Neural Biases to Covert and Overt Signals of Fear: Dissociation by Trait Anxiety and Depression

Leanne M. Williams, Andrew H. Kemp, Kim Felmingham, Belinda J. Liddell, Donna M. Palmer, and Richard A. Bryant

Abstract

Although biases toward signals of fear may be an evolutionary adaptation necessary for survival, heightened biases may be maladaptive and associated with anxiety or depression. In this study, event-related potentials (ERPs) were used to examine the time course of neural responses to facial fear stimuli (versus neutral) presented overtly (for 500 msec with conscious attention) and covertly (for 10 msec with immediate masking to preclude conscious awareness) in 257 nonclinical subjects. We also examined the impact of trait anxiety and depression, assessed using psychometric ratings, on the time course of ERPs. In the total subject group, controlled biases to overtly processed fear were reflected in an enhancement of ERPs associated with structural encoding (120–220 msec) and sustained evaluation persisting from 250 msec and beyond, following a temporo-occipital to frontal topography. By contrast, covert fear processing elicited automatic biases, reflected in an enhancement of ERPs prior to structural encoding (80–180 msec) and again in the period associated with automatic orienting and emotion encoding (230–350 msec), which followed the reverse frontal to temporo-occipital topography. Higher levels of trait anxiety (in the clinical range) were distinguished by a heightened bias to covert fear (speeding of early ERPs), compared to higher depression which was associated with an opposing bias to overt fear (slowing of later ERPs). Anxiety also heightened early responses to covert fear, and depression to overt fear, with subsequent deficits in emotion encoding in each case. These findings are consistent with neural biases to signals of fear which operate automatically and during controlled processing, feasibly supported by parallel networks. Heightened automatic biases in anxiety may contribute to a cycle of hypervigilance and anxious thoughts, whereas depression may represent a “burnt out” emotional state in which evaluation of fear stimuli is prolonged only when conscious attention is allocated.

INTRODUCTION

Vigilance for threat is crucial for survival, and neural systems may have evolved to give precedence to arousing signals of potential danger (Williams, 2006). Both behavioral and brain function studies provide evidence of a “negativity bias” toward aversive, emotionally arousing stimuli (e.g., Williams, Palmer, Liddell, Song, & Gordon, 2006; Cacioppo, Gardner, & Berntson, 1999; Taylor, 1991). However, excessive biases toward such stimuli may contribute to affective disorders (Williams, 2006). Consistent with this view, cognitive models propose that there is an exaggerated bias in the automatic processing of fear-related stimuli in anxiety disorder, and a distinctive negativity bias involving more controlled processing in depression (Mogg & Bradley, 1998; Williams, Watts, MacLeod, & Mathews, 1997). To date, this proposal has been tested only with behavioral measures. Event-related potentials (ERPs) provide an index of neural activity with millisecond precision (Mangun & Hillyard, 1995), well suited to examining the time course of processing biases. In this study, we used ERPs to investigate the time course of neural biases to fear stimuli presented under conditions of both automatic and controlled processing, and whether high trait anxiety is associated with a specific exacerbation of these biases relative to both low anxiety and depression.

In behavioral studies, preferential attention to negatively valenced, particularly fear-related, stimuli has been interpreted as a negativity bias (Cacioppo et al., 1999; Ohman, 1997; Gur et al., 1992; Pratto & John, 1991; Hansen & Hansen, 1988). Enhanced negativity biases in anxiety disorder are reflected in faster reaction times to fear-related stimuli in visual probe tasks using both word and face stimuli (Bryant & Harvey, 1997; Bryant, Harvey, Gordon, & Barry, 1995). These biases may generalize to the greater expectation of aversive events (Smith & Bryant, 2000; Warda & Bryant, 1998). Several findings indicate that biases toward fear stimuli in anxiety may arise from automatic processing, without the need for conscious awareness (Bradley, Mogg, Millar, & White, 1998).
Although contrary findings have also been reported (Mathews, Ridgeway, & Williamson, 1996), excessive automatic biases toward fear cues could feasibly contribute to an ongoing cycle in which vigilance for even low probability sources of danger increases, and anxious thoughts are intensified (Beck, 1976).

In depression, negativity biases are thought to be apparent only when there is sustained stimulus evaluation, or stimuli are particularly salient (Mogg et al., 1995). These biases have been revealed on modified Stroop, visual probe, and facial emotion recognition tasks, when sustained evaluation is promoted by long stimulus durations (Gur et al., 1992; Mogg et al., 1993, 1995; Segal, Gemar, Truchon, Guirguis, & Horowitz, 1995; Gotlib & Cane, 1987). Although these studies have focused on stimuli related to sadness, the explicit recognition of fear is also reduced in depressed individuals (Lenti, Giacobbe, & Pegna, 2000). Excessive activation of the amygdala, associated with arousing and aversive stimuli, has also been observed in depressed patients (Siegle, Steinhauer, Thase, Stenger, & Carter, 2002; Dreverts, 2001; Sheline et al., 2001). Moreover, depression has been associated with particularly sustained amygdala activation, and it has been suggested that the time course of this activity may mediate the negativity biases observed in behavioral studies (Siegle et al., 2002).

Unlike behavioral measures, event-related brain potentials (ERPs) provide a direct window onto the time course of neural activity, which may help distinguish automatic versus controlled biases toward fear stimuli, and whether anxiety is associated with a specific exaggeration of automatic biases versus an exaggeration of controlled biases in depression. Facial expressions of fear have been found to enhance ERPs within the first 200 msec of stimulus processing, and then again in a more persistent manner after 250 msec, consistent with biases in automatic orienting, and subsequent sustained evaluation (Williams, Palmer, et al., 2006; Ashley, Vuilleumier, & Swick, 2004). When awareness is restricted by masking, enhancements in early ERPs remain, but sustained activity is absent (Liddell, Palmer, Gordon, & Williams, under review; Liddell, Williams, Rathjen, Shevrin, & Gordon, 2004; Williams, Liddell, et al., 2004). Masked fearful faces have also been found to elicit enhancements in the N250 and early P300 associated with automatic orienting and encoding of significant stimuli (Liddell et al., 2004). The N250 has also been related to encoding emotional expressions and integrating salient stimuli into a gestalt (Han, He, & Woods, 2000; Streit, Wößler, Brinkmeyer, Ihl, & Gaebel, 2000). Functional magnetic resonance imaging (fMRI) studies, undertaken with equivalent behavioral tasks, suggest that parallel visual pathways to the amygdala may support these responses to fear at differing levels of awareness: a direct extrastriate pathway for automatic orienting and a slower, indirect striate occipito-temporal pathway for sustained conscious evaluation (Williams et al., 2006; Liddell et al., 2005; Morris, Ohman, & Dolan, 1999; Whalen et al., 1998).

ERPs have been used to study negativity biases in anxiety in only two studies to date. One study focused on anticipatory biases prior to stimulus onset, which were enhanced to negatively valenced stimuli in participants with high state (and combined state–trait) anxiety, consistent with greater vigilance to these stimuli (Carretti et al., 2004). Another study reported larger and faster early ERPs to fear-related face stimuli in particular, during an attention-shifting task in 13 “high anxious” compared to “low anxious” participants (Bar-Haim, Lamy, & Glickman, 2005). Other techniques provide convergent evidence for an association between higher anxiety and enhancements in early or automatic biases to signals of fear. Individuals with generalized anxiety show faster initial eye movements to fear-related facial expressions (Mogg, Millar, & Bradley, 2000), those with posttraumatic stress preferentially allocate their initial fixations to trauma-relevant words (Bryant et al., 1995), and trait anxiety in otherwise healthy subjects predicts amygdala activity during covert (nonconscious) perception of fearful faces (Etkin et al., 2004).

Lane and Schwartz (1987) have highlighted the importance of considering levels of awareness in integrating cognitive and neuroscientific theories of psychopathology. Manipulation of awareness provides a means to elicit preferentially automatic versus controlled processing of fear stimuli, yet ERPs have not yet been used with such a manipulation to examine the impact of trait anxiety relative to depression on biases to these stimuli. In this study, backward masking was used to define automatic (covert, nonconscious) fear processing, compared to unmasked (overt, conscious) processing. We expected covert fear to be distinguished by automatic neural biases (reflected in enhancements in early ERPs and those associated with automatic orienting), and overt fear by neural biases in controlled and sustained evaluation (reflected in enhancements in later ERPs). Drawing on behavioral findings, we expected higher trait anxiety to be associated with an augmentation of early ERP biases to covert fear. It was further expected that these biases would distinguish high anxiety not only from low anxiety but also from high trait depression, and its association with an exaggeration of later ERP biases to overt fear.

**METHODS**

**Subjects**

Subjects comprised 257 healthy individuals, recruited in collaboration with the Brain Resource International Database (www.brainresource.com; Gordon, Cooper, Rennie, Hermens, & Williams, 2005; Gordon, 2003a, 2003b). Strict and standardized exclusion criteria were history of mental illness, brain injury, neurological disorder or other serious medical condition, and history of substance abuse. Mean
age was 32.49 years (SD = 12.37 years) and sex was relatively evenly distributed (124 women, 133 men).

Participants provided written informed consent in accordance with national health and medical research council guidelines.

These participants included a large subset (n = 156) of the total of 219 subjects who took part in a previous study (Williams, Palmer, et al., 2006) which focused on the independent contrasts and source localization of ERPs to overt fear versus both happiness and neutral.

**Psychometric Assessment**

All subjects completed the Depression and Anxiety Stress Scale (DASS; Lovibond & Lovibond, 1995b) to provide a measure of trait anxiety and depression. The DASS is based on a dimensional construct of depression and anxiety. The DASS Anxiety scale correlates highly with the Beck Anxiety Inventory and assesses features which correspond to the clinical construct of anxiety disorder, whereas the Depression scale correlates highly with the Beck Depression Inventory and relates to major depressive illness (Lovibond & Lovibond, 1995a). Within its dimensional structure, the DASS yields cutoff scores to assist in the interpretation of scores within this clinical context. Participants in this study spanned the range of scores from “normal” to “severe” on each scale, but no individual scored in the extremely severe category.

**Behavioral Task**

Face stimuli for eight different individuals displaying both neutral and evoked expressions of fear were selected from a standardized set of facial emotion stimuli (Gur et al., 2002). Facial expressions of fear are one of the most salient and universally recognized signals of potential threat (Ekman, Friesen, & Ellsworth, 1972). We selected those stimuli rated independently as the most accurate representations of fear, and equated the stimuli in terms of size, gray-scale parameters, and central alignment of the face within the image (with eyes as the midpoint reference). Stimuli were presented under both covert and overt conditions, and in each condition there were eight blocks containing the eight different individuals in pseudorandom order, making a total of 128 stimuli. Half of the blocks were fear stimuli and half were neutral, such that there were 64 stimuli per expression.

In the overt condition, stimuli were presented for 500 msec, and it has been observed that even at this relatively short duration the subjective experience of the emotion may be elicited (Wild, Erb, & Bartels, 2001). The interstimulus interval was 700 msec, making a total stimulus onset asynchrony of 1200 msec.

In the covert condition, stimuli were presented in a backward masking protocol. Target stimuli (fear or neutral faces) were presented for 10 msec, followed immediately by a neutral face mask for 150 msec. The masking stimulus was superimposed over the target stimulus, but spatially offset by 1 degree in one of the four diagonals, randomly allocated. Target stimuli were synchronized with the refresh rate (100 Hz) of the stimulus presentation computer which ensured that each stimulus was presented for 1 screen refresh (10 msec) only using a customized stimulus presentation program. The interstimulus interval was 1040 msec, ensuring that the total stimulus onset asynchrony (1200 msec) was equivalent to that in the conscious condition.

Parameters used for covert presentation were verified in a series of psychophysical tasks (Williams, Liddell, et al., 2004). In these tasks, subjects indicated whether or not a face stimulus or a blank stimulus was presented prior to the immediate onset of the neutral face mask. Using signal detection criteria (Macmillan, 1986), detection accuracy was shown to be significantly above chance for stimulus durations of 30, 40, 50 msec, only marginally different to chance at 20 msec but no different from chance for 10 msec (Williams, Liddell, et al., 2004).

Conditions were presented in the order of covert followed by overt processing, following previous studies, to avoid the potentially confounding effects of supra-threshold perception on subsequent subthreshold perception (Williams, Liddell, et al., 2004; Bernat, Bunce, & Shevrin, 2001; Wong, Shevrin, & Williams, 1994). Subjects were instructed to attend to the faces in preparation for posttesting briefings, thereby ensuring active attention to the stimuli. Posttest behavioral assessment showed that participants were able to consistently distinguish fear (83%) and neutral (75%) with well above-chance accuracy, indicating that any differential effects in ERPs were unlikely to be due to visual processing difficulties.

**ERP Data Acquisition**

Participants were seated in a sound- and light-attenuated room, with temperature controlled at 24°C. They were asked to refrain from smoking and caffeine for 2 hours prior to testing, given that these factors can have an acute impact on ERPs. Data were acquired continuously at 500 Hz with skin resistance of <5 kΩ from 32 EEG channels, with four EOG channels to allow for detection of any eye movement artifacts using a Quikcap and NuAmps system according to the international 10–10 electrode system. The focal sites of interest for this study were those implicated in face processing, and established in a previous study (Williams, Palmer, et al., 2006): temporal (left, T5; right, T6), occipital (left, O1; right, O2), and medial fronto-central (Fz and Cz). Data were recorded relative to the virtual ground, and referenced off-line to linked mastoids. Eye movement correction was undertaken off-line using the procedure of Gratton, Coles, and Donchin (1983).
ERP Data Reduction and Analysis

Average ERPs were calculated for each emotion stimuli. Individual single-trial ERP epochs were filtered with a low-pass Tukey (cosine taper) filter function that attenuated frequencies above 25 Hz. Single trials were then averaged to form conventional ERPs, and peak components were identified within defined latency windows, validated by visual inspection across individual subjects for each recording site. At the temporal, occipital, and midline regions of focal interest, the following components (and latency windows) were identified: temporal (T5, T6) N80 (50–120 msec), P120 (80–180 msec poststimulus), N170 (120–220 msec), P230 (180–280 msec), and N250 (230–350 msec); occipital (O1, O2) N80 (50–120 msec), P120 (80–180 msec), N170 (120–220 msec), P230 (180–280 msec), and N250 (230–350 msec). The corresponding medial (Fz, Cz) components were the P80 (40–120 msec; concomitant of the N80), N120 (80–150 msec; concomitant of the P120), VPP (120–220 msec; concomitant of the N170), N200 (180–280 msec; concomitant of the P230), early P300 (230–330 msec; concomitant of the N250), and late P300 (300–450 msec).

ERP components were scored using a baseline to peak method, such that peak amplitude and peak latency were determined. Outliers were defined as values beyond 2.5 standard deviations from the mean, and formed only 1.5% of data. These values were excluded and replaced with age-appropriate (within 1 year) group means. The exception to this procedure was the early P500, which, by definition, is elicited less frequently than other components (Squires, Squires, & Hillyard, 1975). In this study, the early P500 was elicited for approximately 50% of stimuli, and missing values were therefore not replaced, resulting in lower degrees of freedom in focal analyses.

Repeated-measures multivariate analyses of variance (MANOVAs) within a general linear model were undertaken for the amplitude and latency of each ERP component. Within-subjects factors with two repeated measures each were condition (overt vs. covert), emotion (fear vs. neutral), and site (Fz, Cz, or T5, T6 or O1, O2). Focal effects of interest were condition and emotion main effects, and in particular, the interaction of condition and emotion. Of secondary interest, we examined instances in which condition and/or emotion varied according to site. The repeated-measures model included planned contrast to elucidate each of these effects. Given the inclusion of three regions, we used a corrected alpha level of .017. We also reported effect size (using eta-squared; $\eta^2$). The Greenhouse–Geisser epsilon method (relevant to repeated-measures models) was used to ensure that homogeneity of variance assumptions was met.

Analysis of ERPs with Anxiety and Depression Groups

We formed groups of “high anxiety” and “high depression,” defined by the top quartile of scores on the DASS Anxiety and Depression scales, respectively, following previous studies (Bar-Haim et al., 2005). “Low anxiety” and “low depression” subjects were those who scored zero on these respective scales, representing an absence of negative mood. Table 1 provides the descriptive data for combined subjects and each of these groups, relative to the DASS categories of “normal” to “extremely severe.” ANOVA confirmed that “high anxiety” differed significantly and substantially from “low anxiety” subjects on mean level of anxiety [$F(1, 187) = 1599, p < .0001$] and “high depression” differed significantly from “low depression” subjects on level of depressed mood [$F(1, 152) = 934, p < .0001$].

Consistent with the comorbidity of anxiety and depression, the correlation between DASS depression and anxiety scores in this study was .43 ($p < .0001$). Reflecting this association, the “high anxiety” and “high depression” groups also differed significantly on respective levels of depression [$F(1, 187) = 31.54, p < .0001$] and anxiety [$F(1, 152) = 45.58, p < .0001$]. Thus, analysis of covariance within a general linear model was used to compare “high anxiety” to “low anxiety” and “high depression” to “low depression,” while controlling for

<table>
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<tr>
<th>Table 1. Summary of Descriptive Data for Combined Subject Group, and “High Anxiety,” “High Depression,” “Low Anxiety,” and “Low Depression” Groups on DASS Anxiety and Depression Scales</th>
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<tr>
<td><strong>DASS Anxiety Scale</strong></td>
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<tr>
<td><strong>Mean</strong></td>
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<td>Combined subjects ($n = 257$)</td>
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<tr>
<td>“High anxiety” ($n = 31$)</td>
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<tr>
<td>“Low anxiety” ($n = 129$)</td>
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<tr>
<td>“High depression” ($n = 57$)</td>
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<td>“Low depression” ($n = 97$)</td>
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Anxiety scale categories: Normal 0–7; Mild 8–9; Moderate 10–14; Severe 15–19; Extremely severe 20+. Depression scale categories: Normal 0–9; Mild 10–13; Moderate 14–20; Severe 21–27; Extremely severe 28+. 
the respective level of depressed mood and anxiety. To determine the specificity of effects for fear, these analyses were undertaken for fear relative to a neutral baseline for both the overt and covert conditions for ERP component amplitude and latency measures. Given the different numbers in each group, Levene’s Test (relevant to ANOVA models) was applied to ensure that homogeneity of variance assumptions was met. We also used a corrected alpha level (for 3 regions) of .017 in these analyses.

In addition, we undertook a parallel set of ANOVAs which did not include a covariate, to provide complementary information on the specificity of effects due to anxiety versus depression, and as a check that covariance analysis was not introducing potentially misleading results.

**RESULTS**

The ERP waveforms elicited during conscious and non-conscious fear (versus) neutral perception are presented in Figure 1.

Focal MANOVA effects of interest were main effects for emotion and condition, and their interaction, as well as the three-way interactions between these factors and laterality. These effects are outlined, in turn, in the

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**Figure 1.** ERP waveforms elicited in response to overt fear versus neutral (indicated by red and orange, respectively) and in response to covert fear relative to neutral (indicated by dark blue and light blue, respectively), recorded over medial fronto-central (Fz and Cz), temporal (T5 and T6), and occipital (O1 and O2) sites. Sites indicated by odd numbers are located in the left side of each region, and those indicated by even numbers are located in the right side. ERP components of focal interest are indicated for each left-sided site, but the equivalent set of components were also scored for the right-sided sites. The y-axis presents microvolts and the x-axis, milliseconds.
sections below, and effects involving emotion are summarized in Table 2.

We first examined condition (covert vs. overt) and emotion (fear vs. neutral) main effects.

Main Effects for Condition

Condition effects reflecting enhanced processing in the overt relative to covert condition were revealed across regions within the first 150 msec: medial fronto-central N120 (80–150 msec) \( [F(1, 256) = 92.72, p < .0001, \eta^2 = .27] \) (Figure 1) and concomitant temporo-occipital P120 [temporal, \( F(1, 256) = 75.13, p < .0001, \eta^2 = .28 \); occipital, \( F(1, 256) = 48.72, p < .0001, \eta^2 = .16 \) (Figure 1). The later P230 (180–280 msec) was also enhanced during overt processing over the temporal region \( F(1, 256) = 107.82, p < .0001, \eta^2 = .30 \).

On the other hand, condition effects representing greater amplitude in the covert relative to overt condition were apparent for 40 msec and beyond. Over the occipital region, the N80 (40–120 msec) was enhanced during overt versus covert processing \( F(1, 256) = 10.16, p = .002, \eta^2 = .04 \). The temporo-occipital N170 (120–220 msec) was also comparatively enhanced during covert processing \( F(1, 256) = 41.46, p < .0001, \eta^2 = .14 \); occipital, \( F(1, 256) = 118.75, p < .0001, \eta^2 = .32 \), as was the concomitant medial fronto-central VPP (120–220 msec) \( [F(1, 256) = 231.13, p < .0001, \eta^2 = .47] \). The subsequent temporo-occipital N250 (230–330 msec) [temporal, \( F(1, 256) = 112.54, p < .0001, \eta^2 = .31 \); occipital, \( F(1, 256) = 113.71, p < .0001, \eta^2 = .31 \) and fronto-central late P300 (300–450 msec) \( F(1, 256) = 155.27, p < .0001, \eta^2 = .38 \) were similarly enhanced for covert relative to overt processing (Figure 1).

Condition effects for ERP latency revealed significantly faster responses to covert processing (regardless of valence) across the time course; for the N80 [temporal, \( F(1, 256) = 9.57, p = .002, \eta^2 = .04 \); occipital, \( F(1, 256) = 44.89, p < .0001, \eta^2 = .15 \) and concomitant fronto-central P80 \( [F(1, 256) = 127.60, p < .0001, \eta^2 = .33] \), P120 [temporal, \( F(1, 256) = 144.22, p < .0001, \eta^2 = .31 \); occipital, \( F(1, 256) = 182.92, p < .0001, \eta^2 = .42 \) and concomitant fronto-central N120 \( F(1, 256) = 504.04, p < .0001, \eta^2 = .66 \), N170 [temporal, \( F(1, 256) = 94.84, p < .0001, \eta^2 = .27 \); occipital, \( F(1, 256) = 104.59, p < .0001, \eta^2 = .29 \) and concomitant fronto-central VPP \( [F(1, 256) = 361.12, p < .0001, \eta^2 = .59] \) and fronto-central early P300 \( [F(1, 134) = 11.15, p = .001, \eta^2 = .08 \) and subsequent late P300 \( F(1, 256) = 136.14, p < .0001, \eta^2 = .35 \) (Figure 1).

Main Effects for Emotion

Enhanced responses to fear relative to neutral were reflected in significant emotion effects for the fronto-central VPP (120–220 msec) \( F(1, 256) = 31.10, p < .0001; \eta^2 = .11 \) (Table 2; Figures 1 and 2).

There was also a trend toward a main effect for emotion for the temporal N250 (230–350 msec) \( F(1, 256) = 4.53, p = .03, \eta^2 = .02 \) (Table 2; Figures 1 and 2), and

### Table 2. Summary of Focal MANOVA Results (\( df = 1,256 \)) for ERP Amplitude, Showing Interactions between Condition (Overt vs. Covert) and Emotion (Fear vs. Neutral) Shaded Blue and Red and Main Effects for Emotion Shaded Yellow and Green

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<thead>
<tr>
<th>Event</th>
<th>40–100 msec</th>
<th>80–180 msec</th>
<th>120–220 msec</th>
<th>180–280 msec</th>
<th>230–330 msec</th>
<th>300–450 msec</th>
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<tr>
<td>N80/P80</td>
<td>Right N120</td>
<td>Right N120</td>
<td>N170/P120</td>
<td>P120/P230</td>
<td>N250/Early P300</td>
<td>Late P300</td>
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<tr>
<th>Event</th>
<th>N120/P120</th>
<th>N170/P120</th>
<th>P230</th>
<th>Early P300</th>
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<td>Temporal</td>
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<tr>
<td>Medial fronto-central</td>
<td>VPP*</td>
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<tr>
<th>Event</th>
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<td>Covert Fear</td>
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<tr>
<td>Medial fronto-central</td>
<td>N120</td>
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Where condition and emotion also interacted with laterality, the right or left side of greatest effect is indicated.

- **Interaction:** Condition-specific increase to fear
- **Interaction & Main effect:** Common increase to fear, greater for conscious fear
- **Main effect:** Common increase to fear across conditions
- **Interaction:** Condition-specific decrease to fear
- **Interaction & Main effect:** Common decrease to fear, greater for conscious fear
- **Main effect:** Common decrease to fear across conditions

*There was a trend toward higher VPP responses to overt versus covert fear (\( p = .05 \)), in addition to the main effect for higher VPP responses to fear versus neutral across conditions.*
Condition by Emotion Interaction Effects due to Modulation by Overt Processing of Fear

ERPs indexing structural encoding and subsequent sustained evaluation were particularly enhanced for overt (but not covert) fear, relative to neutral (Table 2; Figure 2). There was a three-way interaction for the occipital N170 (120–220 msec), indexing structural face encoding, which was due to an enhancement in responses to overt (but not covert) fear, particularly over the right relative to left occipital region \([F(1, 256) = 10.38, p = .001; \eta^2 = .04]\) (Table 2; Figures 1 and 2). The corresponding temporal N170 (120–220 msec) showed similarly enhanced responses to overt (but not covert) fear versus neutral \([F(1, 256) = 6.78, p = .01; \eta^2 = .05]\) (Table 2; Figures 1 and 2).

There was a parallel trend toward a Condition by Emotion interaction for the medial VPP (120–220 msec) component of the N170/VPP complex associated with facial emotion encoding \([F(1, 256) = 3.86, p = .05; \eta^2 = .02]\) (Table 2; Figures 1 and 2). Although the VPP was enhanced for both overt and covert fear (relative to neutral), as indicated by the emotion main effects above, the enhancement was most pronounced in the overt condition (Figure 1).

The subsequent medial fronto-central early P300 (230–330 msec), reflecting automatic orienting, also showed a Condition by Emotion interaction \([F(1, 134) = 7.02, p = .01; \eta^2 = .05]\), as did the following fronto-central late P300 (300–450 msec) \([F(1, 256) = 5.98, p = .01; \eta^2 = .04]\). These interactions were due to similarly greater activity for overt (but not covert) fear versus neutral (Table 2; Figures 1 and 2).

The specific enhancement of temporal N170 and fronto-central VPP as well as early and late P300 responses to overt fear revealed by these interactions was observed against the background of generally reduced activity for these components in the overt compared to covert condition, reflected in the above condition main effects (Table 2; Figures 1 and 2).

By contrast, overt perception of fear was also associated with lower ERP activity relative to neutral, for the P120 index of early perceptual processing. The occipital P120 (80–180 msec) showed a three-way Condition by Emotion by Laterality interaction, due to particularly reduced right occipital responses to overt (but not covert) fear \([F(1, 256) = 9.37, p = .002; \eta^2 = .04]\) (Table 2; Figures 1 and 2). This interaction was observed in the context of a generally enhanced occipital P120 for overt relative to covert processing, as indicated by the above condition main effect.

Condition by Emotion Interaction Effects due to Modulation by Covert Processing of Fear

In contrast to overt fear, covert fear processing was distinguished by enhancements in early ERPs indexing initial perceptual analysis and in ERPs reflecting automatic orienting.

There was a two-way Condition by Emotion interaction for the medial N120 (80–150 msec), due to enhanced early responses to covert (but not overt) fear \([F(1, 256) = 4.99, p = .01; \eta^2 = .02]\) (Table 2; Figures 1 and 2). This interaction was observed in the context of comparatively reduced activity for covert perception, as reflected in the above main effect.

Figure 2. Statistical parameter maps illustrate the spatio-temporal distribution of neural activity for the contrast of fearful relative to neutral face stimuli for overt and covert conditions. The time course of activity is indexed by ERP components N80/P80 to the late P300, elicited in the latency windows of 40–120 msec to 300–450 msec poststimulus. The legend shows the scale of significant effects; regions colored in the red spectrum indicate an increase in activity to fear (versus neutral) at an alpha threshold of at least .01, across medial, temporal, or occipital regions, whereas those colored in the blue spectrum indicate a reduction in activity at an alpha threshold of at least .01 and green indicates no significant difference. Colors between green and red or blue indicate trend level effects at an alpha level of between .05 and .01.

Concomitant medial fronto-central early P300 \([F(1, 134) = 6.72, p = .01, \eta^2 = .05]\), reflecting higher activity for fear relative to neutral.

By contrast, the temporal P230 (180–280 msec) was reduced for fear relative to neutral across both awareness conditions \([F(1, 256) = 11.40, p = .001, \eta^2 = .04]\) (Table 2; Figures 1 and 2).
Additional Condition by Emotion interactions were observed for the N250 (230–350 msec) over both temporal \( [F(1, 256) = 17.18, p < .0001; \eta^2 = .06] \) and occipital regions \( [F(1, 256) = 16.97, p < .0001; \eta^2 = .06] \) (Table 2; Figures 1 and 2). These interactions were due to a greater enhancement for responses to covert than overt fear versus neutral. Although there was a trend main effect for emotion for the temporal N250, the interaction confirms the particularly enhanced responses for covert fear. In this case, these interactions were present in the context of generally enhanced N250 activity in the covert condition, as indicated by the above condition main effects.

**“High Anxiety” versus “Low Anxiety” Groups for Overt and Covert Processing of Fear**

ANCOVA showed that there was a distinctive profile of neural activity, indexed by ERPs, in the “high anxiety” compared to “low anxiety” group, across the time course of overt and covert fear (relative to neutral) processing.

For overt processing, “high anxiety” subjects showed faster processing of fear (relative to neutral) than “low anxiety” subjects. A comparative speeding of ERP latency in the “high anxiety” group was apparent over the temporal cortex for the N80 bilaterally \( [F(1, 157) = 4.30, p = .015, \eta^2 = .04; \text{right}, F(1, 157) = 8.43, p = .004, \eta^2 = .05], \) the P120 bilaterally \( [\text{left}, F(1, 157) = 6.49, p = .012, \eta^2 = .04; \text{right}, F(1, 157) = 7.77, p = .006, \eta^2 = .06], \) and the right-sided P230 \( [F(1, 157) = 6.25, p = .013, \eta^2 = .04] \) (Figure 3).

In the covert processing condition, the “high anxiety” group showed similarly faster ERP latency compared to the “low anxiety” group over the right temporal cortex for N80 \( [F(1, 157) = 8.74, p = .004, \eta^2 = .06] \) and N170 \( [F(1, 157) = 4.43, p = .013, \eta^2 = .04] \) (Figure 3). In addition, the “high anxiety” group showed comparatively enhanced ERP amplitude for the frontal N120 \( [F(1, 157) = 4.48, p = .016, \eta^2 = .03], \) which may have contributed to the subsequent reduction in fronto-central VPP responses \( [\text{frontal}, F(1, 157) = 4.73, p = .013, \eta^2 = .04; \text{central}, F(1, 157) = 6.61, p = .008, \eta^2 = .05] \) (Figure 3).

In these analyses, depression was a significant covariate \( (p = .015) \) only for left temporal (T6) P230 latency for covert fear. However, the relationship between depression and P230 latency was in the opposite direction to that for anxiety, such that it did not account for the anxiety effect.

**Comparison of “High Depression” and “Low Depression” Groups**

The specificity of the findings for high anxiety was further indicated by the findings for comparison of “high depression” and “low depression” groups. Higher depression was found to impact overt processing of fear (vs. neutral) only, and in an opposing direction to that for higher anxiety.

The “high depression” group was distinguished from “low depression” by a slowing of temporol-occipital responses to overt fear, reflected in comparatively slowed latency for the left occipital N170 \( [F(1, 157) = 4.72, p = .015, \eta^2 = .04] \) and N250 \( [F(1, 157) = 4.58, p = .013, \eta^2 = .05] \) and the right temporal P230 \( [F(1, 157) = 6.82, p = .010, \eta^2 = .05] \) (Figure 4).

The “high depression” group showed comparatively enhanced ERP amplitude for the frontal N120 \( [F(1, 157) = 5.59, p = .014, \eta^2 = .04], \) followed by a reduction in the fronto-central VPP \( [\text{frontal}, F(1, 157) = 5.48, p = .012, \eta^2 = .05; \text{central}, F(1, 157) = 5.30, p = .019, \eta^2 = .04] \) (Figure 4). Although this profile corresponded to that observed for “high anxiety” in terms of timing and components involved, it was apparent in the overt fear condition, whereas the changes with anxiety were apparent in the covert condition. In addition, the “high depression” group showed comparatively reduced late P300 responses to overt fear across fronto-central regions \( [\text{frontal}, F(1, 157) = 10.88, p = .001, \eta^2 = .07; \text{central}, F(1, 157) = 16.01, p < .0001, \eta^2 = .07] \) (Figure 4).

Anxiety was not found to covary significantly with these effects of “high” versus “low” depression.

**DISCUSSION**

Consistent with the view that neural systems give precedence to signals of potential danger, facial signals of fear were found to elicit increases in neural activity relative to neutral across the processing time course. The timing and topographical distribution of these enhancements differed with level of awareness. Overt fear elicited global enhancements around the period of structural and emotion encoding (120–200 msec) followed by frontal enhancements associated with orienting and sustained context evaluation (230–450 msec), whereas covert fear elicited earlier frontal increases (80–180 msec) and a more localized later increase in temporol-occipital activity associated with automatic emotion encoding (230–330 msec). Within this framework, the impact of higher anxiety on neural processing of fear, and the specificity of this impact compared to depressed mood, was examined. Higher anxiety was characterized by a speeding of neural activity early in the time course, regardless of awareness, and an early enhancement of responses to covert fear. The specificity of this profile was indicated by a slowing and enhancement of responses with higher depression, apparent only during overt fear processing.

**Time Course of Neural Activity during Overt Processing of Fear**

During overt fear processing, enhancements in neural activity to fear commenced with encoding of the structure
and emotional content of face stimuli (120–220 msec) over all regions (including temporo-occipital), and were subsequently most pronounced during the later periods of orienting and sustained contextual evaluation (230–450 msec) over fronto-central regions. This profile of neural activity to overt fear may reflect a controlled neural negativity bias toward potential threat (relative to neutral), elicited by the explicit processing of these stimuli.

In addition, localized reductions in neural activity to overt fear were observed earlier in the time course (for the occipital P120, peaking around 100 msec) as well as for the subsequent fronto-temporal-occipital N200/P230 (peaking around 230 msec). These reductions accord with previous findings (Williams, Palmer, et al., 2006; Sokolov & Boucsein, 2000; Streit et al., 2000), and might be due to the incipient onset of the N170 and its subsequent offset. The N170 is a key index of structural encoding of face stimuli and is robustly elicited by these stimuli (Williams, Palmer, et al., 2006; Streit et al., 2000). Although previous studies have also revealed localized increases to overt fear within the first 120 msec (Williams, Palmer, et al., 2006), their absence in this study suggests that they are not reliably elicited.
Time Course of Neural Activity during Covert Processing of Fear

By contrast, covert fear elicited increases in activity prior to structural encoding over frontal regions for the N120 component (80–180 msec), consistent with previous evidence (Liddell et al., 2004, under review). Covert fear also elicited enhancements in the frontal VPP relative to neutral around 120–220 msec, although this enhancement was not as great as that observed for overt fear, feasibly due to the absence of modulation from conscious attention. Importantly, there was an absence of the temporo-occipital N170 effect seen for overt fear over this period, consistent with a lack of explicit encoding of stimulus features for covert fear. Yet, there was a later enhancement of neural activity to covert fear across these temporo-occipital regions, in this case, associated with automatic emotion encoding (230–330 msec).

This profile of neural activity suggests that implicit processing of fear elicits an automatic neural negativity bias.
which arises earlier in the processing sequence than for explicit processing. The distinctive spatio-temporal pattern of neural responses to overt versus covert fear accords with functional neuroimaging evidence that parallel neural pathways support threat processing at different levels of awareness (Williams et al., 2006; Morris et al., 1999). Indeed, there were generally faster responses across the time course of covert compared to overt processing, consistent with a direct pathway for relaying sensory input in the absence of awareness. In contrast to the relatively substantial effect sizes (50–60%) for such condition main effects, the effects differentiating overt versus covert processing according to fear versus neutral were subtle (around 5%), highlighting the value of larger subject numbers to elucidate such effects.

Neural Negativity Biases? Impact of Higher Anxiety on Neural Activity during Overt and Covert Processing of Fear

The distinct time course of neural activity to overt versus covert fear provided a pertinent framework for understanding the impact anxiety on this time course, and the specificity of this impact compared to depressed mood. Moderate correlations between anxiety and depression in this study accord with previous findings, yet it has been noted that anxiety, nonetheless, represents a separate construct from depression in factor analytic studies of otherwise healthy individuals (Lovibond & Lovibond, 1995a). Features of anxiety indexed by the DASS used in this study reflect acute fear responses and their link with a relatively enduring state of anxiety. Depression, on the other hand, is thought to involve a more chronic and generalized sensitization of responses to potential threat (Lovibond & Lovibond, 1995a).

“High anxiety” subjects according to the DASS were distinguished by a speeding of neural responses over the temporal cortex to both overt and covert fear (vs. neutral), relative to “low anxiety” subjects (Figure 3). For overt fear, this speeding of responses was apparent bilaterally from as early as 40 msec post-stimulus (for the N80 and P120 components), and was additionally apparent over the right temporal region for 230–330 msec (P230 component). During covert fear processing, the speeding of the N80 in the “high anxiety” group was similarly apparent over the right temporal region, as was a speeding of the subsequent N170 in the period 120–220 msec. In the covert fear condition, “high anxiety” subjects were also distinguished by an exacerbation of the frontal enhancement (80–180 msec, N120 component) observed for covert fear processing in the previous section (Time Course of Neural Activity during Covert Processing of Fear), which may indicate automatic hypervigilance to threat in these subjects (Figure 3). The exacerbation of this component may account for the subsequent attenuation of fronto-central VPP responses (120–220 msec) in the “high anxiety” relative to “low anxiety” group, suggesting that hypervigilance may lead to deficits in emotion encoding.

Together, these findings point to an exaggerated neural bias toward potential threat with higher anxiety, which operates automatically. Notably, the effect sizes of the results for “high” versus “low” anxiety were small, indicating that subclinical anxiety has a subtle impact on neural fear processing, which might be detected only with relatively large subject numbers. This new evidence for a neural negativity bias in anxiety is compatible with previous behavioral evidence of an excessive bias toward threat that is present only under conditions of automatic processing (Bradley et al., 1995; Mogg et al., 1993, 1995). It also accords with functional neuroimaging evidence for enhanced temporal cortex amygdala activity with higher trait anxiety during covert processing of fear (Etkin et al., 2004), and for a hyperresponsive medial prefrontal cortex (including anterior cingulate) and amygdala during covert fear processing in the anxiety disorder of posttraumatic stress disorder (Bryant et al., 2007; Williams, Kemp, et al., 2006). Both behavioral and brain function markers may reflect aspects of hypervigilance in anxiety, which occurs at a physiological level that is beyond conscious control. Without control, an ongoing cycle of vigilance may contribute to the development and maintenance of the symptoms of anxiety disorder as well as interfere with the ability to effectively encode the emotional content of stimuli.

Specificity of the Impact of Higher Anxiety: A Comparison with the Impact of Higher Depression on Neural Activity during Overt and Covert Processing of Fear

The specificity of these findings was indicated by the presence of an opposing pattern of results with higher depression, which were apparent only for overt fear processing. In “high depression” relative to “low depression” subjects, neural responses were slowed (rather than speeded) over temporo-occipital regions. Slowed responses to overt fear were apparent later in the time course, from structural encoding and beyond (120–330 msec). A pattern of enhanced early responses to fear (80–180 msec) over the frontal region, followed by a deficit in emotion encoding, was also apparent for “high depression” subjects, but this pattern was present for overt fear processing, and not for covert processing as it was for higher anxiety. Moreover, higher depression was also associated with a marked attention of the fronto-central late P300 to overt fear, suggesting additional deficits in sustained context evaluation.

These findings provide evidence of a distinctive neural negativity bias in depression, which may prolong orienting to and evaluation of signals of potential threat when controlled processing is engaged. This proposal accords
with evidence for impaired orienting and impaired frontal P150 during working memory updating in a large investigation of subclinical depression (Kemp et al., 2005). Moreover, models of depression based on functional neuroimaging evidence also highlight the role of the medial prefrontal regions in poor control of emotion (e.g., Mayberg, 1997). The findings are also consistent with the suggestion from behavioral studies that depression impacts the bias toward fear only when sustained processing is engaged (Mathews et al., 1996; Bradley et al., 1995; Mogg et al., 1993, 1995), as distinct from the presence of alterations to covert fear processing with higher anxiety.

**Conclusion and Future Research**

Findings from this study indicate that trait anxiety is distinguished by a speeding of neural activity from the earliest phase of both overt and covert fear processing, in contrast to a more localized slowing of activity during overt fear processing, commencing later in the time course, for depression. In addition, trait anxiety was found to enhance initial orienting to covert fear, compared to an enhancement to overt fear for depression, which in both cases may disrupt subsequent emotion encoding. These findings accord with our integrative neuroscience model of emotion processing, in which the mechanisms of anxiety involve an excessive automatic bias, associated with hypervigilance, to sources of potential fear, whereas depression reflects the chronic result of hypervigilance in which a controlled evaluation of fear stimuli is protracted (Williams, 2006).

These findings also point to several areas of future investigation. Because the present results refer to normal variations in anxiety and depression, it would be important to determine whether these effects are present (or even more pronounced) in clinically diagnosed individuals. A related issue is whether emotion-related neural activity is modulated in similar ways by state as well as trait variations in mood. ERP studies using mood induction suggest that state changes in mood bias facial emotion processing within the first 500 msec in ways that may be similar to those for trait aspects of anxiety and depression (Gotlib, Yue, & Joormann, 2005; Esslen, Pascual-Marqui, Hell, Kochi, & Lehman, 2004; Joormann, 2004). A third issue is the question of whether altered biases in anxiety generalize to other emotionally salient stimuli, given evidence for biases to anger and significant stimuli which are not explicitly threat-related in posttrauma anxiety conditions (Bryant et al., 2005; Felmingham, Bryant, & Gordon, 2005). Investigation of the role of antidepressants in normalizing neural negativity biases is also warranted, given that serotonin modulates the neural time course of negative emotion processing (Kemp, Gray, Line, Silberstein, & Nathan, 2003).

**Notes**

1. The effects for higher anxiety were also largely confirmed in parallel ANOVAs without level of depression as a covariate, providing complementary information to indicate that variation in levels of depression did not account for the effects in “high” compared to “low” anxiety. In these analyses, significance was slightly higher in some cases (covert P120 latency at T5) and slightly lower in others (N80 latency at T5 for both covert and overt conditions), but in the remainder it remained equivalent to ANCOVA results.

2. Similarly, results from the ANOVAs (without anxiety as a covariate) paralleled those for the ANCOVAs, with only slight variations in level of significance for two components (slightly more significant for overt N250 latency at O1, slightly less for overt P250 latency at T6), and equivalent levels of significance for the other components.

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