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LOCALIZING AMYGDALA STRUCTURE DIFFERENCES IN LATE-LIFE DEPRESSION

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ABSTRACT

The amygdala is critical for processing emotional information and plays an important role in late-life depression (LLD). Volumetric studies of the amygdala have been inconclusive with reports of increased, decreased, and no volume changes. This study investigates amygdala shape morphemetry to test the hypothesis that if structural changes are specific to certain nuclei, then shape changes may be apparent even when overall volume changes are inconsistent. We have developed a method of shape morphometry based on the work of [1] to localize regions of structural differences. The method relies on generating surface meshes for segmented amygdalae, calculating distances from surface points to the medial manifold, and comparing the distance measures at corresponding surface points between groups. Resulting statistical maps revealed significant structural differences in multiple regions of both amygdalae. Shape morphometry can potentially relate local structure variation to underlying neuroanatomy for a better understanding of LLD neuropathology.

1. INTRODUCTION

Depression is a widespread psychiatric illness that affects an estimated $10 - 15\%$ of the U.S. elderly population [2]. Individuals suffering from late-life depression (LLD) exhibit common depressive symptoms such as prolonged changes in mood and behavior, and are likely to become cognitively impaired. Diagnosis of LLD is often difficult due to variable clinical presentation and treatment response is variable. Symptoms are sometimes dismissed as part of the aging process or attributed to medical illness, physical disability, or medication. Additionally, affected individuals may typically describe only physical symptoms and are reluctant to discuss feelings of sadness and disinterest in pleasurable activities. Undiagnosed and untreated LLD leads to excess morbidity and mortality for the individual. It is known that depressed elderly individuals are more disabled, recover slower from medical illness and surgery, and are more likely to die than non-depressed elderly individuals. Therefore diagnosis is extremely critical considering treatment is available in the form of medication and psychotherapy; both of which have been shown to effectively reduce the symptoms of LLD and improve physical and social function. Better understanding of the underlying neuropathology could lead to improved diagnosis and treatment.

Neuroimaging has emerged as a valuable tool for exploring associations between neuroanatomy and psychiatric illnesses to aid in early diagnosis and treatment solutions for LLD. Many depression studies have utilized Magnetic Resonance Imaging (MRI) to conduct morphometric analysis of various brain structures. The amygdala is of particular interest because it is central to processing emotional information. Several studies have investigated volume morphometry of the amygdala in adult psychiatric illnesses with inconsistent findings. There have been reports of both increased, e.g., [3, 4] and decreased amygdalae volumes in depression, e.g., [5] and mild dementia, e.g., [6]. Volume decrease in the left amygdala has been reported in depression with memory problems, e.g., [7]. Other studies have found no volume differences in major depression, e.g., [8, 9], questionable dementia, e.g., [6], and recurrent depression, e.g., [10]. This discrepancy in findings may be partially attributed to the difficulty in measuring the amygdala. The amygdala consists of multiple nuclei and boundaries are difficult to delineate because many areas merge with surrounding tissue. Therefore results will depend on the the specific delineation protocol and boundaries used. Moreover we believe that these discrepancies may be a limitation in considering volume rather than shape morphometry. Shape morphometry provides better specificity over volume morphometry delivering a spatial map of regions affected by disease, which may be used to link local structural differences to cognitive measures.

In this study, we investigate both volume and shape morphometry to test the hypothesis that if structural changes are specific to certain nuclei, then shape changes may be apparent even when overall volume changes are inconsistent. To assess amygdala shape differences, we have developed a method based on work by Thompson et al. [1] for quantifying local structural differences with the goal of isolating specific regions of surface deformations. This method relies on first tessellating the surface of manually delineated amygdalae. The distance from each surface point to the medial manifold is calculated for each subject. To calculate the medial manifold an algorithm using a framework called Shells and Spheres [11] is used to implicitly link surface points to the medial manifold. Distance measures for corresponding surface points across subject groups are compared via permutation testing, which yields a statistical map consisting of p-values at each surface point. The surface map thus allows for visualizing local structure variation that can be potentially related to underlying neuroanatomy or cognitive deficits for a better understanding of LLD neuropathology.

2. METHODS

2.1. Subject Demographics

To investigate amygdala shape morphometry in LLD, MRI data were acquired from LLD diagnosed patients and healthy elderly controls. A total of 25 subjects were included in this study and all but one were right-handed. All of the subjects received a Structured Clinical Interview for DSM-IV (SCID-IV) evaluation, which were reviewed in a diagnostic consensus conference. Exclusion criteria included all Axis I psychiatric disorders except for major depressive disorder

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(for subjects in the LLD group) and the anxiety disorders. Subjects were also excluded for a prior history of stroke or significant head injury, or Alzheimer's, Parkinson's, or Huntington's disease. Subjects with co-morbid anxiety disorders were also included due to the high prevalence (48%) of anxiety disorders in subjects with LLD [12]. Cognitive status of the subjects was assessed using the Mattis Dementia Rating Scale.

The LLD subject group consisted of 14 elderly patients; 9 males and 5 females with an age of 69.8 ± 5.1 years (throughout this paper measures are reported as mean $\pm$ standard deviation). These patients had a typical clinical presentation of LLD and were diagnosed with the SCID-IV evaluation. The control group consisted of 11 healthy elderly subjects; 7 males and 4 females with an age of 67.2 ± 6.8 years. These subjects presented with no clinical presentation of LLD and did not meet any of the exclusion criteria.

2.2. MRI Data Acquisition

Each subject in the study had their head scanned using the same MRI scanning protocol. High resolution, 3D data were acquired on a 1.5 Tesla Signa Scanner (General Electrics Medial Systems, Milwaukee, WI) full body scanner. A spoiled GRASS imaging sequence was used with the following acquisition parameters: TR/TE = $5/25$ ms, flip angle = 40°, and FOV = 24×18 cm. Data were acquired with the subject in the prone position and images were reconstructed to be $236 \times 192 \times 171$ voxels in size with a resolution of 0.9375×0.9375 mm in the axial plane and 1.5 mm in the inter-slice dimension.

2.3. Amygdala Segmentation and Image Processing

To isolate the amygdalae for morphometric analysis, they were manually segmented from each subject image. All images were initially aligned to the same orientation with the AC-PC (anterior commisure-posterior commisure) alignment routine (rigid body, landmark-based transformation) available in the Automated Functional NeuroImaging (AFNI) software suite. Following AC-PC alignment, an expert rater manually delineated the boundaries of each amygdala following a protocol in which adequate intra- and inter-rater reliability has been previously established [13] (posterior boundary: the alveus of the hippocampus; anterior boundary: 2 mm from the temporal horn of the lateral ventrical; superior boundary: ventral horn of the subarachnoid space (SS); inferior boundary: most dorsal finger of the white matter tract under the horn of the SS; lateral boundary: 2 mm from the surrounding white matter; mesial: 2 mm from the SS). An example segmentation is shown as a 3D surface model overlayed on the corresponding MRI image in Figs. 1 A $-$ C. The segmentations of each subject's amgdalae were saved as a binary image and bisected into separate images such that the left and right amygdala could be analyzed independently. Amygdala volumes were measured by counting the number of voxels within the delineated region.

Variability in subject brain size was normalized by registering each image to a reference subject image using a mean squares intensity metric. To prepare the images for size normalization, the brain was separated from extraneous tissue with the Brain Extraction Tool (FMRIB Software Library) to permit reliable registration results. Intensity correction was unnecessary since all subject images were acquired with the same MRI scanner sequence and differences in field strength were negligible. Registration was performed with an anisitropic scale transform to preserve amygdala shape as much as possible. The transform matrix computed for each subject image was then applied to the subject amygdala segmentations. Implementation of the image registration was performed with software

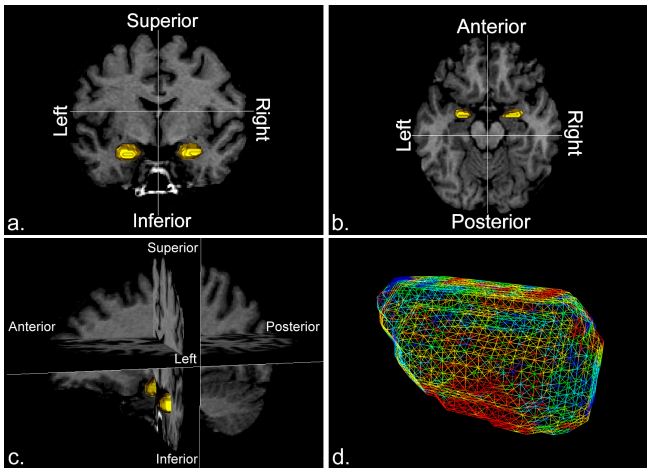

Fig. 1. A. Coronal, B. axial, and C. orthogonal cross-sections of a subject's MRI image are shown with 3D renderings of their segmented amygdalae. D. A mesh tessellated from an amygdala segmentation.

developed utilizing the Insight Toolkit (ITK).

2.4. Shape Morphometry

The method of shape morphometry developed by Thompson, et al., [1] relies on generating parametric surface meshes for each segmented structure, calculating the distance from each surface point to the medial manifold, and comparing inter-group distance measures at each corresponding surface point. Resulting statistical maps consist of p-values at each surface point. Our implementation for each of these steps is now detailed.

2.4.1. Amygdala Surface Tessellation

In [1], a surface mesh is parametrically defined with a fixed number of vertices. The mesh is then warped to match the surface of each amygdala, where each amygdala segmentation has been previously mapped to a stereotaxic space to establish mesh correspondences. In our method, the surface of each segmented amygdala was tessellated using ITK's implementation of a marching cubes algorithm resulting in triangular meshes with a variable number of vertices and cells. A mesh generated from a subject amygdala segmentation is shown in Fig. 1 D with colors indicating statistical significance (see Section 3 for explanation). Surface point correspondences were determined using an iterative closest point algorithm and thus not requiring the segmented images to be mapped to stereotaxic space.

2.4.2. Computing Medial Distance Maps

The next step of the method requires computing, at each surface point, the medial distance, which is defined as the Euclidean distance from each surface point to the medial manifold. A medial distance map is a mesh with medial distances stored at the vertices corresponding to the location of the surface point used to compute the medial distance. The medial manifold is comprised of the locus of points extending along the center of the object of interest such that the points are equidistant to at least two object boundaries.. Many methods exist for extracting the medial manifold of an

object. In this study, we developed an algorithm based on a framework called Shells and Spheres [11]. Shells and Spheres is based on a sphere map, which consists of a set of spheres centered at each voxel. Available to the framework are a set of powerful statistics called Variable-Scale Statistics that are calculated for voxel populations within spheres and within adjacent and overlapping spheres. The goal in the Shells and Spheres framework is to optimize the sphere map such that each sphere is grown to touch but not cross an object boundary. Spheres that touch at least two boundaries are medial and the locus of centers of those spheres are on the medial manifold. Optimization of the sphere map can be guaranteed with binary images since object boundaries are known. An optimized sphere map is equivalent to a distance map with the positive ridges forming the medial manifold. An advantage of using the Shells and Spheres framework is that points along the medial manifold are automatically linked to their respective boundary points making the medial distance from boundary points trivial to compute. The algorithm employed in this study initializes all spheres to a radius of 0 and increases their radii until intra-sphere variance is detected, i.e., the sphere begins to cross an object boundary.

2.4.3. Group Comparison of Medial Distance Maps

Medial distances calculated for each subject's amygdalae are stored in the respective subject's meshes resulting in a medial distance map for each amygdala. For the amygdalae in each subject group, the point-wise mean and standard deviation of the medial distance maps were calculated. An unpaired t-test statistic was then calculated to compare medial distance across subject groups for determining regions of structural difference, both contraction and expansion (LLD subjects relative to control subjects). Statistical maps were generated with permutation testing to correct for Type I error at each surface point to indicate significant regions of localized structural differences. The permutation test measures the distribution that would be observed if subjects were randomly assigned to a group. The computed test statistics are then compared, as a ratio, to the test statistics computed with subjects assigned to the correct group. This ratio is the chance of the observed test statistic occurring by accident, which provides a corrected p-value at each surface point if a very large number of permutations are permitted.

3. RESULTS

Statistical maps were generated with 1 million permutations to correct for Type I error at each surface point and arrive at p-values. The statistical maps indicate regions of structural differences in LLD versus control subjects. The statistical maps for each amygdala are shown in Fig. 2 as surface mesh renderings of a reference subject's amygdalae with p-values interpolated between vertices. Final p-values are color-coded from 0 to 1 with significant differences ($p < 0.05$) of contraction shown as bright red and expansion shown as magenta (circled). The entire range of p-values for expansion and contraction are included to illustrate any trend toward significance. As can be seen in each view there are isolated regions of structural differences, which indicate either contraction or expansion. Regions of contraction and expansion were isolated and p-values were thresholded at 0.05. The thresholded statistical maps are shown in Fig. 3 with red regions indicating contraction and blue regions (circled) indicating expansion. Green regions indicate no statistical difference between controls and LLD patients. Interestingly, there is a single region on the medial tip of right amygdala that exhibits expansion. However, it should be noted that small clusters such as this region of expansion may have potentially occurred by chance given the large number of t-tests performed (the number of vertices in the reference mesh). As such in future work we will implement a method to correct for multiple comparisons across the statistical map as in [1].

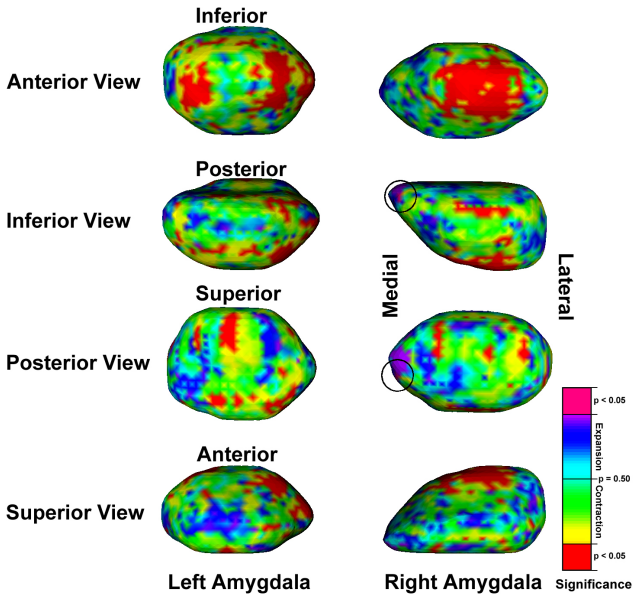

Fig. 2. Amygdalae statistical maps with p-values indicating structural differences between the LLD group and the healthy control group rendered on a reference subject's amygdalae. Bright red indicates significant contraction and magenta (circled) indicates significant expansion.

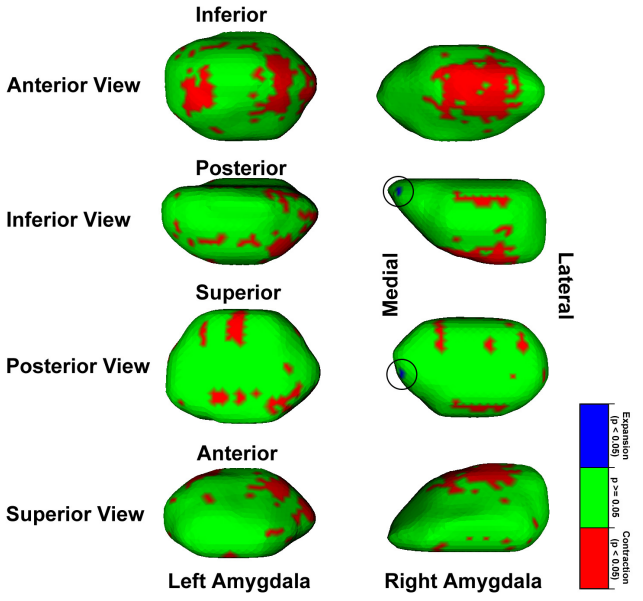

Fig. 3. Surface meshes shown with thresholded p-values that indicate significant contraction (red) and expansion (blue, circled) rendered on a reference subject's amygdalae. Green indicates regions in which the difference is not statistically significant.

Volumetric analysis was performed on both amygdalae of the subject groups by counting the number of segmented voxels. The volume mean and standard deviation are reported for the subject groups in Table 1. A two-tailed t-test statistic was computed between groups to arrive at a p-value. At the $p < 0.05$ significance level there was no significant volumetric difference for either amygdala, although there was a statistical trend toward significance for volume decrease in the left amygdala of the LLD group ($p = 0.07$). It is clear that shape morphometry yields more detailed, localized, and specific information than volume morphometry.

Group	**Volume (mean $\pm$ SD, voxels)**	p**-value**
R. (Control)	1553 ± 293	0.40
R. (Patient)	1433 ± 404	
L. (Control)	1547 ± 329	0.07
L. (Patient)	1295 ± 325	

Table 1. Right (R.) and Left (L.) amygdala volumetrics for subjects and p-values (unpaired, two-tailed t-test) computed between groups.

4. CONCLUSIONS AND DISCUSSION

In this study, we investigated amygdala shape morphometry to determine if local structural differences exist between healthy elderly subjects and patients diagnosed with LLD. Our interest in the amygdala and LLD derives from the amygdala's critical role in processing emotional information. Other studies have examined volume morphometrics of the amygdala in patients with various psychiatric illnesses and results from these studies have been inconclusive. We believe that these conflicting results may be attributed to a limitation in considering volume rather than shape. In this study, we tested the hypothesis that if structural changes are specific to certain nuclei, then shape changes may be apparent, even when overall volumes are inconsistently altered.

Our localized statistical maps revealed significant structural differences at multiple regions of both amygdalae. Specifically, there were dispersed regions of contraction and a single isolated region of expansion on the medial tip of the right amygdala. Volumetric analysis resulted in statistical insignificance for both amygdalae with a trend toward volume decrease in the left amygdala. The statistical maps revealed regions of structural difference despite insignificant volumetric findings. The anterior boundary was the primary region exhibiting contraction and has traditionally been associated with the basolateral nucleus, which plays a key role in emotion recognition in neurobiologic models of depression. One suggested theory for decreased amygdala volume in depression suggests neuronal degeneration as a result of glutamate excitotoxicity associated with hypercortisolemia [14]. It can therefore be inferred that basolateral nucleus of the amygdala is affected by neuronal degeneration in LLD, but further investigation is warranted. Shape morphometry has given us the capability of localizing specific regions of structural differences, which may correlate with anatomical, physiological, or cognitive data. In future work, we will increase the sample size to identify covariates such as gender and education that may also contribute to local structural differences.

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