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Acute stress potentiates anxiety in humans

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Abstract

Background—Stress is an important factor in the development and maintenance of anxiety disorders. Stress also potentiates anxiety-like response in animals, but empirical evidence for a similar effect in humans is still lacking.

Methods—To test whether stress increases anxiety in humans, we examined the ability of a social stressor (speech and a counting task) to potentiate the facilitation of startle in the dark. Measures of subjective distress and of hypothalamic-pituitary-adrenal axis and autonomic nervous system activity (e.g., salivary cortisol, alpha-amylase, blood pressure and hear rate) were also taken to confirm the effectiveness of the stress manipulation.

Results—Startle was significantly facilitated in the dark. This effect was potentiated by prior exposure to the social stressor. The social stressor induced increases in salivary cortisol and alpha amylase, as well as increases in blood pressure, heart rate, and subjective distress.

Conclusion—The findings indicate that stress potentiates anxiety. Animal studies suggest that such an effect may be mediated by glucocorticoid effects on corticotropin-releasing hormone in limbic structures.

Keywords

Fear-potentiated startle; anxiety; stress; cortisol

Introduction

Despite abundant evidence of a role of stress in mood and anxiety disorders (1;2), the underlying mechanisms remain elusive. Preclinical studies provide potential insight into such mechanisms. In animals, stress exacerbates or sensitized subsequent anxiety-like responses in a number of anxiety models involving severe or chronic the stressor (3-6), but sensitized anxiety can be found even immediately after a single acute stressor (6-8).

Despite the wealth of preclinical data on the stress sensitization of anxiety, empirical evidence for a similar effect in humans is lacking. Stress facilitates fear conditioning (9) and eyeblink

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conditioning (10) in humans, like is does in animals (11;12), but this facilitation is mediated by a stress-induced effect on associative learning mechanisms (9;13) rather than on fear/anxiety. Given the relevance of stress-sensitization of anxiety to psychopathology, our main objective was to examine whether stress increases unconditioned fear in humans.

The startle reflex is a sensitive tool to evaluate anxiety-like responses; it is potentiated by aversive events in humans and animals (14). Darkness increases the startle reflex in humans, an effect attributed to anxiety rather than attention (15), suggesting that darkness is unconditionally aversive. We have suggested that the facilitation of startle in the dark (FSD) in humans, a diurnal species, is equivalent to the "light-enhanced startle" in rats (16), a nocturnal species naturally afraid of brightly illuminated environments (15). In the rat, light-enhanced startle is mediated by corticotropin releasing hormone (CRH) receptors in the bed nucleus of the stria terminalis (BNST) (17;18). Because glucocorticoid potentiation of CRH at extra-hypothalamic sites may be responsible for the stress-induced sensitization of anxiety (19), we hypothesized that FSD would be facilitated by a prior social stressor, which activates hypothalamic-pituitary-adrenal (HPA) activity (20). We also measured salivary cortisol, alpha-amylase, and autonomic reactivity to investigate the potential link between stress-related increase in FSD and autonomic nervous system (ANS) and HPA activation.

Methods and Materials

Participants were 20 medically and psychiatrically healthy volunteers (9 males) ages 28.1 years (SD = 8.3 years) who gave written informed consent.

The FSD was investigated in two sessions a week apart, one after a social stressor (stress) and the other after no stressor (control) in a between-subject design counterbalanced across subjects. The 10-min social stressor consisted of delivering a speech followed by a backward counting task (see Fig. 1 for details). The FSD test started twenty-five minutes after the end of the social stressor and consisted three alternating 60-sec blocks of startle stimuli delivered under lighted conditions or in complete darkness, counterbalanced across subjects. There were three startle stimuli per block, two of a high intensity and one of a low intensity.

The saliva samples and the blood pressure (BP) were taken at the five time points specified in Fig 1. Subjective distress was measured at two time points. Heart rate (HR) was averaged within three 5-min periods, at baseline, during the stress challenge, and during the FSD test.

The startle stimuli were 40-ms duration high (103 dB(A)) or low (96 dB(A)) intensity white noise presented through headphones. The eyeblink reflex was recorded with electrodes placed under the left eye. Amplifier bandwidth was set to 30-500 Hz. HR was monitored with two electrodes placed on each side of the chest. Blood pressure was measured by an automatic BP measurement device (Dinamap, Critikon, USA). Saliva samples were collected with the use of plain cotton Salivettes (Sarstedt, Leicester, UK) (see the Appendix for details).

Peak magnitude of the blink reflex was determined in the 20-120-ms time frame following stimulus onset and were averaged within light and dark conditions. Because preliminary analyses indicated no difference between stress or illumination conditions between startle intensities and because there was no order effect for the stress/no stress condition, startle intensity and condition order were not considered in the statistical analysis. The amylase data were square root transformed to reach normality. The data were analyzed with analyses of variance (ANOVAs) with repeated measures. Greenhouse–Geisser epsilon corrections were implemented when appropriate.

Pearson's correlations were calculated in the stress condition to examine correlations 1) between FSD (percent change from light to dark) and neuroendocrine measures (difference

between baseline and stress levels of salivary cortisol, alpha-amylase, systolic and diastolic BP, and HR) and 2) within neuroendocrine measures.

Results

Startle was facilitated by darkness and this facilitation increased after stress (Fig. 2). A Stress Condition (2) × Illumination (2) × Sex (2) ANOVA revealed an Illumination main effect ($F(1,18)=15.9$, $p<.0009$) and an Stress Condition × Illumination interaction ($F(1,18)=6.4$, $p<.02$). Follow up tests showed significant facilitation of startle in the dark in the control ($F(1,19)=6.8$, $p<.02$) and stress condition ($F(1,19)=18.5$, $p<.0009$).

The autonomic, endocrine, cardiovascular and subjective responses confirmed that the social stressor generated a stress response (Table 1). There was an increase in cortisol (time +32, +41, +55) and a sharp increase in alpha amylase (time 2). These data were analyzed with Stress Condition (2) × Time (5) × Sex (2) ANOVAs. Cortisol levels increased after the speech (Condition × Time quadratic trend;($F(1,18)=4.7$, $p<.04$), showing a trend for higher cortisol in the stress condition at t +32, $F(1,19)=3.7$, $p<.07$), and, t +41 ($F(1,19)=3.6$, $p<.07$). For alpha amylase, there was a Condition × Time interaction ($F(4,72)=7.5$, $p<.0009$, epsilon=1) due to significantly elevated alpha amylase at t +23 after the social stressor ($F(1,18)=14.5$, $p<.0009$).

There was a strong cardiovascular reactivity to the stressor (Table 1). The BP data were analyzed using the same ANOVA as the cortisol and alpha amylase data. The HR data were analyzed with a Condition (2) × Time (3) × Sex (2) ANOVA. For systolic and diastolic BP and for HR, there was a significant Condition × Time interaction (all $p<.0009$) due to increased systolic and diastolic BP after the social stressor (t+23) and increased HR during the social stressor (t+16; all $p<.0009$).

Subjects felt substantially distressed during the social stressor (Table 1). A Condition (2) × Time (2) × Sex (2) ANOVA revealed a significant Condition × Time interaction ($F(2,36)=14.4$, $p<.0009$, epsilon=1) due to greater distress at t +11, ($F(1,19)=17.0$, $p<.001$), and t +23, ($F(1,19)=21.1$, $p<.0009$). None of the stress reactivity measures differed between males and females.

No significant correlations were found between the potentiation of startle in the dark and neuroendocrine activation during stress (Table 2). Extensive correlations were observed between measures of neuroendocrine activation themselves. In particular, cortisol levels correlated positively with measures of ANS activation, including alpha amylase, and alpha amylase correlated with other measures of ANS activation.

Discussion

To our knowledge this is the first report showing that unconditioned anxiety is enhanced by prior stress. Consistent with animal data (7;8), anxiety as measured with FSD was sensitized in humans exposed to a social stressor.

The light-enhanced startle effect in the rat is mediated by CRH in the BNST (18), suggesting that the effect of changes in background illumination on startle (i.e., FSD) is also mediated by CRH acting on receptors in the BNST. Sensitized FSD by stress in humans may ultimately rely on an enhancement of CRH effects in the BNST. Indeed, CRH antagonists can abolish sensitized anxiety in rodents (3).

What are the potential mechanisms for the sensitization of CRH effects? Prime candidates are glucocorticoids. Evidence for the role of glucocorticoids comes from two sources; 1) the stress-sensitization of anxiety in rats is believed to depend on glucocorticoids (3) and 2) glucocorticoids can potentiate fear via feed-forward regulation of CRH by glucocorticoids in

the amygdala and in the BNST (19). Indeed, corticosterone (the principal glucocorticoid in rats), despite its well-known inhibitory effects on subsequent release of hypothalamic CRH, also has excitatory effects on CRH at extra-hypothalamic sites (21), including the BNST (22).

An alternative possibility is the involvement of the stress-sensitive noradrenergic input (23) into the BNST (24). Acute stress increases noradrenaline in the lateral BNST (25;26), possibly via stimulation of CRH in the BNST (3;27). In the present study, noradrenergic activity may have promoted alertness and arousal following the social stressor, leading to sensitized FSD via its action on CRH in the BNST.

Our stressor activated two major stress systems, the HPA axis and the ANS. The stress-induced increase in cortisol significantly correlated with increases in HR, systolic BP and salivary alpha-amylase, indicating a coordinated activation of both HPA axis and sympathetic ANS. The absence of correlation between cortisol and the magnitude of FSD may be due to the fact that salivary cortisol does not reflect accurately cortisol in the BNST or may reflect our inability to determine the cortisol response to the stressor because of individual differences in baseline cortisol caused by anticipatory anxiety.

The use of an experimental model and a measure of anxiety derived from animal research provide us with a fairly good understanding of the mechanisms underlying FSD. Whether glucocorticoids mediate the stress-induced sensitization of FSD is speculative, but it is a testable hypothesis. The availability of glucocorticoid receptor antagonists such as mifepristone will help test the role of glucocorticoids in mediating or modulating these responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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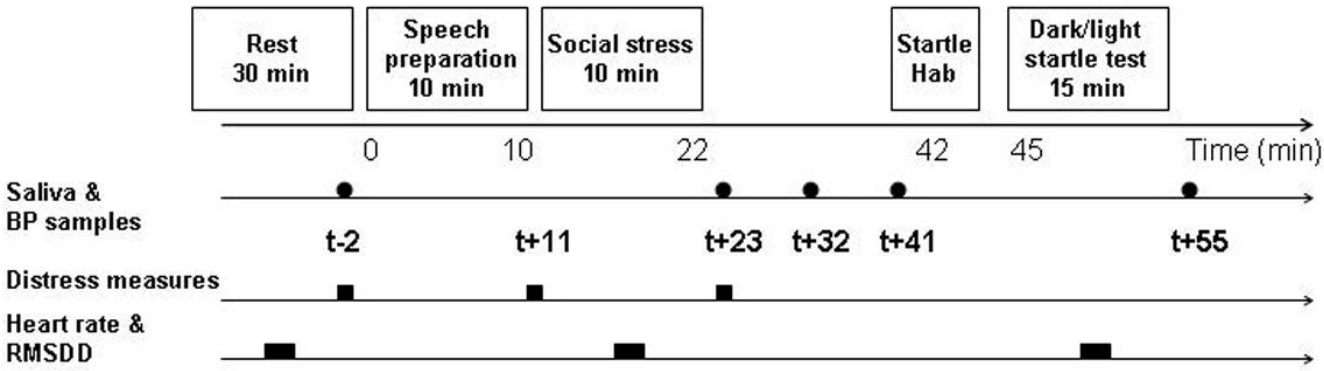

Figure 1.

Schematic representation of the procedure. Following a 10-min preparation, participants in the social stressor condition gave an 8-min unstructured speech on abortion after which they counted backwardly from 1000 in decrements of 13 for 2 min in front of a male and a female "judge" in white lab coats (total duration of social stressor = 10 min). A video camera relayed the speaker's image to a TV screen that the speaker could see while talking. In the control condition, participants rested for about 20 min. The startle test was initiated twenty minutes after the end of the social stressor. It started with six habituation startle stimuli, immediately followed by the FSD test. The FSD test started with an additional six startle stimuli (under lighted conditions) followed by three alternating 60-sec blocks of startle stimuli delivered under lighted conditions or in complete darkness, counterbalanced across subjects. The saliva samples were collected and BP was measured at the five following time points; prior to speech preparation (t -2 min), immediately after and 9 min after the social stressor (t +23 min and t + 32 min), before startle habituation (t +41 min), and after the dark/light startle test (t + 55 min). In addition, subjects were asked to indicate their level of distress on a Likert scale ranging from 1 (not at all distressed) to 10 (extremely distressed) prior to the speech preparation (t -2 min), just before (t +11 min) and after (t +23 min) the social stressor.

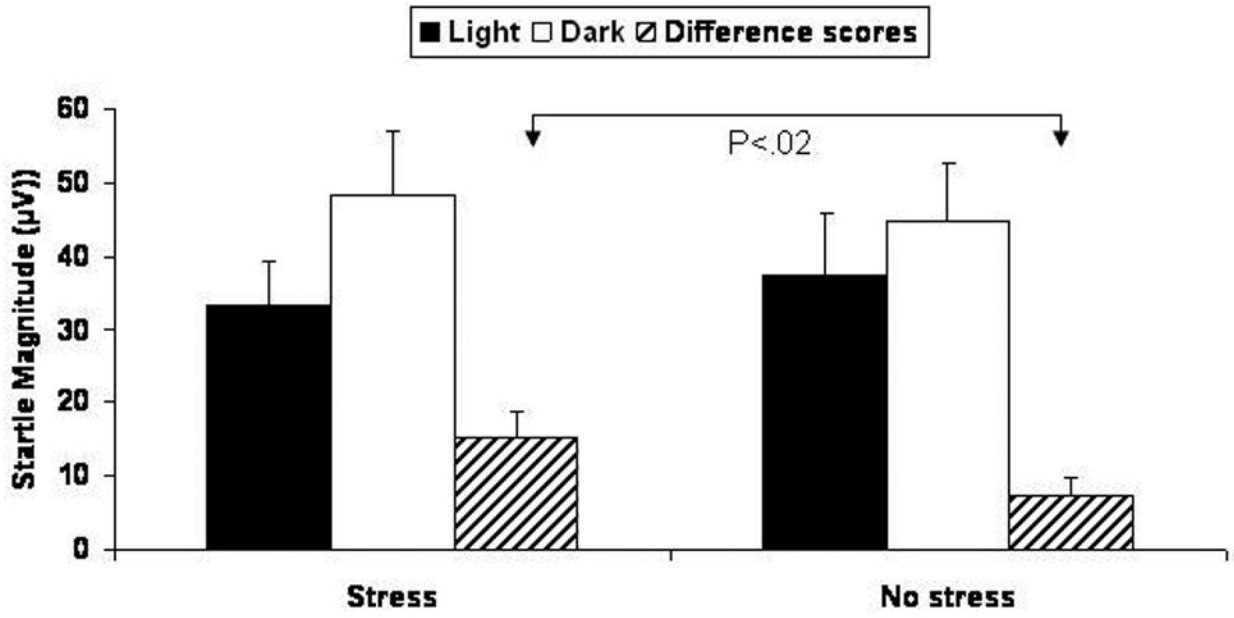

Figure 2.
Startle magnitude and facilitation of startle in the dark (difference scores) during light and dark conditions following the stressor or no stressor.

Table 1

Mean (SEM) salivary cortisol concentration (μg/dL) and alpha-amylase activity (U/ml)), systolic and diastolic blood pressure (BP; mm Hg), heart rate (beats per minute), and distress scores at predetermined time points before and after the social stressor or the control condition.

Measures	Conditions	Baseline^	T +11 min After speech preparation	T +16 min During stress	T +23 min Immediately after stress	T +32 min 10 min after stress	T +41 min Before startle habituation	T +51 min During dark/light startle test
Cortisol	No stress Stress	.20 (.03) .22 (.03)	-	-	.18 (.03) .21 (.02)	.15 (.02) .22 (.03)#	.13 (.02) .18 (.02)#	-
Alpha-amylase	No stress Stress	8.2 (.8) 8.0 (.7)	-	-	8.3 (.9) 11.6 (1.0)*	8.0 (.8) 8.3 (.6)	7.9 (.8) 8.1 (.7)	-
Systolic BP	No stress Stress	112.7 (3.6) 113.8 (3.9)	-	-	108.9 (3.7) 136.2 (3.1)*	111.6 (3.1) 116.8 (4.5)	110.6 (3.4) 114.2 (4.4)	-
Diastolic BP	No stress Stress	68.6 (2.1) 68.0 (2.4)	-	-	69.0 (2.2) 86.6 (2.1)*	68.9 (2.5) 70.5 (2.2)	69.9 (1.9) 69.4 (2.3)	-
Distress	No stress Stress	1.3 (.3) 1.3 (.3)	1.4 (.3) 4.0 (.5)*	-	1.4 (.3) 3.7 (.5)*	-	-	-
Heart rate	No stress Stress	73.7 (2.8) 74.3 (2)	-	72.8 (2.8) 93 (3.1)*	-	-	-	68.1 (2.4) 71.8 (1.8)

#p<.07

*p<.001

^Baseline taken at T-2 min for cortisol, alpha-amylase, BP, distress, and at T-10 min for heart rate

Table 2

Pearson correlations calculated in the stress condition A) between facilitation of startle in the dark (percent change from light to dark) and measures of neuroendocrine activation (difference between baseline and stress levels of salivary cortisol, alpha-amylase, systolic and diastolic blood pressure, heart rate) and B) within neuroendocrine measures.

		Cortisol	Alpha-amylase	Systolic BP	Diastolic BP	Heart rate
A	**Startle**	- 0.22	- 0.18	- 0.09	- 0.02	- 0.14
B	**Cortisol**	.	0.48*	0.57*	0.44	0.66**
	Alpha-amylase	.	.	0.47*	0.23	0.53*
	Systolic BP	.	.	.	0.62**	0.44
	Diastolic BP	.	.	.	.	0.08
	Heart rate	.	.	.	.	.

* $p < 0.05$.

** $p < 0.01$.