A Critical Comparison of Different Methods of Analysis for Individual Variability Studies Utilizing fMRI Regions of Interest

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Introduction: The fMRI literature presents a wide array of different methods in the analyses of neural data. The purpose of this study was to determine whether these methods are equivalent and, if not, which analysis method is most consistent with clinical and physiological data in evaluating data from individual subjects, which requires greater sensitivity than group analyses. Neuroscience research on humans and animals has elucidated the neural circuitry associated with emotion. This emotional circuitry is known as the "limbic system," which includes the amygdala as the region of interest associated with the arousal response, and the medial prefrontal cortex and hippocampus, which are regions of interest associated with inhibition of the arousal response (LeDoux, 1995). To better understand individual vulnerability and resilience to stress, we compared different methods of ROI analysis with clinical and physiological markers for emotional arousal.

Methods: We imaged 34 subjects (12 male, 22 female) with mean age 26.4 years (standard deviation of 8.9) using a Philips 1.5T Intera scanner. These were acquired using two blocks of 136 T2*-weighted echoplanar images covering the frontal and limbic areas of the brain, with TR=2500ms, SENSE factor =2, TE=45ms, Flip angle = 90º Matrix=64x64, 3.9 mm X 3.9 mm X 4 mm voxels, and 30 contiguous oblique coronal slices. During these scans, subjects viewed blocked presentations of faces depicting angry, happy, neutral and fear emotions alternating with a fixation cross (Ekman and Friesen, 1977). ROI masks for the amygdala, hippocampus and the medial prefrontal cortex were traced on a standard anatomical template. We compared five different methods for the ROI analyses:

1. Maximum BOLD method: Maximum BOLD value (% signal change) was extracted in the ROI for each of the contrast-weighted images using the ROI mask.
2. Maximum t-value method: Maximum t score (ratio of contrast signal mean/standard deviation) was extracted in the ROI from each of the t maps.
3. T on average BOLD method: Average BOLD signal in the ROI from the preprocessed EPI images was obtained for each of the five conditions (angry, neutral, happy, fearful & rest). A t-test was performed comparing the averages between different conditions.
4. Average BOLD around maximum activated voxel method: Random effects analysis over all the subjects was done (See figure 1). Using this analysis the voxel with maximum t value in the ROI was identified and an average of BOLD values over 8 contiguous voxels around this voxel was computed for individual subjects.
5. Average BOLD in cluster method: Clusters containing at least 4 contiguous voxels above a threshold (p = 0.05) were identified using t maps. An average of BOLD value over the voxels in the largest cluster identified was taken.

Results were compared, using Pearson bivariate correlations, with both clinical (subjective) measures of anxiety (Spielberger, 1983) and salivary measurements of cortisol, a stress-hormone.

Results: The second ("Maximum t-value method") showed greater consistency than any of the other methods when compared to both clinical and physiological measures of anxiety. Since the amygdala is associated with the excitatory arousal response, we expected to see correlations between anxiety and amygdala activation. However, only the Maximum t-value method showed correlations between level of anxiety and (left) amygdala activation for the Neutral-Rest contrast (r=0.357; p=0.038). Correlational analyses also demonstrated a consistent association of waking cortisol (stress-hormone) and bilateral amygdala activation during the Fear-Rest contrast for the Maximum t-value method (left amygdala: r = 0.464, p = 0.022; right amygdala: r = 0.419, p = 0.042). Maximum t-value for the Fear-Rest contrast (left amygdala) and cortisol continued to be associated (trend) for the 8am sample (r = 0.383, p = 0.059) and 9am sample (r = 0.0366, p = 0.072). The diminished correlation over time is consistent with diurnal physiological changes. We also found a relationship between 9am cortisol (right amygdala: r = 0.550, p = 0.007) and 4pm cortisol (right amygdala: r = 0.457, p = 0.019) for the Neutral-Rest contrast for the T on average BOLD method.

Discussion: While group analyses are often used to increase signal/noise, the necessity for group analyses severely constricts the possibilities for study design; in particular, eliminating important research on the factors responsible for variability between individuals. We believe that, while BOLD % signal change may be adequate for group analyses, use of t-scores, which incorporate not only mean signal but also standard deviation, may introduce the added sensitivity needed for single-subject ROI analyses. The use of maximum, rather than average, values certainly carries an additional risk of false positive because its value is dependent upon only one voxel. However, we believe that maximum values may be necessary in evaluating small structures such as the amygdala, in which the size of the structures involved may only be 1-2 voxels, to prevent activated areas being "washed out" by averaging over a larger number of voxels. Correlations over larger numbers of subjects (n>20) can then be used to prevent false positives from tainting the results.

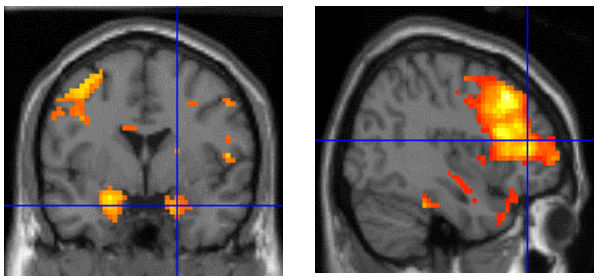

Figure 1: Random Effect Analysis Neutral-Rest (p=0.05, N=34) showing bilateral amygdalar (left) and medial prefrontal cortex (right) activation.

References:

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