Brain regions showing increased activation by threat-related words in panic disorder

Richard J. Maddock,[CA] Michael H. Buonocore,[1] Shawn J. Kile and Amy S. Garrett[2]

Department of Psychiatry, University of California Davis, 2230 Stockton Blvd.; [1]Department of Radiology, University of California Davis, 4801 X St., Sacramento, California 95817; [2]Department of Psychiatry, Stanford University, 401 Quarry Road, Stanford, California 94305, USA

[CA]Corresponding Author: rjmaddock@ucdaris.edu

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Threat-related stimuli consistently activate the posterior cingulate cortex in normal subjects and have exaggerated effects on memory in patients with panic disorder. We hypothesized that panic patients would show increased response to threat-related stimuli in the posterior cingulate cortex. While undergoing fMRI, six panic patients and eight healthy volunteers made valence judgements of threat-related and neutral words. Both groups showed threat-related activation in the left posterior cingulate and left middle frontal cortices, but the activation was significantly greater in panic patients. Panic patients also had more right > left asymmetry of activation in the mid-parahippocampal region. The increased responsivity observed in the posterior cingulate and dorsolateral prefrontal cortices is consistent with the hypothesis that panic disorder patients engage in more extensive memory processing of threat-related stimuli. *NeuroReport* 14:325–328 © 2003 Lippincott Williams & Wilkins.

Key words: Affect; Anxiety; Arousal; Fear; Retrosplenial

INTRODUCTION

Panic disorder is a common and potentially disabling anxiety disorder characterized by recurrent, unprovoked attacks of intense somatic and cognitive symptoms of anxiety. While it is clear that both pharmacological and cognitive–behavioral interventions are effective in the treatment of panic disorder, the specific mechanisms underlying the vulnerability to recurrent panic attacks are not well understood. Many investigators have shown that threat-related stimuli have distinctive effects on cognition and memory in patients with panic disorder and have suggested that these effects may provide insight into the cognitive components of vulnerability to this disorder. Panic patients, in contrast to patients with social phobia or generalized anxiety disorder, exhibit significantly better episodic memory for threat-related words than healthy comparison subjects [1]. They also show greater slowing on color naming and lexical decision tasks involving threat-related words. In contrast to other anxiety disorders, panic patients show this slowing in response to a range of negatively valenced words, not just disease-specific words [2–4]. The mechanisms underlying these effects are unknown, but they may include more extensive neural processing of the threat-related meanings of the stimuli. Functional neuroimaging studies might reveal characteristic brain responses to threat-related stimuli in panic patients and thus lead to more specific hypotheses about the neurocognitive aspects of vulnerability to this illness.

The posterior cingulate cortex is consistently activated when normal volunteers make valence judgments of emotionally salient stimuli, including threat-related words [5–7]. Animal and lesion data indicate that the posterior cingulate cortex has an important role in episodic memory [6]. We have proposed that this region influences interactions between emotion and memory [6,7]. We now report the first functional imaging study comparing the brain responses of panic patients and healthy comparison subjects to threat-related words. Since panic patients show enhanced episodic memory for such words, we hypothesized they would show increased responsivity to threat-related words in a brain circuit involving the posterior cingulate cortex.

Abnormalities have often been observed in the medial temporal region in panic disorder [8–10]. The most consistently replicated finding in PET studies of panic disorder has been significantly more right greater than left metabolic asymmetry in the parahippocampal region in panic patients [11–13]. fMRI has the spatial resolution to more precisely localize this asymmetry within the parahippocampal region. Thus, as a secondary goal of this study, we looked for evidence of this asymmetry in the response to threat-related words in patients with panic disorder.

MATERIALS AND METHODS

Subjects: Six patients (four female, mean age 34 years) meeting DSM-IIIR criteria for panic disorder with ($n = 5$) or

without ($n = 1$) agoraphobia and eight normal volunteers (five female, mean age 35 years) free of psychiatric symptoms were recruited from the patients and staff at UC Davis and gave their written informed consent, as approved by our local Institutional Review Board. Diagnoses were made using the Structured Clinical Interview for DSM-IIIR [14]. No panic patients met diagnostic criteria for any additional current psychiatric diagnoses. All subjects were right-handed and had not taken medications affecting psychiatric or cerebrovascular function for ≥ 14 days. Panic patients were rated on the Hamilton Anxiety scale [15] by a psychiatrist (RJM) and kept panic diaries for 2 weeks.

Stimuli and procedures: The threat-related stimuli were 10 words with meanings suggesting a threat to survival (terror, victim, injury, cancer, panic, dangerous, threatening, emergency, violence, destroyed). The control stimuli were 10 emotionally neutral words (detect, locate, track, border, margin, measurement, impression, pertinent, arrangement, translation) matched for word length and frequency of usage [16]. Each word was presented once in pseudorandom order in each 16 s block of 10 words of the same type. Sixteen alternating blocks of threat-related and neutral words were presented over 256 s following a 32 s baseline. Subjects were instructed to make a silent judgment of the valence (unpleasant, pleasant, or neutral) of each word. Tape recorded stimuli were presented via a Resonance Technology, Inc. audio system (Van Nuys, CA) through sound attenuating earphones to subjects with eyes closed. After scanning, subjects were questioned about stimulus audibility, task performance, and their emotional state during the scan.

Image acquisition: Images were obtained with a General Electric Signa Advantage 1.5 T system, with a local gradient coil (Medical Advances, Inc., Milwaukee, WI, USA). For each subject, a coronal high-resolution fast spin echo sequence was obtained for anatomical localization. Scan parameters were: TR 3100, effective TE 17 and 136, echo train 8, matrix 256 $\times$ 256, field of view 22 cm, slice thickness 6 mm, gap 2 mm, 24 slices from -96 to $+88$ mm. Subsequently, a T2* weighted, gradient recalled echo–echo planar imaging sequence was obtained for functional images. Parameters were: TR 2000 ms, TE 40 ms, FA 90°, matrix 64 $\times$ 64, field of view 22 cm, slice thickness 6 mm, slice gap 2 mm, 16 slices from -70 to $+56$ mm.

Data analysis: After motion detection, motion correction (if motion exceeded 20% of a voxel width), and high pass filtering, individual Z-score maps in Talairach space were created for each subject using Medx software (Sensor Systems, Inc., Sterling, Virginia) as described previously [17]. Group differences were assessed with a random effects analysis using an unpaired *t*-test (df = 12). Significant group differences were defined with threshold criteria for both peak and extent. Significant voxels were required to have a Z value = 3.09 ($p = 0.001$, uncorrected) and to be within a contiguous cluster of 4 voxels all having $Z = 2.33$ ($p = 0.005$, uncorrected) [18].

Asymmetry of the parahippocampal response to threat-related words was analyzed in native brain space for each subject using BrainMRI software [19]. Bilateral parahippocampal ROIs, including all brain voxels inferior and medial to the temporal horn of the lateral ventricle and superior and medial to the collateral sulcus (excluding the amygdala), were defined blind to activation data on six coronal slices (-8 to -48). Time series data were used to calculate the amplitude of the blood oxygen level dependent (BOLD) response to threat-related minus neutral words (as percentage change) in each ROI. Asymmetry was calculated as right minus left response amplitude. Based on prior studies [11–13], we predicted more right greater than left asymmetry in the panic patients. Group differences were analyzed with unpaired *t*-tests at each coronal slice, using a Bonferroni-corrected one-tailed $\alpha = 0.05$ (0.0083 uncorrected).

RESULTS

Mean Hamilton Anxiety Scale score was 22.3 for the panic disorder patients. The patients recorded an average of four panic attacks/week in their panic diaries. Four of six panic patients but no control subjects reported feeling anxious during the scan. No subjects described symptoms of a panic attack during the scan. No subjects reported difficulty hearing the words or judging their valence. Two scans had motion $> 20\%$ of a voxel width (both control subjects, maximum = 24%) and were corrected. Average motion was similar in patients and controls (0.53 mm and 0.44 mm respectively, $t = 0.78$, df = 12, NS).

Both patients with panic disorder and control subjects showed significantly greater responses to threat-related words than neutral words in the bilateral posterior cingulate, bilateral anterior cingulate, bilateral precuneate, left middle frontal, left parahippocampal, left visual (area 17), and right fusiform cortices, and the left medial and lateral cerebellum. Brain regions in which the responses to the threat-related words were significantly different between the two groups are shown in Table 1. Panic patients had significantly greater activation in the left posterior cingulate cortex (BA 23, 30) and the left dorsolateral prefrontal cortex (BA 46; Fig. 1). Several brain regions showed significantly less activation in the panic patients (Table 1).

Group differences in the parahippocampal response asymmetry are shown in Table 2. Panic patients had significantly more right greater than left asymmetry in the mid-parahippocampal region ($Y = -24$) and a trend toward more asymmetry in the anterior parahippocampal region ($Y = -8$).

DISCUSSION

As in prior studies of emotional stimuli [5–7], the normal subjects activated the posterior cingulate cortex during the evaluation of threat-related words. As predicted, patients with panic disorder showed significantly greater activation in the posterior cingulate cortex than normal subjects. This region has reciprocal connections with both emotion and memory related regions, including rostral and subgenual anterior cingulate, medial orbital, entorhinal and parahippocampal cortices [20–24]. We have proposed that this region mediates interactions between emotion and memory

Table I. Areas with significantly different activation in panic disorder patients compared with control subjects evaluating threat-related and neutral words.

Brain region[a] (BA)[b]	Talairach coordinates of peak effect[c]	No. voxels in cluster	Z score of peak effect
Panic patients > controls			
L middle frontal (46)	−45,41,10	8	3.53
L posterior cingulate (23,30)	−3,−48,3	5	3.38
Controls > panic patients			
R ventrolateral thalamus	14,−14,14	7	3.75
L medial cerebellum	−14,−45,−21	4	3.73
L parrahippocampus (36)	−34,−41,−3	4	3.71
L superior temporal and inferior parietal (22,40)	−38,−48,21	133.71	
L dentate of cerebellum	−14,−45,−21	10	3.67
R superior temporal sulcus (22)	52,−38,7	5	3.45
R precuneus and cuneus (31,17)	3,−65,27	4	3.31
R inferior parietal (40)	52,−34,48	4	3.10

[a]Brain regions containing clusters with significantly different activation by threat-related (compared with neutral) words in the panic patients compared with the control subjects. Significantly activated clusters are defined as a peak $p=0.001$ within a cluster of 4 voxels with $p=0.005$. L, left; R, right; B, bilateral.
[b]BA, Brodmann's Area in the region of observed activation.
[c]Highest local maximum in each region.

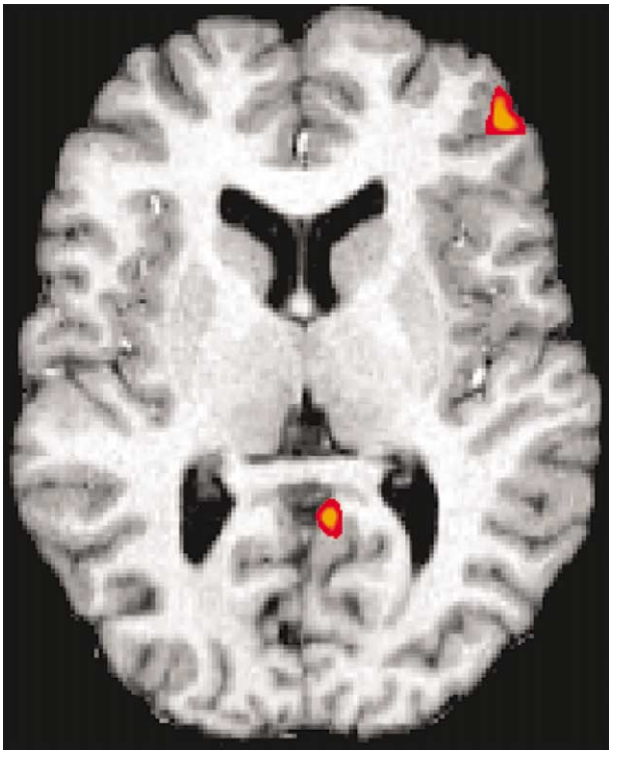

Fig. 1. Significantly greater activation in panic patients than control subjects during the evaluation of threat-related compared to neutral words is shown in the dorsolateral prefrontal cortex (BA 46) and the posterior cingulate cortex (BA 23 and 30). Significantly activated clusters (defined as a peak $p = 0.001$ within a cluster of 4 voxels with $p = 0.005$) are superimposed on a high-resolution axial MR image at $Z = +7$ in Talairach space.

[6,7]. The finding of greater posterior cingulate cortex activation by threat-related words is consistent with the observation that panic patients demonstrate enhanced memory for threat-related words [1]. An exaggerated influence of threat-related stimuli on cognition and memory may be a significant component of the vulnerability to panic disorder. The current results suggest that increased posterior cingulate cortex responsivity to threat-related stimuli may contribute to this cognitive vulnerability.

Panic disorder patients also showed greater activation by threat-related words in the left dorsolateral prefrontal cortex in Brodmann's area 46. Activation in this region qhas been associated with retrieval from memory of semantic and episodic verbal information [25]. Area 46 has reciprocal connections with the posterior cingulate cortex [20,22,26,27] and interconnects with the amygdala, the entorhinal cortex and the parahippocampal cortex [20,27,28]. Greater activation in a circuit involving area 46 and the posterior cingulate cortex is consistent with the proposal that more extensive mnemonic processing of threat-related stimuli is a characteristic feature of panic disorder.

Unlike the posterior cingulate cortex, the amygdala rapidly habituates to repeated threat-related stimuli [5]. Because the stimuli used in this study were 20 words repeated eight times each, the absence of significant findings in the amygdala was not unexpected. fMRI studies using unrepeated threat-related stimuli may be more useful for testing hypotheses about abnormal amygdala responses in panic disorder [7].

The fact that more patients with panic disorder than control subjects reported anxiety during the scan raises the possibility that acute anxiety could account for some of the differences shown in Table 1. The potential for anxiety-induced hypocapnia is a particular concern. Hypocapnia results in a global decrease in brain blood flow and a reduction in the size of BOLD responses in fMRI studies [29]. Although pCO_2 was not measured in this study, hypocapnia is frequently observed in panic patients undergoing stressful procedures [30,31], and could have reduced the BOLD responses of the panic patients in this study. While it is possible that areas showing significantly less activation in the panic patients (Table 1) reflect true differences in neural responses, the possibility that the panic patients were hypocapnic during the scan confounds interpretation of these observations. However, this consideration suggests that the greater activation observed in the posterior cingulate cortex and area 46 may be particularly robust. Measurement of pCO_2 levels or calibration of BOLD responses with a simple sensory or motor task should be incorporated into future fMRI studies of patients with panic disorder.

Table 2. Right > left asymmetry[a] of parahippocampal region activation by threat-related words in panic patients and control subjects.

	Y = −8[b]	Y = −16[b]	Y = −24[b]	Y = −32[b]	Y = −40[b]	Y = −48[b]
Panic patients ($n = 6$)	0.121	−0.028	0.070	−0.053	−0.072	0.028
Control subjects ($n = 8$)	0.003	−0.070	−0.070	−0.061	0.060	0.018
t-score (df = 12)	1.87	−0.21	2.97	0.09	−1.36	0.14
Uncorrected *p*	0.043[c]	NS	0.006[d]	NS	NS	NS

[a]Percentage increase in right parahippocampal region minus percentage increase in left parahippocampal region during evaluation of threat-related *vs* neutral words.
[b]Talairach coordinate of coronal slice through parahippocampal region.
[c]Trend, NS after correction for six comparisons.
[d]Significant after correction for six comparisons, $p = 0.035$, one-tailed.

The panic patients had significantly more right greater than left asymmetry of the BOLD response to threat-related words in the parahippocampal region. This is consistent with the results of three prior PET studies, conducted under resting conditions. Reiman *et al*. [11] found this effect in the mid-parahippocampal region, at the same anterior-posterior location as in the current study (−24 mm). The same asymmetry was observed in untreated, symptomatic panic patients [12] and in clinically remitted panic patients on imipramine [13], suggesting it might represent a trait feature of the disorder. It should be noted that the verbal task used in this study was associated with significant left, but not right, parahippocampal activation in both groups. However, the panic patients had significantly more right > left parahippocampal asymmetry relative to the control subjects, superimposed on the overall left-sided activation of this region induced by the task. Abnormalities of the parahippocampal region have often been observed in panic disorder [8–10]. This region may be a fruitful target for histologic or gene expression studies of post mortem tissue from patients with panic disorder.

This fMRI study of threat-related words in panic disorder shows hyperresponsivity in a circuit involving the posterior cingulate cortex and dorsolateral prefrontal area 46. This finding is consistent with the hypothesis that patients with panic disorder engage in more extensive memory processing of threat-related stimuli. This study also extends previous findings of abnormal parahippocampal asymmetry in panic disorder. Larger studies with concurrent cognitive measures and including both pre- and post-treatment data are warranted to expand upon these initial observations.

REFERENCES

1. Coles ME and Heimberg RG. *Clin Psychol Rev* **22**, 587–627 (2002).
2. Carter CS, Maddock RJ and Magliozzi JR. *Psychopathology* **25**, 65–70 (1992).
3. Cloitre M, Heimberg RG, Holt CS and Liebowitz MR. *Behav Res Ther* **30**, 609–617 (1992).
4. Maidenberg E, Chen E, Craske M *et al*. *J Anxiety Disord* **10**, 529–541 (1996).
5. Maddock RJ and Buonocore MH. *Psychiatry Res Neuroimag* **75**, 1–14 (1997).
6. Maddock RJ. *Trends Neurosci* **22**, 310–316 (1999).
7. Maddock RJ, Garrett AS and Buonocore MH. *Hum Brain Mapp* **18**, 30–41 (2003).
8. Dantendorfer K, Prayer D, Kramer J *et al*. *Psychiatry Res* **68**, 41–53 (1996).
9. Galderisi S, Bucci P, Mucci A *et al*. *Brain Res Bull* **54**, 427–435 (2001).
10. Massana G, Gasto C, Junque C *et al*. *NeuroImage* **16**, 836–842 (2002).
11. Reiman EM, Raichle ME, Robins E *et al*. *Am J Psychiatry* **143**, 469–477 (1986).
12. Nordahl TE, Semple WE, Gross M *et al*. *Neuropsychopharmacology* **3**, 261–272 (1990).
13. Nordahl TE, Stein MB, Benkelfat C *et al*. *Biol Psychiatry* **44**, 998–1006 (1998).
14. Spitzer RL and Williams JBW. *Structured Clinical Interview for DSMIII*, 3rd revised edn. New York: New York State Psychiatric Institute; 1987.
15. Hamilton M. *Br J Med Psychol* **32**, 50–55 (1959).
16. Kucera H and Francis WN. *Computational Analysis of Present-day American English*. Providence, RI: Brown University Press; 1967.
17. Maddock RJ, Garrett AS and Buonocore MH. *Neuroscience* **104**, 667–676 (2001).
18. Forman SD, Cohen JD, Fitzgerald M *et al*. *Magn Reson Med* **33**, 636–647 (1995).
19. Buonocore MH, Maddock RJ, Antognini JF and Aleinov I. *BrainMRI Software Package. Satellite Workshop on Functional MRI, Cognitive Neuroscience Society Annual Meeting*. San Francisco, 1995.
20. Goldman-Rakic PS, Selemon LD and Schwartz ML. *Neuroscience* **12**, 719–743 (1984).
21. Suzuki WA and Amaral DG. *J Comp Neurol* **350**, 497–533 (1994).
22. Morris R, Petrides M and Pandya DN. *Eur J Neurosci* **11**, 2506–2518 (1999).
23. Carmichael ST and Price JL. *J Comp Neurol* **363**, 615–641 (1995).
24. Van Hoesen GW, Morecraft RJ and Vogt BA. Connections of the monkey cingulate cortex. In: Vogt BA and Gabriel M (eds). *Neurobiology of Cingulate Cortex and Limbic Thalamus*. Boston: Birkhauser; 1993, pp. 345–365.
25. Cabeza R and Nyberg L. *J Cogn Neurosci* **12**, 1–47 (2000).
26. Morris R, Pandya DN and Petrides M. *J Comp Neurol* **407**, 183–192 (1999).
27. Petrides M and Pandya DN. *Eur J Neurosci* **11**, 1011–1036 (1999).
28. Amaral DG, Price JL, Pitkanen A and Carmichael ST. Anatomical organization of the primate amygdaloid complex. In: Aggleton JP (ed.). *The Amygdala: Neurobiological Aspects of Emotion*. Chichester: Wiley-Liss; 1992, pp. 1–66.
29. Kolbitsch C, Schocke M, Hormann C *et al*. *Br J Anaesthesia* **83**, 835–838 (1999).
30. Maddock RJ and Carter CS. *Biol Psychiatry* **29**, 843–854 (1991).
31. Maddock RJ, Carter CS, Tavano-Hall L and Amsterdam EA. *Psychosom Med* **60**, 52–55 (1998).

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