

A Single Dose of the Selective Serotonin Reuptake Inhibitor Citalopram Exacerbates Anxiety in Humans: A Fear-Potentiated Startle Study

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Serotonin reuptake inhibitors may increase symptoms of anxiety immediately following treatment initiation. The present study examined whether acute citalopram increased fear-potentiated startle to predictable and/or unpredictable shocks in healthy subjects. Eighteen healthy subjects each received two treatments, placebo and 20 mg citalopram in a crossover design. Participants were exposed to three conditions including one in which predictable aversive shocks were signaled by a cue, a second in which unpredictable shocks were anticipated, and a third in which no shocks were administered. Changes in aversive states were investigated using acoustic startle stimuli. Citalopram did not affect baseline startle. However, the phasic startle potentiation to the threat cue in the predictable condition was robustly increased by acute citalopram. The sustained startle potentiation in the unpredictable conditions was also increased by citalopram, but only when the drug was given during the first session. These results indicate that a single dose of citalopram is not anxiogenic in itself, but can exacerbate the expression of fear and anxiety.

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INTRODUCTION

Serotonin reuptake inhibitors (SSRIs) have become the medication of choice for anxiety and depression, partly because they cause fewer side effects compared to other psychopharmacological treatments. Several studies report symptom improvement in various anxious patient populations following chronic SSRIs. However, the influence of these drugs is not uniform over time. Shortly after treatment initiation, a substantial number of patients report exacerbation characterized by ‘jitteriness’, anxiety, nervousness, restlessness, agitation, irritability, accompanied by sleep and gastrointestinal disturbance (Gorman *et al*, 1987; Ramos *et al*, 1993). This exacerbation of symptoms can be an obstacle to treatment continuation; dropout rates as high as 50% have been reported (Gorman *et al*, 1987).

The mechanisms involved in the anxiolytic and anxiogenic effects of SSRIs are currently unclear. The initially anxiogenic-like effects may result from an acute increased availability of serotonin on inhibitory 5HT_{1A} autoreceptors,

leading to a reduced firing of ascending serotonin raphe neurons, until adaptive changes ensue following chronic treatment (Piñeyro and Blier, 1999). Alternatively, serotonin could affect multiple mechanisms of anxiety. In animals, acute serotonin can increase (Bagdy *et al*, 2001; Griebel *et al*, 1994) or decrease (Inoue *et al*, 1996, 2004; Sanchez and Meier, 1997) anxiety-like responses, depending on the model used. One potential interpretation of these findings is that different animal models represent qualitatively different types of fear or anxiety. This would be consistent with the hypothesis that serotonin has effects on multiple brain structures that mediate anxiety via multiple pathways and receptors (Graeff *et al*, 1997).

Little is known about the effect of SSRIs on experimental anxiety in humans. Acute citalopram facilitates the cortical processing of pleasant stimuli, while reducing the processing of unpleasant stimuli, thus shifting attentional bias from negative to positive stimuli (Kemp *et al*, 2004). Acute citalopram enhances the processing of facial expression of fear and happiness, but not sadness, anger, and disgust, suggesting a selective effect (Harmer *et al*, 2003b). However, the manner in which SSRIs affect emotional responses to more evocative threat cues remains unclear.

Increased understanding of the effect of SSRIs on aversive states may elucidate the psychopharmacological bases of aversive behaviors. As an initial step towards this aim, we investigated the effects of a single dose of citalopram on fear-potentiated startle in humans. A verbal threat proce-

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cedure was implemented instead of a conditioning procedure given that abnormal responses to verbal threat more consistently characterize clinical anxiety states, relative to conditioning procedure (Lissek *et al*, 2005a,b). Moreover, this design focuses on modulatory influences on expressions of aversive responses rather than learning and memory processes inherently associated with associative learning procedures (Grillon and Baas, 2003).

Following up on works by Davis and collaborators using the startle reflex (Walker *et al*, 2003), we identified two threat responses: a phasic fear-potentiated startle response associated with a cue and a more sustained startle potentiation generated by less explicit threat (Grillon *et al*, 2005, 2004) by administering predictable (ie signaled by a cue) or unpredictable (ie unsignaled) shocks, respectively. The main objective of this study was to examine the effect of citalopram on these two types of responses.

It was expected that acute citalopram would facilitate phasic cued fear-potentiated startle. This hypothesis was based on converging evidence that the processing of and reaction to threat cues is modulated by SSRIs. Thus, acute citalopram enhances cued fear conditioning in rodents (Burghardt *et al*, 2004) and facilitates the processing of fear faces in humans (Harmer *et al*, 2003b). In addition, acute administration of serotonin-receptor blockers resanaterin decreases conditioned responses to aversive loud noises (Hensman *et al*, 1991; Silva *et al*, 2001). No hypothesis was formulated concerning the effect of acute citalopram on sustained potentiated startle to unpredictable shocks because the pre-clinical literature has provided conflicting evidence. For example, SSRIs have different effects on two models of sustained anxiety, contextual fear, and light-enhanced startle. Citalopram impairs context conditioning (Inoue *et al*, 1996, 2004) but fluvoxamine does not affect light-enhanced startle (de Jong *et al*, 2002).

METHODS

Participants

Participants were healthy volunteers who gave written informed consent approved by the NIMH Human Investigation Review Board. Inclusion criteria included (1) no past or current psychiatric disorders as per Structured Clinical Interview for DSM-IV (SCID: (First *et al*, 1995)), (2) no medical condition that interfered with the objectives of the study as established by a physician, and (3) no use of elicitor drugs or psychoactive medications as per urine screen.

Participants underwent a screening session that consisted of a SCID, a physical exam, and a shock workup procedure to establish a level of shock that was 'highly annoying but not painful.' In addition, subjects were screened for baseline startle reactivity with nine startle stimuli (40-ms duration, 103 dB). Subjects who displayed small startle responses (a mean of less than 5 μ V over nine startle responses), or no startle response on at least one trial were not invited to participate in the study. Four to 10 days after screening, participants returned for the first of two testing sessions. Eighteen subjects (nine males) with a mean age of 26.5 years (SD = 7.9 years) were ultimately included in the study with a mean trait anxiety score based on Spielberger's

State and Trait Anxiety Inventory (Spielberger, 1983) of 25.8 (SD = 3.8).

Drug Manipulation

The treatments were 20 mg citalopram and placebo, tested in a double-blind crossover design (within-subjects). The order of treatment was counterbalanced across subjects. Half the subjects were given placebo during the first session.

Procedure

The procedure was similar to that of a recent study that tested the effect of alprazolam (Grillon *et al*, in press). On the test day, the electrodes to measure the eyeblink were applied. Next, subjects ingested a capsule containing citalopram or placebo before resting for 2 h to allow drug absorption, after which the shock electrodes were attached.

To test the effect of citalopram on startle reactivity, two blocks of nine startle stimuli (inter-startle stimulus interval: 18–25 s) were presented. One block was given just before ingestion of the drug (pre-drug startle) and the other 2 h later (post-drug startle), just before application of the shock electrodes. Participants were then given explicit instructions regarding the conditions under which they would and would not receive a shock. Following the instructions, the threat experiment began. The experiment consisted of three different conditions, a no shock condition (N), and two conditions during which shocks were administered either predictably (P), that is, only in the presence of a threat cue, or unpredictably (U). Each condition lasted approximately 150 s. In each 150-s condition, an 8-s cue was presented four times. The cues were different geometric colored shapes in the different conditions (eg blue square for N, red circle for P). The cues signaled the possibility of receiving an aversive stimulus only in the P condition, but had no signal value in the N and U conditions. Participants were verbally instructed regarding the risk of shock in the different conditions and they were informed of the contingency or lack of contingency between shock and cues in the P and U conditions. In addition, instructions were also shown on a computer monitor throughout the experiment displaying the following information: 'no shock' (N), 'shock only during shape' (P), or 'shock at any time' (U). During each predictable and unpredictable condition, one shock was administered, during the cue in the predictable condition and in the absence of the cues in the unpredictable condition. In each N, P, and U condition, six acoustic startle stimuli were delivered, three during inter-trial intervals (ITI; that is, between cues) and one during three of the four cues, 5–7 s following cue onset. The threat experiment consisted of two recording blocks with a 5–10 min rest between blocks. Each block started with the delivery of six startle stimuli (pre-test startle) and consisted of three N, two P, and two U conditions in one of the following two orders: P N U N U N P or U N P N P N U. Each participant was presented with the two orders, with half the participants starting with the P condition. One shock was administered in each individual P and U condition for a total of four shocks in the four P conditions and four shocks in the four U conditions. The shock was delivered 7.5 s following cue onset in the P condition. It was

administered either 7 or 10 s following cue offset in the unpredictable condition. No startle stimuli could follow a shock by less than 10 s.

Questionnaires were given just before taking the treatments and 2 h later to assess the effects of the treatment. They included Spielberger's state portion of the state-trait anxiety inventory questionnaire (Spielberger, 1983), and a mood rating scale that evaluated subjective feelings of mental and physical sedation. In addition, after each recording block, subjects retrospectively rated how anxious they felt in the presence and absence of the cue in each condition (N, P, U) on an analog scale ranging from 0 (not at all anxious) to 10 (extremely anxious).

Stimuli and Physiological Responses

Stimulation and recording were controlled by a commercial system (Contact Precision Instruments, London, UK). The acoustic startle stimulus was a 40-ms duration, 103 dB (A) burst of white noise with a near instantaneous rise time presented binaurally through headphones. The eyeblink reflex was recorded with electrodes placed under the left eye. Amplifier bandwidth was set to 30–500 Hz. The electric shock was produced by a constant current stimulator and administered on the left wrist.

Data Analysis

Peak amplitude of the blink reflex was determined in the 20–100-ms time frame following stimulus onset relative to baseline (average baseline EMG level for the 50 ms immediately preceding stimulus onset) and averaged within each condition. The startle data and retrospective measures of subjective anxiety were analyzed with analyses of variance (ANOVA) with repeated measures. Preliminary analyses indicated no sex difference for the startle and subjective measures. Hence, sex was not entered as a factor in the data analysis. Alpha was set at 0.05 for all statistical tests. Greenhouse-Geisser corrections (GG- ϵ) were used for main effects and interactions involving factors with more than two levels.

RESULTS

Startle Data

Effect of citalopram. The pre-drug and post-drug baseline startle magnitudes are shown in Table 1. Baseline startle was not affected differently by placebo and citalopram. A Treatment (placebo, citalopram) \times Time (pre-drug, post

drug) \times Order (placebo first session, citalopram first session) ANOVA revealed no significant main effect or interaction effect (all $p > 0.1$) on baseline startle.

Early contextual effect. Placing the shock electrodes and starting a threat experiment can potentiate startle (Grillon and Ameli, 1998). To examine the effect of citalopram on this type of startle potentiation, post-drug startle magnitude was compared to the pre-test startle (ie in responses to the leading startle stimuli delivered at the beginning of the first threat block). Table 1 shows that the increase in startle magnitude from post-drug to pre-test was greater with citalopram compared to placebo. Statistical analyses using a Treatment (placebo, citalopram) \times Time (post-drug, pre-test) \times Order (placebo first session, citalopram first session) ANOVA confirmed this finding. There was a significant Time main effect ($F(1,16) = 9.8, p < 0.006$) that was qualified by a significant Time \times Treatment interaction ($F(1,16) = 6.1, p < 0.02$), with no significant Treatment \times Order or Time \times Treatment \times Order effect ($p > 0.1$). Follow-up t -tests revealed that the increased startle magnitude was significant with citalopram ($+14.5 \mu\text{V}$, $\text{SEM} = 3.6 \mu\text{V}$; $t(17) = 3.9, p < 0.001$) but not with placebo ($+3.4 \mu\text{V}$, $\text{SEM} = 3.6 \mu\text{V}$; $t(17) = 0.9, \text{NS}$).

Effect of citalopram of fear-potentiated startle to the threat cue. Startle magnitudes in each condition and in each treatment are presented in Table 1. As expected (Grillon et al, 2004), the increase in startle during the cues relative to ITI (fear-potentiated startle) was larger in the predictable condition compared to the two other conditions (Condition \times Cue; $F(2,32) = 21.2, p = 0.001$). Startle was potentiated by the cue relative to ITI in the predictable condition ($F(1,17) = 31.1, p < 0.0009$) and to a lesser extent in the unpredictable condition ($F(1,17) = 8.4, p < 0.01$), but not in the no shock condition. Importantly, the magnitude of fear-potentiated startle to the cues was affected differently by the two treatments (Treatment \times Condition \times Cue interaction: $F(2,32) = 3.2, p < 0.05, \text{GG-}\epsilon = 0.97$). (There was no significant effect with the factor Order.) To facilitate the analysis of the three-way interaction, fear-potentiated startle scores were computed by calculating the difference scores between startle magnitude during the cues and startle magnitude during ITI (Figure 1). Planned comparisons revealed significantly greater fear-potentiated startle effects with citalopram compared to placebo in the predictable condition ($F(1,17) = 4.7, p < 0.04$). No differential effect of treatment was found in the unpredictable condition ($F(1,17) = 0.1, \text{NS}$).

Table 1 Mean (SEM) Startle Magnitude (μV) before and after Treatment and during the Cue and ITI Across Treatments and Conditions

	Pre-drug	Post-drug	Pre-test	Neutral		Predictable		Unpredictable	
				Cue	ITI	Cue	ITI	Cue	ITI
Placebo	30.6 (9.1)	38.3 (10.7)	41.7 (11.3)	27.6 (5.3)	30.0 (6.1)	53.6 (8.1)	39.8 (8.1)	52.1 (8.0)	46.6 (6.8)
Citalopram	32.7 (11.3)	33.4 (11.4)	47.4 (13.1)	24.5 (5.2)	23.9 (5.3)	58.6 (9.8)	35.2 (6.3)	52.0 (8.6)	46.8 (7.9)

ITI, inter-trial interval; SEM, standard error of mean.

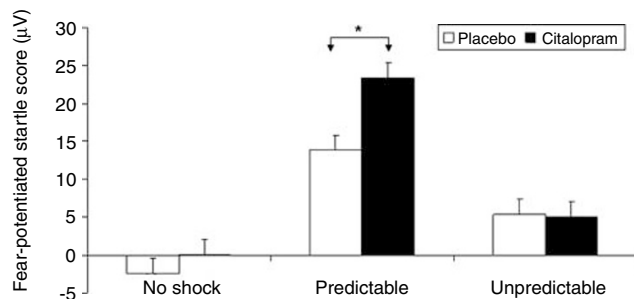


Figure 1 Phasic fear-potentiated startle response in each treatment: Difference scores reflecting cue minus ITI startle magnitudes in the no shock, predictable, and unpredictable conditions. *Significant ($p < 0.05$) difference between placebo and citalopram.

Contextual anxiety. Contextual anxiety was evaluated using the ITI startle data (Table 1), which were entered into a Treatment (placebo, citalopram) \times Condition (N, P, U) \times Order (placebo first session, citalopram first session) ANOVA. This analysis contrasts with the earlier context analysis by focusing on ITI startle responses throughout the entire experiment. There was a Condition main effect ($F(2,32) = 16.8$, $p < 0.0009$, $GG-\epsilon = 0.83$), owing to a progressive increase in ITI startle amplitude from the no shock, to the predictable, to the unpredictable condition (Linear trend: ($F(1,16) = 19.6$, $p < 0.0009$), a trend for a Treatment \times Condition ($F(2,32) = 2.7$, $p < 0.08$, $GG-\epsilon = 0.83$) interaction, and a significant Treatment \times Condition \times Order interaction ($F(2,32) = 3.9$, $p < 0.03$, $GG-\epsilon = 0.83$). To better understand the nature of the three-way interaction, we calculated the increase in ITI startle magnitude from the neutral condition to the predictable and unpredictable conditions (predictable minus neutral and unpredictable minus neutral). The resulting scores were entered into separate Treatment (placebo, citalopram) \times Condition (P, U) session ANOVAs for subjects who were given placebo in the first session (and citalopram in the second session) and for subjects who were given citalopram in the first session (and placebo in the second session). Results showed that startle was significantly increased by citalopram when subjects received citalopram during the first session ($F(2,16) = 3.9$, $p < 0.04$, $GG-\epsilon = 0.85$), not when subjects were given placebo in the first session ($F(2,16) = 0.6$, NS). These effects are shown in Figure 2.

Mood Ratings

The mental and physical rating scores of one subject were lost. Subjects showed no significant effect of citalopram on state anxiety, and on mental and physical sedation (Table 2). Data were analyzed using separate Treatment (placebo, citalopram) \times Time (pre-drug, post drug) \times Order (placebo first session, citalopram first session) ANOVAs. For state anxiety and mental sedation, there were significant Time effect, due to a slight increase in state anxiety ($F(1,16) = 5.5$, $p < 0.03$), and mental ($F(1,15) = 4.8$, $p < 0.04$) and physical ($F(1,15) = 13.6$, $p < 0.0009$) sedation after both treatments. However, there was no significant differential effect of treatment on these ratings (no significant Condition \times Treatment).

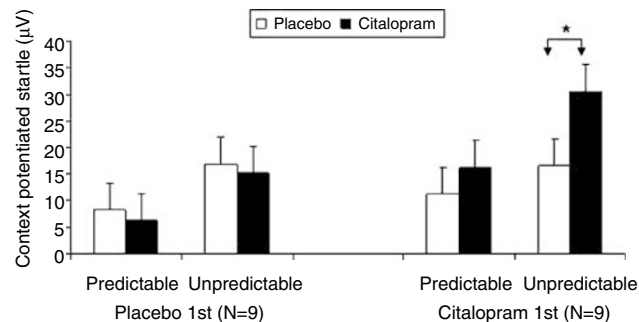


Figure 2 Sustained potentiated startle responses in each treatment: Difference scores between ITI startle magnitudes in the threat conditions (predictable and unpredictable) and ITI startle magnitude in the no shock condition. *Significant ($p < 0.05$) difference between placebo and citalopram.

Table 2 Mean (SEM) State Anxiety, and Physical and Mental Sedation before and after Treatment

	Physical sedation		Mental sedation	
	Pre-drug	Post-drug	Pre-drug	Post-drug
Placebo	9.5 (0.9)	11.8 (1.2)	11.0 (1.3)	12.1 (1.4)
Citalopram	10.8 (1.3)	13.1 (1.5)	12.4 (1.5)	13.6 (1.7)

SEM, standard error of mean.

Retrospective Ratings of Anxiety

The subjective anxiety ratings were analyzed in a similar way as the eyeblink data.

Cued fear. The retrospective anxiety rating scores are shown in Table 3. Subjective anxiety to the cues was differentially affected by the contexts (Condition \times Stimulus Type; $F(2,30) = 17.2$, $p < 0.0009$, $GG-\epsilon = 0.91$). Subjective anxiety was higher during the cue compared to ITI in the predictable condition ($F(1,17) = 17.3$, $p < 0.001$), but not in the unpredictable ($F(1,17) = 2.8$, NS) or the no shock ($F(1,17) = 0.1$, NS) conditions. The increased anxiety in the predictable condition was not affected by the treatment ($F(1,17) = 0.9$, NS).

Contextual anxiety. Like ITI startle amplitude, subjective anxiety increased linearly from the no shock, to the predictable, and to the unpredictable condition ($F(1,16) = 146.3$, $p < 0.0009$). Relative to placebo, citalopram did not significantly affect subjective anxiety, as reflected by a lack of Treatment main effect ($F(1,16) = 0.7$, NS) or Condition \times Treatment interaction ($F(2,32) = 2.5$, NS).

DISCUSSION

The present study found that startle amplitude was not significantly affected by citalopram before the threat test was initiated. In contrast, citalopram increased the magnitude of multiple forms of startle potentiation, including fear potentiated to a discrete threat cue, sustained potentiated

Table 3 Mean (SEM) Retrospective Rating of Anxiety during the Cue and ITI Across Treatments and Conditions

	Neutral		Predictable		Unpredictable	
	Cue	ITI	Cue	ITI	Cue	ITI
Placebo	1.7 (0.2)	1.9 (0.3)	6.7 (0.6)	4.5 (0.6)	5.8 (0.6)	6.1 (0.6)
Citalopram	2.2 (0.5)	1.9 (0.3)	6.4 (0.6)	4.0 (0.5)	5.8 (0.6)	6.5 (0.6)

ITI, inter-trial interval; SEM, standard error of mean.

startle to unpredictable shock, and the contextual startle potentiation in the early phase of the threat experiment. These results suggest that citalopram was not anxiogenic *per se* in the absence of danger, but that it can exacerbate anxiety experienced in responses to threat. This later effect is consistent with clinical evidence of anxiogenic reactions, shortly following treatment initiation with SSRIs in patients with anxiety disorders.

In rodents, acute citalopram increases the acquisition of cued fear conditioning (Burghardt *et al*, 2004). This effect was attributed (Burghardt *et al*, 2004) to better learning, based on the finding that acute citalopram facilitates recall and recognition of verbal material in humans (Harmer *et al*, 2002). Acute citalopram also facilitates the recognition of fear from facial expression, suggesting enhancement of the processing of threatening cue in the environment independent of any effect on memory acquisition (Harmer *et al*, 2003b). It is unlikely that the facilitation of cued fear-potentiated startle in the present study was due to either better learning/memory process or enhanced recognition of threat cues. Subjects were instructed of the contingency between the shock and the threat cue, and instructions concerning the various threat conditions were clearly displayed on the monitor facing the subjects throughout testing (See Methods). Prior studies with this paradigm show that threat cues are very easy to discriminate from safe cues (Grillon *et al*, 1991). The findings also cannot be due to enhanced recognition of the threat signal because fear was probed with the startle reflex several seconds after cue onset, leaving subjects ample time to recognize the non-ambiguous threat cue. The most straightforward interpretation of the results is that acute citalopram facilitated the expression of an aversive response. Taken together, these results indicate that acute SSRIs can affect multiple levels (eg perception, expression) of the aversive motivational system(s). Hence, acute SSRIs may act to enhance fear/anxiety independent of its facilitative effects on more intermediary encoding and retrieval processing stages. All these effects may be causally related to the multitude of symptoms experienced by patients following treatment initiation with SSRIs.

The subjective data did not match the startle findings. Similar dissociations between objective measures and subjective reports have been reported. The increased recognition of fearful faces with a single dose of citalopram arises in the absence of subjective mood changes (Harmer *et al*, 2003b). Similarly, acute citalopram affects behavioral, cortical, and physiological responses to emotional stimuli in the absence of conscious changes in subjective feelings (Kemp *et al*, 2004). One obvious reason for the differential effect of citalopram on physiological and subjective reports in the present study is that startle was used to probe anxiety

online, whereas the subjective anxiety measures were retrospective. The passage of time may have obscured subtle differences in responding because of the complexity of the design. Alternatively, startle and subjective ratings may reflect distinct processes differently sensitive to acute citalopram. Startle is a low-level response and verbal report of emotions involves more elaborate cognitive activity. Potentially, startle may be able to uncover early drug effects before changes in subjective feelings. This would be consistent with findings that the amygdala can be activated without alteration in conscious mood (Morris *et al*, 1998).

Phasic responses to a predictable threat cue and sustained responses to unpredictable shocks were investigated because of evidence that these responses are activated by distinct neural mechanisms in rodents (Walker *et al*, 2003). More specifically, fear-potentiated startle to a discrete cue is mediated by the amygdala, whereas the more sustained startle potentiation is mediated by both the amygdala and the bed nucleus of the stria terminalis (BNST) (Walker *et al*, 2003). We recently reported a psychopharmacological dissociation of responses of these two types of responses in humans. The benzodiazepine alprazolam reduced potentiated startle to unpredictable shocks, but not to predictable shocks (Grillon *et al*, in press). In the present study, there was no strong differential effect of citalopram on responses to predictable and unpredictable shocks. Although acute citalopram led to a robust increase in cued fear independent of treatment order, it facilitated the sustained startle potentiation in the unpredictable condition only in subjects who received citalopram during the first session (and placebo in the second session). In addition, citalopram increased baseline startle during the early phase of the threat experiment (when subjects were not at imminent risk of receiving a shock), reflecting an initial potentiation of contextual fear. This effect did not last as suggested by the fact that there was no effect of treatment on ITI startle in the no shock condition. One common factor between these two aversive situations (unpredictable condition and early contextual anxiety) is that the intensity of anxiety elicited diminished with time. Anxiety to unpredictable shocks is usually the strongest during the first of multiple sessions (unpublished observation) and contextual anxiety is stronger earlier compared to later in the experiment. Hence, one potential interpretation for the selective effect of citalopram is that this drug exacerbated anxiety responses only when these responses were sufficiently intense, but not when they were weaker. This explanation should be tested by examining the effects of citalopram on different levels of fear.

Serotonin modulation in the amygdala alone or in combination with serotonin modulation in the BNST

following a single dose of citalopram could explain startle potentiation to the different types of threat. The amygdala, which receives serotonergic innervations from the median raphe nucleus (Vertes *et al*, 1999), is involved in fear to verbal threat (Phelps *et al*, 1998; Funayama *et al*, 2001). Consistent with this hypothesis, the amygdala is also involved in other types of responses that are facilitated by acute citalopram, that is, cued fear conditioning (Burghardt *et al*, 2004) and the processing of fearful faces (Harmer *et al*, 2003a). The amygdala is also involved in the initiation of sustained aversive responses (Walker *et al*, 2003). Furthermore, acute treatment with SSRIs increases c-fos immunoreactivity in the amygdala (Lino-de-Oliveira *et al*, 2001). It is also possible that serotonin modulation in the BNST mediates the anxiogenic effects of citalopram on responses to unpredictable shocks given that this structure, which modulates response to unpredictable stressors, also receives serotonergic innervations from the raphe nucleus (Phelix *et al*, 1992). However, at the present time, little is known about the role of the BNST in humans.

The anxiogenic effects of acute citalopram are likely to be mediated by action on the reuptake of serotonin because citalopram has minimal effect on dopamine and noradrenalin transporters and has little affinity for a variety of neurotransmitter receptors (Noble and Benfield, 1997; Pollock, 2001). An acute reduction in serotonin availability secondary to activation of 5HT_{1A} autoreceptors has been proposed as a potential mechanism (Piñeyro and Blier, 1999). However, this may not be the case because lowering serotonin availability does not always increase responses to threat. For example, depleting serotonin precursor does not increase, but decreases the recognition of fearful faces (Harmer *et al*, 2003b). Alternatively, results from two animal models suggest that the anxiogenic effect of SSRIs could be mediated by activation of 5HT_{2C} (Bagdy *et al*, 2001). An indirect mechanism could also be responsible. Acute citalopram can increase cortisol levels (Attenburrow *et al*, 2001). Given that cortisol can facilitate fear in the amygdala and the BNST (Schulkin *et al*, 2005), it is possible that increased fear in the present study was not due to a direct effect of serotonin, but to the facilitation of amygdala- and/or BNST-mediated fear by cortisol.

To summarize, acute citalopram increased the expression of fear-related responses to different types of stimuli. This effect is consistent with clinical reports of symptom exacerbation following treatment initiation (Gorman *et al*, 1987; Ramos *et al*, 1993) and with evidence of facilitation of processing threat cues in experimental models following acute SSRIs (Burghardt *et al*, 2004; Harmer *et al*, 2003b). Fear-potentiated startle is therefore a useful tool to examine the effects of SSRI agent. Because the efficacy of SSRIs develops over time, future studies should examine whether the expression of fear-potentiated startle is reduced by chronic SSRIs in both humans and animals. Such an integrative approach will provide insight into the mechanisms underlying the therapeutic effects of SSRIs.

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