Archival Report

The Startle-Evoked Potential: Negative Affect and Severity of Pathology in Anxiety/Mood Disorders

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ABSTRACT

BACKGROUND: The National Institute of Mental Health Research Domain Criteria initiative encourages a search for dimensional biological measures of psychopathology unconstrained by current diagnostic categories. Consistent with this aim, the presented research studies a large sample of anxiety and mood disorder patients, assessing differences in principal diagnoses and comorbidity patterns, clinicians' ratings, and questionnaire measures of negative affect and life dysfunction as they relate to a potential brain marker of pathology: the amplitude of the event-related potential (ERP) elicited by a startle-evoking stimulus.

METHODS: Patients seeking evaluation or treatment for anxiety and mood disorders (N = 208) participated in two tasks at the University of Florida (Gainesville, FL): 1) imagining emotional and neutral events and 2) viewing emotional and neutral pictures while acoustic startle probes were presented and the ERP was recorded. For a comparison patient group (N = 120), startle probes were administered and ERPs recorded at the University of Greifswald (Greifswald, Germany) while performing the same imagery task.

RESULTS: Reduced positive amplitude of a centroparietal startle-evoked ERP (156–352 ms after onset) significantly predicted higher questionnaire scores of anxiety/depression, reports of increased life dysfunction, greater comorbidity, and clinician ratings of heightened severity and poorer prognosis. The effect was general across principal diagnoses, found for both the Florida and German samples, and consistent in pattern despite differences in the tasks administered.

CONCLUSIONS: The startle-evoked ERP reliably predicts severity and breadth of psychopathology, independent of task context. It is a potential significant contributor to a needed array of biological measures that might improve classification of anxiety and mood disorders.

Keywords: Anxiety disorders, Comorbidity, ERP, Mood disorders, RDoC, Startle reflex

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This investigation is part of an ongoing multimeasure study of anxiety and mood disorders. The broad research aim is to test biological measures that might objectively discriminate levels of dysfunction across this highly comorbid patient population. This effort is consonant with the National Institute of Mental Health Research Domain Criteria goal to develop measure clusters that could collectively define patient groups with a common physiology of distress, potentially providing more specific biological targets for treatment interventions than current diagnostic systems.

Previous studies have reported reduced P3 amplitudes over centroparietal electrodes in a wide range of mental disorders, prompting the hypothesis that this blunted response is a general factor, signaling cognitive dysfunction that is widespread and nonspecific to diagnostic categories. Thus, the current research assesses the event-related potential (ERP) evoked by an acoustic startle probe in a large sample of patients, testing the proposition that a diminished startle ERP reliably predicts severity and breadth of anxiety/mood pathology, measured here by heightened negative affectivity on a battery of questionnaires, incidence of comorbidity, clinician evaluations of disorder severity, treatment prognosis, and reported life dysfunction.

The ERP response to an auditory cue includes positive-going components that peak between 250 and 500 ms after presentation (e.g., P2, P3a, and P3b; or globally, the P3), most evident over centroparietal sensors (1–3). Research with healthy participants suggests that the P3 has good retest reliability (4) and both topographical and temporal stability (5). The majority of ERP research with patient populations has studied "oddball" tasks (6), in which the same cue is presented over multiple trials and occasionally a tone of a different frequency occurs. The novel, oddball tone yields a significantly larger P3 than the standard stimuli. Variations in the task often include an overt response (button press to a target cue), distracter stimuli, manipulation of cue expectancy, or differences in cue complexity and discrimination difficulty. Previous patient studies using the ERP oddball task found significant P3 amplitude reduction (relative to controls) and sometimes increased latency of response in a wide range of mental disorders (7–9),

including schizophrenia (10–12), risk for psychosis (13), depression and anxiety (14–16), Alzheimer's disease (17), alcoholism (18,19), and externalizing psychopathology (20,21).

A recent opinion piece considering the assessment of depression (22) lamented the fact that P3 measurement is not more frequently used as a tool in determining diagnosis and prognosis and in assessing treatment response. Achieving this laudable aim is difficult in consideration of our still limited understanding of the neural foundation of the P3 (8,23) and, importantly, the fact that the oddball paradigm used for its evocation with clinical populations varies widely across experiments, e.g., various sensory stimuli are used, working memory is often engaged, a response may or may not be required, and there may be different target cues or an additional novel, unanticipated stimulus. It is clear that measurement would be easier to implement in a clinical assessment context if no instructed task was required.

The Research Paradigm

The following research explores a method for evoking an ERP that appears to be unrelated to instructed expectations, response requirements, novelty, or target appearance within a stimulus series. The stimulus is a brief, intense noise burst (instantaneous rise time) that reliably prompts a startle response in humans, a reflexive reaction to survival threat in many organisms (24). Considering basic startle research suggesting that the centroparietal P3 component is an index of defensive activation (25,26), the current study examines this measure as it varies in a large sample of patients seeking treatment for anxiety and depression.

The broad aim is to determine whether the startle-evoked ERP varies systematically with questionnaire measures of psychopathology, differences in principal diagnosis, incidence of comorbidity, severity of disorder, and reported life dysfunction as these measures are assessed in a large sample of patients diagnosed with principal anxiety and mood disorders. In the primary University of Florida (Gainesville, FL) sample, we assessed the impact of context: startle probes are presented during two different tasks, instructed imagery and picture viewing, as well as during no-task interstimulus intervals. We first assessed the relationship between startle ERP amplitude and pathology-related dependent variables in the overall sample, and separately for men and women. Second, based on the project's ultimate research aim, which is to develop biologically based classifiers of disorder (27), we explored differences among five patient subgroups of increasing startle ERP amplitude, based on a quintile division of the entire sample. Third, we examined the ERP's relation to the blink reflex and to a reaction time response to the startle probe. Finally, we assessed the relationship between startle ERP amplitude and psychopathology in a replication test sample of patients studied at the University of Greifswald (Greifswald, Germany).

METHODS AND MATERIALS

Participants

The primary research sample included 235 participants (208 patients evaluated for treatment and 27 healthy control subjects) seen at the University of Florida Fear and Anxiety Disorders Clinic who were recruited for a multi-session, multitask study of anxiety, mood, and related functional disorders (Table 1). The University of Florida Institutional Review Board approved the research, and all participants consented. A second comparison sample was composed of 120 institutional review board–consented patients (Table 1) at the Psychology Clinic at the University of Greifswald.

Diagnostic Classification, Clinicians' Ratings, and Questionnaire Assessment

DSM-IV diagnoses were established in a structured interview (Anxiety Disorders Interview Schedule IV) (28) assessing current anxiety, mood, substance use, and somatoform disorders, and screening out psychosis and major physical disease. In the case of multiple disorders, diagnostic primacy was determined by clinician-rated severity [Clinical Global Impressions Scale (29,30)] (ranging from 1 [no features present] to 7 [diagnosis present; severe]), reflecting both distress and interference. Before the clinical interview/research day, participants completed a series of widely used psychopathology questionnaires, measuring anxiety, depression, life dysfunction, and trauma history, including the Beck Depression Inventory-II (BDI-II) (31), the State-Trait Anxiety Inventory (STAI) (32), the Illness Intrusiveness Rating Scale (IIRS) (33), and the Mood and Anxiety Symptom Questionnaire (MASQ) (34), which has three subscales measuring general distress specific to depressive, anxious, or mixed symptoms, and two subscales measuring anxious arousal and anhedonic depression. On the research day, patients also completed the state version of the STAI (32) and the Subjective Units of Distress Scale (35).

Table 1. Diagnostic and Demographic Information for Clinical Patients in the Samples From Gainesville, Florida, and Greifswald, Germany

Principal Diagnosis	Gainesville, FL			Greifswald, Germany		
	n	% Female	Age, Year	n	% Female	Age, Year
Social Phobia	47	40 (0.07)	30.0 (1.8)	25	60 (0.10)	29.6 (1.9)
Panic Disorder[a]	26	62 (0.10)	35.0 (2.4)	28	75 (0.00)	39.8 (2.8)
Mood Disorder[b]	16	63 (0.13)	40.0 (3.8)	35	57 (0.08)	38.5 (2.4)
GAD	36	78 (0.07)	31.3 (1.8)	7	57 (0.20)	35.0 (5.4)
PTSD	24	92 (0.06)	37.2 (2.6)	5	80 (0.20)	41.6 (7.8)
Specific Phobia	17	70 (0.15)	45.0 (4.0)	9	100 (–)	37.7 (3.8)
OCD	17	47 (0.12)	27.9 (2.4)	4	75 (0.25)	38.5 (5.7)
Adjustment Disorder	10	82 (0.10)	29.7 (3.0)	5	80 (0.20)	43.6 (7.8)
Other Anxiety[c]	15	47 (0.13)	33.4 (3.7)	2	50 (0.50)	49.5 (0.5)
All Patients	208	63 (0.03)	33 (0.9)	120	68 (0.04)	37.2 (1.2)

Values in parentheses are SD.

GAD, generalized anxiety disorder; OCD, obsessive-compulsive disorder; PTSD, posttraumatic stress disorder.

[a]Includes patients with and without agoraphobia.

[b]Includes patients diagnosed with major depressive disorder and dysthymia.

[c]Includes anxiety not otherwise specified (n = 15) (Florida) and hypochondriasis (n = 2) (Germany).

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Procedure

Imagery Task. Participants were seated in a quiet, dimly lit room and electrodes were attached. Participants wore headphones for communication during the procedure, and participants were told "From time to time, you may hear brief noises over the headphones. You can ignore these." As in previous studies (36–38), participants were instructed to vividly imagine various scenarios, presented here as text on a computer screen, describing emotional (e.g., threat, panic, positive, or erotic) and quotidian neutral events. Each of 32 imagery trials consisted of a 3-second baseline, a 9-second script read period, a 12-second period to imagine the scene, and an intertrial interval (ITI) ranging from 12 to 14 seconds. As in research by McTeague et al. (39), one or two startle probes were presented on each trial during imagery either at 2.5, 4.5, 8.5, or 10.5 seconds after script onset (48 probes in total), and at 8 seconds after offset of the imagery period on 16 ITIs.

Picture Viewing Task. As in research by Wangelin et al. (40), 54 pictures were presented—18 pleasant, neutral, or unpleasant scenes from the International Affective Picture System (41) and 54 pictures of 18 angry, neutral, or happy faces from the Karolinska Directed Emotional Faces set (42). The picture viewing task (188 participants) occurred postimagery, after a rest interval. Pictures were presented for 3 seconds, followed by an ITI ranging from 5 to 10 seconds in duration. Startle probes were presented during every picture at 250, 750, or 2500 ms after picture onset. In total, 108 probes were presented during picture viewing and 18 during the ITI (5 seconds after picture offset). Participants were instructed to make a rapid button-press whenever a startle probe was delivered.

Data Collection and Reduction

In Florida, electroencephalograms were recorded at 250 Hz (0.01–100 Hz online filter; 3-dB cutoff) using a 129-sensor Electrical Geodesics, Inc., system (Eugene, OR) with a vertex reference and reduced offline using Electromagnetic Encaphalography Software (43), which included 20-Hz low-pass filtering, artifact correction based on statistical parameters (44), conversion to the average reference, and a 100-ms preprobe baseline correction. In Germany, electroencephalograms were recorded at 250 Hz (0.16–35 Hz bandpass filter; 3-dB cutoff) using an Isolated Bioelectric AC/DC Amplifier (San Diego Instruments, San Diego, CA) and reduced offline for three midline electrodes (Fz, Cz, and Pz) with a linked-ear reference using BrainVision Analyzer 2.0 software (Brain Products GmbH, Gilching, Germany) with artifact detection (visual inspection; range > 100 μv), and a 100-ms preprobe baseline correction.

The startle probe was a 50-ms, 96-dB white noise probe (instantaneous rise-time), and the blink reflex was recorded at 1000 Hz beginning 50 ms before to 300 ms after probe onset from two electrodes placed over the left orbicularis oculi muscle. The raw signal was bandpass filtered (90–250 Hz), amplified, rectified, and integrated (20-ms time constant) online (Coulbourn Instruments, Whitehall, PA). A computer running VPM Software (45) controlled physiological data recording and offline scoring of blinks in a 20- to 150-ms window after probe onset.

Data Analysis

Figure 1 illustrates the ERP prompted by an acoustic startle probe for the University of Florida sample. As in previous research (23), the predominant ERP feature is a continuous positive waveform, maximal over the centroparietal cortex (top right) that begins around 156 ms after probe onset and continues until about 352 ms after probe onset (see description of ERP components, time window selection, and Supplemental Table S1). Thus, ERP amplitude in this window was averaged for the illustrated sensors (Figure 1, bottom right) to define the startle ERP in subsequent analyses.
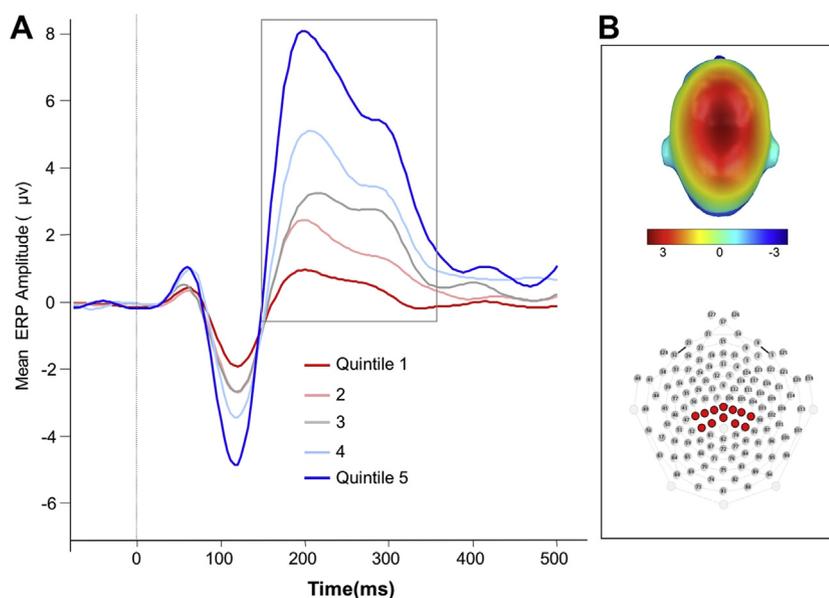

Figure 1. The event-related potential (ERP) to a startling probe is illustrated for patients divided into five quintiles based on increasing startle ERP amplitude. Following a 100-ms baseline preceding stimulus onset (gray line beginning at 0), a dominant positive going waveform (gray box) has a centroparietal maximal topography (top right) and the ERP was averaged in a window from 152 to 356 ms over a group of 12 centroparietal sensors (bottom right).

In the primary multivariate analysis of variance (MANOVA), startle ERP amplitude served as a between-subject independent variable and questionnaire (e.g., BDI-II, STAI) as a repeated factor in analysis of the standardized z scores (computed for each questionnaire across participants). In a separate analysis, significant main effects of age and gender were found for startle ERP amplitude itself, prompting follow-up analyses using residual startle ERP amplitude (following covariance using age and gender) as the independent variable in the same MANOVA, as well as separate MANOVAs for men and women assessing the relationship of startle ERP amplitude to questionnaire scores.

The 208 University of Florida patients were then rank ordered and divided into quintile groups of increasing ERP amplitude similar in number (Figure 1) (n = 41, 42, 42, 42, and 41, respectively) to DSM-IV patient groups previously studied (36). Bivariate correlations and linear trend tests using quintile as a group factor assessed questionnaire scores and clinical ratings as they varied over startle ERP quintile. In addition, ERP differences were tested between the healthy control subject sample and each patient quintile. Effects of medication on startle ERP amplitude were not found (Supplement).

RESULTS

Imagery Task

Startle ERP amplitude strongly predicted participants' overall responses on the questionnaire battery (MANOVA main effect $F_{1,200}$ = 13.9, p = .0003), with highly significant effects of startle ERP amplitude found for each measure of psychopathology (BDI-II [$F_{1,206}$ = 22.5, $p <$ .0001], STAI [$F_{1,206}$ = 10.1, p = .002], and the MASQ subscales of anxious arousal [$F_{1,206}$ = 16.3, $p <$ .0001], anhedonic depression [$F_{1,206}$ = 9.9, p = .002], general distress mixed [$F_{1,206}$ = 14.2, p = .0002], general stress depressive [$F_{1,206}$ = 7.1, p = .008], and general distress anxious

[$F_{1,206}$ = 5.5, p = .02]) as well as for the life dysfunction measure IIRS ($F_{1,206}$ = 17.5, $p <$ .0001). Age and gender differences on questionnaire scores were modest and did not change the relationship between startle ERP amplitude and questionnaires scores (Supplement).

In Figure 2, the sample is divided into quintiles of increasing ERP amplitude, showing that questionnaire scores are highest for patients with the smallest ERP amplitude (quintile 1), with the least reported distress for patients in quintile 5. The bivariate correlations between the mean startle ERP at each quintile and questionnaire score were high for each measure (Table 2), and linear trend tests using quintile as an independent group factor were highly significant for each questionnaire ($F_{1,206}$ = 5.5–21.5, p values ranging from $<$ .0001–.02). When scores were averaged over all of the questionnaires to define a measure of general negative affect, there was a reliable pattern of increasing psychopathology as startle ERP amplitude decreases (Figure 2, inset).

A separate ANOVA on startle ERP amplitude itself, however, showed effects of both age ($F_{1,204}$ = 9.1, p = .0003) and gender ($F_{1,204}$ = 34.8, $p <$ .0001), with smaller ERPs for women compared with men and larger ERPs for younger compared with older patients. To ascertain that the significant relationship between startle ERP amplitude and questionnaire scores were not simply related to differences in age or gender, a follow-up MANOVA used residual ERP amplitude (following covariance removing effects of age and gender) in the analysis of the questionnaire battery, and continued to find a significant main effect of startle ERP amplitude on questionnaire scores ($F_{1,200}$ = 14.5, $p <$ .0001) and similar bivariate correlations (Table 2).

To further assess possible gender differences, separate MANOVAs were conducted for men and women, with startle ERP amplitude continuing to significantly relate to scores on the questionnaire battery for men ($F_{1,73}$ = 10.7, p = .001) and women ($F_{1,127}$ = 4.5, p = .04). In addition, when quintiles were
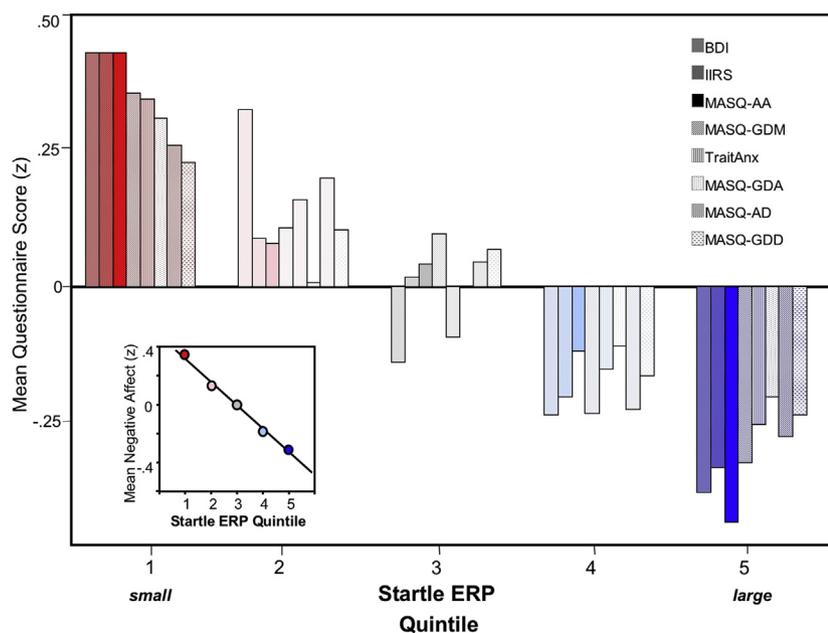

Figure 2. Mean (standardized) questionnaire scores measuring negative affect are largest for patients with the smallest startle event-related potential (ERP) amplitude (first quintile) and decrease as startle ERP amplitude increases. The questionnaire measures included the Beck Depression Inventory (BDI), the State-Trait Anxiety Inventory (TraitAnx), the Illness Intrusiveness Rating Scale (IIRS), and five Mood and Anxiety Symptom Questionnaire (MASQ) subscales measuring general distress specific to depressive symptoms (GDD), anxious symptoms (GDA), or mixed symptoms (GDM) as well as anxious arousal (AA) and anhedonic depression (AD). Higher scores reflect greater levels of symptomology (46). Inset: Mean (standardized) negative affect score (averaged over all questionnaires) shows a strong inverse relationship with startle ERP quintile.

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Table 2. Bivariate Correlations Between Mean Startle ERP Amplitude at Each Quintile and Trait and State Measures of Negative Affect and Clinician Ratings for Startle ERPs Measured During Imagery or Picture Viewing and for Residual Startle Amplitude Following an Analysis in Which Gender and Age Served as Covariates

| | Correlation With Startle ERP at Each Quintile | | | | | | Mean Score | |
| | All Patients | | | | Women Img | Men Img | | |
	Img	Resid Img	PicView	Resid Pic			Women	Men
Trait Measures of Negative Affect								
Overall negative affect[a]	−0.96	−0.96	−0.96	−0.96	−0.86	−0.93	−0.14 (0.8)	0.08 (0.8)
Beck Depression Inventory-II	−0.91	−0.92	−0.91	−0.91	−0.94	−0.90	20.7 (0.9)	17.2 (1.2)
IIRS	−0.94	−0.94	−0.95	−0.95	−0.82	−0.85	53.0 (1.8)	46.7 (2.0)
MASQ subscale								
AA	−0.98	−0.98	−0.91	−0.90	−0.85	−0.85	32.0 (1.1)	28.5 (1.1)
AD	−0.94	−0.94	−0.96	−0.95	−0.98	−0.92	71.4 (1.5)	73.3 (1.8)
GDA	−0.90	−0.90	−0.90	−0.88	−0.38	−0.70	27.8 (0.8)	24.7 (0.9)
GDD	−0.96	−0.96	−0.91	−0.99	−0.92	−0.79	33.0 (1.0)	31.6 (1.3)
GDM	−0.95	−0.95	−0.88	−0.87	−0.83	−0.95	42.3 (1.1)	37.3 (1.3)
STAI-Trait Anxiety	−0.92	−0.92	−0.95	−0.94	−0.90	−0.79	53.8 (0.9)	52.8 (1.4)
State Measures of Distress								
STAI-State Anxiety	−0.91	−0.92	−0.91	−0.91	−0.95	−0.83	50.6 (1.0)	47.1 (1.5)
Distress rating (0,10,…100)	−0.94	−0.94	−0.95	−0.95	−0.96	−0.58	26.5 (1.5)	21.6 (1.9)
Clinician Ratings								
Prognosis (1–7)	−0.83	−0.82	−0.89	−0.89	−0.90	−0.64	4.9 (0.1)	4.7 (0.1)
Global impression (1–7)	−0.96	−0.96	−0.92	−0.92	−0.93	−0.82	2.9 (0.1)	2.7 (0.2)

Bivariate correlations are also listed in which the ERP amplitude quintile was determined separately for men and women for startle probes presented during imagery. Mean raw scores on each measure are also listed for men and women.

AA, anxious arousal; AD, anhedonic depression; ERP, event-related potential; GDA, general distress anxiety; GDD, general distress depression; GDM, general distress mixed; Img, imagery; IIRS, Illness Intrusiveness Rating Scale; MASQ, Mood and Anxiety Symptom Questionnaire; PicView, picture viewing; Resid Img, residual analysis, imagery; Resid Pic, residual analysis, picture viewing; STAI, State-Trait Anxiety Inventory.

[a]Mean z score across trait measures of negative affect.

formed separately for men and women, the bivariate correlations (Table 2) showed the same relationship with questionnaire scores increasing as startle ERP amplitude decreased.

Intertrial ERP Probes. Startle ERP amplitude for probes delivered during active imagery and probes delivered during the ITI (i.e., in the absence of the imagery task) were highly correlated ($r = .89$), and a significant main effect of startle ERP amplitude during the ITIs was again found for scores on the questionnaire battery in both the raw ($F_{1,200} = 11.2$, $p = .001$) and residual ($F_{1,200} = 11.6$, $p = .008$) analyses. Because the identical pattern of high questionnaire scores and smaller startle ERP amplitude was found for ITI probes, the startle ERP/psychopathology covariation is not dependent on the task.

Startle Reflex Blink. Consistent with previous data (25), startle ERP amplitude and the magnitude of the blink response did not significantly covary, and blink magnitude was not significant in a MANOVA using questionnaire scores ($F_{1,200} < 1$).

Picture Viewing Task

The amplitude of the startle ERP during picture viewing was again significant in analysis of questionnaire scores, using either raw ($F_{1,180} = 13.5$, $p = .0003$) or residual (age and gender as covariates) ERP amplitude as the independent variable ($F_{1,180} = 14.4$, $p = .0001$). Paralleling imagery, there were significant effects of startle ERP amplitude for most of the

psychopathology measures (BDI-II [$F_{1,188} = 21.9$, $p < .0001$], STAI [$F_{1,188} = 14.3$, $p = .0002$], and the MASQ subscales of anxious arousal [$F_{1,188} = 6.5$, $p = .01$], anhedonic depression [$F_{1,188} = 14.9$, $p = .0002$], general distress mixed [$F_{1,188} = 10.7$, $p = .001$], general stress depressive [$F_{1,188} = 10.3$, $p = .002$], general distress anxious [$F_{1,188} = 1.4$, not significant], and IIRS [$F_{1,188} = 4.0$, $p = .046$]).

Bivariate correlations (Table 2) again indicated that increased psychopathology was strongly associated with reduced startle ERPs. Furthermore, startle ERPs collected between picture presentations (during the ITIs) similarly predicted questionnaire responses (MANOVA $F_{1,180} = 12.2$, $p = .0006$, residual MANOVA $F_{1,180} = 12.9$, $p = .0004$) (Table 2).

Reaction Time. During the picture viewing session, participants were instructed to press a response key as fast as possible whenever a startle stimulus was presented, both during the task and during the ITI. Startle ERP amplitude was significantly related to reaction time ($F_{1,178} = 5.9$, $p = .0001$), with longer reaction time latency as ERP amplitude decreased. However, reaction time latency did not predict scores on the questionnaire battery in a subsequent MANOVA ($p = .21$, not significant).

Clinical Diagnosis, Assessed Severity, State Distress, and Control Subjects

Principal Diagnosis. Overall, patients with different principal DSM-IV diagnoses varied widely in startle ERP amplitude.

A median split dividing patients into those with smaller or larger ERPs, however, suggested that the proportion of patients in each subgroup varied by clinical diagnosis (Figure 3). Thus, four diagnostic groups tended to show predominantly larger ERPs (specific phobia, adjustment disorder, not otherwise specified, and obsessive-compulsive disorder) and four diagnostic groups showed predominantly small ERPs (panic, depression, posttraumatic stress disorder, and generalized anxiety disorder). χ^2 analysis confirmed a significant difference in these frequencies ($\chi^2_7 = 14.8$, $p < .05$), suggesting that reduced ERP amplitude is more prevalent among the anxious misery diagnoses (46–48).

Comorbidity. The incidence of comorbidity increased significantly as ERP amplitude decreased ($\chi^2_4 = 13.4$, $p = .001$) (Supplemental Figure S1). Those with the smallest ERP amplitude (quintile 1) had the greatest number of comorbid disorders, significantly greater than patients with the largest ERP amplitude in the fourth ($p = .006$) and fifth ($p = .001$) quintiles. The incidence of secondary diagnoses is reported in Supplemental Table S2.

State Measures and Clinicians' Evaluation. In addition to trait measures of psychopathology, startle ERP amplitude significantly predicted responses on state measures of the patients' distress on the day of visit, including the STAI questionnaire ($F_{1,206} = 22.3$, $p = .0001$) and the Subjective Units of Distress Scale ($F_{1,206} = 11.0$, $p < .001$), with scores again increasing as ERP amplitude decreased (Table 2). Similarly, reduced ERP amplitude predicted clinicians' judgments of poorer prognosis ($F_{1,198} = 7.8$, $p = .006$) (8 participants missing data) and greater severity of disorder (Clinical Global Impressions Scale [$F_{1,204} = 12.4$, $p < .0005$]) made after the clinical interview (Table 2 and Supplemental Figure S2).

Healthy Control Subjects vs. Diagnosed Patients. As expected, control participants had markedly lower psychopathology scores than patients, differing significantly from patients at each quintile on all questionnaire measures (all p values < .005). As Figure 4 illustrates, startle ERPs for the control participants fell midway between the third and fourth quintiles and did not differ significantly from these adjacent patient groups. Startle ERPs for healthy control subjects, however, were markedly larger than for the patients in the first ($F_{1,66} = 53.2$, $p < .0001$) and second ($F_{1,67} = 16.7$, $p < .0001$) quintiles and, in contrast, dramatically smaller than for the patients in the fifth quintile ($F_{1,66} = 51.9$, $p < .0001$).

Comparison Sample: Germany

The 120 patients at the University of Greifswald participated in the identical imagery task, responding to the same text prompts (in German), in the same temporal sequence, with recording of startle probe ERP amplitude during the imagery and ITI periods. The distribution of anxiety diagnoses generally paralleled the University of Florida sample (Table 1).

The startle ERP was averaged over central and parietal sensors, showing similar positive components as the University of Florida sample (Supplemental Figure S2). In consideration of the smaller sample size, a median split divided participants into groups based on small and large ERP amplitude (mean 156–352 ms post–startle onset). The questionnaire battery again included the MASQ subscales, the BDI-II, the STAI, and the IIRS. The primary MANOVA assessing probe P3 amplitude and the questionnaire battery was consistent in finding that patients with smaller probe P3 amplitude reported greater overall negative affect ($F_{1,118} = 2.9$, $p = .045$ [one-tailed]), which was more pronounced when the MASQ subscales alone were assessed ($F_{1,118} = 4.5$, $p = .036$) (Supplemental Figure S2).
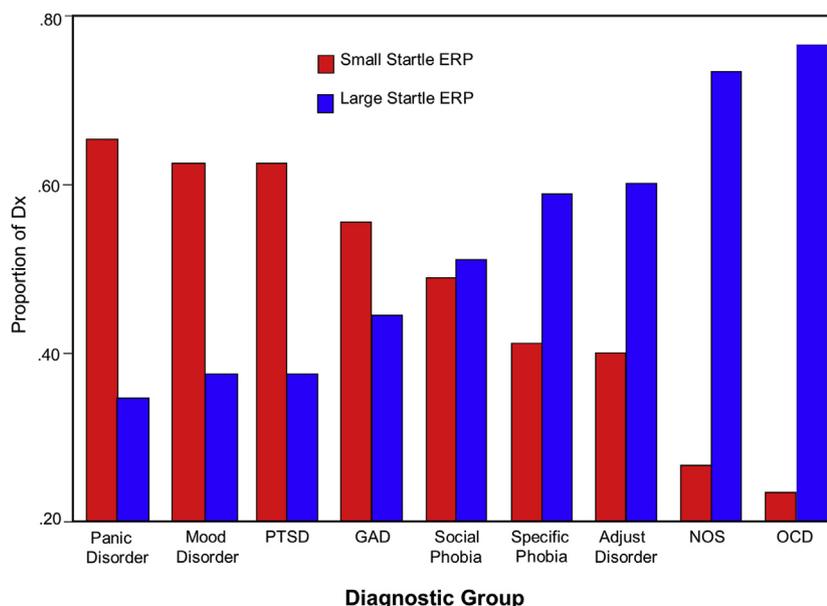

Figure 3. The proportion of patients in each primary diagnostic group with small or large startle event-related potential (ERP) amplitude, based on a median split. Dx, diagnosis; GAD, generalized anxiety disorder; NOS, not otherwise specified; OCD, obsessive-compulsive disorder; PTSD, posttraumatic stress disorder.
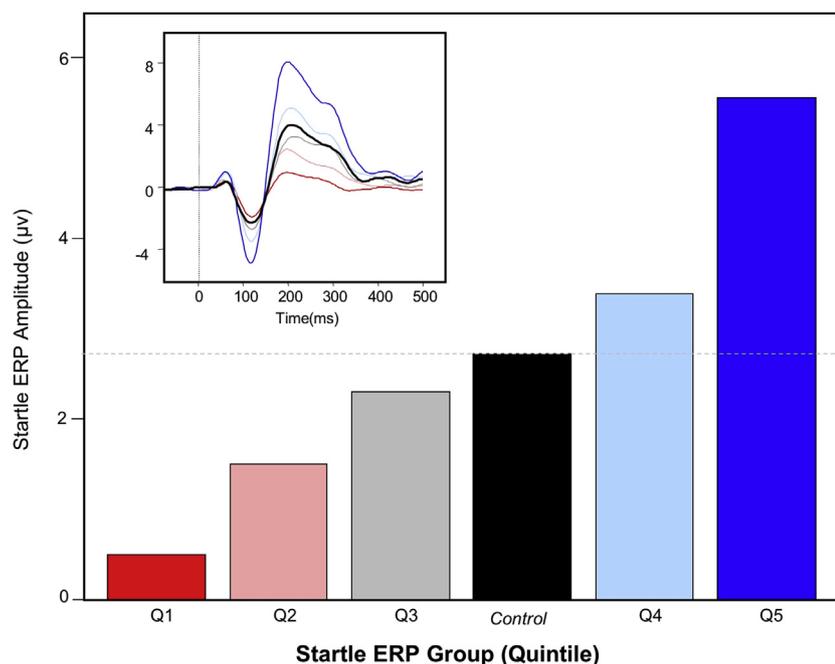
Figure 4. Startle event-related potential (ERP) waveform (inset) for healthy control subjects and patients in each quintile and mean startle ERP amplitude for control participants and anxiety patients.

DISCUSSION

Startle ERP amplitude predicts severity of psychopathology in anxiety and mood disorder patients, as measured by multiple questionnaires assessing anxiety, depression, and the impact of pathology on life function. For these measures, as the degree of reported pathology increased, startle ERP amplitude correspondently diminished. Importantly, this relationship did not depend on instructed (motivated) attention, and it occurred in the context of different tasks, as well as in the absence of a task. Patients with smaller ERP amplitude also reported greater current anxiety and distress on the interview day and, importantly, were rated higher in transdiagnostic severity, had more diagnosed comorbidities, and were judged by the attending clinician to have the poorest treatment prognosis.

The neural determinants of this novel, ERP task–independent finding are unclear (49). The circuit mediating the involuntary blink reflex, on the other hand, is well understood; an abrupt auditory noise activates cochlear root neurons, projecting to the nucleus-reticularis-pontis-caudalis, engaging neurons in the spinal cord that activate the body's flexor muscles (50,51). The overall magnitude of the obligatory reflex response did not predict psychopathology, or, replicating Putnam and Roth (25), covary with startle ERP amplitude, supporting their conclusion that "the amplitude of the automatically elicited P300 is not governed by the same mechanisms as startle amplitude" (see previous research and the Supplement). On the other hand, during picture viewing, attention was directed to the startle probe by requiring a voluntary motor response, and reaction time did covary with startle ERP amplitude. That is, when "effortful control" (52) was required, a voluntary motor response was inversely correlated with ERP amplitude, as found in previous P3 studies (53). Nevertheless, startle reaction time was not independently

predictive of state or trait psychopathology as startle ERP amplitude clearly was.

Reduced P3 amplitude with increasing age has been reported in both healthy and patient participants (54,55), and a modest age effect was found here for startle ERP amplitude. Residual analyses that controlled for age, however, found the same highly significant relationship between reduced startle ERP amplitude and increased psychopathology. Gender has also sometimes been reported to modulate the ERP (56), and women had smaller ERPs than men in the University of Florida sample. Again, however, startle ERP amplitude significantly predicted the psychopathology measures in a gender-controlled residual analysis, as well as in separate tests for men and women. Moreover, in the German replication sample, women rather than men had significantly larger ERPs, suggesting that gender differences are unreliable.

Not surprisingly, startle ERPs for the healthy control sample were significantly larger than for patients with the smallest ERPs (e.g., first and second quintiles), which included patients with the most generalized principal diagnoses, greater number of comorbidities, and the highest scores on questionnaires measuring anxiety and depression. In addition, however, a group of patients (quintile 5) showed larger ERPs than healthy control subjects. This patient group was distinctive because it included the fewest anxious misery disorders, reported the least negative affect, and in general was judged by clinicians to have more positive treatment prognosis. As a group, they were hyperalert participants showing an uncompromised, strong neural reaction to a "defense response" cue (25).

The German comparison sample provides important replication evidence, because despite the different language and cultural context, smaller sample size, and distribution of diagnoses, smaller startle ERP amplitude again predicted higher scores on the same psychopathology questionnaires. These

findings encourage the view that the startle ERP could have practical clinical utility, because it can be obtained with a limited (and quickly applied) electrode array, conceivably in the context of a normal clinical interview.

Smaller P3 amplitude has been reported in many functional and neurophysiological disorders, generally presumed to reflect a breakdown in cognitive operations of attention allocation. Considering that the startle ERP findings reported here are not dependent on a task context, they may well reflect a broader state of brain dysfunction. Startle ERP amplitude did not target specific, principal DSM-IV diagnoses; rather, when smaller in amplitude, it is a reliable neural signal of severe pathology, prolonged distress, and significant disruption in basic life functions (in the workplace, social activities, and in family and sexual relations).

The breadth of these effects recalls the "general psychopathology dimension," or p factor, described by Caspi et al. (57). This concept emerged in factor analysis of a developmental study with more than 1000 participants, based on a large battery of pathology measures, and assessed multiple times from 3 to 38 years of age. Three factors of internalizing, externalizing, and thought disorder were obtained. However, a superordinate, single dimension, the p-factor, also accounted for significant variance—conceptually summarizing multiple measures, as the g-factor is a measure of general intelligence. Importantly, "higher p scores are associated with more life impairment…worse development histories and more compromised early-life brain dysfunction" (p. 119).

The findings reported here suggest that the startle-evoked response may be indexing a similar p-factor, and furthermore that this diminished neural response (to an event prompting a species-wide, obligatory defense reflex) likely reflects disorder severity, broad dysfunction, and a realized poor prognosis across the full range of mental disorders. Determining the underlying brain processes, the integrity of circuit structures, and their functional and white matter circuit connectivity is clearly a high priority and major target for future research.

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