Amygdalar activation associated with positive and negative facial expressions

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Most theories of amygdalar function have underscored its role in fear. One broader theory suggests that neuronal activation of the amygdala in response to fear-related stimuli represents only a portion of its more widespread role in modulating an organism's vigilance level. To further explore this theory, the amygdalar response to happy, sad, angry, fearful, and neutral faces in 17 subjects was characterized using 3 T fMRI. Utilizing a random effects model and hypothesis-driven analytic strategy, it was observed that each of the four emotional faces was associated with reliable bilateral activation of the amygdala compared with neutral. These findings suggest a broader role for the amygdala in modulating the vigilance level during the perception of several negative and positive facial emotions. *NeuroReport* 13:1737–1741 © 2002 Lippincott Williams & Wilkins.

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INTRODUCTION

The primate amygdala is thought to be involved in social behavior, emotion, and the processing of facial expressions specifically governing emotional and social responses to the face [1] and processing the affective information conveyed by the face [2]. In humans, the evidence to date indicates that the amygdala plays an important role in how a person evaluates social cues [3] and processes facial expressions [4]. Lesions to the amygdala greatly impairs an individual's processing and recognition of fearful faces [4], but responses to other emotional faces are less clear.

Functional neuroimaging studies also have been consistent in demonstrating that the amygdala is involved in the perception of fearful faces [5]. There have been fewer or less conclusive neuroimaging studies with amygdalar responses to the perception of other emotions such as anger and sadness [6,7] or to positive emotions such as happiness.

In an fMRI study, Breiter *et al.* [8] unexpectedly found that the amygdala responded to the perception of happy *vs* neutral faces, suggesting a possible generalized response of the amygdala to emotionally valenced stimuli. Because these findings were unexpected, Breiter *et al.* stated that these findings should be replicated.

Whalen *et al.* [9] proposed that the amygdala should be considered as an integral component of a constant vigilance system which is preferentially invoked during ambiguous learning situations of biological relevance. Thus, the amygdala should be activated by a stimulus that requires additional information to be understood. Whalen *et al.* suggest that fearful faces stimulate the amygdala because the source of the threat is perceived as ambiguous. Based upon this theory, we hypothesized that other negative and positive facial expressions that might be interpreted as ambiguous should also activate the amygdala. For instance, a sad facial expression might indicate that the person is sad due to some personal misfortune (i.e., the failing of an examination) or that some terrible event has occurred that could have a negative effect on everyone (i.e. the start of the next World War). Similarly, a happy facial expression might also be ambiguous. A happy facial expression might indicate that something wonderful has happened for everyone (i.e. a good harvest with food for everyone) or that your enemy is happy because something dreadful is about to happen to you. In both examples, additional information is needed in order to understand the possible biological relevance of the perceived emotion to the observing person.

The purpose of the present fMRI study was to assess whether amygdalar activation is specific to the perception of a negative facial emotion such as fear or whether it has a possible broader role in the perception of several negative and positive facial expressions. Therefore, we examined the amygdalar response to the perception of happy, sad, angry, and fearful facial expressions compared to neutral.

On the basis of the PET study showing an enhanced response to sad facial expressions in the left amygdala [10], fMRI studies demonstrating amygdalar response to angry [9] and fearful faces [5,8], it was hypothesized that the

human amygdala would show significant activation during an individual's perception of fearful, angry, sad, and possibly happy faces. In addition to the fMRI study by Breiter *et al.* [8], unit recordings in humans [11] suggest that the amygdala may play a role in processing positive facial expressions. Thus, based upon these findings, clearly defined individual regions of interest (ROIs) were selected *a priori* in order to measure changes in activation of the left and right amygdalae.

The strengths of the current study include the use of (a) a large number of carefully rated faces, (b) a relatively large number of subjects, (c) a behavioral task that allowed us to monitor performance measures such as accuracy and reaction time, (d) a hypothesis-driven analysis using data from individually drawn amygdala ROIs and (e) a voxel-by-voxel random effects model analysis. Using this model ensured that only brain voxels that are consistently activated across subjects, rather than within subjects, would emerge as significant population activation. Hence, the results generated from using this model provide a better generalization to the (normative) population from which the sample was acquired [12].

MATERIALS AND METHODS

Subjects and stimuli: Seventeen healthy, right-handed subjects (11 females and six males, ages 18–32 years, mean ($\pm$ s.d.) 23 ± 3.69 years) participated in this study after giving written informed consent.

A set of over 1600 photographs of faces of people posing different emotions was assembled from several sources, including photograph collections of other researchers (Laura Carstensen, Ruben Gur, Paula Niedenthal, Stephen Nowicki, and Robert Zajonc), standardized sets of emotional faces developed by Ekman and his colleagues [13,14], and sets of photographs developed by Lang and his colleagues [15]. In addition, photographs were taken of 27 undergraduate, graduate, and postdoctoral student volunteers posing different emotions. All images were digitized and edited to be monochromatic and of the same size (260×300 pixels, or $\sim 9 \times 10$ cm).

All photographs were independently rated by 14 right-handed, healthy students (seven females and seven males, ages 15–38 years, mean 21 ± 5.93 years) with respect to happiness, sadness, anger, and fear, using scales ranging from 1 (no emotion) to 7 (extreme emotion) (Table 1). Faces were categorized as a particular target negative expression if they received a mean rating of > 4 (with 4 representing moderate intensity) on the target scale, < 4 on the other two negative expression scales, and < 2 on the happiness scale. Faces were categorized as happy if they received an average rating of > 4 on the happiness scale and < 2 on the sadness, anger, and fear scales. Faces were categorized as neutral if they received an average rating of < 2 on all four emotion scales (happiness, sadness, anger, and fear).

Following the procedures of Bradley *et al.* [16], pairs of one emotional and one neutral photograph of the same poser were used as stimuli. Using the same poser ensured that the pictures in each pair were matched exactly with respect to age, gender, race, physical appearance, attractiveness, etc., and that the only difference between the two pictures was the emotional expression. In contrast to Bradley *et al.* [16], however, who used only two types of picture pairs (angry–neutral and happy–neutral), four types of picture pairs were used in this study: fearful–neutral, angry–neutral, sad–neutral, and happy–neutral. Within each emotion face category, half of the pictures were male and half were female. The pictures were carefully matched with respect to the intensity of the dominant emotion displayed in the picture, both across the gender of the poser and across the different types of emotional expression.

Experimental design: The functional imaging experiment was divided into two scans composed of 17 24 s blocks in order to minimize subject fatigue. In each scan, two valenced expressions (happy and sad (first) scan or fearful and angry (second) scan) and the neutral expression were each presented for four blocks. Within each block of faces posing a particular target emotion, eight faces were presented contiguously for 3 s each with no ISI. A rest, neutral, or scrambled block was placed between each affect block. This was done to allow the blood oxygenation level-dependent (BOLD) signal to decay to baseline levels between the presentation of different affective stimuli. The happy (H), sad (S), neutral (N), scrambled (SC), and rest (R) blocks were presented in the following order: R-H-N-S-SC-H-N-S-R-H-N-S-SC-S-N-H-R. The angry (A), fearful (F), neutral (N), scrambled (SC), and rest (R) blocks were presented in a similar pattern: R-A-N-F-SC-A-N-F-R-A-N-F-SC-F-N-A-R. No comparisons with the scrambled blocks were used in this study.

Subjects were instructed to perform a gender discrimination task while inside the scanner to assure attention to the stimuli.

Image acquisition and analysis: Images were acquired on a 3 T GE Signa scanner (General Electric, Milwaukee, WI) with EchoSpeed gradients using the standard GE coil. A spiral sequcnce was employed in order to reduce susceptibility-related loss of signal and warping in/near the amygdala. A custom-built head holder was used to minimize head movement. Eighteen axial slices (4 mm thick, 0.5 mm skip) parallel to the anterior and posterior commissure covering the whole brain were imaged with a temporal resolution of 3 s using a T2*-weighted gradient echo spiral pulse sequence (TE = 30 ms, TR = 3000 ms, TR = 2000 ms for last seven subjects, flip angle = 89° and 1 interleave). Number of slices (28), slice thickness (4.0 mm), epoch length, and voxel size remained the same for all subjects. Field of view was 200 mm and the in-plane spatial resolution was 3.125 mm. To aid in localization of functional data, high resolution T1-weighted spoiled grass gradient recalled (SPGR) 3D MRI sequence with the following parameters was used: TR = 35 ms; TE = 6 ms; flip angle = 45°; 24 cm field of view; 124 slices in coronal plane; 256×192 matrix; acquired resolution = $1.5 \times 0.9 \times 1.2$ mm. The images were reconstructed as a $124 \times 256 \times 256$ matrix with a $1.5 \times 0.9 \times 0.9$ mm spatial resolution. An automated high-order shimming method based on spiral acquisitions was employed to reduce B_0 heterogeneity [17].

fMRI analysis: fMRI data from each subject were analyzed using SPM99b (www.fil.ion.bpmf.ac.uk/spm). Prior

to statistical analysis, images were corrected for movement using least square minimization without higher-order corrections for spin history, normalized to stereotaxic Talairach coordinates [18], resampled every 2 mm using sinc interpolation, and smoothed with a 4 mm Gaussian kernel to eliminate spatial noise. Further details are published elsewhere [19].

Brain activation was determined for each of the four facial emotions (i.e., Happy, sad, angry, and fearful) contrasted with the neutral condition. Because this study was hypothesis-driven and used a more stringent statistical model (random effects), a threshold of $Z > 1.67$ ($p < 0.05$) was used to identify significantly activated voxels.

Amygdala ROIs: Amygdala ROIs were separately drawn for each of the 17 individual subjects using a highly reliable method described elsewhere [20].

Behavioral data analysis: The percentage of correct and incorrect button presses was computed for each of the five facial expressions: happy, sad, angry, fearful, and neutral. Reaction times for the correct and incorrect responses were also recorded. Friedman tests were conducted to test for differences in accuracy and reaction times across the five facial expressions.

RESULTS

Behavioral data: All 17 subjects performed the gender discrimination task with a high level of accuracy. The average percentage correct ranged from 0.91 to 0.98 (happy: 0.98 ± 0.03; sad: 0.96 ± 0.05; angry: 0.94 ± 0.04; fearful: 0.91 ± 0.04; neutral: 0.97 ± 0.02). The average reaction times ranged from 829 to 865 ms (happy: 826 ± 98; sad: 865 ± 108; angry: 864 ± 137; fearful: 863 ± 136; neutral: 830 ± 96). Non-parametric Friedman tests were conducted to examine differences in accuracy and reaction times across the five facial expressions. Statistically significant differences in accuracy were observed, ($\chi^2 = 31.1$, $df = 4$, $p < 0.0001$). Follow-up comparisons using the Wilcoxon test indicated that accuracy for fearful faces was significantly lower than accuracy for happy faces ($p = 0.007$), angry faces ($p = 0.019$), and neutral faces ($p = 0.01$). Accuracy for the sad faces was lower but not significantly lower than fearful faces ($p = 0.09$). The Friedman test conducted on reaction times did not indicate any significant differences for the five conditions ($\chi^2 = 5.7$, $df = 4$, $p = 0.22$).

Amygdalar activation: Compared with neutral faces, all four facial emotions were associated with significant activation in the amygdala. As shown in Fig. 1, Z score test statistics exceeded 1.67 ($p < 0.05$) for all affect conditions. ROI analyses also demonstrated significant amygdalar activation for each of the four facial emotions relative to neutral (Fig. 2). A repeated-measures ANOVA with factors affect (happy, sad, angry, fearful) and hemisphere (left, right) was used to directly compare the extent and magnitude of activation in the amygdala. Using percentage of voxels activated in the amygdala as the measure of activation, no significant differences were found across the four affect conditions ($F(3,48) = 0.68$, $p = 0.57$) or between the two hemispheres ($F(1,3) = 0.09$, $p = 0.77$). No significant affect $\times$ hemisphere interaction was detected ($F(3,48) = 0.08$, $p = 0.97$).

DISCUSSION

Using a within-subject design and a random effects analysis, we found that all four facial emotions, including happy faces, activated the amygdala compared to neutral faces. These findings demonstrate that the amygdala is activated by the perception of several negative and positive facial emotions. They furthermore suggest that the amygdala may be involved with modulating the vigilance level during the perception of several negative and positive facial emotions. In a 1.5 T fMRI experiment using stimuli selected from the International Affective Picture System (IAPS), the amygdala was found to respond to both positively and negatively valenced stimuli [21]. The results from the current study support the finding by Garavan *et al.* [21] that the role of the amygdala in processing emotional stimuli extends beyond just negative and fearful stimuli.

We also found that accuracy on the gender discrimination task was lower for fearful faces than for any of the other facial emotions. This result suggests that fearful faces may have been more engaging of attention and that the subjects were less able to suppress the emotional information during the presentation of fearful faces. The findings of Ohman *et al.* [22] support this possibility. In a series of experiments, Ohman's group demonstrated that facial stimuli implying threat were more effective than nonthreatening stimuli in capturing the attention in subjects.

The present investigation found amygdalar activation to the perception of sad faces compared to neutral. This finding is consistent with reports that human subjects with

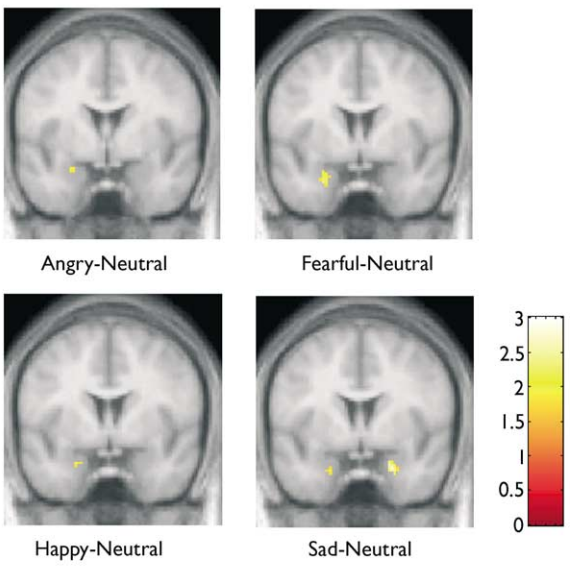

Fig. 1. Coronal sections through the amygdala demonstrating activation for each of the four facial emotions (angry, fearful, sad, happy) compared with neutral faces. The Talairach coordinates for each of the activations seen in this figure are as follows: angry *vs* neutral (−25, −1, −16), fearful *vs* neutral (−22, −5, −15), happy *vs* neutral (−23, −1, −19), sad *vs* neutral (−21, −1, −23) (left), sad *vs* neutral (22, −1, −18) (right).

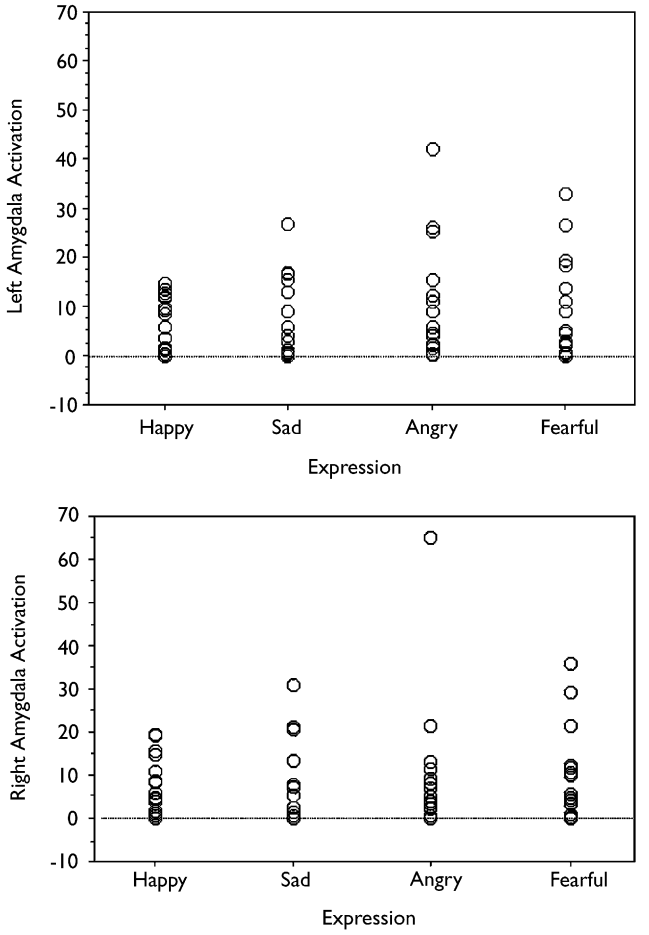

Fig. 2. Scatterplot showing percentage of voxels activated in the left and right amygdalae for each of the four facial emotions (happy, sad, angry, fearful) compared to neutral faces. All activations shown here exceeded a Z-score threshold of 1.67 ($p < 0.05$).

amygdalar lesions are impaired in recognizing negative emotions, especially fear and sadness [23]. This result also agrees with the PET study by Blair *et al.* [10]. Since Blair *et al.* [10] only examined males, the present study extends the amygdalar response to the perception of sad faces to include both genders. In their fMRI experiment, Phillips *et al.* [6] found no amygdalar activation in response to sad facial expressions. However, they had hypothesized that the perception of sad facial expressions would specifically activate bilateral limbic structures. It is possible that they failed to observe amygdalar activation in response to sad facial expressions because of the rapid habituation of the amygdala [8], and they suggested that the use of more frequent stimulus presentations might help to clarify this issue. In contrast to their study, in which subjects viewed alternating sad and neutral faces that were each presented for 30 s, each face was presented for only 3 s in the present study. Thus, it is possible that amygdalar activation was observed in the present study in response to sad faces because we used a more frequent stimulus presentation paradigm.

Although previous neuroimaging studies have failed to find amygdalar activation to angry faces, the finding of amygdalar activation in response to angry faces relative to neutral faces agrees with one other recent 3 T fMRI study that examined human amygdalar responses to fearful and angry facial expressions [9]. The current study confirms that the amygdala is activated by angry faces and provides additional information by including behavioral data and utilizing a random effects model. These results are also consistent with several human lesion studies involving the amygdala that have found partial impairment in recognizing anger [24].

Table 1. Ratings for dominant and non-dominant emotions.

Face type	Emotion rating[a]	Mean	s.d	Range
Angry	**Angry**	**5.20**	**1.77**	**1–7**
	Fearful	1.97	1.45	1–6
	Happy	1.06	0.35	1–5
	Sad	2.06	1.46	1–7
Fearful	Angry	2.02	1.38	1–7
	Fearful	**5.15**	**1.84**	**1–7**
	Happy	1.09	0.47	1–6
	Sad	2.95	1.86	1–7
Happy	Angry	1.32	0.73	1–3
	Fearful	1.6	1.03	1–3
	Happy	**5.87**	**1.46**	**1–7**
	Sad	1.35	0.77	1–5
Sad	Angry	2.8	1.78	1–6
	Fearful	2.49	1.52	1–7
	Happy	1.18	0.66	1–7
	Sad	**5.05**	**1.81**	**1–7**

Dominant emotions (e.g., angry emotion for angry faces) are shown in bold.
[a]Emotions were rated on a scale from 1 (no emotion) to 7 (extreme emotion).

This study agrees with Breiter *et al.*'s [8] finding of amygdalar activation in response to happy faces compared to neutral faces. While most human lesion studies have shown that damage to the amygdala greatly impairs the processing and recognition of fearful faces, these results are consistent with the findings of at least one human lesion study in which facial processing was examined following amygdalotomy. In this study, Young *et al.* [25] found that in the facial expression matching and recognition tasks the patient was significantly impaired in matching and identifying several emotions, including happiness. In addition to this human lesion study, unit recordings in humans [11] suggest that the amygdala may play a role in processing positive facial expressions.

Limitations: As one component of examining amygdalar activation, this study employed the use of voxel counting in the amygdala ROIs. Although a probability threshold of $Z > 1.67$ was applied in order to minimize false positives, there still exists the possible confound of spatial extent with height of activation. Future studies may wish to take an alternative approach by computing the mean intensity within each predefined ROI, and then analyzing those time series.

CONCLUSION

Although the role of the amygdala in processing fearful stimuli has been well established, its possible broader role in

other emotions has been much less clear. The present 3 T fMRI study helps to clarify the role of the human amygdala by using a random effects model and within-subjects design to present a well-standardized set of stimuli to 17 normal subjects. This study demonstrated amygdalar activation in response to the perception of happy, angry, sad, and fearful faces compared to neutral faces. These results do not support the conclusion that amygdalar activation is specific to the perception of fear. Rather, they support Whalen *et al.*'s [9] wider theory that the amygdala is involved with processing stimuli that have some biologically relevant, but presently unclear, predictive value. These results also extend the findings of Garavan *et al.* [21] to include amygdalar response to positive and negative facial expressions as well as positively and negatively valenced IAPS stimuli. Finally, these results establish a baseline against which amygdalar response to emotional stimuli in clinical conditions such as depression may be compared.

REFERENCES

1. Rolls ET. In: Aggleton JP (ed.) *The Amygdala*. Oxford: Oxford University Press; 2000, pp. 447–478.
2. Tovee MJ. *Curr Biol* **5**, 480–482 (1995).
3. Phelps EA and Anderson AK. *Curr Biol* **7**, R311–314 (1997).
4. Adolphs R, Tranel D, Damasio H *et al. Nature* **372**, 669–672 (1994).
5. Phillips ML, Young AW, Scott SK *et al. Proc Soc Lond B Biol Sci* **265**, 1809–1817 (1998).
6. Phillips ML, Bullmore ET, Howard R *et al. Psychiatry Res* **83**, 127–138 (1998).
7. Sprengelmeyer R, Rausch M, Eysel UT and Przuntek H. *Proc R Soc Lond B Biol Sci* **265**, 1927–1931 (1998).
8. Breiter HC, Etcoff NL, Whalen PJ *et al. Neuron* **17**, 875–887 (1996).
9. Whalen PJ, Shin LM, McInerney SC *et al. Emotion Special Issue* **1**, 70–83 (2001).
10. Blair RJ, Morris JS, Frith CD *et al. Brain* **122**, 883–893 (1999).
11. Kreiman G, Koch C and Fried I. *Nature Neurosci* **3**, 946–953 (2000).
12. Friston KJ, Holmes AP, Price CJ *et al. Neuroimage* **10**, 385–396 (1999).
13. Ekman P and Friesen W. *Pictures of Facial Affect*. Palo Alto, CA: Consulting Psychologists Press; 1976.
14. Matsumoto D, Ekman P. *Japanese and Caucasian Facial Expressions of Emotion (JACFEE) and Japanese and Caucasian Neutral Faces (JACNeuF) (slides)*. San Francisco: Intercultural and Emotion Research Laboratory, Department of Psychology, San Francisco State University, 1988.
15. Lang P, Bradley M and Cuthbert B. *International Affective Picture System (IAPS). Technical Manual and Affective Ratings*. Boston: NIMH Center for the study of emotion and attention, 1997.
16. Bradley BP, Mogg K, Millar N *et al. Cogn Emotion* **11**, 25–42 (1997).
17. Kim DH, Adalsteinsson E, Glover G and Spielman S. SVD regularization algorithm for improved high-order shimming. *8th Annual Meeting of ISMRM*. Denver, 2000: 1685.
18. Talairach J, Tournoux P. *Co-Planar Stereotaxic Atlas of the Human Brain: 3-Dimensional Proportional System: An Approach to Cerebral Imaging*. New York: Thieme; 1988.
19. Menon V, White CD, Eliez S *et al. Hum Brain Mapp* **11**, 117–129 (2000).
20. Kates WR, Abrams MT, Kaufmann WE *et al. Psychiatry Res* **75**, 31–48 (1997).
21. Garavan H, Pendergrass JC, Ross TJ *et al. Neuroreport* **12**, 2779–2783 (2001).
22. Ohman A, Flykt A and Lundqvist D. In: Lane RD and Nadel L, (eds.) *Cognitive Neuroscience of Emotion*. Oxford: Oxford University Press; 2000, 296–327.
23. Adolphs R, Damasio H, Tranel D, Damasio AR. *J Neurosci* **16**, 7678–7687 (1996).
24. Broks P, Young AW, Maratos EJ *et al. Neuropsychologia* **36**, 59–70 (1998).
25. Young AW, Aggleton JP, Hellawell DJ *et al. Brain* **118**, 15–24 (1995).

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