grey and white matter. Second, we adopted the framework mentioned above, categorizing the connectivity data by fiber class (association, commissural, and projection fibers). By filtering the tractograms using these categories, we could tackle the two remaining issues together: it reduced the occurrence of fiber-crossing areas and allowed the examination of the cognitive networks across their different components (and fiber tracts) separately.

The results significantly improved the Functionnectome priors and the analysis workflow. They also offer some general advice for the field of structural-functional analysis.

## Methods

The processing and analysis workflow is summarized in Fig. 1 below.



**Figure 1:** Graphical summary of the processing and analysis. In orange, each processing step. In green, the new priors now provided with the Functionnectome to improve the integration of functional and structural information on the white matter. Here, “class” refers to the classes of white matter fibers included in the tractograms (as listed below the “Filtered tractograms” box). “n = 100” denotes the number of individual tractograms used to generate the priors.

### Dataset, acquisition parameters, and preprocessing

The dataset used in this study comes from the young-adult cohort of Human Connectome Project (HCP) (Van Essen et al. 2013). It consists of 3T MRI scans with anatomical, diffusion (dMRI), and functional (fMRI) MRI acquisitions. The anatomical and diffusion scans are from a subset of 100 randomly selected participants and were used to generate normative white matter priors (containing structural connectivity information) used in the Functionnectome analyses. The functional scans are from a subset of 46 participants, the same participants as the one used in the original Functionnectome paper (Nozais et al. 2021) and independent from the 100 participants used for the priors. The HCP data was acquired by the WU-Minn Consortium with IRB approval, all participants gave their informed consent, and the WU-Minn HCP Consortium Open Access Data Use Terms were respected in the study.

The detailed acquisition parameters for each scan can be found on the HCP website (<https://www.humanconnectome.org/hcp-protocols-ya-3t-imaging>) and were discussed in an HCP article (Ugurbil et al. 2013). We briefly summarize them here.

**Anatomical scans:** T1-weighted (T1w) acquisitions (3D MPRAGE), with TR of 2400 ms, TE of 2.14 ms, T1 of 1 ms, flip angle of 8°, 0.7 x 0.7 x 0.7 mm<sup>3</sup> isotropic voxels, and a bandwidth of 210 Hz/Px.

**Diffusion scans:** Spin-echo EPI (Echo-planar imaging) acquisitions, with TR of 5520 ms, TE of 89.5 ms, flip angle of 78°, 1.25 x 1.25 x 1.25 mm<sup>3</sup> isotropic voxels, a Multiband factor of 3, echo spacing of 0.78 ms, bandwidth of 1488 Hz/Px, and three shells of b-values of 1000, 2000, and 3000 s/mm<sup>2</sup>. In total, 90 diffusion directions were acquired per shell and 18 b=0 images for a total of 288 diffusion-weighted images.

**Functional scans:** Gradient-echo EPI acquisitions, with TR of 720 ms, TE of 31.1 ms, flip angle of 52°, 2 x 2 x 2 mm<sup>3</sup> isotropic voxels, Echo spacing of 0.58 ms, and bandwidth of 2290 Hz/Px.

All the data were downloaded from the HCP website in their preprocessed form. This preprocessing was done using the HCP's Minimal Preprocessing Pipelines (MPP) (Glasser et al. 2013). Briefly, the structural preprocessing consisted of linear and non-linear registration to a standard MNI space, and a brain segmentation using FreeSurfer (Reuter et al. 2010); the dMRI preprocessing consisted of b0 intensity normalization, correction of EPI distortions, correction of Eddy currents and motion effects, correction of gradient non-linearity, and a registration to the T1w image in the participant's space; the fMRI preprocessing consisted of gradient distortion correction, motion correction, EPI distortion correction, and registration to MNI space.

## Tractography

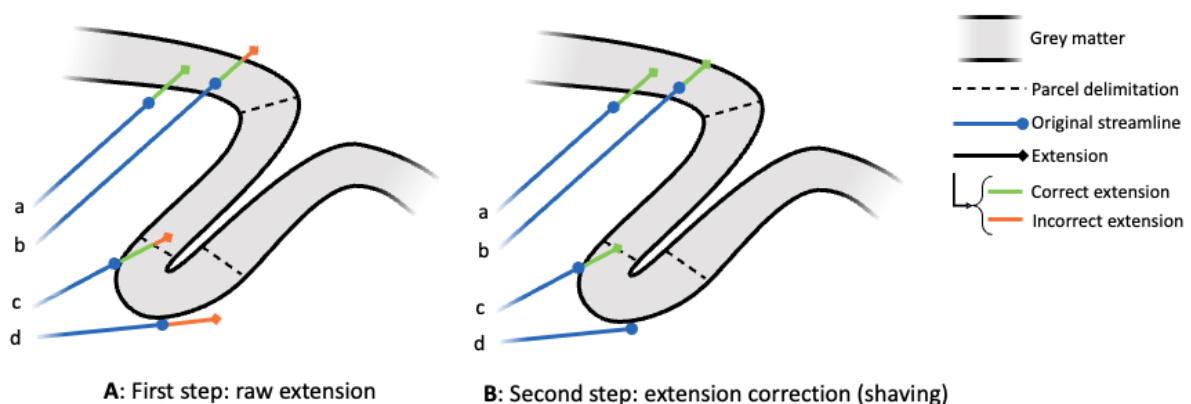
The tractography was performed using a probabilistic tractography approach. We used TractoFlow (Theaud et al. 2020), an automated dMRI processing pipeline for probabilistic tractography. TractoFlow was run with its two tracking algorithms: a classical local tracking and the Particle Filter Tracking (PFT) (Girard et al. 2014). The streamlines generated by the two probabilistic tracking algorithms were combined to form each whole-brain probabilistic tractogram. Most of the settings used for the tracking were the default settings proposed by TractoFlow: the shape factor for the elongated symmetric diffusion tensor of the FRF was  $1.5 \times 10^{-3} \text{ mm}^2 \cdot \text{s}^{-1}$ ; spherical harmonics order was 8; all the shells were used (b = 1000, 2000, and 3000) for the fODF; step-size was 0.5 mm; and maximal angle between steps was 20°. The seeding was done on the whole white matter, with 10 seeds per voxel for the PFT and 5 seeds per voxel for the local tracking. Each tractogram was then registered to the MNI space by applying the linear and nonlinear transformations used to register the anatomical scan.

## Streamline extension

When combining volumetric grey matter data with tractography, a significant risk lies in the streamlines not properly reaching the grey matter voxels. More specifically, the stopping criteria of the tractography algorithm (i.e., the criteria defining when and where a streamline should stop) may not allow the streamlines to reach deep enough into the grey matter. Note that we are not discussing the challenge of

properly connecting the whole grey matter using tractography. This topic and the related gyral bias (Rheault et al. 2020; St-Onge et al. 2018) are mentioned in the discussion but they go beyond the current proposed improvements. In the present study, we focus on the termination location of streamlines and their relation to structural-functional coupling. Indeed, in methods combining grey matter functional and tractography (like the Functionnectome), this coupling could be underestimated if the streamlines stop before properly reaching the grey matter voxel they should be related to. To test this effect, we devised a simple post-hoc lengthening procedure that was applied on each tractogram, adding up to 5 mm at each end (depending on local grey matter thickness), effectively allowing the streamlines to fully reach the grey matter if it was close to its termination points. The extension procedure is as follows: all streamlines are linearly extended by 5 mm at both ends in the same direction as the streamline’s ending segments; if the extension doesn’t reach the grey matter, the extension is canceled; if the extension reaches the grey matter but changes area (as per the FreeSurfer grey matter parcellation) or gets out of the grey matter, the extended part is cut, keeping only the part in the first grey matter area (Fig. 2). This last point reduces the risk that the procedure extends the streamline to a different functional domain. The code for this extension method is the “scil\_tractogram\_GM\_extension.py” script available as part of a branch of scilpy: <https://github.com/NotaCS/scilpy>.

Such a procedure is similar to tractography toolboxes when building connectomes, like the tck2connectome function from MRtrix3 (Smith et al. 2015; Tournier et al. 2019). However, the current method differs from these approaches as they usually only allow the association of a streamline to a whole grey matter region, while our method was specifically tailored to our needs by associating the streamlines to the grey matter voxels they reach. It was necessary for testing the effect of such extension on Functionnectome analyses.



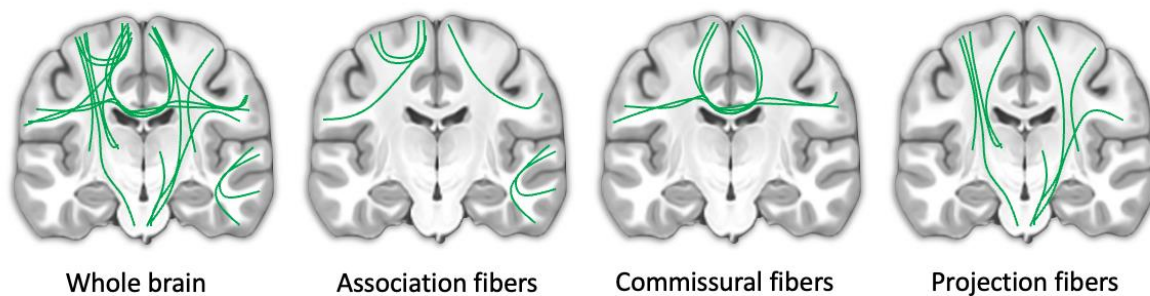
**Figure 2:** Streamline lengthening procedure. **A:** All the streamlines are lengthened by a specific amount (5 mm here). **B:** The parts of the extension considered incorrect are removed. **a:** Correct extension. **b:** Part of the extension leaves the grey matter. **c:** Part of the extension crosses to another grey matter parcel. **d:** The extension doesn’t reach the grey matter.



## Streamline filtering and categorization

When combining functional information directly on the white matter using connectivity information from whole-brain tractography, as the Functionnectome does, two potential issues arise: First, the resulting activation maps do not differentiate between fiber tracts, which limits our ability to interpret the data and precisely identify the tracts involved in the circuit. Second, whole-brain tractograms present crossing-fibers regions (i.e., regions of the brains where multiple white matter tracts, part of different functional networks, intersect). Using structural-functional combination methods in these areas can mix unrelated functional signals, artificially lowering the local signal-to-noise ratio and thus the ability to precisely estimate the involvement of the white matter in specific cognitive functions.

To circumvent these problems, the streamlines in the whole brain tractogram underwent a filtering process using a method called ExTractor\_flow. This method, described comprehensively in (Petit et al. 2023), automatically classifies the streamlines as either anatomically plausible or implausible. We then utilized ExTractor\_flow to first isolate the anatomically plausible streamlines and further categorized them into three distinct types: association, commissural, and projection fibers (Fig. 3). To further reduce the occurrence of crossing fibers, association streamlines were also split into three length categories: “short fibers” with streamlines below 40 mm in length, “medium fibers” with streamlines between 40 and 80 mm, and “long fibers” with streamlines longer than 80 mm (Schüz and Braitenberg 2002; Shastin et al. 2022).



**Figure 3:** Schematic illustration of the three different classes of white matter fibers (and streamlines). Association fibers link different parts of the same hemisphere. Commissural fibers link the two hemispheres. Projection fibers comprise all the fibers connected to subcortical structures and the brainstem.

## Functionnectome, white matter priors, and activation maps

To evaluate the effect of splitting the tractograms by fiber class on a structural-functional analysis, we used each set of tractograms in Functionnectome analyses (Nozais et al. 2021). The whole procedure is described in the original paper, but we summarized it below. First, the white matter priors are computed using the tractograms ( $n = 100$ ). These priors correspond to maps, one per brain voxel, giving the

probability of structural connectivity from this voxel to the other brain voxels. These priors can then be used by the Functionnectome program (available at <http://www.bcblab.com>) to project grey matter BOLD signals onto the related white matter. Essentially, for a given white matter voxel, the Functionnectome uses the structural connectivity information from the white matter priors to do a weighted average of the BOLD time-series from the grey matter voxels connected to the said white matter voxel. This results in a new functional volume (with 4 dimensions), with functional time-series on the white matter voxels, that can then be statistically analyzed in the same way as a classical fMRI volume. Here, we used the Functionnectome program to project the signal of two task-fMRI datasets onto the white matter (finger-tapping dataset,  $n = 46$ ; working-memory dataset,  $n = 45$ ). The functionnectome volumes of each participant were then statistically analyzed by generalized linear modeling (GLM) to reveal the corresponding activation maps. Both first- and second-level analyses were done using FEAT (FMRI Expert Analysis Tool, v6.00), from the FMRIB Software Library (FSL), yielding a group-level activation z-map per task.

In the present study, we created one set of white matter priors per set of tractograms, for a total of eight sets of priors, namely: two whole-brain sets, extended and non-extended; four association extended sets (all fibers, and separately short, medium, and long association extended fibers); one commissural extended set; and one projection extended set. Together, the association, commissural, and projection priors are referred to as the *split* priors, in opposition to the *whole-brain* priors. Note that all the *split* priors we generated with tractograms that underwent the streamline-extension procedure. The functional analyses described above (for the two functional datasets) were repeated for each of these 8 sets of priors.

## Maps analyses and statistics

All the activation z-maps were analyzed and displayed after applying a white matter mask to keep only white matter voxels, and using an arbitrarily high threshold of  $z = 3$  (equivalent to  $p < 0.0013$ , uncorrected). We kept this uncorrected threshold to make the displayed map clearer and easier to read, as applying a false discovery rate correction did not change the significance of the results with the chosen threshold. The white matter mask was defined as the voxels attributed to white matter in at least 10% of the study's participants. All the presented maps are z-maps, displaying voxelwise z-scores for the task activation being studied. The slices shown in the results were selected to display the main white matter functional pathways revealed by the Functionnectome activation mapping. The unthresholded z-maps are freely available on Neurovault (<https://identifiers.org/neurovault.collection:13538>).

To compare the results obtained from using whole-brain priors and those obtained using split priors, the corresponding z-maps (association, commissural, and projection) were combined, keeping, for each voxel, the maximum z-score across the three maps. These maps are referred to as the z-max maps. Similarly, the activation z-maps generated using the three association priors split by length (short, medium, and long) were recombined in the same manner to create association z-max maps. Note that

the whole-brain z-max map mentioned above uses these association z-max maps instead of the basic association z-map.

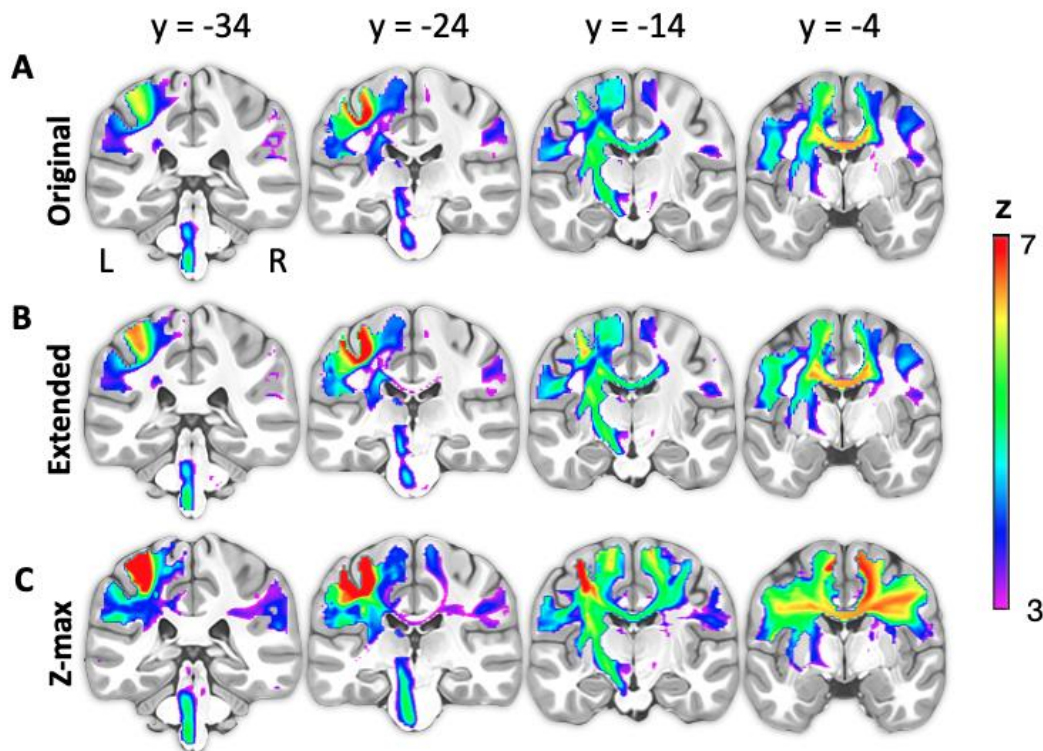
The statistical comparison between the maps was done on the voxels with z-scores above 3 in at least one of the maps, to only compare regions that are considered activated. The p-values displayed are the results from the two-sided paired t-test on the selected voxels.

## Results

### Enhancement of the activity detection in the white matter

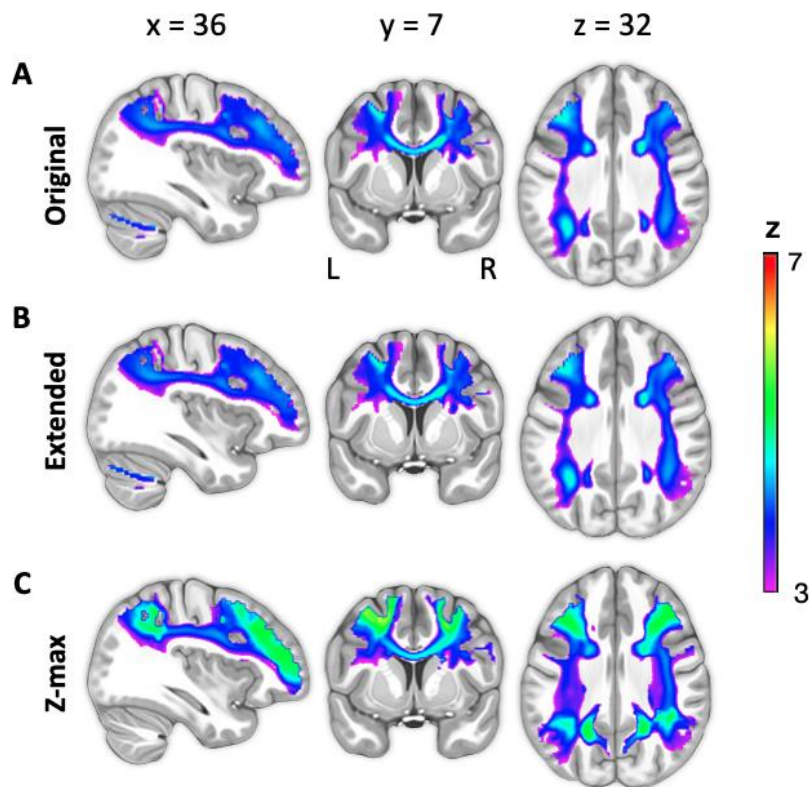
We first explored the effect of extending the WM streamlines in the GM on the structural-functional analysis. We therefore analyzed the involvement of the white matter in the right-hand finger-tapping task by contrasting the activation maps from the Functionnectome analysis using the priors generated with and without streamline extension. The resulting maps (Fig. 4 A-B) showed that using the extension method significantly improved the statistics of the activated regions (i.e., with  $z > 3$ ), resulting in an average increase of 12% ( $p < 0.0001$ ) of the z-values across the map. The analysis was repeated with the working-memory dataset (Fig 5 A-B), which also demonstrated a significant increase of 4% ( $p < 0.0001$ ) in z-values across the map when using the extended tractograms.

We interpreted these increases as proof that using our simple method to extend the streamlines improved the structural-function coupling in the analysis. As a result, we used the extension method in all the following analyses.



**Figure 4:** Effect of streamline extension and fiber categorization on the group-level functionnectome z-map of the right-hand finger-tapping task. **A:** Z-map generated using whole-brain priors from non-extended tractograms. **B:** Z-map generated using whole-brain priors from extended tractograms. **C:** Z-max map generated by recombining the z-maps obtained from using the priors split by fiber class.

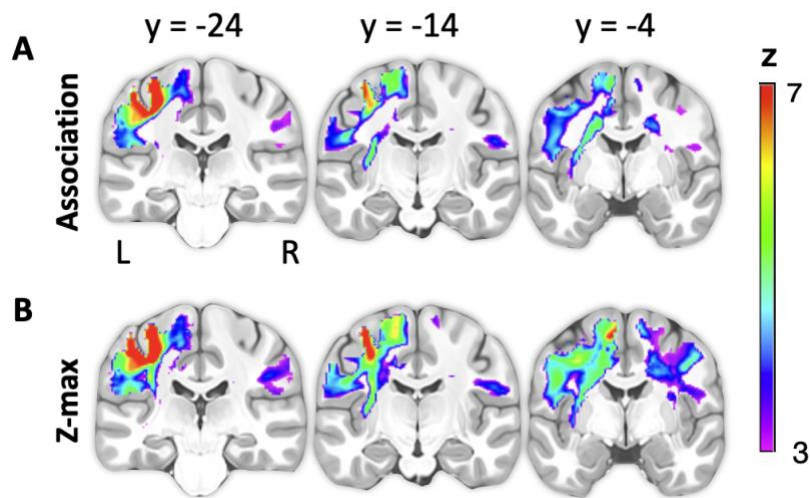
While the increase in z-values from the extension procedure is relatively modest, the improvement from splitting the white matter priors and recombining the activation maps are quite striking. First, compared to the z-maps from the extended priors, we note an 83% increase ( $p < 0.0001$ ) in the mean z-values of the z-max map for the finger-tapping task (Fig. 4 B-C), and a 31% increase ( $p < 0.0001$ ) for the working memory task (Fig. 5 B-C). The z-max map from the finger-tapping task also shows a clear recovery of the signal in areas of crossing fibers, very distinct from the disappearance of the “gaps” in the activation map. This is especially obvious around the *centrum semiovale*, where the superior longitudinal fasciculus (SLF, not involved in the task) intersects with callosal fibers and the cortico-spinal tract (both involved in the task).



**Figure 5:** Effect of streamline extension and fiber splitting on the group-level functionnectome z-map of the working memory task. **A:** Z-map generated using whole-brain priors from non-extended tractograms. **B:** Z-map generated using whole-brain priors from extended tractograms. **C:** Z-max map generated by recombining the z-maps obtained from using the priors split by fiber class.

Likewise, when focusing on the split priors for the association fibers, splitting them again by length category allowed reducing further the effect of the remaining crossing regions. In the z-maps from the finger-tapping task (Fig 6), using the z-max approach with the three split association priors increases the z-values by 103% on average ( $p < 0.0001$ ). For the working-memory task, we observe a 34% increase on average ( $p < 0.0001$ ) with the z-max approach. Also, similarly to what is shown above with the whole-brain z-max map on the finger-tapping task, the association z-max map displays a recovery of the

activation in fiber-crossing regions, filling the visible gap where the SLF intersects with shorter association fibers.



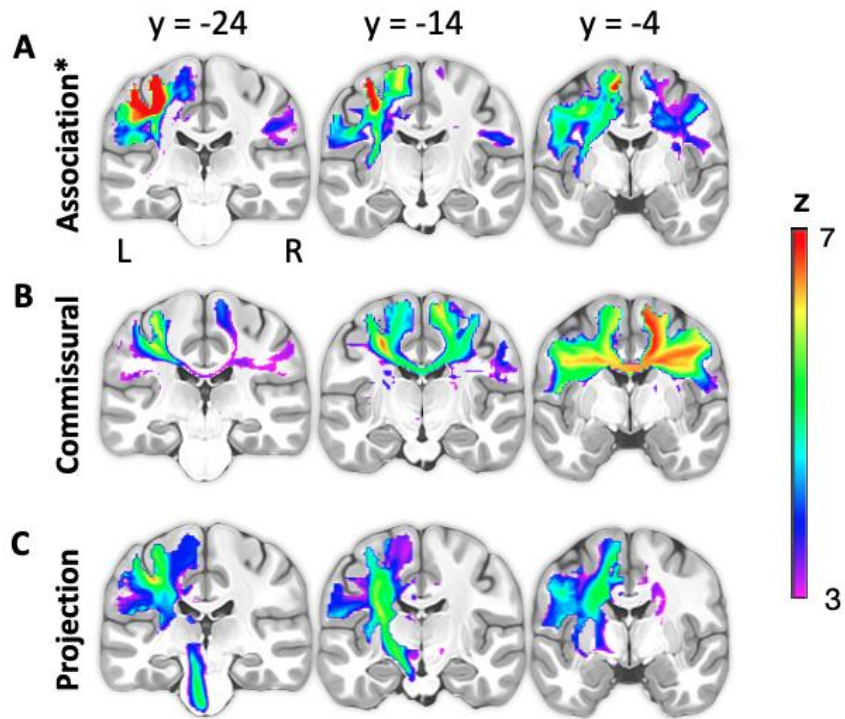
**Figure 6:** Effect of categorization by the length of association fibers on the group-level fonctionnectome z-map of the right-hand finger-tapping task. **A:** Z-map generated using association priors. **B:** Z-max map generated by recombining the z-maps obtained from using the association priors split according to fiber length (short, medium, and long association fibers).

## Clarifying the structure of the networks

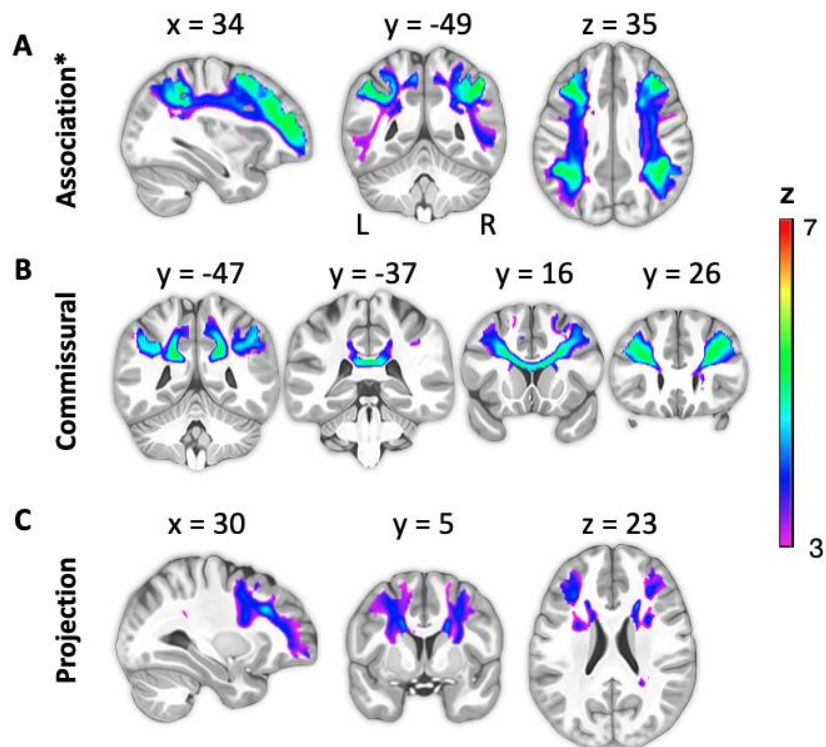
Using the split priors also allows us to differentiate between the functional integration mediated by different classes of fibers and, thus, different tracts. In the case of the finger-tapping task (Fig. 7), using the split priors helps differentiate the functional involvement of multiple fiber tracts, both near grey matter hubs and in the deep white matter. For example, we can see that while most of the fibers involved in activations around the thalamic nuclei are from the projection category, some of this activity is also related to association fibers connecting the insula with the motor cortex. Similarly, this separation helps determine that the strong activation observed near the motor cortex from the left hemisphere is mostly related to association fibers.

Likewise, in the activation maps from the working-memory task (Fig. 8), we can better differentiate between the strong activations related to either the association or commissural fibers. Most interestingly, the split also reveals the involvement of the projection fibers, which is not obvious when using whole-brain priors.





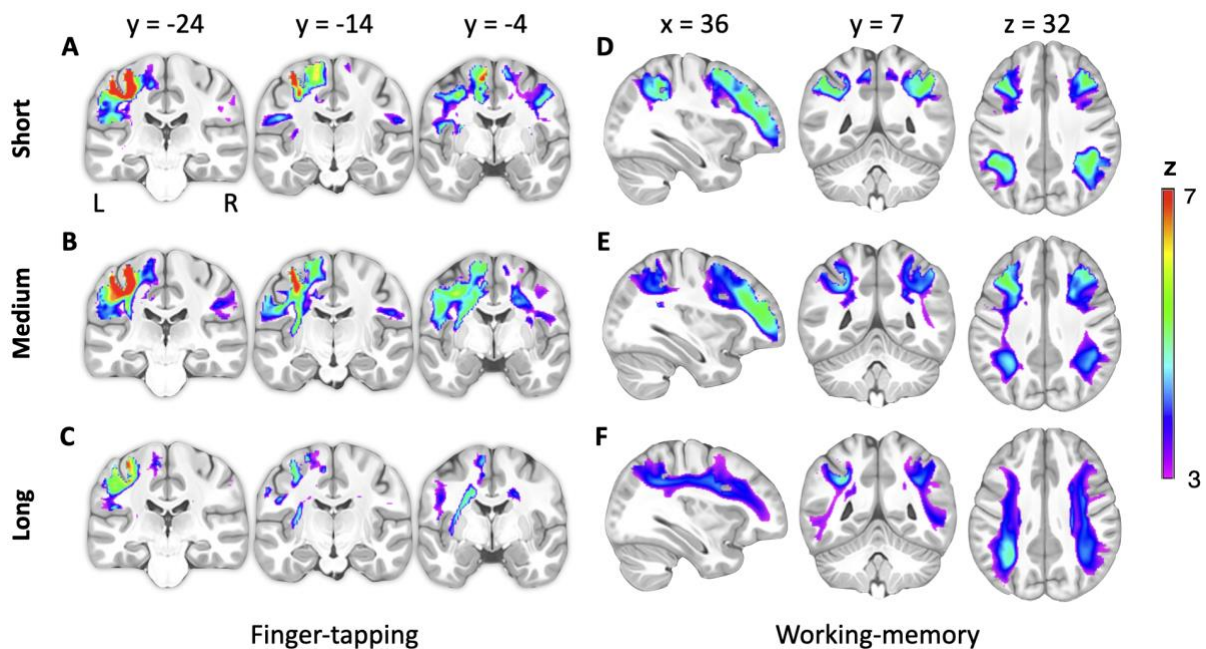
**Figure 7:** White matter activation maps for each class of fibers in the right-hand finger-tapping task. **A:** Z-max map generated by recombining the z-maps generated using the three sets of association priors (split by length), the asterisk (\*) indicates that this is a Z-max map, contrary to the other two maps. **B:** Z-map generated using commissural priors. **C:** Z-map generated using projection priors.



**Figure 8:** White matter activation maps for each class of fibers in the working-memory task. **A:** Z-max map generated by recombining the z-maps generated using the three sets of association priors (split by length), the asterisk (\*) indicates that this is a Z-max map, contrary to the other two maps. **B:** Z-map generated using commissural priors. **C:** Z-map generated using projection priors.

Similarly, to the split between fiber classes, splitting the association fibers by length category reveals the different scales in connectivity that govern the functional organization of the cognitive networks. In the case of the finger-tapping task (Fig. 9 A-B-C), we observe that most of the strong activation near the motor cortex is related to short and medium-length (U-shaped) fibers. Also, the connectivity of the insula to the other parts of the network is mostly mediated by medium-length fibers, but also in part by long association fibers.

As for the working-memory task (Fig 9 D-E-F), the split reveals the strong activations related to small and medium-length fibers, especially in the frontal lobe, likely driving the local integration of functional information. It also unveils the exact connectivity profile specifically related to the long-distance association in the network, with both fronto-parietal and parieto-temporal connectivity.



**Figure 9:** White matter activation maps for each length category of association fibers in the right-hand finger-tapping task and working-memory task. **A-B-C:** Finger-tapping Z-maps generated using priors for the short, medium, and long fibers category, respectively. **D-E-F:** Working-memory Z-maps generated using priors for the short, medium, and long fibers category, respectively.



## Discussion

We explored two approaches to significantly enhance structural-functional analysis to study the function of white matter and improved the Functionnectome framework accordingly. Our results highlight three main factors that influence the analyses and their interpretation: elongating the streamlines at the interface between grey matter voxels and white matter tracts improves the structural-functional integration; splitting the fibers by category upstream of the analysis, then recombining the resulting maps vastly increase the ability to detect the involvement of the white matter in cognition; and the same split also strikingly clarify the structural-functional architecture of the studied networks. Moreover, these improvements should be equally applicable to other structural-functional methods (besides the Functionnectome), which would give our approach a global impact on the domain.

Structural-functional analyses in neuroimaging, combining cortical functional information and tractography data, have been successfully used to explore the structure-function relationship between the brain's grey and white matter. However, the association between the two is not a trivial step, and two notable approaches have been used. One consists in using functionally defined ROI as a seed for the tractography (Javad et al. 2014), while the other consists in doing whole brain tractography and then selecting the streamlines terminating in or near the studied grey matter areas (Wang et al. 2015). The method defining the termination point of the streamlines also plays a major role in shaping the association between streamlines and grey matter voxels. Here, three main methods exist: a streamline can stop when it reaches the edge of a predefined white matter mask (Theaud et al. 2020); it can stop when it reaches a voxel associated with specific diffusion metrics (e.g., fractional anisotropy lower than a given threshold) (Thiebaut de Schotten et al. 2011), or can be guided to the cortical surface by a geometrical model when approaching the grey matter (St-Onge et al. 2018; St-Onge et al. 2021). In each case, there is no guarantee that the streamline will actually fully reach the grey matter it should be linked to. Even in the case when streamlines are guided to the cortical surface, which will improve the estimation of cortical connectivity (St-Onge et al. 2021), the streamlines may not reach all the functionally related grey matter voxels. This is especially true for voxels on the outer side of the cortex or deep inside subcortical nuclei.

In our study, we showed that a simple elongation of the streamline (constricted by grey matter boundaries) is enough to correct this bias and to significantly improve the results of a structural-functional analysis. While the increase in the significance of the activations was somewhat modest, it should be noted that the tractography used in the present study was very “aggressive”, attempting to maximize the estimation of the brain connectivity by generating a vast number of streamlines. It is entirely possible that the streamline-extension procedure would have a much stronger effect on less dense tractograms. This method has thus the potential to become a standard step applied to tractograms when used in this framework. It should be noted, however, that for the same region the connectivity changes according to the layer of the cortex studied (Pandya et al. 2015). While such estimation is

beyond the resolution available nowadays, future endeavors based on higher resolution diffusion weighted imaging datasets should adapt streamline elongation to the anatomical principles of the cortical architecture. Additionally, to ensure a proper streamline elongation, the orientation of the terminal segments of a streamline must be correct. This may not always be the case, as can be illustrated by the existence of the gyral bias (Schilling et al. 2018). Ideally, this elongation step should be part of the tractography itself and coupled with a method that ensures the proper orientation of the streamlines (*e.g.*, (St-Onge et al. 2018; St-Onge et al. 2021) with surface-enhanced tractography) and not done post-hoc.

The other avenue we explored to improve structural-functional analyses was to split the analysis by fiber class, independently studying association, commissural and projection fibers. Depending on the method used to perform the structural and functional integration, a detection bias may arise from fibers crossing and mixing unrelated functional signals together. Splitting the analysis by fiber category and reassembling the resulting maps can correct this bias. In the case of the Fonctionnectome analysis, which directly combines the functional information from grey matter sources onto white matter voxels, we showed that this correction effect is present and very strong. Here, splitting the analysis enabled us to both fill the gap in the activation maps located in regions of crossing fibers, and to increase the significance of the studied activation globally. These two points are very beneficial from a technical standpoint and a clear improvement in our ability to map the involvement of white matter in cognitive processes fully. Nevertheless, the aspect that may be the most important when splitting the analysis by fiber category is the ability it gives us to disentangle different fibers' roles in a white matter activation. Indeed, each set of split priors, whether by fiber class or fiber length, revealed details about the organization of the studied cognitive circuits, which would have been obscured in the analyses using whole-brain priors. For example, the working memory task revealed projection fibers connecting the frontal lobe with thalamic nuclei. This activity was effectively hidden by the cortico-cortical parts of the circuit in the whole-brain analysis, and is extremely interesting, especially in the light of the mounting evidence of the involvement of the thalamus in working memory (Piras et al. 2010; Roy et al. 2022; Watanabe and Funahashi 2012). Additionally, the short association fibers could be used as a proxy for U-shaped fibers, which are receiving increasing attention for their role in cognitive processes (Ouyang et al. 2017; Schilling et al. 2023; Shastin et al. 2022) and thus could unlock new investigations, especially in healthy participants.

We expect the improvement derived from splitting the tractograms to be generalizable to other volumetric structural-functional methods that integrate information directly on white matter voxels, such as track-weighted imaging (Calamante 2017) or white-matter interpolation of fMRI (Tarun et al. 2020). However, fiber crossing can still happen in split tractograms, especially with association fibers (*e.g.*, the SLF crossing the frontal aslant tract). To further improve this aspect, one conceivable approach would be to use an approach akin to using fixels (Dhollander et al. 2021) instead of voxels in the analysis. With a type of volumetric data that keeps the information about the direction of the fibers from

which the signal was extracted, it should help separate unrelated signals when integrating them onto the white matter, which in turn would remove the need to split the tractograms. However, this type of analysis would require a complete remake of the current methods, is more complicated to implement, would likely be much more computationally intensive, and its results would definitely be harder to interpret.

The seven sets of extended priors (whole-brain, association, commissural, projection, and short-/medium-/long-association fibers), are all freely available online and are now part of the updated Functionnectome. These new priors, and especially the split priors, will help disentangle the involvement of different fiber tract populations in cognitive functions. The priors can be easily selected and downloaded from the Functionnectome interface, then the analysis has to be run separately for each set of priors.

## **Conclusion**

We showed that structural-functional analyses combining fMRI and tractography can be dramatically improved through two relatively easy-to-implement steps: By ensuring a proper reach of each streamline in the grey matter and by grouping streamlines into classes that minimize streamline crossings. In the present study, we used the Functionnectome as an example of structural-functional analysis, but we believe our results are generalizable to many other similar methods and could thus help improve results obtained in this field. All the improvements presented here are now part of the Functionnectome toolbox and are freely available online.

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