

Families at High and Low Risk for Depression: A Three-Generation Startle Study

Christian Grillon, Virginia Warner, Jeffrey Hille, Kathleen R. Merikangas, Gerard E. Bruder, Craig E. Tenke, Yoko Nomura, Paul Leite, and Myrna M. Weissman

Background: Anxiety symptoms might be a vulnerability factor for the development of major depressive disorder (MDD). Because elevated startle magnitude in threatening contexts is a marker for anxiety disorder, the present study investigated the hypothesis that enhanced startle reactivity would also be found in children and grandchildren of individuals with MDD.

Methods: The magnitude of startle was investigated in two tests (anticipation of an unpleasant blast of air and during darkness) in children (second generation) and grandchildren (third generation) of probands with (high risk) or without (low risk) MDD (first generation).

Results: Startle discriminated between the low- and high-risk groups. In the probands' children, the high-risk group showed increased startle magnitude throughout the fear-potentiated startle test. In the probands' grandchildren, a gender-specific abnormality was found in the high-risk group with high-risk girls, but not boys, exhibiting elevated startle magnitude throughout the procedure.

Conclusions: Increased startle reactivity in threatening contexts, previously found in patients with anxiety disorder and in children of parents with an anxiety disorder, might also constitute a vulnerability marker for MDD. These findings suggest that there might be common biologic diatheses underlying depression and anxiety.

Key Words: High risk, major depressive disorder, anxiety, startle, psychophysiology

Numerous studies show a two- to threefold increase of major depressive disorder (MDD) in the first-degree adult relatives of individuals with MDD (e.g., [Hammen et al 1990](#); [Weissman et al 1987, 1997](#)). The investigation of offspring of parents with MDD is, therefore, a powerful strategy to identify premorbid risk factors and early signs of expressions of this condition. In the present study, we used a prospective high-risk design to examine vulnerability factors for the development of MDD as a part of a multigenerational longitudinal family study of the familial aggregation of mood and other psychiatric disorders across generations (see [Warner et al 1999](#)).

The mechanism by which parental MDD influences offspring psychopathology has not been established. Several studies suggest that anxiety might act as a vulnerability factor to recurrent, early-onset, major depression. First, there is a significant degree of comorbidity between MDD and anxiety disorders in both the parents and offspring, and depression in the parents is strongly associated with an increased risk for anxiety disorders and anxiety comorbid with depression in the offspring ([Avenevoli et al 2001](#); [Breslau et al 1988](#); [Weissman et al 1997](#); [Wickramaratne and Weissman 1998](#)). Second, the adult outcome of childhood anxiety disorders is frequently MDD ([Beidel and Turner 1997](#); [Breslau et al 1995](#); [Pine et al 1998](#)). Third, in both children and adults the onset of anxiety disorders usually precedes the onset of MDD ([Kovacs et al 1997](#); [Merikangas et al 2003](#); [Parker et al 1997](#)).

These findings raise the possibility that the early presence of

From the Mood and Anxiety Disorder Program (CG, KRM), National Institute of Mental Health, Bethesda, Maryland; the Department of Psychiatry (GEB, CET, YN, MMW), College of Physicians and Surgeons of Columbia University, New York; Division of Biopsychology (GEB, CET, PL) and Division of Clinical and Genetic Epidemiology (VW, MMW), New York State Psychiatric Institute, New York, New York; and Department of Psychiatry (JH), Yale University School of Medicine, New Haven, Connecticut.

Address reprint requests to Christian Grillon, Ph.D., NIMH/NIH/DHHS/MAP, 15K North Drive, Building 15K, Room 113, MSC 2670, Bethesda, MD 20892-2670; E-mail: Christian.Grillon@nih.gov.

Received June 8, 2004; revised January 25, 2005; accepted January 28, 2005.

anxiety symptoms is a vulnerability factor for the development of MDD. Because MDD preceded by early-onset anxiety disorder is characteristically more severe, with recurrent episodes and poor treatment outcome ([Fava et al 1997](#); [Kendler 1995](#)), identification of early warning signs or markers in these cases would be extremely important for early detection of vulnerable individuals. Identification of vulnerability markers would also assist in reducing etiologic heterogeneity and might also provide pathophysiologic clues.

An intriguing question, however, is whether the nature of anxiety in individuals with a family history of MDD is similar to anxiety in individuals with a family history of anxiety disorders. One possible vulnerability factor for anxiety disorders is a negative affectivity or neuroticism ([Clark and Watson 1991](#)), which can be conceptualized as a temperamental sensitivity to negative stimuli ([Tellegen 1985](#)). We have investigated the predisposition to react fearfully to mildly challenging stimuli with the startle reflex. The startle reflex is a cross-species response highly sensitive to fear and anxiety in children and adults, as well as in animals (see [Grillon and Baas \[2003\]](#) and [Davis \[1998\]](#) for reviews). In rats, the central nucleus of the amygdala is critically involved in startle potentiation to phasic threat cues, whereas another structure, the bed nucleus of the stria terminalis, has been linked to sustained startle potentiation in the presence of long-duration aversive stimuli, such as exposure to aversive contexts or bright lights ([Davis 1998](#)).

Increased startle reactivity has been reported in anxiety disorders ([Grillon et al 1994, 1998b](#)). For example, patients with panic disorder and Vietnam veterans with posttraumatic stress disorder (PTSD) show a sustained elevation in "baseline" startle throughout an experiment that involves the administration of unpleasant shocks signaled by a cue ([Grillon et al 1994, 1998b](#)). This elevation in baseline startle was attributed to increased anxious apprehension of threatening experimental contexts ([Grillon et al 1998b](#)). A similar sensitivity to threatening contexts was recently reported in police officers with PTSD ([Pole et al 2003](#)).

With respect to vulnerability to anxiety, we have documented a gender-specific elevation in startle magnitude in a high-risk study for anxiety disorders ([Grillon et al 1998a](#)). More specifically, daughters of parents with an anxiety disorder

der showed the similar pattern of startle abnormality reported in adults with anxiety disorders (i.e., sustained elevation in baseline startle) in a threat experiment in which subjects anticipated an unpleasant intense blast of air (airblast) to the larynx. In contrast, sons of anxious parents exhibited an abnormally elevated fear-potentiated startle but normal baseline startle. Taken together, these results suggest that increased anxious apprehension in threatening contexts, as evidenced by elevated baseline startle, might be a vulnerability marker for anxiety, at least in female subjects.

In the present study, we examined whether a similar vulnerability characterized individuals at high risk for MDD. This study was a part of a longitudinal investigation of risk factors for MDD initiated more than 20 years ago. The offspring (second generation) of parents with MDD (first generation) are now adults and have children (third generation), the grandchildren of the original cohort. Results from a 10-year follow-up of the first two generations and preliminary results on a sample of grandchildren (Warner et al 1999; Weissman et al 1997; Wickramaratne and Weissman 1998) showed that children and grandchildren of the original MDD probands were at high risk for anxiety disorders in childhood. Results also showed that anxiety was a precursor of MDD in the second generation (Weissman et al 1997).

Two tests were selected: fear-potentiated startle to threat of an aversive event and darkness-facilitation of startle. The fear-potentiated startle test was selected because of our previous findings in patients with anxiety disorders (Grillon et al 1994, 1998c) and in children at high risk for anxiety disorders (Grillon et al 1998a). On the basis of our previous high-risk study (Grillon et al 1998a), we hypothesized increased baseline startle in third-generation females (grandchildren) at high-risk for MDD. On the basis of our studies in patients with anxiety disorders (Grillon et al 1994; 1998b), we hypothesized increased baseline startle in second-generation males and females (children) at high risk for MDD.

The second test was exploratory. The darkness-facilitation of startle refers to the increase in startle in complete darkness. We have argued that the facilitation of startle in the dark in humans, a diurnal species, is similar to the enhancement of startle in the dark in rodents, a nocturnal species (Grillon and Baas 2003). This test was selected because it produces a sustained state of anxiety, which might be mediated by the same structures involved in contextual fear (e.g., the bed nucleus of the stria terminalis) (Davis 1998; Grillon and Baas 2003). Although we have reported overall increase in startle reactivity in an experiment in which startle was tested in the dark and under illumination in veterans with PTSD, we have not found a specific increase in startle facilitation in the dark in these individuals compared with non-PTSD veterans (Grillon et al 1998c).

Methods and Materials

Participants

In the original study initiated in 1982, Caucasian probands (first generation) with major depression were selected from outpatient clinics for the treatment of mood disorders at Yale University. They had moderate to severe MDD that resulted in impairment. Nondepressed probands were also selected at the same time from a sample of adults from the same community. They were required to have no lifetime history of psychiatric illness, on the basis of several direct interviews. The present study tested children (second generation) and grandchildren (third generation) (Table 1) of these first-generation probands. Specific details regarding methods used in the family study are described in earlier publications (see Warner et al 1999; Weissman et al 1982, 1992, 1997). Assessments of offspring and parents were conducted at baseline (wave 1), 2 years later (wave 2), and 10 years later (wave 3). A fourth wave of assessments was obtained 17 years after baseline (see Weissman et al 2005).

The diagnostic interviews across all waves were conducted with a semistructured diagnostic assessment (the Schedule for Affective Disorders and Schizophrenia–Lifetime Version for adults [Mannuzza et al 1986] and the child version modified for DSM-IV for subjects between the ages of 6 and 17 years [Orvaschel et al 1982]). Psychophysiological tests were obtained during the fourth wave assessments. To be eligible for these tests, the proband's children and grandchildren had to be more than 7 years old, living in the geographic area of the study, and without a hearing impairment or history of seizures, epilepsy, head trauma, or psychosis. Of the 182 eligible offspring of the second generation, 110 participated in the psychophysiological tests, and 108 had analyzable startle data. Of the 137 eligible offspring of the third generation, 75 participated in the psychophysiological tests, and 70 had analyzable data. The main reason for eligible subjects not to participate in the study was that they declined to be tested. Subjects not participating and subjects included in the startle study in the second and third generations did not differ significantly in terms of age, gender, or depression status of the first generation.

Clinical Diagnosis and Medication Status

None of the subjects tested had a current psychiatric disorder. Tables 2 and 3 give the percentage of second- and third-generation participants with lifetime diagnoses of MDD, anxiety disorders, and other psychiatric disorders. There were higher rates of anxiety disorders, phobias, and MDD in the second-generation high-risk compared with low-risk groups (Table 2). There was a higher rate of anxiety disorders in the third-generation high-risk females compared with the low-risk females (Table 3). There were higher rates of drug abuse/dependence and dysthymia in the third-generation high-risk males compared

Table 1. Age and IQ in the Low-Risk and High-Risk Groups

	Second Generation			Third Generation		
	<i>n</i>	Age (y)	IQ	<i>n</i>	Age (y)	IQ
Females Low Risk	24	35.3 (1.5)	102.4 (2.9)	17	12.2 (1.1)	100.0 (3.4)
Females High Risk	41	36.7 (1.2)	94.8 (2.9)	16	17.3 (1.2)	105.7 (3.0)
Males Low Risk	16	36.2 (1.9)	104.0 (3.0)	20	12.0 (1.0)	103.3 (3.2)
Males High Risk	27	33.0 (1.4)	103.7 (2.8)	17	16.8 (1.1)	112.8 (3.6)

Age and intelligence quotient (IQ) data presented as mean (SEM).

Table 2. Rates of Lifetime Disorders in the Second Generation Participants, Based on Gender and Proband MDD Status

	Females				Males			
	High Risk (n = 41)	Low Risk (n = 24)	χ^2	p	High Risk (n = 27)	Low Risk (n = 16)	χ^2	p
Anxiety Disorders ^a	26 (63.4)	6 (25.0)	8.9	.003	16 (55.5)	2 (12.5)	7.8	.005
Phobias	20 (48.7)	3 (12.5)	8.7	.003	9 (33.3)	1 (6.2)	4.1	.06 ^b
Major Depressive Disorders	25 (61.0)	8 (33.3)	4.6	.03	51.8 (35)	18.7 (10)	4.6	.03
Conduct Disorders	11 (26.8)	3 (12.5)	1.8	.17	10 (37.0)	3 (18.7)	1.5	.30 ^b
Disruptive Disorders	12 (29.2)	4 (16.6)	1.3	.20	11 (40.7)	3 (18.7)	2.2	.13
Alcohol Abuse/Dependence	10 (24.4)	4 (16.6)	.53	.46	9 (33.3)	5 (31.2)	.2	.8
Drug Abuse/Dependence	10 (24.3)	4 (16.6)	.53	.46	6 (22.2)	3 (18.7)	.07	1.0 ^b
Attention-Deficit Disorder	2 (4.8)	1 (4.1)	.01	.89	5 (18.5)	1 (6.2)	1.2	.38 ^b
Dysthymia	7 (24.3)	10 (29.1)	.2	.6	5 (18.5)	1 (6.2)	1.2	.38 ^b

Data are presented as n (%). MDD, major depressive disorder.

^aIncluding phobias.

^bFisher's exact test two-tail probability

with low-risk males. There were also trends for increased anxiety disorders, phobias, MDD, and alcohol abuse/dependence in the third-generation high-risk males. The elevated rates of psychiatric disorders in the high-risk third-generation group were attributable to the large age difference between the high- and low-risk groups. An additional analysis restricted to subjects between 10 and 20 years old (resulting in a mean [SEM] age of 13.4 [.61] years and 14.2 [.61] years in the low- and high-risk groups, respectively) revealed no significant difference in the rates of psychiatric disorder among the high- and low-risk subjects. In this restricted group, there were three and two cases of lifetime MDD in the high- and low-risk groups, respectively. There were seven and three lifetime cases of anxiety disorders in the high- and low-risk groups, respectively. There was no case of alcohol/drug abuse/dependence in either of the two groups.

Use of current medication did not differ significantly among groups. In the second-generation group, five low-risk subjects (12.5%) and nine high-risk subjects (13.2%) (χ^2 : $p = .9$) were taking antidepressants, and two low-risk subjects (5%) and four high-risk subjects (7.3%) (χ^2 : $p = .6$) were taking minor tranquilizers. In the third generation, one high-risk subject was taking an antidepressant.

Description of the Experiments

The procedure started with an assessment of resting electroencephalogram (Bruder et al 2005), which was followed by three

tests: startle habituation, consisting of the delivery of nine acoustic startle stimuli (every 20–25 sec), fear-potentiated startle to a threat stimulus, and darkness-facilitation of startle. These three tests were run in that order. Startle habituation was used to reduce initial startle reactivity and to examine the magnitude of startle in the absence of explicit threat. Fear-potentiated startle was always tested after startle habituation to give priority to this test over the darkness-facilitation of startle, owing to specific a priori hypotheses for fear-potentiated startle. We were concerned that with a long testing procedure the quality of the startle reflex would be reduced and that the motivation of the participants would lessen over time.

The threat study consisted of examining startle potentiation during the threat of an airblast directed to the larynx. The threat of airblast is a very efficient and replicable way of potentiating startle and is well tolerated by children (Grillon et al 1999). The experiment consisted of three conditions: safe, threat, and inter-trial interval (ITI). The safe and threat conditions were signaled by 8-sec visual cues presented on a monitor (e.g., blue circle for safe and green square for threat, with the association cues/conditions being reversed from one subject to the next). Only the threat cue signaled the possibility of receiving an airblast. Half the threat cues were reinforced with an airblast delivered at cue offset. There were six threat and six safe conditions, presented in three blocks of two safe and two threat conditions per block. The interval between cues varied from 18 sec to 40 sec. In approxi-

Table 3. Rates of Lifetime Disorders in the Third Generation Participants, Based on Gender and Proband MDD Status

	Females				Males			
	High Risk (n = 16)	Low Risk (n = 17)	χ^2	p	High Risk (n = 17)	Low Risk (n = 20)	χ^2	p
Anxiety Disorders ^a	6 (37.5)	1 (5.8)	4.9	.04 ^b	8 (47.0)	4 (20.0)	3.1	.07
Phobias	3 (18.7)	1 (5.8)	1.2	.33 ^b	8 (47.5)	3 (15.0)	4.5	.03
Major Depressive Disorders	1 (6.25)	0 (0)	1.09	.49 ^b	6 (35.3)	2 (10.0)	3.4	.10 ^b
Conduct Disorders	0 (0)	0 (0)			2 (11.7)	1 (5.0)	.56	.58 ^b
Disruptive Disorders	1 (6.2)	3 (17.6)	1.0	.60 ^b	3 (17.6)	3 (15.0)	.04	1.0 ^b
Alcohol Abuse/Dependence	1 (6.2)	0 (0)	1.0	.48 ^b	3 (17.6)	0 (0)	3.8	.09 ^b
Drug Abuse/Dependence	0 (0)	0 (0)			5 (29.4)	0 (0)	6.8	.01 ^b
Attention Deficit Disorder	0 (0)	0 (0)			1 (5.8)	2 (10.0)	.20	1.0 ^b
Dysthymia	2 (12.5)	0 (0)	2.2	.22 ^b	4 (23.5)	0 (0)	5.5	.04 ^b

Data are presented as n (%). MDD, major depressive disorder.

^aIncluding phobias.

^bFisher's exact test two-tail probability

mately half the subjects, the threat cue appeared first, whereas the safe cue appeared first in the remaining subjects.

Six acoustic startle stimuli were delivered at the beginning of the threat experiment (before presentation of the safe/threat cue). Acoustic startle stimuli were also delivered 1) 5–7 sec after the onset of each of the six threat and safe cues; and 2) during ITI (i.e., between cues). Hence, each condition (ITI, safe, threat) was probed six times with a startle stimulus.

The darkness-facilitation of startle examined startle reactivity during periods of light and complete darkness. There were three alternating 60-sec light and dark phases. For approximately half the subjects, the light phase was presented first, whereas the dark phase was presented first in the remaining subjects. Two acoustic startle stimuli were delivered during each light/dark phase, with the first startle stimulus being delivered 13–18 sec after the onset of a phase. Four acoustic startle stimuli were presented at the beginning of the experiment with the light turned on. The interval between startle stimuli varied from 26 sec to 34 sec.

Materials and Recording

Stimulation and recording were controlled by a commercial system (Contact Precision Instrument, London, United Kingdom). The physiologic measures included the startle reflex, skin conductance, and heart rate, but only the startle data are presented here. The skin conductance and heart rate data will be reported in a separate report. The acoustic startle stimulus was a 40-msec, 102-dB(A) burst of white noise with a near instantaneous rise time presented binaurally through headphones. Left and right orbicularis oculi electromyogram (EMG) eyeblink reflexes were recorded with miniature electrodes placed under each eye. Impedance levels were kept below 5 K Ω . The EMG signal was amplified, filtered (30–500 Hz), and rectified and integrated (100-msec time constant). The unpleasant threat stimulus was an airblast, an intense (60-psi) jet of air delivered by plastic tubing and delivered at the level of the larynx (Grillon and Ameli 1998).

Questionnaires

Participants were asked to rate the unpleasantness of the acoustic startle and the airblast stimuli on a scale of 0 (not at all unpleasant) to 10 (extremely unpleasant) at the end of each test. They also rated their level of subjective anxiety/nervousness in each condition (i.e., safe/threat and light/dark) on a scale of 0 (not at all anxious/nervous) to 10 (extremely anxious/nervous).

Procedure

A research assistant telephoned the families and gave a description of the topic and data collection procedures. After preliminary verbal consent was obtained, the laboratory session was scheduled. Coffee, alcohol, and tobacco were prohibited within 1 hour of the testing procedure. On the day of the laboratory assessment, all offspring received a brief reminder of the study topic and signed a consent (assent for minors) form approved by the investigation review boards of both Yale University and Columbia University.

The electrodes and airblast-delivery system were then attached and the testing started. Participants were informed that their physiologic reaction to various types of external stimuli was recorded. After the startle habituation procedure and before the threat experiment, a sample airblast was delivered. Participants were then told that during testing they could receive an airblast only when the threat signal was on and that they would receive an airblast on half the threat trials. At the end of the threat

experiment, the tubing delivering the airblast was removed. Participants were told that no more airblast would be administered. They were then informed that startle stimuli would be delivered during alternating periods of light and darkness.

Data Analysis

Peak amplitude of the blink reflex was determined in the 20–120-msec time frame following stimulus onset relative to baseline (average baseline EMG level for the 50 msec immediately preceding stimulus onset) (Grillon et al 1998a, 1998b). The data were averaged for each condition over blocks. The magnitude of the eyeblink was also analyzed after standardization within subjects with z scores. Because similar results were obtained with the raw scores and with the z scores, only results of the raw scores are presented. Statistical analysis was conducted with repeated-measures analyses of variance (ANOVA). The p value was set at $<.05$. Greenhouse-Geisser corrections were used for main effects and interactions involving factors with more than two levels. Significant interactions were followed up by focused contrasts (Rosenthal and Rosnow 1985).

Results

Probands' Children/Second Generation

Demographic. There was no difference in age or intelligence quotient (IQ) between the low- and high-risk groups (Table 1). Additional demographic information can be found in Weissman et al (2005).

Startle Habituation. Startle magnitudes averaged over the three habituation blocks are shown in Table 4. A Group (low-risk, high-risk) \times Gender (male, female) \times Block (3 blocks) ANOVA revealed a Block main effect [$F(1,103) = 23.8, p < .0009$; linear trend: $F(1,103) = 38.8, p < .0009$], reflecting a decrease in startle magnitude (habituation) across blocks. There was also a trend for the magnitude of startle to be larger in the high-risk compared with the low-risk group [$F(1,103) = 2.9, p < .09$]. There was no significant gender effect.

Fear-Potentiated Startle. Figure 1 shows the startle data during the ITI, safe, and threat conditions. Results were analyzed with a Group (low-risk, high-risk) \times Gender (female, male) \times Condition (ITI, safe, threat) ANOVA. The overall magnitude of startle was significantly larger in the high-risk compared with the low-risk group [$F(1,102) = 4.1, < .05$]. As expected, startle varied as a function of the different conditions [$F(2,204) = 25.3, p < .0009$], owing to greater startle magnitude (startle potentiation) in the threat condition compared with both the safe [$F(4,102) = 9.6, p < .0009$] and the ITI [$F(4,102) = 10.1, p < .0009$] conditions. There was no significant difference between the safe and the ITI conditions [$F(4,102) = 1.6$]. The degree of startle potentiation during the threat condition did not differ significantly between the low- and high-risk groups [Group \times Condition: $F(2,204) = .4$].

Table 4. Second and Third Generation: Eyeblink Magnitude in Microvolts During Habituation

	Second Generation	Third Generation ^a
Females Low Risk	382 (52)	315 (73)
Females High Risk	525 (69)	638 (75)
Males Low Risk	372 (83)	261 (69)
Males High Risk	452 (54)	298 (72)

Data are presented as mean (SEM).

^aAdjusted means for group difference in age in the Third generation.

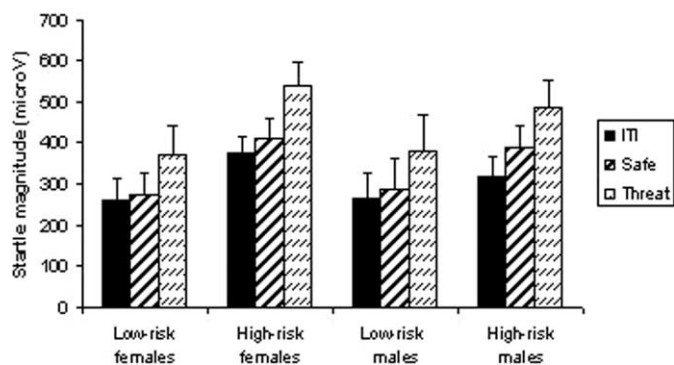


Figure 1. Magnitude of startle during intertrial interval (ITI) and during the safe and threat signals in the fear-potentiated startle experiment in females and males at low- and high-risk for major depressive disorder in the second generation. Error bars are standard errors.

There was no significant main effect or interaction involving Gender.

Questionnaires (Threat Experiment). Retrospective ratings of anxiety during the threat experiment are shown in Table 5. Subjective anxiety was higher in the threat compared with the safe condition [$F(1,98) = 88.1, p < .0009$]. Anxiety was also higher in the high-risk compared with the low-risk group [$F(1,98) = 9.5, p < .003$], but this effect did not vary as a function of conditions [$F(1,98) = 2.6$]. Rating of unpleasantness of the airblast and the white noise (startle stimulus) did not differ significantly between risk groups.

Influence of Lifetime Diagnosis. Because there were differences in the lifetime rates of MDD and anxiety disorders between the low- and high-risk groups (Table 2), we examined the influence of lifetime diagnosis of each of these disorders on startle reactivity, using Group (low-risk, high-risk) \times Diagnosis (anxiety disorder, no anxiety disorder, or MDD, no MDD) \times Condition (ITI, safe, threat) ANOVA. There was no significant Diagnosis main effect or Diagnosis interaction effect (all $p > .1$), but the Group main effect remained significant ($p < .05$).

Darkness Facilitation of Startle. Results were analyzed with Group (low-risk, high-risk) \times Gender (female, male) \times Condition (light, dark) ANOVA. The results are presented in Table 6. There was a trend for overall startle magnitude to be greater in the high-risk group compared with the low-risk group [$F(1,100) = 3.0, p < .08$]. Startle was facilitated by darkness [$F(1,100) = 16.3, p < .0009$]. This facilitation did not differ between the two groups [$F(1,100) = .1$]. There was no significant Main effect or interaction involving the factor Gender.

Questionnaires (Light/Dark Experiment). Overall ratings of anxiety during the light/dark experiment were higher in the high-risk group compared with the low risk group [$F(1,97) = 5.1, p < .03$], and in the dark compared with the light phase [$F(1,97) = 8.7, p < .004$].

Table 6. Second and Third Generation: Eyeblink Magnitude in Microvolts During Dark/Light Experiment

	Second Generation		Third Generation ^a	
	Light	Dark	Light	Dark
Females Low Risk	138 (32)	193 (39)	122 (30)	184 (41)
Females High Risk	238 (25)	266 (30)	315 (33)	383 (44)
Males Low Risk	153 (39)	141 (47)	161 (29)	213 (38)
Males High Risk	166 (31)	236 (38)	135 (30)	198 (41)

Data are presented as mean (SEM).

^aAdjusted means for group difference in age in the third generation.

Probands' Grandchildren/Third Generation

A female in the high-risk group was not included in the data analysis because she had an unusually high startle magnitude (more than two times higher than the next highest score). Overall findings were not changed by the removal of this subject.

Demographic. The high-risk group was older [$F(1,66) = 19.2, p < .0009$] and had higher IQ (age-normed Peabody Picture Vocabulary Test) [$F(1,66) = 19.2, p < .0009$] compared with the low-risk group. Ages ranged from 9 to 28 years in the high-risk group and 8 to 21 years in the low-risk group. Because age can influence startle reactivity, statistical analyses used age as a covariate. Intelligence quotient did not influence startle results and was not used as a covariate.

Startle Habituation. The habituation data are shown in Table 4. Startle was larger in the high-risk group and in females, leading to significant Group [$F(1,65) = 9.3, p < .003$] and Gender [$F(1,65) = 8.0, p < .0006$] main effects. The Group \times Gender interaction was also significant [$F(1,65) = 4.3, p < .04$]. Focused contrasts revealed significant greater startle magnitude in the female high-risk compared with the female low-risk group [$F(1,64) = 8.8, p < .004$], but there was no significant high-risk group difference in the males [$F(1,64) = .12$].

Fear-Potentiated Startle. Figure 2 shows the startle data during the ITI, safe, and threat conditions. Startle magnitude tended to be greater in the high-risk compared with the low risk group [$F(1,63) = 3.7, p = .058$] and was greater in females compared with males [$F(1,63) = 6.7, p < .01$]. There was, however, also a trend for a Group \times Gender interaction [$F(1,63) = 3.6, p = .06$]. Because of our a priori hypothesis concerning gender differences, additional analyses were conducted within each gender. As in the habituation condition, overall startle magnitude was greater in the high-risk females compared with the low-risk females [$F(1,63) = 6.8, p < .01$]. There was no group difference in the males [$F(1,63) = .03$].

Across groups, startle magnitude was potentiated in the threat condition [$F(2,126) = 20.1, p < .0009$]. Post hoc tests indicated that startle magnitude was greater in the threat condition compared with both the safe [$F(5,63) = 4.4, p < .002$] and the ITI [$F(5,63) = 7.8, p < .0009$] conditions. The safe and ITI conditions

Table 5. Second Generation: Subjective Rating (on Scale of 0 to 10) of Anxiety (Safe and Threat signal, Light and Dark Phase) and Unpleasantness of the Airblast and White Noise

	Safe	Threat	White Noise	Airblast	Light	Dark
Females Low Risk	2.2 (.4)	4.3 (.3)	5.6 (.4)	3.6 (.4)	2.5 (.3)	3.0 (.4)
Females High Risk	2.6 (.2)	5.6 (.4)	5.6 (.3)	4.7 (.4)	2.9 (.3)	3.6 (.4)
Males Low Risk	1.8 (.5)	3.7 (.4)	4.8 (.5)	3.3 (.6)	1.9 (.5)	2.3 (.6)
Males High Risk	2.7 (.3)	5.4 (.3)	5.2 (.4)	3.5 (.5)	3.1 (.4)	3.7 (.4)

Data are presented as mean (SEM).

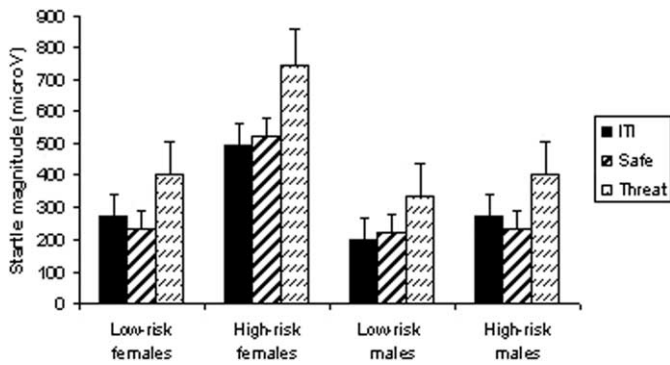


Figure 2. Magnitude of startle during intertrial interval (ITI) and during the safe and threat signals in the fear-potentiated startle experiment in females and males at low- and high-risk for major depressive disorder in the third generation. Error bars are standard errors.

did not significantly differ from each other [$F(5,63) = 1.0$]. The magnitude of startle potentiation during the threat condition did not differ significantly between groups [Group \times Condition: $F(2,126) = .1$]. There was no Gender effect.

An additional analysis was conducted for controlling for the effect of age. We analyzed data of subjects of equivalent age in the high- and low-risk groups. To this aim, only subjects between 10 and 20 years old were included in the analysis, resulting in a mean (SEM) age of 13.4 (.61) and 14.2 (.61) years in the low- and high-risk groups, respectively. Results for the startle data were unchanged. The Group \times Gender interaction remained significant [$F(1, 36) = 5.8, p < .02$], reflecting greater startle magnitude the high-risk females compared with the low-risk females [$F(1,35) = 8.1, p < .007$]. There was no group difference in the males [$F(1,35) = .04$].

Influence of Lifetime Diagnosis. Because they were differences in the lifetime rates of anxiety disorders in the low- and high-risk females (Table 3), we examined the influence of lifetime diagnosis of anxiety disorders on startle reactivity in the females. Given the small number of children with a lifetime diagnosis (e.g., one subject in the low-risk females), we conducted an analysis that excluded females with a lifetime diagnosis of anxiety disorders, using a Group (low-risk, high-risk) \times Condition (for fear-potentiated startle) ANOVA. The results confirmed the main analysis, showing increased startle magnitude in the high-risk compared with the low-risk females [$F(1,23) = 5.6, p < .03$].

Questionnaires (Threat Experiment). Retrospective ratings of anxiety during the threat experiment are shown in Table 7. There was a trend for a main Group effect [$F(1,50) = 3.7, p < .057$] and a significant Group \times Condition interaction [$F(1,50) = 8.5, p < .005$]. Focused contrasts showed greater rating of anxiety in the presence of the threat signal, but not the safe signal, in the high-risk group compared with the low-risk group [$F(1,50) = 41.6, p < .0009$].

Rating of unpleasantness of the airblast and the white noise (startle stimulus) did not differ significantly between risk groups.

Darkness-Facilitation of Startle. The results are presented in Table 6. Both the Group and the Gender main effect were significant [$F(1,63) = 5.9, p < .01$ and $F(1,64) = 5.2, p < .02$] as was the Group \times Gender interaction [$F(1,63) = 11.1, p < .001$]. As in the habituation and fear-potentiated startle tests, the Group \times Condition interaction was due to greater startle magnitude in high-risk females compared with low-risk females [$F(1,63) = 15.4, p < .0009$]. In the males, startle magnitude did not differ between the high-risk and the low-risk groups [$F(1,63) = .2$].

Startle was facilitated by darkness [$F(1,63) = 5.9, p < .02$]. This facilitation did not differ between the two groups [$F(1,63) = .6$] or by Gender [$F(1,63) = .7$].

Questionnaires (Light/Dark Experiment). Overall rating of anxiety was higher in the high-risk group (Table 7) compared with the low-risk group [$F(1,97) = 5.1, p < .03$] and in the dark compared with the light phase [$F(1,97) = 8.7, p < .004$], with no differential effect between groups.

Discussion

The major finding of this study, the first study of startle among individuals at high risk for MDD, is that in children of probands, startle was greater among the high-risk compared with the low-risk group during the threat experiment, regardless of gender. Among the grandchildren of the probands, there was a gender-specific difference in startle reactivity between the high- and low-risk groups. Startle was substantially and significantly increased in the high-risk females compared with the low-risk females throughout the experiment. Of note, fear-potentiated startle to the threat cue did not distinguish among the low- and high-risk individuals (males or females), nor was there a difference in startle magnitude between the high- and low-risk males. Finally, these results were unaffected by the level of lifetime psychopathology or by the use of medication of the subjects.

Results in the high-risk third generation present important similarities with previous findings from a "threat of airblast" experiment in children and adolescent offsprings of parents with an anxiety disorder (Grillon et al 1998a). In both studies, the high-risk girls exhibited a sustained elevation in startle magnitude that lasted the entire experiment and a normal fear-potentiated startle to the threat cue. These results are also reminiscent of findings in adult females and males with an anxiety disorder, who exhibit elevated baseline startle in the context of normal fear-potentiated startle in threat experiments (Grillon and Morgan 1999; Grillon et al 1994, 1998b).

Startle, including fear-potentiated startle, did not differentiate between the low- and high-risk boys in the third generation. This result differs from our previous findings, in which we showed normal baseline startle but increased fear-potentiated startle in boys at high risk for anxiety disorder (Grillon et al 1998a). The

Table 7. Third Generation: Subjective Rating (on Scale of 0 to 10) of Anxiety (Safe and Threat Signal, Light and Dark Phase) and Unpleasantness of the Airblast and White Noise

	Safe	Threat	White Noise	Airblast	Light	Dark
Females Low Risk	2.2 (.5)	4.0 (.5)	4.6 (.6)	5.3 (.6)	1.6 (.3)	2.9 (.5)
Females High Risk	2.0 (.5)	6.5 (.5)	6.0 (.6)	6.8 (.6)	2.0 (.3)	4.0 (.5)
Males Low Risk	2.8 (.5)	4.3 (.5)	6.0 (.6)	5.8 (.6)	2.2 (.3)	3.5 (.5)
Males High Risk	2.4 (.5)	5.6 (.6)	5.2 (.6)	4.5 (.6)	2.2 (.3)	2.8 (.4)

Data are presented as mean (SEM).

reason for this discrepancy between the two studies is not clear, given the similarities of results in the two studies in the high-risk girls and given that anxiety is a risk factor for both boys and girls at high risk for depression (Breslau et al 1995; Weissman et al 1992). One possibility is that the nature of underlying vulnerability to anxiety in boys at high-risk for anxiety disorders differs from that in boys at high risk for MDD. A more likely alternative is that our previous result of increased fear-potentiated startle in the boys at high risk for anxiety was a false positive. Indeed, we have never found elevated fear-potentiated startle in males (or females) with anxiety disorders (Grillon and Morgan 1999; Grillon et al 1994, 1998b).

It is also unclear why increased startle reactivity during fear-potentiated startle and darkness facilitation of startle was found in the female and male second-generation high-risk groups and in the female third-generation high-risk group but not in the male third-generation high-risk group. It is possible that increased startle in threatening contexts emerges at a later age in high-risk boys compared with girls. Alternatively, given the lower rate of anxiety disorder and major depression in males compared with females, it is possible that it is more difficult to demonstrate an effect in high-risk males than in high-risk females.

It is unknown how the present results compare with those of patients with current MDD, because startle to threat has not been investigated in such patients. The literature in startle in MDD is still in its infancy, with no clear finding emerging. Baseline startle is normal in MDD patients during prepulse inhibition studies (Ludewig and Ludewig 2003; Perry et al 2004). Dichter et al (2004) examined patients with MDD in a protocol of affective modulation of startle by neutral, pleasant, and unpleasant pictures. Depressed patients showed normal baseline startle but, unlike the control subjects, startle was not modulated by the valence of the pictures. In a similar experiment, Allen et al (1999) reported reduced baseline startle in severely depressed patients. These investigators also reported an abnormal modulation of startle, consisting of an increased startle to pleasant pictures, instead of the normal inhibition that characterizes healthy subjects. Allen et al (1999) suggested that pleasant stimuli were experienced as aversive because these stimuli were seen as a signal for frustrative nonreward. These studies provide no evidence of increased baseline startle in patients with current MDD.

Both our previous study in children of anxious parents (Grillon et al 1998a) and the present study show that contextual fear is a vulnerability factor for depression and anxiety. The grandchildren of the high-risk grandparents in the present study had higher rates of anxiety disorders compared with the low-risk grand children (see also Weissman et al 2005). The increase in anxiety disorder in the high-risk grandchildren is consistent with the findings from their parents when they were the same age as the grandchildren (Warner et al 1999; Wickramaratne and Weissman 1998); however, a past history of anxiety disorders was not associated with abnormal startle reactivity in the high-risk group. Indeed, enhanced startle was found in high-risk subjects with and without a history of anxiety disorders. Taken together, these results raise the possibility that elevated anxious apprehension in threatening contexts is the expression of a similar vulnerability in MDD and in anxiety disorders.

A number of findings in the third-generation high-risk group were not replicated in the second-generation high-risk group. Although startle was elevated in the second-generation high-risk group, it reached significance only in the threat experiment. The fact that a statistical difference was found only during the most

stressful period (i.e., fear-potentiated startle test) is consistent with the view that difference in startle reactivity among the low-risk and the high-risk participants was mediated by the experimental context. It is likely that this difference in startle reactivity between the two high-risk groups was due to the experiment being not as stressful for the second generation as for the third. Indeed, airblasts might be less anxiogenic to adults (second generation) than to children and adolescents (third generation). A subsequent analysis of the subjective ratings supports this hypothesis. A comparison of the anxiogenic effect of the airblast between the second and third generation showed that whereas there was no significant group difference in rating of the acoustic startle stimuli ($p = .8$), the airblast was rated as less anxiogenic in the second generation compared with the third generation ($p < .009$; see Tables 5 and 7). It is possible that findings similar to those in the third generation would have been found in the second generation if a more aversive stimulus (e.g., shock) had been used in place of the airblast.

The limitations of the present study must also be considered when interpreting the present results. The high-risk group was heterogeneous with respect to the development of psychopathology; that is, whereas some high-risk individuals might possess risk factors for anxiety or mood disorders, others might not. The sample size was also relatively small. This might have prevented us from truly assessing gender differences. Finally, the third-generation participants varied greatly in age. Although the results were confirmed when only data for high- and low-risk individuals of the same age were compared, this limited our ability to assess the impact of developmental changes on risk factors.

The present findings have implications for identifying neuropsychological factors associated with risk for MDD. High-risk individuals exhibit enhanced anxious apprehension in stressful contexts, while displaying appropriate responses to imminent threat. This suggests ill-timed anticipatory anxiety response in the absence of an impending danger. Future studies with the present third-generation cohort will examine whether high-risk individuals with elevated startle are more likely to develop MDD compared with high-risk people who do not show such an increased startle. In addition, brain imaging studies of high-risk individuals selected according to their deviant psychophysiological responses to threat might help identify neurobiological concomitants of risk for MDD. These studies are now underway in this sample in the fifth wave.

This research was support by National Institute of Mental Health (NIMH) grants MH36197 (to MMW) and MH36295 (to GEB) and by the Intramural Research Program at the NIMH (to CG and KRM).

- Allen NB, Trinder J, Brennan C (1999): Affective startle modulation in clinical depression: Preliminary findings. *Biol Psychiatry* 46:542–550.
- Avenevoli S, Stolar M, Li J, Dierker L, Merikangas KR (2001): Comorbidity of depression in children and adolescents: Models and evidence from a prospective high-risk family study. *Biol Psychiatry* 49:1071–1081.
- Beidel DC, Turner SM (1997): At risk for anxiety: I. Psychopathology in the offspring of anxious parents. *J Am Acad Child Adolesc Psychiatry* 36:918–924.
- Breslau N, Davis GC, Prabucki K (1988): Depressed mothers as informants in family history research—are they accurate? *Psychiatry Res* 24:345–359.
- Breslau N, Schultz L, Peterson E (1995): Sex differences in depression: A role for preexisting anxiety. *Psychiatry Res* 58:1–12.
- Bruder GE, Tenke CE, Warner V, Nomura, Y, Grillon C, Hille J, et al (2005): Electroencephalographic measures of regional hemispheric activity in offspring at risk for depressive disorders. *Biol Psychiatry* 57:328–335.

- Clark LA, Watson D (1991): Tripartite model of anxiety and depression: Psychometric evidence and taxonomic implications. *J Abnorm Psychol* 100:316–336.
- Davis M (1998): Are different parts of the extended amygdala involved in fear versus anxiety? *Biol Psychiatry* 44:1239–1247.
- Dichter GS, Tomarken AJ, Shelton RC, Sutton SK (2004): Early- and late-onset startle modulation in unipolar depression. *Psychophysiology* 41:433–440.
- Fava M, Uebelacker LA, Alpert JE, Nierenberg AA, Pava JA, Rosenbaum JF (1997): Major depressive subtypes and treatment response. *Biol Psychiatry* 42:568–576.
- Grillon C, Ameli R (1998): Effects of threat and safety signals on startle during anticipation of aversive shocks, sounds, or airblasts. *J Psychophysiol* 12:329–337.
- Grillon C, Ameli R, Goddard A, Woods S, Davis M (1994): Baseline and fear-potentiated startle in panic disorder patients. *Biol Psychiatry* 35:431–439.
- Grillon C, Baas JM (2003): A review of the modulation of startle by affective states and its application to psychiatry. *Clin Neurophysiol* 114:1557–1579.
- Grillon C, Dierker L, Merikangas KR (1998a): Fear-potentiated startle in adolescent offspring at risk for anxiety disorders. *Biol Psychiatry* 44:990–997.
- Grillon C, Merikangas KR, Dierker L, Snidman N, Arriaga RI, Kagan J, et al (1999): Startle potentiation by threat of aversive stimuli and darkness in adolescents: A multi-site study. *Int J Psychophysiol* 32:63–73.
- Grillon C, Morgan CA (1999): Fear-potentiated startle conditioning to explicit and contextual cues in Gulf war veterans with posttraumatic stress disorder. *J Abnorm Psychol* 108:134–142.
- Grillon C, Morgan CA, Davis M, Southwick SM (1998b): Effects of experimental context and explicit threat cues on acoustic startle in Vietnam veterans with posttraumatic stress disorder. *Biol Psychiatry* 44:1027–1036.
- Grillon C, Morgan CA, Davis M, Southwick SM (1998c): Effect of darkness on acoustic startle in Vietnam veterans with PTSD. *Am J Psychiatry* 155:812–817.
- Hammen C, Burge D, Burney E, Adrian C (1990): Longitudinal study of diagnoses in children of women with unipolar and bipolar affective disorder. *Arch Gen Psychiatry* 47:1112–1117.
- Kendler KS (1995): Is seeking treatment for depression predicted by a history of depression in relatives? Implications for family studies of affective disorder. *Psychol Med* 25:807–814.
- Kovacs M, Devlin B, Pollock M, Richards C, Mukerji P (1997): A controlled family history study of childhood-onset depressive disorder. *Arch Gen Psychiatry* 54:613–623.
- Ludewig S, Ludewig K (2003): No prepulse inhibition deficits in patients with unipolar depression. *Depress Anxiety* 17:224–225.
- Mannuzza S, Fyer AJ, Klein DF, Endicott J (1986): Schedule for Affective Disorders and Schizophrenia-Lifetime Version modified for the study of anxiety disorders (SADS-LA): Rationale and conceptual development. *J Psychiatr Res* 20:317–325.
- Merikangas KR, Zhang H, Avenevoli S, Acharyya S, Neuenschwander M, Angst J (2003): Longitudinal trajectories of depression and anxiety in a prospective community study: The Zurich Cohort Study. *Arch Gen Psychiatry* 60:993–1000.
- Orvaschel H, Puig-Antich J, Chambers W, Tabrizi MA, Johnson R (1982): Retrospective assessment of prepubertal major depression with the Kiddie-SADS-e. *J Am Acad Child Psychiatry* 21:392–397.
- Parker G, Wilhelm K, Asghari A (1997): Early onset depression: The relevance of anxiety. *Soc Psychiatry Psychiatr Epidemiol* 32:30–37.
- Perry W, Minassian A, Feifel D (2004): Prepulse inhibition in patients with non-psychotic major depressive disorder. *J Affect Disord* 81:179–184.
- Pine D, Cohen P, Gurley D, Brook J, Ma Y (1998): The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Arch Gen Psychiatry* 55:56–64.
- Pole N, Neylan TC, Best SR, Orr SP, Marmar CR (2003): Fear-potentiated startle and posttraumatic stress symptoms in urban police officers. *J Trauma Stress* 16:471–479.
- Rosenthal R, Rosnow RL (1985): *Contrast Analysis: Focused Comparisons in the Analysis of Variance*. New York: Cambridge University Press.
- Tellegen A (1985): Structures of mood and personality and their relevance to assessing anxiety, with an emphasis on self-report. In: Tuma AH, Maser J, editors. *Anxiety and the Anxiety Disorders*. Hillsdale, NJ: Lawrence Erlbaum Associates, 681–706.
- Warner V, Weissman MM, Mufson L, Wickramaratne PJ (1999): Grandparents, parents, and grandchildren at high risk for depression: A three-generation study. *J Am Acad Child Adolesc Psychiatry* 38:289–296.
- Weissman M, Wickramaratne PJ, Nomura Y, Warner V, Verdelli H, Pilowsky DJ, et al (2005): Family at high and low risk for depression: A 3-generation study. *Arch Gen Psychiatry* 62:29–36.
- Weissman MM, Fendrich M, Warner V, Wickramaratne P (1992): Incidence of psychiatric disorder in offspring at high and low risk for depression. *J Am Acad Child Adolesc Psychiatry* 31:640–648.
- Weissman MM, Gammon GD, John K, Merikangas KR, Warner V, Prusoff BA, et al (1987): Children of depressed parents: Increased psychopathology and early onset of major depression. *Arch Gen Psychiatry* 44:847–853.
- Weissman MM, Kidd KK, Prusoff BA (1982): Variability in rates of affective disorders in relatives of depressed and normal probands. *Arch Gen Psychiatry* 39:1397–1403.
- Weissman MM, Warner V, Wickramaratne P, Moreau D, Olfson M (1997): Offspring of depressed parents: Ten years later. *Arch Gen Psychiatry* 54:932–940.
- Wickramaratne PJ, Weissman MM (1998): Onset of psychopathology in offspring by developmental phase and parental depression. *J Am Acad Child Adolesc Psychiatry* 37:933–942.