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NeuroReport 7, 1765–1769 (1996)

Human amygdala activation detected with echo-planar functional magnetic resonance imaging

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ECHO-PLANAR functional magnetic resonance imaging (EP-fMRI) was used to study the activity of the amygdalae while three normal female subjects viewed alternating blocks of affectively neutral and affectively negative still pictures. Bilateral activation in the amygdala that was significantly correlated with the changing valence of the visual stimuli was found in all three subjects. These findings are consistent with the large corpus of data from non-human studies suggesting that the amygdala is a key structure for extracting the affective significance from external stimuli. This is the first known report of phasic amygdala activation detected with EP-fMRI in normal human subjects responding to affective stimuli.

Key Words: Human; Amygdala; Echo-planar; Functional magnetic resonance imaging

Introduction

Disruptions in affective behavior resulting from temporal lobe insults were first documented more than a century ago.[1] Brown and Schafer noted disruptions in emotional behavior, including tameness, increased oro-alimentary exploration, reduced feeding and abnormal sexual behavior, in several species of monkeys following temporal, but not occipital lobe resections. Kluver and Bucy[2,3] rediscovered this phenomenon (the Kluver–Bucy syndrome) in rhesus monkeys following gross temporal lobe lesions. Non-human studies underscore the centrality of one temporal lobe structure, the amygdala, in affective processes. Weiskrantz[4] performed the first specific study of the neuroanatomical basis of the Kluver–Bucy syndrome. Bilateral amygdala ablation in rhesus monkeys produced the classic hypoemotional response to threatening stimuli. In a unique study, Downer[5] prepared split-brain monkeys with a unilateral amygdalectomy and a lesion to the optic chiasm. When presented with threatening visual stimuli to the eye (hemisphere) with the amygdala, the monkey showed an appropriate fear response. When the same stimuli were presented to the eye (hemisphere) without the amygdala, no response was produced. In rodents, the amygdalae have been shown to be necessary for involuntary affective behavior such as the conditioned fear response.[6,7] Without the amygdalae, rats can neither learn, nor once learned, express a conditioned fear response.

Evidence related to the function of the amygdalae in human affective processes is inconclusive and largely derived from brain-damaged patients. Bechara and colleagues[8] compared human patients with either complete amygdala, hippocampus or amygdala and hippocampus damage on performance in a conditioning task. Using skin conductance to index conditioned affective associations, the patient with amygdala damage failed to acquire stimulus–affect associations, but correctly identified the target stimuli (the conditioned stimuli). The patient with hippocampus damage acquired the affective associations, but could not identify the target stimuli. The patient with both amygdala and hippocampus damage failed in both respects. Patients with damage to the amygdalae also show impairment in the recognition of facial expressions of fear.[9] However, Tranel and colleagues[10] showed that a patient with complete bilateral destruction of the amygdalae was able to acquire non-conscious associations between strangers and the affective valence of their behavior.

Several studies have used positron emission tomography (PET) to examine regional cerebral blood flow or glucose metabolism in the amygdalae in response to affective manipulations.[11–15] With the exception of

two recent studies that used MRI-PET co-registration techniques to isolate activity in the amygdalae,[14,15] the findings from other studies are questionable due to constraints in the spatial resolution of PET which make it impossible to isolate amygdala activity from that of other nearby structures.

Functional magnetic resonance imaging (fMRI) has better spatial resolution than PET and a temporal resolution that better approximates the time course of affective responses. The current study examined human amygdalae activation in response to viewing affective visual stimuli using fMRI in a normal human sample. We hypothesized that bilateral activation in the amygdalae would increase when subjects viewed affectively negative rather than neutral pictures.

Materials and Methods

fMRI uses the principles of MRI to non-invasively detect local changes in blood oxygenation coincident with changes in local neuronal activity.[16] Implementation of this technique requires a paradigm that produces alternating brain states every tens of seconds, during which functional images are obtained every few seconds. Changes in MR signal between alternating states reflect changes in neuronal activity. In the current study, the alternating states were negative affect (produced by viewing aversive pictures) and no affect (produced by viewing neutral pictures).

Subjects: Six female right-handed subjects (age 18–32 years) were recruited through advertisements in the Department of Psychology and paid $50.00 (US) for their participation. All subjects signed a consent form which was approved by the Institutional Review Board. The experiment took place in three sessions. Session 1 acquainted subjects with the procedures using a mock scanner. Session 2 was the actual scanning session. In Session 3, subjects made ratings of the pictures to which they were exposed.

Stimulus presentation: Still pictures were selected from the International Affective Picture System (IAPS).[17] Based on the large-sample valence (positive–negative) ratings, pictures were selected as neutral (e.g. a book) or negative (e.g. a mutilated face). Moderately positive pictures (e.g. sunset) were selected for a control trial. Pictures were presented on a screen located at the foot of the scanner bed and subjects used a mirror built into a standard head coil to see the screen while in the supine position. Pictures were presented under computer control and synchronized with the start of the scanner for each trial. A trial consisted of 11 blocks of five valence-constant pictures, with each picture presented for 6.6 s. The first trial consisted of blocks of only positive pictures. The second trial consisted of alternating blocks of neutral and negative pictures, always beginning and ending with a neutral block.

Scanning protocol: An initial anatomical scan was used to locate the amygdalae in each subject with reference to a standard atlas.[18] Three contiguous 5 mm coronal slices with the middle slice centered on the amygdalae were prescribed from which functional data were acquired. A gradient-echo echo-planar pulse sequence (TE = 50 ms, TR = 1500 ms, flip angle = 90°, matrix = 128 × 64, FOV = 40 × 20, NEX = 1) was used to acquire functional images (246 per slice per trial) using a GE Signa 1.5T (General Electric, Waukesha, WI) scanner equipped with Advanced Nuclear Magnetic Resonance (ANMR, Boston, MA) whole-body gradients. A three-dimensional Spoiled GRASS pulse sequence (TE = 14 ms, TR = 35 ms, flip angle = 35°, matrix = 256 × 256, FOV = 20 × 20, NEX = 1) was used to acquire whole-brain anatomical images.

Results

Manipulation check: The subjects' picture ratings confirmed that the intended manipulation of affective response was successful. The difference in valence ratings across subjects between the neutral (5.18 ± 0.58, mean ± s.d.) and negative (2.45 ± 0.57) pictures was significant ($t(53) = 17.60$, $p < 0.001$; 1 = negative to 9 = positive).

Head motion detection and correction: The image data were examined for signs of head movement. This included both visual examination for signs of gross head movement, and examination of correlational patterns. The latter was accomplished by cross-correlating the MR time course of pixels in the region of interest (ROI), the amygdala, with all other pixels in the image. High correlations (positive or negative) between the ROI and the rim of the image are suggestive of head movement.[19] Three of the six subjects had activation patterns consistent with movement artifact. Image data were then motion corrected with the automated image registration (AIR)[20] algorithm using the first image in the time series as the reference image. The inferred head movement based upon the correlation procedure in the three subjects was corroborated by comparing the results of the time course analysis (described below) between the uncorrected and AIR-corrected data. For the three subjects whose data we present, there were no correlational patterns suggestive of head movement, and analysis of the uncorrected and AIR-corrected data yielded virtually identical results. The head movement in the other three subjects was judged to be too severe for motion correction.

Time course analyses: Time course analyses were conducted on the motion-corrected data by

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computing a three-parameter (i.e. amplitude, slope, mean) least-squares fit between a reference function and the MR image pixel values which yields a correlation coefficient indexing the goodness of fit.[21] The reference function was a box-car corresponding to the presentation of alternating picture blocks. The hemodynamic delay between change in picture blocks and change in MR signal was 3 s (i.e. two functional images). The first picture block was 6 s (four functional images) longer than subsequent blocks to ensure an accurate measure of the baseline MR signal. Thus, a phase offset of six functional images was used for all analyses. The Student's t threshold for the correlation coefficient was set at 2.3 [$p < 0.01$, df = 237; (246 images–6 offset images–3 parameters)]. *Anatomical co-registration and subject averaging:* The least-squares analysis yields a statistical parametric map (SPM) identifying those regions which have significant paradigm-correlated activation. Analysis of Functional NeuroImages (AFNI)[22] was used to co-register the SPMs with the anatomical images. This allows precise anatomical localization of the areas of activation, as well as transformation into Talairach[23] coordinates, which then permits comparisons across subjects.

Figure 1 shows representative time courses from the pixel with the maximum value of Student's t from the right and left amygdala of one subject. For purpose of visualization, these time courses were convolved with a 22-point (width of the picture blocks) Hamming window to remove high frequency components which are a function of image-to-image fluctuations (e.g. shot-to-shot variability) during acquisition. The fitting procedure for these pixels yielded a Student's t of 4.99 and 2.83, and activation (percentage change in signal from neutral to negative picture blocks) of 1.86% and 0.85% for the right (top) and left amygdala (bottom), respectively. The patterns of these time courses are consistent with the hypothesis that there was an increase in activation in the amygdalae when subjects were viewing negative rather than neutral pictures. There were no pixels with significant paradigm-correlated activity in the amygdalae during the trial where subjects saw only positive pictures. Significant activation in the amygdalae was found in all three subjects, though not in the exact location and not at the same levels. The maximum Student's t ranged from 2.75 to 4.99 with activation ranging from 0.75% to 1.86% for the right, and 2.50 to 3.53 with activation ranging from 1.14% to 1.36% for the left amygdala.

Figure 2 shows areas of common activation across two different pairings of the subjects. The activation is presented co-registered with anatomical images in Talairach coordinates with coronal, axial and sagittal views shown (the left side of the coronal and axial

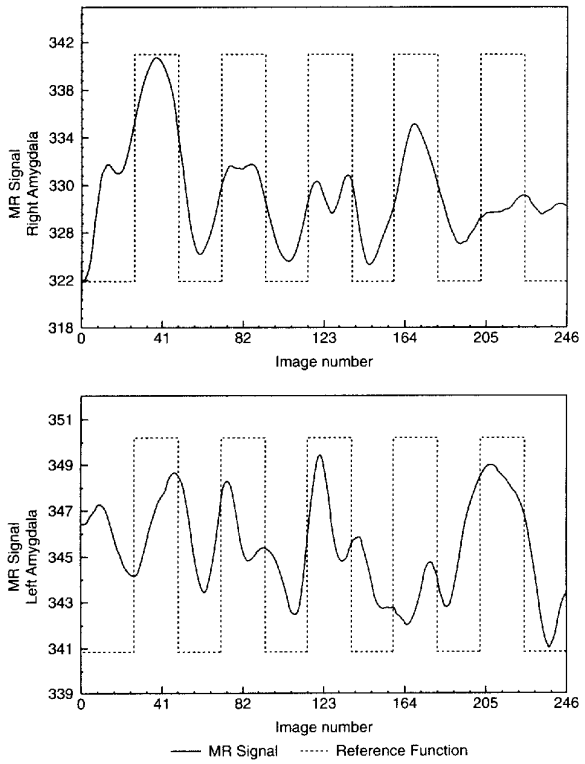

FIG. 1. Reference function (broken line) and MR signal (solid line) from a single pixel in the right (top) and left (bottom) amygdala from Subject 1. Beginning and ending with neutral picture blocks, the MR signal reveals an oscillation that is in good correspondence with the alternation of picture blocks.

images is the subject's right). Figure 2, left, shows bilateral foci of activation in the medial region of the amygdalae (Talairach coordinates: x = ±15, y = –6, z = –11; Subjects 1 and 2). The average activation for these pixels ranges from 0.50% to 1.50%. Figure 2, right, shows a focus of activation in the lateral region of the right amygdala (Talairach coordinates: x = 26, y = –6, z = –12; Subjects 1 and 3). The average activation for these pixels ranged from ~0.50% to 1.85%. The values of the color bars indicate the upper bin boundaries. Note that this figure displays only pixels in these planes that were common across subjects, but bilateral activation in the amygdala was found in all subjects.

Because our hypothesis was about amygdalae activation, the planes shown in Figure 2 were selected to display activation in the amygdalae to the exclusion of other planes. However, common activation in other regions in these planes is evident. For example, in addition to the robust amygdalae activation, the coronal view in the left panel in Figure 2 reveals a small focus of activation (yellow) in the region of left insular cortex as well as a small focus of activation (red) in the lateral right temporal lobe.

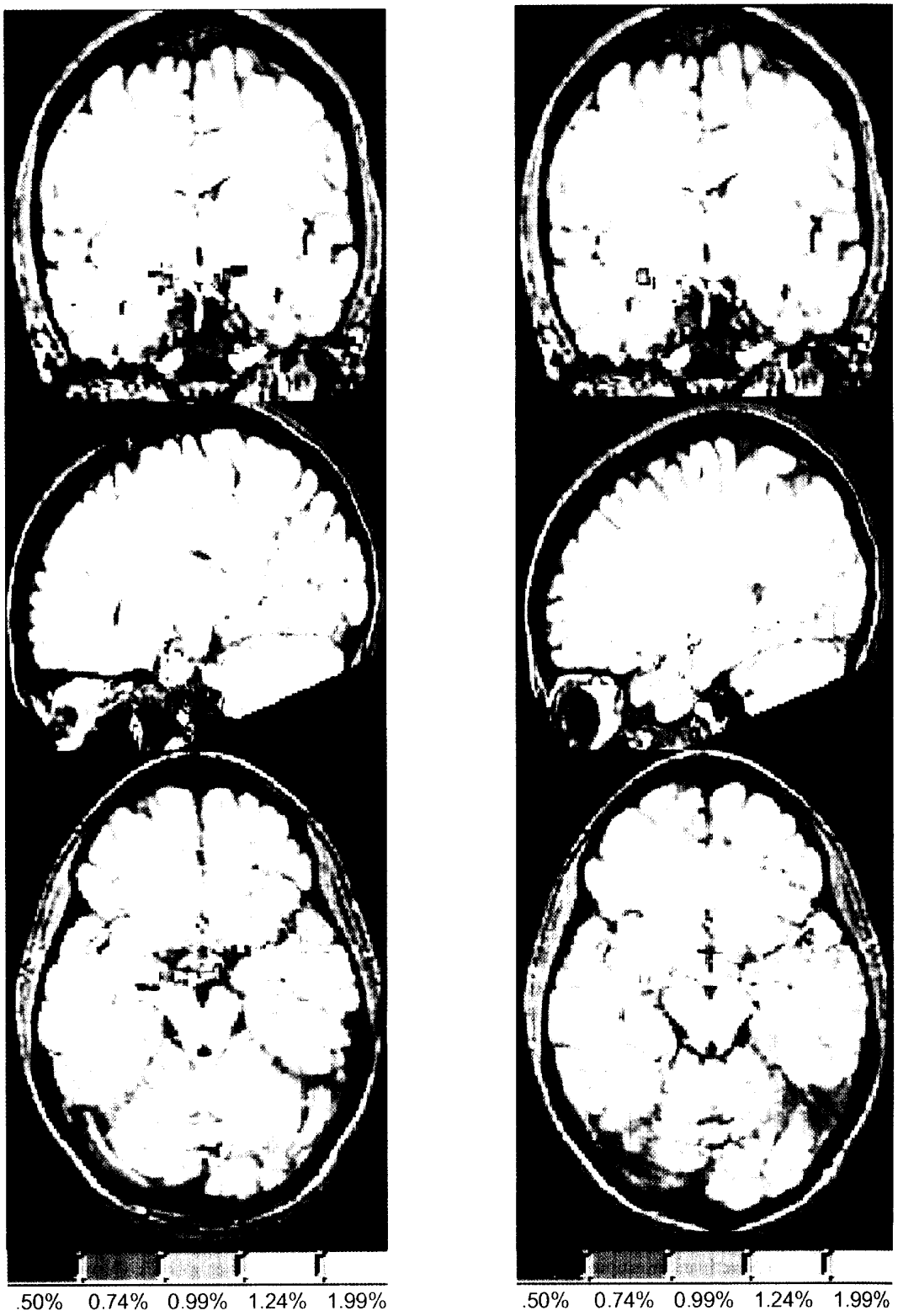

FIG. 2. Coronal (top), sagittal (middle) and axial (bottom) views of anatomical images showing coregistered activation foci. The images are presented in the Talairach coordinate system. Left: bilateral amygdala activation common to Subjects 1 and 2, with coordinates for the foci in the right and left amygdala being x = -15, y = -6, z = –11, and x = -15, y = -6, z = –11, respectively. Right: unilateral (right) amygdala activation common to Subjects 1 and 3, with the coordinates of the focus being x = -26, y = –6, z = -12. Color scale indicates average percentage increase in signal between neutral and negative picture blocks where the values are the upper boundaries of the bins.

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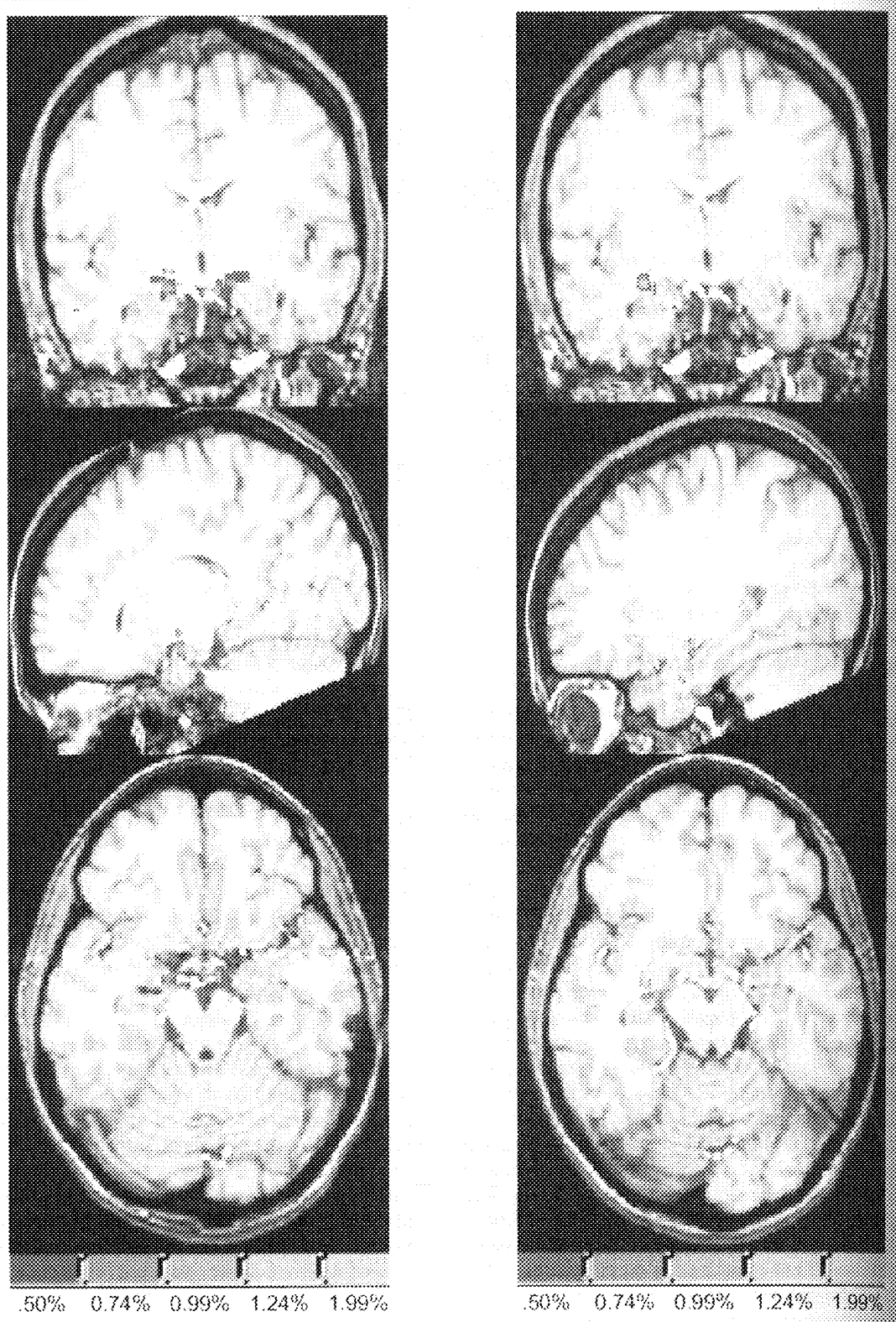

FIG. 2. Coronal (top), sagittal (middle) and axial (bottom) views of anatomical images showing coregistered activation foci. The images are presented in the Talairach coordinate system. Left: bilateral amygdala activation common to Subjects 1 and 2, with coordinates for the foci in the right and left amygdala being x = -15, y = -6, z = -11, and x = -15, y = -6, z = -11, respectively. Right: unilateral (right) amygdala activation common to Subjects 1 and 3, with the coordinates of the focus being x = -26, y = -6, z = -12. Color scale indicates average percentage increase in signal between neutral and negative picture blocks where the values are the upper boundaries of the bins.

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If the viewing of the negative pictures resulted in a significant increase in non-specific arousal, one would expect other regions of activation to emerge. In fact, in the limited region (1.5 cm) from which we obtained functional images such activation was not found.

Discussion

Using a non-invasive technique with the temporal resolution necessary to capture the time course of a brief affective response and a spatial resolution capable of localizing the activation of small subcortical structures, we have demonstrated for the first time in a normal human sample activation in the amygdalae in response to affectively negative visual stimuli. Activation in the amygdalae was increased when subjects viewed negative rather than neutral still pictures. This finding is consistent with, and builds upon, the findings from non-human and human lesion studies that have identified the amygdala as the neural structure responsible for extracting the affective content from stimuli in the environment. In this study, it was the affective saliency (valence) of the stimuli which changed between picture blocks. This change was correlated with variations in MR signal intensity in the amygdalae, reflecting changes in local neuronal activity. The magnitude of the observed MR signal increase in the amygdalae ranged between ~0.50 and 1.85%. Other cortical and subcortical regions are obviously involved in the processing of these types of stimuli, but those regions are active in the processing of both neutral and negative pictures and, therefore, not revealed as significant changes between these conditions in activation levels.

We do not know from this study whether our findings are associated with the activation of affect *per se* or with negative affect in particular. Some investigators have claimed a very general role for the amygdalae in all affective behavior,[24] while others have implied a specific role for fear-related affect.[9] Until a comparison in the same subjects is made between amygdalae activation in response to negative and positive affective stimuli, this issue will remain unresolved.

Finally, the fact that we observed activation in the amygdala should not be interpreted as showing that activation in response to negative pictures is restricted to this region. In this study, we only imaged a limited region of brain activity. Our laboratory is now conducting studies that include whole-brain acquisition while both negative and positive affective states are generated. Only with such data will we be in a position to describe the circuitry that participates in affective processes.

Conclusion

This study is the first to show MR signal changes in the human amygdalae produced in response to affective stimuli. Our findings are consistent with the results from non-human and human brain damage studies on the role of the amygdala in affect and extend those studies to normal humans. Because only negative affect was manipulated in our paradigm, we cannot address whether amygdala activation is a non-specific component of the generation of all affect, independent of valence, or whether it is specific to negative affect. Future studies will utilize whole-brain fMRI during both positive and negative affective states in both normal individuals and patients with affective disorders so that the detailed circuitry that subserves affect, differentiates between positive and negative affect, and is abnormal in psychopathology, can be better understood.

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ACKNOWLEDGEMENTS: This work was supported by a NSF Graduate Fellowship to W.I., a NIMH Research Scientist Award (MH00875), NIMH grants P50-MH52354, MH40747 and MH43454, a grant from the John D. and Catherine T. MacArthur Foundation and a NARSAD Established Investigator Award to R.J.D., a NRSA training grant (T32-CA09206) to the Department of Radiology and NICHD core grant HD03352 to the Waisman Center.

Received 11 April 1996;
accepted 30 April, 1996