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Acute SSRIs Increase Conditioned Fear Expression: Blockade with a 5-HT$_{2C}$ Receptor Antagonist

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Abstract

Background— SSRIs effectively treat various anxiety disorders, although symptoms of anxiety are often exacerbated during early stages of treatment. We previously reported that acute treatment with the SSRI citalopram enhances the acquisition of auditory fear conditioning, which is consistent with the initial anxiogenic effects reported clinically. Here, we extend our findings by assessing the effects of acute SSRI treatment on the expression of previously acquired conditioned fear.

Methods— Rats underwent fear conditioning drug-free. Tone-evoked fear responses were tested after drug treatment the following day. This protocol more closely resembles the clinical setting than pre-conditioning treatment, since it evaluates effects of treatment on a pre-existing fear, rather than on the formation of a new fear memory.

Results— A single pre-testing injection of the SSRIs citalopram or fluoxetine significantly increased fear expression. There was no effect of the antidepressant tianeptine, or the norepinephrine reuptake inhibitor, tomoxetine, indicating that this effect is specific to SSRIs. The SSRI induced enhancement in fear expression was not blocked by tropisetron, a 5-HT$_3$ receptor antagonist, but was blocked by SB 242084, a specific 5-HT$_{2C}$ receptor antagonist.

Conclusions— Enhanced activation of 5-HT$_{2C}$ receptors may be a mechanism for the anxiogenic effects of SSRIs observed initially during treatment.

Keywords

fear conditioning; citalopram; 5-HT$_{2C}$ receptor; amygdala; serotonin; 5-HT$_3$ receptor

Introduction

Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed to treat depression (Bondareff et al 2000; Stahl 2000) as well as a range of anxiety disorders, such as panic disorder, obsessive compulsive disorder, post-traumatic stress disorder, and social anxiety disorder (Kent et al 1998; Pollack and Doyle 2003; Stein and Stahl 2000). Typically, several weeks of treatment with SSRIs are necessary before patients experience the therapeutic effects (Feighner and Boyer 1991), and symptoms of anxiety or agitation are frequently exacerbated when treatment is first initiated (Mir 1997; Spigset 1999). To minimize this initial “anxiogenic”

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effect, drug dose is titrated (Gorman et al 1987) and benzodiazepines are often prescribed concomitantly (Bingefors and Isacson 1998; Gregor et al 1996). However, benzodiazepines can lead to adverse effects (O'Brien 2005; Verster and Volkerts 2004), and some evidence indicates they may decrease the therapeutic effects of SSRIs (Martin and Puech 1996). Thus, it is important to develop our understanding of the mechanisms underlying this anxiogenic effect, since advances could lead to alternative treatment options.

A number of animal studies using various tests of anxiety, such as the social interaction test, elevated-plus maze, and the two-compartment black and white box also report an anxiogenic-like effect of SSRIs following acute treatment (Dekeyne et al 2000; Griebel et al 1994; Matto et al 1996; Sanchez and Meier 1997). Also, in our previous study we found that acute SSRI treatment increases fear when administered prior to fear learning (Burghardt et al 2004).

The advantage of using auditory fear conditioning is that it is a model of fear for which the neural circuitry has been elucidated in detail (LeDoux 2000; Maren 2001). In this procedure, a neutral conditioned stimulus (CS), such as a tone, elicits defensive responses after being paired with an aversive unconditioned stimulus (US), typically a footshock. An extensive body of evidence indicates that the acquisition and expression of fear conditioning depends on the amygdala (LeDoux 2000; Maren 2001; Muller et al 1997), a brain region that has been implicated in a variety of anxiety disorders (Britton et al 2005; Cannistraro et al 2004; Milham et al 2005). Imaging and electrophysiological studies reveal that amygdala activity is modulated by the serotonin transporter gene (Canli et al 2005; Hariri et al 2002) and serotonin neurotransmission (Stutzmann et al 1998). Furthermore, a single systemic SSRI injection leads to an increase in amygdala extracellular serotonin (Bosker et al 2001), an increase in amygdala Fos-like immunoreactivity (Morelli et al 1999; Veening et al 1998), and changes in amygdala activity in healthy humans (Del-Ben et al 2005; McKie et al 2005). Together, these studies, as well as our previous fear conditioning study (Burghardt et al 2004), indicate that the amygdala may be an important site of action for the anxiogenic effects of acute SSRI treatment.

As a means of gaining further insight into how acute SSRI treatment alters amygdala-dependent fear, the present study extends our previous findings by assessing the effects of acute SSRI treatment on the expression of conditioned fear. Unlike the previous study, rats were trained to associate the CS and US drug-free, and were injected with drug the next day, prior to exposure to the fear provoking CS. Given that patients are typically treated with SSRIs for their anxiety symptoms after the disorder has already developed, the present focus on fear expression more closely resembles the clinical setting. We evaluated the effects of two SSRIs, citalopram and fluoxetine, on conditioned fear expression, and compared their effects to those of tianeptine, an effective antidepressant that is proposed to be a serotonin reuptake enhancer, and tomoxetine, a norepinephrine reuptake inhibitor.

In an effort to better understand the mechanisms through which SSRIs affect fear circuits, we also explored the role of specific serotonin receptor subtypes in mediating the effects of citalopram on conditioned fear expression. We focused on the 5-HT_{2C} and 5-HT_3 receptor subtypes because previous studies have shown that their presence in the amygdala influences its excitability (Stein et al 2000), and blocking them systemically with selective antagonists alters fear in several animal models (Costall 1991; Martin et al 2002), including fear conditioning (Hensman et al 1991; Yoshioka et al 1995). We therefore tested whether blocking these receptors with their respective antagonists, SB 242082 and tropisetron, alone or in combination with acute citalopram treatment, affects the expression of conditioned fear.

Materials and Methods

Subjects

Adult male Sprague-Dawley rats (Hilltop Laboratories, Scottdale, PA) weighing 350–400g were housed individually in clear plastic cages in a thermally controlled colony room. They were placed on a 12 hr light/dark cycle and food and water were provided ad libitum throughout the duration of the experiment. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Experimental Animals and were approved by the New York University Animal Care and Use Committee.

Drugs

Citalopram hydrobromide, fluoxetine hydrochloride, tomoxetine, SB 242084, and tropisetron were purchased from Sigma-Aldrich Co. (St. Louis, MO). Tianeptine sodium salt was provided by Servier (Courbevoie, France). SB 242084 and tomoxetine were dissolved in pure water and all other drugs were dissolved in 0.9% sterile saline. Drugs were administered at the following doses, which were chosen based on previous work that found these drugs to be effective in a fear conditioning task and/or another anxiety-related tasks: Citalopram (10 mg/kg) (Burghardt et al 2004; Matto and Allikmets 1999); Fluoxetine (10 mg/kg) (Bodnoff et al 1989; Silva and Brandao 2000); Tianeptine (10 mg/kg) (Burghardt et al 2004; Conrad et al 1999); Tropisetron (0.1 mg/kg) (Yoshioka et al 1995); SB 242084 (0.2 mg/kg) (Bagdy et al 2001). Tomoxetine was administered at 1 mg/kg, which has been shown to effectively increase extracellular norepinephrine levels (Bymaster et al 2002). Previous studies indicate that tropisetron and SB 242084 selectively antagonize 5-HT_3 and 5-HT_{2C} receptors, respectively, in vivo at the doses used in this study (Higgins et al 1993; Kennett et al 1997). All drugs were injected intraperitoneally (i.p.) in a volume of 1 ml.

Apparatus

Rats were fear conditioned in a Plexiglas rodent conditioning chamber that was brightly lit with three white house lights, and contained a metal grid floor (ENV-001; Med Associates, Inc. Georgia, VT). During tone testing, the context of this chamber was altered with a fitted flat black Formica floor cover scented with peppermint soap and was dimly lit with two house lights covered with red lenses. Previous studies have shown that this testing environment is distinct enough to minimize generalization from the training environment (Nader and LeDoux 1999; Schafe et al 1999). Behavior during training and testing was videotaped with a camera mounted at the top of the chamber.

Habituation, Auditory Fear Conditioning, and Testing

Rats were habituated to the conditioning context for 20 minutes, as well as to handling. Fear conditioning occurred the next day, during which rats were trained in a single conditioning session involving two pairings of a 20-sec tone CS (10 kHz, 75 dB) that co-terminated with a footshock US (0.5 sec, 0.7 mA), with an inter-trial interval that varied randomly between 90 and 120 sec. After conditioning, rats were returned to their home cages and freezing was scored (see below) so that natural variability in acquisition could be counterbalanced across treatment groups the next day. This particular training protocol was selected because pilot studies determined that it produced approximately 50% of maximal freezing during the first tone presentation in vehicle-treated rats at test. This allowed for the detection of increases in freezing resulting from drug treatment, and was the protocol used in our previous study, which found that acute citalopram treatment increases fear when administered before conditioning (Burghardt et al 2004).

Twenty-four hours after conditioning, separate groups of rats were weighed and injected with citalopram, fluoxetine, tianeptine, tomoxetine, or their respective vehicle. Each drug treated group was tested with a separate vehicle group. In the experiment involving the 5-HT_3 antagonist, rats received a single injection of citalopram, tropisetron, citalopram + tropisetron, or saline. In the experiment with the 5-HT_{2C} antagonist, we followed a protocol similar to that used by Dekeyne et al (2000), and rats were given two injections. The first injection of SB 242084 or water was given 15 minutes before the second injection of citalopram or saline (Dekeyne et al 2000). All treatment began 60 minutes before testing.

During testing, rats were presented with ten 20-sec CS tones (10 kHz, 75 dB; inter-trial interval, 90–120 sec), without the US. The inter-trial interval and the properties of the tones presented during training and testing were identical. The experimenter, who was blind to the treatment group, measured fear expression from the videotape using a timer to count the number of seconds rats spent freezing during each 20 second tone. Freezing was expressed as a percentage of the total tone presentation time. Baseline freezing was measured by scoring freezing during the 20-sec interval prior to the first tone onset on the testing day. Freezing was defined as the absence of all movement, with the exception of breathing. Data were analyzed using Student's *t*-test for independent samples, or a two- or three-way ANOVA followed by Tukey's HSD post hoc test, and significance levels were set at $p<0.05$.

Results

Acute SSRI Treatment Enhances Conditioned Fear Expression

Animals given an injection of citalopram (10 mg/kg, i.p.) 60 minutes before testing showed enhanced fear expression (Figure 1). We performed a two-way ANOVA with factors: SSRI group (citalopram vs. saline) and tone trial (1–10; repeated measures). There was a significant main effect of group [$F_{1,20}=14.97$, $p<.01$], indicating that citalopram-treated animals froze significantly more than control animals during testing. There was a significant effect of tone trial [$F_{9,180}=7.37$, $p<.01$], indicating that fear responses extinguished over the course of 10 CS presentations. The group × tone trial interaction was not significant [$F_{9,180}=.96$, $p=.47$], suggesting that citalopram treatment did not alter the rate of fear reduction over the trials. Rats showed virtually no freezing prior to the initial CS, and a *t*-test on baseline freezing indicated no significant difference between groups [$t_{20}=.46$, $p=.65$].

Animals treated acutely with fluoxetine (10 mg/kg, i.p.) showed a similar enhancement in conditioned fear expression (Figure 1). An ANOVA with factors: SSRI group (fluoxetine vs. saline) and tone trial (1–10; repeated measures) revealed a significant effect of group [$F_{1,18}=8.93$, $p<.01$], a significant effect of tone trial [F9,162=162, $p<.05$], and no significant group × tone trial interaction [F9,162=.35, $p=.96$]. This indicated that acute fluoxetine treatment also significantly increased freezing compared with control animals across tones. There was no significant difference between groups in baseline freezing [$t_{18}=1.21$, $p=.24$], indicating that the enhancement in freezing was specific to the tone. Together, these findings indicate that acute treatment with either citalopram or fluoxetine increases the expression of conditioned fear.

An Acute Injection of Tianeptine Does Not Affect the Expression of Conditioned Fear

In contrast, animals that received a single pre-testing injection of tianeptine (10 mg/kg, i.p.) showed no significant difference from control animals in their levels of freezing during testing (Figure 2). The ANOVA with factors: drug group (tianeptine vs. saline) and tone trial (1–10; repeated measures) revealed no significant effect of group [$F_{1,19}=2.0$, $p=.66$] and no significant group × tone trial interaction [$F_{9,171}=.20$, $p=.99$]. A significant effect of tone trial [$F_{9,171}=12.1$,

$p<.01$] was observed, indicating that fear extinguished throughout the test session. A *t*-test revealed no significant difference between groups in baseline freezing [$t_{19}=1.74$, $p=.10$].

Acute Treatment with Tomoxetine Does Not Affect Conditioned Fear Expression

A pre-testing injection of the norepinephrine reuptake inhibitor, tomoxetine (1 mg/kg, i.p.), did not significantly affect conditioned fear expression (Figure 2). The ANOVA with factors: drug group (tomoxetine vs. water) and tone trial (1–10; repeated measures) revealed no significant main effect of group [$F_{1,15}=1.51$, $p=.24$] and no significant group × tone trial interaction [$F_{9,135}=1.10$, $p=.37$]. The significant effect of tone trial [$F_{9,135}=8.16$, $p<.01$] indicated extinction in both groups during the test session. There was also no significant effect of tomoxetine on baseline freezing compared to controls [$t_{15}=1.42$, $p=.18$].

An Injection of a 5-HT_3 Antagonist Does Not Block the Effect of Citalopram on Conditioned Fear Expression

Injecting the 5-HT_3 antagonist, tropisetron (0.1 mg/kg, i.p.), with citalopram (10 mg/kg, i.p.) did not alter the enhancing effects of citalopram (10 mg/kg, i.p.) on conditioned fear expression (Figure 3). We performed a three-way ANOVA with factors: antagonist (tropisetron vs. saline), SSRI (citalopram vs. saline), and tone trial (1–10; repeated measures). There was a significant main effect of SSRI [$F_{1,45}=29.12$, $p<.01$] and tone trial [$F_{9,405}=16.85$, $p<.01$] but no significant main effect of antagonist [$F_{1,45}=.14$, $p=.71$]. There was a significant SSRI × tone trial interaction [$F_{9,405}=2.39$, $p<.05$], but the other two-way interactions were not significant and the three-way interaction was also not significant. An analysis on baseline freezing revealed no differences between any of the groups.

Pretreatment with a 5-HT_{2C} Antagonist Blocks the Effect of Citalopram on Conditioned Fear Expression

Pre-treatment with the 5-HT_{2C} antagonist, SB-242084 (0.2 mg/kg, i.p.), significantly blocked the enhancing effects of citalopram (10 mg/kg, i.p.) on conditioned fear expression (Figure 4). We performed a three-way ANOVA with factors: antagonist (SB 242084 vs. water), SSRI (citalopram vs. saline), and tone trial (1–10; repeated measures). There were significant main effects of antagonist [$F_{1,52}=8.61$, $p<.01$], SSRI [$F_{1,52}=11.35$, $p<.01$] and tone trial [$F_{9,468}=15.05$, $p<.01$] and a significant antagonist × SSRI interaction [$F_{1,52}=4.48$, $p<.05$]. The other two-way interactions were not significant and the three-way interaction was also not significant. Post hoc comparison using Tukey's HSD confirmed the previously described significant effect of citalopram ($p<.01$). It also indicated that SB 242084 had no significant effect on its own ($p=.90$), but did reverse the effects of citalopram ($p<.05$). An analysis on baseline freezing revealed no differences between any of the groups.

Discussion

Effects of SSRIs on Conditioned Fear Expression

Previously, we reported that a single pre-training injection of the SSRI citalopram enhanced the acquisition of auditory fear conditioning (Burghardt et al 2004). Here we show that a single pre-testing injection of citalopram or the SSRI fluoxetine enhanced conditioned fear expression. Given that animals in the present set of experiments were given drug treatment after fear acquisition, and patients are typically treated with SSRIs for their anxiety symptoms after the disorder has already developed, the current findings examine the effects of SSRIs in a manner that resembles the clinical setting. Although fear and anxiety are distinct, our results showing that acute SSRI treatment increases fear are consistent with reports that symptoms of anxiety are often exacerbated during early stages of SSRI treatment (Mir 1997; Spigset 1999).

The acute effects of SSRIs have also been explored in a number of different animal models of anxiety. While our findings are consistent with the anxiogenic-like effects of acute SSRI treatment reported using the social interaction test (Bagdy et al 2001; Dekeyne et al 2000), the elevated plus maze (Silva and Brandao 2000), and the open field test (Matto and Allikmets 1999), these effects are not found in all animal models of anxiety (Matto and Allikmets 1999; Poltronieri et al 2003; Schreiber et al 1998).

Interestingly, pre-testing citalopram treatment decreases freezing to a fear conditioned context, using the same dose as that used in the present study (Hashimoto et al 1996). Therefore, acute SSRI treatment may have the opposite effect on contextual and auditory fear conditioning. A similar discrepancy was described for the effects of acute SSRI treatment on the acquisition of conditioned fear. While we found that a single pre-training injection of citalopram enhanced the acquisition of auditory fear conditioning (Burghardt et al 2004), others report that the same drug treatment impaired the acquisition of contextual fear conditioning (Inoue et al 1996a). Although there are a number of factors that may account for these differential effects (see below), the enhanced conditioned fear expression we report using auditory fear conditioning more closely resembles the initial anxiogenic effects reported clinically. As a result, auditory fear conditioning, but not contextual fear conditioning, can be used as a model for understanding the anxiogenic effects of SSRI treatment.

These differential effects of acute SSRI treatment on auditory and contextual fear conditioning may be attributable to differences in the neural circuits that are recruited by these tasks and their respective serotonergic inputs. Lesion studies indicate that both the hippocampus and amygdala are involved in the processing of complex, polymodal stimuli required for contextual fear conditioning (Kim and Fanselow 1992; Phillips and LeDoux 1992), while the amygdala, but not the hippocampus, is involved in the processing of simple, modality-specific information required for auditory fear conditioning (Phillips and LeDoux 1992). The hippocampus receives its serotonergic input primarily from the median raphe (Vertes et al 1999), while the serotonergic inputs to the amygdala arise primarily from the dorsal raphe (Vertes 1991; Vertes et al 1999). Consequently, differences in the effects of acute SSRI administration on contextual and auditory fear conditioning may reflect differences in the primary sources of serotonin to the brain regions that underlie these tasks.

An analysis of freezing behavior prior to tone onset indicates that there were no differences in baseline freezing between animals treated with citalopram or fluoxetine and their respective control groups. Therefore drug treatment did not increase freezing in a non-specific manner. A number of other studies assessing the effects of citalopram or fluoxetine on movement found no evidence of a motor impairment with the doses used in this study (Belzung et al 2001; Hashimoto et al 1996). The low levels of freezing across groups during the pre-CS time period also indicate that there was no generalization of fear from the training to the testing context. Rather, the increase in freezing resulting from SSRI treatment appears to tone-specific. Furthermore, our earlier study (Burghardt et al 2004) shows that citalopram does not increase freezing responses to a tone before the tone is associated with a shock, suggesting that the increase in freezing reported here reflects enhanced expression of conditioned fear and not a non-specific change in reactivity to the tone.

Effects of Tianeptine on Conditioned Fear Expression

Tianeptine is purported to be a serotonin reuptake enhancer (Datla and Curzon 1993; Fattaccini et al 1990), although recent studies suggest that it is involved in modulating glutamatergic transmission (Kole et al 2002; Reagan et al 2004). Clinically, tianeptine has been found to be just as effective as SSRIs in treating depression and symptoms of anxiety in depressed patients (Lepine et al 2001). Unlike fluoxetine, tianeptine has been associated with a decrease in the requirement for concomitant anxiolytic prescription (Alby 1993). Although such findings

indicate that it may have anxiolytic properties, tianeptine treatment has not been explored in patients with anxiety disorders. Animal studies have found that daily treatment with tianeptine (10 mg/kg) prevents stress-induced dendritic atrophy in the hippocampus (Conrad et al 1999), and stress-induced spatial memory impairments in the Y-maze (Conrad et al 1996).

Using the same dose shown to be sufficient in the remodeling of dendrites, we found no effect of a single pre-testing injection of tianeptine (10 mg/kg) on conditioned fear expression. Similarly, we found no effect of a pre-training tianeptine on the acquisition of auditory fear conditioning in our previous study (Burghardt et al 2004), and others have found no acute tianeptine effects using the social interaction test (File and Mabbutt 1991), immobility time test, or tests of spatial memory (Morris et al 2001; Nowakowska et al 2000). Although we found no acute effect of tianeptine, the anxiolytic potential of chronic administration at this dose is suggested by our previous findings that daily tianeptine treatment decreases the acquisition of auditory fear conditioning (Burghardt et al 2004). If tianeptine is found to effectively treat anxiety disorders, then the lack of an acute effect on fear expression suggests that tianeptine may be preferable to SSRIs.

Effects of Tomoxetine on Conditioned Fear Expression

Serotonin/norepinephrine reuptake inhibitors (SNRIs) have been shown to be at least as effect as SSRIs in the treatment of anxiety disorders (Silverstone 2004). Interestingly, SNRIs have not been found to exacerbate symptoms of anxiety in patients with anxiety disorders or depression, despite the expectation that such side effects would result from activation of the norepinephrine system (Silverstone 2004). To further evaluate the role of norepinephrine in modulating the fear system, we tested the effects of tomoxetine, a selective norepinephrine reuptake enhancer, on the expression of conditioned fear. We found that a pre-testing injection of tomoxetine, at a dose that significantly increases norepinephrine levels without affecting serotonin (Bymaster et al 2002), had no significant effect on freezing responses to the tone. Similarly, it was reported that a pre-testing injection of other norepinephrine reuptake inhibitors did not affect contextual fear conditioning (Hashimoto et al 1996). These findings indicate that selectively increasing extracellular norepinephrine does not have an anxiogenic effect. Rather, this effect seems to be specifically mediated by increases in serotonin resulting from SSRI treatment.

Effects of a 5-HT_3 Receptor Antagonist on Conditioned Fear Expression

Preclinical studies indicating that selective 5-HT_3 antagonists are anxiolytic (Costall and Naylor 1992; Kilfoil et al 1989), although the results of additional studies have been less consistent (Cutler et al 1997; Rodgers et al 1997). Using a dose that others found to significantly reduce contextual fear conditioning (Yoshioka et al 1995), we found no effect of pre-testing tropisetron, a 5-HT_3 antagonist, on auditory fear conditioning. Perhaps these differential effects can be accounted for by the aforementioned differences in the way serotonin modulates the dissociable neural circuits that mediate these tasks.

To examine the functional relationship between SSRIs and the 5-HT_3 receptor we administered citalopram and tropisetron together and tested their effects on conditioned fear expression. We found that blocking the 5-HT_3 receptor did not reverse the citalopram-induced enhancement of fear expression, indicating that the 5-HT_3 receptor may not be involved in mediating this effect.

The Effects of 5-HT_{2C} Receptor Antagonists on Conditioned Fear Expression

Clinical and preclinical studies suggest a role for 5-HT_2 receptors in anxiety. For example, the non-selective 5-HT_2 agonist mCPP increases symptoms of anxiety in patients with panic disorder and agoraphobia (Charney et al 1987). Conversely, the mixed 5-$HT_{2A/2C}$ antagonist

ritanserin is anxiolytic in patients with generalized anxiety disorder (Ceulemans et al 1985) and agoraphobia (Humble 1986), and decreases conditioned fear responses in healthy humans (Hensman et al 1991). Animal studies indicate a similar anxiogenic effect of mCPP administration (Bagdy et al 2001; Guitton and Dudai 2004), and an anxiolytic effect of the selective 5-HT_{2C} receptor antagonist, SB 242084 (Martin et al 2002), implicating the involvement of this specific receptor subtype in anxiety.

We tested the effects of SB 242084 on the expression of auditory fear conditioning using the minimal dose shown to be effective in increasing social interaction (Bagdy et al 2001), and found that it did not significantly reduce freezing. Similarly, others report that 5-HT_2 antagonists had no effect on contextual fear conditioning (Inoue et al 1996b), or tail suspension responses (Cremers et al 2004).

The Involvement of 5-HT_{2C} Receptors in the Acute Effects of Citalopram

It has been shown that selective blockade of the 5-HT_{2C} receptor with SB 242084 blocks the anxiogenic effects of SSRIs in the social interaction test (Bagdy et al 2001; Dekeyne et al 2000), although 5-HT_2 receptor antagonists do not reverse the anxiogenic effects of SSRIs in the free-exploration test. To address whether the relationship between 5-HT_{2C} receptors and SSRIs described using the social interaction test can be generalized to other fear/anxiety related behaviors, we assessed the effects of 5-HT_{2C} receptor blockade on acute SSRI-induced increases in conditioned fear expression. We found that pretreatment with the selective 5-HT_{2C} antagonist, SB 242084, reversed the citalopram-induced enhancement of fear expression. It is unlikely that these results are attributable to non-specific effects of the antagonist, since there were no differences between any treatment groups in baseline freezing, no effects of SB 242084 alone on conditioned fear expression, and other groups report that the dose of SB 242084 used in this study does not impair movement (Kennett et al 1997; Martin et al 2002). The lack of an effect of SB 242084 alone indicates that 5-HT_{2C} blockade may only have effects on auditory fear conditioning under conditions of elevated serotonin. Our results suggest that 5-HT_{2C} receptors may play a significant role in mediating the anxiogenic effects of SSRI treatment found clinically, and that blockade of these receptors could be therapeutically advantageous.

Our past and present results showing that systemic SSRI treatment modulates auditory fear conditioning implicate the amygdala as a possible site of action for these drugs. Given that direct infusions of a 5-HT_2 receptor agonist into the amygdala has anxiogenic effects (Campbell and Merchant 2003), it is possible that the enhancement we find in conditioned fear expression following SSRI treatment is attributable to activation of these particular amygdala serotonin receptors. Future studies involving intra-amygdala infusions of 5-HT_{2C} receptor antagonists, in animals treated systemically with an SSRI, are required before this conclusion can be drawn.

Summary and Conclusions

In summary, we extended our previous findings that acute SSRI treatment enhances the acquisition of auditory fear conditioning by showing that acute SSRIs also enhance the expression of conditioned fear. This effect was found with two different SSRIs, citalopram and fluoxetine, but not with tianeptine, a purported serotonin reuptake enhancer, or tomoxetine, a norepinephrine reuptake inhibitor. Therefore the effect seems to be specific to SSRIs. We also show that the SSRI-induced enhancement in fear expression is blocked by systemic administration of a 5-HT_{2C}, but not a 5-HT_3, receptor antagonist. These findings indicate that the 5-HT_{2C} receptors may be a mechanism through which the anxiogenic effects of SSRIs are mediated in humans. Clinically, our findings suggest that co-administration of 5-HT_{2C} receptor antagonists with SSRIs may help prevent the increase in symptoms of anxiety often reported during early stages of treatment.

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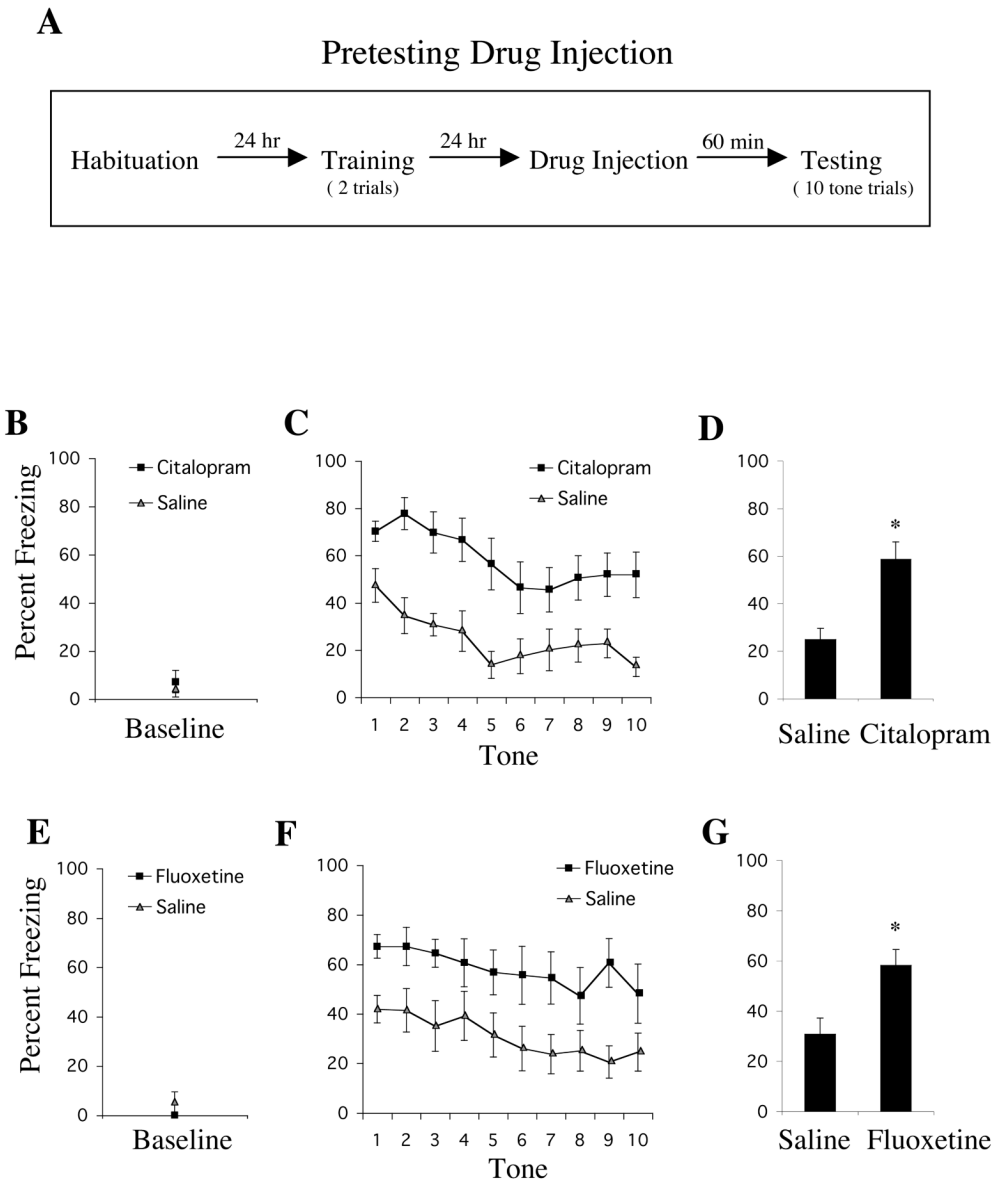

Figure 1.

Animals given a pre-testing injection of an SSRI showed enhanced conditioned fear expression. **(A)** General behavioral procedures: 24 hours after habituation, rats were fear conditioned with two tone-shock pairings. The next day, animals were given an injection of drug or vehicle and tested 60 minutes later to ten presentations of the tone alone. **(B)** Mean ± SEM percent freezing of rats treated with citalopram (n=11) or saline (n=9) during the baseline period prior to the first tone trial. **(C)** Mean ± SEM percent freezing of citalopram-treated (10 mg/kg, i.p.) or saline-treated animals during each tone trial. **(D)** Mean ± SEM percent freezing of each group (citalopram vs. saline) averaged across all 10 tones. * $p<.01$ versus saline. **(E)** Mean ± SEM percent freezing of rats given a single injection of fluoxetine (n=9) or saline (n=11) during the baseline period prior to the first tone trial. **(F)** Mean ± SEM percent freezing of fluoxetine-treated (10 mg/kg, i.p.) or saline-treated animals during each tone trial. **(G)** Mean ± SEM percent freezing of each group (fluoxetine vs. saline) averaged across all 10 tones * $p<.01$ versus saline.

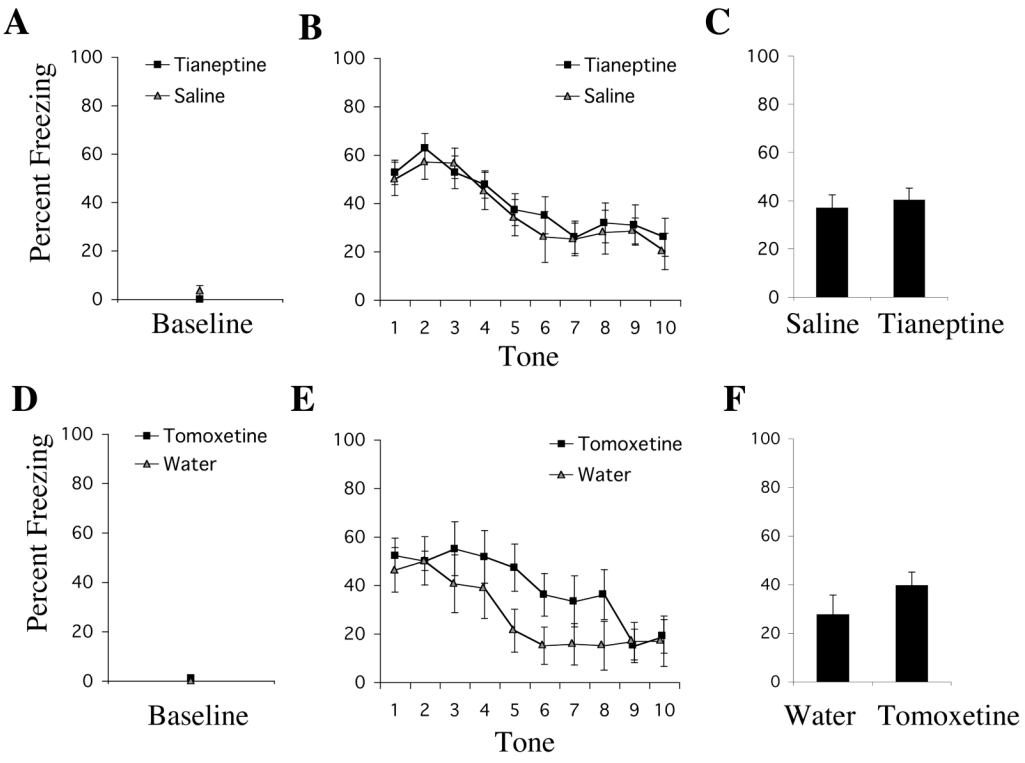

Figure 2.

Using the same general behavioral procedures outlined in Figure 1, acute treatment with tianeptine (10 mg/kg, i.p.) or tomoxetine (1 mg/kg, i.p.) did not affect conditioned fear expression. **(A)** Mean ± SEM percent freezing of rats treated with tianeptine (n=11) or saline (n=10) during the baseline period prior to the first tone trial. **(B)** Mean ± SEM percent freezing of tianeptine-treated or saline-treated animals during each tone trial. **(C)** Mean ± SEM percent freezing of each group (tianeptine vs. saline) averaged across all 10 tones. **(D)** Mean ± SEM percent freezing of rats treated with tomoxetine (n=9) or water (n=8) during the baseline period prior to the first tone trial. **(E)** Mean ± SEM percent freezing of tomoxetine-treated or water-treated animals during each tone trial. **(F)** Mean ± SEM percent freezing of each group (tomoxetine vs. water) averaged across all 10 tones.

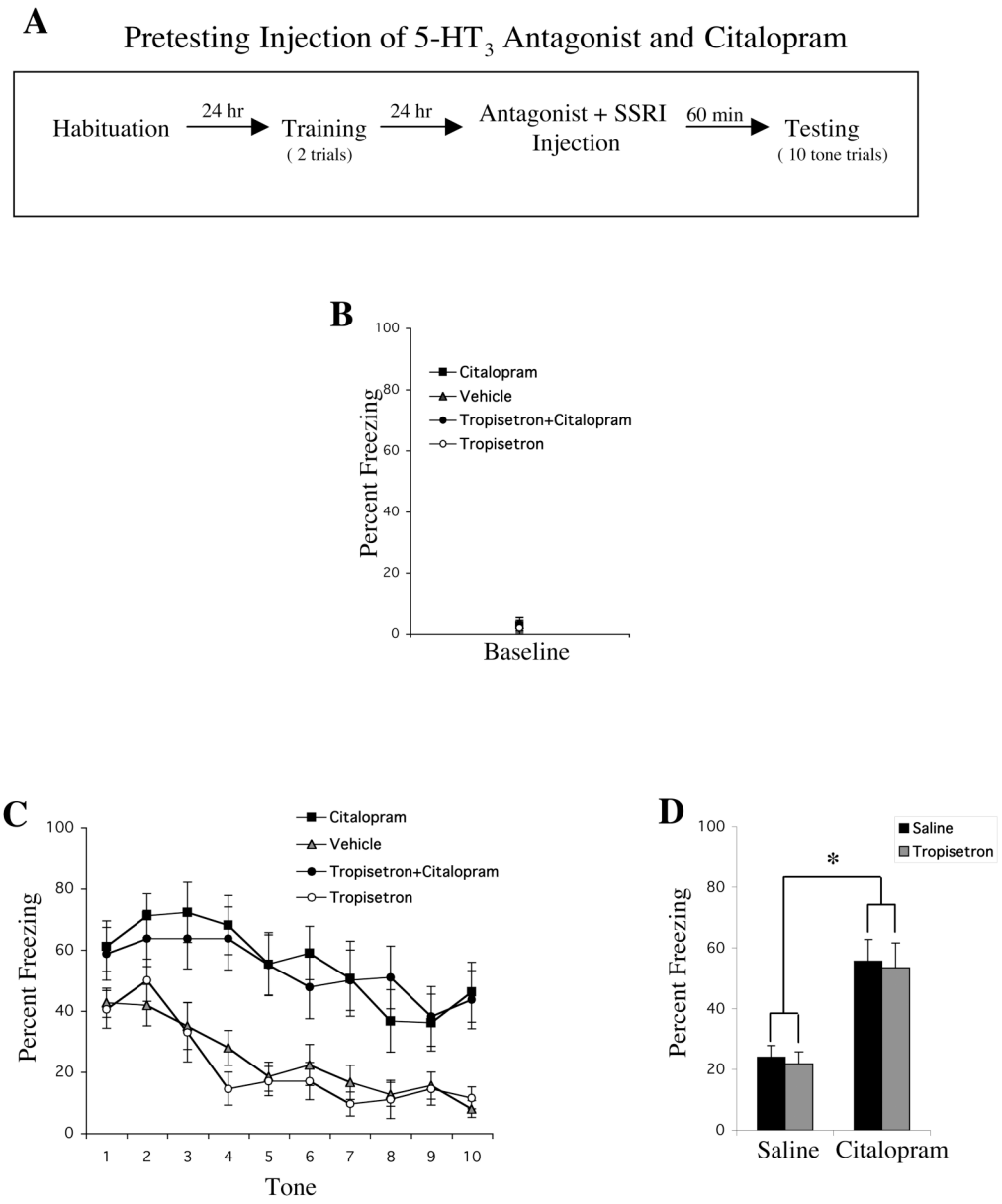

Figure 3.

The 5-HT$_3$ receptor antagonist, tropisetron (0.1 mg/kg, i.p.), did not block the enhancing effects of citalopram (10 mg/kg, i.p.) on conditioned fear expression. **(A)** General behavioral procedures: 24 hours after habituation, rats were fear conditioned with two tone-shock pairings. The next day, animals were given a single injection containing citalopram, tropisetron, citalopram+tropisetron, or saline 60 minutes before they were tested to 10 presentations of the tone alone. **(B)** Mean ± SEM percent freezing of rats treated with citalopram (n=9), vehicle (n=19), tropisetron+citalopram (n=11), or tropisetron (n=10) during the baseline period prior to the first tone trial. **(C)** Mean ± SEM percent freezing of each group during each tone trial. **(D)** Mean ± SEM percent freezing of each group averaged across all 10 tones. * indicates main effect of citalopram, $p<.01$.

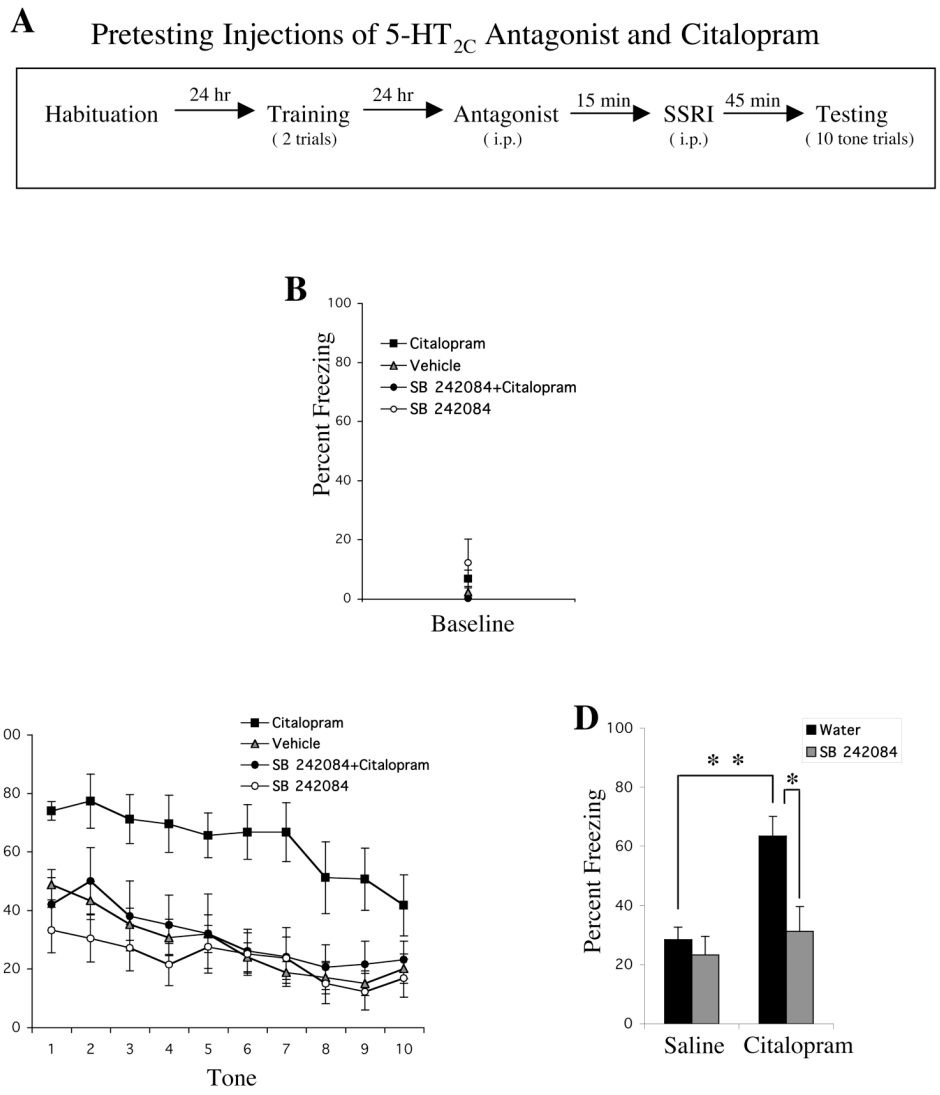

Figure 4.

Pre-treatment with the 5-HT2C receptor antagonist, SB 242084 (0.2 mg/kg, i.p.), blocked the enhancing effects of citalopram (10 mg/kg, i.p.) on conditioned fear expression. **(A)** General behavioral procedures: 24 hours after habituation, rats were fear conditioned with two tone-shock pairings. The next day, animals were given 2 injections. The first injection of SB 242084 or water was given 15 minutes before the second injection of citalopram or saline. Forty-five minutes later, rats were tested to 10 presentations of the tone alone. When one of the two injections contained vehicle, only the drug condition is indicated in Figures B and C. **(B)** Mean ± SEM percent freezing of rats treated with citalopram (n=9), vehicle (n=23), SB 242084 +citalopram (n=10), or SB 242084 (n=14) during the baseline period prior to the first tone trial. **(C)** Mean ± SEM percent freezing of each group during each tone trial. **(D)** Mean ± SEM percent freezing of each group averaged across all 10 tones. * indicates that $p<.05$ and ** indicates $p<.01$ using Tukey's HSD post hoc test.