Differential effectiveness of tianeptine, clonidine and amitriptyline in blocking traumatic memory expression, anxiety and hypertension in an animal model of PTSD

Phillip R. Zoladz a, Monika Fleshner b, David M. Diamond c, d, e, f, *

a Department of Psychology, Sociology & Criminal Justice, Ohio Northern University, Ada, OH, USA
b Department of Integrative Physiology & Center for Neuroscience, University of Colorado, Boulder, CO, USA
c Medical Research Service, VA Hospital, Tampa, FL, USA
d Department of Psychology, University of South Florida, Tampa, FL, USA
e Department of Molecular Pharmacology & Physiology, University of South Florida, Tampa, FL, USA
f Center for Preclinical & Clinical Research on PTSD, University of South Florida, Tampa, FL, USA

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ABSTRACT

Individuals exposed to life-threatening trauma are at risk for developing post-traumatic stress disorder (PTSD), a debilitating condition that involves persistent anxiety, intrusive memories and several physiological disturbances. Current pharmacotherapies for PTSD manage only a subset of these symptoms and typically have adverse side effects which limit their overall effectiveness. We evaluated the effectiveness of three different pharmacological agents to ameliorate a broad range of PTSD-like symptoms in our established predator-based animal model of PTSD. Adult male Sprague–Dawley rats were given 1-h cat exposures on two occasions that were separated by 10 days, in conjunction with chronic social instability. Beginning 24 h after the first cat exposure, rats received daily injections of amitriptyline, clonidine, tianeptine or vehicle. Three weeks after the second cat exposure, all rats underwent a battery of behavioral and physiological tests. The vehicle-treated, psychosocially stressed rats demonstrated a robust fear memory for the two cat exposures, as well as increased anxiety expressed on the elevated plus maze, an exaggerated startle response, elevated heart rate and blood pressure, reduced growth rate and increased adrenal gland weight, relative to the vehicle-treated, non-stressed (control) rats. Neither amitriptyline nor clonidine was effective at blocking the entire cluster of stress-induced sequelae, and each agent produced adverse side effects in control subjects. Only the antidepressant tianeptine completely blocked the effects of psychosocial stress on all of the physiological and behavioral measures that were examined. These findings illustrate the differential effectiveness of these three treatments to block components of PTSD-like symptoms in rats, and in particular, reveal the profile of tianeptine as the most effective of all three agents.

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1. Introduction

Individuals who are exposed to intense trauma that threatens physical injury or death are at significant risk for developing post-traumatic stress disorder (PTSD). People who develop PTSD respond to a traumatic experience with intense fear, helplessness or horror (American Psychiatric Association, 1994) and may endure chronic psychological distress by repeatedly reliving their trauma through intrusive, flashback memories (Ehlers et al., 2004; Hackmann et al., 2004; Reynolds and Brewin, 1998, 1999; Speckens et al., 2006, 2007). The re-experiencing and avoidance symptoms of the disorder may hinder everyday functioning in PTSD patients and can foster the development of debilitating symptoms, such as persistent anxiety, exaggerated startle and cognitive impairments (Brewin et al., 2000; Elzinga and Bremner, 2002; Nemeroff et al., 2006; Newport and Nemeroff, 2000; Stam, 2007).

The finding that a subset of people with PTSD exhibit an improvement in their symptoms following treatment with selective serotonin reuptake inhibitors (SSRIs) suggests a role of the serotonergic system in the disorder (Assis et al., 2004; Davis et al., 2006; Hidalgo and Davidson, 2000; Ipser et al., 2006; Stein et al., 2006). Indeed, two SSRIs, paroxetine and sertraline, are the only FDA-approved pharmacological treatments for the disorder (Albucher and Liberzon, 2002; Van der Kolk, 2001; Vaswani et al., 2003). However, response rates to SSRIs in PTSD patients rarely exceed 60% and full remission from the disorder following SSRI treatment is achieved only 20–30% of the time (Stein et al., 2002). In addition, some forms of PTSD, such as combat-related PTSD, are resistant to SSRI treatment (Jakovljevic
et al., 2003; Rothbaum et al., 2008; Stein et al., 2002). Moreover, SSRIs tend to blunt the depressive components of PTSD, while having little effect on the memory- and anxiety-related symptoms of the disorder (Annis et al., 2004; Boehnlein and Kinzie, 2007; Brady et al., 2000). It is important to note that SSRIs tend to exert anxiogenic effects on individuals early in the treatment phase (Browning et al., 2007; Burghardt et al., 2004; Humble and Wistedt, 1992). Given the caveats to the efficacy of SSRIs in treating PTSD, there is a need for additional research in people with PTSD and in animal models of PTSD to facilitate the discovery of more effective treatments for the disorder.

Other work has examined the efficacy of first-generation antidepressants, the tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), in treating PTSD. TCAs inhibit the reuptake of serotonin and norepinephrine. Research has shown that one such antidepressant, amitriptyline, ameliorates a subset of the symptoms of PTSD and, in some cases, may be more effective than SSRIs (Celik et al., 2011; Davidson et al., 1990, 1993). Thus, in the present study, we included amitriptyline as a positive control, as we expected it to block anxiety-like symptoms in rats. MAOIs, on the other hand, interfere with the catabolism of monoamine transmitters. Overall, studies that have been conducted on TCAs and MAOIs have generally shown that they can reduce a subset of symptoms associated with PTSD (Baker et al., 1995; Bisson, 2007; Burstein, 1984; Davidson et al., 1990, 1993; Frank et al., 1988; Katz et al., 1994; Kosten et al., 1991; Neal et al., 1997; Reist et al., 1989; Shestatzky et al., 1988). However, both classes of drugs are rarely used as the first line of treatment for PTSD and, instead, are typically employed only when SSRIs are ineffective (Albucher and Liberzon, 2002). Due to the numerous effects of both drug classes, the dropout rates for these agents are high (e.g., 30–50%). Additionally, patients who take MAOIs adhere to a special low tyramine diet to avoid a potential life-threatening hypertensive crisis. Thus, despite some beneficial features of TCAs and MAOIs in the treatment of PTSD symptoms, overall, these drugs are not ideal for the treatment of people with anxiety disorders.

An alternative pharmacological approach is to target the elevated baseline and stress-induced elevations of sympathetic activity which are known to occur in PTSD (Buckley and Kaloupek, 2001; Pole, 2007). One indication of the accentuated sympathetic activity in PTSD patients is the hyper-responsivity they exhibit to the administration of yohimbine, an α2-adrenergic receptor antagonist that inhibits noradrenergic autoreceptors and leads to increased central norepinephrine activity (Rasmussen et al., 2000; Southwick et al., 1993, 1999a, 1999b, 1999c). These findings, along with those of greater baseline norepinephrine levels in PTSD patients (Strawn and Geracioti, 2008), have implicated a major role of the noradrenergic system in the hyperarousal component of PTSD.

Based on observed hyperactivity of the noradrenergic system in PTSD, recent work has examined the efficacy of drugs that reduce central noradrenergic activity in treating the disorder. Studies have found some support for the use of propranolol, a β-adrenergic receptor antagonist, to reduce a subset of symptoms of PTSD when administered after a traumatic event or with re-experiencing a traumatic memory (Brunet et al., 2011; Hoge et al., 2012; Pitman et al., 2002; Taylor and Raskind, 2002; Vaiva et al., 2003). Related work has shown that clonidine, an α2-adrenergic receptor (noradrenergic autoreceptor) agonist, and prazosin, an α1-adrenergic receptor antagonist, ameliorated symptoms of heightened anxiety and hyperarousal in people with PTSD (Boehnlein and Kinzie, 2007). Though many studies have examined the effects of clonidine on PTSD and found it to be effective at reducing intrusive memories and hyperarousal (Harmon and Rigg, 1996; Porter and Bell, 1999; Viola et al., 1997), no randomized, placebo-controlled studies of clonidine’s effects on PTSD have been performed (Boehnlein and Kinzie, 2007). Recent work has shown that prazosin is an effective treatment for hyperarousal symptoms, intrusive thoughts, recurrent distressing dreams and sleep disturbances in PTSD (Brikanac et al., 2003; Peskind et al., 2003; Raskind et al., 2002, 2003; Taylor and Raskind, 2002; Taylor et al., 2006). Collectively, these studies suggest that pharmacological reduction of adrenergic activity may be incorporated into the treatment of PTSD patients.

Whereas the antidepressant tianeptine is commonly known for its effectiveness in ameliorating symptoms of major depression, one pilot study provided evidence that it has beneficial effects in the treatment of PTSD (Onder et al., 2006). Tianeptine’s primary mechanism of action involves the stabilization of glutamatergic neurotransmission and the enhancement of synaptic plasticity, particularly under stress conditions (Brink et al., 2006; Kasper and McEwen, 2008; Kole et al., 2002; McEwen et al., 2010; Zoladz et al., 2008b). Preclinical studies have shown that stress significantly increases glutamate levels (Bagley and Moghaddam, 1997; Lowy et al., 1993, 1995; Moghaddam, 1993; Reznikov et al., 2007), inhibits glutamate uptake (Yang et al., 2005), increases the expression and binding of glutamate receptors (Bartanusz et al., 1995; Krugers et al., 1993; McEwen et al., 2002) and increases calcium currents (Joels et al., 2003). Tianeptine prevents the deleterious effects of stress on brain and behavior by normalizing these alterations in glutamatergic neurotransmission. Extensive preclinical research has shown that tianeptine reverses stress-induced alterations of neuronal morphology and synaptic plasticity and blocks the effects of stress on learning and memory (Campbell et al., 2008; Diamond et al., 2004; Kasper and McEwen, 2008; McEwen et al., 2010; Shakesby et al., 2002; Vouima et al., 2006; Zoladz et al., 2008b). Overall, the clinical and preclinical findings suggest that tianeptine could be an effective pharmacological treatment for individuals diagnosed with PTSD.

Our understanding of the mechanisms underlying PTSD may be enhanced by animal models that mimic the experiences of traumatized individuals. To this end, we have developed an animal model that includes conditions known to maximize the likelihood of PTSD developing in people, including a threat to survival, a lack of control, an intrusive reminder of the traumatic experience and a lack of social support (Roth et al., 2011; Zoladz et al., 2008a, 2012). Specifically, we have produced PTSD-like effects in rats by exposing them to two 1-h periods of inescapable confinement in close proximity to a cat, in conjunction with daily social stress, over a period of 1 month. We demonstrated that rats that were administered this psychosocial stress paradigm exhibited heightened anxiety, exaggerated startle, impaired cognition, increased cardiovascular reactivity and an exaggerated response to yohimbine administration, all of which are commonly observed in people with PTSD. The purpose of the present experiment was to investigate the influence of chronic administration of amitriptyline, clonidine or tianeptine on physiological and behavioral sequelae induced by this predator-based psychosocial stress regimen. To emphasize that the design of this experiment has clinical relevance, administration of the pharmacological agents did not begin until 24 h post-trauma. Our findings, therefore, are potentially relevant toward optimizing clinically relevant outcomes based on a person seeking treatment soon after an intense traumatic experience occurs.

2. Method

2.1. Animals

Experimentally naïve adult male Sprague–Dawley rats (225–250 g upon delivery) obtained from Charles River laboratories (Wilmington, MA) were used in the present experiment. The rats were housed on a 12-h light/dark schedule (lights on at 0700) in standard Plexiglas cages (two per cage) with free access to food and water. Upon arrival, all rats were given 1 week to acclimate to the housing room environment and cage changing procedures before any experimental manipulations took place. All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Florida.
2.2 Psychosocial stress procedure

2.2.1. Acute stress sessions

Following the 1-week acclimation phase, rats were brought to the laboratory and randomly assigned to “psychosocial stress” or “no psychosocial stress” groups (N = 10 rats/group). Rats from these groups were first exposed to a chamber for 3 min. During the last 30 s of the 3-min chamber exposure, a 74-dB, 2500 Hz tone was presented to the rats. The chamber (26 × 30 × 29 cm; Coulbourn Instruments; Allentown, PA) consisted of two aluminum sides, an aluminum ceiling, and a Plexiglas front and back. The floor of the chamber consisted of 18 stainless steel rods, spaced 1 cm apart. The sole purpose of exposing rats to the chamber was to allow rats in the psychosocial stress groups to associate the chamber (contextual fear conditioning) and tone [auditory (cue) fear conditioning] with the acute stress experience (i.e., immobilization plus cat exposure, as described below) and measure their memory for the experience (via an assessment of immobility in the chamber) during behavioral testing (see Zoladz et al., 2012 for comparable methodology). Locomotor activity in the chamber was measured during the acute stress sessions and behavioral testing by a 24-cell infrared activity monitor (Coulbourn Instruments; Allentown, PA) mounted on the top of the chamber, which used the emitted infrared body heat image (1300 nm) from the animals to detect their movement. Immobility was defined as periods of inactivity lasting at least 7 s. Pilot data from our lab indicated that the 7-s cut-off most closely represented off-line scoring of freezing behavior in rats.

Following the 3-min chamber exposure, rats in the psychosocial stress groups were removed from the chamber and immediately immobilized in plastic DecapiCones (BrainTree Scientific; BrainTree, MA), and then they were placed in a perforated wedge-shaped Plexiglas enclosure (BrainTree Scientific; BrainTree, MA; 20 × 20 × 8 cm). The rats, immobilized in the plastic DecapiCones within the Plexiglas enclosure, were taken to the cat housing room where they were placed in a metal cage (61.5 × 53.5 cm) with an adult female cat for 1 h. The Plexiglas enclosure prevented any contact between the cat and rats, but the rats were still exposed to all non-tactile sensory stimuli associated with the cat. Canned cat food was smeared on top of the Plexiglas enclosure to direct the cat’s attention toward the rats. An hour later, the rats were returned to the laboratory. Rats in the no psychosocial stress groups were not immobilized or exposed to the cat; rather, they remained in their home cages in the laboratory during the 1-h period the stressed group was exposed to the cat.

Psychosocial stressed rats were exposed to 2 acute stress sessions which were separated by 10 days. The first acute stress session took place during the light cycle, between 0800 and 1300 h, and the second acute stress session took place during the dark cycle, between 1900 and 2100 h. The two acute stress sessions took place during different times of the day to add an element of unpredictability as to when the rats might re-experience the traumatic event (Zoladz et al., 2008a). A lack of predictability in one’s environment is a major factor in the development and expression of PTSD, at least in a subset of susceptible people (Orr et al., 1990; Regehr et al., 2000; Solomon et al., 1988, 1989).

2.2.2. Daily social stress

Beginning on the day of the first acute stress session, and continuing for the next 31 days, rats in the psychosocial stress groups were exposed to daily unstable housing conditions, as described previously (Roth et al., 2011; Zoladz et al., 2008a, 2012). Rats in the psychosocial stress groups were housed two per cage, but every day, their cohort pair combinations were changed. Therefore, no rat in the psychosocial stress groups had the same cage mate on two consecutive days during the 31-day stress period. All rats in the stress groups were administered the two cat exposures in conjunction with daily social instability because we found previously that the complete PTSD-like profile was generated only in rats that were exposed to the combination of predator exposure and social stress (Zoladz et al., 2008a). Rats in the control groups were housed with the same cohort pair for the duration of the experiment.

2.3. Pharmacological agents

Twenty-four hours after the first acute stress session (i.e., Day 2), all rats began receiving daily intraperitoneal (i.p.) injections of amitriptyline (5 or 10 mg/kg), clonidine (0.01 or 0.05 mg/kg), tianeptine (10 mg/kg) or vehicle (0.9% saline). The injections occurred every day throughout the 31-day period of psychosocial stress and also throughout behavioral testing. Drug administration continued during behavioral testing to prevent possible drug withdrawal effects from influencing rat behavior. The injections were always administered in the morning (between 0900 and 1200 h) at a volume of 1 ml/kg. Ten rats from each of the psychosocial stress and no psychosocial stress groups were randomly assigned to each of the drug conditions. Amitriptyline and clonidine were obtained from Sigma-Aldrich (St. Louis, MO), and tianeptine was provided by Servier Pharmaceuticals (France). The doses of amitriptyline and clonidine were chosen based on the findings of preclinical studies demonstrating the efficacy of similar doses in preventing stress–induced sequelae in rats (Blanc et al., 1991; Ferretti et al., 1995; Katz and Hersh, 1981; Murphy et al., 1996; Sobolsky and Thurmond, 1986). The single dose of tianeptine was based on extensive findings from our laboratory and others which have demonstrated the effectiveness of this dose in preventing acute and chronic stress effects on the brain and behavior (Campbell et al., 2008; Conrad et al., 1999; Diamond et al., 2004; Reagan et al., 2004; Vouimba et al., 2006; Zoladz et al., 2010).

2.4. Behavioral testing

Three weeks after the second acute stress session (Day 32), behavioral testing began; all rats were given tests to measure their fear memory, anxiety, startle, learning and memory, cardiovascular activity and corticosterone levels. The three-week delay from the second acute stress session to behavioral testing was based on comparable time periods employed in other studies on the effects of stress on brain and behavior (Adamec and Shallow, 1993; Cook and Wellman, 2004; Magarinos et al., 1996; McLaughlin et al., 2007; Park et al., 2001; Watanabe et al., 1992a, 1992b, 1992c; Zoladz et al., 2008a). On the first 4 days of behavioral testing (Days 32–35), rats were taken to the procedure room across from the rat housing rooms, where they received i.p. injections of the drug appropriate to the condition to which they had been assigned. Then, they were taken to the laboratory and left undisturbed for 30 min before testing began. All behavioral testing took place during the light cycle, between 0800 and 1500 h.

2.4.1. Contextual and cue fear memory

On Day 32, rat behavior in response to the chamber (context test) and tone (cue test) that had been previously paired with the acute stress sessions was examined. Rats were placed in the same chamber that they were exposed to during each of the two acute stress sessions for 5 min, and their immobility was recorded. One hour after the 5-min context test, following methods and timing in our previous work (Zoladz et al., 2008a, 2012), the rats were placed in a novel chamber that had different lighting, walls and flooring from the chamber in which they were placed during each of the two acute stress sessions. The rats were placed in the novel chamber for a total of 6 min (cue test). Three minutes into the cue test, the rats were presented with a 74-dB, 2500 Hz tone that continuously played for the remainder of the 6-minute testing period. The amount of immobility recorded during the first 3 min of the cue test (i.e., no tone) provided a measure of the general fear of a novel place, while
the amount of immobility recorded during the last 3 min of the cue test (i.e., tone) provided a measure of the fear response to the cue that was, in the psychosocial stress group, specifically associated with the two acute cat exposures.

2.4.2. Elevated plus maze

The elevated plus maze (EPM) is a routine test of anxiety in rodents and consisted of two open arms (11 × 50 cm) and two closed arms (11 × 50 cm) that intersect each other to form the shape of a plus sign. On Day 33, the rats were placed on the EPM for 5 min, and their behavior was scored by 48 infrared photobeams located along the perimeter of the open and closed arms, which were connected to a computer program (Motor Monitor, Hamilton-Kinder, San Diego, CA). The primary dependent measures of interest were the percent of time rats spent in the open arms and the number of ambulations (i.e., an indication of overall movement) made by each rat.

2.4.3. Startle response

Acoustic startle testing was administered one hour after the EPM assessment following the methodology from our previous work (Zoladz et al., 2008a, 2012). The rats were placed inside a small Plexiglas box (19 × 10 × 10 cm), which was inside a larger startle monitor cabinet (Hamilton-Kinder; San Diego, CA; 36 × 28 × 50 cm). The small Plexiglas box within this cabinet contained a sensory transducer on which the rats were placed at the beginning of the trial. The sensory transducer was connected to a computer (Startle Monitor; Hamilton-Kinder; San Diego, CA), which recorded the startle responses by measuring the maximum amount of force (Newtons) that rats exerted on the sensory transducer for a period of 250 ms after the presentation of each auditory stimulus. To control for any differences in body weight, the sensitivity of the sensory transducer was adjusted prior to each trial via a Vernier adjustment with a sensitivity range of 0–7 arbitrary units. The startle trial began with a 5-min acclimation period, followed by the presentation of 24 bursts of white noise (50 ms each), eight from each of three auditory intensities (90, 100, and 110 dB). The noise bursts were presented in sequential order, and the time between each noise burst varied pseudorandomly between 25 and 55 s. Upon the commencement of the first noise burst, the startle apparatus provided uninterrupted background white noise (57 dB).

2.4.4. Novel object recognition

The novel object recognition (NOR) task was based on methods described previously (Baker and Kim, 2002). On Day 34, the rats were placed in an open field (Hamilton-Kinder; San Diego, CA; 40 × 47 × 70 cm) for 5 min to acclimate to the environment. Their behavior was monitored by a Logitech camera that was mounted on the ceiling overlooking the open field. This camera was connected to a computer program known as ANY-Maze (Stoelting; Wood Dale, IL), which scored rat behavior. Twenty-four hours later (Day 35), the rats were placed in the same open field with two identical (plastic/metal) objects for 5 min. The objects were in opposite corners of the open field and secured to the flooring to prevent the rats from displacing them. The objects were counterbalanced across rats, as were the corners in which the objects were placed. Three hours later, the rats were returned to the open field for a final 5-min test trial, but this time the open field contained a replica of the object that had been there before and a novel object. During this testing session, greater time spent by the rats in proximity to the novel versus familiar object was an indication of intact memory for the familiar object. The time that each rat spent with the objects during training and testing was quantified by specifying a zone around the objects for the ANY-maze software to score the duration of investigatory behavior.

2.4.5. Blood sampling, cardiovascular activity & post-mortem dissections

On the final day of behavioral testing (Day 36), rats were brought, one cage at a time, to a nearby procedure room for blood sampling.

The saphenous vein of each rat was punctured with a sterile, 27-gauge syringe needle. A 0.2 cm³ sample of blood was then collected from each rat. This first blood sample was collected within 2 min after the rats were removed from the housing room. After obtaining this sample, the rats were immobilized in plastic DecapiCones for 20 min. Then, the rats were removed from the DecapiCones, and another 0.2 cm³ sample of blood was collected via saphenous vein puncture. Blood sampling at the 20 min time point was based on our previous work (Zoladz et al., 2008a, 2012), and the well-established finding that corticosterone reaches its maximum level approximately 20 min after stress onset (e.g., Ferland and Schrader, 2011). Immediately after collecting this blood sample, the rats were placed in Plexiglas tubes within a warming test chamber (~32 °C) for 5 min to increase their body temperature. This enhanced blood flow to their tails and allowed heart rate (HR) and blood pressure (BP) to be assessed using a tail cuff fitted with photoelectric sensors (IITC Life Science; Woodland Hills, CA). Three HR and BP recordings were obtained from each rat (these three recordings were averaged to create single HR and BP data points for each rat). Following HR and BP measurements, the rats were returned to their home cages. An hour later, one last blood sample (trunk blood) was collected following rapid decapitation. Then, the adrenal glands and thymuses were removed and weighed. Once all of the blood had clotted at room temperature, it was centrifuged (3000 rpm for 8 min), and the serum was extracted and stored at −80 °C until assayed by M.F. at the University of Colorado at Boulder.

2.5. Statistical analyses

The present study utilized a between-subjects, 2 × 6 factorial design. The independent variables were psychosocial stress and drug. In most cases, two-way, between-subjects ANOVAs were used to analyze the data from the physiological and behavioral assessments, with psychosocial stress and drug serving as the between-subjects factors. When repeated measures variables were involved, mixed-model ANOVAs were utilized to analyze the data. Planned comparisons (independent samples t-tests) were conducted between groups that were predicted to differ a priori. For all analyses, alpha was set at 0.05, and Holm Sidak post hoc tests were employed when necessary.

3. Results

In all figures, the specific drug groups are identified by the following abbreviations: vehicle (VEH), amitriptyline — 5 mg/kg (AMI-5), amitriptyline — 10 mg/kg (AMI-10), clonidine — 0.01 mg/kg (C-0.01), clonidine — 0.05 mg/kg (C-0.05), tianeptine — 10 mg/kg (TIA-10).

3.1. Fear memory expression

3.1.1. Acute stress sessions (Days 1 and 10)

During the first session, there was little evidence of freezing (immobility) in any group, and no significant group differences (p > 0.05). This absence of freezing was evident because all rats were naive to the chamber, which had not yet been paired with an adverse stimulus (cat exposure) in the psychosocial stress group. During the second acute stress session, which took place 10 days after the first, there was a significant main effect of psychosocial stress, indicating that the psychosocial stress groups spent significantly more time immobile than the no psychosocial stress groups, \( F(1,103) = 7.55, p < 0.01 \) (Fig. 1).

3.1.2. Fear conditioning context memory test (Day 32)

There were significant main effects of psychosocial stress, \( F(1,97) = 11.96 \), and drug, \( F(5,97) = 3.90 \), and the Psychosocial Stress × Drug interaction was significant, \( F(5,97) = 2.56 \) (p < 0.05).
The vehicle-treated psychosocial stress group spent significantly more time immobile than the vehicle-treated no psychosocial stress group (Fig. 1). Chronic treatment with 5 or 10 mg/kg of amitriptyline or 10 mg/kg of tianeptine in groups that were psychosocially stressed prevented the chronic stress-induced increase in immobility, as evidenced by significantly less immobility than the vehicle-treated psychosocial stress group and a lack of statistical significance relative to each of the group’s respective drug-treated no psychosocial stress group. The group of psychosocially stressed rats treated with 0.01 mg/kg of clonidine also did not exhibit significantly greater immobility than its respective drug-treated control group; however, the amount of immobility displayed by this group was not statistically different from that of the vehicle-treated psychosocial stress group.

3.1.3. Fear conditioning pre-cue (novel environment) test (Day 32)
There were significant main effects of psychosocial stress, $F(1,100) = 9.40$, and drug, $F(5,100) = 2.72$, and the Psychosocial Stress × Drug interaction was significant, $F(5,100) = 5.73$ ($p < 0.05$). Chronic treatment with 0.05 mg/kg of clonidine in rats that were psychosocially stressed led to significantly greater immobility than all other groups.

3.1.4. Fear conditioning cue memory test (Day 32)
There was a significant main effect of psychosocial stress, $F(1,100) = 12.04$, and the Psychosocial Stress × Drug interaction was significant, $F(5,100) = 2.71$ ($p < 0.05$). The vehicle-treated psychosocial stress group spent significantly more time immobile than the
vehicle-treated no psychosocial stress group. Additionally, chronic treatment with 10 mg/kg of amitriptyline in groups that were not psychosocially stressed led to significantly greater immobility than the vehicle-treated no psychosocial stress group. Chronic treatment with 5 or 10 mg/kg of amitriptyline or 10 mg/kg of tianeptine in groups that were psychosocially stressed blocked the fear-conditioned increase in immobility, as evidenced by a lack of statistical significance relative to each of the group’s respective drug-treated no psychosocial stress group.

3.2. Elevated plus maze

3.2.1. Percent time in open arms

A two-way ANOVA revealed no significant main effects or interactions \((p>0.05)\). Therefore, planned comparisons were conducted between groups that were predicted to differ a priori. The vehicle-treated psychosocial stress group spent significantly less time in the open arms of the EPM than the vehicle-treated no psychosocial stress group, \(t(15)=2.44, p<0.05\) (Fig. 2). Chronic treatment with 10 mg/kg of amitriptyline, 0.01 or 0.05 mg/kg of clonidine or 10 mg/kg of tianeptine in groups that were psychosocially stressed blocked the stress-induced decrease in open arm exploration. These groups exhibited significantly greater percent time spent in the open arms than the vehicle-treated psychosocial stress group \((p<0.05)\).

3.2.2. Ambulations

There was a significant main effect of drug, \(F(5,100)=4.48, p<0.001\). Groups treated with 10 mg/kg of amitriptyline or 10 mg/kg of tianeptine exhibited significantly more ambulations than the vehicle-treated group. Planned comparisons were conducted between groups that were predicted to differ a priori. There was no significant difference between the number of ambulations made by the vehicle-treated psychosocial stress group and the vehicle-treated no psychosocial stress group on the EPM, \(t(16)=0.16, p>0.05\). Chronic treatment with 10 mg/kg of amitriptyline, \(t(15)=2.67\), or 10 mg/kg of tianeptine, \(t(16)=2.38\), in groups that were not psychosocially stressed led to a significantly greater number of ambulations on the EPM, relative to the vehicle-treated no psychosocial stress group \((p<0.05)\).

3.3. Startle response

The Psychosocial Stress × Drug interaction was significant for the startle responses to 90 and 100 dB stimuli \((90 \text{ dB}: F(5,102)=2.74; 100 \text{ dB}: F(5,98)=2.32; p<0.05)\). For the 110 dB stimuli, no significant effects were observed in the ANOVA, so planned comparisons were conducted between groups that were predicted to differ a priori. For each stimulus intensity, except for 110 dB which was marginally significant \((p=0.056)\), the vehicle-treated psychosocial stress group exhibited significantly greater startle responses than the vehicle-treated no psychosocial stress group (Fig. 3). All of the chronically administered pharmacological agents blocked the psychosocial stress-induced increase in startle response, except for 5 mg/kg of amitriptyline and 0.01 mg/kg of clonidine in response to 100 dB stimuli.

3.4. Novel object recognition

3.4.1. Habituation

3.4.1.1. Overall locomotor activity. There were significant main effects of psychosocial stress, \(F(1,104)=8.25\), and drug, \(F(5,104)=9.27\) \((p<0.01)\). In general, rats that had been psychosocially stressed traveled significantly less distance in the open field than rats that had not been psychosocially stressed. In addition, rats that were treated with 0.05 mg/kg of clonidine, independent of psychosocial stress, traveled significantly less distance than all other groups.

3.4.1.2. Time spent in each area of open field. For this analysis, the open field was divided into four square quadrants via the ANY-Maze computer program, and the amount of time that rats spent in each of the quadrants was analyzed. No significant main effects or interactions were observed \((p>0.05)\).

3.4.2. Training

Within-group comparisons (i.e., dependent \(t\) tests) of the amount of time that rats spent with each object replica (to rule out object preference effects) indicated that most groups spent a comparable amount of time with each of the objects that were placed in the open field during object recognition training, suggesting that no object preference effects were present \((p>0.05)\). Only one group, the 0.01 mg/kg clonidine-treated no psychosocial stress group, spent more time with one object than the other \((t(9)=2.78, p<0.05)\). A between-groups comparison of the total amount of time spent with both objects during training revealed that all main effects and interactions were not significant \((p>0.05)\). These findings indicated that all groups spent an equivalent amount of time with both objects during training.

3.4.3. Testing

A “ratio time” score was calculated for each group by taking the time that rats spent with the novel object and dividing it by the

![Fig. 2. Amount of time spent in the open arms (left) and overall locomotor activity (right) exhibited by rats on the elevated plus maze. The vehicle-treated psychosocial stress group spent significantly less time in the open arms, without exhibiting a significant reduction of overall locomotion, compared to the control group. Clonidine, tianeptine and the higher dose of amitriptyline were all effective in preventing the anxiogenic effects of psychosocial stress on the elevated plus maze. The data are presented as the mean ± SEM. * \(p<0.05\) relative to the vehicle-treated no psychosocial stress group; β = \(p<0.05\) relative to the vehicle-treated psychosocial stress group.](image-url)
time that rats spent with the familiar object. The analysis comparing the ratio times of all groups revealed no significant main effects or interactions (ps > 0.05). Overall, this behavioral assessment revealed no evidence of a preference by the rats for the novel object, nor was there an effect of psychosocial stress or drug treatment.

3.5. Corticosterone levels

There was a significant main effect of time point, $F(2,194) = 487.29$, $p < 0.001$. Corticosterone levels, overall, increased following 20 min of acute immobilization stress, and these levels declined, but remained significantly elevated relative to baseline, 1 h later (Fig. 4). There was also a significant main effect of drug, $F(5,97) = 24.00$, $p < 0.001$. Rats treated with 5 mg/kg of amitriptyline displayed significantly lower corticosterone levels, overall, than all other groups, except for those treated with tianeptine or 10 mg/kg of amitriptyline. In addition, rats treated with 10 mg/kg of amitriptyline exhibited significantly lower corticosterone levels, overall, than all other groups, except for those treated with 5 mg/kg of amitriptyline. Lastly, rats that were treated with tianeptine had significantly lower corticosterone levels, overall, than rats treated with vehicle or 0.01 mg/kg of clonidine. The Time Point × Drug interaction was significant, $F(10,194) = 8.91$, $p < 0.001$. Both doses of amitriptyline, particularly the 10 mg/kg dose, significantly blunted the acute immobilization-induced increase in serum corticosterone levels, independent of exposure to chronic psychosocial stress. Tianeptine had a similar effect, although not as pronounced as that of amitriptyline. The Psychosocial Stress × Drug, $F(3,97) = 2.70$, and Time Point × Psychosocial Stress × Drug, $F(10,194) = 2.21$, interactions were also significant (ps < 0.05). Psychosocially stressed rats treated with 10 mg/kg of amitriptyline exhibited significantly lower corticosterone levels than the controls treated with 10 mg/kg of amitriptyline an hour following 20 min of immobilization. In contrast, 0.01 mg/kg of clonidine prevented the reduction in corticosterone levels an hour following immobilization in the psychosocial stress group only.

3.6. Cardiovascular activity

3.6.1. Heart rate

There was a significant main effect of drug, $F(5,86) = 11.80$, $p < 0.01$. The vehicle-treated psychosocial stress group exhibited significantly greater HR than the vehicle-treated no psychosocial stress group (Fig. 5). The Psychosocial Stress × Drug interaction was significant, $F(5,81) = 3.99$, $p < 0.01$. Chronic treatment with 5 or 10 mg/kg of amitriptyline, 0.01 or 0.05 mg/kg of clonidine or 10 mg/kg of tianeptine in groups that were psychosocially stressed prevented the chronic stress-induced increase in HR, as evidenced by the presence of significantly lower HR than the vehicle-treated psychosocial stress group or a lack of statistical significance relative to each of the group’s respective drug-treated no psychosocial stress group.

3.6.2. Systolic blood pressure

There was a significant main effect of drug, $F(5,86) = 3.60$, ps < 0.01. The vehicle-treated psychosocial stress group had significantly higher systolic BP than the vehicle-treated no psychosocial stress group (Fig. 5). Chronic treatment with 5 or 10 mg/kg of amitriptyline in groups that were not psychosocially stressed led to significantly greater systolic BP than the vehicle-treated no psychosocial stress group. Chronic treatment with 5 or 10 mg/kg of amitriptyline,
0.01 or 0.05 mg/kg of clonidine or 10 mg/kg of tianeptine in groups that were psychosocially stressed prevented the chronic stress-induced increase in systolic BP, as evidenced by the presence of significantly lower systolic BP than the vehicle-treated psychosocial stress group or a lack of statistical significance relative to each of the group’s respective drug-treated no psychosocial stress group.

3.6.3. Diastolic blood pressure

There were significant main effects of psychosocial stress, $F(1,86) = 9.67$, and drug, $F(5,86) = 21.78$, and the Psychosocial Stress × Drug interaction was significant, $F(5,86) = 6.11$ ($p < 0.001$). The vehicle-treated psychosocial stress group had significantly higher diastolic BP than the vehicle-treated no psychosocial stress group (Fig. 5). Additionally, chronic treatment with 5 or 10 mg/kg of amitriptyline in groups that were not psychosocially stressed led to significantly greater diastolic BP than the vehicle-treated no psychosocial stress group. Chronic treatment with 10 mg/kg of amitriptyline, 0.01 or 0.05 mg/kg of clonidine or 10 mg/kg of tianeptine in psychosocially stressed rats led to significantly lower diastolic BP than the vehicle-treated psychosocial stress group. However, psychosocially stressed rats that were treated with 10 mg/kg of amitriptyline still displayed significantly greater diastolic BP than the vehicle-treated no psychosocial stress group, and psychosocially stressed rats that were treated with 0.01 mg/kg of clonidine still exhibited significantly greater diastolic BP than its respective drug-treated no psychosocial stress group.

3.7. Growth rate

Growth rate, expressed as an increase in grams per day (g/day), was calculated for all rats by taking their total body weight gained from the day of the first stress session through the first day of behavioral testing and dividing this by the total number of days elapsed (i.e., 31 days). There was a significant main effect of drug, $F(5,100) = 11.78$, and the Psychosocial Stress x Drug interaction was significant, $F(5,100) = 13.42$ ($p < 0.001$). The vehicle-treated psychosocial stress group had a significantly lower growth rate than the vehicle-treated no psychosocial stress group (Fig. 6). Chronic treatment with 5 or 10 mg/kg of amitriptyline or 0.05 mg/kg of clonidine in groups that were not psychosocially stressed led to significantly lower growth rates than the vehicle-treated no psychosocial stress group. Chronic treatment with 5 or 10 mg/kg of amitriptyline, 0.01 mg/kg of clonidine or 10 mg/kg of tianeptine prevented the chronic stress-induced reduction of growth rate, as evidenced by the presence of significantly greater growth rates than the vehicle-treated psychosocial stress group or a lack of statistical significance relative to each of the group’s respective drug-treated no psychosocial stress group (Fig. 6). However, the psychosocial stress group treated with 5 mg/kg of amitriptyline exhibited a significantly lower growth rate than the vehicle-treated no psychosocial stress group.

3.8. Adrenal gland weight

The adrenal glands were weighed and expressed as milligrams per 100 g of body weight (mg/100 g b.w.). There was a significant main effect of drug, $F(5,97) = 10.53$, and the Psychosocial Stress × Drug interaction was significant, $F(5,97) = 3.26$ ($p < 0.01$). The vehicle-treated psychosocial stress group had significantly greater adrenal gland weight than the vehicle-treated no psychosocial stress group (Fig. 6). Chronic treatment with 10 mg/kg of amitriptyline or 0.05 mg/kg of clonidine in groups that were not psychosocially stressed led to significantly lower adrenal gland weight than the vehicle-treated no psychosocial stress group or a lack of statistical significance relative to each of the group’s respective drug-treated no psychosocial stress group.
greater adrenal gland weight than the vehicle-treated no psychosocial stress group. Chronic treatment with 5 mg/kg of amitriptyline, 0.01 or 0.05 mg/kg of clonidine or 10 mg/kg of tianeptine in groups that were psychosocially stressed prevented the chronic stress-induced hypertrophy of the adrenal glands, as evidenced by a lack of a statistically significant increase in adrenal gland weight relative to each of the group's respective drug-treated no psychosocial stress group. Interestingly, the psychosocial stress group treated with 10 mg/kg of amitriptyline exhibited significantly reduced adrenal gland weight than its respective drug-treated control group.

3.9. Thymus weight

The thymus was weighed and expressed as milligrams per 100 g of body weight (mg/100 g b.w.). There was a significant main effect of psychosocial stress, F(1,91) = 17.12, and the Psychosocial Stress × Drug interaction was significant, F(5,91) = 6.49 (p < 0.001). The difference in thymus weight between the vehicle-treated psychosocial stress group and the vehicle-treated no psychosocial stress group was marginally significant (p = 0.051). Chronic treatment with 10 mg/kg of amitriptyline in groups that were not psychosocially stressed led to significantly reduced thymus weight than the vehicle-treated no psychosocial stress group. Chronic treatment with 5 mg/kg of amitriptyline or 0.01 or 0.05 mg/kg of clonidine in groups that were psychosocially stressed led to significantly reduced thymus weight than the vehicle-treated no psychosocial stress group. Chronic treatment with 10 mg/kg of amitriptyline or 10 mg/kg of tianeptine prevented the slight decrease in thymus weight induced by chronic psychosocial stress, as evidenced by significantly greater thymus weight than the vehicle-treated psychosocial stress group and/or a lack of a statistically significant decrease in thymus weight relative to each of the group's respective drug-treated no psychosocial stress group (Fig. 6).

4. Discussion

We have extended our established animal model of PTSD by evaluating the influence of conventional, as well as potentially novel, pharmacological treatments on psychosocial stress-induced sequelae in psychosocially rats. We have emphasized the clinical relevance in our design by initiating treatment 24 h after initial stress exposure and continuing the treatment daily throughout the duration of the experiment. Treatment beginning 24 h after exposing rats to an intense stressor is potentially relevant to treatment applications initiated in people who seek treatment within 24 h of a traumatic experience. The relative effectiveness of the treatments described here may highlight the importance of beginning a treatment regimen soon after a person experiences intense trauma. Our approach may be useful not only for understanding the physiological and behavioral mechanisms underlying PTSD, but also for discerning how certain treatments act on the brain to ameliorate PTSD-like symptom development.

Consistent with our previous work (Zoladz et al., 2008a, 2012), vehicle-treated psychosocially stressed rats exhibited reduced growth rate, greater adrenal gland weight, heightened anxiety, an exaggerated startle response, greater BP reactivity to an acute stressor and strong, fear conditioned “traumatic” memory for the context and cue that were associated with the two cat exposures. While amitriptyline and clonidine were effective in ameliorating a subset of the PTSD-like...
symptoms, tianeptine was the only pharmacological agent that prevented the effects of psychosocial stress on all physiological and behavioral endpoints without having adverse effects of its own in control animals. Thus, our findings highlight tianeptine, more than the other agents, as a potential component of pharmacotherapy to include in early post-trauma treatment. In Table 1 we have provided a comparison of the effectiveness of each pharmacological agent in ameliorating the psychosocial stress effects on the behavioral and physiological measurements under study.

### Table 1

Efficacy of amitriptyline, clonidine and tianeptine in ameliorating PTSD-like sequelae in rats.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>AMI-5</th>
<th>AMI-10</th>
<th>C-0.01</th>
<th>C-0.05</th>
<th>TIA</th>
</tr>
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<tr>
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<td>+</td>
<td>+</td>
<td>N</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cue test</td>
<td>N</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EPM — open arms</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Startle — 90 dB</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Startle — 100 dB</td>
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<td>N</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>C</td>
<td>C</td>
<td>N</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth rate</td>
<td>C</td>
<td>C</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Adrenal gland weight</td>
<td>+</td>
<td>C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thymus gland weight</td>
<td>-</td>
<td>C</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = prevented chronic psychosocial stress effects on endpoint; − = did not prevent chronic psychosocial stress effects on endpoint; N = insufficient basis for determining if chronic psychosocial stress effects on endpoint were prevented; C = compromised assessment due to adverse side effects in controls.

#### 4.1. Amitriptyline

Both doses of the TCA amitriptyline blocked the expression of traumatic memory in psychosocially stressed rats in response to the context and cue that were paired with the two cat exposures (see the 5 mg/kg dose response for a caveat; Table 1). These fear responses served as a measure of memory for the acute stress experiences and rat analogs of a traumatic memory in humans (Zoladz et al., 2012). As traumatic memories are a source of psychological distress in people with PTSD, these findings suggest that amitriptyline could serve to effectively reduce the strength of traumatic memories and consequentially diminish the intrusion and re-experiencing symptoms endured by PTSD patients. One caveat to this interpretation, however, is that extensive work has reported amitriptyline-induced memory impairments in both humans (Kerr et al., 1996; Liljequist et al., 1978; Mattila et al., 1978; Spring et al., 1992; van Laar et al., 2002) and rodents (Everss et al., 2005; Gonzalez-Pardo et al., 2008; Kumar and Kulkarni, 1996), findings that may be related to the drug’s anti-cholinergic side effects (Pavone et al., 2005; Gonzalez-Pardo et al., 2008; Kumar and Kulkarni, 1996). Therefore, the attenuation of contextual and cue fear conditioning in psychosocially stressed rats treated with amitriptyline could be due to its amnestic side effects, rather than a specific amelioration of the chronic stress-induced behavioral sequelae. On the other hand, studies reporting amitriptyline-induced memory impairments have administered the drug prior to learning. In the present experiment, amitriptyline treatment did not begin until 24 h after the first pairing of the context and cue with the cat exposure. Therefore, a more likely explanation of the present findings is that amitriptyline blunted the augmentation of contextual and cue fear conditioning in psychosocially stressed rats that occurred in response to the second cat exposure on
Day 11 of the paradigm. Another possible explanation of these findings is that amitriptyline increased general locomotor activity, thus reducing overall immobility. Amitriptyline-treated control animals did display a significantly greater amount of motor activity on the EPM than vehicle-treated animals, but the same effect was not observed during the open field habituation period on the following day.

Both doses of amitriptyline were at least partially effective in preventing the chronic stress-induced increase in startle response, but only the 10 mg/kg dose of amitriptyline blocked the effects of chronic psychosocial stress on anxiety, as measured by rat behavior on the EPM. These findings are consistent with several studies in the rodent literature reporting that chronic amitriptyline administration results in significantly reduced basal and stress-induced levels of adrenocorticotropic hormone (ACTH) and corticosterone in rats (Barden, 1999; Reul et al., 1993). Amitriptyline appears to accomplish these effects by enhancing the negative feedback inhibition of the hypothalamus–pituitary–adrenal (HPA) axis. Investigators have shown that chronic administration leads to an up-regulation of glucocorticoid receptor expression and enhanced glucocorticoid receptor binding in several brain regions (Barden, 1999; Pariante and Miller, 2001; Przegalinski and Budziszewska, 1993; Reul et al., 1993). Interestingly, in the present study, there was an additive effect of amitriptyline and psychosocial stress on the recovery of serum corticosterone levels following immobilization. Psychosocially stressed rats that were treated with amitriptyline, particularly the 10 mg/kg dose, exhibited significantly lower corticosterone levels at the 80 minute time point than amitriptyline-treated controls. In theory, chronic amitriptyline and psychosocial stress (Zoladz et al., 2012) synergistically facilitated the production of enhanced negative feedback of the HPA axis, which led to a more rapid recovery of stress-induced serum corticosterone levels in these rats.

Despite the positive effects of amitriptyline on the chronic stress-induced physiological and behavioral sequelae in rats, there were adverse side effects of the drug that should be considered. For instance, chronic amitriptyline treatment resulted in significantly greater acute stress-induced increases in systolic and diastolic BP than vehicle. In addition, the mortality rate for amitriptyline-treated rats (3/40, 7.5%) was greater than the mortality rates for rats treated with clonidine (0/40, 0%) or tianeptine (0/20, 0%). Thus, despite its prevention of effects of psychosocial stress on anxiety-like behavior, startle response and fear memory, the adverse physiological side effects of amitriptyline could be a major limitation to its use in the treatment of people with PTSD.

4.2. Clonidine

Neither dose of clonidine prevented the expression of traumatic memory in psychosocially stressed rats in response to the context and cue that were paired with the two cat exposures. Although psychosocially stressed rats chronically treated with 0.01 mg/kg of clonidine did not display significantly greater immobility during the context test than vehicle-treated control rats, the within-drug contrast (i.e., clonidine-treated psychosocial stress group vs. clonidine-treated controls) was marginally significant. However, these findings should be interpreted cautiously. Since clonidine is an α2-adrenergic receptor agonist and significantly reduces central noradrenergic activity, it can have sedative side effects at higher doses (Millan et al., 2000). In the present study, the higher dose of clonidine did result in a significant reduction of locomotor activity in the open field during NOR habituation. On the other hand, clonidine had no significant effects on motor activity on the EPM, and psychosocially stressed rats treated with 0.05 mg/kg of clonidine still produced significantly more fecal boli during the context test than the no psychosocial stress group treated with 0.05 mg/kg of clonidine (data not shown). One study also reported that clonidine’s sedative effects are not observed until doses greater than 0.1 mg/kg are employed (Millan et al., 2000). Therefore, the present data support the notion that clonidine is ineffective in blunting the expression of a traumatic memory in rats.

Both doses of clonidine, and in particular the 0.05 mg/kg dose, blocked the effects of psychosocial stress on anxiety and startle, as well as cardiovascular responses to acute immobilization. However, the higher dose of clonidine led to a significantly reduced growth rate in controls, which was exacerbated by chronic psychosocial stress. It also resulted in significantly increased adrenal gland weight in control animals. Lastly, neither dose of clonidine prevented the chronic psychosocial stress-induced decrease in thymus weight. Thus, despite its amelioration of a subset of the chronic stress-induced behavioral and cardiovascular sequelae, clonidine was ineffective at preventing the remaining physiological changes induced by the psychosocial stress regimen.

Since people with PTSD have significantly elevated baseline NE levels and demonstrate adverse reactions (e.g., panic attacks, flashbacks) to agents that increase these levels (e.g., yohimbine), pharmacological agents that reduce noradrenergic activity could be effective treatments for people with PTSD (Boehnlein and Kinzie, 2007; Strawn and Geraciotti, 2008). Some studies have reported that propranolol, a β-adrenergic receptor antagonist, may be an effective treatment for PTSD if administered after the traumatic event or after the re-experiencing of a traumatic event (Brunet et al., 2011; Hoge et al., 2012; Pitman et al., 2002; Taylor and Raskind, 2002; Vaiva et al., 2003). Other work has found that prazosin, an α1-adrenergic receptor antagonist, reduces hyperarousal symptoms, intrusive thoughts, recurrent distressing dreams and sleep disturbances in PTSD (Bikac et al., 2003; Peskind et al., 2003; Raskind et al., 2002, 2003; Taylor and Raskind, 2002; Taylor et al., 2006). The present findings suggest that...
clonidine, as well as propranolol and prazosin, can serve as adjunct contributions to pharmacotherapy, specifically in targeting the anxiety, hyperarousal (e.g., exaggerated startle response) and cardiovascular components of PTSD.

4.3. Tianeptine

Tianeptine was the only pharmacological agent that prevented the effects of chronic psychosocial stress on all physiological and behavioral measures and did not produce adverse side effects in control animals. Tianeptine completely blocked the expression of fear-related behaviors in psychosocially stressed rats in response to the context and cue that were paired with the two cat exposures. It also prevented the effects of psychosocial stress on anxiety, startle, cardiovascular reactivity to an acute stressor, growth rate, adrenal gland and thymus weights.

The present findings are consistent with previous work which reported that chronic, but not acute, administration of tianeptine significantly reduced the expression of conditioned fear in rats (Burghardt et al., 2004). These effects appear to be more related to a reduction of fear expression by tianeptine, rather than a memory-impairing effect, per se, as numerous studies have shown that tianeptine treatment enhances, rather than impairs, hippocampus-dependent learning and memory (Jaffard et al., 1991; Meneses, 2002; Munoz et al., 2005). Indeed, we have reported that tianeptine enhanced spatial memory and synaptic plasticity by blocking the adverse effects of acute stress on both measures (Campbell et al., 2008; Diamond et al., 2004; Vouimba et al., 2006; Zoladz and Diamond, 2010; Zoladz et al., 2008b). Thus, tianeptine appears to demonstrate long-term anxiolytic effects that are similar to SSRI's without the initial anxiogenic effects typically induced by SSRIs (Burghardt et al., 2004, 2007; Ravinder et al., 2011; Robert et al., 2011; Zimowicz et al., 2006).

We also observed that tianeptine, unlike clonidine, significantly attenuated the immobilization-induced increase in serum corticosterone levels. This finding is consistent with previous work indicating that chronically administered tianeptine reduced the stress-induced activation of the HPA axis (Delbende et al., 1994). In contrast, we have reported that acute treatment with tianeptine blocked predator stress effects on spatial memory without affecting corticosterone levels (Campbell et al., 2008). Furthermore, we have demonstrated that tianeptine blocked the memory-impairing effects of predator stress in adrenalectomized rats (Zoladz et al., 2008b). This observation confirmed that stress effects on memory are not dependent on a stress-evoked increase in corticosterone levels, and more importantly, that the memory-protective effect of tianeptine is independent of corticosterone levels. The current work adds to our understanding of tianeptine-HPA interactions by demonstrating that chronic tianeptine treatment attenuated HPA axis activity in rats exposed to a novel acute stressor.

In a related finding, tianeptine, relative to vehicle, resulted in significantly lower systolic BP in control animals following 20 min of immobilization stress. Few studies have examined the effects of tianeptine on cardiovascular activity, but those that have investigated the phenomenon have reported either no effect on HR or BP (Juvent et al., 1990; Lasnier et al., 1991) or reduced diastolic BP (Lechin et al., 2006). The effects of tianeptine on cardiovascular activity in the present study are not likely due to acute effects of the drug. In recent work we have found that tianeptine does not prevent an acute stress-induced increase in BP (unpublished findings). In this pilot study, rats were treated with 10 mg/kg of tianeptine or vehicle 30 min prior to a 15-min exposure to predator stress. Rats exposed to the cat for 15 min exhibited significant elevations of systolic and diastolic BP which were unaffected by tianeptine treatment. Hence, tianeptine did not exert a direct influence on the acute stress-induced increase in BP. Therefore, the tianeptine-mediated prevention of the effects of chronic psychosocial stress on cardiovascular reactivity to an acute stressor is more likely attributable to its attenuation of anxiety in response to stress, rather than a direct effect on blood pressure regulation. It is also possible that the tianeptine effects on stabilizing blood pressure (as well as the HPA axis) under stress may be mediated indirectly via a strengthening of coping strategies involving the tianeptine-mediated enhancement of higher cortical functioning (Dupin et al., 2006; Qi et al., 2009; Sacchetti et al., 1993).

Chronic treatment with tianeptine also prevented the effects of chronic psychosocial stress on all of the other physiological endpoints, including growth rate, adrenal gland weight and thymus weight. Previous work in rodents has reported that tianeptine had no effect on the chronic stress-induced adrenal gland hypertrophy or reduction in growth rate (Magarinos et al., 1999; Watanabe et al., 1992b). However, these studies utilized a different stressor (restraint stress, 6 h per day for 21 days) than that which was employed here, which could account for the discrepancies. The finding that tianeptine prevented chronic stress-induced atrophy of the thymus is consistent with work demonstrating that tianeptine insulates the immune system from adverse effects of stress. For instance, several studies have shown that tianeptine prevents the adverse effects of cytokines on brain biochemistry and peripheral measures of inflammation in the rat (Castanon et al., 2001; Plaisant et al., 2003a, 2003b). Thus, an important avenue of study in clinical trials would involve the administration of tianeptine to block auto-immune related pathology, which is commonly observed in traumatized people (Boscarino, 2004, 2010; Heim et al., 2000).

Recent work has suggested that tianeptine's therapeutic effects may be associated with enhanced neuroplasticity and stabilization of glutamatergic neurotransmission (Brink et al., 2006; Kasper and McEwen, 2008; Cole et al., 2002; McEwen et al., 2010; Zoladz et al., 2008b). For example, the deleterious effects of stress on brain structure and function are, at least in part, a result of glutamatergic hyperactivity (Bagley and Moghaddam, 1997; Bartanusz et al., 1995; Joels et al., 2003; Krugers et al., 1993; Lowy et al., 1993, 1995; McEwen et al., 2002; Moghaddam, 1993; Reznikov et al., 2007; Yang et al., 2005), and tianeptine appears to normalize this alteration (Kole et al., 2002; Reznikov et al., 2007), resulting in normal physiology and behavior during stress (Campbell et al., 2008; Diamond et al., 2004; Kasper and McEwen, 2008; McEwen et al., 2010; Shakesby et al., 2002; Vouimba et al., 2006; Zoladz et al., 2008b). It is therefore likely that the PTSD-like sequelae observed in our rats were associated with alterations of glutamate function which were normalized by daily tianeptine treatment. In related work, investigators have suggested that abnormalities in glutamatergic function could underlie the pathology of PTSD (Chambers et al., 1999; Nair and Singh, 2008; Reul and Nutt, 2008). Together, these findings highlight the potential importance of stabilizing glutamate neurotransmission in future research on PTSD pharmacotherapy.

4.4. Caveats

In contrast to our previous work (Zoladz et al., 2008a, 2012), vehicle-treated psychosocially stressed rats did not display lower levels of baseline corticosterone, a significant impairment of object recognition memory or significantly reduced thymus weights, relative to vehicle-treated control animals. One possible explanation for the lack of statistical significance is that the chronic injections in the present study acted as a persistent mild stressor in control rats, which added variability to their data on these measures. Indeed, other work in rodents has reported that chronic mild stress in the form of repeated injections can significantly alter rat physiology and behavior (Weaver et al., 2005; Wellman, 2001). In addition, we have previously reported significantly reduced baseline corticosterone levels in rats that were exposed to our psychosocial stress paradigm (Zoladz et al., 2012), a finding that is consistent with much of the PTSD literature on HPA axis alterations (Yehuda, 2005). However, we observed this effect only when psychosocially stressed rats had
not been exposed to repeated injections prior to blood sampling. Thus, in the present study, it is likely that the chronic injections and numerous behavioral manipulations that were performed on rats hindered our ability to detect some of the previously-observed effects on stress on rat physiology and behavior.

Another finding that diverges from our previous work is that the vehicle-treated psychosocially stressed rats demonstrated significantly greater HR than vehicle-treated control rats following exposure to an acute stressor on the final day of testing. Previously, we reported that the present psychosocial stress paradigm resulted in significantly lower HR, compared to controls, following acute stress on the final day of testing. Nevertheless, the HR exhibited by psychosocially stressed rats in the present study (409.85 ± 7.30 bpm) was very similar to the HR exhibited by psychosocially stressed rats in our previous work (413.25 ± 9.93 bpm). The basis of the inconsistent effects between the findings of the present study and those of our previous work is the HR exhibited by the control animals in each case. Vehicle-treated control rats displayed lower HR in the present study (385.61 ± 8.11 bpm) than that which was displayed by controls in the prior study (462.88 ± 11.43 bpm). In theory, vehicle-treated controls could have exhibited lower HR in the present study because the chronic mild stress of repeated injections desensitized them from responding as strongly to the acute stressor as the more naïve animals that were utilized in our prior work.

The design of the present experiment did not afford us the opportunity to make within-group comparisons (i.e., pre-stress vs. post-stress) on measures of physiology and behavior in rats that were exposed to chronic psychosocial stress and administered the various pharmacological agents. We chose not to expose rats in the present study to any pre-stress physiological or behavioral testing to eliminate any influence pre-stress manipulations could have had on the battery of tests administered at the conclusion of the experiment. Moreover, we did not want to expose rats to the same apparatus multiple times, as prior exposures could influence behavior on subsequent tests. For example, in the current study the no Psychosocial Stress Vehicle- and drug-treated groups were exposed twice to the fear conditioning chamber without reinforcement (cat exposure). This repeated unreinforced exposure resulted in the rats in these groups developing greater immobility with each exposure, which indicates that their initial exploratory response habituated with repeated exposures to the same environment. Although this observation is of value to the study of habituation behavior, it illustrates the problem with repeatedly exposing rats to the same environment where subsequent critical behavioral testing, specifically fear memory-induced immobility, will occur. Overall, our findings should be interpreted within the context of a design involving the strictest control over factors that might have influenced how the various drug treatments interacted with psychosocial stress.

5. Summary and conclusions

A subset of people with PTSD shows an improvement in their symptoms following treatment with SSRIs. However, SSRIs tend to blunt the depressive components of PTSD, while having insufficient effect on the memory- and anxiety-related symptoms of the disorder. In addition, some forms of PTSD, such as combat-related PTSD, are highly resistant to SSRI treatment. Moreover, these agents can have anxiogenic effects early in the treatment phase and exert their antidepressant effects only after a substantial delay. Thus, there is an urgent need to develop alternative pharmacotherapeutic interventions for the treatment of PTSD.

We have found that the TCA amitriptyline was effective in reducing the magnitude of the fear-conditioned memory for the context and cue that were associated with the acute cat exposures, and it ameliorated the stress-induced increase in anxiety and startle. However, amitriptyline had adverse side effects, as it significantly increased cardiovascular reactivity in control animals and led to adverse physiological reactions, including reduced growth rate, increased adrenal gland weight and internal adhesions. Thus, despite some positive effects, the adverse side effects of amitriptyline could be a significant limitation to its use in the treatment of people with PTSD.

Clonidine blocked the effects of chronic psychosocial stress on anxiety and startle, and in contrast to amitriptyline, prevented the stress-induced changes in cardiovascular reactivity to the acute stressor, as well. However, clonidine did not blunt the expression of conditioned fear memory in psychosocially stressed rats in response to re-exposure to the context and cue that were paired with the acute cat exposures. Clonidine had adverse side effects as well, including a significant reduction in overall growth rate and an idiosyncratic increase in adrenal gland weight in the control (non-stress) group. Thus, the current work indicates that clonidine can serve as an effective anxiolytic agent and reduces the hyperarousal (e.g., exaggerated startle response) and cardiovascular components of PTSD, but it would have little effect on the strength (and intrusiveness) of a traumatic memory.

Tianeptine was the only pharmacological agent to prevent the effects of chronic psychosocial stress on our entire battery of physiological and behavioral endpoints. Specifically, tianeptine blocked the expression of fear-conditioned traumatic memory in psychosocially stressed rats when they were re-exposed to the context and cue that were paired with the two cat exposures. Tianeptine also prevented the effects of psychosocial stress on anxiety, startle, cardiovascular reactivity to an acute stressor, growth rate, and adrenal gland and thymus weights. These salutary effects of tianeptine occurred in the absence of any observed adverse side effects.

In summary, the present experiment examined the influence of amitriptyline, clonidine and tianeptine on the expression of PTSD-like sequelae in rats. Our findings provide guidance in the development of treatments for traumatized people by demonstrating the differential effectiveness of each of these three agents in blocking the behavioral and physiological consequences of psychosocial stress in rats.

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