

A Functional Anatomy of Anticipatory Anxiety

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Anticipatory anxiety is a complex combination of a future-oriented cognitive state, negative affect, and autonomic arousal. A dual-task paradigm of anticipation of electric shocks and a motor-learning task was used to examine the changes in neural patterns of activation associated with modulation of the cognitive state in anxiety by a distracting motor task. We used positron emission tomography (PET) and ¹⁵O-water to measure regional cerebral blood flow (rcbf) in 10 healthy male volunteers. A 2 × 2 factorial design (shock vs no shock) × (low vs high distraction) was used with three scans per condition. Twelve PET scans were performed on each subject. In six of these scans, subjects were given electric shocks. In all scans, subjects also simultaneously performed a motor repetition (low distraction) or learning (high distraction) task. Galvanic skin conductance (GSR), Spielberger State and Trait Anxiety Inventory (STAI), and self-report data were also collected. In comparisons between the shock and no-shock conditions, the main finding was of increased rcbf in the left insula (−38,8,8) ($z = 4.85$, $P < 0.05$ corrected) and a homologous area in the right insula at a lower threshold ($z = 3.20$, $P = 0.001$ uncorrected). Other areas activated were the right superior temporal sulcus, left fusiform, and left anterior cingulate. Using the STAI-state scores as a covariate of interest, significant correlations with rCBF were seen in the left orbitofrontal cortex, left insula, and left anterior cingulate cortex. There was no significant distraction effect as measured by the STAI, self-report, GSR response or interactional analysis of the PET data. These findings support the role of paralimbic structures as neural substrates of anticipatory anxiety. The failure to demonstrate behavioral and neurophysiological changes with the distractor task may reflect the modest increases in anxiety with the shock, the relatively simple distractor task, and small sample size. © 1999 Academic Press

INTRODUCTION

Anxiety is best conceptualized as a future-oriented cognitive-affective-somatic state, the prominent feature being “a sense of uncontrollability focused on possible future threat, danger, or other upcoming, potentially negative events” (Barlow *et al.*, 1996). Associated with this is a negative affective state of a sense of fear (Izard and Youngstrom, 1996), helplessness (Barlow *et al.*, 1996), and accompanying somatic components of central nervous system arousal. Various models of anxiety have attempted to explain the neuropsychological mechanisms (Barlow *et al.*, 1996; Gray and McNaughton, 1996) and neuroanatomical substrates of anxiety. For example, functional neuroimaging techniques have demonstrated that the neural substrates of emotions are not confined to one specific “emotional” area of the brain but consist of complex interactions between different brain structures. It is noteworthy that most of these studies have focussed on anxiety disorders such as simple phobia (Fredrickson *et al.*, 1993; Mountz *et al.*, 1989; O’Carroll *et al.*, 1993; Wik *et al.*, 1993; Rauch *et al.*, 1995), panic disorder (Reiman *et al.*, 1984; Reiman, 1987; Reiman *et al.*, 1989; Nordahl *et al.*, 1990), and obsessive-compulsive (Baxter *et al.*, 1987a,b, 1988, 1992; Swedo *et al.*, 1989; Horwitz *et al.*, 1991; Nordahl *et al.*, 1989; Martinot *et al.*, 1990; Azari *et al.*, 1993; Perani *et al.*, 1995; McGuire *et al.*, 1994; Rauch *et al.*, 1994) disorder rather than in anxiety in normal individuals.

A common element in anxiogenic stimuli in studies is the production of an unpleasant sensation such as pain (Reiman *et al.*, 1989; Rodriguez *et al.*, 1989). The aim of this study was to investigate one of the most basic forms of anxiety, namely anticipatory anxiety which is experienced by normal individuals and which may play a role in anxiety disorders such as panic disorder (Barlow *et al.*, 1996). We applied several electric shocks to the hand to induce a state of anticipatory anxiety and manipulated the subject’s attention to these anxiogenic

stimuli using a distracting motor-learning task. Attentional focus in exposure-based treatments for fear and anxiety has been hypothesized to interact with the mechanisms for fear reduction (Craske and Street, 1991), although controversy remains in regards to the direction of fear modification and the immediate versus long-term effects. Specifically, a dual-task paradigm of electric shocks and motor-learning task can potentially tease apart the neural substrates involved in cognitive aspects of anxiety (i.e., attention), from those responsible for affective and physiological components. A non-scanning pilot study prior to the current study had indicated that this type of motor-learning task can reduce subjective anxiety.

METHODS

Subjects

The subjects were 10 normal healthy male volunteers with a mean age of 30.4 years (range = 21–40 years). All were right handed. None of the subjects had a history of major neurological or psychiatric disorders including substance abuse. None were using psychotropic medications. The study was approved by the Ethics Committee of the Institute of Neurology. Each subject gave informed consent.

Experimental Design

All subjects underwent a PET (Positron Emission Tomography) and MRI (Magnetic Resonance Imaging) scan. The MRI scan was for the purpose of anatomical localization of PET data after coregistration with the data. For the PET scan, the dual-task paradigm consisted of electric shocks to induce anticipatory anxiety and the use of a motor-learning task as the distractor from the anxiety. The factorial design consisted of four conditions: (1) shock and motor learning, (2) no shock and no motor learning, (3) shock and no motor learning, and (4) no shock and no motor learning. Twelve sequential scans using ^{15}O -water as a tracer and measure of regional cerebral blood flow (rCBF) were performed for each subject. There were three scans per condition and the four conditions were randomized within and between subjects across the 12 scans.

In the shock conditions, electric shocks were administered to the dorsal aspect of the right hand. An electrical stimulator box delivered variable voltage and current to the subject within a safety limit. Two Neuroline disposable neurology electrodes with gel and adhesive were attached to the dorsal aspect of the subjects right hand. The size of the delivered shock was determined individually for each subject. Prior to each scan, subjects were given electric shocks to determine the highest threshold which they could tolerate and the electrical stimulator was set using this value as the minimum

setting. During the PET scan sessions, electric shocks were delivered randomly prior to the onset of the PET scanning interval such that the subject was unable to predict when the next electric shock was going to be delivered. No electric shocks were delivered during the scanning window. The background color of the computer monitor screen was either red or blue to warn the subject whether there were going to be shocks given during the task or not. A red screen color indicated that shocks would be given at any time and a blue screen color indicated that shocks would not be given. Subjects were shown this in the training session prior to the PET scan.

A motor learning task was used as a distractor task. In the learning condition, the subject had to learn a new sequence of key presses by trial and error. The keypad consisted of four keypresses corresponding to the four fingers of the right hand. The movements were paced by the computer screen at a rate of one every 2.5 s. A circle appearing on the computer screen every 2.5 s prompted the subject to make the next finger movement. The subject would try to identify the first movement. If their response was correct, a tick would appear. If it was incorrect, a cross would appear instead. This method of trial and error continued until the subject learned an eight-movement sequence. When a sequence was learned, subjects were then instructed that they would be given a new sequence to learn. There were different sequences for the six learning conditions for each subject. Subjects were specifically told not to remember the sequence by naming or numbering the digits.

In the no motor-learning condition, subjects performed a repetitive sequence which was learned prior to the scans. The eight finger presses for the repetitive no motor-learning sequence consisted of index, middle, ring, little, index, middle, ring, and little. The movements were still paced by a circle on the computer screen at the same rate of 2.5 s. Subjects still received a feedback of a tick for a correct response and a cross for an incorrect response. Subjects were taught both the motor learning and no motor learning tasks prior to the scans.

Galvanic skin response (GSR) was measured using Neuroscan and a skin conductance coupler providing a constant 0.5 V across electrodes. The GSR was measured using two electrodes attached to the palmar aspect of the left index and left middle fingers. Prior to the placement of the electrodes, the fingers were wiped clean with distilled water and dried. Data was collected prior to the onset of PET scanning and finished after the PET scan ended. The timing of the delivered shocks were manually marked onto the Neuroscan data.

Prior to the PET scans, subjects were administered the Spielberger State and Trait Anxiety (STAI) questionnaires and asked to give a rating of their anxiety on a

scale from 0 to 100. An intravenous cannula was inserted into the left cubital fossa and electrodes were attached to the dorsal aspect of the right hand. The PET scans were performed in a darkened room with the subjects lying supine. Both arms were supported by arm rests. The keypad was placed within comfortable reach of the right hand and secured to the right arm rest. Subjects received a total of 9 mCi over 20 s through an automatic delivery system. After each PET scan, subjects were administered the STAI-state questionnaire and asked to give a rating of their anxiety on a scale from 0 to 100. A debriefing session after the last scan confirmed that subjects were not able to predict when they were going to be given the next electric shock.

PET and MRI Data Acquisition

A Siemens/CTI ECAT EXACT HR+ PET scanner operating in high sensitivity 3D mode was used. The transaxial field of view was 15.5 cm. The scanner was coordinated with the automated ^{15}O -water delivery system and was activated when counts in the field of view reached thirty thousand counts per second (cps). The activation frame was 90 s. The scanner operated on an 8-min cycle from the beginning of one scan to the next. Images were reconstructed into 63 planes using a Hanning filter. This resulted in a 6.4-mm transaxial and 5.7-mm axial resolution.

The structural MRI scan was obtained using a Siemens/Magneton vision scanner operating at 2 Tesla and a maximum gradient field strength of 25 Tesla per meter. A Magnetized Prepared-Rapidly Acquired Gradient Echo (MPRAGE) volume acquisition T1-weighted sequence was used to obtain good anatomical details. Inplane resolution was 1 mm and slice thickness was 1.5 mm. The sequence took 7.5 min. Images were acquired in the axial planes.

Data Analysis

The data was processed and analyzed using Statistical Parametric Mapping (SPM) 96 software (Wellcome Department of Neurology, London) implemented in Matlab (Mathworks, Sherborn, MA). The PET scans were realigned to correct for involuntary movements between scans. The mean realigned PET scan was coregistered with the structural MRI scan. The realigned PET and coregistered structural MRI scans were then transformed through the process of normalization into standard stereotactic space. The PET images were then filtered with a low-pass Gaussian filter at 12 mm. Differences in global blood flow were adjusted to a global mean of 50 ml/dl/min by using subject-specific analysis of covariance. Blood flow changes between conditions were assessed using the t statistic. The resultant SPM(t) map identified areas of

statistically significant blood flow changes as a result of the differences in rcbf between the different conditions. The localization of the maxima of the SPM(t) statistics are identified in terms of Talairach and Tournoux (1988) coordinates. A mean MRI of the 10 subjects was used also as a template to improve the localization of these activations. Two separate analyses were also carried out using the STAI scores and the rCBF value from the left insula as covariates of interest in the analysis of the PET data. In the latter analysis, the rCBF values in the left insula were used to examine whether the activity in the left insula predicted responses in other brain regions, specifically the amygdala, under the condition of shock versus no shock.

The GSR data was analyzed using Neuroscan and Acknowledge software. The GSR data was edited using the Edit module in the Neuroscan software. The data obtained in the time interval from the onset of GSR recording to 4 s from the administration of the last shock was collected for further analysis by the Acknowledge software. In the conditions where no shocks were delivered, the mean time interval of relevant data collection, as defined above, was calculated for each subject and a similar time interval of GSR data collection was chosen for the no shock conditions. Using the Acknowledge software, the range, i.e., from the minimum to the maximum peaks, was calculated for each of the scans.

RESULTS

Behavioral Self-Report Measures

The findings from the STAI-state scores indicated that although the increases in the scores were modest, the averaged group STAI-state score was significantly higher in the shock (50.5 ± 12.3) compared to no shock conditions (35.5 ± 6.6) ($P < 0.001$). The distraction effect, whereby the STAI scores were one standard deviation lower in the shock and motor learning condition compared to the shock and no motor learning conditions, was seen in only two subjects. Four other subjects showed a degree of distraction but the difference in the scores did not reach one standard deviation significance.

In the self-report scale of 100, 7 of 10 subjects had higher scores in the shock state compared to the no shock state at a significance level $P < 0.05$; four of these achieved differences in the scores at $P < 0.001$. Three subjects were distracted by the motor learning task as evident in the difference of one standard deviation in their self-report anxiety scores. One other subject demonstrated a small level of distraction but did not reach one standard deviation significance.

GSR Measures

GSR was collected for 9 of the 10 subjects. The GSR peak to peak range (units-mSiemens) was greater in the shock compared to the no shock conditions in eight of the subjects, the difference reaching a significance level of $P < 0.05$ in five of the eight subjects. There was no significant effect of distraction evident in the GSR data.

PET Data

Analysis of the data comprised results from 10 subjects to demonstrate the main effect of anticipatory anxiety. A further analysis of nine subjects was undertaken whereby the STAI scores were used as covariates of interest. One subject was excluded as he did not have balanced number of scans.

Main effects of shock. The main effect of shock was determined by comparison of the neural response in the conditions where the subjects were expecting compared to not expecting shock (Fig. 1). In the shock condition, the sole area where there is increased neural activity, which survived correction, was in the left insula ($z = 4.85$, $P < 0.05$ corrected). A homologous area in the right insula also showed a significant increase in neural response at a lower threshold ($z = 3.20$, $P = 0.001$ uncorrected). The rCBF data for the pixel of maximal significance in the left insula for each of the four conditions is shown in Fig. 1. A number of other areas which did not survive stringent correction are presented for descriptive purpose in Table 1. These areas comprise the right superior temporal sulcus, left anterior cingulate, and left fusiform gyrus.

Main effects of learning. The main effect of learning was determined by contrasting the motor learning with the no motor learning conditions. The areas of activation comprised the prefrontal cortex bilaterally, left cingulate cortex, left premotor cortex, bilateral insula, bilateral parietal cortex, left thalamus, and bilateral cerebellum. (Table 2).

STAI as a Covariate of Interest

To determine the relationship between the presence of anticipatory anxiety and the underlying neural response, the anxiety scores on the STAI were used as covariates of interest. Nine subjects were used in this analysis as one subject did not have an equal number of scans across all four conditions. The regions which showed significant covariance at a significant level of $P < 0.001$ (uncorrected) included the left insula, left

TABLE 1

Coordinates of the Pixels Where the Most Significant Differences in rcbf Were Identified at a Threshold of $P = 0.001$ for Shock Conditions in Ten Subjects

Area activated	Talairach coordinates of peak activation	Z score of peak activation
Insula (L)	-38, 8, 8	4.85
Superior temporal sulcus (R)	58, -48, 26	3.41
Fusiform (L)	-42, -16, -30	3.34
Anterior cingulate (L)	-8, 40, 16	3.42

Note. The coordinates refer to the stereotaxic atlas of Talairach and Tournoux (Talairach and Tournoux, 1988). The z score is a measure of the degree of significance of the difference and is the number of standard deviations from the mean t value in the (t) statistical map of the t value for the most significant pixel in the plane. L, left. R, right.

orbitofrontal, left inferior frontal gyrus, and left anterior cingulate (Table 3). The SPM(t) map of the contrast and the rCBF for the left orbitofrontal cortex is shown in Fig. 2.

Psychophysiological Interaction with the Left Insula

There was no evidence that activity in the left insula predicted activity in the amygdala in the context of shock compared to the no shock condition.

Effect of Distraction

For the 10 subjects, no areas survived stringent correction in the analysis for the interaction effects of shock with and without motor learning at a threshold of $P < 0.001$.

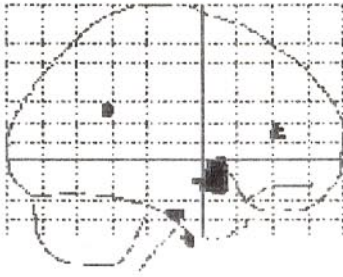
DISCUSSION

The main result from the study is that anticipatory anxiety induced by the expectancy of electric shocks is associated with increased neural activity in the left insula, right temporal sulcus, left fusiform, and left anterior cingulate cortex. When STAI scores are used as a covariate of interest, the significant areas of increased activity are in the left orbitofrontal cortex, left insula, and left anterior cingulate cortex. Interactional analysis of shock and learning was used to tease out the effect of distraction (i.e., which regions had greater activity during the shock and learning conditions compared to the shock and no learning conditions) but failed to demonstrate an effect. This is re-

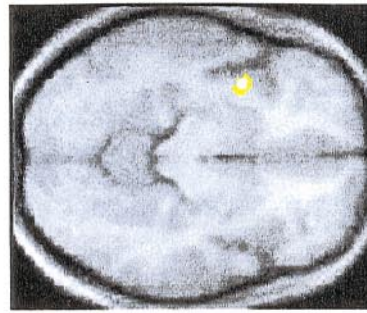
FIG. 1. Statistical Parametric Map demonstrating significant changes in cerebral blood flow between Shock and No Shock conditions at a height threshold of $p = 0.001$ for the 10 subjects. rCBF in the left insula for the 10 subjects in the shock condition at a threshold of $p = 0.001$

FIG. 2. Statistical Parametric Map demonstrating significant changes in cerebral blood flow between Shock and No Shock conditions at a height threshold of $p = 0.001$, using STAI as a covariate of interest. rCBF in the left orbitofrontal cortex.

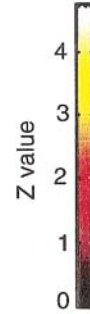
Sagittal



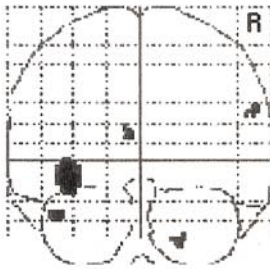
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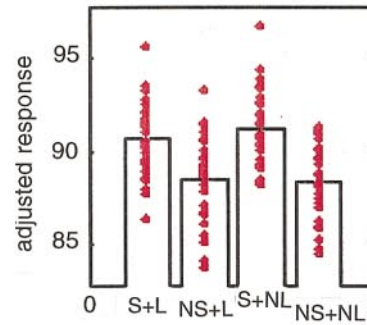
R



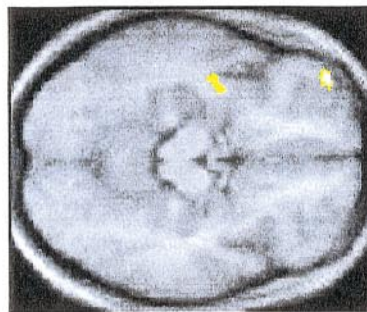
Coronal



Z=4.85, p=0.000 (0.026 corrected)



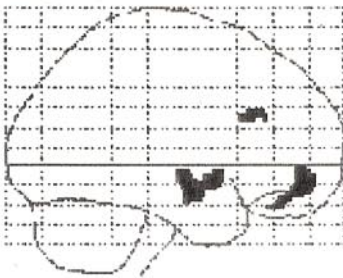
transverse



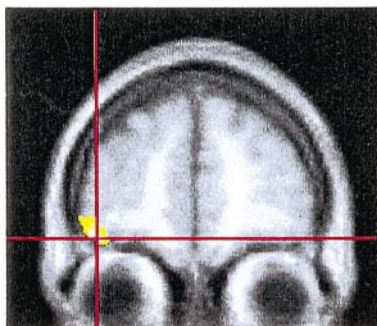
R



Sagittal



Coronal



R

Z=4.47, p=0.000 (0.122 corrected)

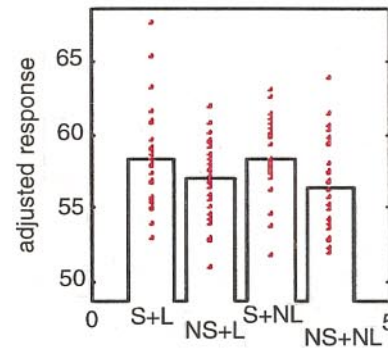


TABLE 2

Coordinates of the Pixels Where the Most Significant Differences in rcbf Were Identified at a Threshold of $P = 0.001$ for Learning vs No Learning Conditions in Learning and Nonlearning Environments

Area activated	Talairach coordinates of peak activation	Z score of peak activation
Prefrontal cortex		
Orbitofrontal (L)	-32, 60, -10	4.84
Orbitofrontal (R)	34, 60, -4	5.27
Superior frontal (R)	28, -2, 66	4.11
Middle frontal (L)	-48, 22, 32	4.50
Middle frontal (R)	40, 30, 32	4.21
Inferior frontal (L)	-44, 48, 16	4.18
Inferior frontal (R)	56, 12, 20	4.56
Cingulate cortex		
Anterior (L)	-14, 8, 50	5.02
Premotor cortex (L)	-24, -6, 46	5.17
Precentral (L)	-46, -2, 34	4.83
Precentral (R)	48, 4, 34	4.03
Postcentral (L)	-68, -4, 26	3.59
Insula (R)	38, 22, 0	5.25
Insula (L)	-38, 18, 2	3.71
Parietal cortex		
Intraparietal sulcus (L)	-44, -36, 40	6.75
Intraparietal sulcus (R)	36, -60, 46	5.72
Superior parietal gyrus (L)	-14, -72, 58	6.26
Superior parietal gyrus (R)	10, -74, 52	6.30
Thalamus (L)	-20, -20, 6	3.32
Cerebellum		
Cortex (L)	-32, -56, -30	5.76
Cortex (R)	36, -68, -26	4.78
Vermis (L)	-6, -76, -26	4.37
Vermis (R)	8, -72, -22	3.79

Note. L, left. R, right.

flected by the lack of significant distraction as measured by the STAI, self-report anxiety, and GSR response. There was no evidence of context-dependent interactions between the left insula and amygdala under the condition of shock compared to no shock. The results from the main effect of the motor learning is similar to those in previous studies of motor learning (Jueptner *et al.*, 1997).

These findings on anticipatory anxiety differ in some respects from those in previous studies. In a similar PET study, Reiman *et al.* (1989) used anticipation of a painful electric shock to induce anticipatory anxiety and found significant increases in the rCBF in bilateral temporal poles. Although this finding was not without controversy (Drevets *et al.*, 1992), the temporal pole has been implicated in other studies on anxiety (Fredrickson *et al.*, 1993; Wik *et al.*, 1993; Rauch *et al.*, 1995; Fredrikson *et al.*, 1995). The subjects in Reiman *et al.*'s (1989) study were scanned before they were given any shocks, unlike the current study where they were scanned after receiving shocks and consequently probably had greater anticipatory anxiety. Rodriguez *et al.*

TABLE 3

Coordinates of the Pixels Where the Most Significant Differences in rcbf Were Identified at a Threshold of $P = 0.001$ for Shock vs No Shock Conditions Using the STAI as a Covariate of Interest

Area activated	Talairach coordinates of peak activation	Z score of peak activation
Prefrontal cortex		
Inferior frontal (L)	-42, 52, -12	4.47
Orbitofrontal (L)	-40, 44, -18	3.63
Insula (L)	-40, -6, -12	4.00
Anterior Cingulate (L)	-12, 28, 26	3.50

Note. L, left. R, right.

found a negative correlation between rCBF as measured by Xenon-133 and anxiety measures (Rodriguez *et al.*, 1989) when the insertion of an intravenous line was used as a means of inducing anxiety. A similar relationship between regional cerebral glucose metabolism (CMRglu) and anxiety has been noted in previous studies (Gur *et al.*, 1987; Reivich *et al.*, 1984). Performance on a verbal analogy task was positively correlated with the rCBF as measured with Xenon-133, both of which had an inverted-U relationship with the level of anxiety in a later study by Gur *et al.* (1988). Benkelfat *et al.* (1995) used a bolus injection of cholecystokinin tetrapeptide to elicit an anxiogenic response and found rcbf increases in the anterior cingulate, claustrum-insular-amygdale region, and cerebellar vermis.

Our main findings in the anticipatory anxiety conditions are consistent with current views on the neural substrates of anxiety. Rauch *et al.* (1997) reported on data pooled from three PET studies on three different anxiety disorders—simple phobia, obsessive-compulsive disorder, and posttraumatic stress disorder. Using symptom provocation paradigms specific to each disorder, common areas of activation across the three anxiety disorders were in the right posterior medial orbitofrontal cortex, bilateral insular cortex, bilateral lenticulate nuclei, and bilateral brainstem. In our study, the left insula and left anterior cingulate cortex were common to the effect of shock versus no shock and when the STAI scores were used as a covariate of interest emphasizing their role in the mediation of anxiety.

These areas of the insula, lateral orbitofrontal cortex, and cingulate cortex that we identify constitute what is termed paralimbic cortex where there is transition from more rudimentary allocortex to more complex granular isocortex (Mesulam and Mufson, 1982). The juxtaposition of these structures between the neocortex and limbic areas of the brain, and its architecture, are in keeping with the view that one of the functions of the

paralimbic structures is to integrate the internal and external milieu (Mesulam and Mufson, 1982). In this regard, the insula has been described as a limbic integration cortex (Augustine, 1996). The insula has connections with portions of the frontal, parietal, temporal lobes, cingulate gyrus, basal nuclei, amygdala, and other structures of the limbic system and dorsal thalamus (Augustine, 1985, 1996). The richness of these connections lend support to its multiple role as a visceral sensory, visceral motor, motor association, vestibular, somatosensory, and language area. The insula has also been directly implicated in PET studies of emotion (Lane *et al.*, 1997) and anxiety (Curtis, 1991). In particular, the region in the insula which was activated in our study was centred on the anterior insula. It has been suggested that the functional role of the anterior insula may participate in evaluating potentially distressing cognitive and interoceptive sensory information (Reiman *et al.*, 1997; Reiman, 1997).

The anterior cingulate has been subdivided into areas specific for affect (Brodmann area 24, 25, 33) and cognition (Brodmann area 24' and 32') (Devinsky *et al.*, 1995). The areas implicated in affect have extensive connections with the amygdala, periaqueductal grey, and autonomic brainstem motor nuclei (Devinsky *et al.*, 1995). Lesion and functional neuroimaging studies have implicated the anterior cingulate in the modulation of autonomic activity and emotional responses. For example, in a conditioning experiment on rats whereby a neutral conditioning stimulus was paired with an aversive unconditioned response, i.e., footshock, lesions of the anterior cingulate cortex reduced the autonomic responses during the conditioning stimulus phase (Fryszak and Neafsey, 1991). The anterior cingulate and insula have also been implicated in previous imaging studies in pain (Derbyshire *et al.*, 1994; Rainville *et al.*, 1997; Howland *et al.*, 1995). Rainville *et al.* (1997) used hypnotic suggestions to alter, selectively, the unpleasantness of the painful stimuli. The results revealed a modulation of pain-evoked activity in the anterior cingulate but not in the primary somatosensory cortex. This suggests a representation of the painful stimuli within the anterior cingulate which can be modified by cognitive means, i.e., hypnotic suggestions.

Regional specialization has also been reported within orbitofrontal cortex, which is divided into medial and lateral divisions by the medial orbital gyrus and olfactory groove (Morecraft *et al.*, 1992), each division having connections with different paralimbic structures. It receives afferents from a diverse group of structures, including the hypothalamus, amygdala, hippocampus, olfactory cortex, midline and anterior thalamic nuclei, unimodal association areas, secondary somatosensory areas, and gustatory cortex. In this context, it is noteworthy that the left orbitofrontal

cortex is activated when STAI scores are used as a covariate of interest. This suggests a possible role for this area in the "conscious" experience of anxiety given that the STAI consists of a series of statements about the subject's subjective experience. In a PET study of visualizing and perceiving aversive stimuli, it has been reported that rCBF increases in bilateral insula with negative, relative to neutral imagery but no such increases in comparison of negative and neutral perception (Kosslyn *et al.*, 1996). The greater activation in the imagery conditions relative to the perception conditions suggest the insula may play a role in mental representation.

The evidence reviewed lends support to a proposal that anterior cingulate and insula have a role in mental representation of sensory and autonomic experience associated with anticipating painful electric shock. This is in line with Damasio's model of emotion, which he defined as a collection of changes in bodily and brain states in response to the content of one's thoughts relative to a particular entity and the experience of a particular cognitive mode (Damasio, 1995). Central to the model is the idea of representations of these bodily changes. The insula, anterior cingulate, and orbitofrontal cortex could conceivably act as the system through which sensory, autonomic, and cognitive information in relation to anticipatory states are integrated and represented.

In view of the extensive literature on the role of the amygdala in fear and conditioning (Maren and Fanselow, 1996; Morris *et al.*, 1996), further analysis of the data examining for psychophysiological interactions between the insula and amygdala was performed but failed to find any interactional effects between these regions. The absence of the amygdala in the our study, and other studies of anxiety, may be explained by several factors. First, the time course of the amygdala response may be rapid with an attenuation of response that would not be resolved by PET (Breiter *et al.*, 1996; Whalen *et al.*, 1998). It has now been demonstrated that the amygdala response to fear-related contexts is time limited and, consequently, blocked experimental designs may be insensitive to regions which show profound time by condition interactions (Buchel *et al.*, 1998). Second, while it is evident that the amygdala is important in recognition of fearful faces (Morris *et al.*, 1996) and fear conditioning (Maren and Fanselow, 1996), fear and anxiety are not necessarily synonymous (Charney and Deutch, 1996; Barlow *et al.*, 1996; Izard and Youngstrom, 1996). Fear is generally stimulus-bound and temporally demarcated. Anxiety has similar features but is less stimulus-bound and is not closely bound in time to the actual stimulus. Third, in our study where the conditions were randomized, it is also possible that anxiety levels had not subsided sufficiently between scans. Indeed some subjects dem-

onstrated a degree of anxiety related to the motor learning task. This may also explain the lack of significant distraction effect in the motor learning task compared with the motor repetition task. Indeed, the psychological effects of distraction tasks during exposure to phobic stimuli can be ambiguous and in some, increases anxiety measures on reexposure to the stimulus (Rodriguez and Craske, 1993). A nonscanning pilot study on a different set of subjects had demonstrated that the level of anxiety could be reduced by using a motor learning task. Due to the large habituation effects in these type of paradigms, pilot data looking for a distraction effect was not specifically obtained on subjects involved in the scanning study prior to the scan. Fourth, failure to demonstrate an interaction between shock and learning may reflect the fact that similar structures, i.e., left insula, left orbitofrontal cortex, left anterior cingulate, demonstrated increased activity in the shock condition and motor learning conditions when considered individually. A final consideration to explain the lack of interaction between distraction and shock is that the neural responses to anticipatory anxiety may be obligatory in the sense that they are not influenced by other cognitive processes, i.e., the availability of attentional resources.

In summary, the main findings from this study indicate the importance of several paralimbic structures—namely the left insula, left anterior cingulate, and left orbitofrontal cortex—in the mediation of anticipatory anxiety in normal subjects. Of significance is the overlap of this finding with previous neuroimaging studies of anxiety disorders and suggests a common pathway in the functional neuroanatomy of pathological anxiety and the experience of anticipatory anxiety in normal subjects.

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