Variation of Human Amygdala Response During Threatening Stimuli as a Function of 5'HTTLPR Genotype and Personality Style

Alessandro Bertolino, Giampiero Arciero, Valeria Rubino, Valeria Latorre, Mariapia De Candia, Viridiana Mazzola, Giuseppe Blasi, Grazia Caforio, Ahmad Hariri, Bhaskar Kolachana, Marcello Nardini, Daniel R. Weinberger, and Tommaso Scarabino

Background: In the brain, processing of fearful stimuli engages the amygdala, and the variability of its activity is associated with genetic factors as well as with emotional salience. The objective of this study was to explore the relevance of personality style for variability of amygdala response.

Methods: We studied two groups (n = 14 in each group) of healthy subjects categorized by contrasting cognitive styles with which they attribute salience to fearful stimuli: so-called phobic prone subjects who exaggerate potential environmental threat versus so-called eating disorders prone subjects who tend to be much less centered around fear. The two groups underwent functional magnetic resonance imaging (fMRI) at 3T during performance of a perceptual task of threatening stimuli and they were also matched for the genotype of the 5' variable number tandem repeat (VNTR) polymorphism in the serotonin transporter.

Results: The fMRI results indicated that phobic prone subjects selectively recruit the amygdala to a larger extent than eating disorders prone subjects. Activity in the amygdala was also independently predicted by personality style and genotype of the serotonin transporter. Moreover, brain activity during a working memory task did not differentiate the two groups.

Conclusions: The results of the present study suggest that aspects of personality style are rooted in biological responses of the fear circuitry associated with processing of environmental information.

Among basic emotions, fear plays an important role in the process of adaptation of the individual to the environment. Individual differences exist in nearly every aspect of human behavior, and fear is no exception; different individuals experience and express fear with different intensity when presented with similar threatening stimuli. The role played by the amygdala in fear processing has been extensively investigated. Studies in rats using the fear-conditioning paradigm have identified a primary role for the amygdala (LeDoux 1998), which provides an ideal interface between external stimuli and behavioral responses (LeDoux 1998). Other studies have also suggested that the amygdala is critically involved in learning/memory processes associated with fear conditioning (Muller et al 1997), especially if the stimuli have emotional valence (LeDoux 1996). Neurons in the amygdala of nonhuman primates have been shown to respond to the affective significance of sensory stimuli, and lesions of the amygdala affect socioemotional functioning (Amaral 2002), rendering the animal insensitive to stimuli that normally evoke fear (Posamentier and Abdi 2003). Other studies in animals have also demonstrated that the effects of amygdala lesions may be dependent on the environment in which the behavior is observed and on the size of social groups. Convergent findings have been reported in human research. Lesion studies have indicated that the amygdala is involved in the acquisition of fear conditioning (Bechara et al 1995; LaBar et al 1995), as well as in the perception of fear (Adolphs et al 1995; Young et al 1995). In addition, studies with functional imaging in healthy individuals have shown activation of the amygdala during fear conditioning (Buchel et al 1998; Critchley et al 2002; LaBar et al 1998) and while processing faces and other emotional stimuli (Breiter et al 1996; Morris et al 1996). Increasing intensity of fear is associated with increased activity of the amygdala, which is also specifically activated if the stimuli are processed implicitly (Morris et al 1998) and when the subjects do not know that the stimuli are being presented (Whalen et al 1998). Furthermore, amygdala engagement reflects moment-to-moment subjective emotional experience and it enhances memory in relation to the emotional intensity of an experience (Cahill et al 1996; Canli et al 2000), suggesting that activity in the amygdala is possibly associated with variability in the individual experience of fear.

This variability may also be associated with the effects of specific genes. For example, Hariri et al (2002) have recently reported that a functional polymorphism of the serotonin transporter (5-HT) gene is associated with differential activity of the amygdala during incidental processing of fearful stimuli. Along with and possibly related to genes, psychological factors may be associated with variability in the individual experience of fear. Once again, the amygdala may be involved in explaining at least part of this variability (Everitt and Robbins 1992; LeDoux 1996). Consistent with this idea, recent studies have highlighted the association between activity in the amygdala during emotion processing and personality characteristics such as extraversion and neuroticism (Canli et al 2001, 2002) or inhibited tempera-

ment (Schwartz et al 2003). These studies in humans along with other studies in animals are converging on a broader view that the amygdala's role in emotion processing is a special case of its more general role in directing attention to affectively salient stimuli and issuing a call for further processing of stimuli that have major significance for the individual (Goldsmith and Davidson 2003). Therefore, it can be hypothesized that the response of the amygdala to fearful stimuli can vary as a function of the salience and the significance of these stimuli to the individual.

It is widely accepted that personality develops through the interaction of hereditary dispositions and environmental influences. The notion of genetic canalization (or "epigenetic landscape") has been revised to include the reciprocal necessity of genetic endowment and environmental stimulation in the development of behaviors. Recognizing an ontological value to the attachment relationship (Ainsworth et al 1978; Bowlby 1973a, 1973b, 1979, 1982, 1985, 1988; Bretherton 1995), a cognitivist model has been developed in which the concept of personality style (Arciero et al 2004; Arciero and Guidano 2000; Guidano 1991) was initially elaborated based on the relationship between cognitive styles and attachment patterns in some psychopathological conditions. Current models of personality style (Arciero et al 2004; Arciero and Guidano 2000) emphasize a gradual construction of personality and of the sense of self through interactions with others and through the regulation of emotions. Based on the organization of cognitive and emotional processes and their interaction with attachment, two basic categories of constructing identity and of regulating cognitive and emotional processes are identified according to this cognitivist scheme: the "inward" (more focused on the inner experience, not to be confounded with introversion–see below) and the "outward" (more focused on the outer experience, not to be confounded with extraversion–see below) (Arciero et al 2004). Within these two general categories, four dimensions (which are not mutually exclusive) are identified as personality styles among which two are particularly orthogonal: 1) phobic prone individuals (inward), and 2) eating disorders prone individuals (outward). It is necessary to underline that the terms phobic prone and eating disorders prone do not necessarily implicate that these subjects are at higher risk of pathological phobias or of eating disorders. Phobic prone individuals are characterized by a sense of self linked to the sensorial reading of emotional states; basic emotions (especially fear) play a central role in the development of personality and they are usually perceived with immediacy (Arciero et al 2004; Arciero and Guidano 2000; Guidano 1991). Emotions generated through automatic appraisal (Ekman 2003) seem to be more meaningful to these individuals (emotions begin without the individuals being aware of the processes involved). Therefore, the emotion of fear and its control are centrally salient to these individuals to regulate their emotional life. On the other hand, eating disorders prone individuals are characterized by a lesser defined sense of self as a tendency to select internal states and opinions based on an external point of reference (either a meaningful figure of reference or situations). Their basic emotions tend to follow reflective appraisal (Ekman 2003); these individuals are more consciously aware of the evaluative processes generating an emotion. Their emotional life is much less centered around fear. Therefore, these two personality styles seem to differ prominently in terms of the immediacy with which they process basic emotions such as fear. Thus, it is possible to hypothesize that different personality styles may be associated with variability in processing of fearful stimuli and with variability in the response of the amygdala. More specifi-

cally, we hypothesized that phobic prone healthy individuals may have more pronounced responses of the amygdala during perceptual processing of threatening stimuli.

We used functional magnetic resonance imaging (fMRI) during threatening stimuli to explore the relationship between personality style and the response of the amygdala in phobic prone and in eating disorders prone healthy subjects. The two groups of healthy subjects were otherwise matched for a series of variables that may be associated with variability in the activity of the amygdala.

Methods and Materials

Subjects

Twenty-eight subjects were selected from a larger cohort of 36. The 28 subjects were selected to match the groups for a series of variables, including age, gender, intelligence quotient (IQ), parental education, level of education, handedness, and genotype of the variable number of tandem repeats (VNTR) polymorphism in the promoter of the 5HTT gene (detailed below). All subjects were diagnosed (with a semistructured interview) for personality style independently by two investigators (G.A. and V.M.) who were highly trained in this cognitivist model and blind to each other's results. The interview was structured in three consecutive steps:

1. A detailed account of two episodes (involving fear and/or anger) that were meaningful to the subject was required to elicit the following two steps.
2. The subject was asked to provide a detailed description of the emotional experience of anger and fear to assess the style of emotional activation and regulation. Phobic prone subjects have a more rapid and intense emotional response, which might be associated with changes in physiological responses. In other words, they tend to be more focused on bodily aspects of emotional activations ("inwardness"). On the other hand, eating disorders prone subjects tend to generate emotional responses based on cognitive evaluation (which may also be automatic) of the stimulus at hand. This cognitive evaluation generates acknowledgment of emotional experiences by focusing on an external reference, avoiding focalization of internal, bodily states. Thus, emotional experiences will be slower and less intense, whereas control of these emotions will be centered on the external reference ("outwardness"). This step is intended to evaluate the patterns of personal meaning and the style of appraisal (reflective or automatic). Phobic prone subjects preferentially show a type of emotional appraisal called "automatic appraisal" (Ekman 2003). They become aware of being afraid or angry only after the emotion has begun and not during the generating stimulus. Furthermore, they modify these emotions by cognitive processes only after they have been triggered, as they have a subjective perception of an inability to think while angry or afraid. In eating disorders prone subjects, instead, basic emotions are more preferentially associated with reflective appraisal (Ekman 2003). This appraisal allows the interpretation of the feeling to be modified, modulating it and changing its emotional tone. Anger and fear are, in these subjects, more cognitively mediated through reflective appraisal, which is a cognitive evaluation followed by the development of an emotion.
3. An analysis of onset, manifestations, and extinction of the emotional experience was conducted. Usually, fear is related to the perception of immediate and subjectively

uncontrollable danger in phobic prone individuals. Fear in these subjects lasts just as long as the perception of not being in control. The length with which this emotion is expressed is variable. In eating disorders prone subjects, the trigger is usually related to a subjective perception of being judged/criticized or of not being considered important or considered at all. Their fear is perceived among an almost intact cognitive activity: they maintain the ability to think clearly while they are afraid, also trying to see the other's point of view. In these subjects, there is a heightened tendency to evaluate and appraise soon after the stimulus has appeared. Unlike the phobic prone group, this group acts only after the evaluation has been made, and the type of action depends on such evaluation. Intensity and length of time in which the feeling is expressed are also variable in this group.

Fourteen subjects were phobic prone (PP) (9 female subjects, mean age ± SD: 32.7 ± 9.6) and 14 were eating disorders prone (EDP) (9 female subjects, mean age ± SD: 34.3 ± 7.6). Concordance of diagnosis between the two investigators was 100%. All subjects completed the Personality Meaning Questionnaire (PMQ) (Picardi 2003) evaluating the key cognitive themes characterizing different personality styles. The questions in which PP subjects tend to score higher identify a score of need for emotional overcontrol in situations that may be felt as potentially dangerous (PP score) (Picardi 2003). The questions in which EDP subjects score higher identify a score for need for consent and approval, sensitivity to judgment, and vulnerability to criticism (EDP score) (Picardi 2003). Subjects also completed a series of questionnaires identifying different personality characteristics, such as the NEO Five Factors Inventory (Costa and McRae 1992), the Temperament and Character Inventory (TCI) (Cloninger et al 1994), the Positive and Negative Attitude Scale (PANAS) (Watson et al 1988), the Eysenck Personality Inventory (EPI) (Eysenck and Eysenck 1968), and the Big Five Questionnaire (BFQ) (Caprara et al 1993). Other demographic variables included years of education, parental socioeconomic status (Hollingshead and Redlich 1958), total IQ (assessed with the Wechsler Adult Intelligence Scale-Revised [WAIS-R]), and handedness (Oldfield 1971) (Table 1). Exclusion criteria included any psychiatric diagnosis (assessed with the Structured Clinical Interview for DSM-IV, Axis I and II), history of significant drug or alcohol abuse (no active drug use in the past year), head trauma with loss of consciousness, and any significant medical condition.

The present study was approved by the Comitato Etico Indipendente Locale of the Azienda Ospedaliera "Ospedale Policlinico Consorziale" of Bari. Moreover, after complete description of the study to the subjects, written informed consent was obtained from each subject.

Genetic Analysis of the Serotonin Transporter Gene

Subjects in the two groups were also specifically chosen to be matched for a genetic variation of the promoter region of the serotonin transporter gene (Lesch et al 1996). DNA isolation and analysis was conducted on blood samples obtained from all subjects. Polymerase chain reaction (PCR) amplification and gel based separation of alleles was accomplished using the methods of Heils et al (1997). Based on this assay, individuals possessing either one (n = 6 in each group) or two copies (n = 3 in each group) of the s allele and those homozygous for the l allele (n = 5 in each group) were included in the analysis.

Table 1. Demographics of the Two Groups of Subjects

	Phobic Prone n = 14		Eating Disorders Prone n = 14	
	Mean	SD	Mean	SD
Age	32.7	(9.6)	34.3	(7.6)
Gender	9 F		9 F	
Handedness	.9	(.1)	.9	(.3)
Hollingshead	47.4	(15.6)	45.7	(18.4)
Total IQ	108.1	(18.2)	123.2	(12.8)
PP Score (PMQ)	61.3	(5.3)	49.5	(7.07)
EDP Score (PMQ)	43.6	(6.2)	55.8	(7.4)
Temperament and Character Inventory				
Harm avoidance	9.1	(3.5)	9.6	(4.1)
Novelty seeking	9.5	(3.8)	10.2	(3.9)
Reward dependence	10.2	(6.5)	9.3	(3.2)
Persistence	2.4	(1.7)	1.8	(1.4)
Positive and Negative Attitude Scale				
Positive	33.1	(3.4)	31.0	(8.7)
Negative	19.1	(9.0)	20.0	(7.2)
Eysenck Personality Inventory				
Psychoticism	2.4	(2.4)	5.0	(3.2)
Extraversion	14.4	(4.2)	13.6	(3.0)
Neuroticism	8.8	(5.0)	10.3	(5.9)
Big Five Questionnaire				
BFQ_energy	79.1	(11.9)	79.5	(11.0)
BFQ_friendliness	78.7	(8.9)	84.4	(8.1)
BFQ_conscienciousness	81.7	(8.3)	81.5	(8.1)
BFQ_emotional Stability	64.4	(14.8)	67.1	(12.2)
BFQ_openness	84.5	(5.0)	87.5	(7.3)
NEO Five Factors Inventory				
Neuroticism	19.9	(6.6)	22.2	(5.9)
Extraversion	30.8	(6.3)	28.0	(4.7)
Openness	29.6	(4.4)	31.8	(4.0)
Agreeableness	28.0	(4.8)	31.1	(6.4)
Conscientiousness	31.3	(6.3)	28.9	(5.5)

IQ, intelligence quotient; PP, phobic prone; EDP, eating disorders prone; PMQ, Personality Meaning Questionnaire; BFQ, Big Five Questionnaire.

Cognitive Tasks and Physiology

Emotion Task. This fMRI paradigm consisted of two blocks of an emotion task interleaved with three blocks of a sensorimotor control task (Hariri et al 2002). During the emotion task, subjects viewed a trio of unfamiliar faces and had to match one of two simultaneously presented images with an identical target image. Each emotion block consisted of six images, three of each gender and target affect (angry or afraid) all derived from a standard set of pictures of facial affect (Ekman and Friesen 1976), presented sequentially for 5 seconds. During the sensorimotor control, subjects viewed a trio of simple geometric shapes (circles, vertical and horizontal ellipses) and selected one of two shapes (bottom) identical to the target shape (top). Each control block consisted of six different images presented sequentially for 5 seconds. We acquired two blocks of the emotion task and three blocks of the sensorimotor control task. Subject performance was measured as accuracy (percent correct responses) and reaction time (milliseconds).

Working Memory Task. To exclude the possibility that exaggerated responses to the emotion task in phobic prone subjects reflected a nonspecific tendency to overactivate a complex brain response, we studied 9 PP subjects and 11 EDP subjects (of the 28 subjects studied with the emotion task) with a working

memory task. The other subjects were not studied for technical difficulties. Working memory was assessed with the N-Back task, as in earlier reports (Bertolino et al 2004). Briefly, N-Back refers to how far back in the sequence of stimuli the subject had to recall. The stimuli consisted of numbers (1–4) shown in random sequence and displayed at the points of a diamond-shaped box. There was a nonmemory guided control condition (0-Back) that presented the same stimuli but simply required subjects to identify the stimulus currently seen. The working memory task required the recollection of a stimulus seen two stimuli (2-Back) previously while continuing to encode additionally incoming stimuli. Performance data were recorded as the number of correct responses (accuracy) and as reaction time.

Physiology. We measured skin conductance load (SCL) during the acquisition of functional scans in 18 of the 28 subjects. For the other 10 subjects, we did not have SCL, as we did not have access to fMRI-compatible equipment or we could not measure it for technical reasons (4 subjects). Skin conductance load was recorded as in Hariri et al (2003). Briefly, mean changes in SCL in the emotion and the adjacent blocks of the sensorimotor control task were determined and then standardized (for example, mean of the emotion task − total mean/ total standard deviation).

Neuroimaging

Emotion Task. Each subject was scanned using a GE Signa 3T scanner (Milwaukee, Wisconsin). Blood oxygenation-level dependent (BOLD) functional images were acquired with a gradient-echo echo planar imaging (EPI) sequence, with 24 axial contiguous slices (5-mm thick, no gap) encompassing the entire cerebrum and the majority of the cerebellum (repetition time/ echo time [TR/TE] = 3000/30 milliseconds, field of view [FOV] = 24 cm, matrix = 64 x 64) (Hariri et al 2002). Whole-brain image analysis was completed using Statistical Parametric Mapping (SPM99; http://www.fil.ion.ucl.ac.uk/spm; Wellcome Department of Imaging Neuroscience, London, United Kingdom). All fMRI data were reconstructed, registered, linear detrended, globally normalized, and then smoothed (10 mm Gaussian kernel) before analysis within SPM99. Functional magnetic resonance imaging data were then interrogated for data quality (scan stability) prior to inclusion in any further analysis. The registration parameters were extracted and used to exclude subjects with excessive interscan motion (>1 voxel translation, >1° rotation). Predetermined condition effects at each voxel were calculated using a t statistic, producing a statistical image for the contrast of the emotion task versus the sensorimotor control for each subject. These individual contrast images were then used in second-level random effects models that account for both scan-to-scan and subject-to-subject variability, to determine task-specific regional responses at the group level with one sample t test (main effects of task, $p < .05$, k = 3) and analysis of variance (ANOVA) (direct comparisons). Because of our strong a priori hypothesis regarding the differential response of the amygdala and our use of a rigorous statistical model, a statistical threshold of $p < .001$ (extent 8 voxels) was used to identify significant responses for ANOVA comparisons. The task simply contrasts the response of the amygdala (and extended circuitry) to biologically salient and arousing stimuli (faces) and their opposite (shapes). To evaluate whether the response in amygdala was related to autonomic measures of fear, we performed in the whole sample a simple regression within SPM between activity in the amygdala and SCL during the emotion task ($p < .05$, small volume correction, k = 3).

Multiple Linear Regression Analysis. To further explore the results of the ANOVA and to evaluate the contributions of genotype of the serotonin transporter and personality to activation of the amygdala, we used multiple linear regression within SPM99. To restrict the search for significant correlations and avoid spurious results, we first created a functional mask using the results of the ANOVA (same statistical threshold and cluster size), thus restricting the search to the amygdala, and then we entered the single subject contrasts with the number of short alleles of the serotonin transporter genotype and personality style (either PP or EDP personality) as predictors. To corroborate this analysis, we also performed another multiple linear regression with serotonin transporter genotype, PP score, and EDP score (as assessed with the PMQ) as regressors.

Working Memory Task. The N-Back task was performed as previously described (Bertolino et al 2004). Using the same magnetic resonance imaging (MRI) scanner, gradient-echo EPI BOLD fMRI data were acquired (TE = 30 milliseconds, TR = 2 seconds, 20 contiguous slices, voxel dimensions = 3.75 × 3.75 × 5 mm). We used a simple block design in which each block consisted of eight alternating 0-Back and rest (subjects were instructed to fixate the diamond on the screen) or 2-Back and 0-Back conditions (each lasting 30 seconds). Each task combination was obtained in 4 minutes and 8 seconds, 120 whole-brain scans. Data analysis was performed as previously described (Bertolino et al 2004) and it was similar to that performed for the emotion task. Single subject contrast maps were created by contrasting 2-Back and 0-Back tasks with t statistics. The resultant contrast images were then entered into second-level (random effects) analyses consisting of ANOVA ($p < .001$ uncorrected, cluster size eight voxels). For anatomical localization, statistical maxima of activation were converted to conform to the standard space of Talairach and Tournoux.

Results

Demographics and Questionnaires

T tests and χ^2 indicated that the two groups of subjects were well matched for age, gender, IQ, parental education, years of education, handedness (all $p > .2$), and, of course, genotype of the polymorphism in the promoter region of the serotonin transporter gene (three ss, six ls, five ll in each group). Consistent with the diagnosis based on the semistructured interview, an ANOVA with personality style as a between-subjects factor and with PP and EDP scores as within-subjects factor showed no main effect of personality ($df = 1,26$, $F = .4$, $p > .5$), a significant effect of scores ($df = 1,26$, $F = 6.4$, $p < .02$), and a significant interaction between personality and scores ($df = 1,26$, $F > 15$, $p < .001$). Post hoc analysis with Tukey Honestly Significant Difference (HSD) test indicated that PP subjects had higher PP scores ($p < .001$) and that EDP subjects had higher EDP scores ($p < .001$). Similar ANOVAs were performed with personality style as a between-subjects factor and the various subscores (those identifying different aspects of personality) of the different questionnaires (NEO, TCI, PANAS, EPI, and the BFQ) as within-subjects factor. These ANOVAs did not indicate any significant effect of personality style ($df = 1,26$, all $F < 1.3$, all $p > .3$) or any interaction between personality style and subscores ($df = 1,26$, all $F < 1.4$, all $p > .2$), suggesting that the two groups of subjects did not significantly differ on other aspects of personality identified by these questionnaires (see Table 1).
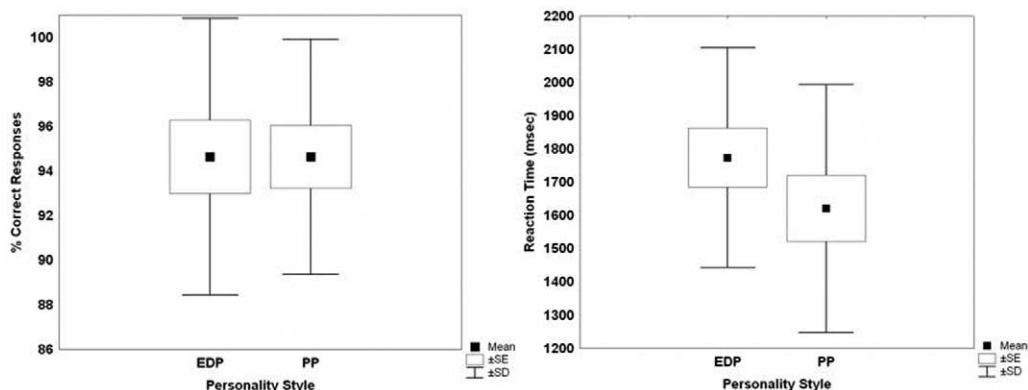
Figure 1. Box and whisker plot showing mean, standard error, and standard deviation (as indicated in the graph) of performance accuracy and reaction time on the emotion task in the two groups of subjects: eating disorders prone (EDP) and phobic prone (PP). No significant difference was found (for statistics, see text).

Emotion Task: Behavior and Physiology

All subjects performed well on the sensory motor control task and the emotion task. Analysis of variance with personality style (PP vs. EDP) as the independent variable and percent correct responses on the two tasks (emotion and sensorimotor control task) as the dependent repeated measures factors did not show any significant main effect or interaction ($df = 1,26$, all $F < .7$, all $p > .4$, Figure 1). A similar ANOVA to investigate reaction time during the two tasks showed no significant effect of personality style ($df = 1,26$, $F = .9$, $p > .3$), a significant effect of task ($df = 1,26$, $F = 40.8$, $p < .0001$), and no interaction ($df = 1,26$, $F = .9$, $p > .3$, Figure 1). Post hoc analysis with Tukey HSD indicated that regardless of personality styles, reaction time (RT) during the sensory motor control task was faster than that during the emotion task (respectively, 1204 milliseconds vs. 1697 milliseconds, $p < 0.001$), suggesting that the emotion task requires more complicated neural processing.

An ANOVA on SCL data indicated a main effect of task [higher mean SCL during the emotion task compared with the control task, $F(1,16) = 7.2$, $p < .01$], while no main effect of personality style and no interaction [all $F(1,16) = 0.6$, all $p > 0.4$].

Emotion Task: Neuroimaging

The two groups of subjects did not differ in the degree of residuals of motion correction as assessed with SPM99 (all $p > .1$). The main effect of task revealed a similar and significant BOLD response in the fear network, including the amygdala, the posterior fusiform gyrus, inferior parietal lobules, and frontal eye fields ($p < .05$, $k = 3$, Table 2). Direct comparisons (ANOVA) revealed that the response of the right amygdala was greater in the PP group ($p < .001$ uncorrected, $k = 8$, Talairach coordinates: x 29, y −4, z −10) (Figure 2). These results also survive correction for multiple comparisons across a small volume of interest ($p < .01$). There was no other significant difference within the distributed perceptual network, implicating a regionally specific difference in the response of the amygdala to threatening stimuli in PP subjects. The inverse analysis did not reveal any area in which EDP subjects had greater activation than PP subjects ($p < .001$ uncorrected, $k = 8$). Using an anatomically defined region of interest (ROI) within the amygdala, the interaction analysis showed a significant difference in amygdala with the PP ss subjects activating the most and the EDP ll subjects activating the least (x 22, y −8, z −15, $p = .001$, $k = 10$, small volume correction $p = .02$; x −29, y −4, z −14, $p = .001$, $k = 5$, small volume correction $p = .02$).

Consistent with the results of the ANOVA, the results of the multiple linear regression analysis (with personality style and genotype as regressors) indicated that the number of s alleles as well as phobic prone personality predict response in the amygdala (respectively, $p < .03$, uncorrected, x 25, y −8, z −14; $p < .02$, small volume correction for multiple comparisons, x 29, y −4, z −9, Figure 3). The results of the multiple linear regression analysis with genotype, PP score, and EDP score as regressors indicated very similar results. The number of s alleles in the serotonin tranporter genotype as well as PP score (respectively, $p < .03$, uncorrected, x 18, y −8, z −9; $p < .01$, small volume correction for multiple comparisons, x 25, y −4, z −9) predicted response in the amygdala, while the EDP score did not (no voxel crossing the $p < .05$ threshold). This approach allows for the unbiased determination of the contribution of independent variables to activation of the amygdala, suggesting that both factors are independently associated with it.

Working Memory: Behavioral and Imaging Data

No significant difference between PP and EDP subjects emerged in terms of accuracy or reaction time of the working memory task (all $df = 1,18$, all $F < 1.7$, all $p > .2$). Furthermore, using the same statistical threshold ($p < .001$, uncorrected, k = 8) as for the emotion task, there was no anatomical area in which PP subjects had higher activation than EDP subjects (data not shown). Because of the slightly smaller sample sizes in this imaging experiment as compared with the face-processing task, we repeated the analysis, lowering the statistical threshold by a factor of five to $p < .005$ with no change in the results.

Discussion

Consistent with earlier experiments, the results of the present study indicate that perceptual processing of threatening stimuli qualitatively engages a neural network focused around the amygdala and that this response is also associated with evidence of autonomic arousal in the SCL response. We also have shown that this pattern of activation is qualitatively similar in individuals with two different personality styles, phobic prone subjects and eating disorders prone subjects. However, there were quantitative differences; phobic prone subjects engage the amygdala to a greater degree than eating disorders prone subjects during perceptual processing of threatening stimuli. Moreover, the 5' VNTR polymorphism of the serotonin transporter and phobic proneness appear to act independently, as well as to interact in

Table 2. One Sample T Test

	Tal			T Value	BA
	x	y	z		
Phobic Prone Subjects					
	26	−8	−10	8.96	Right lentiform nucleus, putamen
	25	−4	−10	5.88	Right amygdala
	−19	−33	−3	5.85	Left parahippocampal gyrus
	−21	−5	−15	4.14	Left amygdala
	−34	−55	−12	6.88	BA 37 left fusiform gyrus
	30	−76	4	6.71	Right middle occipital gyrus
	48	22	21	4.26	BA 46 right middle frontal gyrus
	−44	3	55	3.91	BA 6 left middle frontal gyrus
	−34	28	−11	3.7	BA 47 left inferior frontal gyrus
	−48	22	21	3.56	BA 9 left inferior frontal gyrus
	−26	−70	48	3.11	BA 7 left superior parietal lobule
	−11	55	19	2.82	BA 9 left superior frontal gyrus
	11	49	36	2.64	BA 8 right superior frontal gyrus
	4	28	54	2.6	BA 6 right superior frontal gyrus
	−44	40	3	1.91	BA 46 left inferior frontal gyrus
Eating Disorders Prone Subjects					
	−38	−59	−12	10.12	BA 37 left fusiform gyrus
	30	−77	−6	6.81	BA 18 right middle occipital gyrus
	48	30	15	4.69	BA 46 right inferior frontal gyrus
	−34	7	60	4.55	BA 6 left middle frontal gyrus
	−15	−63	58	3.14	BA 7 left precuneus
	26	51	3	2.88	BA 10 right middle frontal gyrus
	40	2	33	2.73	BA 6 right precentral gyrus
	−19	−37	2	2.67	BA 30 left parahippocampal gyrus
	26	−4	66	2.47	BA 6 right superior frontal gyrus
	11	−75	42	2.37	BA 7 right precuneus
	−38	−51	58	2.24	BA 40 left inferior parietal lobule
	4	−33	−3	2.21	Right brainstem, midbrain
	−4	13	55	2.11	BA 6 left superior frontal gyrus
	−19	−8	−10	1.93	Left amygdala, parahippocampal gyrus

Coordinates of the voxel with the highest T value relative to standard stereotactic space (Talairach and Tournaux, Tal) during the emotion task in the two groups of subjects.

BA, Brodmann Area.

determining the response of the amygdala. The two groups of subjects were otherwise matched for a series of demographic variables, for scores assessing different aspects of personality, for performance (accuracy and reaction time) during the emotion task, and for SCL responses. The subjects in the two groups were also matched for genotype of the promoter region of serotonin transporter gene that has been previously shown to affect activity of the amygdala during perceptual processing of threatening stimuli, which we have now confirmed (Hariri et al 2002). Furthermore, this differential response of the amygdala did not appear to reflect a nonspecific tendency to overactivate a complex brain response since phobic prone subjects did not have any exaggerated response during a working memory task.

A close link has been established between fear and the amygdala. A large body of literature in animals and in humans suggests that the amygdala may be a protection device, being designed to detect and avoid danger (LeDoux 1998). A primary function of the amygdala is to evaluate objects or organisms in the environment prior to interacting with them (Amaral 2002). A basic tenet of this hypothesis is that the emotional salience ("the danger") of the stimulus is appreciated once the amygdala is involved. Although the precise mechanism by which the amygdala assesses the threat of a stimulus is at present unknown, the "set-point" of what is dangerous can be certainly governed both by innate predilections as well as learned associations (Amaral

2002). In this sense, cognitive evaluation of fear processing in humans is affected by the appraisal that individuals give about environmental signals (Phan et al 2004). These evaluative judgments help determine the significance of a stimulus to the individual (salience). Stimuli may have different salience to an individual in several ways. Exogenous characteristics of a stimulus (i.e., simple physical characteristics of a stimulus like bright colors) stand in contrast with endogenously determined responses to a stimulus that depend on an appraisal by the organism beyond basic perception. Among others, two main types of endogenous salience may be distinguished: one based on the intrinsic value of the stimulus (a snake), either innate or learned, and the other based on the recall of a personal event or emotional memory elicited by the stimulus (Phan et al 2004). While the latter type of endogenous salience seems to be associated with activity in cortical structures, stimuli with intrinsic value of salience seem to be associated with activity of the amygdala (Phan et al 2004). In fact, patients with bilateral lesions of the amygdala lose the ability to judge facial expressions of fear (Adolphs et al 1995). Furthermore, recent studies in healthy subjects have demonstrated that activity of the amygdala is associated with endogenous salience of emotional intensity, suggesting that it is implicated differently in different individuals in detection or attribution of emotional value (Phan et al 2004). Therefore, the amygdala seems to be a central structure in the
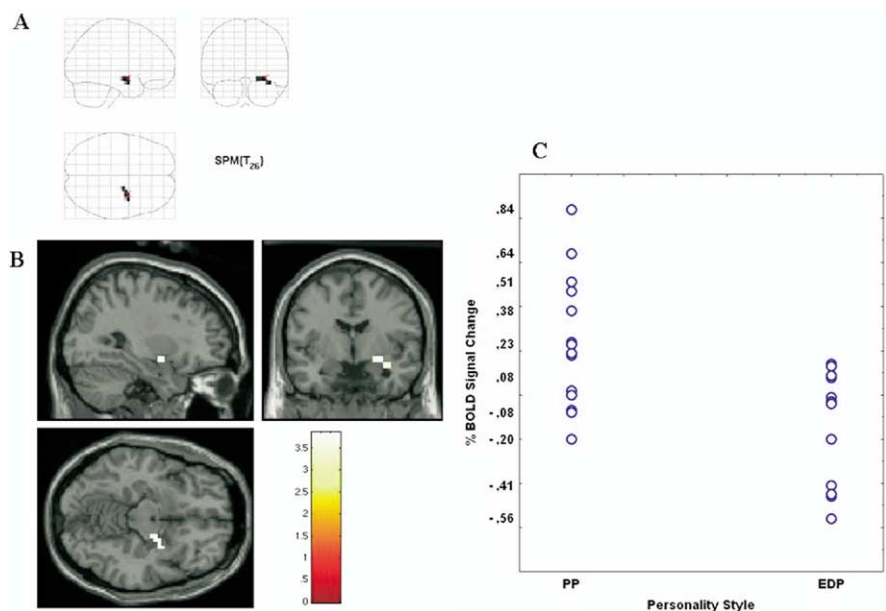
Figure 2. (A) SPM99 glass brain showing the results of the ANOVA ($p < .001$, k = 8) of the emotion task for the comparison phobic prone (PP) > eating disorders prone (EDP). In the amygdala (maximal voxel, Talairach coordinates: x 29, y −4, z −10), PP subjects had a greater fMRI response compared with EDP subjects. **(B)** The same statistics as in **(A)** overlaid onto an average structural MRI in all three planes. **(C)** Effect of personality style on right amygdala activity. Individual circles represent the activity for each subject in the maximal voxel, as above. SPM99, Statistical Parametric Mapping; ANOVA, analysis of variance; MRI, magnetic resonance imaging.

attribution of endogenous salience for emotional stimuli. Since endogenous salience can also be learned, the specific attachment bonds established with the caregiver during development might be instrumental in determining differential responses of the amygdala to different stimuli based on different environmental stimuli learned through the attachment figure. Mammalian infants display affiliative behaviors and form "attachment bonds" with their caregivers. The attachment process between the infant and the caregiver is guided by emotional exchanges so that they subsequently influence how information is processed and emotions are regulated (Grossman and Grossman 1990). Consistently, it has been demonstrated that development of the amygdala and that individual differences in maternal care in terms of prenatal and postnatal environments influence each other in the ability to evaluate potentially dangerous situations in social contexts (Joseph 1999; Francis et al 1999, 2003; Baumann et al 2004).

Since attachment is a process by which children also learn to regulate their emotions (Carstensen et al 2003), activity of the amygdala in humans may be cognitively primed during early development by the attachment figure to respond more robustly to fearful stimuli. Indeed, the attachment figure of phobic prone individuals tends to regulate the attachment relationship through anticipation of potential dangers so that the child learns to estimate his/her world and the environment by evaluating all dangerous aspects of stimuli (Arciero et al 2004; Arciero and Guidano 2000; Guidano 1991). In other words, the amygdala might be primed to respond more robustly to threatening stimuli compared with other subjects (like the eating disorders prone) in whom threatening stimuli do not have the same endogenous salience. This interpretation of our findings is speculative in its nature, and it is, indeed, possible that some other yet unidentified and more specific independent variable may contribute to explain our results. However, our interpretation is consistent

Figure 3. (A) Multiple linear regression within SPM99 illustrating significantly linear positive relationships between activity in the right amygdala and the number of s alleles of the serotonin transporter genotype (which includes the statistics overlaid onto an average structural MRI passing through the amygdala as well as the bargraph with mean ± SE of signal change in amygdala in the three different SERT genotypes) as well as personality style **(B)**. See text for statistics. SPM99, Statistical Parametric Mapping; SERT, serotonin transporter.
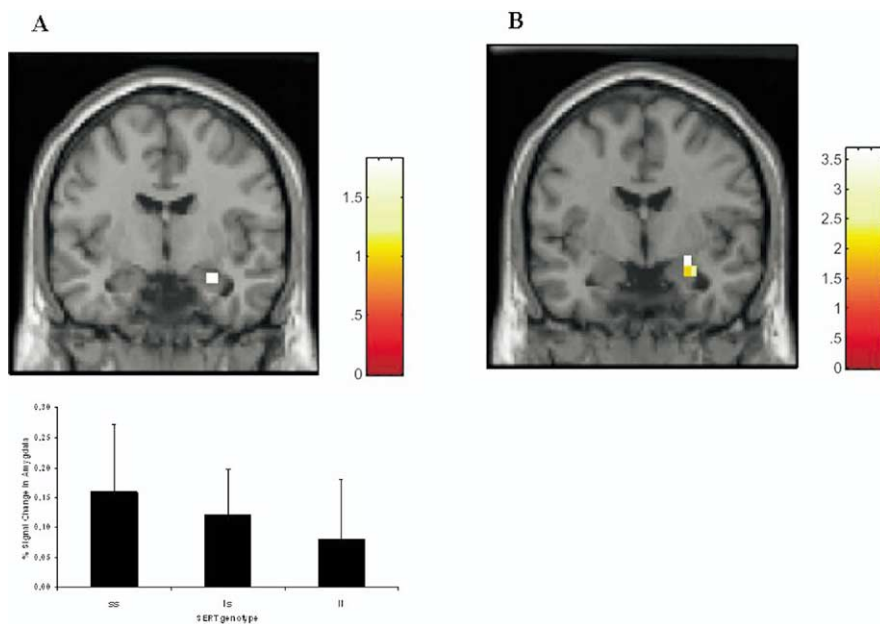
with the robust literature we have detailed above and with studies that have implicated different aspects of personality being associated with activity of the amygdala. Furthermore, this interpretation is consistent with the results of the regression analysis performed in the two groups of subjects. Activity in the amygdala is independently predicted by diagnosis of personality style and by genotype of the serotonin transporter, further suggesting that response in the amygdala might arise as an emergent property from the association between genetic and psychological factors. In other words, the 5-HTT s allele predisposes for a more reactive arousal system that, through experience as well as other genetic and environmental moderators, manifests as phobic proneness in our subjects. Simply put, these subjects may be phobic prone because of a heightened amygdala response and this response bias is, at least in part, mediated by genetic variation in 5-HT signaling. This interpretation is consistent with recent studies providing evidence of a gene-by-environment interaction, in which an individual's response to environmental insults is moderated by his or her genetic makeup (Caspi et al 2003).

Limitations

In the present study, we did not evaluate the full spectrum of basic emotions and we did not have a neutral face as a baseline. Thus, we cannot address the specificity of differences in amygdala response to threatening stimuli. Therefore, it is theoretically possible that phobic prone subjects might engage to a greater degree the amygdala also during processing of other emotions because of a higher level of arousal. However, we think this seems unlikely based on the other data we have. The selectivity of the differential response in the amygdala may suggest that this finding is not based on a nonspecific tendency to overactivate a complex brain response. Consistently, even though the samples studied are slightly smaller, phobic prone subjects do not engage the working memory cortical network to a higher degree compared with eating disorders prone subjects.

Another limitation of our study is that we cannot rule out whether habituation (to both emotion stimuli and to the scanner setting) had a role in determining the differential response of the amygdala in the two groups of subjects (Zald and Pardo 2002). Also, the emotion task was always acquired before the working memory task. Therefore, there is a theoretical possibility that the difference in amygdala activation between the two groups is exaggerated by the fact that PP subjects might be more intimidated by the scanner setting as a whole. On the same note, there is a theoretical possibility that lack of difference between the two groups at the N-Back is a function of the lack of randomization for acquisition of the tasks. However, we do not believe these confounds have a major effect on our results for several reasons. The first is that the difference between the two groups during perceptual emotion processing is very selective, being significant only at the level of the amygdala. We would expect that nonspecific intimidation effect might be evident in different brain regions associated with nonspecific arousal. Second, if the amygdala results in the emotion task are a reflection of less habituation to a threatening situation in the PP subjects, even though more nonspecific, this would still be consistent with the idea that the amygdala in phobic prone subjects is primed to respond more robustly (less habituation) to threatening stimuli and that PP subjects are more sensitive to threatening situations.

Earlier studies in the literature have indicated that fear processing is associated with increases in a series of bodily responses, including changes of the autonomic nervous system.

Therefore, one might hypothesize that phobic prone subjects might express increased skin conductance load during perceptual processing of fear. However, we failed to find a significant difference between the two groups of subjects in terms of skin conductance load. This negative result might be associated with several factors: less sensitivity of this parameter to the difference in personality style compared with the robust statistical evidence provided by fMRI; the standard deviation of this measurement is somewhat large and this is possibly associated with technical difficulties intrinsic to obtaining electric measurements in a nuclear magnetic resonance (NMR) environment; we designed the paradigm to elicit perceptual processing of threatening stimuli, which is certainly not apt to induce direct and robust fearful responses; and not all subjects had SCL measurements.

Statistically speaking, the results of the relationship between genotype of the serotonin transporter and activity of the amygdala would not survive correction for multiple comparisons. However, we do not believe such correction is necessary, as the results obtained by Hariri et al (2002) have already been replicated by Furmark et al (2004) and by Hariri et al (2005) in another independent cohort of subjects.

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