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Chronic Antidepressant Treatment Impairs the Acquisition of Fear Extinction

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Abstract

Background—Like fear conditioning, the acquisition phase of extinction involves new learning that is mediated by the amygdala. During extinction training, the conditioned stimulus is repeatedly presented in the absence of the unconditioned stimulus and the expression of previously learned fear gradually becomes suppressed. Our previous study revealed that chronic treatment with a selective serotonin reuptake inhibitor (SSRI) impairs the acquisition of auditory fear conditioning. To gain further insight into how SSRIs affect fear learning, we tested the effects of chronic SSRI treatment on the acquisition of extinction.

Methods—Rats were treated chronically (22 days) or subchronically (9 days) with the SSRI citalopram (10 mg/kg/day) before extinction training. The results were compared to those following chronic and subchronic treatment with tianeptine (10 mg/kg/day), an antidepressant with a different method of action. The expression of the NR2B subunit of the NMDA receptor in the amygdala was examined after behavioral testing.

Results—Chronic but not subchronic administration of citalopram impaired the acquisition of extinction and downregulated the NR2B subunit of the NMDA receptor in the lateral and basal nuclei of the amygdala. Similar behavioral and molecular changes were found with tianeptine treatment.

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Conclusions—These results provide further evidence that chronic antidepressant treatment can impair amygdala-dependent learning. Our findings are consistent with a role for glutamatergic neurotransmission in the final common pathway of antidepressant treatment.

Keywords

fear conditioning; SSRI; citalopram; amygdala; NR2B subunit; extinction

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are widely prescribed for treating a range of anxiety disorders (1–5). Although the known pharmacological actions of these drugs take place immediately (6), patients require several weeks of continuous treatment before experiencing their therapeutic effects (7). Despite extensive experimental work focused on understanding this delay in therapeutic onset, the relevant underlying mechanisms remain unknown.

Evidence indicates that the amygdala may be one site of action for long-term SSRI treatment (8–13). Our previous study in rats revealed that chronic SSRI treatment impairs the acquisition of auditory fear conditioning (14), a task that depends on the amygdala (15, 16). In fear conditioning, a neutral conditioned stimulus (CS), such as a tone, acquires the capacity to elicit fear-related behavioral responses after being associated with an aversive unconditioned stimulus (US), such as a footshock. When the CS is repeatedly presented in the absence of the US, conditioned fear responses diminish, a process called extinction.

Extinction does not destroy original fear memories, but instead involves learning new information about the relationship between the CS and US (17–19). Learning during both initial fear conditioning and extinction depends upon activation of N-methyl-D-aspartate receptors (NMDARs) in the lateral and basal nuclei of the amygdala. The finding that both types of learning are impaired by selective blockade of the NR2B subunit of the NMDAR in the amygdala, indicate a distinct role for this subunit in the underlying plasticity (20, 21).

The extinction phases of fear conditioning are thought to be important from a therapeutic standpoint. Extinction-based cognitive behavioral therapy (CBT) is widely used in the treatment of anxiety disorders (22, 23), during which patients systematically confront feared objects, situations, autonomic responses, or memories in the absence of an aversive event. The therapeutic value of targeting extinction is exemplified by studies using the drug *D*-cycloserine, which facilitates extinction in animals (24) and enhances the outcome of exposure therapy (25–30).

Given that antidepressants and extinction-based CBT are each effective in treating anxiety disorders (1–3, 23, 31), the expectation has been that combining these treatments would further enhance outcome. However, numerous studies reveal that combination treatment leads to few advantages when compared to either treatment modality alone (32–38), prompting the suggestion that medication may interfere with the beneficial effects of CBT (39, 40). Understanding how SSRIs affect the cognitive mechanisms underlying extinction-based therapies will provide important insight into how combination treatment affects clinical outcome.

We tested the effects of chronic treatment with the SSRI citalopram on the acquisition of fear extinction in rats. In contrast to our previous study, rats were fear conditioned drug-free and SSRI treatment began the next day, a protocol that better mimics the clinical setting. To evaluate the length of treatment time required for changes in extinction to be detected, the

effects of subchronic citalopram treatment were also measured. In an effort to reveal some of the neuroadaptive changes underlying SSRI-mediated modifications in extinction learning, NR2B protein levels in the amygdala were quantified upon completion of behavioral testing. The results were compared to those following chronic and subchronic treatment with tianeptine, an antidepressant with a different method of action. Unlike citalopram, which increases the synaptic availability of serotonin by binding to the serotonin transporter protein, tianeptine does not bind to any monoaminergic receptors or transporters (41, 42) and does not affect extracellular levels of serotonin (43). Instead, the effects of tianeptine have been linked to changes in the glutamatergic system (44, 45).

METHODS AND MATERIALS

Subjects

Adult male Sprague-Dawley rats (Hilltop Laboratories, Scottsdale, Pennsylvania) weighing 280–310g were housed individually in clear plastic cages in a thermally controlled colony room with a 12-hour light/dark cycle, where food and water were provided ad libitum. All procedures were performed at New York University, 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 (Sigma, St. Louis, MO) and tianeptine sodium salt (courtesy of Servier, Courbevoie, France) were dissolved in 0.9% sterile saline and injected intraperitoneally (i.p.) at a dose of 10 mg/kg (14). Based on studies measuring plasma drug levels in rats (46–48) and humans (49, 50), the human dose equivalence is 25 mg for citalopram and 37 mg for tianeptine. Drugs were made fresh daily in a volume of 1 mL and administered at approximately the same time each day. All animals were weighed daily so the appropriate dose could be calculated.

Behavioral Procedures

Apparatus—Fear conditioning took place in a brightly lit Plexiglas rodent conditioning chamber with a metal grid floor and three white house lights, which was enclosed within a sound-attenuating cubicle (ENV-001; Med Associates, Georgia, Vermont). The context of the chamber was changed during extinction training by covering the metal rods on the floor with a flat black Formica plate scented with peppermint soap and putting red lenses on two of the house lights (51, 52). An overhead camera videotaped behavior.

Auditory Fear Conditioning—Rats were habituated to handling and the conditioning context for 20 minutes. The next day, they were trained with a single conditioning trial involving two presentations of a tone CS (20 sec, 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 seconds. Freezing (see below) was scored during training so that natural variability in acquisition could be counterbalanced across treatment groups the next day.

Twenty-four hours after conditioning, separate groups of rats were weighed and injected with citalopram, tianeptine, or saline. Animals treated chronically were injected once/day for 22 consecutive days and animals treated subchronically were injected once/day for 9 consecutive days. There was a separate control group for each type of treatment (subchronic or chronic) and for each drug (citalopram or tianeptine). Rats received their final injection one hour before extinction training, and were on drug when extinction training began. Extinction training involved ten presentations of the CS tone in the absence of a US shock (Tones 1–10). The next day, drug was not administered and long-term memory of extinction

was tested with 10 additional CS tones (Tones 11–20). The properties of the tones and the inter-trial interval were identical during fear conditioning and each extinction trial.

Freezing, defined as the cessation of all movement unrelated to respiration (53), was used as a measure of conditioned fear. An experimenter blind to treatment group watched the videotapes and measured the number of seconds rats froze during each 20-second tone with a digital timer. Freezing is reported as a percentage of total tone presentation time.

Determination of NR2B Protein in the Amygdala

Immediately following presentation of the final tone (Tone 20), a subset of rats in each condition were deeply anesthetized with Nembutal (120 mg/kg, i.p.) and decapitated. Brains were removed, frozen on dry ice and stored at -80°C until processed. A 1 mm punch tool (Fine Science Tools, Foster City, CA) was used to obtain the lateral and basal nuclei of the amygdala from 400 μm -thick sections cut on a freezing microtome. Portions of the lateral central nucleus and cortical tissue directly lateral to the external capsule may have been included in the punches. Tissue samples were homogenized in 50 μl of ice-cold buffer (0.35 M sucrose, 0.5 mM EGTA, 2 mM EDTA, 2 mM Na^3VO_4 , and 1 mM PMSF, in 10 mM Tris-HCl, pH 7.4), centrifuged at 2000 X *g*, and the pellet was removed. The homogenate was sonicated for 10 mins, boiled for 5 mins with added sample buffer, electrophoresed on 7.5% SDS-polyacrylamide gels and blotted onto Immobilon-P membranes (Millipore, Bedford, MA). Western blots were blocked in 5% milk in TTBS buffer (50 mM Tris-HCl, pH 7.5, 150mM NaCl, and 0.05% Tween 20) and incubated with anti-NR2B (1:250; BD Biosciences). Blots were incubated with anti-mouse conjugated to horseradish-peroxidase (Jackson Immunoresearch, West Grove, PA) and visualized using chemiluminescence (Pierce, Rockford, IL). Actin was used as a loading control (1:1000; Sigma, St Louis, MO). To control for variance between gels, NR2B signal of each sample was divided by its actin signal and expressed as a percentage of that in the vehicle-treated group on the same blot.

Statistical Analysis

Data were analyzed with Student *t* test for independent samples or a one- or three-way ANOVA. A one-way ANOVA *a priori* test was used for planned mean comparisons on freezing during the first tone trial of extinction training. JMP Version 5 software (SAS Institute Inc., Cary, NC) was used for the analyses and significance was accepted for $p < 0.05$; p -values > 0.05 but < 0.1 were considered trends.

RESULTS

During extinction training, freezing during the 20-second period before the first presentation of the tone (Pre-CS) was used as an indicator of the drug's effects on motor activity. Freezing during the first presentation of the tone was a measure of the drug's effects on fear expression and freezing during subsequent presentations of the tone indicated extinction learning. Data were analyzed with a three-way ANOVA with factors drug group (drug vs. saline), day (day 1 vs. day 2), and tone trial (1–20; repeated measures).

Chronic SSRI Treatment Impairs the Acquisition of Fear Extinction

Rats treated chronically with citalopram or saline (Figure 1A) exhibited similar low levels of Pre-CS freezing (Cit: 5.67 ± 2.92 %; Sal: 1.79 ± 1.45 %) ($F_{(1,27)} = 1.35$, $p = 0.25$) and similar CS-elicited freezing during the first trial of extinction training ($F_{(1,27)} = 0.10$, $p = 0.75$). With subsequent tone presentations, saline-treated rats showed a gradual reduction in freezing, while citalopram-treated rats displayed sustained freezing, indicating impaired extinction learning (Figure 1B, left). The next day, vehicle-treated rats exhibited low levels of freezing, indicating successful retrieval of extinction learning. Freezing elicited by the

citalopram-treated group was still higher than controls, but gradually diminished to control levels by the end of the session (Figure 1B, right).

The three-way ANOVA revealed significant effects of drug group ($F_{(1,27)} = 11.32, p < 0.01$), day ($F_{(1,27)} = 39.74, p < 0.01$), tone trial ($F_{(9,243)} = 28.94, p < 0.01$), and a significant drug group \times tone trial interaction ($F_{(9,243)} = 2.59, p < 0.01$). When each day was analyzed separately, citalopram significantly enhanced freezing during the first ten tones ($F_{(1,27)} = 10.05, p < 0.01$) (Figure 1C) and the subsequent ten tones the following day ($F_{(1,27)} = 7.93, p < 0.01$) (Figure 1D). Citalopram enhanced freezing during tone 10 ($t_{(27)} = 3.02, p < 0.01$) but not tone 20 ($t_{(27)} = 0.96, p = 0.34$). Since citalopram-treated rats were impaired through the end of the initial day of extinction training, the deficit detected during the second day only confirmed the impairment in extinction learning found the previous day and cannot be used to address the effects of drug treatment on the consolidation and/or retrieval of extinction. These findings indicate that chronic citalopram treatment impaired the acquisition of extinction across two days of training.

Subchronic SSRI Treatment Does Not Impair the Acquisition of Fear Extinction

Rats treated subchronically with citalopram or saline (Figure 1A) exhibited similar low levels of Pre-CS freezing (Cit: 5.00 ± 4.21 %; Sal: 2.29 ± 1.47 %) ($F_{(1,41)} = 0.44, p = 0.51$) and similar CS-elicited freezing during the first trial of extinction training ($F_{(1,41)} = 0.17, p = 0.69$). With subsequent tone presentations, both groups showed a gradual reduction in freezing, although citalopram transiently increased freezing during two tone trials in the middle of the training session (Figure 1E, left). The next day, both groups exhibited low levels of freezing (Figure 1E, right).

The three-way ANOVA revealed no significant effect of drug group ($F_{(1,41)} = 0.48, p = 0.49$). The effects of day ($F_{(1,41)} = 92.44, p < 0.01$), tone trial ($F_{(9,369)} = 37.86, p < 0.01$) and the drug group \times day \times tone trial interaction ($F_{(9,369)} = 2.18, p < 0.05$) were significant. When each day was analyzed separately, groups exhibited similar freezing responses to the first ten tones ($F_{(1,41)} = 1.86, p = 0.18$) (Figure 1F) and the subsequent ten tones the next day ($F_{(1,41)} = 0.09, p = 0.76$) (Figure 1G). There were no differences between groups during tones 10 ($t_{(41)} = 1.41, p = 0.17$) and 20 ($t_{(41)} = 1.24, p = 0.22$). Significant group differences during tones 4 ($t_{(41)} = 2.46, p < 0.05$) and 5 ($t_{(41)} = 2.66, p < 0.05$) supported the impression that subchronic citalopram treatment led to a transient increase in freezing during extinction training.

Chronic Tianeptine Treatment Impairs the Acquisition of Fear Extinction

Rats treated chronically with tianeptine or saline (Figure 2A) exhibited similar low levels of Pre-CS freezing (Tian: 9.17 ± 4.09 %; Sal: 9.35 ± 4.29 %) ($F_{(1,45)} = 0.0009, p = 0.98$) and similar CS-elicited freezing during the first trial of extinction training ($F_{(1,45)} = 0.11, p = 0.74$). With subsequent tone presentations, both groups showed a gradual decrease in freezing, although tianeptine-treated rats did not reach control levels by the end of the training session (Figure 2B, left). The next day, saline-treated rats exhibited low levels of freezing, while tianeptine-treated rats continued to freeze more than controls (Figure 2B, right).

A three-way ANOVA revealed significant effects of drug group ($F_{(1,45)} = 10.44, p < 0.01$), day ($F_{(1,45)} = 115.54, p < 0.01$) and tone trial ($F_{(9,405)} = 48.62, p < 0.01$). There was no significant drug group \times tone trial interaction ($F_{(9,405)} = 0.58, p = 0.81$). When each day was analyzed separately, tianeptine significantly enhanced freezing during the first ten tones ($F_{(1,45)} = 7.45, p < 0.01$) (Figure 2C) and the subsequent ten tones the following day ($F_{(1,45)} = 10.75, p < 0.01$) (Figure 2D). Tianeptine also increased freezing during tones 10 ($t_{(45)} = 3.60,$

$p < 0.001$) and 20 ($t_{(45)} = 2.41$, $p < 0.05$). Since tianeptine-treated rats were impaired through the end of the initial day of extinction training, the deficit detected during the second day of training only confirmed the impairment in extinction learning found the previous day and cannot be used to address the effects of drug treatment on the consolidation and/or retrieval of extinction. These results indicate that like chronic citalopram treatment, chronic tianeptine treatment impaired extinction learning across two days of training.

Subchronic Tianeptine Treatment Does Not Impair the Acquisition of Fear Extinction

Rats treated subchronically with tianeptine or saline (Figure 2A) exhibited similar low levels of Pre-CS freezing (Tian: 0.71 ± 0.52 %; Sal: 1.25 ± 0.86 %) ($F_{(1,43)} = 0.26$, $p = 0.61$). Subchronic tianeptine treatment reduced CS-elicited freezing during the first trial of extinction training ($F_{(1,43)} = 8.21$, $p < 0.01$), indicating reduced fear memory expression. With subsequent tone presentations, the saline group showed a gradual decrease in freezing, while freezing levels in the tianeptine group remained low (Figure 2E, left). The next day, both groups exhibited low levels of CS-elicited freezing (Figure 2E, right).

The three-way ANOVA revealed no significant effect of drug group ($F_{(1,43)} = 0.45$, $p = 0.51$). The effects of day ($F_{(1,43)} = 81.56$, $p < 0.01$), tone trial ($F_{(9,387)} = 32.78$, $p < 0.01$), and the drug group \times day \times tone trial interaction ($F_{(9,387)} = 4.33$, $p < 0.01$) were significant. When each day was analyzed separately, groups exhibited similar freezing responses to the first ten tones ($F_{(1,43)} = 0.005$, $p = 0.94$) (Figure 2F) and the subsequent ten tones the next day ($F_{(1,43)} = 2.84$, $p = 0.10$) (Figure 2G). There were no group differences during tones 10 ($t_{(43)} = 0.97$, $p = 0.34$) and 20 ($t_{(43)} = 0.54$, $p = 0.59$). These results indicate that subchronic tianeptine treatment did not impair the acquisition of extinction.

Chronic SSRI Treatment Decreases NR2B Expression Levels in the Amygdala

Rats treated chronically with citalopram had significantly less NR2B protein in the lateral and basal nuclei than animals treated chronically with saline ($t_{(12)} = 2.41$, $p < 0.05$) (Figure 3A, B). The amount of NR2B protein expression correlated negatively with the amount of freezing animals exhibited across two days of extinction training ($r = -0.61$). In contrast, animals treated subchronically with citalopram or saline had similar levels of NR2B protein ($t_{(6)} = 1.03$, $p = 0.34$) (Figure 3C, D), indicating that downregulation of the NR2B subunit requires more than nine days of citalopram treatment. Chronic tianeptine treatment also appeared to reduce NR2B protein in the amygdala, but the decrease did not reach significance ($t_{(21)} = 1.87$, $p = 0.076$) (Figure 3E, F). A different mechanism of action may underlie the impairment in extinction following chronic tianeptine treatment.

DISCUSSION

Main Findings

It has long been understood that extinction does not erase conditioned fear memories, but is instead a form of new learning (17, 18). Here we show that chronic but not subchronic administration of citalopram and tianeptine impairs the acquisition of fear extinction in rats. In a previous study, we showed that chronic but not acute administration of these same drugs impairs the acquisition of a conditioned fear memory (14). Hence, two drugs with proven antidepressant efficacy adversely affect two types of learning tested with auditory fear conditioning when administered chronically. In contrast, citalopram and tianeptine modulate fear circuits differently when administered subchronically. While subchronic citalopram treatment briefly impairs the acquisition of extinction, subchronic tianeptine treatment decreases expression of a fear memory. An investigation of the neuroadaptive changes that accompany chronic SSRI treatment reveals that the citalopram-induced impairment in extinction learning is associated with downregulation of the NR2B subunit of

the NMDA receptor in the lateral and basal nuclei of the amygdala. The time course of these behavioral and molecular effects in rodents mirrors the delay in onset of therapeutic action found among clinical populations.

A Role for Amygdala NMDA Receptors in Mediating Antidepressant-Induced Impairments in Extinction Learning

Numerous studies implicate a role for the amygdala in extinction learning. The acquisition of extinction is blocked by inactivation of the basal nucleus of the amygdala (54) and impaired by intra-amygdala infusions of NMDAR antagonists (55–58). A subset of cells in the basal nucleus show an increase in CS-evoked activity during extinction training (54). Interestingly, the amygdala is not required for extinguishing the association between two neutral stimuli (58).

NMDA receptors are heteromeric complexes comprised of the NR1 subunit and different NR2 subunits (59, 60), each of which confers distinct biophysical and pharmacological properties of the receptor (61, 62). Compared to the NR2A subunit, the NR2B subunit is important for learning and memory and associated synaptic plasticity (63–68). Selective blockade of the NR2B subunit in the lateral amygdala impairs both the acquisition of auditory fear conditioning and the acquisition of extinction, without affecting expression of the fear memory (20, 21). Here and in our previous study (14), we found the same pattern of learning impairments following chronic citalopram treatment. Similarly, chronic fluoxetine treatment impairs the reacquisition of fear responses (69), which is also dependent upon NMDA receptors in the amygdala. A direct role of the NR2B subunit in mediating these effects is suggested by our finding that impaired extinction learning was associated with downregulation of the NR2B subunit in the amygdala. Although it is not known how chronic SSRI treatment leads to this downregulation, studies in the prefrontal cortex implicate the involvement of the 5-HT_{1A} and 5-HT_{2A} receptors (70, 71). Our results are in line with earlier work showing that chronic antidepressant treatment modulates the structure and function of NMDA receptors (72–74) and indicate that a functional consequence of such a change is impaired fear extinction learning.

In contrast to the cognitive deficits that result from reduced glutamatergic neurotransmission, improvements in mood and anxiety are also found when glutamatergic transmission is antagonized. Inhibiting the glutamatergic system with the NMDA receptor antagonist ketamine or the presynaptic glutamate release inhibitor riluzole produces an antidepressant effect (75–77) and may improve symptoms of anxiety (78–80). Selectively blocking the NR2B subunit is sufficient to produce an antidepressant response (79, 81, 82) and anxiolytic-like effects in animals (79). These beneficial effects of reducing glutamatergic transmission, along with previous work identifying changes in NMDA and AMPA receptors with antidepressant treatment (42, 72–74, 83), and our finding that chronic citalopram and tianeptine administration similarly downregulate the NR2B subunit, all support the hypothesis that adaptive changes in the glutamatergic system within different brain regions represent a final common pathway for the therapeutic action of antidepressants (84–86). Unfortunately, such adaptive changes block the facilitating effects of the partial NMDA agonist D-cycloserine on extinction (87), indicating that the opposing effects of glutamatergic neurotransmission on fear learning and mood/anxiety may pose a problem when combining different types of treatment.

The impairment in extinction learning that we find following twenty-two days of administration with two independent antidepressants contrasts with a recent study reporting that fourteen days of fluoxetine treatment facilitates learning to extinguish a conditioned response (88). One difference between these studies is that the latter was conducted in mice, whereas we used rats, which is the primary species used in Pavlovian fear conditioning

studies (89–93). Rats and mice respond differently to several drugs (94, 95), including MDMA, which has a neurodegenerative effect on serotonin in rats but not mice (96). Species differences in baseline corticosterone levels (97), the expression of some serotonin, estrogen and corticosteroid receptors (98–100), and risk assessment (101) could contribute to a differential drug response. Alternatively, the discrepant results might be attributable to the use of different experimental conditions. In our study, rats were weighed and injected for twenty-two consecutive days and were habituated to the training context the day before fear conditioning. Consequently, they were handled significantly more than the mice used in the other study, which were given fluoxetine in the drinking water and were not habituated prior to training. While handling significantly elevates physiological correlates of stress in rats and mice (102), weighing a rat and administering seven daily injections decreases anxiety compared to non-handled controls (103). The potential relevance of an altered stress response is implicated by the finding that brief handling in conjunction with administration of the partial NMDA receptor agonist, D-cycloserine, enhances the consolidation of extinction learning (104), while a decrease in corticosterone impairs the acquisition of extinction tested with contextual fear conditioning (105). Although it is expected that daily handling of our rats decreased non-specific responses to the experimental procedure, it is possible that consequent changes in stress contributed to the effects we report. These and other methodological differences, including the use of different SSRIs for different periods of time, may account for the discrepant findings.

Clinical Implications

Numerous studies have investigated whether the combination of antidepressant treatment and extinction-based CBT is more effective than either monotherapy alone for treating anxiety disorders. While some find a modest benefit of combining treatment in the short-term (32–35), others report no short-term advantage (36–38, 106, 107). However there is evidence that combined treatment impedes the long-term benefits of exposure therapy. For example, when panic disorder patients were evaluated after treatment discontinuation, the outcome of those previously treated with CBT and imipramine (32) or alprazolam (33) was worse than patients who received CBT alone. Similarly, when social anxiety disorder patients were evaluated after treatment ended, the outcome of patients previously treated with both CBT and sertraline (108) was worse than patients who received CBT alone. It has been suggested that the limited improvements seen with combined treatment are the result of both the beneficial anxiolytic effects of antidepressants and their disruptive effects on exposure-based therapy (39, 40). One way in which the inclusion of antidepressant is thought to be disruptive is by creating distinct internal states during treatment and follow-up (40). Since extinction is context-dependent (109) and internal state is a contextual cue, fear that is extinguished on-drug could be renewed off-drug (110). Such a change in internal state provides one potential explanation for why any advantage of combined treatment is lost upon discontinuation of medication (40). Data presented here indicates that the blunted effects of medication on extinction learning during exposure-based CBT may also contribute to the disruptive effects of antidepressants on CBT found over time.

Summary

In summary, we found that chronic but not subchronic administration of two antidepressants with distinct pharmacological properties produces impaired extinction learning in rats. This impairment was associated with downregulation of the NR2B subunit of the NMDA receptor in the amygdala. These and our previous findings demonstrate that chronic antidepressant administration impairs amygdala-dependent learning. Our work supports a role for glutamatergic neurotransmission in the final common pathway of antidepressant treatment.

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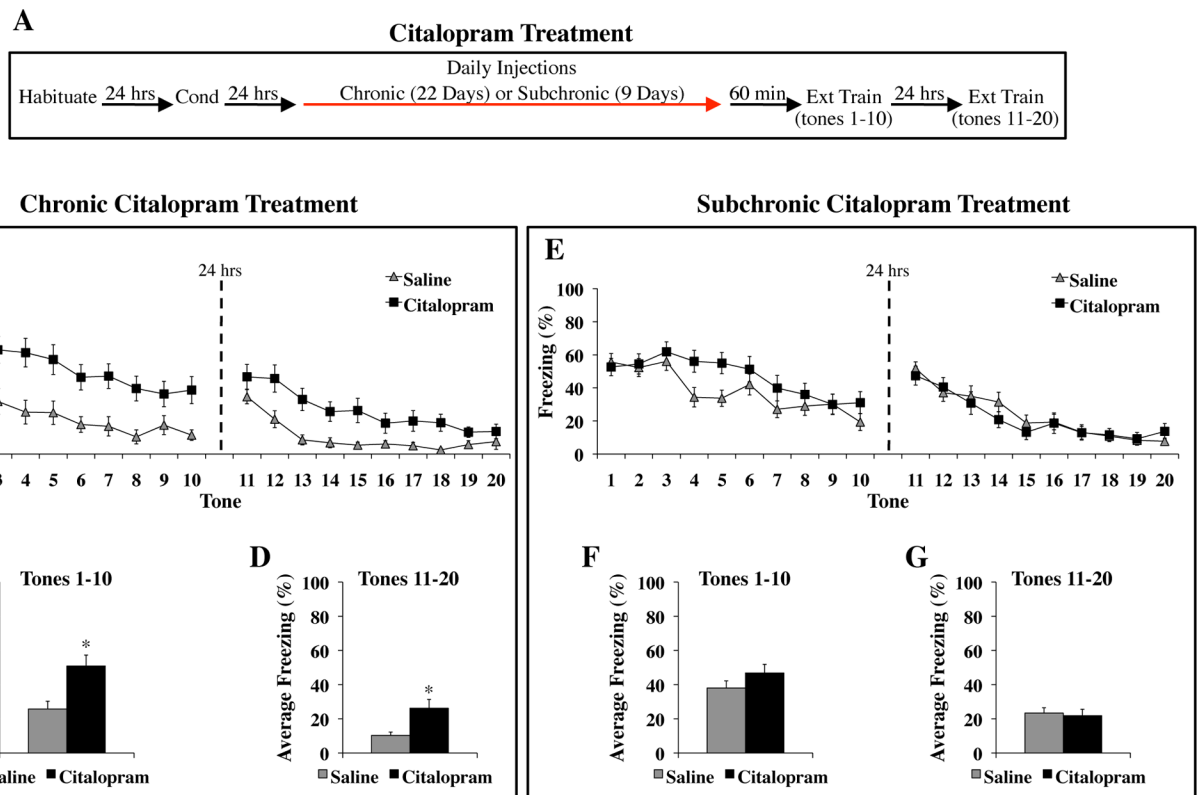


Figure 1. Chronic but not subchronic citalopram treatment impairs the acquisition of fear extinction

(A) General behavioral procedures: 24 hours after habituation, rats were fear conditioned with two tone-shock pairings. The next day, animals began receiving daily injections of citalopram (10 mg/kg, i.p.) or saline. Chronic treatment lasted for 22 consecutive days. Subchronic treatment lasted for 9 consecutive days. Extinction training began the same day the final injection was administered and involved 20 presentations of the tone alone over the course of two days. (B–D) The effects of chronic citalopram treatment on extinction learning. (B) Mean \pm SEM percent freezing of citalopram-treated ($n=15$) and saline-treated ($n=14$) rats during each trial of extinction training. The average response of each group was not significantly different during tone 1, indicating that chronic citalopram treatment did not affect expression of the fear memory. (C) Mean \pm SEM percent freezing of each group averaged across the first 10 tones, which were presented on the first day of extinction training. (D) Mean \pm SEM percent freezing of each group averaged across the last 10 tones, which were presented on the second day of extinction training. (E–G) The effects of subchronic citalopram treatment on extinction learning. (E) Mean \pm SEM percent freezing of citalopram-treated ($n=19$) and saline-treated ($n=24$) rats during each trial of extinction training. The average response of each group was not significantly different during tone 1, but was significantly different during tones 4 and 5 ($p<0.05$). (F) Mean \pm SEM percent freezing of each group averaged across the first 10 tones, which were presented on the first day of extinction training. (G) Mean \pm SEM percent freezing of each group averaged across the last 10 tones, which were presented on the second day of extinction training. * $p<0.01$ versus saline.

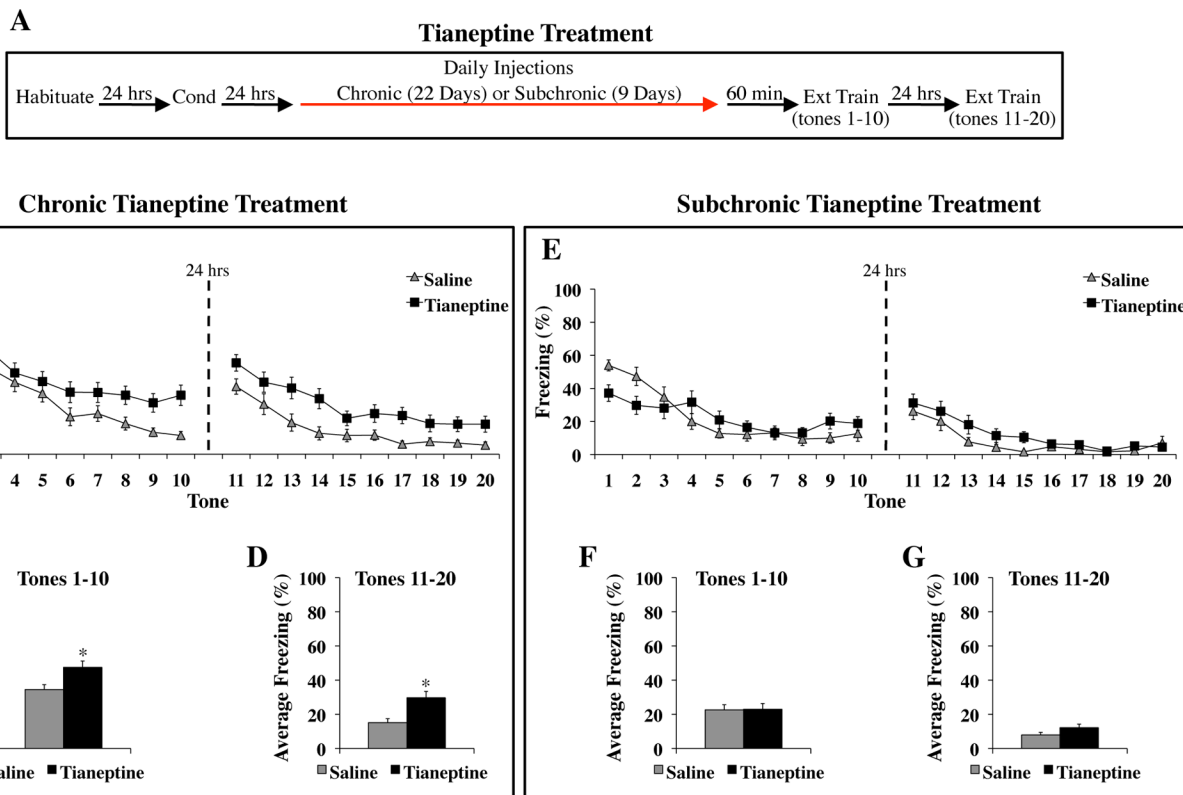


Figure 2. Chronic but not subchronic tianeptine treatment impairs the acquisition of fear extinction

(A) General behavioral procedures: 24 hours after habituation, rats were fear conditioned with two tone-shock pairings. The next day, animals began treatment with tianeptine (10 mg/kg, i.p.) or saline. Chronic treatment lasted for 22 consecutive days. Subchronic treatment lasted for 9 consecutive days. Extinction training began the same day the final injection was administered and involved 20 presentations of the tone alone over the course of two days. (B–D) The effects of chronic tianeptine treatment on extinction learning. (B) Mean ± SEM percent freezing of tianeptine-treated (n=24) and saline-treated (n=23) rats during each trial of extinction training. The average response of each group was not significantly different during tone 1, indicating that chronic tianeptine treatment did not affect expression of the fear memory. (C) Mean ± SEM percent freezing of each group averaged across the first 10 tones, which were presented on the first day of extinction training. (D) Mean ± SEM percent freezing of each group averaged across the last 10 tones, which were presented on the second day of extinction training. (E–G) The effects of subchronic tianeptine treatment on extinction learning. (E) Mean ± SEM percent freezing of tianeptine-treated (n=21) and saline-treated (n=24) rats during each trial of extinction training. Tianeptine-treated rats exhibited significantly less tone-elicited freezing than saline-treated rats during the first tone trial, indicating that subchronic tianeptine treatment reduced fear expression (p<0.01). (F) Mean ± SEM percent freezing of each group averaged across the first 10 tones, which were presented on the first day of extinction training. (G) Mean ± SEM percent freezing of each group averaged across the last 10 tones, which were presented on the second day of extinction training. *p<0.01 versus saline.

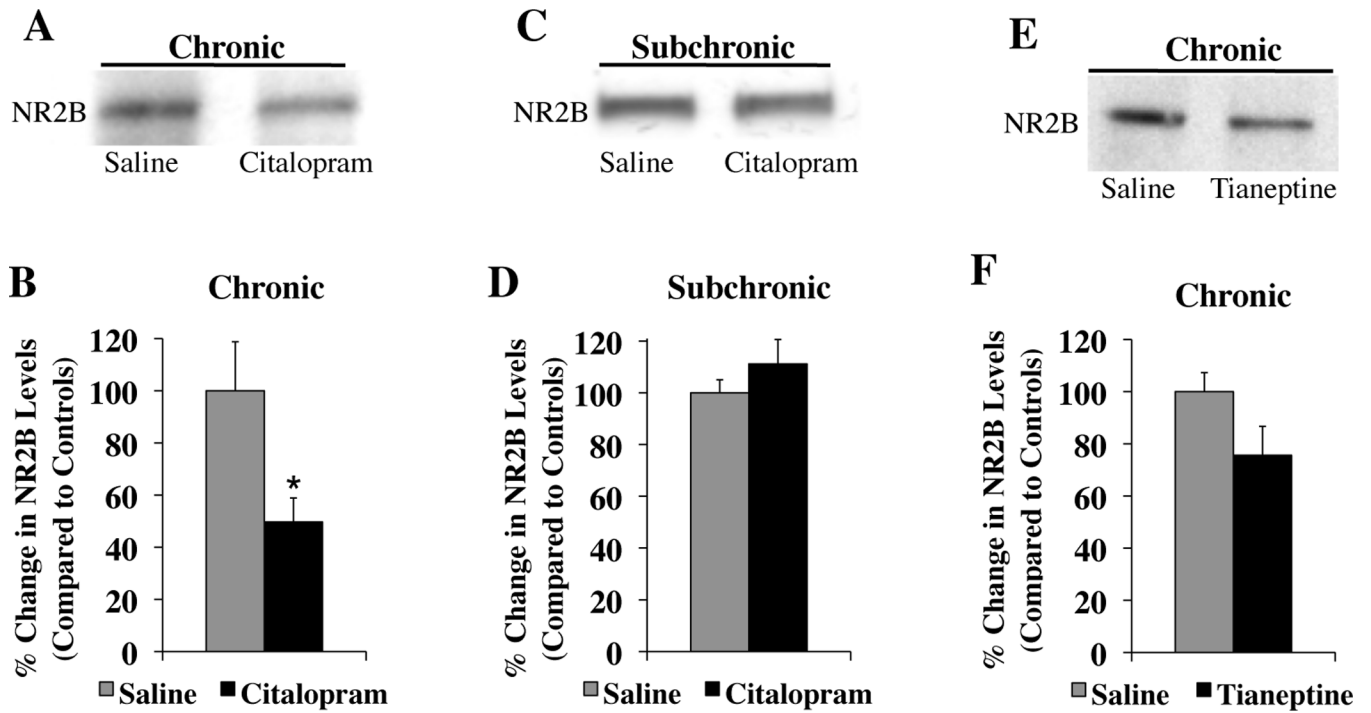


Figure 3. NR2B protein expression in the amygdala (A, C, & E)

Representative western blots of rat tissue containing the lateral and basal nuclei of the amygdala. **(B & D)** Bar graphs summarizing the effects of chronic (citalopram, n=7; saline, n=7) **(B)** and subchronic (citalopram, n=4; saline, n=4) **(D)** citalopram treatment on NR2B subunit expression. **(F)** Bar graph summarizing the effects of chronic (tianeptine, n=11; saline, n=12) tianeptine administration on NR2B subunit expression. All samples were normalized to actin levels and then compared to controls samples. *p<0.05 versus saline.