*Additional Supplemental Material for “****A high-performance computing voxel-based analysis pipeline for the rodent brain with a formal validation framework.”***

***S1. Details of the VBA Pipeline***

We present an automated pipeline for VBA, designed to handle multi-modal data, which may or may not need to be co-registered. In practice, the multiple contrasts provided by DTI are most often used. The three types of input required by the pipeline are the images, the matrix of predictors, and the headfile. The latter two of these merit further discussion here.

*S1.1. Matrix of predictors*

While an ideal pipeline would be fully automated, certain aspects can be problematic in the absence of human judgment. In anticipation of this, we have designed the format of the inputs to help mitigate interruptive processes. The user can include custom pre-processing options for individual subjects by way of the matrix of predictors. A simple example of this is image orientation, to account for occasional differences in specimen positioning within the imaging device. It is difficult for rigid registration to overcome initial rotational mismatches greater than 90°, and these must be handled during the data cleaning stage. A pre-pipeline quality assurance review of the input is recommended, so that outliers can be visually identified and noted as such in the matrix of predictors (to be rotated during Stage 1). Additionally, subpar performance during skull-stripping and masking can critically affect all subsequent results. The parameters of the histogram-based stripping algorithm can be tuned on an individual basis during quality assurance and passed via the matrix.

*S1.2. Input headfile*

Quality assurance of the inputs helps minimize user effort by dealing with potential points of failure before occupying significant resources. An extensive check is performed when the pipeline is initialized to ensure that all files needed throughout the process are available, and in the proper format. Furthermore, each module will inspect all required variables for validity and consistency. Any of these that are not specified in the headfile are set to the default value, the user being notified in each instance. In general, one only needs to specify a handful of these to effectively run the pipeline. However, any of them can be set to a custom value, providing additional flexibility. Once the check runs to completion, any errors found will kill the process and be reported to the user in tabulated form, allowing them to correct as many issues as possible before restarting the pipeline. Otherwise Stage 1 will be initiated if no errors are found. The headfile will dynamically evolve as the pipeline runs and is written to a separate file after every stage for both recordkeeping and troubleshooting purposes.

*S1.3. Stage 1: Data Cleaning and Preparation*

Preprocessing ensures all input images and external atlases are consistent. Typically, the header information of the input images will be modified such that the origin is in the center of the data, and the data itself can be rotated to the desired orthogonal orientation. The skull and other extraneous tissues surrounding the brain can be stripped using an algorithm that produces an initial mask created via histogram thresholding, and then refines it through dilation and erosion ([Badea et al., 2007](#_ENREF_2)). These individual masks can be applied to the corresponding images, or can be used as a target to which an atlas-based mask is affinely registered. In this case, the atlas mask is transformed according to the resultant warps and is used instead. If multiple, non-coincident contrasts are used, then one is chosen as a fixed target and all other contrasts are affinely registered to the target on a per-subject basis. The masking and co-registration steps can be performed in whichever order is most appropriate for the contrasts involved. Bias-field correction, when necessary, is performed by calculating the correction on the unmasked image with *N4BiasFieldCorrection* ([Tustison et al., 2010](#_ENREF_7)), applying the correction and running the masking algorithm, and then running *N4BiasFieldCorrection* again.

The final preprocessing step is to set a global reference space (GRS) such that all pipeline images have the same bounding box, voxel spacing, and array size. Here, the bounding box and voxel spacing are used to uniquely identify the GRS. The GRS is indirectly specified by the user and can be derived from any of the images in the study (“native GRS”), a standard atlas such as Waxholm Space (WHS) ([Johnson et al., 2010](#_ENREF_4)), or an arbitrarily specified image. Any image inconsistent with the GRS is appropriately converted by applying an identity matrix transform via the ANTs antsApplyTransforms command using the GRS-defining image as the reference for the output. If the voxel spacing is that of the GRS, nearest neighbor interpolation is used to avoid unintentional smoothing that might occur due to half-voxel re-gridding. If there are images in a bounding box larger than the GRS, then a rectangular mask will be created in the middle of a GRS image, and an *antsRegistration* call will be initialized between this centered dummy image and the dissimilar image with an initial alignment based on image intensities. The resulting translation matrix will be applied instead of the identity matrix to ensure that these images are centered as best as possible before being confined to the bounding box of the GRS.

*S1.4. Stage 2: Rigid Alignment to Atlas*

In many cases, it is advantageous to produce images in an established coordinate system or frame of reference, such as Waxholm space ([Johnson et al., 2010](#_ENREF_4)). The user has the option to specify a standard atlas or other arbitrary image, which is used as the target for rigid alignment of all co-registered sets of image. The only degrees of freedom allowed are rotation and translation; any scaling or sheering could artificially bias the results towards the target image and is reserved for Stage 3. The transform matrices are not immediately applied but rather used to initialize future stages of the pipeline. When transforming an image, all transforms and/or warps are applied at once to avoid unnecessary floating-point errors that would arise from applying them serially ([Avants et al., 2011](#_ENREF_1)). If no external alignment is desired, this stage uses identity matrices for all co-registered sets.

*S1.5. Stage 3: Affine Target Creation and Alignment*

Non-linear registration is the most resource-intensive process in the pipeline, and poor initial alignment can result in a dramatic increase in the number of iterations required for convergence. It is imperative that bona fide affine alignment has been achieved before beginning such processes. Full affine registration, including sheering and scaling, is performed between each co-registered set and the selected target. By separating the affine registration from the rigid registration of Stage 2, global volumetric differences between the study’s subjects are isolated. This is useful when performing Label-Based Analysis (LBA, Stage 6).

*S1.6. Stage 6: Segmentation and Label-Based Analysis*

Although VBA is the primary goal of the pipeline, label based analysis (LBA) is achieved at relatively low computational cost. One method of automatically generating a label field for a given anatomical structure is finding a set of transforms that relate the native space of the subject in question to that of a high-quality atlas that has been manually labeled ([Badea et al., 2007](#_ENREF_2); [Johnson et al., 2010](#_ENREF_4)). The atlas label field can then be aligned to the subject by applying these transforms and/or their inverses. At this point, registering the MDT to the desired atlas is all that is needed to complete the transform chains. The only prerequisite is that the MDT exists, and so after an affine registration between it and the atlas, it is virtually identical to the diffeomorphic registration processes between the non-MDT subjects and the MDT, and can be run in parallel with that group of jobs. In practice, this rarely adds to the time needed for the pipeline to complete, yet provides a substantial benefit.

Once the appropriate affine and diffeomorphic atlas-MDT transforms have been calculated, it is trivial to produce label fields for all subjects and the MDT. A MATLAB script is used to calculate a variety of image parameters for each label within each subject. Further study-wide statistics are then computed in conjunction with the matrix of predictors. The MDT labels serve several purposes. First, it should be recognized that the MDT is a study-specific atlas. One can convert the MDT into a fully functioning labeled atlas by manually correcting those imported from the external atlas. It is also practical to have the VBA results displayed on top of the MDT labels whilst interpreting them. There are three notable options for the space of the label fields: the original “pre-rigid” space of each subject, the common “post-rigid”/”pre-affine” space, and the “post-affine” ALT space, the latter of which is globally normalized. The pre-affine and post-affine options are achieved by respectively omitting the inverse rigid or rigid + affine components from the transform train when creating the labels. The forward versions of the unused transforms will then be applied to all the images to create a set over which the labels can be overlaid. Regions of Interest (ROIs) can be derived from the label sets for purposes such as calculating a structural connectivity matrix, or synthetically induce changes in structures of our choosing to create VBA phantoms.

*S1.7. Stage 7: VBA Pre-processing*

At this point, all images have been spatially normalized, the normalizing warps calculated, and MDT images for all contrasts have been produced. A binary mask is generated from the MDT of an appropriate contrast and morphologically eroded three voxels using the *ImageMath* command (ME). This will be applied during VBA analysis, primarily for the purpose of removing edge effects that might appear in the VBA results due to Gaussian smoothing. For VBM, the log-Jacobian is calculated by using the *CreateJacobianDeterminantImage* command using Subject-to-MDT as input. The log is used instead of the Jacobian itself to symmetrically linearize the quantities of volume change. Calculating the Jacobians that are induced by the “to-MDT” warps ensure that the resultant images are spatially normalized in MDT space, and that positive values correspond to voxel-wise increases in volume (relative to the MDT). This is consistent with previous work of similar nature such as [Lau et al. (2008)](#_ENREF_6). Lastly, the ANTs SmoothImage command smooths all image contrasts with a Gaussian kernel of 3 voxels. Other kernel sizes, in either voxels or mm, can be specified via the headfile. [Jones et al. (2005)](#_ENREF_5) showed that the matched-filter theorem must be kept in mind when smoothing images for VBA. If there is no prior knowledge of the expected spatial extent of the treatment effect, this parameter may need tuning. If one is unsure of the proper smoothing kernel size, several smoothing parameters can be specified and analysis will be done for each of them.

*S1.8. Stage 8: Voxel-based Analysis*

The software with which to perform VBA is left to the preference of the user. The pipeline supports simple analyses with SurfStat and ANTsR without user interaction. Two single-tailed t-tests are performed in opposite directions, i.e. with contrasts “Group *x* greater than Group *y”,* and vice versa. Statistical maps are generated for the *t*-value, uncorrected *p*-value, *q*-value, and effect size. The *q*-value is the result of the multiple-comparison correction using False-Discovery Rate (FDR) ([Genovese et al., 2002](#_ENREF_3)). If interactive analysis via SPM is desired, the images are sorted in their folders by contrast, and further by comparison groups.

**References**

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