Virtual definition of neuronal tissue by cluster analysis of multi-parametric imaging (virtual-dot-com imaging)

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Introduction

Cyto-architecture and myelo-architecture are histological features that reveal the microscopic arrangements of tissue compartments (cells and fibers) (Amunts and Zilles, 2001; Paxinos, 1990; Garey, 1999; Sakissov et al., 1955, Morel et al., 1997). Since Brodmann first used this approach in 1909 to segment the cortex into 52 histologically distinct regions (Garey, 1999), dozens of cyto- and myelo-architecture-based atlases defining fine structures in the brain have been published. Different staining methodologies, such as Nissl stain, myelin basic protein stain (MBP), calcium binding proteins stain and many others, provide contrast to the histological sections that allow differentiation between sub-regions (Amunts and Zilles, 2001; Paxinos, 1990; Garey, 1999; Sakissov et al., 1955, Morel et al., 1997). The histology-based atlases have been the basis for stereotactic brain surgeries, functional brain mapping references, as well as educational textbooks. Most of them are based on data from one subject despite the wide variability between subjects; thus, individual subject mapping is needed. The non-invasiveness, high resolution and sensitivity of magnetic resonance imaging (MRI) make it potentially useful for brain mapping for the individual subject. Indeed, combining high anatomical MRI with functional MRI has been used for some years now for pre-surgical brain mapping (Lundquist et al., 1997; Schuler et al., 1997).

Despite its advantages, many brain sub-structures (e.g. cortical layers, thalamus sub-nuclei structures) are hidden from conventional human MRI. In vitro high resolution MRI has been successful in detecting the cortical layers arrangement (Bendersky et al., 2003; Barbier et al., 2002; Fatterpekar et al., 2002; Kruggel et al., 2003), but the long scan time required for such high resolution images prevents it from being implemented in vivo. Resolution and contrast limits of MRI appear to be the main factors restricting our ability to segment and define certain tissues in vivo. While typical human brain image resolution lies in the order of 1–2 mm, many central nervous system structures are much smaller than that. In addition, contrast differences within a specific region might be close to noise level, preventing accurate definition of sub-regions within it.

MRI’s greatest advantage lies in its multi-contrast modality, the most conventional of which are the T\textsubscript{1}, T\textsubscript{2} and T\textsubscript{2*} relaxation mechanisms (Stark and Bradley, 1999). Brain tissue will appear differently in each of these contrasts: e.g. white matter will appear hypointense on a T\textsubscript{1}-weighted image and hyperintense on a T\textsubscript{2}-weighted image. The contrast differences between gray matter,
Table 1

<table>
<thead>
<tr>
<th></th>
<th>TR (ms)</th>
<th>TE (ms)</th>
<th>Exp. time</th>
<th>Misc.</th>
</tr>
</thead>
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<tr>
<td>1. FLAIR</td>
<td>9000</td>
<td>140</td>
<td>4:50</td>
<td>TI=2100 ms</td>
</tr>
<tr>
<td>2. T2-weighted</td>
<td>7000</td>
<td>150</td>
<td>3:00</td>
<td>ETL=32</td>
</tr>
<tr>
<td>3. Proton density</td>
<td>7000</td>
<td>6</td>
<td>3:00</td>
<td>ETL=32</td>
</tr>
<tr>
<td>4. T1-weighted</td>
<td>550</td>
<td>8</td>
<td>5:00</td>
<td></td>
</tr>
<tr>
<td>5. T1+MgT</td>
<td>550</td>
<td>8</td>
<td>6:20</td>
<td>IF=1.2 kHz</td>
</tr>
<tr>
<td>6. T2*</td>
<td>600</td>
<td>2</td>
<td>5:00</td>
<td>FA=20°</td>
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<tr>
<td>7. T2*</td>
<td>600</td>
<td>15</td>
<td>5:00</td>
<td>FA=20°</td>
</tr>
<tr>
<td>8. T2*</td>
<td>600</td>
<td>32</td>
<td>5:00</td>
<td>FA=20°</td>
</tr>
<tr>
<td>9. STIR</td>
<td>5000</td>
<td>25</td>
<td>3:00</td>
<td>TI=130 ms</td>
</tr>
<tr>
<td>10. SPGR</td>
<td>400</td>
<td>2</td>
<td>2:30</td>
<td>FA=12°</td>
</tr>
</tbody>
</table>

FLAIR=fluid level attenuated inversion recovery, TR=time to repeat, TE=time to echo, MgT=magnetization transfer, STIR=short tau inversion recovery, SPGR=spoiled gradient recalled echo, TI=time to invert, ETL=echo train length, IF=irradiation frequency, FA=flip angle.

Subjects

Nine healthy male subjects aged 25–30 years underwent MRI in a 3T scanner (GE) using an 8-channel head coil. The institutional review board approved the research protocol and each subject signed an informed consent. Each volunteer was subjected to 10 different image contrasts (summarized in Table 1). All images were created in an axial plane with FOV of 20×20 cm², 48 slices of 1.5 mm with no gap, image matrix of 128×128 providing a cubic resolution of 1.5×1.5×1.5 mm³. The total MR acquisition time was 40 min.

Pre-processing

The volumes of the different imaging methods in each subject were realigned to each other to correct for head motions. This was done using SPM2 (UCL, London, UK) with a 4th degree b-spline interpolation with no wrap and no mask. After realignment, the volumes of all the subjects were co-registered and re-sliced to a single subject template (one of the subjects). This was done to ensure similar slice locations across all subjects for purposes of comparison. Registration was done using SPM2 by a rigid body affine 12 mode linear transformation using the mutual information cost-function and trilinear algorithm for re-slicing with no wrapping or image masking. As opposed to the realignment, this step was not necessary for the algorithm, but facilitated comparing subjects.

Virtual-dot-com algorithm

Following pre-processing, an in-house algorithm (virtual.com imaging) was applied to automatically segment the thalamus. The algorithm was written in Matlab© (mathworks, USA) and includes the following 4 main steps (also presented in Fig. 1):

1. Selection of region of interest (ROI)
2. Contrast enhancement via stretching of the intensity dynamic range
3. Transformation of the data into its principal component (PC) space
4. Executing a clustering algorithm

Selecting an ROI

The thalamus ROIs were selected in a semi-automated manner using the virtual.com imaging algorithm itself. The full algorithm was executed on each slice, with the whole brain taken as the ROI. This provided different clusters for gray matter, white matter and CSF. Using these clusters, both thalami could be easily marked manually (see Fig. 1B).

Contrast dynamic range stretching

This procedure is done separately for each slice and for each imaging method to remove nonhomogeneous sampling of different slices, and to exclude outlier pixels. This procedure sorts the pixels based on their intensity (see Fig. 1D), and using the derivative of the sorted pixel function, the algorithm calculates the desired intensity window. Next, the image intensities within this window are normalized in a linear way to be between zero and one (Fig. 1D).

white matter and CSF have been the basis for many brain segmentation algorithms (Ashburner and Friston, 1997; Atkins and Mackiewich, 1998; Wu et al., 2005; Lemieux et al., 2003; Stokking et al., 2000; Lemieux et al., 1999, Fischl et al., 2002). Most of these algorithms use single contrast data – typically T1-weighted images via a spoiled gradient echo sequence, SPGR – to segment the brain into its three main compartments. Recent works demonstrated that tissue segmentation using more than one contrast data can considerably improve results (Zavali̇jevski et al., 2000). These multi-contrast segmentation routines provided more reliable tissue segmentation into gray matter, white matter, CSF and a few sub-cortical structures. However, none of them succeeded to define different cortical regions or sub-cortical nuclei segmentation, mainly because the appearance of the gray matter (both cortical and sub-cortical) is iso-intense throughout all sub-regions.

In this work, we devised an algorithm, which we call virtual.com imaging, that detects and defines small sub-cortical regions based on contrast dynamic range stretching and cluster analysis of a multi-contrast set of MRI data. We tested the algorithm on MRI slices of the thalamus obtained by as many as 10 different MRI-based contrast mechanisms. The thalamus, part of the diencephalon, consists of two nuclear masses of gray matter situated on each side of the third ventricle. Each 3–4 cm long thalamus gathers sensory and conceptual signals and coordinates them. Histology shows that the thalamus is composed of at least 9 different nuclei groups, each with its own cyto-architectonics and functions (Talairach and Tournoux, 1988, also given in: http://ric.uthscsa.edu/projects/talairachdaemon.html and in: http://www.ihb.spb.ru/~pet_lab/TSU/TSUMain.html). Each of these nuclei groups can be further divided based on cyto-architecture features to smaller sub-nuclei groups (Talairach and Tournoux, 1988; Hirai and Jones, 1989; Hassler, 1982; Schaltenbrand and Wahren, 1977). We will refer in this paper to 9 sub-nuclei groups identified on the Talairach brain atlas (namely Pulvinar (Pul), Medial–Dorsal (MD), Lateral–Posterior (LP), Lateral–Dorsal (LD), Ventral–Posterior/Lateral/ Medial (VPL/M), Ventral Lateral (VL), Ventral Anterior (VA) and Anterior (A)). These nuclei groups are likely to be represented by an adequate number of pixels in the resolution limits of this study. It is expected that the different cyto- and myelo-architecture of the thalamus nuclei will be differentially weighted in a multi-contrast MRI protocol. We found that within the thalamus, contrast differences combined with clustering algorithm enable us to segment and characterize its sub-nuclei.

Materials and methods

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Transforming the data into its PC space

In this step, the eigenvalues and eigenvectors of the covariance matrix of the data are calculated. The data are then transformed into its PC space by projecting it on the eigenvectors. The number of dimensions is usually reduced in this step by manually excluding the eigenvectors with the lowest eigenvalues (typically to 6 dimensions). As a consequence, the data are presented along its maximum variance axes. Other benefits of this procedure are noise reduction and computation simplification due to the reduction of the dimensions of the data.

Clustering

In this step we used iterations of the “k-means” clustering algorithm starting with a low number of clusters (k) given as input. The obtained clusters are tested to be significantly different from each other using ANOVA on the original data (after contrast enhancement). The k-means algorithm keeps running with an increasingly larger number of clusters (each iteration k increments by 1) as an input until the clusters cease to differ significantly. It should be emphasized that all pixels (in all slices) are equally treated in this step, ensuring a real 3D clustering.

Statistical analysis

Each pixel in each cluster was regarded as an individual observation, which resulted in around 300 individual observations in each cluster. The data were then tested for statistical significance using a repeated measure ANOVA, with the clusters as the independent factor and the 10 methods as the repeated variable. Post hoc analysis was performed on the interaction between method and cluster using the Tukey test. The analysis was done using statistica 6.0©. The correlation between the mean vectors of the clusters in different subjects was calculated to compare the similarity between the clusters that were found.
Rat data

To validate the human results, similar experiments were also performed on rats (in vivo, \(n=2\)). The experiments were performed on a 7T/30 BioSpec MRI scanner (Bruker, Germany) equipped with a 40 G/cm gradient system. The rats were anesthetized with Ketamine (50 mg/kg) and Xylazine (5 mg/kg). The MRI protocol included only some of the human multi-contrast data set: T2-weighted fast-spin echo (TR/TE=7000/90 ms), Spin echo proton density weighted (TR/TE=7000/24 ms), T1-weighted (TR/TE=750/7 ms), T2*-weighted (TR/TE=1500/3 ms, flip angle of 30°), T2*-weighted (TR/TE=1500/10 ms, flip angle of 30°) and FLASH sequence (similar to SPGR with TR/TE=100/2 ms and flip angle of 30°). All experiments were done with a cubic resolution of 0.4 mm\(^3\). Experimental time was approximately 1.5 h.

Results

Fig. 1 shows the different steps of the virtual.com algorithm for clustering sub-regions in the thalamus. The contrast information embedded in the multi-parametric MRI data becomes evident following the dynamic range stretching (compare Figs. 1C and E).

Inter-subject variability

The most trivial estimator for the inter-subject variability would be the spatial locations of the clusters across subjects. For that purpose, we need to assign and match the clusters of the different subjects. This task is not trivial as the number of clusters and their assignment was not the same across subjects. This problem stems from the need to pre-define the number of clusters (\(k\)) and their initial centers (\(k\)-vectors), when using the \(k\)-means. In its most simple form, \(k\)-means is executed with pre-determined number of clusters (\(k\)). In order to determine the optimal number of clusters, we repeated the clustering procedure with an increasing number of \(k\) clusters.

![Figure 2: Single slice of the thalamus segmented with k-means](image)

Fig. 2. A single slice of the thalamus segmented with \(k\)-means using (A) \(k=3\), (B) \(k=5\), (C) \(k=9\) and (D) \(k=15\). Note that the yellow cluster in panel A divides into two clusters in panel B (yellow and orange), four clusters in panel C (yellow, orange, red and brown) and more than 5 clusters in panel D. The graphs at the second row show the contrast profiles for the clusters identified in the region of the yellow cluster in panel A. Notice that the profiles differ from each other when the \(k\) is 5 and 9 but not when \(k=15\). Also note that the standard deviation decreases when the number of clusters increase due to higher resembles of pixels assigned to a certain cluster.
subject. The algorithm detected between 8 and 10 clusters in the thalamus of each subject, each cluster significantly different from the others per subject (repeated measure ANOVA, $p<0.05$).

**Fig. 2** shows an example for clustering of one thalamus slice using 3, 5, 9 or 15 clusters for a typical subject. In this example, the clusters ceased to differ from each other when $k$ was set to 10. Indeed, in **Fig. 2D1**, where 15 clusters were used, some of the clusters are not statistically different, although recognized as separate clusters by $k$-means. In addition, for each cluster, we could plot the MRI contrast profile vs. MRI methods. These contrast profiles are given in **Fig. 2** (plates A2, B2, C2 and D2) for one cluster identified with $k=3$ (yellow cluster in A2) which was further divided into smaller structures with $k=5, 9$ and 15 (B2, C2 and D2). When the clustering was done with a higher number of clusters ($k=5$, B2 and $k=9$, C2), the contrast profiles of the sub-clusters still differ significantly from each other implying that this sub-division is a real feature of the data. When the clustering for this slice was done with $k=15$, some of the clusters seem to have very similar contrast profile implying that these clusters might belong to same area. Another point worth mentioning while examining the profiles of the clusters from left to right is that the standard deviation decreases while the number of cluster rises. This is due to the fact that when the number of clusters is smaller than the actual number, $k$-means finds large clusters that are actually a combination of several clusters.

Once we determined the optimal number of clusters, the comparison across subjects is still problematic as each subject has different number of clusters and those not necessarily match. Matching of clusters can be done by using initial conditions for the $k$-means algorithms. By entering initial conditions (vectors), the $k$-means algorithm will find the cluster that is the most similar to the entered vector. In that sense, clusters that are found similar to a certain initial vector across subjects are matched and can be compared. In our algorithm, we used the cluster centers of a single subject (referred to as the template subject) with the highest number of obtained clusters (10) as initial vectors. Following that analysis, we were able to test the correlation between matched clusters across subjects. This analysis found that 7 of the 10 “template” clusters can be found in all subjects. The rest 3 clusters were correlated across subjects and their analysis will be described below (see Cluster assignment section below). For the matched 7 clusters, both method and cluster were found significant ($p<0.05$) and there was a significant interaction ($p<0.001$) between cluster and MRI method. When the mean contrast vectors of the template subject were correlated with those of the rest of the subjects, there was a significant ($p<0.02$) correlation between the mean vectors of the clusters belonging to the same nuclei (following assignment, see below) in different subjects.

Following these analysis procedures (defining number of clusters and matching clusters over subjects), we could spatially compare the results across subjects. **Fig. 3**, showing the segmentation of the thalami of 3 of the 9 subjects superimposed on an SPGR scan, demonstrates the robustness of the segmentation: it depicts high symmetry between the two thalami and resemblance between subjects. Quantitative spatial comparison was done as well but will be described below in Cluster assignment. Aside from visual inspection of the clusters, each one of the cluster is characterized by the unique contrast profile (or contrast vector) presented in **Fig. 4**. The figure shows the large differences between cluster’s profiles reflecting the contrast differences of the clusters. The small error bars (standard deviation) indicate the similarity of these contrast profiles across subjects. It should be emphasized that contrast profile similarity does not indicate spatial location similarity. Indeed clusters having the same contrast profile might be located in slightly different areas of the thalamus of different subjects (see Cluster assignment). However, as described qualitatively above, the cluster profiles might reflect the morphological architecture that assembles the cluster. For instance, cluster number 2 has low T1 contrast while cluster number 4 has reversed contrast, implying different

![Fig. 3. Applying the virtual-dot-com algorithm for similar slice locations of 3 subjects. The algorithm was able to segment the thalamus with high similarity across subjects and show symmetry between the left and right thalami and high resemblance between subjects.](image-url)
involvement of gray and white matter within these clusters. The sizes of the clusters are very similar across subjects. Their relative sizes and assignments (see below) are summarized and compared with atlas data in Table 2.

Cluster assignment

Before performing the assignment, we had to deal with the three clusters that were unmatched across subjects (see previous section). These three clusters were not significantly different from other clusters in all subjects. Concomitantly, analyzing their correlation with the rest of the clusters showed that they are highly correlated with two of the seven matched clusters (explicitly, clusters 1 and 2 which were later on visually and quantitatively assigned to the Pulvinar and Medio-dorsal nuclei, see below). This is shown in Fig. 6 where two thalamus slices are depicted divided to 7 clusters (Figs. 5A and B). One unmatched cluster can be identified in Fig. 5 as a dark-blue area (similar to cluster 1) with light blue border (similar to the color of cluster 2). The contrast profiles of this unmatched cluster as well as of clusters 1 and 2 are shown in Fig. 5C. Observing the profiles, it seems that this cluster is a weighted average of clusters one and two. When examining correlation, we found that this profile has a higher correlation with cluster 1 than cluster 2 and thus we decided to merge it with cluster 1. This same procedure was repeated for all subjects and resulted in nine subjects with seven clusters.

The assignment of the clusters to the different sub-thalamus nuclei was done based on three brain/thalamus atlases: a radiology-based (Kikinis et al., 1996, shown in: [http://splweb.bwh.harvard.edu](http://splweb.bwh.harvard.edu)) and histology-based (Morel et al., 1997 and Talairach and Tournoux, 1988). The assignment was done in two ways: (1) Qualitatively—the virtual.com thalamus and atlas thalamus were co-registered and compared by visual inspection. (2) Quantitatively—the virtual.com was registered and normalized to a standard brain coordinate system (e.g. Talairach) and the cluster centers were...
identified and assigned according to their atlas spatial locations. The qualitative assignment of the clusters was done by digitizing and registering two atlases (Kikinis et al., 1996 and Morel et al., 1997) to the template subject (using SPM2, UCL, London, UK). The atlas sub-divisions were indexed with the same color scale as the virtual.com clustering to ease the comparison. Each cluster was assigned, visually, to the appropriate nucleus according to its size (see Table 2) and spatial position (Fig. 6), although the atlases show considerable variance.

The quantitative assignment was done by normalizing our brain volumes with the Talairach coordinate system. Following normalization (which was applied on the segmented thalamus as well), the cluster’s centers were calculated, averaged across subjects and their averaged location was identified on the atlas. These data are summarized in Table 3. To visualize this assignment, we produced a probabilistic segmented thalamus from the different subjects data shown in Fig. 7 compared with the Talairach coordinates of each specific nucleus. In this figure, for each cluster, the left column represents an averaged thalamus in which each pixel is colored with the matching probability (i.e. 100% means this pixel was matched to a specific cluster in all subjects). In addition, for each cluster, the right column depicts in color the assigned nuclei of this cluster in the Talairach coordinate system. On each of these nuclei, the white cross represents the center of the assigned cluster according to our results. This quantitative comparison shows that the centers of clusters one through five (Figs. 7A–E) fall within the territories of the visually assigned nuclei, thus supporting our visual assignment (namely the pulvinar, medial–dorsal, lateral posterior and lateral–dorsal complex, ventral–posterior lateral and ventral–lateral). Figs. 7F and G show the probabilistic virtual.com assignment.

<table>
<thead>
<tr>
<th>Cluster number</th>
<th>Assignment</th>
<th>Relative size (%) (mean±SD) (virtual.com)</th>
<th>Relative size (%) (mean±SD) (Morel Atlas*a)</th>
<th>Relative size (%) (mean±SD) (Kikinis Atlasb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pulvinar (Pul)</td>
<td>22±2</td>
<td>25.4</td>
<td>22.3</td>
</tr>
<tr>
<td>2</td>
<td>Medio-dorsal (MD)</td>
<td>18±2</td>
<td>18.4</td>
<td>13.3</td>
</tr>
<tr>
<td>3</td>
<td>Lateral posterior (LP)+ Lateral Dorsal (LD)</td>
<td>10±1</td>
<td>7.5</td>
<td>12.9</td>
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<tr>
<td>4</td>
<td>Ventral postero-lateral (VPL)</td>
<td>10±2</td>
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<td>13.4</td>
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<tr>
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<td>Ventral lateral (VL)</td>
<td>11±2</td>
<td>22.2</td>
<td>11.6</td>
</tr>
<tr>
<td>6</td>
<td>Ventral anterior (VA)</td>
<td>10±1</td>
<td>9.2</td>
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<tr>
<td>7</td>
<td>Anterior+ Lamina</td>
<td>8±4</td>
<td>12.9</td>
<td>13.1</td>
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</table>

*a Morel et al. (1997).

*b Kikinis et al. (1996).
The main findings of this study are that multi-parametric MRI can detect and define brain sub-structures that previously were undetectable by conventional MRI, and that each detected structure displays a unique contrast fingerprint that can be related to the morphological arrangement of its tissue compartments. Based on this approach, we developed virtual.com imaging, a framework for analyzing high-resolution multi-parametric MR images. Its main features are acquisition of multi-contrast data set, definition of region of interest, contrast dynamic range stretching and clustering. Using this methodology, we were able to segment the thalamus into some of its sub-nuclei; we detected 7 clusters representing histologically distinct areas, with high reliability and reproducibility. The assignment of the algorithm clusters was done by visual comparison to thalamic atlases and by normalization and registration with the Talairach coordinates system. The strength
of the methodology was demonstrated by comparing rat and human data on which 4 independently assigned nuclei were highly similar between the rats and human.

**Implications of virtual.com imaging on neuro-imaging**

The proposed methodology has wide-ranging implications for neuro-imaging research, bearing in mind that its applications go beyond the thalamus. First, the ability to obtain subject-specific mapping of sub-cortical nuclei: it is common knowledge that size and location of brain structures vary greatly between subjects, depending on personal genetic and developmental factors. Nevertheless, studies often refer to a single subject atlas to strengthen and reference their findings. This is done through brain normalization and registration pre-processing procedures to provide voxel-based morphometry (VBM). Although it still needs to be studied, it is agreed that brain normalization and registration errors might lead to significant artifacts in VBM analysis (Uylings et al., 2005; Bookstein, 2001; Salmond et al., 2002; Ashburner and Friston, 2001). While these errors might be acceptable for certain brain areas (e.g. cortical localization in fMRI), for smaller regions, their identification is ill-posed by these procedures. In many aspects, this is the case of the thalamus. We used the normalization and registration procedures in order to provide quantitative validation of our algorithm segmentation (Fig. 7). While for the large sub-nuclei structures (e.g. MD, Pul) high similarity was found between the Talairach coordinate system and our segmentation, for the smaller structures partial similarity was observed. This might be a result of poor clustering and assignment of our algorithm but also of poor normalization of the data which for small regions (∼3–4 mm) might result in complete dislocation of the region.

The virtual.com algorithm, once validated, should not suffer from registration and normalization artifacts. Using virtual.com imaging, one can create, at least for the healthy brain, a subject-specific atlas; and, as each cluster is characterized by a unique, statistically significant contrast profile, it is possible to mark its morphological signature. As was shown in Fig. 4, different contributions of gray and white matter might lead to opposite

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**Fig. 7.** Comparison with Talairach atlas: (A–G) represent each of the 7 visually assigned clusters following normalization to the Talairach coordinate system. The left column represents the averaged virtual.com thalamus and the color-scale represents the cluster probability. For example, this means that pixels with white color belong to the specific cluster in all subjects. The right column in each of the figure plates (A–G) depicts the Talairach spatial location of specific thalamic sub-nuclei (A—Pul, B—MD, C—LP/LD, D—VPL/M, E—VL, F—VA, G—A) that were visually assigned to each of the clusters in Fig. 6. One can visually compare the locations of the averaged clusters and locations of the sub-nuclei according to Talairach atlas. To ease the comparison and provide quantitative measures, we also calculated the centers of averaged clusters. Those are given by the white crosses in the right column of each of the figure plates (also summarized in Table 3). For the Pul, MD, LP/LD, VPL/M and VL, the cluster centers fall within these sub-nuclei territories. For the VA and A, the cluster centers fall outside the sub-nuclei territories which might indicate wrong assignment or normalization errors and poor registration across subjects.
The segmentation of the thalamus described here has challenged neuro-anatomists and MRI researchers for years (Morel et al., 1997; Niemann et al., 2000; Spinks et al., 2002; Hirai and Jones, 1989). Some segmentation studies relied on histological analysis of brain sections while others used subjective marking of the sub-nuclei borders. The non-invasive nature of MRI, combined with the automated manner in which the algorithm works, gives it an advantage over previous thalamus segmentation methodologies and opens up the possibility to explore thalamus-related diseases such as Parkinson’s and Kreuzfeld–Jacob with higher sensitivity and specificity. Recently, it was shown that the thalamus can be segmented based on diffusion tensor imaging data (Behrens et al., 2003; Wiegell et al., 2003). In these works, the authors describe how the thalamus–cortex connections can be reconstructed. The main difference between our study and the DTI studies is that here we create a unique contrast profile for each thalamus nuclei that quantitatively characterizes this nuclei. Moreover, our method can be applied to other cortical regions whereas DTI works best in white matter as well as in the thalamus as it contains significant portion of white matter.

The contrast value

One of MRI’s greatest advantages is its multi-contrast modality. This property is used radiologically for qualitative comparison of methods to diagnose specific brain pathological conditions. On the other hand, some of the contrast mechanisms have been the basis for brain mapping and parcellation of brain structures. For instance, the majority of brain segmentation studies use T1 contrast to anatomically map brain structures because the gross anatomical structures (gray and white matter) can be easily defined. Functional
MRI uses the blood oxygenation level dependent (BOLD) contrast to define activity in brain regions for mapping functional areas. Zavalaevskiy et al. (2000) suggested using multi-contrast data to enhance gray/white matter segmentation, and specifically employed T1, T1+Gadolimun, T2, PD and Perfusion in that study. We took their approach one step forward and used 10 MRI-based contrast mechanisms. Indeed, with more than one technique, we were able to reproduce Zavalaevskiy et al.’s observations and obtain excellent brain segmentation into gray matter; white matter and CSF (see Fig. 1B).

We then tested the approach’s capability to segment sub-cortical regions. The idea to use a large number of imaging modalities stems from the expected minor contrast differences in the sub-cortical regions; in other words, the number of imaging methods needed to detect tissues with different characteristics is a function of the extent of the differences between the tissues. If we consider similar signal to noise ratios (SNRs) in the different methods, it is obvious that the more methods used the better the differentiation, since each method supplies more data. In this work, we tried to use as many methods as possible, with the limiting factor being only reasonable acquisition time. We tested the effect of reducing the number of acquired methods by repeating the analysis a few times, each time removing one method, and checking whether the clusters still significantly differ from each other. The clusters became insignificant for all the methods except two-T2, and FLAIR. When the data from one of these two methods were excluded, the data were still significant, implying that each of them separately is not needed for defining the clusters in the thalamus. When the data from both methods were excluded, most of the clusters became insignificant. This analysis does not mean that 9 is the minimum number of methods needed to define the thalamus. It is possible that including other imaging protocols will enable reducing the number of methods. Furthermore, the fact that the interaction between methods and cluster was found to be significant across subjects implies that some clusters can be detected using only part of the methods.

The clustering algorithm

The disadvantages of $k$-means as a clustering function are well described. The need to define the number of clusters in advance is probably the main problem in our case, since many tissues and organs can be divided into a few or more subdivisions, depending on the scale being examined. We dealt with this limitation by running the algorithm with an ever-increasing cluster number and setting as the stop condition the requirement that clusters are significantly different from each other. However, one can claim that this is an irrelevant limitation since there is no need for different sub-organs to differ significantly, given the number of methods used.

Another issue in cluster analysis is the importance of distance between adjacent pixels. We added the spatial information as additional input to the algorithm so as to include its contribution to the final output. We were confident doing this because there is a much greater chance that a particular pixel belongs to its neighboring pixels’ cluster than distant pixels.

Resolution

The virtual.com algorithm can be executed in different levels of resolution. Due to contrast dynamic stretch and PCA decomposition, the effect of the algorithm on different scales of ROI will be different. This actually helped in defining the thalamus ROI: once the algorithm was applied on the entire slice and disclosed the borders of the thalamus, it could then be applied on a smaller scale region. The scaling freedom enables us to dictate the focus of our spot. By using different ROI scales, we can look for segmentations of white/grey matter and CSF, or limit ourselves to smaller regions and obtain segmentations of smaller structures such as sub-cortical nuclei. This will eventually reach a resolution limit, as we will not be able to define structures smaller than our voxel size. Perhaps higher resolution can enable the identification of larger numbers of clusters.

Conclusions

This work describes an acquisition and analysis framework for MRI-based multi-contrast data that may enhance the ability of MRI to cope with small structure segmentation and definition that to date has been done subjectively. The results point to the possibility to create subject-specific cortical segmentation similar to Brodmann’s atlas, and segmentation of other neuronal structures. The approach may also be used to define a pathology-specific contrast profile that can be searched semi-automatically within the brain, making the method a radiological tool for assessing pathological brain conditions. For the time being, the potential of multi-contrast imaging for the thalamus nuclei has been demonstrated. Future work should include a more comprehensive study of animal thalamus which could be compared with the same tissue histology. This will define the limits and abilities of the virtual.com framework.

References


