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Morphometry of human insular cortex and insular volume reduction in Williams syndrome

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ABSTRACT

Functional imaging in humans and anatomical data in monkeys have implicated the insula as a multimodal sensory integrative brain region. The topography of insular connections is organized by its cytoarchitectonic regions. Previous attempts to measure the insula have utilized either indirect or automated methods. This study was designed to develop a reliable method for obtaining volumetric magnetic resonance imaging (MRI) measurements of the human insular cortex, and to validate that method by examining the anatomy of insular cortex in adults with Williams syndrome (WS) and healthy age-matched controls. Statistical reliability was obtained among three raters for this method, supporting its reproducibility not only across raters, but within different software packages. The procedure described here utilizes native-space morphometry as well as a method for dividing the insula into connectivity-based sub-regions estimated from cytoarchitectonics. Reliability was calculated in both ANALYZE ($N = 3$) and BrainImageJava ($N = 10$) where brain scans were measured once in each hemisphere by each rater. This highly reliable method revealed total, anterior, and posterior insular volume reduction bilaterally (all p's < .002) in WS, after accounting for reduced total brain volumes in these participants. Although speculative, the reduced insular volumes in WS may represent a neural risk for the development of hyperaffiliative social behavior with increased specific phobias, and implicate the insula as a critical limbic integrative region. Native-space quantification of the insula may be valuable in the study of neurodevelopmental or neuropsychiatric disorders related to anxiety and social behavior.

1. Introduction

A variety of methods have been described for assessing insular morphometry, each of which has inherent, specific limitations. Previous attempts to measure the insula using an indirect method of measuring the cerebrospinal fluid (CSF) space in the Sylvian fossa (Foundas et al., 1996, 1997) revealed inferred reductions in insular volume in Alzheimer's disease. Insular volume reductions were inferred because the measurements were of CSF space in the Sylvian fossa, and increased CSF was assumed to equate to reduced insular cortex. Subsequently, this method yields no direct quantification of insular volumes. Efficiency is the main advantage of semi-automated methods, such as voxel-based morphometry (VBM) or FreeSurfer (Desikan et al., 2006). While VBM has been used to show changes in insular morphometry (Karas et al., 2003, 2004), individuals with a clinical or developmental disorder may have variations in anatomical landmarks, such as gyrification, that are critical for normalization algorithms and automated measurements (Thompson et al., 2000a,b). Voxel-based morphometry is also a whole-brain statistical approach (Mechelli et al., 2005), and the current study was focused specifically on insular morphometry. FreeSurfer has difficulty correctly identifying the insula due to its complex boundaries, and does not label this region. It should be noted that the anatomical boundaries, gyri and sulci are visible in post-mortem brains and can be visualized on magnetic resonance imaging (MRI) scans (Naidich et al., 2004). One previous study that measured insula volume directly using volumetric MRI methodology

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(Crespo-Facorro et al., 2000), but this research group designed their own software package to measure the insula which runs only on a Linux platform. Therefore, a novel method was developed and used in the reported study. This method is similar in approach to the Crespo-Facorro method in its use of insular boundaries and native-space (i.e. non-warped, ACPC-aligned) morphometry. It differs from previous methods by including a procedure to attempt to estimate the two main insular sub-regions based on connectivity (Mesulam and Mufson, 1982a,b,c) and is not restricted to any particular software package. The insula is a multifunctional region of cortex, but its connectivity with other brain regions is topographically organized (Mesulam and Mufson, 1982a,b,c; Augustine, 1985, 1996; Craig, 2003; Dronkers, 1996; Oppenheimer et al., 1992; Yaxley et al., 1990). While the current method is based primarily on anatomical data from non-human studies (Mesulam and Mufson, 1982a,b,c), more recent studies in humans provide strong homology between insular organization (i.e. cytoarchitectonic sub-regions) in non-human primates and humans (Bonthius et al., 2005; Shaw et al., 2008). The design of methods to specifically demarcate these connectivity-based regions will likely enhance the understanding of insular involvement in general function and in clinical disorders.

Williams syndrome (WS) is a genetic condition associated with the deletion of approximately 20 contiguous genes on chromosome 7. Most individuals with WS have general intellectual disability as well as particular cognitive deficits in visual–spatial, mathematical, and problem-solving abilities (Bellugi et al., 2000). Individuals with WS also typically display hyperaffiliative behavior, atypical expressive language, and enhanced musical interest. WS individuals also express increased incidence of specific phobias, anxiety disorders consisting of an extreme fear of a specific object or situation that is disproportionate to the actual danger or threat. Previous functional imaging studies have shown insular involvement in emotional processing (Damasio et al., 2000; Craig, 2003; Mayer et al., 2006; Rauch et al., 1995; Winkielman et al., 2006; Carr et al., 2003; Phillips et al., 2004) and speech-motor functions (Braun et al., 1997; Corefield et al., 1999; Dronkers, 1996; Price, 2000; Fox et al., 2001; Brown et al., 2005). The insula is involved in reactions to aversive stimuli and representation of aversive experiences (Paulus and Stein, 2006), both physical (i.e. visceral and somatic pain) and emotional (i.e. affect and mood) (Damasio et al., 2000; Zald and Pardo, 2002; Mayer et al., 2006). In particular, the right anterior insula has been identified as a key region of interest in specific phobias (Wright et al., 2003; Paulus and Stein, 2006). While Wright and colleagues examined small animal specific phobia, it is reasonable to assume that insular activity is generalizable to most, if not all, types of specific phobias.

It was hypothesized that the anatomy of insular cortex would be atypical in individuals with WS as compared to healthy matched controls based on their characteristic anomalous social-emotional processing, and exacerbation of specific phobias especially. Atypical anatomy can be defined by atypical size of the region-of-interest (ROI) in the left and/or right cerebral hemisphere, or atypical asymmetry patterns. Examples of atypical ROI volume and asymmetry have been found in other clinical populations. Atypical brain region volume may represent a change in morphology specific to a clinical population (Foundas et al., 2003), while atypical asymmetry could be related to specific behavioral attributes (Foundas et al., 2004), such as stuttering severity. Given the predominant right hemispheric deficits in WS (i.e. emotional anomalies) (Meyer-Lindenberg et al., 2004; Nakamura et al., 2001), it was hypothesized that the WS group would have right hemispheric insular volume reductions. In addition, based on the connectivity of the insular sub-regions and its involvement in specific phobias, it was hypothesized that the anterior sub-region would be more anomalous than the posterior sub-region.

2. Methods

2.1. Insular method reliability

2.1.1. Subjects

Initial reliability was calculated using 3 subjects (6 hemispheres) from Tulane University. All subjects used in this study were right-handed adults. Scans were selected at random from a cohort of neurologically intact adults.

Initial reliability was extended by adding a set of 10 subjects from the Williams syndrome data set that were randomly selected and included in a separate reliability calculation described below.

2.1.2. Data acquisition

Volumetric MR images from Tulane University were acquired for each subject on a GE 1.5 Tesla Signa scanner. T1 weighted images were obtained as a series of 1.5 mm gapless sagittal images. A fast gradient spoil recall was used for the GE scans, with the following parameters: TR = 400, TE = 19, 256 × 256 voxel matrix, 24 cm field of view and 10 degree flip angle. To ensure subject confidentiality and rater blindness, each scan was assigned a subject number. To correct for head position and create a standardized space across images, the MR images were aligned in ANALYZE using the ACPC tool so that the line containing the anterior commissure and posterior commissure, or AC-PC line, was in the horizontal plane.

Coronal brain scans were acquired for each subject from the WS cohort using a GE-Signa 3T scanner (General Electric, Milwaukee, WI) at Stanford University. Coronal brain images were acquired using the following fast 3D volumetric radio frequency spoiled gradient echo pulse sequence parameters: TR = 24 ms, TE = 5 ms, flip angle = 45°, number of excitations = 2, matrix = 256 × 256, field of view = 24 cm, slice thickness = 1.2 mm, 124 contiguous slices.

2.1.3. Image processing

The ANALYZE software package (MAYO Clinic, 1986), version 5.0, was used to process images and determine the volume of the insula and connectivity-based sub-regions in each subject. In order to utilize the program tools, the original scan files for the 3 subjects were stacked using the Import/Export volume tool to create the full brain file in ANALYZE format. All MRI files were aligned along the AC-PC line. Half of the brains were randomly flipped, reversing left and right hemispheres, to insure rater blindness. Within the package, there was a region-of-interest (ROI) function that allowed the rater to create cursor-guided free-hand traces on individual images of desired brain regions. The ROI tracing on each image created an area that was multiplied to slice thickness in order to produce a volume. Region-of-Interest volumes from successive images were then summed to yield a volume, in cubic centimeters, for the full extent of the desired ROI. All summations were calculated within the ANALYZE program.

2.1.4. BrainImageJava

(BIJ) (Ng et al., 2001) (CIBSR.stanford.edu/tools), a freeware program developed in the Center for Interdisciplinary Brain Sciences Research, was used to process images and trace insular ROI volumes in 10 additional subjects. Brain images were stacked, aligned, and skull stripped in BIJ. Insular ROIs were drawn on the spatially aligned images in BIJ, and volumes were determined from the ROI drawings. An insula-specific segmentation tool was built into BIJ that produced the same segmentation capabilities as that used in ANALYZE.

2.1.5. Reliability from ANALYZE and BrainImageJava (BIJ)

Thirteen (13) total brain scans were used to calculate method reliability across two programs, ANALYZE ($N = 3$) and BIJ ($N = 10$).