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Uncontrollable and Unpredictable Stress with a Reminder Experience Induces Long-Lasting Effects on Physiology and Behavior: A Novel Approach to Modeling Post-Traumatic Stress Disorder in Rats

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by

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For my parents, who have been there for me every step of the way…

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Uncontrollable and Unpredictable Stress with a Reminder Experience Induces Long-Lasting Effects on Physiology and Behavior: A Novel Approach to Modeling Post-Traumatic Stress Disorder in Rats

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ABSTRACT

People who endure horrific, life-threatening experiences are at risk for developing post-traumatic stress disorder (PTSD). However, only about 25% of all individuals who experience trauma develop PTSD. Recent research indicates that the presence of certain physiological conditions, such as reduced cortisol and parasympathetic inhibition, during trauma may increase one's susceptibility to developing PTSD. Thus, I attempted to develop a novel animal model of PTSD and test the hypothesis that reduced adrenal and parasympathetic activity during stress would exacerbate its long-term effects on behavior.

In Experiment One, adult male rats were exposed to two stress sessions, each involving one hour of immobilization plus cat exposure. Before each session, rats were injected with vehicle, metyrapone, AF-DX 116, or both drugs. The second session occurred 10 days after the first and served to model a traumatic flashback. Stressed rats endured unstable housing conditions throughout the experiment to add an element of daily anxiety. Three weeks after the second session, all rats underwent a battery of tests to examine the lasting effects of stress on physiology and behavior. The results indicated that stressed rats exhibited heightened anxiety on the elevated plus maze, an exaggerated

startle response, and greater blood pressure, relative to controls. Moreover, metyrapone, when combined with stress, led to significant short- and long-term spatial memory impairments.

Experiment Two assessed the effects of the same stress paradigm on rats' sensitivity to yohimbine, an α_2 adrenergic receptor antagonist. Yohimbine induces flashbacks and panic attacks in patients with PTSD; thus, I hypothesized that stressed rats would react abnormally to this agent. Stressed and unstressed rats were administered vehicle or yohimbine (1 mg/kg) 30 min prior to behavioral testing. The results indicated that stressed rats were hyperresponsive to yohimbine, as evidenced by a greater suppression of rearing, greater avoidance of the center of the open field, and a greater suppression of activity on the elevated plus maze, relative to controls. Collectively, the findings of these studies indicate that uncontrollable and unpredictable psychological stress produces lasting changes in the physiology and behavior of rats that resemble symptoms commonly observed in people with PTSD.

Chapter One: Background

Stress

Since the pioneering research of Hans Selye (for a review, see Selye, 1976, 1973, & 1936), it has been accepted that stress has a number of effects on physiology and behavior, some beneficial and some aversive. Stress can invigorate organisms and provide them with the energy to act rapidly in threatening situations, or stress can be overpowering, debilitating, and lead to both physiological and behavioral impairments. Early work discussed stress as an elicitor of the well known fight-or-flight response, which leads to an increased release of neurotransmitters (e.g., epinephrine and norepinephrine) and stress hormones (e.g., cortisol) throughout the body, which helps prepare an organism for action. Science has since then focused on the adverse consequences of stress, acknowledging that in chronic forms, stress can induce adrenal-gland enlargement, atrophy of the thymus and lymph nodes, increased cardiovascular tone, immune-system suppression, and ulcerations (Sapolsky, 1992; Selye, 1976). Recent work has implicated stress as a major precipitating factor to the development of mental disorders (Boyer, 2000; Esch, Stefano, Fricchione, & Benson, 2002). Considering these findings, it is of primary importance to develop a better understanding of the mechanisms by which stress affects physiology and cognition.

Both chronic stress and a persistent elevation of glucocorticoids (one of the main physiological markers of stress) have detrimental effects on brain morphology, leading to

neuronal atrophy and cell death in the hippocampus, a medial temporal lobe structure that is critical for the formation of memories (Arbel, Kadar, Silberman, & Levy, 1994; Bodnoff, Humphreys, Lehman, Diamond, Rose, & Meaney, 1995; Bremner, 1999; Conrad, Magarinos, LeDoux, & McEwen, 1999; Lambert et al., 1998; Luine, Villegas, Martinez, & McEwen, 1994; Magarinos & McEwen, 1995; Magarinos, McEwen, Flugge, & Fuchs, 1996; Magarinos, Verdugo, & McEwen, 1997). Others have found that stress and elevated glucocorticoids impair synaptic plasticity (Bodnoff et al., 1995; Diamond, Bennett, Fleshner, & Rose, 1992; Diamond & Park, 2000; Garcia, 2001; Garcia, Musleh, Tocco, Thompson, & Baudry, 1997; Garcia, Tocco, Baudry, & Thompson, 1998; Kim, Foy, & Thompson, 1996; Kim, Koo, Lee, & Han, 2005; Kim, Lee, Han, & Packard, 2001; Maroun & Richter-Levin, 2003; Mesches, Fleshner, Heman, Rose, & Diamond, 1999; Pavlides, Nivon, & McEwen, 2002; Sacchetti et al., 2002; Sapolsky, 2003; Shors, Seib, Levine, & Thompson, 1989; Vouimba, Yaniv, Diamond, & Richter-Levin, 2004) and memory in both humans (Elzinga, Bakker, & Bremner, 2005; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Kuhlmann, Piel, & Wolf, 2005; Sauro, Jorgensen, & Pedlow, 2003) and rodents (Conrad, Galea, Kuroda, & McEwen, 1996; de Quervain, Roozendaal, & McGaugh, 1998; Diamond & Park, 2000; Diamond, Park, Heman, & Rose, 1999; Diamond, Park, & Woodson, 2004; Kim et al., 2005; Kim et al., 2001; Luine et al., 1994; Park, Campbell, & Diamond, 2001; Rashidy-Pour, Sadeghi, Taherain, Vafaei, & Fathollahi, 2004; Sandi et al., 2005; Sauro et al., 2003; Woodson, Macintosh, Fleshner, & Diamond, 2003). These impairments have been linked to the effects that stress and glucocorticoids have on N-methyl-D-aspartate (NMDA) receptors (Kim et al.,

1996; Magarinos et al., 1995; Park, Fleshner, & Diamond, 2004), neural cell adhesion molecules (Sandi, 2004; Sandi et al., 2005; Sandi, Merino, Cordero, Kruyt, Murphy, & Regan, 2003), synaptic currents (Karst & Joels, 2003), brain-derived neurotrophic factor (Marmigere, Givalois, Rage, Arancibia, & Tapia-Arancibia, 2003; Radecki, Brown, Martinez, & Teyler, 2005), and amygdala-mediated modulation of hippocampal plasticity (Abe, 2001; Akirav & Richter-Levin, 2002; Akirav & Richter-Levin, 1999; Kim et al., 2005; Kim et al., 2001; Park & Diamond, 2005; Richter-Levin, 2004). Taken together, these studies indicate that acute and chronic stress can lead to permanent modifications of brain biochemistry, physiological functioning, and behavior.

Anxiety Disorders and Post-Traumatic Stress Disorder

Diagnostic Criteria

Anxiety disorders are the most common form of mental illness in the United States (National Institute of Mental Health, 2002). Characterized by extreme fear, panic, and anxiety, the disorders affect nearly 20 million adults nationwide. One of the most notable anxiety disorders is post-traumatic stress disorder (PTSD). This disorder is precipitated by some terrifying life-threatening event, such as war, rape, or a natural disaster, and instills worry, panic, fear, anxiety, and terror in the individual for years thereafter. PTSD is unique relative to other disorders because its diagnostic criteria actually specify an etiologic event: exposure to trauma (McNally, 2003). Importantly, to be considered *traumatic*, the event must pose a threat to the individual's physical well being and cause him or her to feel a sense of horror and helplessness. According to the

Diagnostic and Statistic Manual of Mental Disorders (American Psychiatric Association, 1994, p. 424-429), the diagnostic criteria for PTSD are:

A. The person has been exposed to a traumatic event in which both of the following were present:
    1) The person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of others.
    2) The person's response involved intense fear, helplessness, or horror. In children, this may be expressed instead by disorganized or agitated behavior.

B. The traumatic event is persistently re-experienced in one (or more) of the following ways:
    1) Recurrent and distressing recollections of the event, including images, thoughts, or perceptions. In young children, repetitive play may occur in which themes or aspects of the trauma are expressed.
    2) Recurrent distressing dreams of the event. In children, there may be frightening dreams without recognizable content.
    3) Acting or feeling as if the traumatic event were recurring (includes a sense of reliving the experience, illusions, hallucinations, and dissociative flashback episodes, including those that occur on awakening or when intoxicated). In young children, trauma-specific reenactment may occur.

4) Intense psychological distress at exposure to internal or external cues that symbolized or resemble an aspect of the traumatic event
5) Physiological reactivity on exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event

C. Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness, as indicated by three or more of the following:
1) Efforts to avoid thoughts, feelings, or conversations associated with the trauma
2) Efforts to avoid activities, places, or people that arouse recollections of the trauma
3) Inability to recall an important aspect of the trauma
4) Markedly diminished interest or participation in significant activities
5) Feeling of detachment or estrangement from others
6) Restricted range of affect (e.g., unable to have loving feelings)
7) Sense of a foreshortened future (e.g., does not expect to have a career, marriage, children, or a normal life span)

D. Persistent symptoms of increased arousal, as indicated by two (or more) of the following:
1) Difficult falling or staying asleep
2) Irritability or outbursts of anger
3) Difficulty concentrating

4) Hypervigilance

5) Exaggerated startle response

E. Duration of the disturbance (symptoms in Criteria B, C, and D) is more than one month.

F. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Individuals diagnosed with PTSD are hypervigilant and frequently experience trouble sleeping, concentrating, and functioning regularly in their daily lives. These symptoms are further exacerbated by reminders of the trauma through flashbacks, situational reminders, intrusive memories, and nightmares (Bryant, 2003; Reynolds & Brewin, 1999). In fact, PTSD patients are not just reminded of the traumatic event; rather, they feel as if they actually relive the experience. Accordingly, individuals with PTSD make great efforts to avoid stimuli that remind them of their trauma.

The recent acts of war and terrorism in countries across the world assure that individuals will continue to develop PTSD in the years to come. In fact, recent reports (Heffernan, 2006) have suggested that of the 170,000 Operation Iraqi Freedom veterans, over 34,000 have sought assistance from VA medical centers and been diagnosed with some type of psychological disorder, including PTSD. This is an extremely high prevalence rate of psychological illness (roughly 20%), which supports the need for more research geared towards a better understanding of PTSD.

Do PTSD Patients Exhibit Heightened Startle?

PTSD is the only anxiety disorder where an exaggerated startle response is listed as one of its core symptoms. Although diagnosticians and researchers have emphasized this indication, studies examining the baseline startle response of PTSD patients have presented conflicting results. The startle response is defined as the rapid sequence of flexor motor movements that occurs after the onset of a briefly-presented, intense stimulus (Morgan, 1997). The most frequently utilized method to assess an individual's startle response, in both humans and rodents, has been the acoustic startle reflex. This task consists of exposing a human or a rodent to a loud, rapid noise burst and assessing their startle to the stimulus. In humans, this involves measuring the intensity and speed of one's eye-blink via EMG recordings, while in rats, it consists of measuring the whole body's motor response, typically through some type of sensory transducer. Several studies have examined the baseline startle response in individuals with PTSD; and, while some have found heightened startle in these individuals (Morgan, Grillon, Lubin, & Southwick, 1997; Orr, Lasko, Shalev, & Pitman, 1995; Shalev, Peri, Orr, Bonne, & Pitman, 1997), others have found no differences between PTSD patients and control subjects (Elsesser, Sartory, & Tackenberg, 2004; Grillon, Morgan, Southwick, Davis, & Charney, 1996; Lipschitz, Mayes, Rasmusson, Anyan, Billingslea, Gueorguieva, & Southwick, 2005; Orr, Solomon, Peri, Pitman, & Shalev, 1997; Shalev, Orr, Peri, Schreiber, & Pitman, 1992; Siegelaar et al., 2006). Despite these inconsistencies, research has reliably shown that upon exposure to loud tones, individuals with PTSD do exhibit significantly greater autonomic reactivity (e.g., increases in heart rate, blood pressure,

and skin conductance) than controls (Orr et al., 1997; Orr et al., 1995; Shalev, Peri, Brandes, Freedman, Orr, & Pitman, 2000). This includes a failure to physiologically habituate to the stimuli, in addition to the elicitation of greater autonomic responses from the onset of the tones. Thus, while it is unclear whether or not individuals with PTSD exhibit a heightened baseline startle response, they do tend to display exaggerated autonomic reactivity to sudden, intense stimulation.

Given the inconclusive nature of these studies, Morgan and colleagues (Grillon et al., 1996; Morgan, Grillon, Southwick, Davis, & Charney, 1995; Morgan, Grillon, Southwick, Nagy, Davis, & Charney, 1995) conducted a series of experiments to examine the startle response in PTSD patients. In one study, they examined the individuals' startle response at baseline (no threat condition) and when they were expecting to be shocked (threat condition). In another study, they examined the effects of a noradrenergic drug versus a placebo on their startle response. The investigators found that individuals with PTSD exhibited greater startle responses throughout both experiments. Given that these individuals displayed exaggerated startle in the no threat and placebo conditions of these studies, it could be reasoned that startle is elevated in individuals with PTSD at baseline. However, it could also be that the exaggerated startle in these patients was due to the anxiety associated with the unfamiliar environment (i.e., the laboratories of Yale University) in which the experiments took place. Accordingly, in another study, Grillon et al. (1996) examined the baseline startle response of Vietnam veterans with PTSD in a familiar environment, namely, the VA hospital from which they had been recruited. In this study, the investigators found no differences between the startle responses of

Vietnam veterans with PTSD, Vietnam veterans without PTSD, and healthy control subjects. This finding supports the argument that individuals with PTSD exhibit an exaggerated startle response only when they are already anxious or aroused. In the case of Morgan et al. (1995), the PTSD patients could have endured heightened anxiety due to the novelty of their surroundings. Thus, the exaggerated startle responses that have been reported in PTSD patients could be state-dependent, rather than a stable trait of these individuals. In support of this argument, other studies (Grillon & Morgan, 1999; Grillon, Morgan, Davis, & Southwick, 1998; Pole, Neylan, Best, Orr, & Marmar, 2003) have found that manipulations of the experimental context or the presentation of explicit threat cues consistently leads to enhanced startle responses in PTSD patients. Therefore, these individuals may display exaggerated fear-potentiated startle responses, rather than greater baseline startle responses in general.

Most, if not all, of the data obtained from PTSD patients has been collected after the individuals experienced the trauma. Thus, researchers have been unable to determine PTSD patients' pre-trauma startle responses and compare them with their post-trauma startle responses. It is well known that startle responses are remarkably consistent within subjects, but extremely variable between subjects (Morgan, 1997). Thus, comparing the startle responses of independent samples could be problematic in and of itself. It would be optimal to obtain baseline startle responses in traumatized individuals before and after the trauma to understand whether heightened startle is a cause of or is caused by the onset of PTSD. In light of this reasoning, Guthrie and Bryant (2005) assessed the auditory startle response of firefighters before and after they had been exposed to trauma.

Although none of the firefighters who were exposed to trauma developed PTSD during the course of the study, they did display more symptoms (e.g., intrusive memories, avoidance) of the disorder after the trauma than firefighters who had not been exposed to a traumatic experience. More importantly, the investigators found that the magnitude of the pre-trauma startle response predicted the development of acute PTSD symptoms, as measured by the Impact of Event Scale (IES). The authors emphasized that the individuals' pre-trauma physiological reactivity could have been a risk factor for the development of PTSD symptomatology after exposure to an intense, traumatic event. These findings suggest that PTSD may not necessarily cause an exaggerated startle response; rather, it could be a pre-existing factor that increases one's susceptibility to developing the disorder.

Do PTSD Patients Display Impairments in Cognition?

As noted above, stress can have detrimental effects on hippocampus-dependent learning and memory. Accordingly, several studies (Bremner et al., 1995; Bremner et al., 1993; Gilbertson, Gurvits, Lasko, Orr, & Pitman, 2001; Golier, Yehuda, Lupien, Harvey, Grossman, & Elkin, 2002; Jenkins, Langlais, Delis, & Cohen, 1998; Moradi, Doost, Taghavi, Yule, & Dalgleish, 1999; Sachinvala et al., 2000; Uddo, Vasterling, Brailey, & Sutker, 1993; Vasterling, Constans, Brailey, & Sutker, 1998) have reported declarative and working memory impairments, along with deficits in attention, in PTSD patients. Many of the investigators have attempted to relate their findings to studies reporting smaller hippocampi in individuals with PTSD (described in further detail below). However, as with much of the research in this area, not all studies have provided similar

results. In fact, some researchers have found no differences in cognitive functioning between individuals with PTSD and healthy control subjects (Barrett, Green, Morris, Giles, & Croft, 1996; Crowell, Kieffer, Siders, & Vanderploeg, 2002; Neylan et al., 2004; Zalewski, Thompson, & Gottesman, 1994). One major criticism of the work reporting neurocognitive impairments in PTSD patients is that many of these investigations have reported high rates of major depressive disorder (MDD) and a history of substance abuse in the PTSD groups, which makes it difficult to ascribe the cognitive deficits to PTSD alone. Indeed, MDD (Veiel, 1997) and substance abuse (Goldman, Brown, Christiansen, & Smith, 1991) can have major effects of their own on physiology and cognition. Moreover, many of these studies found that the individuals with PTSD had fewer years of education and a lower IQ than controls. When all of these factors were carefully controlled – that is, when the individuals with MDD or substance abuse were excluded from the experiment, and the individuals with PTSD were well educated –, investigators (Neylan et al., 2004) found no differences between the two groups on any measure of cognitive functioning, including assessments of attention. Collectively, these findings suggest that deficits in cognitive functioning may not be unique to PTSD; rather, they could be due to other factors associated with the disorder (e.g., MDD, substance abuse, less education).

Development of PTSD

Not everyone who is traumatized develops PTSD. In fact, research has indicated that only about 25% of the individuals who are exposed to trauma eventually develop the disorder (Yehuda, 2001). This finding implies that there is some fundamental difference

between those individuals who develop the disorder and those who do not develop the disorder. While specific characteristics of the trauma (e.g., natural disaster, rape, combat, etc.) and developmental experience (see Heim and Nemeroff, 2001 for a review) appear to play a large role in whether or not an individual will develop PTSD, certain physiological predispositions of the person seem to be important as well. For instance, research has shown that people with PTSD exhibit an abnormally large sympathetic [e.g., increased heart rate (HR) and blood pressure (BP)] response (Cohen et al., 1998; Orr, 1990) and reduced adrenal steroid levels (Bremner et al., 2003a) in response to the initial traumatic event than traumatized people who do not develop PTSD. Although this work examined individuals after the trauma, it is possible that the differences existed before the trauma and influenced the development of the disorder.

Autonomic Nervous System

Sympathetic Nervous System and General Arousal

Shalev and colleagues (Shalev et al., 1998) found that following trauma, those individuals who exhibited the highest HRs upon admission to the emergency room were more likely to later develop PTSD. A similar study found that motor-vehicle-accident survivors who displayed higher HRs upon hospital discharge were more likely to express symptoms of PTSD 6 months later (Bryant, Harvey, Guthrie, & Moulds, 2000). According to Cohen et al. (1998), PTSD patients display dysfunctional autonomic activity at two distinct levels: basal tone and reaction to stress-related cues. Compared to control subjects, PTSD patients have been found to exhibit significantly elevated basal HR and BP (Blanchard, 1990; Blanchard, Kolb, Pallmeyer, & Gerardi, 1982; Buckley,

Holohan, Greif, Bedard, & Suvak, 2004; Gerardi, Keane, Cahoon, & Klauminzer, 1994; Kolb, 1987; Muraoka, Carlson, & Chemtob, 1998). Others have presented conflicting results, however, suggesting that PTSD patients do not demonstrate these elevations (McFall, Murburg, Ko, & Veith, 1990; McFall, Veith, & Murburg, 1992; Murburg, McFall, Lewis, & Veith, 1995). Despite these findings, Buckley and Kaloupek (2001) published a meta-analytic review, examining 34 studies that investigated basal cardiovascular activity in PTSD patients. Effect sizes for HR and diastolic BP differences were significant when individuals with PTSD were compared to traumatized individuals without PTSD and healthy controls. However, effect sizes for systolic BP differences were only significant when PTSD patients were compared to healthy controls. Additionally, anticipatory, or priming, anxiety (i.e., uneasiness that participants experience in a laboratory setting partially because of the uncertainty involved) was ruled out as a contributing factor to the basal HR and BP elevations observed in PTSD patients. These findings suggest that individuals with PTSD do exhibit significant elevations in basal HR and BP and that the inconsistent findings across laboratories could be due to differences in environmental surroundings or methodology.

PTSD patients have also been found to react more strongly to trauma-related cues than traumatized people who have not developed the disorder (Kolb & Mutalipassi, 1992; McFall et al., 1990; Orr, 1990). Although this sounds tautological (since the disorder itself is defined by traumatic memories having a greater lasting effect on these individuals), a large amount of effort has been put forth to understand the underlying mechanisms for these results. Combat veterans with PTSD have shown heightened

physiological responses to simulated combat noise, intense scripts describing combat, and hypnotically-induced imagery of combat situations (Blanchard et al., 1982; Malloy, Fairbank, & Keane, 1983; Pitman, Orr, Forgue, de Jong, & Claiborn, 1987). Similarly, veterans have been shown to respond to traumatic combat stimuli with significantly greater elevations of epinephrine (EPI) (McFall et al., 1990) and norepinephrine (NE) (Blanchard, Kolb, Prins, & Gates, 1991) than control subjects. Liberzon et al. (1999c) used single photon emission computerized tomography to show that in response to simulated combat sounds, war veterans with PTSD showed increased activation in the amygdala, an increase that was not observed in control subjects. Using functional magnetic resonance imaging, Lanius and colleagues (Lanius et al., 2003) showed that PTSD patients exhibited significantly less activation of the anterior cingulate gyrus when emotional scripts were read to them. Others have found that when PTSD patients were presented with traumatic scripts, they demonstrated direct influences of the amygdala on the visual cortex, subcallosal gyrus, and anterior cingulate gyrus (Gilboa et al., 2004). Again, these effects were not observed in control subjects. The amygdala is an almond-shaped, temporal lobe structure that plays a major role in emotional memory and is greatly activated during stressful situations (LeDoux, 1998). Past research has suggested that PTSD patients' hyperreactivity to trauma-related cues and their recurrent, intrusive memories of the traumatic event may be related to a failure of cingulate gyrus inhibition over a hyperresponsive amygdala and/or some general dysfunction of limbic system structures (see Gilboa et al., 2004). These findings provide further support for this argument.

Parasympathetic Nervous System

Although investigators have extensively described the relationship between the sympathetic nervous system (SNS) and PTSD, little work has addressed the contribution of the parasympathetic nervous system (PNS) to the disorder. The PNS is often referred to as the "brakes" of the autonomic nervous system (ANS), or the system that slows things down and returns the body to baseline. Hence, it is the negative feedback component of homeostasis (Stern, Ray, & Quigley, 2001). The vagus nerve, the tenth cranial nerve and an important part of the PNS, innervates the sinoatrial (SA) node on the right atrium of the heart, where electrical impulses are generated to trigger cardiac contraction. By modulating the SA node, the vagus nerve slows HR and maintains a balance between the SNS and PNS. Vagal modulation of HR is very important for reactions to and recovery from stressful situations and has been considered a possible mechanism for the differences in basal HR and HR changes in response to trauma-related cues in individuals with PTSD (Sahar, Shalev, & Porges, 2001).

Parasympathetic function has been monitored in numerous studies involving PTSD patients, where heart-rate variability (HRV) was used as the primary measure. HRV is a measure of beat-to-beat alterations in heart rate, or more specifically, the variability of the intervals between R waves. The two main frequency bands that are examined during HRV assessment are the Low-Frequency (LF) band (0.04 to 0.15 Hz) and the High-Frequency (HF) band (0.15 to 0.40 Hz) (Sahar et al., 2001). It is believed that the SNS influences the LF component, while the PNS primarily influences the HF component. Cohen and colleagues (Cohen, Benjamin, Geva, Matar, Kaplan, & Kotler,

2000a; Cohen, Kotler, Matar, Kaplan, Miodownik, & Cassuto, 1997) found that, at rest, PTSD patients displayed significantly lower HRV than control subjects. Further, these patients demonstrated lower HF and higher LF HRV components than controls, suggesting enhanced sympathetic and reduced parasympathetic tone at baseline. However, Cohen and colleagues (Cohen et al., 2000a; Cohen et al., 1998) showed that PTSD patients did not respond to recollection of trauma with elevations of HR and LF. The investigators reasoned that since PTSD patients exhibited autonomic dysregulation at rest, they could not manifest further increases in sympathetic tone in response to the traumatic reminder.

Sahar et al. (2001) examined PTSD patients' vagal modulation of HR in response to a mental challenge by using respiratory sinus arrhythmia (RSA) as their dependent measure. Changes in RSA have been shown to reflect activity of the vagus nerve, as RSA levels positively correlate with parasympathetic influence on the heart (Berntson, Cacioppo, & Quigley, 1993). Sahar and colleagues (Sahar et al., 2001) found that PTSD patients and control subjects, who had previously experienced trauma but had not developed a stress disorder, did not differ on resting levels of parasympathetic activity. However, when faced with a challenging arithmetic task, control subjects showed a significant increase in RSA (which was highly correlated with their HR), while PTSD patients showed no such increase. Thus, vagal mechanisms contributed to control subjects', but not PTSD patients', HR regulation. Similar findings were observed by Sack, Hopper, and Lamprecht (2004), who found that when PTSD patients were divided into low and high RSA groups, individuals with high RSA showed a greater increase in

HR in response to trauma-related script. Together, these studies suggest that the mechanisms responsible for HR regulation in response to stress may be different between individuals who develop PTSD and traumatized people who do not develop PTSD. In particular, HR regulation in PTSD patients, especially in response to stress, may be controlled in part by non-vagal means.

Role of the Noradrenergic System in PTSD

The noradrenergic system influences physiological arousal by supplying NE throughout an organism's central nervous system. Noradrenergic cell bodies are prominently found in hindbrain nuclei and most extensively in an area of the brain known as the locus coeruleus (LC). Studies in animals have shown that increased LC firing leads to alertness (Berridge & Foote, 1991; Foote, Ashton-Jones, & Bloom, 1980), while a decrease in LC firing leads to lethargy (Caballero & de Andres, 1986). Furthermore, direct stimulation of the LC via electrical current or pharmacological agents elicits vigilance and fear responses in rodents (Southwick, Bremner, Rasmusson, Morgan III, Arnsten, & Charney, 1999a).

A vast amount of literature has implicated increased noradrenergic activity in individuals with PTSD (see Southwick et al., 1999a). Geracioti et al. (2001) found significant elevations of baseline cerebrospinal fluid (CSF) NE in PTSD patients, compared to healthy control subjects. Moreover, these individuals' CSF NE levels positively correlated with the severity of their PTSD symptoms. Yehuda and colleagues (Yehuda et al., 1998) also found increased basal plasma NE levels in PTSD, as compared to individuals diagnosed with MDD and nonpsychiatric control subjects. In addition,

Kosten, Mason, Giller, Ostroff, and Harkness (1987) examined urinary NE and EPI levels at two-week intervals in hospitalized patients suffering from PTSD, MDD, bipolar disorder (BD), paranoid schizophrenia, and undifferentiated schizophrenia. In this study, PTSD patients exhibited significantly greater NE levels than all other groups, and they demonstrated significantly greater EPI levels than all groups except for those individuals with BD. Nevertheless, some investigators have been unable to show elevated levels of NE and EPI in PTSD patients (Murburg et al., 1995; Pitman & Orr, 1990). According to Yehuda et al. (1998), the increase in baseline noradrenergic activity may be confined to PTSD patients without comorbid depression. Investigators in this study found that NE levels were significantly associated with severity of depression; when PTSD patients were divided into two groups, those with or without comorbid depression, only the PTSD patients without comorbid depression exhibited significantly elevated plasma NE levels.

The noradrenergic system is regulated in part by α_2 adrenergic receptors, which exert an inhibitory influence on the LC (Southwick et al., 1999a). Alpha-2 adrenergic receptor antagonists, such as yohimbine, block this inhibition, which thereby leads to an increase in LC firing and ultimately enhanced alertness and arousal. PTSD patients, unlike controls, exhibit an exaggerated response to these substances, including flashbacks and panic attacks (Southwick, Morgan III, Charney, & High, 1999b). Southwick and colleagues (Southwick et al., 1993) found that 70% of PTSD patients who were administered yohimbine experienced panic attacks, and 40% experienced flashbacks. PTSD patients also exhibited significantly greater HR, systolic BP, and anxiety-related behavior in response to the drug. Investigators have linked these findings to a reduction

in NE metabolism in patients suffering from PTSD (Bremner et al., 1997a). Accordingly, PTSD patients, in comparison to control subjects, exhibited a significant decrease in the metabolism of NE in neocortical brain regions when administered yohimbine. As a result of persistently elevated catecholamine levels, PTSD patients also exhibit a down-regulation of α_2 adrenergic receptors (Perry, 1994; Perry, Southwick, Yehuda, & Giller, 1990), a finding that is consistent with the evidence for an overactive SNS in these individuals. Comparable findings have been demonstrated in animal studies, where chronic psychosocial stress has led to a down-regulation of α_2 adrenergic receptors in the LC of tree shrews (Flugge, 1996). In theory, fewer α_2 adrenergic receptors could underlie the enhanced sensitivity to yohimbine that has been demonstrated by PTSD patients.

The Hypothalamus-Pituitary-Adrenal Axis

Near the Time of Trauma

Increases in stress cause the hypothalamus to send corticotrophin-releasing hormone (CRH) to the anterior pituitary gland, which subsequently releases adrenocorticotrophin (ACTH), which then stimulates the adrenal cortex to produce and release corticosteroids (CORT) (corticosterone in rodents; cortisol in humans). These neuromodulators help coordinate an individual's ability to cope with stress and divert energy to undersupplied tissues (de Kloet, Oitzl, Joels, 1999). Given the role of CORT as a stress hormone, it would be intuitive to anticipate heightened levels of CORT in PTSD patients. On the contrary, a majority of the research in this area has found that individuals diagnosed with PTSD exhibit attenuated CORT levels shortly after the initial traumatic event. Resnick et al. (1995) examined CORT levels in rape victims only hours after the

trauma and related them to those who did and did not develop PTSD. Those who developed PTSD exhibited lower CORT levels shortly after the trauma, in comparison to those who never developed the disorder. In addition, Delahanty, Raimonde, and Spoonster (2000) collected urine samples 15 hrs after individuals experienced motor vehicle accidents. They assessed the development of PTSD symptomatology in these individuals 1 month later. Their results indicated that those who developed PTSD had significantly lower urinary CORT levels shortly after the traumatic event than accident victims who did not develop PTSD. In addition, cortisol levels within the first few days after the accident were negatively correlated with the presence of intrusive memories of the trauma in these individuals. Together, these findings indicate that traumatized individuals who exhibit attenuated CORT responses shortly after the traumatic event may be more susceptible to developing PTSD symptomatology.

Baseline

Studies comparing the baseline levels of CORT in PTSD patients and control subjects have produced mixed results. Most investigations have found that individuals with PTSD exhibit significantly lower levels of CORT at baseline (Boscarino, 1996; Kanter et al., 2001; King, Mandansky, King, Fletcher, & Brewer, 2001; Mason, Giller, Kosten, Ostroff, & Podd, 1986; Yehuda, Southwick, Nussbaum, Wahby, Giller, & Mason, 1990; Yehuda, Boisoneau, Mason, & Giller, 1993b; Yehuda, Teicher, Trestman, Levengood, & Siever, 1996). Then again, others have found that these levels are elevated (Carrion, Weems, Ray, Glaser, Hessl, & Reiss, 2002; Lemieux & Coe, 1995; Liberzon, Abelson, Flagel, Raz, & Young, 1999a; Lindauer et al., 2006; Lindley, Carlson, &

Benoit, 2004; Maes et al., 1998; Pitman & Orr, 1990) or not different from controls (Baker et al., 1999; Duval et al., 2004; Yehuda, Golier, Halligan, Meaney, & Bierer, 2004). Boscarino (1996) found that, independent of PTSD symptomatology, the levels of CORT in Vietnam veterans were a function of the degree of combat exposure that they had experienced. As the veterans' combat exposure increased, their levels of CORT decreased. Bremner (2001) hypothesized that the differences in basal CORT levels among PTSD patients could be dependent on the length of time that an individual has had the disorder. In other words, individuals with chronic PTSD may have had high CORT levels for such a long period of time that regulation of CORT production eventually became defective. However, Boscarino (1996) only observed low baseline CORT levels in Vietnam veterans who had current PTSD – that is, they had developed it within the past year. Those with a lifetime diagnosis of PTSD did not have lower CORT levels than control subjects. In contrast to Bremner (2001), these investigators reasoned that CORT hyporesponsivity could be related to the recency of PTSD, with more exaggerated effects being apparent closer to the time of PTSD onset. In any case, the HPA abnormalities observed in individuals with PTSD does not lead to impairments in CORT release during stress (Bremner et al., 2003a). In fact, some studies have shown that PTSD patients exhibit an exaggerated CORT response to trauma-related stressors, in comparison to healthy controls (Elzinga, Schmahl, Vermetten, van Dyck, & Bremner, 2003).

Cortisol is released in pulses (Deuschle et al., 1997); its levels are highest upon awakening and lowest late in the evening (Gunnar & Vazquez, 2001). Yehuda et al. (1996b) examined the baseline levels of CORT in combat veterans with PTSD at 30-min

intervals over a 24-hr period of bed rest. Their results revealed that the individuals with PTSD had significantly lower levels of CORT during the late evening and early morning hours, which appeared to result from a prolonged nadir and shorter peak response in the cycle of CORT release. Because further analyses revealed a greater signal-to-noise ratio in PTSD patients, the investigators reasoned that HPA functioning in these individuals could provide optimal conditions for response to stress-related cues. As Yehuda et al. (1996b, p. 86) suggested, the "enhanced signal-to-noise ratio describes a system with a maximally low background and, accordingly, a potentially greater capacity to respond to the environment." This could help explain why individuals diagnosed with PTSD are more reactive to their surroundings. In support of this theory, Liberzon et al. (1999a) found that in response to combat sounds, veterans with PTSD displayed significantly greater plasma CORT than controls.

Investigators have also shown that the production of the precursors to CORT is atypical in PTSD patients. Bremner and colleagues (Bremner et al., 1997b) found significantly elevated levels of CSF CRH in Vietnam veterans with PTSD. By using a serial CSF sampling technique, Baker and colleagues (Baker et al., 1999) replicated the findings of Bremner et al. (1997b) and correspondingly reported greater levels of CRH in combat veterans with PTSD. These findings create a paradox – that is, why do a majority of PTSD patients exhibit lower levels of CORT if they have significantly elevated levels of CRH? Well, Smith and colleagues (Smith et al., 1989) found that in response to CRH treatment, PTSD patients produced significantly less ACTH than healthy control subjects, which would at least help explain the low levels of CORT observed in individuals with

PTSD. It is possible that persistent elevations of CRH desensitize CRH receptors in the anterior pituitary, leading to a blunted ACTH release. In addition, Lerer, Ebstein, Shestatsky, Shemesh, and Greenberg (1987) found lower levels of cyclic adenosine monophophate (AMP) in individuals with PTSD. Since cyclic AMP is the second messenger for CRH stimulation of ACTH in the pituitary, these findings could explain why PTSD patients exhibit a blunted ACTH response to CRH administration and why they can have greater levels of CRH and lower levels of cortisol simultaneously.

Enhanced Negative Feedback of the HPA Axis

A review of the literature suggests that the CORT differences in PTSD patients could be attributed to an enhanced negative feedback of the HPA axis. When CORT is released into the blood, it exerts a negative feedback on the HPA axis by binding to glucocorticoid receptors (GRs) in the brain. One of the primary regions where GR-binding takes place is the hippocampus. In fact, this medial temporal lobe structure has one of the highest numbers of glucocorticoid receptors in the mammalian brain (Kim & Diamond, 2002). Research has shown that PTSD patients display an increased number and sensitivity of glucocorticoid receptors (Yehuda, Boisoneau, Lowy, & Giller, 1995; Yehuda et al., 1993b; Yehuda, Lowy, Southwick, Shaffer, & Giller, 1991). In addition, some studies have found an increased suppression of CORT (Grossman et al., 2003; Stein, Yehuda, Koverola, & Hanna, 1997b; Yehuda et al., 1995; Yehuda, Southwick, Krystal, Bremner, Charney, & Mason, 1993c) and ACTH (Duval et al., 2004; Yehuda et al., 2004) in PTSD patients in response to the administration of dexamethasone (DEX), a synthetic glucocorticoid. Theoretically, the DEX is capable of producing more negative

feedback in PTSD patients, which leads to a greater suppression of CORT and ACTH. Nevertheless, other studies have failed to replicate these findings (Kosten, Wahby, Giller, & Mason, 1990; Lindley et al., 2004).

Investigators have also observed increased pituitary activation in PTSD patients following the administration of metyrapone, a glucocorticoid antagonist that blocks the conversion of 11-deoxycortisol to cortisol (or 11-deoxycorticoterone to corticosterone in rodents) (Kanter et al., 2001; Yehuda, Levengood, Schmeidler, Wilson, Guo, & Gerber, 1996a). Since it prevents the production of cortisol, metyrapone hinders the negative feedback component of the HPA axis and consequentially leads to greater levels of CRH, ACTH, and 11-deoxycortisol. These findings therefore provide further support the theory that PTSD patients have enhanced negative feedback of the HPA axis.

Can CORT Administration Help Treat PTSD?

If a blunted CORT response to stress increases an individual's susceptibility to develop PTSD, then perhaps the administration of CORT could prevent the onset of the disorder. Schelling et al. (1999) tested the hypothesis that CORT administration at the time of trauma would reduce the incidence of PTSD in the sample. These investigators examined the effects of CORT administration on patients with septic shock, a major systemic infection that ultimately leads to multiple organ dysfunctions and a considerable amount of physical and emotional stress. Since a large percentage (upwards of 50%) of individuals who suffer from septic shock eventually develop PTSD, this group of subjects was a premier candidate for testing this hypothesis. In the study, some patients received stress doses of hydrocortisone in addition to the standard treatment for the pathology.

Administration of hydrocortisone during septic shock significantly reduced the incidence of PTSD in patients, as only 19% of the patients who received hydrocortisone as part of their treatment developed the disorder, compared to the 59% of control patients who developed PTSD symptomatology. Other investigators have examined the efficacy of CORT treatment in individuals who have already developed PTSD. Aerni et al. (2004) found that one month of low-dose cortisol administration (10 mg/day) resulted in a reduction of reexperiencing symptoms associated with traumatic memories. In addition, Soravia et al. (2006) even showed that the administration of cortisol reduces phobic fear in humans. Collectively, these studies suggest that the administration of CORT may not only help prevent the onset of PTSD, but also serve as a therapeutic agent as well.

Hippocampal Volume in PTSD

Studies have reported smaller hippocampal volumes in individuals who developed PTSD after combat exposure (Bremner et al., 1995; Gurvits et al., 1996), firefighting (Shin et al., 2004), police work (Lindauer et al., 2006; Lindauer et al., 2004), childhood abuse (Bremner et al., 2003b; Bremner et al., 1997c; Stein et al., 1997a), and mixed types of events, such as motor vehicle accidents and assaults (Villarreal et al., 2002; Wignall et al., 2004). Many of these studies have observed smaller hippocampi in individuals with PTSD even after adjusting for participants' total brain volume and age. The debate over the mechanisms underlying these effects is still very active (Bremner, 2001; Pitman, 2001; Yehuda, 2001), and there is some dispute over the significance of these findings in relation to the disorder. For one, the differences in hippocampal volume between PTSD patients and controls have ranged from 5% to > 20%, and some may question whether or

not these findings are biologically relevant to understanding the disorder. Other major problems with these studies include the comorbidity of PTSD with other disorders and the substance abuse that is often observed in these individuals. For instance, many of these studies have not controlled for comorbidity of PTSD with MDD. Major depressive disorder is in many key ways different from PTSD and could very well influence individuals' hippocampal volumes.

Despite the reports of smaller hippocampi in PTSD patients, some investigations (Bonne et al., 2001; De Bellis, Hall, Boring, Frustaci, & Moritz, 2001; De Bellis et al., 1999; Fennema-Notestine, Stein, Kennedy, Archibald, & Jernigan, 2002; Pederson et al., 2004; Schuff et al., 2001; Yamasue et al., 2003) have been unable to replicate these findings. These investigators, in other words, have found no differences in hippocampal volume between the individuals diagnosed with PTSD and control subjects. The question arises, then, as to whether or not a smaller hippocampal volume is a necessary characteristic of all individuals with PTSD or if it simply increases susceptibility to developing the disorder in select individuals. One study (Wignall et al., 2004) examined the hippocampal volumes of individuals with recent-onset PTSD and compared them to non-traumatized controls. After adjusting for total brain volume and participants' age, the investigators found that the PTSD patients had significantly smaller right hippocampal volume than controls. Given that the average time between the trauma and the experiment was 158 days, these findings suggested that either (1) the hippocampi of PTSD patients atrophies quickly after the onset of the disorder or (2) a smaller hippocampus increases one's susceptibility to developing PTSD.

To help resolve this issue, Gilbertson and colleagues (Gilbertson et al., 2002) examined the hippocampal volumes of identical twins (all males). In each twin pair, one of the individuals had been exposed to the Vietnam war, while the other had not. In addition, some of these individuals developed PTSD and some did not. The investigators found that hippocampal volumes of the traumatized individuals who developed PTSD was not different from those of their twin brothers; however, both of these individuals' hippocampal volumes were smaller than those of the traumatized individuals who did not develop PTSD and their twin brothers. In addition, there was a negative relationship between the hippocampal volumes of the individual with PTSD and his twin brother and PTSD symptom severity of the twin brother with the disorder. Thus, not only did the individual with PTSD have a smaller hippocampus than controls but so did his twin brother. These findings suggest that a small hippocampus is a risk factor for developing PTSD rather than a result of the disorder.

Animal Models of PTSD

Although PTSD remains a disorder that is unique to humans, the limitations of human research have compelled investigators to attempt to model the disorder in non-human animals. A valid animal model of PTSD would allow scientists to study many aspects of the disorder, including (1) the factors that contribute to the disorder's development, (2) the neurobiological progression of the disorder, and (3) the effects of novel therapeutic agents on treatment of the disorder. Indeed, many researchers have used stressors such as electric shock, immobilization (i.e., restraint stress), underwater trauma, and predator stress to produce physiological and behavioral effects in rodents that are

comparable to those observed in humans with PTSD. However, many of these models are inadequate, either because they lack face validity and ethological relevance or because they do not encompass the entire range of symptoms that are observed in PTSD patients. Rachel Yehuda, a well-known researcher who studies PTSD in humans, presented five criteria that all animal models of PTSD should meet before they are accepted by the scientific community:

(1) Even very brief stressors should be capable of inducing biological and behavioral sequelae of PTSD.

(2) The stressor should be capable of producing the PTSD-like sequelae in a dose-dependent manner.

(3) The stressor should produce biological alterations that persist over time or become more pronounced with the passage of time.

(4) The stressor should induce biobehavioral alterations that have the potential for bidirectional expression (i.e., enhanced and reduced responsiveness to different aspects of the environment).

(5) Interindividual variability in response to a stressor should be present either as a function of experience (e.g., prior stress history and poststressor adaptations), genetics, or an interaction of the two (Yehuda & Antelman, 1993a, p. 480-482).

These points are well warranted and should be kept in mind throughout the following review of the most relevant animal models of PTSD.

Although PTSD is a disorder than can develop after very brief exposure to trauma, some investigators have likened the long-term effects of chronic stress in rodents to the disorder. Several studies have shown that chronic restraint stress (6 hrs/day for 21 days) leads to atrophy of hippocampal dendrites (Magarinos et al., 1996; Magarinos et al., 1995) and impairments of hippocampus-dependent, spatial memory (Conrad et al., 1996; Luine et al., 1994). These effects have been linked with the persistent increase in glucocorticoids and excitatory amino acids (EAAs) that accompanies stress (Magarinos et al., 1995). In fact, the effects of restraint stress on hippocampal morphology can be blocked via the administration of NMDA receptor antagonists (Magarinos et al., 1995) or pharmacological agents, such as phenytoin, that reduce extracellular levels of EAAs (Watanabe, Gould, Cameron, Daniels, & McEwen, 1992). Other work has investigated the long-term effects of milder psychosocial stress on rats' functioning. For instance, Park and colleagues (Park et al., 2001) examined the effects of chronic cat exposure and unstable housing conditions on rats' behavior and sensitivity to yohimbine. The investigators found that stress led to a significant impairment in rats' spatial memory and an increased sensitivity to yohimbine, as evidenced by greater immobility in the open field following its administration. Similarly, Song, Che, Min-wei, Murakami, & Matsumoto (2006) found that unpredictable, chronic mild stress led to significant impairments in spatial memory, as did Gerges and colleagues (Gerges, Alzoubi, Park, Diamond, & Alkadhi, 2004), who used 4-6 weeks of unstable housing as the primary stressor in their study.

Other investigators have studied the effects of a small number of stress sessions or a single stress session with periodic reminders of the "trauma" on long-term behavior in rodents. Pynoos and colleagues (Pynoos, Ritzmann, Steinberg, Goenjian, & Prisecaru, 1996) exposed mice to 10 sec of footshock (2 mA) and then assessed their behavioral response 1, 21, or 42 days later. Some of the stressed mice were reminded of the initial experience weekly throughout the experiment by placing them back in the apparatus in which they received the footshocks. The investigators found that all of the stressed mice, regardless of whether or not they received the reminders, exhibited increased locomotor activity 24 hrs after being shocked. However, only those mice that were reminded of the shock on a weekly basis exhibited increased anxiety on the elevated plus maze and a heightened startle response during behavioral testing. In fact, these rats' displayed increased anxiety on the plus maze 1, 21, and 42 days after being shocked, but they did not demonstrate an exaggerated startle response until six weeks post-stress. Similar to these findings, Servatius and colleagues (Servatius, Ottenweller, Bergen, Soldan, & Natelson, 1994; Servatius, Ottenweller, & Natelson, 1995) observed a delayed sensitization of startle following exposure to repeated stress. Servatius et al. (1994) exposed rats to either 1 or 3 days of 2-hr stress sessions that involved being restrained and exposed to 40, 2-mA tailshocks. The investigators found that the rats exposed to 3 days of stress exhibited a heightened startle response 4 days, but not 1 or 10 days, after cessation of the stress. Servatius et al. (1995) used the same methodology to assess the effects of 1 or 3 days of stress on startle 4, 7, and 10 days after the stress. In contrast to their earlier findings, the results indicated that 1 day of stress led to a greater startle

response 7 days post-stress, while the rats exposed to 3 days of stress exhibited exaggerated startle 10 days post-stress. These findings were inconsistent with the investigators' prior findings that 3 days of stress led to an exaggerated startle response 4 days after the stressor. Thus, stress may induce a delayed sensitization of rats' startle response, but the timeline for this effect seems to be unclear.

Robert Adamec and colleagues have extensively investigated the long-term effects of cat exposure on rats' behavior. In a series of experiments, Adamec and Shallow (1993) found that a single 5-min exposure to a cat led to heightened anxiety in rats, as indicated by a reduction in open-arm exploration on the elevated plus maze, up to three weeks later. Further work by Adamec and colleagues (Adamec, Burton, Shallow, & Budgell, 1999; Blundell, Adamec, & Burton, 2005) supported the argument that these effects are mediated, in part, by NMDA-dependent plasticity in the brain. When rats were administered MK-801, AP7, or CPP (all competitive NMDA-receptor antagonists) 30 min prior to cat exposure, the rats did not show a lasting increase in anxiety. However, these drugs were incapable of blocking the stress-induced increase in anxiety if they were administered 30 min after cat exposure, suggesting that they had to be present at the time of the stress to be effective. In theory, it is NMDA-dependent plasticity in the amygdala that is in some ways responsible for the lasting effects of cat exposure on anxiety. Studies have observed NMDA-dependent synaptic plasticity in the amygdala as a result of fear conditioning (Bauer, Schafe, & LeDoux, 2002; Rogan, Staubli, & LeDoux, 1997), and the administration of NMDA receptor antagonists prevents the formation of fear memories in rodents (Fanselow, Kim, Yipp, & De Oca, 1994; Kim, DeCola, Landeira-

Fernandez, & Fanselow, 1991; Kim, Fanselow, DeCola, & Landeira-Fernandez, 1992; Maren, Aharonov, Stote, & Fanselow, 1996). These findings support the idea that stress induces NMDA-dependent plasticity within the amygdala, which results in increased anxiety-like behavior.

To model PTSD, most investigators expose rodents to some form of stress and then assess the effects of that stress on long-term physiology and behavior. The investigators typically present the results as mean values and therefore compare the entire stressed groups to control animals. Cohen, Zohar, and Matar (2003) argued that, in practice, this stressed group of animals is not a homogenous population. Rather, some animals appear to be more vulnerable to the stress than others, which supports the fifth criterion in Yehuda & Antelman's (1993a) manuscript (see above). Given this argument, Cohen et al. (2003) examined the differential response of rats to intense stress. The investigators exposed 150 rats to a cat for a period of 10 min and then examined their behavior on the elevated plus maze one week later. As a group, the stressed rats did exhibit greater levels of anxiety on the elevated plus maze, relative to controls. However, within the stressed group of rats, there were some rats that did not show elevated levels of anxiety and freely explored the open arms of the maze. Therefore, the investigators used cutoff behavioral criteria to divide the stressed rats into well-adapted (WA) or maladapted (MA) rats. A rat was considered WA if it spent $\leq$ 1 min in the closed arms of the plus maze and made $\geq$ 8 entries into the open arms; a rat was considered MA if it spent the entire 5-min trial in the closed arms and made no entries into the open arms. These groups of stressed rats were then compared on additional physiological measures.

Results of these analyses indicated that the MA rats exhibited greater levels of CORT and ACTH shortly after the stress than the WA rats. Moreover, the MA rats displayed lower HRV, with a higher HF and lower LF component of their HRs, indicating greater sympathetic and lower vagal tone, respectively. Cohen and colleagues have replicated and extended this work by reporting similar effects on other behavioral measures, such as the acoustic startle response, and using another type of stress – underwater trauma (Cohen, Zohar, Matar, Kaplan, & Geva, 2005; Cohen, Zohar, Matar, Zeev, Loewenthal, & Richter-Levin, 2004). Collectively, these findings support the notion that stress does not affect all rodents the same; rather, some appear to be more vulnerable to the effects of stress. These studies relate to humans in that not every traumatized individual reacts the same way to the stress.

Other work has focused on developing a model of PTSD that compares well with the HPA alterations observed in human patients. For instance, Liberzon, Krstov, and Young (1997) found that exposing rats to a single prolonged stress session (SPS) and "restressing" them 7 days later led to enhanced negative feedback of the HPA axis. These investigators conducted additional work (Liberzon, Lopez, Flagel, Vazquez, & Young, 1999b) using the SPS procedure and found that it not only led to enhanced negative feedback of the HPA axis but a differential regulation of GR and mineralocorticoid receptor (MR) mRNA in the hippocampus as well. Specifically, there was an up-regulation of GR mRNA and a down-regulation of MR mRNA that lasted for at least 2 weeks in the stressed rats. These results provide insight into the mechanisms that may underlie the enhanced negative feedback of the HPA axis that has been observed in

humans with PTSD. As noted above, these individuals show greater numbers of GRs than controls, which could be responsible for a lower baseline level of CORT.

Another group of researchers used a stress-restress paradigm similar to that of Liberzon et al. (1997) to produce PTSD-like physiological and behavioral symptoms in rats. Harvey, Naciti, Brand, and Stein (2003) exposed rats to several sequential stressors in one day, restressed them 7 days later, and then examined their physiological and behavioral responses one week later. The stressed rats displayed significant learning and memory impairments in a hippocampus-dependent spatial memory task and had lower baseline levels of CORT, compared to controls. This is a finding that coincides with a majority of the literature on HPA functioning in human PTSD patients, providing support for the use of a stress-restress paradigm to model the disorder in non-human animals.

Cohen et al. (2006) recently hypothesized that a blunted HPA axis response to stress may increase the susceptibility of rats to develop PTSD-like symptoms. These investigators tested the hypothesis by taking advantage of a strain of rats (Lewis rats) that naturally fails to show a stress-induced increase in CORT. They exposed the Lewis rats and two other strains of rats (Fisher and Sprague-Dawley rats, both produce the typical stress-induced increase in CORT) to a well-soiled cat litter for 10 min and examined their behavioral responses 7 days later. While the Fisher and Sprague-Dawley rats exhibited a significant increase in CORT levels from baseline to stress exposure, the Lewis rats showed no such elevation. The Lewis rats also exhibited more PTSD-like symptoms than the other strains, including a larger startle response and greater anxiety on the elevated plus maze. Given these findings, the experimenters tested the hypothesis that

administering CORT to the Lewis rats would ameliorate these effects. The results of this manipulation indicated that when the Lewis rats were given CORT prior to stress, they demonstrated fewer PTSD-like symptoms, a lower startle response, and less anxiety on the plus maze than the Lewis rats that were treated with vehicle. These findings suggest that a blunted CORT response during stress increases the susceptibility of rats to develop PTSD-like symptoms.

However, there are some caveats for these results. For one, in this work, the Lewis rats displayed a significantly greater startle response and heightened anxiety, compared to the other rat strains, at baseline. Therefore, the abnormal HPA functioning exhibited by these rats may not just create greater PTSD-like symptoms in response to stress; it could produce them at baseline. In addition, the experimenters could not replicate the stress-induced increase in anxiety-like behavior and startle in the Lewis rats in the second experiment (the one in which CORT was administered to the Lewis rats). These problems, in addition to the short delay between stress and behavioral testing (7 days), demand further consideration.

Hypotheses of the Present Experiments

The following experiments were designed to mimic a subset of physiological features in rats that may contribute to the development of PTSD in humans. In the first experiment, rats were administered the pharmacological agents AF-DX 116 and metyrapone to respectively enhance HR and BP and inhibit the stress-induced rise in circulating CORT prior to two intense stress sessions. Rats' long-term behavioral responses to these manipulations were examined three weeks later. The second

experiment extended the findings of the first experiment and assessed rats' sensitivity to yohimbine three weeks after the second stress session.

I hypothesized that stress alone would result in long-term behavioral abnormalities, including enhancements of anxiety, startle, and fear conditioning and impairments of learning and memory. I also hypothesized that a combination of decreased PNS activity (induced by AF-DX 116) and adrenal activity (induced by metyrapone) would exacerbate the effects, leading to a greater expression of behavioral abnormalities in these rats. Furthermore, I expected the stress to induce dynamic brain changes that would increase rats' sensitivity to yohimbine.

Chapter Two: Experiment One

Does a Combination of Reduced PNS and Adrenal Activity Exacerbate Rats' Long-Term Response to Stress?

The initial experiment was designed to examine the long-term effects of a unique stress paradigm on rats' physiology and behavior. In addition, the study served to assess whether a combination of decreased PNS and adrenal activity at the time of stress could exacerbate these effects. Prior to each of two stress sessions, rats were administered pharmacological agents to reduce their PNS and adrenal activity, conditions which are believed to contribute to the development of PTSD in humans. The second stress experience served to remind rats of the initial stress session and exacerbate the stress-induced effects on brain and behavior. It was also intended to add elements of unpredictability and uncontrollability to the stress experience. Three weeks after the second stress session, all rats were tested for the long-term effects of the stress experience on anxiety, startle, fear, and learning and memory through use of various behavioral assessments.

Methods

Rats

Adult male Sprague-Dawley rats (225-250 g upon delivery) obtained from Charles River laboratories were used for the present experiment. The rats were housed two to a cage (standard Plexiglas – 46 x 25 x 21 cm), maintained on a 12-hr light-dark

cycle (lights on at 0700), and had access to food and water ad libitum. Upon arrival, all rats were afforded a habituation period of one week to acclimate to the housing room and cage changes before any experimental manipulations took place. All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Florida.

Design

The present study employed a 2 x 2 x 2 factorial design. The three manipulated factors were stress (stress, no stress), metyrapone (metyrapone, vehicle), and AF-DX 116 (AF-DX 116, vehicle). Each cell of the factorial had a sample size of n = 10, except for the stressed rats treated with both vehicles, which had a sample size of n = 8.

Heart Rate/Blood Pressure Machine Habituation

The measurement of heart rate and blood pressure (HR/BP) required immobilizing rats in a Plexiglas tube and placing their tails in a tail cuff sensor. The tube was designed to prevent excessive movement during testing, which would help eliminate movement artifacts during HR/BP recording. Since rats find even such subtle immobilization stressful, it was necessary to habituate the rats to the procedure prior to the stress sessions. This would prevent (1) excessive movement artifacts during the testing sessions that could not be eliminated by the restraint alone and (2) misleading increases in the unstressed rats that were simply a result of the stress of the HR/BP measurement. All rats, regardless of group, were therefore transported to the laboratory one day prior to the first stress session and exposed to the HR/BP apparatus for 10 min

each. Afterwards, all rats were returned to the housing room, where they were left undisturbed for the remainder of the day.

Pharmacological Agents

The day after HR/BP habituation, all rats were divided into "stress" and "no stress" groups. The rats were transported to the laboratory where they sat for 30 min. Next, each rat was tail-marked with a black permanent marker and received an i.p. injection of metyrapone (50 mg/kg) or vehicle. Metyrapone is a potent glucocorticoid synthesis inhibitor that blocks the conversion of 11-deoxycorticosterone to corticosterone. Previous work in our lab has shown that when administered at this dose, metyrapone significantly blunts the stress-induced CORT increase in rats (Park, Campbell, Woodson, Smith, Fleshner, & Diamond, 2006). The drug was dissolved in 40% polyethylene glycol solution and administered at a volume of 1 ml/kg. After the injection, all rats were left undisturbed for 1 hr.

Subsequently, each rat received an i.p. injection of AF-DX 116 (2 mg/kg) or vehicle. AF-DX 116 is a well-studied presynaptic muscarinic M_2 receptor antagonist that has been found to significantly elevate heart rate and blood pressure in rats (Hata, Itoh, Funakami, Ishida, & Uchida, 2001). The drug works specifically on cardiac receptors by reducing PNS modulation of heart rate (Giachetti, Micheletti, & Montagna, 1986). AF-DX 116 is poorly soluble in aqueous solutions and was therefore dissolved in 0.1 N HCl. This solution was brought to a stable pH of 7.4 by adding 0.1 N NaOH and 0.9% saline and then administered at a volume of 1 ml/kg. After the injection, all rats were left

undisturbed for 30 min, as research indicates that AF-DX 116 begins having a significant effect on blood pressure and heart rate 30-60 min after administration (Hata et al., 2001).

Stress Manipulations

Thirty minutes after the administration of AF-DX 116 or vehicle, rats in the "stress" group were restrained in plastic DecapiCones (Braintree Scientific; Braintree, MA) and placed in a perforated wedge-shaped Plexiglas enclosure (Braintree Scientific; Braintree, MA; 20 x 20 x 8 cm). Rats in the "no stress" group remained in their home cages until the HR/BP measurements described below. After 15 min, the stressed rats, restrained in the plastic DecapiCones and resting in the perforated, wedge-shaped Plexiglas enclosure, were placed in a metal cage (24 x 21 x 20 in) with an adult female cat for 45 min in a room located adjacent to the rat housing rooms. The cat posed no threat to the physical wellbeing of the rats, as the plastic DecapiCones and Plexiglas enclosure prevented any contact between the two organisms. Also, canned cat food was placed on top of the Plexiglas enclosure to direct cat activity towards the rats. After 45 min had elapsed, the rats were returned to the laboratory.

Blood Sample

Immediately after cat exposure (or a yoked time period of 1 hr in the control animals), a blood sample was taken from all rats to examine the level of circulating CORT. Rats were placed in a wire mesh restrainer, and a 2 mm tail snip was made with a sterile razor blade. A 0.5 cc sample of blood was collected in a microcentrifuge tube. After the blood had been collected, lidocaine and gauze were placed on the tip of the tail, and the rats were removed from the restrainer. All blood samples were collected within

2-3 min after the rats were restrained in order to obtain the samples before a rise in CORT could be detected in the serum. Once the blood had clotted at room temperature, it was centrifuged (3000 rpm for 8 min), and the serum was extracted and stored at -80º C until shipped for assay.

Heart Rate and Blood Pressure

After the blood sample was obtained, all rats' HR and BP were assessed. Rats were immobilized in a Plexiglas tube within a warming test chamber (ambient temperature = ~32º C) to increase their body temperature. This allowed for an enhancement of blood flow to the tail, which permitted HR and BP to be assessed using tail cuffs with photoelectric sensors (IITC Life Science; Woodland Hills, CA). After the rats' body temperature had increased to a level suitable for HR/BP measurement (this took an average of 10 min), I attempted to obtain three HR/BP recordings from each rat. For each recording, the tail cuff was inflated with a manual BP inflator, and the measurements were obtained via computer software that was provided by IITC Life Science, Inc. If three recordings could not be obtained, I attained as many recordings as possible (i.e., one or two recordings). In some instances (e.g., rat would not stop moving, HR/BP equipment was unable to acquire a valid reading, etc.), I could not acquire any recordings. These cases were simply removed from the analysis of HR/BP data.

Completely Unstressed Control Group

After each stress session, all rats were restrained to obtain HR/BP recordings and blood samples for analysis of CORT levels. These manipulations were undoubtedly stressful and could have had lasting effects on the rats in the "no stress" conditions.

Therefore, one group of unstressed rats (n = 10) were not exposed to either of these manipulations and simply received vehicle injections during the stress sessions. Planned comparisons were made between the behavior of this group and the vehicle-treated, unstressed rats that were restrained during the stress sessions to determine the effects of the HR/BP and blood sampling manipulations on the rats' long-term behavior.

Stress Sessions

All of the manipulations during the first stress session were performed during the rats' light cycle, between 0700 and 1500 hrs. All rats that were stressed in the initial session were exposed to a second stress session 10 days later, as research has shown that 10 days of repeated immobilization stress significantly modifies brain morphology (Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002). The second stress session took place during the rats' dark cycle to add elements of unpredictability and uncontrollability to the stress experience, as well as to reinforce stress-induced changes in brain and behavior initiated by the first stress session.

During the second stress session, all rats were transported to the laboratory. Here, the same pharmacological manipulations that were performed prior to the first stress session were performed again. Rats received the same pharmacological agents that they received the first time, depending on the group to which they belonged. Then, the procedure that was followed in the first stress session was employed once more. The "stress" rats were restrained and exposed to the cat, while the "no stress" rats remained in their home cages. Afterwards, another blood sample and HR/BP measurement was obtained from all rats. Upon completion of the stress session, all rats were placed in their

home cages and returned to the vivarium. All manipulations took place between 1900 and 0300 hrs.

Randomized Housing

After the initial stress session, the stressed rats were exposed to unstable housing conditions until the commencement of behavioral testing. Previous research has found that social support aids in recovery from traumatic stress (Solomon, Mikulincer, & Flum, 1989; Tarrier & Humphreys, 2003). I therefore hypothesized that preventing a stable social environment in the stressed rats would prevent them from adapting appropriately to the stress experiences. All stressed rats were still housed two per cage, but every day, the cage mate of each rat was changed. The randomization of the stressed rats occurred within groups, so each rat was exposed to every other rat within its own group between 3 to 4 times prior to behavioral testing. No rat had the same cage mate on two consecutive days, and the randomization manipulations took place between 0800 and 1200 hrs.

Handling, Weight, and Behavioral Testing

Before behavioral testing began, all rats were handled for three consecutive days. The handling procedure involved transporting the rats to the laboratory, placing them in a room with attenuated light and sound, and letting them sit for 1 hr. After 1 hr, each rat was handled for 2-3 min each. On the third and final day of handling, each rat was tail marked again, as the initial markings had worn down considerably by this time. All rats were weighed prior to the first stress session, prior to the second stress session, on the last day of handling, and on the last day of behavioral testing.

3 weeks after the second stress session, all rats were tested for fear, anxiety, and learning and memory through use of several behavioral assessments. I chose this time point because PTSD patients show long-term changes in these types of behaviors, and research involving rats has found that a single exposure to a predator can have significant effects on behavior up to 3 weeks later (Adamec & Shallow, 1993). All behavioral testing took place between 0700 and 1500 hrs.

Behavioral Apparatus

Elevated plus maze (EPM; see Figure 1). Twenty-four hrs after the last day of handling, all rats were transported to the lab and subjected to the EPM assessment. The EPM (Hamilton-Kinder; San Diego, CA) is an apparatus that has been used extensively to study anxiety in rodents (Korte & DeBoer, 2003). It consists of one open arm (10.80 x 51.17 cm) and one closed arm (10.80 x 51.17 cm) that intersect each other to form the shape of a plus sign. The intersection area is 10.80 by 10.80 cm, and the walls of the closed arms are 40.01 cm high. The more time rats spend in the closed arms, the more anxious they are assumed to be. In other words, time spent in the open arms is considered risk-taking behavior, as it places the rat in open view and susceptible to danger. Each rat was placed on the EPM for 10 min, and its behavior was monitored by 48 infrared photobeams connected to a computer program (Motor Monitor) that analyzed the behavior. The program enabled the experimenter to assess the rats' total ambulations, distance traveled in each area of the maze, distance traveled overall, and time spent in each area of the maze. The primary measurement of concern was the percentage of time that each rat spent in the open arms, as compared to the closed arms. The EPM was

wiped down with 25% ethanol solution between each testing session to reduce odor buildup in specific areas of the maze.

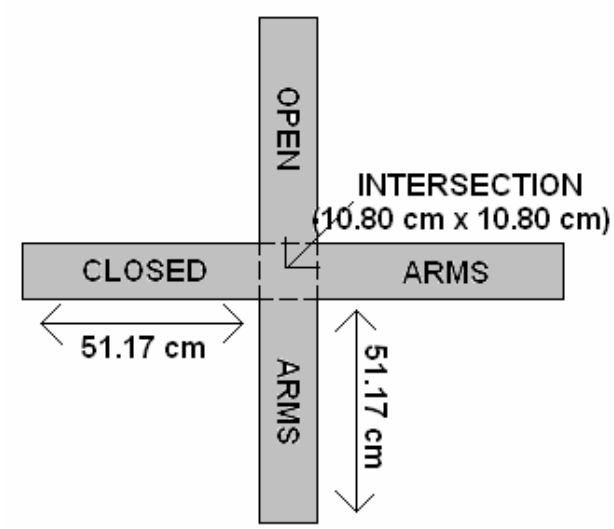

Figure 1. Schematic diagram of the elevated plus maze.

Startle response. Approximately 1 hr after the EPM assessment, all rats were subjected to a test of their startle reflex. Each rat was placed inside a restraint box that was inside a larger startle monitor cabinet (Hamilton-Kinder; San Diego, CA; 35.56 x 27.62 x 49.53 cm). Within the restraint box, the rats sat on a sensory transducer, which recorded their startle reflexes. The startle trial began with a 5-min acclimation period, followed by the presentation of 24 noise bursts, eight from each of three auditory intensities (90, 100, and 110 dB). The noise bursts were presented in sequential order (i.e. 8 bursts at 90 dB, followed by 8 bursts at 100 dB, etc.), and the time between each noise burst varied between 25 and 55 sec. Upon the commencement of the first noise burst, the startle apparatus provided an uninterrupted background noise of 57 dB. Each startle reflex was recorded in Newtons, and the complete session lasted approximately 20 min.

Radial-arm water maze (RAWM; see Figure 2). Twenty-four hrs after the EPM and startle reflex assessments, all rats underwent RAWM training to assess their learning and memory. The RAWM consists of a black galvanized round tank (168 cm diameter, 56 cm height, 43 cm depth) filled with water (21º-22º C). Using 6 V-shaped stainless steel walls (54 cm height, 56 cm length), the tank was divided into six arms radiating from an open central area. A black plastic platform (12 cm diameter) was placed 1 cm below the surface of the water at the end of one arm (the goal arm).

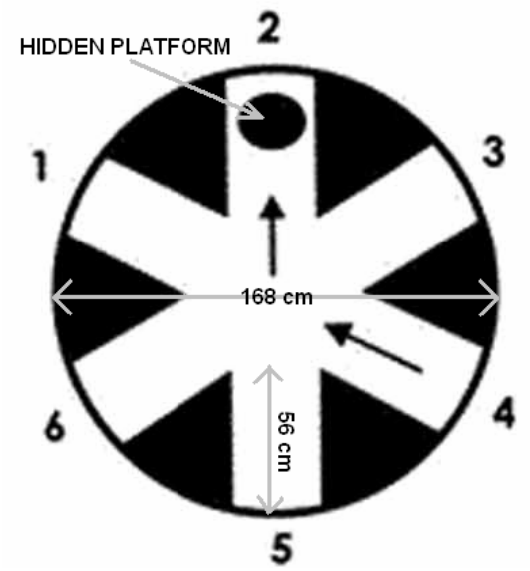

Figure 2. Schematic diagram of the radial-arm water maze. The black arrows represent a path that rats might take to locate the hidden platform.

All rats received a total of 12 acquisition trials to learn the location of the hidden platform. At the beginning of each trial, rats were released at the top of one arm (the start arm) and given 60 sec to find the hidden platform. If the rat could not locate the platform within the 60 sec time frame, I guided it to the platform. Once a rat found or was guided to the platform, it was left there undisturbed for 15 sec. For each trial, I recorded the

number of errors made by each rat and how long the rat took to find the platform. An error consisted of (1) an entry into one of the arms that did not contain the hidden platform or (2) an entry into the arm that contained the hidden platform but an inability to locate it. The latter type of entry error was extremely rare – over 98% of arm entry errors were entries into arms that did not contain the hidden platform. An arm entry was defined as the rat passing halfway down the arm. In addition, if a rat turned around and entered the arm in which it was released (i.e., the start arm), it was not counted as an error. However, if the rat left the start arm (i.e., entered the open area) after being released, then a subsequent entry into the start arm was counted as an arm entry error.

The hidden platform was placed in a different arm for each rat. This eliminated the possibility of odor cues building up in one arm and attracting rats to the goal arm solely for this reason. The goal arms for each rat were assigned randomly without replacement, and between trials, all fecal boli were removed from the water. Additionally, the start arms varied randomly as the trials progressed. All six arms were used as start arms before repeating a start arm a second time. The sequential order of the start arms was also random.

All rats received 12 acquisition trials (T1-T12) to learn the location of the hidden platform. Afterwards, they were dried and returned to their home cages where they spent a 1-hr delay period. The rats were then given a single short-term (1 hr) memory test trial (T13). Then, the rats were dried and returned to their home cages. To assess long-term memory, all rats were returned to the laboratory 24 hrs later and given one retention trial

in the RAWM. Under control conditions, rats have been shown to exhibit excellent 24-hr memory in this training paradigm.

Fear conditioning. Twenty-four hrs after the RAWM retention trial, all rats underwent fear conditioning. Fear conditioning training took place in a dark fear conditioning chamber (25.5 x 30 x 29 cm; Coulbourn Instruments; Allentown, PA) that consisted of two aluminum sides, an aluminum ceiling, and a Plexiglas front and back. The floor consisted of 18 stainless steel rods, spaced 1.25 cm apart, through which shock could be delivered. Auditory stimuli were presented through a speaker located on one side of the chamber. The chamber was wiped down with 25% ethanol solution after each training and retention session.

The fear conditioning training paradigm consisted of placing the rat in a fear conditioning chamber. After a 2-min acclimation period had elapsed, a 10-sec tone (74 dB; 2500 Hz) was presented, which co-terminated with a 2-sec, 0.4 mA footshock. Rats then endured a 50-sec post-shock period, with the presence of no stimulus. Subsequently, another 10-sec tone was presented, which again co-terminated with a 2-sec, 0.4 mA footshock. Afterwards, there was a 30-sec final post-shock period, after which the experimenter removed the rat and returned it to its home cage. The entire fear conditioning training session lasted 240 sec (4 min).

Fear conditioning retention tests were conducted 24 hrs after the training session. Rats' freezing behavior served as an indicator of the rats' memory. Rats' behavior was measured by a 24-cell infrared activity monitor (Coulbourn Instruments; Allentown, PA), mounted on the top of the fear conditioning chamber, which uses the emitted infrared

body heat image (1300 nm) from the animal to detect movement. Freezing was defined as periods of inactivity lasting ≥ 3 sec. A Microsoft Excel spreadsheet with a macro designed to analyze freezing behavior was used to calculate the total number of seconds spent freezing by each animal at 30-sec epochs. These parameters have been employed by laboratories elsewhere (Lee & Kim, 1998) and have been shown to significantly correlate with time sampling observer methods often employed to assess freezing behavior (Kim et al., 1991; Lee & Kim, 1998). This procedure allowed for accurate measurement of the rats' freezing behavior without requiring the presence of the experimenter, which could interfere with the rats' naturalistic behavior.

To assess contextual fear conditioning, which is dependent upon the hippocampus and the amygdala (Phillips & LeDoux, 1992), rats were returned to the same context in which they were originally shocked. All rats remained in the shock context (without receiving shock) for 5 min, and freezing scores were calculated for every 30-sec epoch. Freezing was presented as a percentage score, defined as the time of inactivity divided by the total time. An hour and a half after assessing contextual fear conditioning, the rats' cue-based fear conditioning (i.e., fear of the tone), which is dependent on the amygdala but independent of the hippocampus (Phillips et al., 1992), was assessed. Rats were placed in the light side (25 x 22.5 x 33 cm) of a shuttle box (Coulbourn Instruments; Allentown, PA) that consisted of two aluminum sides, an aluminum ceiling, and a Plexiglas front and back. A house light was turned on, and a metal plate (21.5 x 21.5 cm) was placed on the floor of the shuttle box to eliminate the sensation of the stainless steel rods beneath their paws. The rats remained in the light side of the shuttle box for a total

of 6 min. During the first 3 min, no tone was presented; during the last 3 min, a tone (74 dB; 2500 Hz) was introduced to the rat. All rats were expected to show little or no freezing during the first 3 min (since it was a different context), and their freezing was expected to increase dramatically at the commencement of the tone. Therefore, rats' freezing behavior in response to the tone served as a measure of their memory for the tone-shock association, independent of the context, and the extent of freezing indicated the strength of this memory.

Blood samples, heart rate, and blood pressure. Twenty-four hrs after the fear conditioning retention tests, 3 blood samples and a HR/BP measurement were obtained from all rats to assess the long-term effects of the drug and stress manipulations on ANS and adrenal activity. All rats were placed in a wire mesh restrainer, and a 2 mm tail snip was made with a sterile razor blade. A 0.5 cc sample of blood was collected in a microcentrifuge tube. Lidocaine was then placed on the tip of the tail. The first blood sample was the "baseline" measure of CORT in all rats and was collected within 2-3 min after rats were removed from their home cages to obtain the sample before a rise in CORT could be detected in the plasma. After obtaining this sample, all rats remained in the restrainer for 20 min. Then, the wound on the tip of the tail was gently opened with sterile gauze, thereby making another tail snip unnecessary. Another 0.5 cc sample of blood was collected in a microcentrifuge tube. This "stress" measure of CORT served to examine rats' hormonal response to restraint stress. After collecting the sample, lidocaine and sterile gauze were placed on the tip of the tail, and the rats were removed from the restrainer. Then, the rats were placed in a Plexiglas tube within a warming test chamber

to increase their body temperature. The rats' tails were placed in a tail cuff sensor, and once the rats' body temperature was increased appropriately, I attempted to obtain three HR/BP recordings from each rat. The rats were then returned to their home cages and remained undisturbed for 1 hr. Afterwards, one last blood sample was collected by placing rats in a wire mesh restrainer, opening the tail wound gently with sterile gauze, and obtaining 0.5 cc of blood in a microcentrifuge tube. Lidocaine was placed on the tip of the tail once the blood had been collected. This sample examined how well rats' CORT levels recovered and returned to baseline after restraint stress. 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 -80º C until shipped for assay.

Statistical Analysis

Most data were analyzed with between-subjects, three-way analyses of variance (ANOVAs); mixed-model ANOVAs were employed when repeated measures variables were a part of the behavioral assessment. Post-hoc comparisons were made through use of Bonferroni-corrected *t*-tests.

Since I originally hypothesized that stress alone would have effects on behavior, planned comparisons were made between the stressed rats treated with both vehicles and the unstressed rats treated with both vehicles in cases when the omnibus *F* test did not reveal a suspected difference between these two groups. Independent samples *t*-tests were used for these analyses.

It was also of biological importance to determine whether or not each of the other groups was different from the unstressed rats treated with both vehicles. This group was

considered a "reference group," or primary control group, and therefore enabled differences from baseline to be detected within the unstressed and stressed groups of rats. These differences could highlight specific drug effects of interest for future work. These comparisons were strictly post-hoc and were made using Bonferroni-corrected *t*-tests.

Data points that were greater than three standard deviation units beyond the exclusive mean were considered outliers and removed from the analyses. Less than 1% of the data were outliers, and no more than one data point was removed from any single group of rats. All data are expressed as means ± SEM.

Heart rate and blood pressure. Three separate three-way ANOVAs were utilized to examine group differences in HR, systolic BP, and diastolic BP. Stress (stress, no stress), Metyrapone (50 mg/kg, vehicle), and AF-DX 116 (2 mg/kg, vehicle) served as the between-subjects factors at each time point (first stress session, second stress session, last day of behavioral testing).

Corticosterone. Only those blood samples from groups that provided physiological and/or behavioral effects were assayed for CORT levels. All of the blood samples from stress session one were assayed. Therefore, a three-way ANOVA was employed to compare the CORT levels between the groups. Stress, Metyrapone, and AF-DX 116 served as the between-subjects factors for the analysis. Only some of the blood samples from stress session two were assayed. Since the AF-DX 116 manipulations resulted in little or no behavioral effects, this factor was dropped from the CORT analysis for stress session two. Thus, a two-way ANOVA was employed to compare the CORT levels between the groups. Stress and Metyrapone served as the

between-subjects factors for the analysis. In addition, only some of the blood samples from the final day of testing were assayed. Again, AF-DX 116 was dropped from this analysis. A mixed-model ANOVA was employed to analyze the CORT samples. Stress and Metyrapone served as the between-subjects factors, and time (baseline, stress, return-to-baseline) served as the within-subjects factor. It is important to note that the AF-DX 116 factor was kept in the analysis for stress session one for the sole purpose of determining if administration of the drug affected rats' CORT levels.

Elevated plus maze (EPM). Each dependent measure acquired from the EPM was subjected to a three-way ANOVA, with Stress, Metyrapone, and AF-DX 116 serving as the between-subjects factors.

Startle response. For each rat, there were eight startle responses at each of three auditory intensities. These eight responses were averaged to create one data point per intensity per rat. The data from each auditory stimulus intensity were analyzed using separate three-way ANOVAs, with Stress, Metyrapone, and AF-DX 116 serving as the between-subjects factors for each analysis.

Radial-arm water maze (RAWM). Separate analyses were conducted on the acquisition trials, the 1-hr memory test trial, and the 24-hr retention trial for the RAWM data. A mixed-model ANOVA was used to compare arm entry errors between the groups during the acquisition trials. Stress, Metyrapone, and AF-DX 116 served as the between-subjects factors, and trial (T1-T12) served as the within-subjects factor. Data from the 1-hr and 24-hr retention trials were analyzed using three-way ANOVAs, with Stress, Metyrapone, and AF-DX 116 serving as the between-subjects factors for each analysis.

Fear conditioning. The fear conditioning retention tests (contextual fear conditioning, cue-based fear conditioning) were analyzed separately. Contextual fear conditioning freezing percentages were compared using a three-way ANOVA, with Stress, Metyrapone, and AF-DX 116 serving as the between-subjects factors. The cue-based fear conditioning freezing percentages were compared using a mixed-model ANOVA, with Stress, Metyrapone, and AF-DX 116 serving as the between-subjects factors and tone (no tone, tone) serving as the within-subjects factor.

Weight. Although each group of rats was ordered for the same weight range, a one-way ANOVA indicated there were weight differences between some of the groups at the time of the first stress session, $F(7,71) = 8.65$, $p < .001$. Nevertheless, all of the groups reached adult weight status by the time of stress session one (see Table 1). In order to examine group differences in weight over the course of the experiment, the amount of weight that rats gained between stress session one and each subsequent time point (i.e., stress session two, last day of handling, last day of behavioral testing) was analyzed using a mixed-model ANOVA, with Stress, Metyrapone, and AF-DX 116 serving as the between-subjects factors and time (i.e. first stress session to second stress session, first stress session to the last day of handling, first stress session to the last day of behavioral testing) serving as the within-subjects factor.

Results

Heart Rates and Blood Pressure during Stress Session 1

Heart rates (see Figure 3). The analysis of rats' HRs during stress session one revealed no main effect of stress, $F(1,57) = 0.94$, $p > .33$. There was a main effect of

metyrapone, $F(1,57) = 9.19$, $p < .01$, indicating that metyrapone-treated rats (435.36 ± 6.79 bpm) had lower HRs than vehicle-treated rats (463.32 ± 6.24 bpm). In contrast, rats treated with AF-DX 116 (464.92 ± 6.79 bpm) had higher HRs than vehicle-treated rats (433.77 ± 6.25 bpm), $F(1,57) = 11.41$, $p < .001$. The Stress x Metyrapone, $F(1,57) = 0.59$, $p > .44$, Metyrapone x AF-DX 116, $F(1,57) = 0.04$, $p > .84$, and Stress x Metyrapone x AF-DX 116, $F(1,57) = 2.54$, $p > .11$, interactions were not significant. There was a significant Stress x AF-DX 116 interaction, $F(1,57) = 4.09$, $p < .05$. Post hoc analyses indicated that AF-DX 116 increased HRs of rats that were not stressed (469.76 ± 9.00 bpm), relative to vehicle-injected controls (419.97 ± 9.00 bpm). However, the drug did not lead to a greater increase in HR (460.07 ± 10.16 bpm) than that which was produced by stress alone (447.57 ± 8.66 bpm) (p's < .05).

A planned comparison revealed a trend, suggesting that the stressed rats treated with both vehicles (466.27 ± 12.23 bpm) displayed greater HRs than the unstressed rats treated with both vehicles (431.03 ± 13.90 bpm), $t(18) = 1.90$, $p = .07$.

The stressed rats treated with metyrapone, $t(16) = -0.10$, AF-DX 116, $t(14) = 1.36$, or both drugs, $t(15) = 1.45$, did not differ from the unstressed rats treated with both vehicles (p's > .05). The unstressed rats treated with AF-DX 116 (493.72 ± 10.85 bpm) exhibited greater HRs than the unstressed rats treated with both vehicles, $t(18) = 3.56$, $p < .05$. The unstressed rats treated with metyrapone, $t(15) = -1.27$, or both drugs, $t(15) = 0.83$, did not differ from the unstressed rats treated with both vehicles (p's > .05).

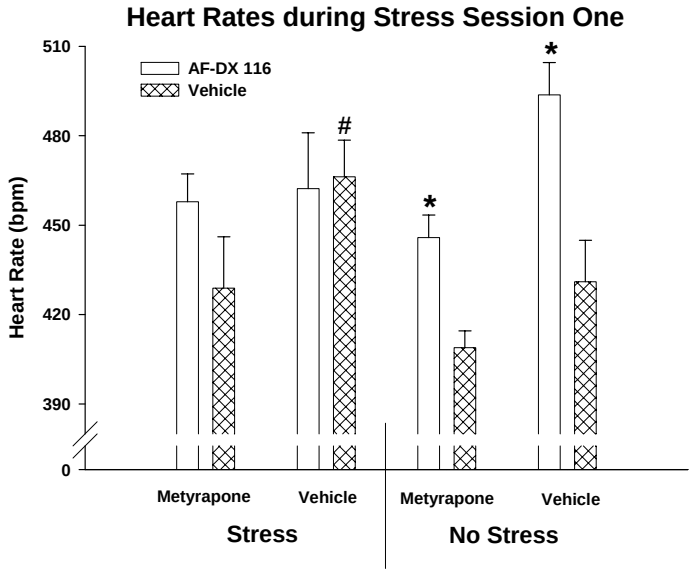

Figure 3. Heart rates (+ *SE*) during stress session one. AF-DX 116 only increased heart rates in the unstressed rats. # $p = .07$ compared to unstressed rats treated with both vehicles; * $p < .05$ compared to respective groups of unstressed rats treated with the vehicle for AF-DX 116.

Systolic blood pressure (see Figure 4). There was a main effect of stress, $F(1,55) = 14.05$, $p < .001$, indicating that the stressed rats (139.46 ± 1.97 mm Hg) had greater systolic BP than the unstressed rats (129.37 ± 1.84 mm Hg). In addition, rats that were treated with AF-DX 116 (141.89 ± 2.01 mm Hg) had greater systolic BP than vehicle-treated rats (126.05 ± 1.79 mm Hg), $F(1,55) = 30.76$, $p < .001$. There was no main effect of metyrapone, $F(1,55) = 0.04$, $p > .84$, and the Stress x Metyrapone, $F(1,55) = 0.05$, $p > .82$, Stress x AF-DX 116, $F(1,55) = 2.06$, $p > .15$, Metyrapone x AF-DX 116, $F(1,55) = 0.01$, $p > .93$, and Stress x Metyrapone x AF-DX 116, $F(1,55) = 1.50$, $p > .22$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (132.73 ± 3.12 mm Hg) exhibited greater systolic BP than the unstressed rats treated with both vehicles (121.48 ± 4.57 mm Hg), $t(17) = 2.07$, $p = .05$.

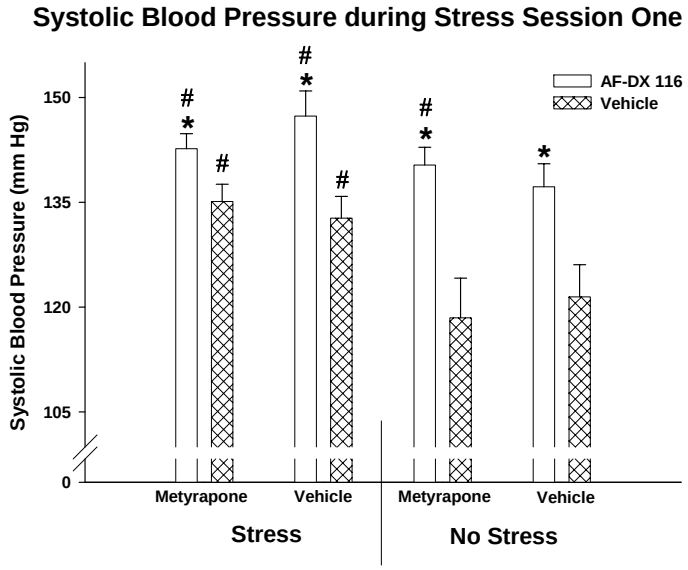

Figure 4. Systolic blood pressure (+ *SE*) during stress session one. Stress and AF-DX 116, in general, led to greater systolic blood pressure. * $p < .05$ compared to respective rats treated with the vehicle for AF-DX 116; # $p \leq .05$ compared to unstressed rats treated with both vehicles.

The stressed rats treated with metyrapone (135.13 ± 2.46 mm Hg), $t(15) = 2.54$, AF-DX 116 (147.33 ± 3.61 mm Hg), $t(13) = 4.06$, or both drugs (142.67 ± 2.15 mm Hg), $t(13) = 3.58$, all displayed greater systolic BP than the unstressed rats treated with both vehicles (p's < .05). The unstressed rats treated with AF-DX 116 (137.21 ± 3.30 mmHg), $t(15) = 2.73$, or both drugs (140.33 ± 2.54 mm Hg), $t(15) = 3.48$, exhibited greater systolic BP than the unstressed rats treated with both vehicles (p's < .05). The unstressed

rats treated with metyrapone did not differ from the unstressed rats treated with both vehicles, $t(15) = -0.42$, $p > .68$.

Diastolic blood pressure (see Figure 5). There was a main effect of stress, $F(1,57) = 45.00$, $p < .001$, indicating that the stressed rats (99.54 ± 1.35 mm Hg) had greater diastolic BP than the unstressed rats (87.32 ± 1.22 mm Hg). In addition, rats that were treated with AF-DX 116 (97.20 ± 1.36 mm Hg) had greater diastolic BP than vehicle-treated rats (89.66 ± 1.21 mm Hg), $F(1,57) = 17.18$, $p < .001$. The Stress x AF-DX 116 interaction was also significant, $F_v = 8.43$, $p < .01$. Post hoc analyses indicated that AF-DX 116 increased diastolic BP in rats that were not stressed (93.74 ± 1.75 mm Hg), relative to vehicle-injected controls (80.91 ± 1.71 mm Hg). However, the drug did not lead to a greater increase in diastolic BP (100.67 ± 2.08 mm Hg) than that which was produced by stress alone (98.41 ± 1.71 mm Hg) (p's < .05). There was no main effect of metyrapone, $F(1,57) = 0.10$, $p > .75$, and the Stress x Metyrapone, $F(1,57) = 0.20$, $p > .65$, Metyrapone x AF-DX 116, $F(1,57) = 0.00$, $p > .96$, and Stress x Metyrapone x AF-DX 116, $F(1,57) = 0.01$, $p > .90$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (98.60 ± 2.86 mm Hg) exhibited greater diastolic BP than the unstressed rats treated with both vehicles (80.07 ± 1.10 mm Hg), $t(18) = 6.05$, $p < .001$.

The stressed rats treated with metyrapone (98.21 ± 3.03 mm Hg), $t(16) = 6.13$, AF-DX 116 (100.72 ± 2.28 mm Hg), $t(14) = 9.19$, or both drugs (100.61 ± 1.70 mm Hg), $t(14) = 10.64$, displayed greater diastolic BP than unstressed rats treated with both vehicles (p's < .05). Unstressed rats treated with AF-DX 116 (93.19 ± 2.41 mmHg), $t(17)$

= 5.13, or both drugs (94.29 ± 2.02 mm Hg), $t(16) = 6.53$, exhibited greater diastolic BP than the unstressed rats treated with both vehicles (p's < .05). The unstressed rats treated with metyrapone did not differ from unstressed rats treated with both vehicles, $t(16) = 0.54$, $p > .60$.

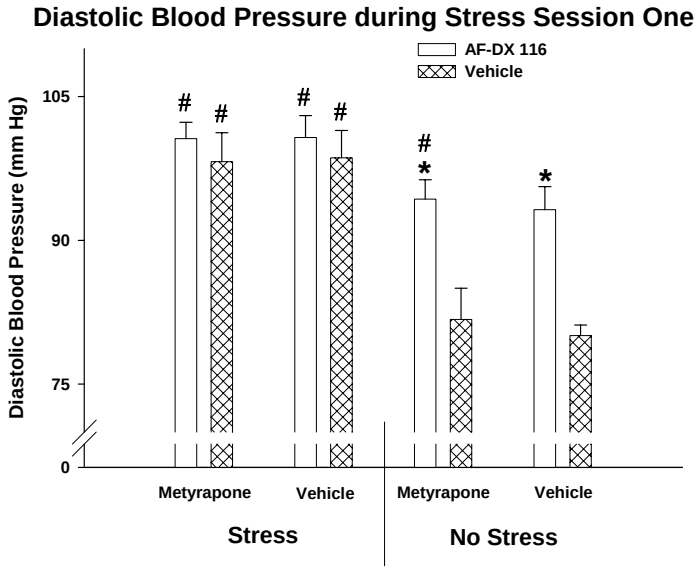

Figure 5. Diastolic blood pressure (+ *SE*) during stress session one. Stress, in general, led to greater diastolic blood pressure, but AF-DX 116 only increased diastolic blood pressure in unstressed rats. * $p < .05$ compared to respective unstressed rats; # $p < .05$ compared to unstressed rats treated with both vehicles.

Heart Rates and Blood Pressure during Stress Session 2

Heart rates (see Figure 6). There was no main effect of stress, $F(1,56) = 0.13$, $p > .71$, or metyrapone, $F(1,56) = 0.45$, $p > .50$. There was a main effect of AF-DX 116, $F(1,56) = 26.10$, $p < .001$, indicating that rats treated with AF-DX 116 (473.26 ± 6.22 bpm) had greater HRs than rats treated with vehicle (431.68 ± 5.25 bpm). The Stress x Metyrapone interaction was not significant, $F(1,56) = 0.08$, $p > .78$. There was a

significant Stress x AF-DX 116 interaction, $F(1,56) = 15.89$, $p < .001$. Post hoc analyses indicated that AF-DX 116 increased HRs of rats that were not stressed (469.76 ± 9.00 bpm), relative to vehicle-injected controls (419.97 ± 9.00 bpm). However, the drug did not lead to a greater increase in HR (455.57 ± 9.84 bpm) than that which was produced by stress alone (446.43 ± 7.21 bpm) (p's < .05). The Metyrapone x AF-DX 116 interaction was also significant, $F(1,56) = 5.12$, $p < .05$. Post hoc analyses indicated that only a combination of the two drugs resulted in greater HRs (479.75 ± 9.61 bpm) than controls (445.29 ± 10.40 bpm) (p's < .05). Lastly, the Stress x Metyrapone x AF-DX 116 interaction was significant, $F(1,56) = 7.42$, $p < .01$. As shown in Figure Four, a general pattern emerged from the results, indicating that AF-DX 116 produced relatively greater HRs compared to the respective vehicle-treated rats. However, this was not the case for the stressed rats that had been administered the metyrapone vehicle. In this case, the opposite pattern was evident – that is, AF-DX 116 actually led to lower HRs than its respective vehicle.

A planned comparison indicated that the stressed rats treated with both vehicles (470.59 ± 11.09 bpm) exhibited greater HRs than the unstressed rats treated with both vehicles (416.63 ± 12.10 bpm), $t(13) = 3.25$, $p < .01$.

The stressed rats treated with both drugs (472.00 ± 17.76 bpm) displayed greater HRs than the unstressed rats treated with both vehicles, $t(10) = 2.61$, $p < .05$. The stressed rats treated with metyrapone, $t(16) = 0.33$, or AF-DX 116, $t(13) = 1.38$, did not differ from the unstressed rats treated with both vehicles (p's > .05). The unstressed rats treated with AF-DX 116 (494.41 ± 13.13 bpm), $t(15) = 4.32$, or both drugs (487.50 ± 4.11 bpm),

$t(14) = 5.55$, exhibited greater HRs than the unstressed rats treated with both vehicles (p's < .05). The unstressed rats treated with metyrapone did not differ from the unstressed rats treated with both vehicles, $t(15) = 0.04$, $p > .96$.

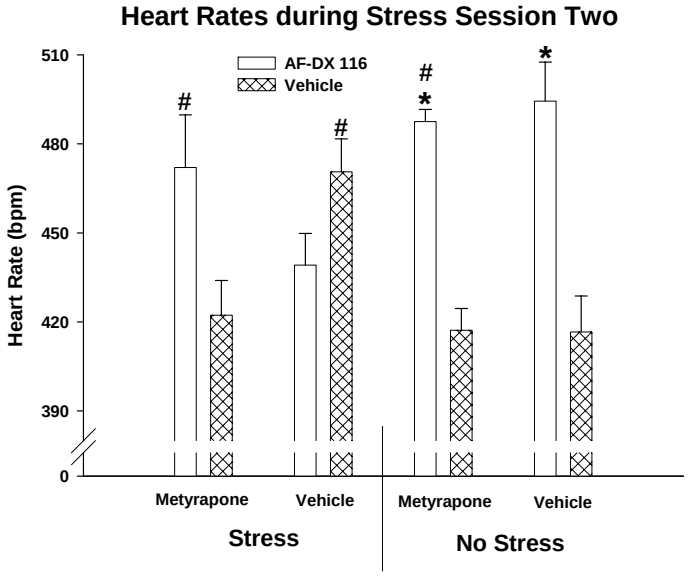

Figure 6. Heart rates (+ *SE*) during stress session two. AF-DX 116 led to greater heart rates in the unstressed rats. # $p < .05$ compared to unstressed rats treated with both vehicles; * $p < .05$ compared to rats treated with the vehicle for AF-DX 116.

Systolic blood pressure (see Figure 7). There was a main effect of stress, $F(1,55) = 4.15$, $p < .05$, indicating that the stressed rats (139.07 ± 2.17 mm Hg) had greater systolic BP than the unstressed rats (133.21 ± 1.90 mm Hg). In addition, rats that were treated with metyrapone (132.66 ± 2.11 mm Hg) had lower systolic BP than vehicle-treated rats (139.62 ± 1.96 mm Hg), $F(1,55) = 5.85$, $p < .05$. There was no main effect of AF-DX 116, $F(1,55) = 1.01$, $p > .31$, and the Stress x Metyrapone, $F(1,55) = 3.16$, $p > .08$, Stress x AF-DX 116, $F(1,55) = 3.26$, $p > .07$, and Metyrapone x AF-DX 116, $F(1,55) = 0.78$, $p > .38$ interactions were not significant. The Stress x Metyrapone x

AF-DX 116 interaction, however, was significant, $F(1,55) = 5.82$, $p < .05$. As shown in Figure 7, the only time that AF-DX 116 produced a significant increase in systolic BP relative to vehicle-treated rats is when unstressed rats were administered the metyrapone vehicle as well (p's < .05).

A planned comparison indicated that the stressed rats treated with both vehicles (148.46 ± 4.80 mm Hg) exhibited greater systolic BP than the unstressed rats treated with both vehicles (125.33 ± 5.64 mm Hg), $t(13) = 3.07$, $p < .01$.

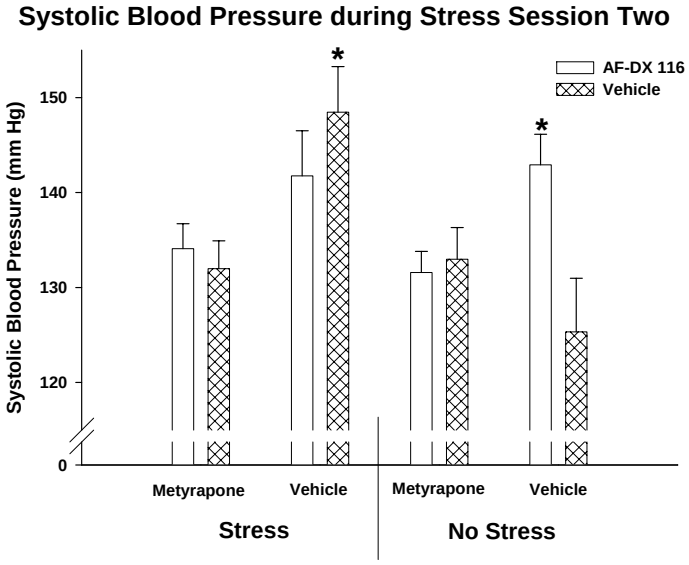

Figure 7. Systolic blood pressure (+ *SE*) during stress session two. Stress, in general, led to greater systolic blood pressure. AF-DX 116 only produced greater systolic blood pressure in the unstressed rats that had been administered the vehicle for metyrapone. * $p < .05$ compared to unstressed rats treated with both vehicles.

The stressed rats treated with metyrapone, $t(16) = 1.11$, AF-DX 116, $t(13) = 2.19$, or both drugs, $t(10) = 1.05$, did not differ from the unstressed rats treated with both vehicles (p's > .05). The unstressed rats treated with AF-DX 116 (142.93 ± 3.21 mm Hg)

exhibited greater systolic BP than the unstressed rats treated with both vehicles, $t(15) = 2.79$, $p < .05$. The unstressed rats treated with metyrapone, $t(16) = 1.20$, or both drugs, $t(14) = 1.03$, did not differ from the unstressed rats treated with both vehicles (p's > .05).

Diastolic blood pressure (see Figure 8). There was a main effect of stress, $F(1,56) = 38.06$, $p < .001$, indicating that stressed rats (100.40 ± 1.28 mm Hg) displayed greater diastolic BP than unstressed rats (89.97 ± 1.10 mm Hg). There was also a main effect of metyrapone, $F(1,56) = 12.31$, $p < .001$, indicating that rats treated with metyrapone (92.22 ± 1.23 mm Hg) exhibited lower diastolic BP than rats treated with vehicle (98.15 ± 1.16 mm Hg). There was no main effect of AF-DX 116, $F(1,56) = 0.34$, $p > .56$. The Stress x Metyrapone, $F(1,56) = 8.55$, $p < .01$, and Stress x AF-DX 116, $F(1,56) = 40.17$, $p < .001$, interactions were significant. Both of these interactions revealed that the stressed rats treated with vehicle (either that of metyrapone or AF-DX 116) had greater diastolic BP than all other groups (p's < .05). The Metyrapone x AF-DX 116 interaction was significant, $F(1,56) = 5.23$, $p < .05$. Post hoc analyses indicated that rats treated with metyrapone and the vehicle for AF-DX 116 (89.79 ± 1.46 mm Hg) had lower diastolic BP than all other groups (p's < .05). The Stress x Metyrapone x AF-DX 116 was also significant, $F(1,56) = 8.53$, $p < .01$. As shown in Figure 8, stressed rats that were treated with both vehicles showed the most prominent increase in diastolic BP (p's < .05).

A planned comparison indicated that the stressed rats treated with both vehicles (115.08 ± 2.66 mm Hg) exhibited greater diastolic BP than the unstressed rats treated with both vehicles (84.08 ± 1.84 mm Hg), $t(13) = 9.31$, $p < .001$.

Stressed rats treated with metyrapone (95.42 ± 2.08 mm Hg), $t(16) = 3.97$, AF-DX 116 (96.57 ± 3.04 mm Hg), $t(13) = 3.62$, or both drugs (94.50 ± 2.57 mm Hg), $t(10) = 3.28$, displayed greater diastolic BP than the unstressed rats treated with both vehicles (p's < .05). Unstressed rats treated with AF-DX 116 (96.85 ± 1.97 mm Hg), $t(15) = 4.70$, or both drugs (94.79 ± 1.44 mm Hg), $t(14) = 4.58$, exhibited greater diastolic BP than the unstressed rats treated with both vehicles (p's < .05). The unstressed rats treated with metyrapone did not differ from the unstressed rats treated with both vehicles, $t(16) = 0.03$, $p > .97$.

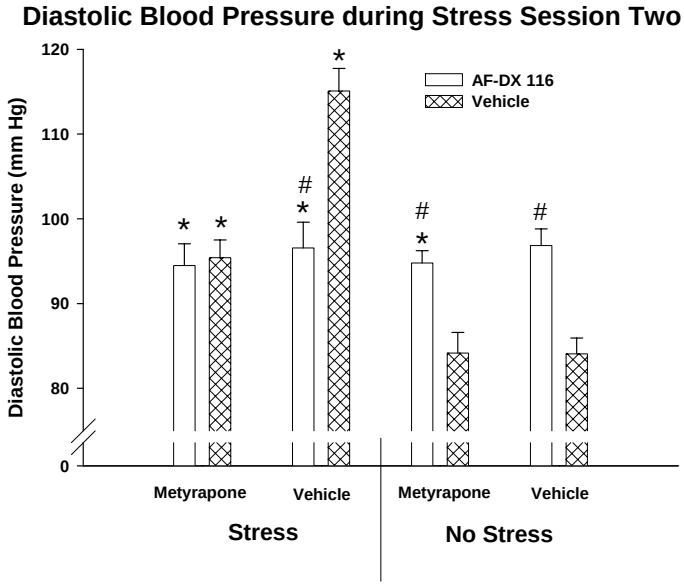

Figure 8. Diastolic blood pressure (+ *SE*) during stress session two. Stress, in general, led to greater diastolic blood pressure. However, AF-DX 116 only produced greater diastolic blood pressure in the unstressed rats. # $p < .05$ compared to respective groups of unstressed rats treated with the vehicle for AF-DX 116; * $p < .05$ compared to unstressed rats treated with both vehicles.

Stress Sessions' Corticosterone Levels

Stress Session 1 Corticosterone Levels (see Figure 9). The analysis of CORT levels during stress session one revealed a main effect of stress, $F(1,67) = 218.58$, $p < .001$, indicating that the stressed rats (29.38 ± 0.84 μg/dL) had greater levels of CORT than the unstressed rats (11.46 ± 0.88 μg/dL). There was a trend effect of metyrapone, $F(1,67) = 3.61$, $p = .06$, suggesting that the rats treated with metyrapone (19.27 ± 0.88 μg/dL) had lower levels of CORT than the rats treated with vehicle (21.57 ± 0.84 μg/dL). There was no main effect of AF-DX 116, $F(1,67) = 0.16$, $p > .69$. The Stress x Metyrapone interaction was significant, $F(1,67) = 62.65$, $p < .001$. Post hoc analyses indicated that the stressed rats treated with metyrapone (23.43 ± 1.20 μg/dL) had lower CORT levels than the stressed rats treated with vehicle (35.33 ± 1.17 μg/dL). However, these rats still had greater CORT levels than the unstressed rats treated with metyrapone (15.11 ± 1.27 μg/dL) or vehicle (7.82 ± 1.20 μg/dL), indicating that metyrapone did not completely block the stress-induced increase in CORT levels. As expected, the stressed rats treated with vehicle exhibited greater CORT levels than the unstressed rats treated with metyrapone or vehicle. Surprisingly, the unstressed rats treated with metyrapone displayed greater CORT levels than the unstressed rats treated with vehicle (p's $< .05$). The Stress x AF-DX 116, $F(1,67) = 0.12$, $p > .73$, Metyrapone x AF-DX 116, $F(1,67) = 0.06$, $p > .80$, and Stress x Metyrapone x AF-DX 116, $F(1,67) = 1.51$, $p > .22$, interactions were not significant.

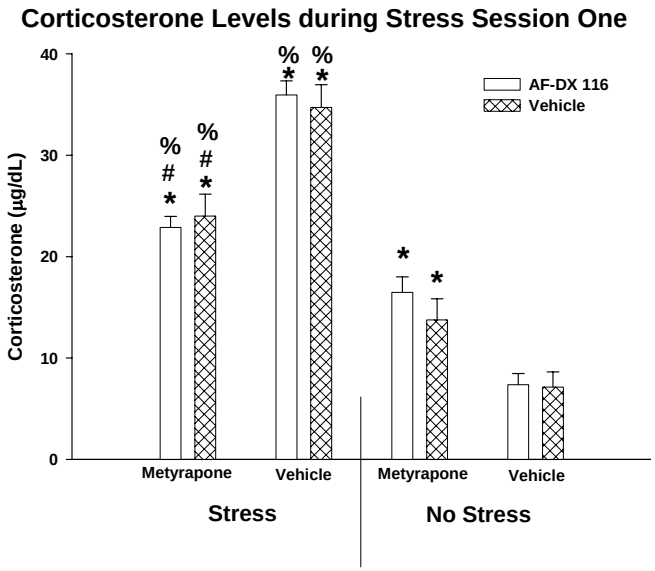

Figure 9. Corticosterone levels (+ *SE*) during stress session one. Stress led to increased levels of corticosterone, and pre-treatment with metyrapone blunted this elevation. * $p < .05$ compared to unstressed rats treated with the both vehicles or AF-DX 116; # $p < .05$ compared to stressed rats treated with vehicle or AF-DX 116; % $p < .05$ compared to unstressed rats treated with metyrapone or both drugs.

Stress session 2 corticosterone levels (see Figure 10). The analysis revealed a main effect of stress, $F(1,32) = 215.37$, $p < .001$, indicating that the stressed rats (31.50 ± 0.97 µg/dL) had greater levels of CORT than the unstressed rats (10.73 ± 1.04 µg/dL).

There was no main effect of metyrapone, $F(1,32) = 0.01$, $p > .90$. The Stress x Metyrapone interaction was significant, $F(1,32) = 49.76$, $p < .001$. Post hoc analyses indicated that the stressed rats treated with metyrapone (26.60 ± 1.40 µg/dL) had lower CORT levels than the stressed rats treated with vehicle (36.42 ± 1.33 µg/dL). However, these rats still had greater CORT levels than the unstressed rats treated with metyrapone (15.80 ± 1.59 µg/dL) or vehicle (5.65 ± 1.33 µg/dL), indicating that metyrapone did not

completely block the stress-induced increase in CORT. As expected, the stressed rats treated with vehicle exhibited greater CORT levels than the unstressed rats treated with metyrapone or vehicle. Surprisingly, the unstressed rats treated with metyrapone again displayed greater CORT levels than the unstressed rats treated with vehicle (*p*'s < .05).

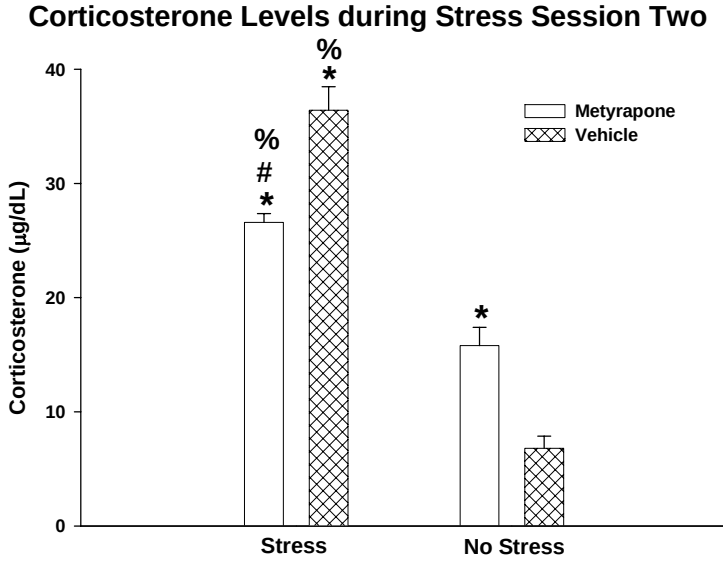

Figure 10. Corticosterone levels (+ *SE*) during stress session two. Stress led to increased corticosterone levels, and pre-treatment with metyrapone blunted this elevation. * $p < .05$ compared to unstressed rats treated with both vehicles; # $p < .05$ compared to stressed rats treated with vehicle; % $p < .05$ compared to unstressed rats treated with metyrapone.

Elevated Plus Maze

Ambulations (see Figure 11). Ambulations consist of beam breaks on the EPM and can serve as a useful measure of general locomotor activity. Analysis of total ambulations revealed a trend effect of stress, $F(1,65) = 3.35$, $p = .07$, suggesting that the stressed rats (374.15 ± 15.67 ambulations) made fewer ambulations than the unstressed rats (414.48 ± 15.48 ambulations). There was also a main effect of metyrapone, $F(1,65) =$

14.03, $p < .001$, indicating that the rats treated with metyrapone (353.06 ± 15.43 ambulations) made fewer ambulations than the rats treated with vehicle (435.57 ± 15.72 ambulations). There was no main effect of AF-DX 116, $F(1,65) = 2.81$, $p > .09$, and the Stress x Metyrapone, $F(1,65) = 0.24$, $p > .87$, Stress x AF-DX 116, $F(1,65) = 2.49$, $p > .12$, Metyrapone x AF-DX 116, $F(1,65) = 0.89$, $p > .34$, and Stress x Metyrapone x AF-DX 116, $F(1,65) = 2.39$, $p > .12$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (387.38 ± 16.52 ambulations) made fewer ambulations on the EPM than the unstressed rats treated with both vehicles (499.90 ± 45.95 ambulations), $t(16) = 2.09$, $p = .05$.

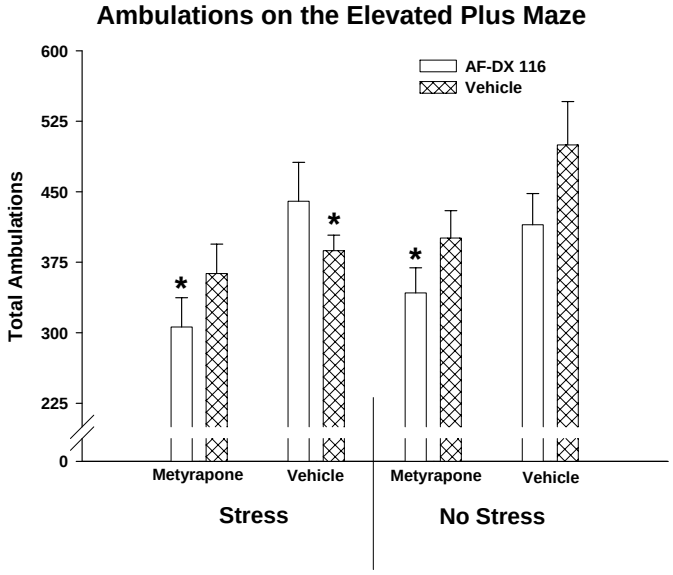

Figure 11. Ambulations (+ *SE*) on the elevated plus maze. Stress tended to result in fewer ambulations on the plus maze, as did the administration of metyrapone. * $p \leq .05$ compared to unstressed rats treated with both vehicles.

The stressed rats treated with both drugs (306.11 ± 31.25 ambulations) made fewer ambulations on the EPM than the unstressed rats treated with both vehicles, $t(17) =$

-3.41, $p < .05$. The stressed rats treated with metyrapone, $t(17) = -2.41$, or AF-DX 116, $t(18) = -0.97$, did not differ from the unstressed rats treated with both vehicles (p's > .05). The unstressed rats treated with both drugs (342.33 ± 26.87 ambulations) made fewer ambulations on the EPM than the unstressed rats treated with both vehicles, $t(17) = -2.87$, $p < .05$. The unstressed rats treated with metyrapone, $t(18) = -1.82$, or AF-DX 116, $t(16) = -1.43$, did not differ from the unstressed rats treated with both vehicles (p's > .05).

Percent time spent in the open arms (see Figure 12). Time spent in the open arms of the EPM was converted into a percent of total time score. The analysis of these scores revealed a main effect of stress, $F(1,68) = 10.09$, $p < .01$, indicating that the stressed rats (14.98 ± 3.26 %) spent less percent time in the open arms than the unstressed rats (29.39 ± 3.16 %). There was no main effect of metyrapone, $F(1,68) = 3.00$, $p > .08$, or AF-DX 116, $F(1,68) = 0.37$, $p > .54$. The Stress x AF-DX 116 interaction was significant, $F(1,68) = 9.03$, $p < .01$. Independent of being stressed (20.42 ± 4.40 %) or not (21.20 ± 4.53 %), rats that were treated with AF-DX 116 spent a comparable amount of time in the open arms. However, stressed rats that were treated vehicle (9.54 ± 4.79 %) spent less percent time in the open arms than unstressed rats that were treated with vehicle (37.59 ± 4.41 %) (p's < .05). The Stress x Metyrapone, $F(1,68) = 0.64$, $p > .42$, Metyrapone x AF-DX 116, $F(1,68) = 0.05$, $p > .82$, and Stress x Metyrapone x AF-DX 116, $F(1,68) = 0.94$, $p > .33$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (9.97 ± 2.09 %) spent less percent time in the open arms than the unstressed rats treated with both vehicles (46.03 ± 5.32 %), $t(16) = 5.75$, $p < .001$.

The stressed rats treated with metyrapone (9.12 ± 3.03 %), $t(18) = -1.76$, or both drugs (16.61 ± 5.54 %), $t(18) = -3.83$, spent less percent time in the open arms than the unstressed rats treated with both vehicles (p's < .05). The stressed rats treated with AF-DX 116 did not differ from the unstressed rats treated with both vehicles, $t(18) = -2.41$, $p > .05$. The unstressed rats treated with AF-DX 116 (15.87 ± 5.39 %), $t(17) = -3.97$, or both drugs (18.15 ± 5.99 %), $t(17) = -3.49$, spent less time in the open arms than the unstressed rats treated with both vehicles (p's < .05). The unstressed rats treated with metyrapone did not differ from the unstressed rats treated with both vehicles, $t(18) = -1.91$, $p > .05$.

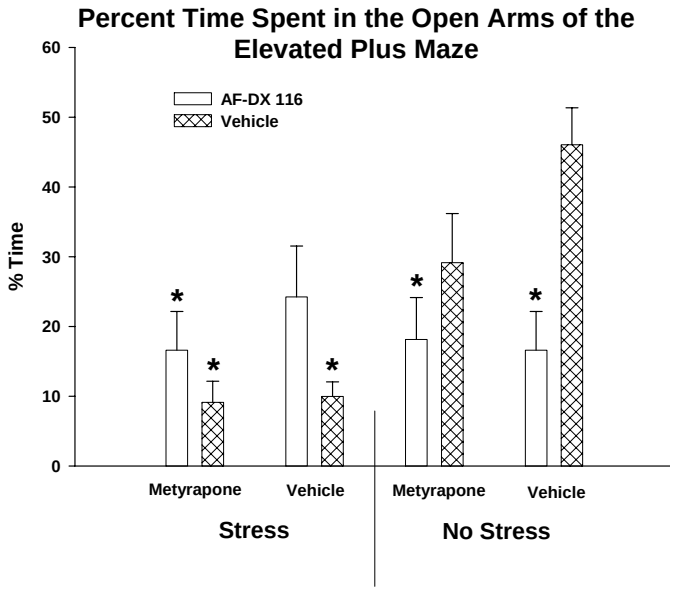

Figure 12. Percent time spent in the open arms (+ *SE*) of the elevated plus maze in Experiment 1. Stress, in general, led to a reduction of open-arm exploration on the plus maze. * $p < .05$ compared to unstressed rats treated with both vehicles.

Percent time spent in the open arms, controlling for ambulations. Since there was a trend effect of stress for the analysis of total ambulations, one could argue that the

stressed rats spent less time in the open arms because they moved less. Therefore, a three-way analysis of covariance (ANCOVA) was used to examine group differences in the percent of time spent in the open arms, with total ambulations on the EPM serving as the covariate. The analysis revealed that the main effect of stress remained significant, $F(1,62) = 5.48$, $p < .05$. There were, however, no main effects of metyrapone, $F(1,62) = 0.01$, $p > .93$, or AF-DX 116, $F(1,62) = 1.61$, $p > .20$. The Stress x AF-DX 116 interaction also remained significant, $F(1,62) = 19.72$, $p < .001$. The Stress x Metyrapone, $F(1,62) = 0.00$, $p > .96$, Metyrapone x AF-DX 116, $F(1,62) = 1.87$, $p > .17$, and Stress x Metyrapone x AF-DX 116, $F(1,62) = 1.28$, $p > .26$, interactions were not significant.

A one-way ANCOVA was also run to control for total ambulations and compare the percent time that the stressed and unstressed rats treated with both vehicles spent in the open arms. The analysis indicated that even when total ambulations were controlled for, the stressed rats treated with both vehicles spent less percent time in the open arms than the unstressed rats treated with both vehicles, $F(1,15) = 21.15$, $p < .001$.

Percent time spent in the closed arms (see Figure 13). Time spent in the closed arms of the EPM was converted into a percent of total time score. The analysis of these scores revealed a main effect of stress, $F(1,68) = 8.58$, $p < .01$, indicating that the stressed rats (74.20 ± 3.06 %) spent more percent time in the closed arms than the unstressed rats (61.54 ± 3.05 %). There were no main effects of metyrapone, $F(1,68) = 1.19$, $p > .27$, or AF-DX 116, $F(1,68) = 3.17$, $p > .08$. The Stress x AF-DX 116 interaction was significant, $F(1,68) = 11.43$, $p < .001$. Independent of being stressed (70.74 ± 4.20 %) or not (72.69 ± 4.43 %), rats that were treated with AF-DX 116 spent a comparable amount of time in the

closed arms. However, stressed rats that were treated with vehicle (77.66 ± 4.46 %) spent more percent time in the closed arms than unstressed rats that were treated with vehicle (50.39 ± 4.20 %) (*p*'s < .05). The Stress x Metyrapone, $F(1,68) = 0.03$, $p > .85$, Metyrapone x AF-DX 116, $F(1,68) = 0.12$, $p > .73$, and Stress x Metyrapone x AF-DX 116, $F(1,68) = 3.04$, $p > .08$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (78.72 ± 3.20 %) spent more percent time in the closed arms than the unstressed rats treated with both vehicles (43.14 ± 4.56 %), $t(16) = 6.07$, $p < .001$.

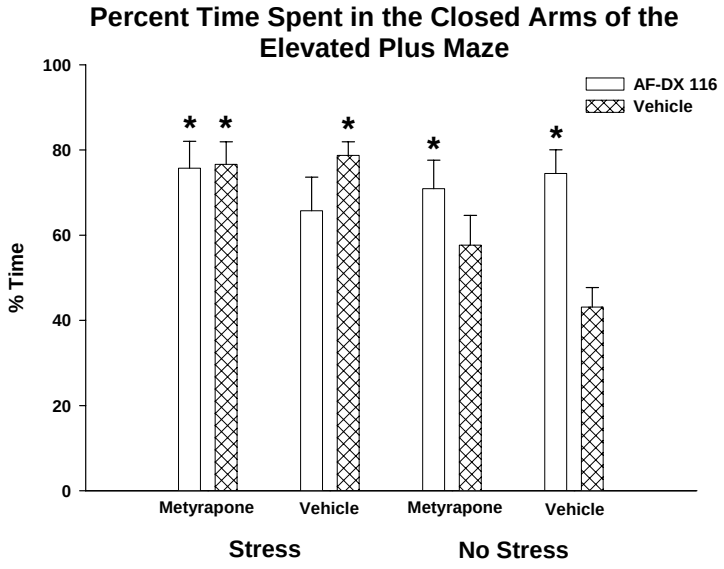

Figure 13. Percent time spent in the closed arms (+ *SE*) of the elevated plus maze in Experiment 1. Stress, in general, increased the amount of time that rats spent in the closed arms. * $p < .05$ compared to unstressed rats treated with both vehicles.

The stressed rats treated with metyrapone (76.61 ± 5.28 %), $t(18) = 4.80$, or both drugs (75.74 ± 6.31 %), $t(18) = 4.19$, spent more percent time in the closed arms than the unstressed rats treated with both vehicles (*p*'s < .05). The stressed rats treated with

AF-DX 116 did not differ from unstressed rats treated with both vehicles, $t(18) = 2.48$, $p > .05$. The unstressed rats treated with AF-DX 116 (74.46 ± 5.59 %), $t(17) = 4.38$, or both drugs, (70.93 ± 6.69 %), $t(17) = 3.49$, spent more percent time in the closed arms than unstressed rats treated with both vehicles (p's < .05). The unstressed rats treated with metyrapone did not differ from unstressed rats treated with both vehicles, $t(18) = 1.74$, $p > .05$.

Movement per unit time in the closed arms (see Figure 14). The distance that each rat traveled in the closed arms was divided by the amount of time it spent in the closed arms to produce a distance/time score. This score represented the distance that each rat traveled in the closed arms per the amount of time that it spent in the closed arms. The analysis of this data revealed a trend effect of stress, $F(1,64) = 3.61$, $p = .06$, suggesting that the stressed rats (11.88 ± 0.39 cm/sec) traveled less distance/time in the closed arms than the unstressed rats (12.92 ± 0.39 cm/sec). Rats that were treated with metyrapone (11.76 ± 0.38 cm/sec) traveled less distance/time in the closed arms than rats that were treated with vehicle (13.04 ± 0.40 cm/sec), $F(1,64) = 5.34$, $p < .05$. There was a main effect of AF-DX 116, $F(1,64) = 4.69$, $p < .05$, indicating that rats treated with AF-DX 116 (11.80 ± 0.38 cm/sec) traveled less distance/time in the closed arms than rats treated with vehicle (13.00 ± 0.40 cm/sec). The Stress x Metyrapone, $F(1,64) = 0.32$, $p > .57$, Stress x AF-DX 116, $F(1,64) = 0.23$, $p > .63$, Metyrapone x AF-DX 116, $F(1,64) = 1.02$, $p > .31$, and Stress x Metyrapone x AF-DX 116, $F(1,64) = 0.80$, $p > .37$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (12.47 ± 0.44 cm/sec) traveled less distance/time in the closed arms than the unstressed rats treated with both vehicles (14.72 ± 0.86 cm/sec), $t(16) = -2.16$, $p < .05$.

The stressed rats treated with metyrapone (11.94 ± 0.59 cm/sec), $t(18) = -2.67$, or both drugs (10.70 ± 0.95 cm/sec), $t(18) = -3.14$, traveled less distance/time in the closed arms than the unstressed rats treated with both vehicles (p's < .05). The stressed rats treated with AF-DX 116 did not differ from the unstressed controls, $t(18) = -1.65$, $p > .05$. The unstressed rats treated with metyrapone, $t(18) = -1.25$, AF-DX 116, $t(17) = -1.35$, or both drugs, $t(18) = -1.76$, did not differ from the unstressed controls (p's > .05).

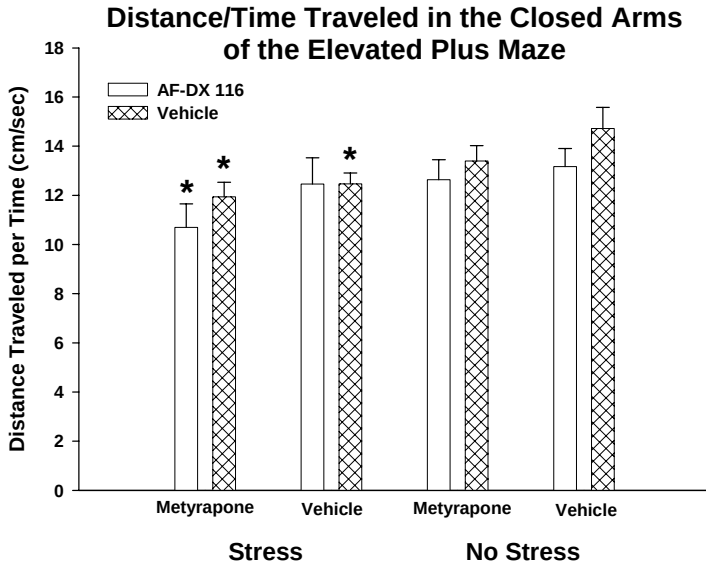

Figure 14. Distance/time traveled in the closed arms (+ *SE*) of the elevated plus maze in Experiment 1. Stress tended to decrease the distance/time that rats traveled in the closed arms, as did metyrapone. * $p < .05$ compared to unstressed rats treated with both vehicles.

Distance on the elevated plus maze (see Figure 15). There was a main effect of stress, $F(1,68) = 5.22$, $p < .05$, indicating that the stressed rats (6926.79 ± 173.31 cm)

traveled less distance on the EPM than the unstressed rats (7485.94 ± 172.74 cm). In addition, rats that were treated with metyrapone (6791.50 ± 170.45 cm) traveled less distance on the EPM than rats treated with vehicle (7621.23 ± 175.56 cm), $F(1,68) = 11.50$, $p < .001$. There was also a main effect of AF-DX 116, $F(1,68) = 6.95$, $p < .01$, indicating that rats treated with AF-DX 116 (6883.89 ± 172.74 cm) traveled less distance on the EPM than rats treated with vehicle (7528.84 ± 173.31 cm). The Stress x Metyrapone, $F(1,68) = 0.23$, $p > .63$, Stress x AF-DX 116, $F(1,68) = 0.11$, $p > .73$, Metyrapone x AF-DX 116, $F(1,68) = 1.69$, $p > .19$, and Stress x Metyrapone x AF-DX 116, $F(1,68) = 0.31$, $p > .57$, interactions were not significant.

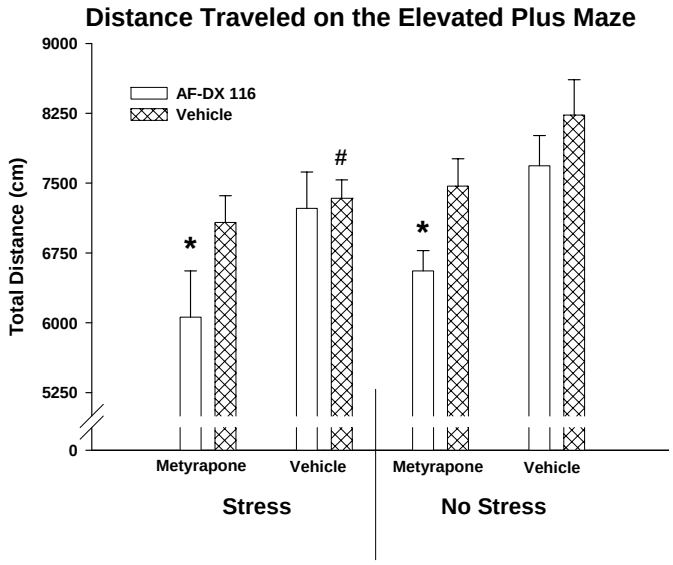

Figure 15. Distance traveled on the elevated plus maze (+ *SE*) in Experiment 1. Stress, AF-DX 116, and metyrapone, in general, resulted in less distance traveled on the elevated plus maze. # $p = .07$ compared to unstressed rats treated with both vehicles; * $p < .05$ compared to unstressed rats treated with both vehicles.

A planned comparison suggested that the stressed rats treated with both vehicles (7337.38 ± 197.61 cm) traveled less distance on the EPM than the unstressed rats treated with both vehicles (8231.60 ± 377.89 cm), $t(16) = 1.94$, $p = .07$.

The stressed rats treated with or both drugs (6061.50 ± 495.46 cm) traveled less distance on the EPM than the unstressed rats treated with both vehicles, $t(18) = -3.48$, $p < .05$. The stressed rats treated with metyrapone, $t(18) = -2.43$, or AF-DX 116, $t(18) = -1.85$, did not differ from the unstressed rats treated with both vehicles, (p's > .05). The unstressed rats treated with both drugs (6558.11 ± 216.76 cm) traveled less distance on the EPM than the unstressed rats treated with both vehicles, $t(17) = -3.73$, $p < .01$. The unstressed rats treated with metyrapone (7467.50 ± 294.29 cm), $t(18) = -1.60$, or AF-DX 116 (7686.56 ± 324.74 cm), $t(17) = -1.08$, did not differ from the unstressed rats treated with both vehicles (p's > .05).

Fecal boli. The amount of fecal boli that a rat deposits in an apparatus is often a good measure of its autonomic reactivity to the test procedure. The analysis of fecal boli deposited on the EPM revealed a main effect of stress, $F(1,69) = 12.81$, $p < .001$, indicating that the stressed rats (3.10 ± 0.27 boli) defecated more than the unstressed rats (1.74 ± 0.27 boli). Moreover, rats that were treated with metyrapone (3.00 ± 0.26 boli) defecated more than rats that were treated with vehicle (1.84 ± 0.27 boli), $F(1,69) = 9.34$, $p < .01$. There was also a main effect of AF-DX 116, $F(1,69) = 8.53$, $p < .01$, indicating that rats treated with AF-DX 116 (1.87 ± 0.27 boli) defecated less than rats treated with vehicle (2.98 ± 0.27 boli). The Stress x Metyrapone, $F(1,69) = 1.46$, $p > .23$, Stress x AF-DX 116, $F(1,69) = 0.06$, $p > .81$, Metyrapone x AF-DX 116, $F(1,69) = 0.06$, $p > .81$,

and Stress x Metyrapone x AF-DX 116, $F(1,69) = 1.07$, $p > .30$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (3.50 ± 0.46 boli) defecated more in the EPM than the unstressed rats treated with both vehicles (1.20 ± 0.51 boli), $t(16) = 3.25$, $p < .01$.

Comparison of the control groups. Independent samples *t*-tests were used to compare the behavior of the completely unstressed controls and the experimental unstressed controls on the EPM. There were no differences between the two groups when comparing their total ambulations, $t(18) = -1.85$, percent time spent in the open arms, $t(18) = -1.22$, entries into the open arms, $t(18) = -1.84$, percent time spent in the closed arms, $t(18) = 1.44$, distance/time traveled in the closed arms, $t(18) = -1.16$, entries into the closed arms, $t(18) = 0.52$, total distance traveled, $t(18) = -1.76$, and fecal boli deposited on the plus maze, $t(18) = 0.11$ (all p's > .05).

Startle Response

90 dB auditory stimuli (see Figure 16). The analysis of startle responses to the 90 dB auditory stimuli revealed a main effect of stress, $F(1,64) = 10.76$, $p < .01$, indicating that the stressed rats (0.27 ± 0.02 Newtons) exhibited a greater startle response than the unstressed rats (0.19 ± 0.02 Newtons). There was also a main effect of AF-DX 116, $F(1,64) = 14.76$, $p < .001$, indicating that the rats treated with AF-DX 116 (0.18 ± 0.02 Newtons) displayed a smaller startle response than the rats that were treated with vehicle (0.27 ± 0.02 Newtons). There was no main effect of metyrapone, $F(1,64) = 2.76$, $p > .10$. The Stress x Metyrapone interaction was marginally significant, $F(1,64) =$

3.58, $p = .06$. Post hoc analyses suggested that the stressed rats that were treated with vehicle (0.31 ± 0.03 Newtons) exhibited greater startle responses than all other groups (p's < .05). The Stress x AF-DX 116 interaction was significant, $F(1,64) = 9.27$, $p < .01$. Post hoc analyses indicated that stressed rats treated with vehicle (0.35 ± 0.03 Newtons) displayed greater startle responses than all other groups (p's < .05). The Metyrapone x AF-DX 116 interaction was significant, $F(1,64) = 12.40$, $p < .001$. Post hoc analyses indicated that the rats treated with both vehicles (0.34 ± 0.03 Newtons) exhibited greater startle responses than all other groups (p's < .05). The Stress x Metyrapone x AF-DX 116 interaction was significant, $F(1,64) = 9.65$, $p < .01$. Post hoc analyses indicated that the stressed rats that were treated with both vehicles (0.47 ± 0.07 Newtons) displayed greater startle responses than all other groups (p's < .05). Collectively, these effects indicated that metyrapone, AF-DX 116, and a combination of both pharmacological agents blocked the stress-induced enhancement of startle.

A planned comparison indicated that the stressed rats treated with both vehicles (0.47 ± 0.07 Newtons) exhibited a greater startle response to the 90 dB auditory stimuli than unstressed rats treated with both vehicles (0.20 ± 0.04 Newtons), $t(15) = 3.55$, $p < .01$.

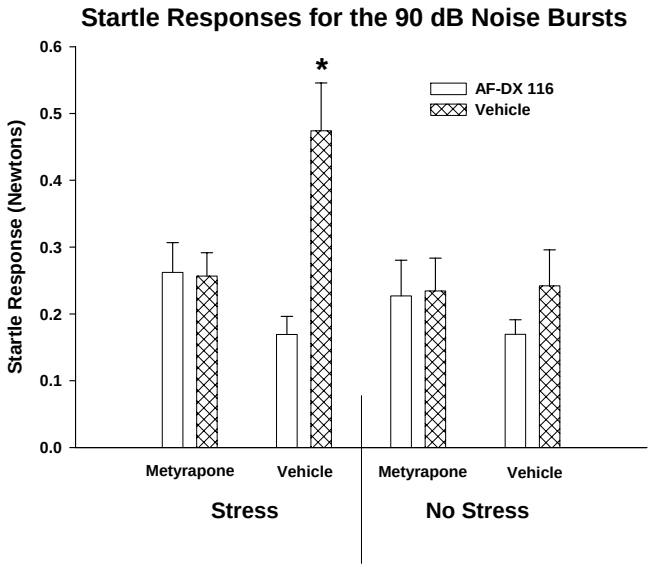

Figure 16. Startle responses (+ *SE*) for the 90 dB noise bursts in Experiment 1. Stressed rats treated with both vehicles show the greatest startle responses. * $p < .05$ compared to all other groups.

100 dB auditory stimuli (see Figure 17). The analysis of startle responses to the 100 dB auditory stimuli revealed a trend effect of stress, $F(1,69) = 3.40$, $p = .07$, suggesting that the stressed rats (1.40 ± 0.12 Newtons) exhibited a greater startle response than the unstressed rats (1.09 ± 0.12 Newtons). There was a main effect of AF-DX 116, $F(1,69) = 4.00$, $p < .05$, indicating that the rats treated with AF-DX 116 (1.08 ± 0.12 Newtons) displayed a smaller startle response than the rats that were treated with vehicle (1.42 ± 0.12 Newtons). There was no main effect of metyrapone, $F(1,69) = 1.85$, $p > .17$. The Stress x Metyrapone, $F(1,69) = 2.87$, $p > .09$, Stress x AF-DX 116, $F(1,69) = 0.21$, $p > .64$, Metyrapone x AF-DX 116, $F(1,69) = 0.02$, $p > .89$, and Stress x Metyrapone x AF-DX 116, $F(1,69) = 3.03$, $p > .08$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (2.03 ± 0.35 Newtons) exhibited a greater startle response to the 100 dB auditory stimuli than the unstressed rats treated with both vehicles (1.06 ± 0.30 Newtons), $t(16) = 2.12$, $p = .05$.

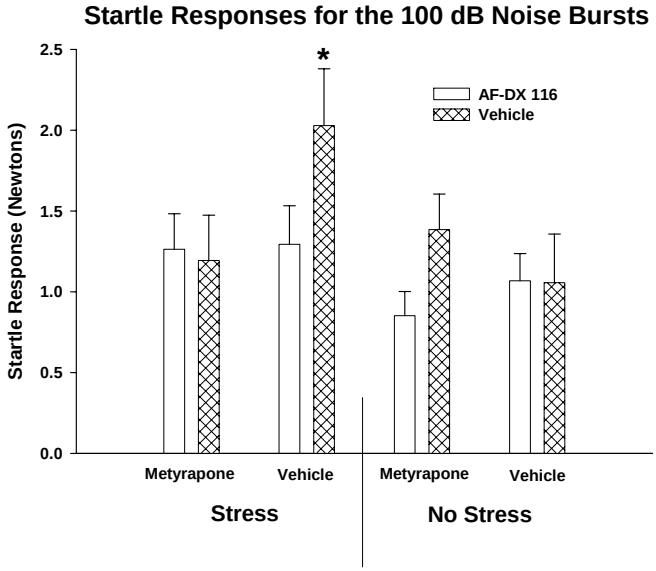

Figure 17. Startle responses (+ *SE*) for the 100 dB noise bursts in Experiment 1. Stressed rats treated with both vehicles show the greatest startle responses. * $p = .05$ compared to unstressed rats treated with both vehicles.

110 dB auditory stimuli (see Figure 18). The analysis of startle responses to the 110 dB auditory stimuli revealed no main effects of stress, $F(1,69) = 2.42$, $p > .12$, metyrapone, $F(1,69) = 2.35$, $p > .13$, or AF-DX 116, $F(1,69) = 0.39$, $p > .53$. The Stress x Metyrapone interaction was significant, $F(1,69) = 4.40$, $p < .05$. Post hoc analyses indicated that while the stressed rats treated with vehicle (3.07 ± 0.23 Newtons) exhibited a greater startle response than the unstressed rats treated with vehicle (2.27 ± 0.21 Newtons), the stressed rats treated with metyrapone (2.28 ± 0.21 Newtons)

did not show this enhancement (p's < .05). The Stress x AF-DX 116, $F(1,69) = 1.46$, $p > .23$, Metyrapone x AF-DX 116, $F(1,69) = 0.06$, $p > .81$, and Stress x Metyrapone x AF-DX 116, $F(1,69) = 0.93$, $p > .33$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (3.35 ± 0.25 Newtons) exhibited a greater startle response to the 110 dB auditory stimuli than the unstressed rats treated with both vehicles (2.07 ± 0.33 Newtons), $t(15) = 2.85$, $p < .01$.

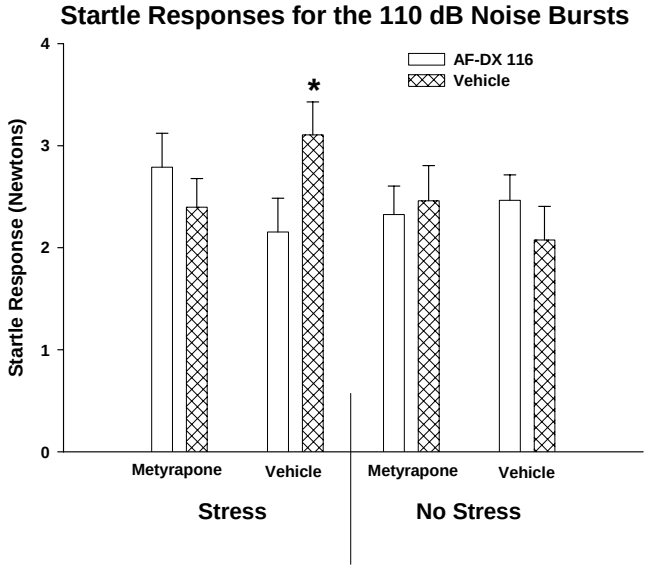

Figure 18. Startle responses (+ *SE*) for the 110 dB noise bursts in Experiment 1. Stressed rats treated with both vehicles show the greatest startle responses. * $p < .05$ compared to unstressed rats treated with both vehicles.

Fecal boli. The analysis of fecal boli deposited in the startle box revealed no main effect of stress, $F(1,67) = 0.01$, $p > .92$. However, there was a main effect of metyrapone, $F(1,67) = 8.83$, $p < .01$, indicating that rats treated with metyrapone (1.81 ± 0.32 boli) defecated less than rats that were treated with vehicle (3.16 ± 0.32 boli). There was no

main effect of AF-DX 116, $F(1,67) = 0.00$, $p > .99$, and the Stress x Metyrapone, $F(1,67) = 0.03$, $p > .85$, Stress x AF-DX 116, $F(1,67) = 1.87$, $p > .17$, Metyrapone x AF-DX 116, $F(1,67) = 1.91$, $p > .17$, and Stress x Metyrapone x AF-DX 116, $F(1,67) = 0.19$, $p > .66$, interactions were not significant.

Comparison of the control groups. A planned comparison indicated that the experimental unstressed controls (0.20 ± 0.04 Newtons) exhibited a greater startle response to the 90 dB auditory stimuli than the completely unstressed controls (0.11 ± 0.01 Newtons), $t(16) = 2.39$, $p < .05$. However, the two groups did not differ in terms of their startle response to the 100 dB, $t(18) = 1.14$, and 110 dB, $t(18) = 0.42$, auditory stimuli (p's > .05).

Radial-Arm Water Maze

Acquisition (see Figure 19). The within-subjects analysis of arm entry errors from the 12 acquisition trials revealed a main effect of trial, $F(11,770) = 43.07$, $p < .001$, indicating that the rats adequately learned the spatial memory task and made less arm entry errors as the trials progressed. The Trial x Stress, $F(11,770) = 1.05$, $p > .39$, Trial x Metyrapone, $F(11,770) = 0.56$, $p > .86$, and Trial x AF-DX 116, $F(11,770) = 1.79$, $p > .05$, interactions were not significant. The Trial x Stress x Metyrapone, $F(11,770) = 0.86$, $p > .58$, Trial x Stress x AF-DX 116, $F(11,770) = 0.75$, $p > .69$, Trial x Metyrapone x AF-DX 116, $F(11,770) = 1.58$, $p > .10$, and Trial x Stress x Metyrapone x AF-DX 116, $F(11,770) = 1.10$, $p > .35$, interactions were not significant.

The between-subjects analysis revealed no main effects of stress, $F(1,70) = 0.09$, $p > .76$ or metyrapone, $F(1,70) = 1.91$, $p > .17$. However, there was a trend effect for

AF-DX 116, $F(1,70) = 3.61$, $p = .06$, suggesting that rats treated with AF-DX 116 (1.18 ± 0.07 errors) tended to make more errors during acquisition than rats treated with vehicle (0.99 ± 0.07 errors). The Stress x Metyrapone, $F(1,70) = 0.02$, $p > .89$, Stress x AF-DX 116, $F(1,70) = 0.67$, $p > .41$, Metyrapone x AF-DX 116, $F(1,70) = 1.26$, $p > .26$, and Stress x Metyrapone x AF-DX 116, $F(1,70) = 0.2$, $p > .89$, interactions were not significant.

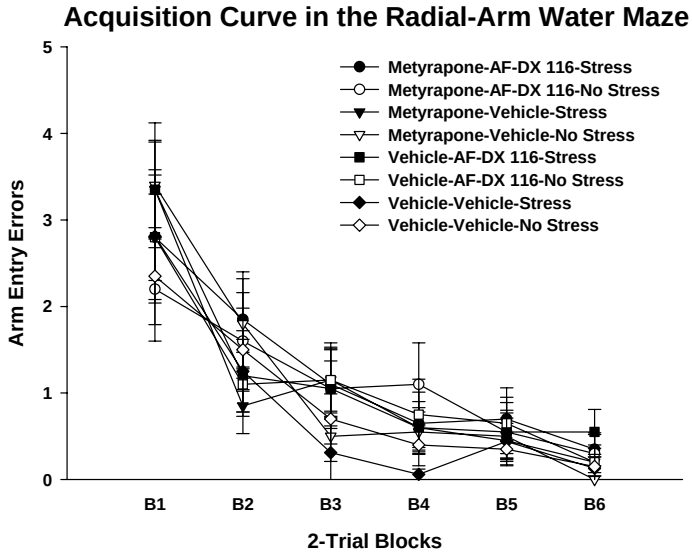

Figure 19. Acquisition curve (+ *SE*) in the radial-arm water maze. All rats adequately learned the task, as evidenced by fewer arm entry errors as the trials progressed.

One-hour memory (see Figure 20). The analysis of arm entry errors on the 1-hr memory test trial revealed no main effects of stress, $F(1,70) = 2.55$, $p > .12$, metyrapone, $F(1,70) = 2.55$, $p > .12$, or AF-DX 116, $F(1,70) = 0.28$, $p > .59$. There was a significant Stress x Metyrapone interaction, $F(1,70) = 4.53$, $p < .05$. Post hoc analyses indicated that the metyrapone-treated, stressed rats (0.45 ± 0.09 errors) made more errors than the vehicle-treated, stressed rats (0.10 ± 0.10 errors) and the unstressed rats that were

administered metyrapone (0.10 ± 0.09 errors) or vehicle (0.15 ± 0.09 errors) (*p*'s < .05). There was also a significant Stress x AF-DX 116 interaction, $F(1,70) = 4.53$, $p < .05$. Independent of being stressed (0.15 ± 0.09 errors) or not (0.20 ± 0.09 errors), the rats treated with AF-DX 116 made a comparable number of arm entry errors. However, when the vehicle-treated rats were stressed (0.40 ± 0.10%), they made more arm entry errors than the unstressed, vehicle-treated rats (0.05 ± 0.09%) (*p*'s <. 05).

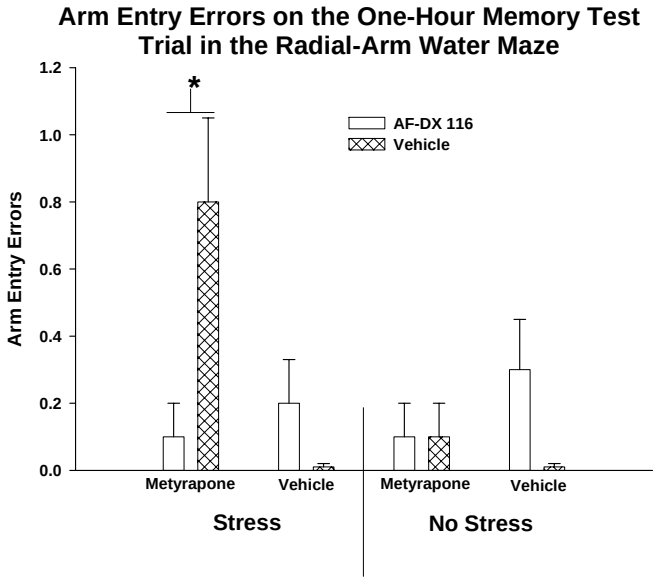

Figure 20. Arm entry errors (+ *SE*) on the 1-hr memory test trial in the radial-arm water maze. Stressed rats treated with metyrapone made more arm entry errors than all other groups. * $p < .05$ compared to all other group combinations.

The Metyrapone x AF-DX 116 interaction was significant, $F(1,70) = 10.18$, $p < .01$. Post hoc analyses revealed that rats treated with AF-DX 116 made a comparable number of arm entry errors, regardless of whether they had been administered metyrapone (0.10 ± 0.09 errors) or its vehicle (0.25 ± 0.09 errors). On the contrary, rats injected with the vehicle for AF-DX 116 made more errors when they were administered

metyrapone (0.45 ± 0.09 errors) than when they were treated with the metyrapone vehicle (0.00 ± 0.00 errors) (*p*'s < .05). The Stress x Metyrapone x AF-DX 116 interaction was not significant, $F(1,70) = 2.55$, $p > .12$.

Twenty-four hour memory (see Figure 21). The analysis of arm entry errors on the long-term memory test trial revealed no main effect of stress, $F(1,64) = 3.04$, $p > .08$. There was a trend effect of metyrapone, $F(1,64) = 3.65$, $p = .06$, suggesting that rats treated with metyrapone (0.68 ± 0.16 errors) made more errors than rats treated with vehicle (0.23 ± 0.17 errors). There was no main effect of AF-DX 116, $F(1,64) = 1.39$, $p > .24$.

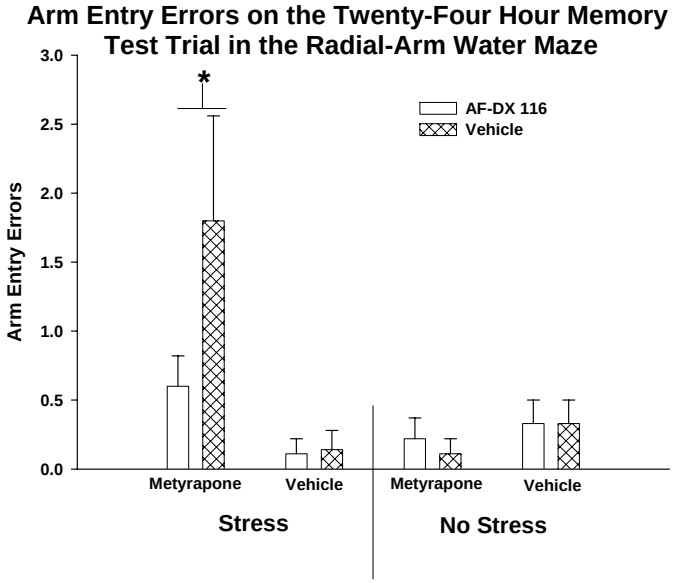

Figure 21. Arm entry errors (+ *SE*) on the 24-hr memory test trial in the radial-arm water maze. Stressed rats treated with metyrapone made more arm entry errors than all other groups. * $p < .05$ compared to other group combinations.

The Stress x Metyrapone interaction was significant, $F(1,64) = 6.82$, $p < .05$. Post hoc analyses revealed that the metyrapone-treated, stressed rats (1.20 ± 0.22 errors) made

more errors than the vehicle-treated, stressed rats (0.13 ± 0.25 errors) and the unstressed rats that were administered metyrapone (0.17 ± 0.24 errors) or vehicle (0.33 ± 0.24 errors) (p's < .05). The Stress x AF-DX 116, $F(1,64) = 2.00$, $p > .16$, Metyrapone x AF-DX 116, $F(1,64) = 1.24$, $p > .27$, and Stress x Metyrapone x AF-DX 116, $F(1,64) = 1.82$, $p > .18$, interactions were not significant.

Comparison of the control groups. A mixed-model ANOVA was utilized to compare arm entry errors on the acquisition trials between the control groups, with trials serving as the within-subjects variable. The analysis revealed a main effect of trial, $F(11,198) = 11.67$, $p < .001$, indicating that all of the rats made fewer arm entry errors as the trials progressed. The Trial x Group interaction was not significant, $F(11,198) = 0.55$, $p > .86$, nor was the between-subjects effect of group, $F(1,18) = 0.56$, $p > .46$.

Independent samples t-tests indicated that the control groups made a comparable amount of arm entry errors on the 1-hr, $t(18) = -1.00$, and 24-hr memory test trials, $t(16) = 1.11$ (all p's > .05).

Fear Conditioning

Contextual fear memory (see Figure 22). The amount of time spent freezing during the 5-min contextual fear memory test was converted into a percent of total time score. The analysis of these scores revealed a main effect of stress, $F(1,70) = 4.34$, $p < .05$, indicating that the stressed rats (68.09 ± 4.55 %) froze more than the unstressed rats (54.89 ± 4.41 %). There were no main effects of metyrapone, $F(1,70) = 3.26$, $p > .07$, or AF-DX 116, $F(1,70) = 1.09$, $p > .30$. There was a significant Stress x Metyrapone interaction, $F(1,70) = 6.49$, $p < .05$. While the rats treated with vehicle exhibited similar

levels of freezing independent of being stressed (65.73 ± 6.62 %) or not (68.67 ± 6.24%), those rats treated with metyrapone froze more if they were stressed (70.44 ± 6.24 %) than if they were not stressed (41.10 ± 6.24 %) (p's < .05). The Stress x AF-DX 116, $F(1,70) = 0.18$, $p > .67$, Metyrapone x AF-DX 116, $F(1,70) = 1.50$, $p > .22$, and Stress x Metyrapone x AF-DX 116, $F(1,70) = 0.01$, $p > .91$, interactions were not significant.

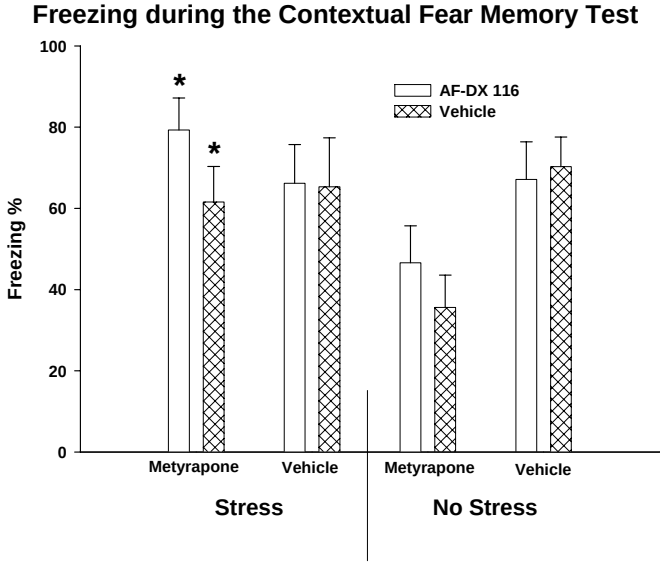

Figure 22. Freezing (+ *SE*) during the contextual fear memory test. * $p < .05$ compared to respective unstressed rats treated with metyrapone.

Context test fecal boli. The analysis of fecal boli deposited during the context test revealed no main effects of stress, $F(1,69) = 0.01$, $p > .93$, metyrapone, $F(1,69) = 0.01$, $p > .93$, or AF-DX 116, $F(1,69) = 0.00$, $p > .96$. The Stress x Metyrapone, $F(1,69) = 0.40$, $p > .52$, Stress x AF-DX 116, $F(1,69) = 2.34$, $p > .13$, Metyrapone x AF-DX 116, $F(1,69) = 0.34$, $p > .56$, and Stress x Metyrapone x AF-DX 116, $F(1,69) = 0.53$, $p > .46$, interactions were not significant.

Cued fear memory (see Figures 23 & 24). The amount of time spent freezing during the 6-min cue fear memory test (no tone, tone) was converted into a percent of total time score. For the within-subjects analysis, there was a main effect of tone, $F(1,67) = 147.51$, $p < .001$, indicating that rats froze more in the presence of the tone (61.65 ± 3.72 %) than when the tone was not presented (23.93 ± 2.45 %). The Cue x Stress interaction was not significant, $F(1,67) = 0.45$, $p > .50$. There was a significant Cue x Metyrapone interaction, $F(1,67) = 7.43$, $p < .01$. When there was no tone presented, rats exhibited similar levels of freezing, independent of being administered metyrapone (24.22 ± 3.31 %) or vehicle (23.63 ± 3.61 %). However, those rats treated with metyrapone (53.48 ± 5.02 %) froze less when the tone was presented than rats treated with vehicle (69.83 ± 5.49 %) (p's < .05).

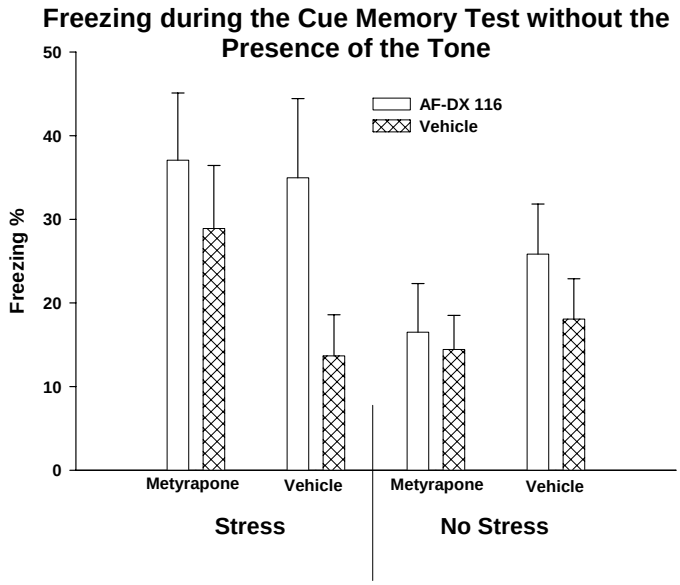

Figure 23. Freezing (+ *SE*) during the cue memory test without the presence of the tone.

The Cue x AF-DX 116 interaction was also significant, $F(1,67) = 4.50$, $p < .05$. When the tone was presented, rats exhibited similar levels of freezing, independent of being administered AF-DX 116 (59.73 ± 5.02 %) or vehicle (63.57 ± 5.49 %). However, those rats treated with AF-DX 116 (28.59 ± 3.31 %) froze more when there was no tone presented than rats treated with vehicle (19.26 ± 3.61 %) (p's < .05). The Cue x Stress x Metyrapone, $F(1,67) = 1.14$, $p > .29$, Cue x Stress x AF-DX 116, $F(1,67) = 0.09$, $p > .76$, Cue x Metyrapone x AF-DX 116, $F(1,67) = 0.21$, $p > .64$, and Cue x Stress x Metyrapone x AF-DX 116, $F(1,67) = 2.59$, $p > .11$, interactions were not significant.

For the between-subjects analysis, there was a main effect of stress, $F(1,67) = 5.23$, $p < .05$, indicating that the stressed rats (49.05 ± 4.00 %) froze more than the unstressed rats (36.53 ± 3.75 %). There were no main effects of metyrapone, $F(1,67) = 2.07$, $p > .15$, or AF-DX 116, $F(1,67) = 0.25$, $p > .61$. The Stress x Metyrapone interaction was at a trend level, $F(1,67) = 3.60$, $p = .06$. While the rats treated with vehicle exhibited similar levels of freezing independent of being stressed (47.79 ± 6.04%) or not (45.66 ± 5.37 %), those rats treated with metyrapone tended to freeze more if they were stressed (50.31 ± 5.23 %) than if they were not stressed (27.39 ± 5.23 %) (p's < .05). The Stress x AF-DX 116, $F(1,67) = 0.41$, $p > .52$, Metyrapone x AF-DX 116, $F(1,67) = 0.26$, $p > .61$, and Stress x Metyrapone x AF-DX 116, $F(1,67) = 0.44$, $p > .51$, interactions were not significant.

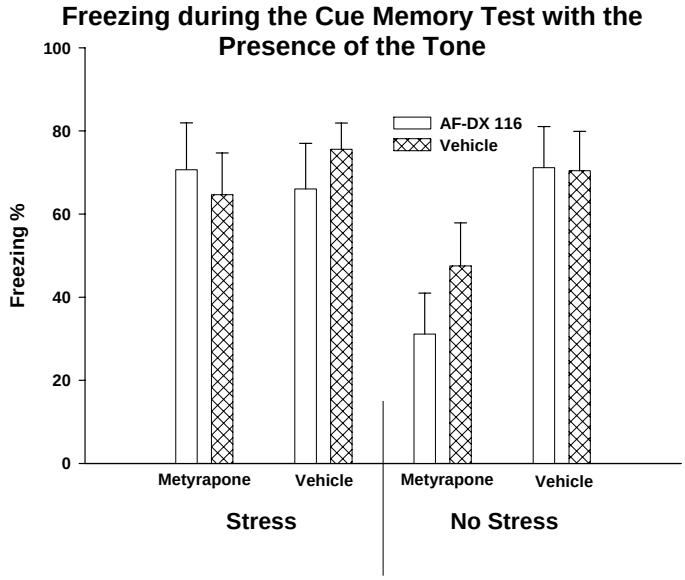

Figure 24. Freezing (+ *SE*) during the cue memory test with the presence of the tone.

Cue test fecal boli. The analysis of fecal boli deposited during the cue test revealed no main effects of stress, $F(1,70) = 1.62$, $p > .20$, metyrapone, $F(1,70) = 0.82$, $p > .36$, or AF-DX 116, $F(1,70) = 0.29$, $p > .59$. The Metyrapone x AF-DX 116 interaction was significant, $F(1,70) = 7.70$, $p < .01$. Post hoc analyses revealed that rats treated with metyrapone and AF-DX 116 (1.70 ± 0.40 boli) defecated less than rats treated with metyrapone (3.05 ± 0.40 boli) or AF-DX 116 alone (3.20 ± 0.40 boli) (p's < .05). The Stress x Metyrapone, $F(1,70) = 2.40$, $p > .12$, Stress x AF-DX 116, $F(1,70) = 0.28$, $p > .59$, and Stress x Metyrapone x AF-DX 116, $F(1,70) = 0.03$, $p > .86$, interactions were not significant.

Comparison of the control groups. Independent samples *t*-tests were used to compare the control groups' freezing and defecation during the context test. The groups did not differ in the amount of freezing they displayed during the context test, $t(18) =$

-0.14, $p > .89$. They also did not differ in the amount of fecal boli that was deposited in the apparatus during the test, $t(18) = 0.11$, $p > .91$.

A mixed-model ANOVA was employed to compare the control groups' freezing during the cue test, with cue (no tone, tone) serving as the within-subjects factor. This analysis revealed a main effect of cue, $F(1,18) = 47.55$, $p < .001$, indicating that the rats froze more when the tone was presented (69.93 ± 6.44 %) than when it was not presented (31.48 ± 6.19 %). However, the Cue x Group interaction was not significant, $F(1,18) = 2.42$, $p > .13$, and there was no main effect of group, $F(1,18) = 0.46$, $p > .50$. An independent samples *t*-test also indicated that the groups did not differ in terms of the amount of fecal boli deposited in the apparatus during the cue test, $t(18) = 0.00$, $p = 1.00$.

Final Day's Heart Rate and Blood Pressure

Heart rates (see Figure 25). The analysis of HRs from the final day of testing revealed a main effect of stress, $F(1,64) = 12.88$, $p < .01$, indicating that the stressed rats (386.13 ± 5.48 bpm) had lower HRs than the unstressed rats (413.50 ± 5.31 bpm). There was also a main effect of metyrapone, $F(1,64) = 11.21$, $p < .01$, indicating that rats treated with metyrapone (387.05 ± 5.15 bpm) had lower HRs than rats treated with vehicle (412.58 ± 5.62 bpm). Moreover, rats treated with AF-DX 116 (386.62 ± 5.29 bpm) had lower HRs than rats treated with vehicle (413.00 ± 5.50 bpm), $F(1,64) = 11.96$, $p < .01$. There was also a significant Metyrapone x AF-DX 116 interaction, $F(1,64) = 4.04$, $p < .05$. Specifically, rats that were treated with metyrapone (392.57 ± 7.19 bpm), AF-DX 116 (391.72 ± 7.57 bpm), or both drugs (381.52 ± 7.38 bpm) had lower HRs than rats that had been administered both vehicles (433.44 ± 8.32 bpm) (*p*'s < .05). The Stress

x Metyrapone, $F(1,64) = 3.06$, $p > .08$, Stress x AF-DX 116, $F(1,64) = 1.47$, $p > .23$, and Stress x Metyrapone x AF-DX 116, $F(1,64) = 1.37$, $p > .24$, interactions were not significant.

Planned comparisons indicated that the stressed rats treated with both vehicles (404.00 ± 4.16 bpm) exhibited lower HRs than the unstressed rats treated with both vehicles (462.88 ± 10.22 bpm), $t(13) = -5.06$, $p < .001$.

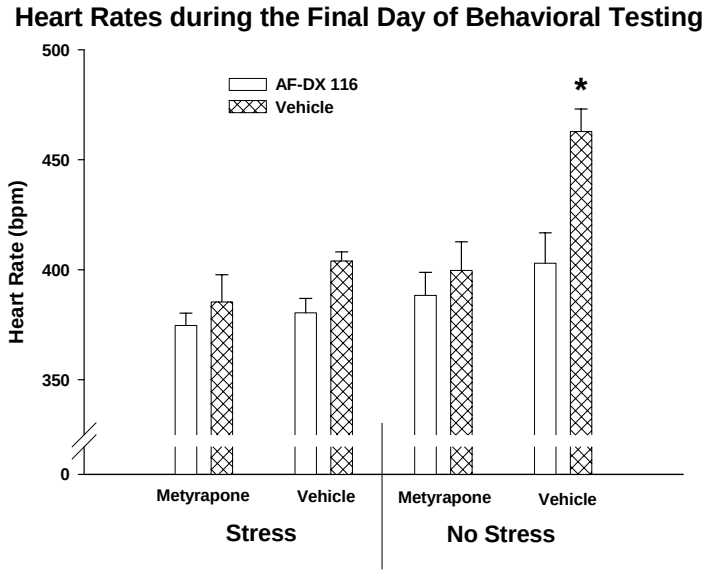

Figure 25. Heart rates (+ *SE*) during the final day of behavioral testing. Unstressed rats treated with both vehicles showed the greatest heart rates. * $p < .001$ compared to all other groups.

The stressed rats treated with metyrapone (385.40 ± 12.36 bpm), $t(16) = -4.50$, AF-DX 116 (380.44 ± 6.56 bpm), $t(15) = -6.45$, or both drugs (374.67 ± 5.62 bpm), $t(15) = -7.18$, displayed lesser HRs than the unstressed rats treated with both vehicles (p's < .01). The unstressed rats treated with metyrapone (399.73 ± 13.03 bpm), $t(16) = -3.54$, AF-DX 116 (403.00 ± 13.84 bpm), $t(15) = -3.29$, or both drugs

(388.38 ± 10.47 bpm), $t(16) = -4.79$, exhibited lesser HRs than the unstressed rats treated with both vehicles (p's < .01).

Systolic blood pressure (see Figure 26). There was a main effect of stress, $F(1,63) = 4.37$, $p < .05$, indicating that the stressed rats (135.60 ± 2.44 mm Hg) exhibited greater systolic BP than the unstressed rats (128.32 ± 2.48 mm Hg). There were no main effects of metyrapone, $F(1,63) = 0.12$, $p > .73$, or AF-DX 116, $F(1,63) = 1.03$, $p > .31$. There was a significant Stress x Metyrapone interaction, $F(1,63) = 9.57$, $p < .01$. Independent of being stressed (130.80 ± 3.33 mm Hg) or not (134.30 ± 3.24 mm Hg), rats that were treated with metyrapone tended to exhibit similar systolic BP. However, stressed rats that were treated with vehicle (140.39 ± 3.57 mm Hg) displayed greater systolic BP than unstressed rats that were treated with vehicle (122.35 ± 3.75 mm Hg) (p's < .05). The Stress x AF-DX 116, $F(1,63) = 0.33$, $p > .56$, Metyrapone x AF-DX 116, $F(1,63) = 0.81$, $p > .37$, and Stress x Metyrapone x AF-DX 116, $F(1,63) = 0.04$, $p > .85$, interactions were not significant.

A planned comparison indicated that the stressed rats treated with both vehicles (136.38 ± 2.57 mm Hg) exhibited greater systolic BP than the unstressed rats treated with both vehicles (119.69 ± 5.11 mm Hg), $t(12) = 2.92$, $p < .01$.

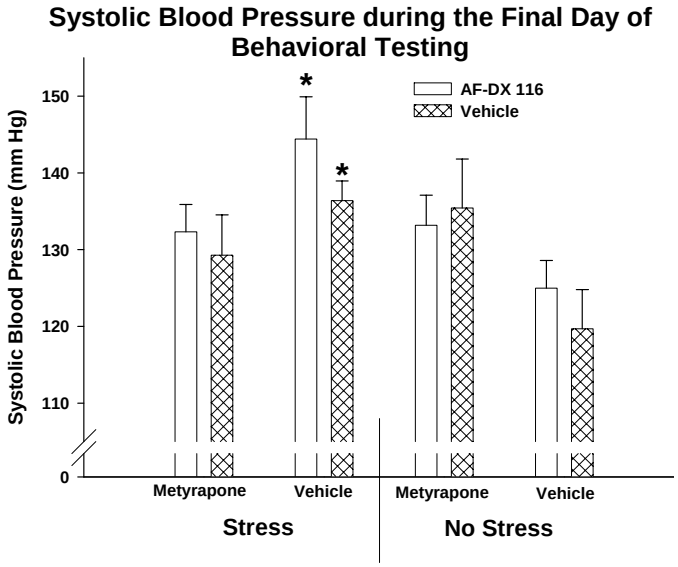

Figure 26. Systolic blood pressure (+ *SE*) during the final day of behavioral testing. Stressed rats, in general, exhibited elevated systolic blood pressure. However, this effect was most pronounced in the stressed rats treated with the vehicle for metyrapone. * $p < .05$ compared unstressed rats treated with both vehicles.

The stressed rats treated with AF-DX 116 (144.40 ± 5.51 mm Hg) displayed greater systolic BP than the unstressed rats treated with both vehicles, $t(15) = 3.14$, $p < .05$. The stressed rats treated with metyrapone, $t(15) = 1.26$, or both drugs, $t(14) = 2.10$, did not differ from the unstressed rats treated with both vehicles (p's > .05). The unstressed rats treated with metyrapone, $t(15) = 1.80$, AF-DX 116, $t(13) = 0.87$, or both drugs, $t(15) = 2.13$, did not differ from the unstressed rats treated with both vehicles (p's > .05).

Diastolic blood pressure (see Figure 27). There was a main effect of stress, $F(1,65) = 21.45$, $p < .001$, indicating that the stressed rats (95.51 ± 1.46 mm Hg) had greater diastolic BP than the unstressed rats (85.71 ± 1.53 mm Hg). There were no main

effects of metyrapone, $F(1,65) = 1.42$, $p > .23$, or AF-DX 116, $F(1,65) = 0.22$, $p > .64$. The Stress x Metyrapone, $F(1,65) = 1.91$, $p > .17$, Stress x AF-DX 116, $F(1,65) = 1.63$, $p > .20$, Metyrapone x AF-DX 116, $F(1,65) = 0.74$, $p > .39$, and Stress x Metyrapone x AF-DX 116, $F(1,65) = 0.04$, $p > .84$, interactions were not significant.

Planned comparisons indicated that the stressed rats treated with both vehicles (96.67 ± 3.82 mm Hg) exhibited greater diastolic BP than the unstressed rats treated with both vehicles (86.24 ± 2.80 bpm), $t(13) = 2.15$, $p = .05$.

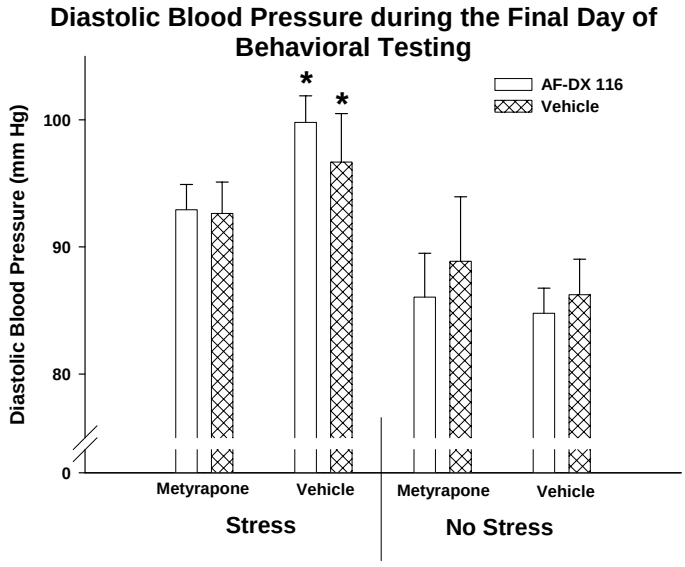

Figure 27. Diastolic blood pressure (+ *SE*) during the final day of behavioral testing. Stressed rats, in general, exhibited elevated diastolic blood pressure. However, this effect was most pronounced in the stressed rats treated with the vehicle for metyrapone.
* $p \leq .05$ compared to unstressed rats treated with both vehicles.

The stressed rats treated with AF-DX 116 (99.80 ± 2.09 mm Hg) displayed greater diastolic BP than the unstressed rats treated with both vehicles, $t(15) = 3.97$, $p < .05$. The stressed rats treated with metyrapone, $t(15) = 1.70$, or both drugs, $t(15) =$

2.01, did not differ from the unstressed rats treated with both vehicle (p's > .05). The unstressed rats treated with metyrapone, $t(15) = 0.40$, AF-DX 116, $t(14) = 0.44$, or both drugs, $t(15) = 0.04$, did not differ from the unstressed rats treated with both vehicles (p's > .05).

Correlation between Cardiovascular Activity and Anxiety

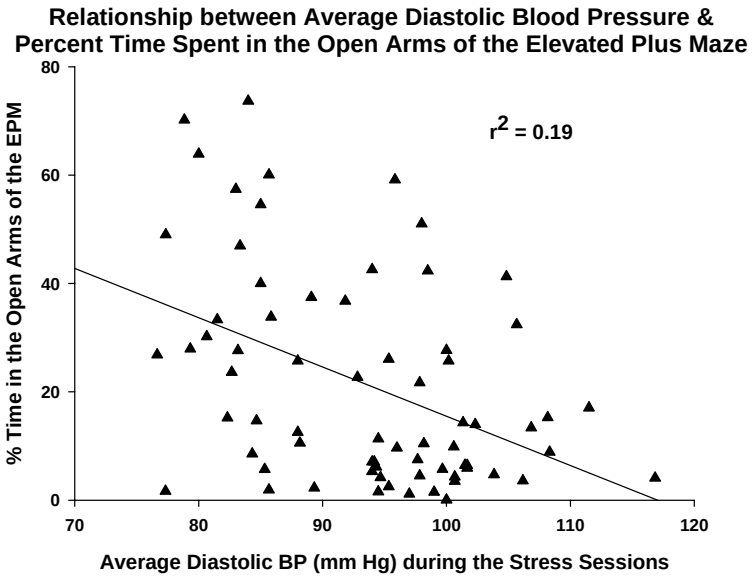

Figure 28. Relationship between average diastolic blood pressure during the stress sessions and percent time spent in the open arms of the elevated plus maze. Greater diastolic blood pressure during the stress sessions was associated with less time spent in the open arms of the elevated plus maze during behavioral testing.

Relationship between cardiovascular activity during the stress sessions and anxiety-like behavior on the elevated plus maze (see Figure 28). Since stress produced a robust increase in rats' diastolic BP during the stress sessions and on the last day of behavioral testing, an analysis was run to examine the correlation between rats' average diastolic BP during the stress sessions and their behavior on the EPM (specifically, the

percent time spent in the open arms). The analysis of this data revealed a significant negative relationship between these two variables, $r(70) = -0.43$, $p < .001$, indicating that as the rats' diastolic BP (during the stress sessions) increased, the amount of time that they spent in the open arms of the EPM (weeks later) decreased.

Correlation between Corticosterone Levels and Average Startle Response

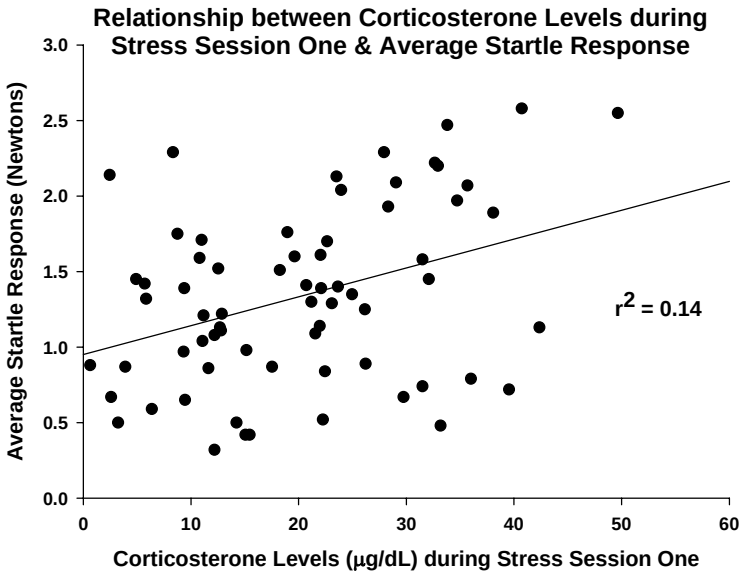

Figure 29. Relationship between corticosterone levels during stress session one and average startle response. Greater corticosterone levels during stress session one was associated with greater startle responses during behavioral testing.

Relationship between corticosterone levels during stress session one and average startle responses (see Figure 29). Since stress produced a robust increase in rats' CORT levels during the stress sessions, an analysis was run to examine the correlation between rats' CORT levels during stress session one and their average startle response (the effect was witnessed for each level of auditory stimulus, so rats' startle responses from the 90, 100, and 110 dB noise bursts were averaged to facilitate the discussion and graphical

representation of the data). The analysis of this data revealed a significant positive relationship between these two variables, $r(68)= 0.37$, $p < .01$, indicating that as the rats' CORT levels (during stress session one) increased, their average startle response (weeks later) also increased.

Final Day's Corticosterone Levels (see Figure 30)

The within-subjects analysis revealed a main effect of time, $F(2,54) = 86.76$, $p < .001$. Post hoc analyses indicated that the rats exhibited greater CORT levels 20 min after the onset of stress (22.82 ± 0.85 μg/dL) than they did at baseline (4.68 ± 0.69 μg/dL). These levels were lower 1 hr after the cessation of stress (12.54 ± 1.47 μg/dL), but they were still elevated relative to baseline levels (p's < .05). The Time x Metyrapone interaction was also significant, $F(2,54) = 5.28$, $p < .01$. While the metyrapone-treated rats showed a reduction in CORT levels 1 hr after the cessation of stress (Stress: 23.20 ± 1.17 μg/dL; Return-to-Baseline: 9.59 ± 2.03 μg/dL), the CORT levels in the vehicle-treated rats remained elevated (Stress: 22.44 ± 1.23 μg/dL; Return-to-Baseline: 15.49 ± 2.14 μg/dL) (p's < .05). The Time x Stress, $F(2,54) = 1.31$, $p > .27$, and Time x Stress x Metyrapone, $F(2,54) = 0.75$, $p > .47$, interactions were not significant.

The between-subjects analysis revealed no main effects of stress, $F(1,27) = 2.28$, $p > .14$, or metyrapone, $F(1,27) = 0.36$, $p > .55$. However, the Stress x Metyrapone interaction was significant, $F(1,27) = 6.40$, $p < .05$. Rats that were treated with vehicle exhibited comparable CORT levels, independent of whether they were stressed (13.05 ± 1.56 μg/dL) or not (14.47 ± 1.28 μg/dL). However, rats that were treated with

metyrapone exhibited greater CORT levels when they had been stressed (15.74 ± 1.36 μg/dL) than when they had not been stressed (10.12 ± 1.36 μg/dL) (*p*'s < .05).

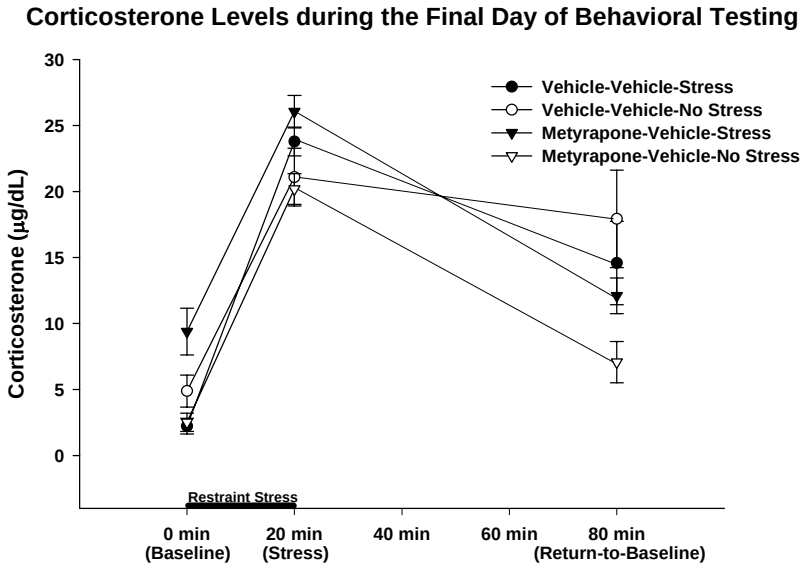

Figure 30. Corticosterone levels (± *SE*) during the final day of behavioral testing in Experiment 1. All rats showed a significant increase in corticosterone levels following restraint stress. However, only the metyrapone-treated rats showed a return-to-baseline of corticosterone levels 1 hr later. The corticosterone levels of those rats treated with vehicle remained elevated.

Weight (see Table 1)

The within-subjects analysis revealed a main effect of time, $F(2,138) = 2999.26$, $p < .001$, indicating that all of the rats gained a significant amount of weight over the course of the experiment. The Time x Stress, $F(2,138) = 2.32$, $p > .10$, Time x Metyrapone, $F(2,138) = 0.14$, $p > .77$, and Time x AF-DX 116, $F(2,138) = 1.20$, $p > .30$, interactions were not significant.

The Time x Stress x Metyrapone interaction was significant, $F(2,138) = 4.12$, $p < .05$. Post hoc analyses indicated that the unstressed, metyrapone-treated rats (57.90 ± 2.11 g) gained more weight from stress session one to stress session two than the stressed, metyrapone-treated rats (45.90 ± 2.11 g). However, the vehicle-treated rats gained a comparable amount of weight from stress session one to stress session two, regardless of whether they were stressed (50.14 ± 2.24 g) or not (51.19 ± 2.17 g). By the start of behavioral testing, these differences were not evident. However, they resurfaced by the final day of testing, indicating that testing may have had a greater impact on the stressed, metyrapone-treated rats than the unstressed, metyrapone-treated rats (p's < .05).

The Time x Stress x AF-DX 116 interaction was also significant, $F(2,138) = 7.18$, $p < .001$. According to post hoc analyses, rats that were stressed and treated with AF-DX 116 gained less weight than all other groups from stress session one to stress session two (46.90 ± 2.11 g), from stress session one to the last day of handling (130.70 ± 4.46 g), and from stress session one to the last day of testing (135.30 ± 4.50 g) (p's < .05).

The Time x Metyrapone x AF-DX 116 interaction was significant, $F(2,138) = 9.99$, $p < .001$. Post hoc analyses indicated that while there were no differences between the groups in terms of body weight gained from stress session one to stress session two, the metyrapone-treated rats that were also administered the vehicle for AF-DX 116 gained more weight than all of the other groups from stress session one to the last day of handling (148.20 ± 4.46 g) and to the last day of behavioral testing (153.75 ± 4.50 g) (p's < .05). The Time x Stress x Metyrapone x AF-DX 116 interaction was not significant, $F(2,138) = 0.76$, $p > .47$.

Table 1

Average Raw Weights for all Groups in Experiment 1

Group	Raw weights (g ± *SE*)			
	Stress session 1	Stress session 2	Last day of handling	Final day of testing
Stress				
Metyrapone				
AF-DX 116	275.90 (1.04)	323.10 (2.66)	401.30 (5.86)	401.10 (6.37)
Vehicle	278.00 (3.24)	322.60 (3.83)	424.20 (6.35)	423.50 (6.56)
Vehicle				
AF-DX 116	275.80 (2.39)	322.40 (3.65)	411.80 (5.61)	421.20 (6.36)
Vehicle	275.45 (3.11)	330.90 (6.05)	418.13 (10.60)	421.50 (10.00)
No Stress				
Metyrapone				
AF-DX 116	287.50 (3.36)	340.10 (5.64)	425.10 (10.44)	434.80 (10.42)
Vehicle	266.10 (2.31)	329.30 (3.08)	416.30 (6.89)	428.10 (6.57)
Vehicle				
AF-DX 116	282.90 (3.27)	333.50 (4.20)	426.40 (8.01)	428.40 (8.50)
Vehicle	292.78 (2.60)	344.56 (4.22)	419.33 (6.41)	429.33 (5.46)
Completely unstressed				
controls	300.25 (2.10)	376.60 (7.79)	470.00 (18.09)	478.85 (20.33)

The between-subjects analyses revealed no main effect of stress, $F(1,69) = 1.84$, $p > .18$, metyrapone, $F(1,69) = 0.17$, $p > .67$, or AF-DX 116, $F(1,69) = 2.61$, $p > .11$. The Stress x Metyrapone interaction was significant, $F(1,69) = 5.17$, $p < .05$. Post hoc analyses indicated that while the rats that were treated with vehicle gained a comparable amount of weight, regardless of being stressed (112.41 ± 3.75 g) or not (109.08 ± 3.64 g), the metyrapone-treated rats that were stressed (105.68 ± 3.54 g) gained less weight than the metyrapone-treated rats that were not stressed (118.82 ± 3.54 g) (p's < .05). The Stress x AF-DX 116, $F(1,69) = 1.02$, $p > .31$, Metyrapone x AF-DX 116, $F(1,69) = 3.63$, $p > .06$, and Stress x Metyrapone x AF-DX 116, $F(1,69) = 0.96$, $p > .33$, interactions were not significant.

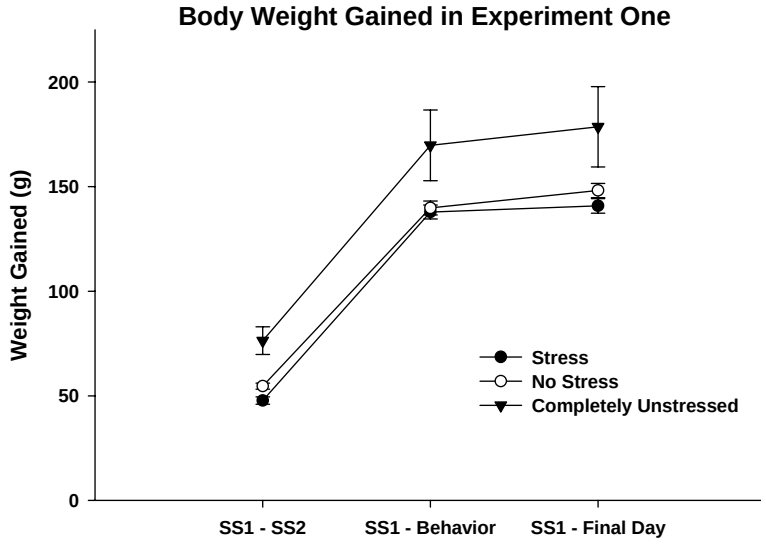

Figure 31. Body weight gained (± *SE*) throughout the course of Experiment 1. Both the experimental controls and the stressed rats gained less weight than the completely unstressed controls throughout the course of the experiment.

Comparison of the control groups. A mixed-model ANOVA was run on the weights that the rats in the control groups gained, with time serving as the within-subjects variable. The analysis revealed a significant effect of time, $F(2,28) = 114.23$, $p < .001$, indicating that all rats gained a significant amount of weight over the course of the experiment. The Time x Group interaction was not significant, $F(2,28) = 1.93$, $p > .16$. However, there was a main effect of group, $F(1,14) = 4.58$, $p = .05$, indicating that the completely unstressed controls (141.57 ± 11.37 g) gained a more weight than the experimental controls (101.83 ± 14.68 g).

Discussion

Major Findings and Significance

The primary finding of this study is that stress alone increased rats' cardiovascular and hormonal activity at the time of stress and led to heightened anxiety, an exaggerated startle response, and a sensitized blood pressure response to acute stress 3 weeks later. Although stress alone did not affect learning and memory in the water maze, the rats that had been administered metyrapone in addition to being stressed exhibited significant memory impairments on the 1-hr and 24-hr memory test trials. These findings suggest that a reduced amount of CORT at the time of stress may exacerbate its long-term effects on cognition. Despite the fact that metyrapone and AF-DX 116 blunted the stress-induced increases in CORT (Figures 9 and 10) and led to greater HRs and BP (Figures 3 through 8), respectively, there was little evidence to indicate that these agents exacerbated the effects of stress on rats' anxiety-like behavior. In fact, there was actually some indication that the drugs, particularly metyrapone, actually *blocked* the stress-induced increase in

startle. Although the present pharmacological manipulations were unsuccessful in exacerbating the stress-induced effects on anxiety, this study does provide evidence for a novel model of PTSD in rats. Specifically, it demonstrates that exposure to unpredictable and uncontrollable stress, in conjunction with a reminder experience, produces behavioral and physiological symptoms in rats that are comparable to those observed in humans with PTSD. In contrast to some animal models of PTSD, the present model used an ethologically relevant stressor, cat exposure, to produce the effects. Moreover, it consists of a sequence of events, including a traumatic reminder experience and daily mild stress, which closely resembles the daily life of a human subject with the disorder.

Stress-Induced Enhancements of Anxiety and Acoustic Startle Response

The finding that stress led to a significant reduction of open-arm exploration on the EPM is consistent with the findings of other animal models of PTSD (Adamec & Shallow, 1993; Cohen et al., 2006; Cohen et al., 2003). When rats are placed on the EPM, they face a decision: (1) to enter the open arms and explore novel places (which they naturally do in experimental settings) or (2) to stay in the closed arms and remain out of harm's way. Following stress in the present study, the rats appeared to choose the latter, inhibiting their natural tendency to explore novel places. However, there was evidence indicating that the stressed rats exhibited less activity (i.e., fewer ambulations) than the unstressed rats on the plus maze. To control for these findings, an ANCOVA was run on the open arms' data (i.e., percent time spent in the open arms) with ambulations serving as the covariate. The results revealed that the effects of stress on open-arm exploration remained significant after controlling for the rats' general activity. Thus, it would appear

that the effects of stress on open-arm exploration, at least in the present paradigm, are independent of its effects on general locomotor activity.

The finding that stressed rats tended to move less on the EPM is consistent with previous research. Many studies have shown that stress can lead to a general decrease in activity on the EPM and other behavioral apparatuses, such as the open field (Adamec et al., 1999; Adamec & Shallow, 1993; Bowman, Ferguson, & Luine, 2002; Katz, Roth, & Carroll, 1981; Ottenweller, Natelson, Pitman, & Drastal, 1989). In fact, when Adamec and Shallow (1993) controlled for the effects of predator stress on general activity, cat exposure no longer influenced open-arm exploration in the plus maze. Nevertheless, the finding that stress leads to a reduction in locomotor activity should not negate the conclusion that stressed rats are exceedingly anxious. For one, their avoidance of the open arms leaves them with less area to travel (i.e., just the closed arms). Thus, one would expect these rats to move less and not incessantly pace back and forth from one closed arm to the other. This argument is supported by the fact that the stressed rats in the present study traveled less distance per time in the closed arms than the unstressed rats. In general, rats' reluctance to enter the open arms can be related to the behavior often observed in PTSD patients. These individuals often face the choice of (a) going out into the world and running the risk of experiencing a panic attack or (b) remaining home and avoiding any threat of traumatic reminders. The avoidant behavior that is exhibited by individuals with PTSD often leads to agoraphobia, a disorder that is commonly diagnosed in individuals with PTSD (Maes, 2000). The findings of the current study relate nicely to these observations.

Although it is not clear if individuals with PTSD exhibit an exaggerated startle response, the stressed rats in the present study exhibited significantly greater startle during behavioral testing. However, their startle response appeared to be elevated only when they had received vehicle injections during the stress sessions. The rats that had been stressed and administered one or both drugs did not show this enhancement. It is possible that low CORT at the time of stress prevents long-lasting increases in startle. In support of this argument, there was a positive correlation between CORT levels at the time of stress session one and rats' startle responses during behavioral testing. That is to say, greater CORT levels during stress predicted greater startle responses weeks later. The finding of an enhanced startle response in the stressed rats is consistent with previous work showing that a single exposure to a predator can lead to enhanced startle one week later (Adamec, 1997). The present study extends this finding, suggesting that predator stress can have longer-lasting effects on this type of behavior.

Although the primary hypothesis of the present study was that a blunted CORT response at the time of stress would exacerbate its effects on behavior, it is possible that a blunted CORT response to stress actually ameliorates some of these effects. In support of this argument, Cohen and colleagues (Cohen, Benjamin, Kaplan, & Kotler, 2000b) found that the administration of ketoconazole, a steroid synthesis inhibitor, *prevented* the effects of stress on anxiety-like behavior in rats. In the present study, metyrapone appeared to block the stress-induced increase in startle, but it did not alleviate the effects of stress on open-arm exploration in the EPM. In addition, metyrapone actually exacerbated the effects of stress on learning and memory in the RAWM. Therefore, a reduced amount of

CORT at the time of trauma may differentially affect, rather than simply exacerbate or ameliorate, rats' long-term response to stress.

Heightened startle in PTSD patients has been linked with an elevated state of anxiety at the time of testing, thus raising the possibility that individuals with PTSD do not have an exaggerated *baseline* startle response, but rather a greater *fear-potentiated* startle response. It is also possible that PTSD patients do not exhibit heightened startle at baseline because they are already anxious and cannot manifest a larger response to the acoustic stimuli. This is related to work conducted by Cohen and colleagues (Cohen et al., 2000a; Cohen et al., 1998), who failed to find a significant increase in the autonomic activity of PTSD patients when they were exposed to a recollection of their trauma. The investigators reasoned that since the PTSD patients displayed autonomic dysregulation at rest, they could not manifest further increases in sympathetic tone in response to the traumatic reminders. The same argument could provide an alternative explanation for why not all of the stressed rats displayed an enhanced startle response in the present study. A heightened state of anxiety in these rats could have led to a greater overall state of arousal, making a larger response relative to this state of arousal unlikely.

Spatial Learning & Fear Memory

Stress alone did not have any long-term effects on learning and memory in the RAWM. However, the stressed rats that had been administered metyrapone during the stress sessions exhibited significant memory impairments on the 1-hr and 24-hr memory test trials. These findings suggest that a reduced amount of CORT at the time of trauma may have long-lasting effects on cognitive processing. Moreover, it is possible that

certain physiological responses at the time of trauma are responsible for different long-term effects of stress on behavior. While a reduction in CORT at the time of stress may be more predictive of long-term cognitive deficits, it may not lead to an exacerbation of the stress-induced effects on anxiety, given the lack of heightened startle in the same groups of rats. Regardless, this is the first study to suggest that metyrapone, in conjunction with intense stress, can have lasting consequences on spatial learning and memory in rats.

Although the stressed rats generally froze more than the unstressed rats on the contextual and cue fear memory tests, the reduction of freezing in the metyrapone-treated, unstressed rats was primarily responsible for this effect. The unstressed rats treated with both vehicles froze just as much as the stressed rats treated with both vehicles during both the context and cue tests. Thus, it would not appear that the stress during trauma had a long-term effect on rats' fear conditioning. Since all of the rats in the present study were stressed during the stress sessions when they were restrained for the measurement of HR/BP and the collection of blood samples, it is possible that the unstressed rats exhibited *enhanced* fear conditioning. Indeed, research has shown that even a single stressful experience can lead to the enhancement of contextual fear conditioning (Cordero, Venero, Kruyt, & Sandi, 2003). However, since the completely unstressed controls were not significantly different from the experimental controls, there is little evidence to support this claim. Another possibility is that the shock amplitude used in the present study was too high, especially since the rats were most likely stressed during the behavioral testing that occurred prior to fear conditioning training.

Long-Term Stress Effects on the Cardiovascular System

Of particular interest is the finding that stress led to a long-term sensitization of rats' cardiovascular response to acute stress. Rats that had previously been stressed exhibited a significant elevation of systolic BP and diastolic BP on the final day of behavioral testing. These elevations were apparent almost an entire month after the second stress session, indicating that intense stress, in conjunction with daily mild stress, can have lasting influences on how the cardiovascular system responds to future stressors.

Similar work (Bruijnzeel, Stam, Croiset, & Wiegant, 2001) found that a single stressful experience could lead to long-term sensitization of rats' cardiovascular stress response. Bruijnzeel et al. (2001) exposed rats to a 15-min session of scrambled electric footshocks (ten 6-sec footshocks @ 0.5 mA) and then left them undisturbed for 2 weeks. After this period of time, the experimenters exposed rats to a novel environment (Day 14) and a repeated shock prod procedure (Days 15 and 16). The repeated shock prod procedure consisted of placing an electrified prod into the rats' home cages on Day 15 and then reinserting an unelectrified prod into the home cages on Day 16 and observing their behavior. During Days 14-16, the investigators continuously recorded rats' cardiovascular response to the stressors. Baseline HR and BP did not differ between the pre-shocked and control rats on Days 14-16. However, rats that were shocked two weeks earlier exhibited significantly greater elevations in mean arterial BP than controls in response to the novel stressors. They did not demonstrate a greater HR response to the stressors than the control rats. These results coincide with the current findings in that the

stressed rats demonstrated a significant increase in BP, but not HR, when exposed to acute stress four weeks after the second stress session.

Although elevated systolic BP and diastolic BP have both been associated with an increased risk for the development of cardiovascular disease (Joint National Committee on the Detection, Evaluation, and Treatment of High Blood Pressure, 1997; Sesso et al., 2000), elevations of systolic BP appear to generate a greater risk for developing this type of ailment (Haider, Larson, Franklin, & Levy, 2003; Izzo, Levy, & Black, 2000). A considerable amount of research on humans has also indicated that a range of life stressors (e.g., job stress, anxiety disorders, personality characteristics – hostility for instance, lack of social support, depression) are strongly associated with an increased risk for developing cardiovascular disease (see Rozanski, Blumenthal, & Kaplan, 1999 for a review), and the present study provides evidence for the long-term effects of stress on cardiovascular reactivity in rats. The stressed rats in this study demonstrated a robust increase in BP during both stress sessions and during the final day of behavioral testing. Since individuals with PTSD display heightened baseline HR and BP and greater autonomic reactivity to stress, these findings further validate the use of the current paradigm as an animal model of PTSD. They also suggest that the current paradigm may be useful in examining the long-lasting effects of stress on the cardiovascular system.

Stressed Controls and the Effectiveness of Metyrapone and AF-DX 116

It is important to bear in mind that the unstressed rats in the present study were exposed to moderate amounts of stress during the stress sessions when they were restrained for HR/BP measurements and the collection of blood samples. Therefore, the

stressed and unstressed rats in this study were in all actuality exposed to traumatic and mild-to-moderate stress, respectively. Given these circumstances, it is not surprising that the stressed rats did not gain less weight, relative to the unstressed rats, throughout the course of the experiment. There is evidence to suggest that as little as 20 min of immobilization stress can reduce the amount of food that a rat ingests for several days thereafter (Valles, Marti, Garcia, & Armario, 2000). In addition, the completely unstressed controls gained more weight throughout the study than the experimental unstressed controls, providing further support for this argument.

Many investigations involving chronic stress paradigms have reported a smaller amount of weight gain in the stressed rats, relative to controls (Karst & Joels, 2003; Magarinos & McEwen, 1995; Park et al., 2001; Sandi, Merino, Cordero, Touyarot, & Venero, 2001). However, a majority of studies that have used a small number of stress exposures (Servatius et al., 1994) or even just a single stress exposure (Adamec et al., 1999; Adamec & Shallow, 1993; Blundell et al., 2005; Cohen et al., 2003) to model PTSD in rodents have not reported, or have reported a quick recovery in, such differences. In addition, Gerges et al. (2004) exposed rats to unstable housing conditions (similar to those employed in the present study) for 4-6 weeks and found no weight differences between the stressed and unstressed rats at the end of the study. This finding provides support for the weight effects in the current study – that is, it is likely that the unstable housing conditions were not stressful enough to sustain the smaller weight gain in the traumatized rats, relative to those rats that were still mildly stressed during the stress sessions.

Based on the results of this study, the ability of metyrapone and/or AF-DX 116 to exacerbate rats' long-term response to stress should not be dismissed. Again, the unstressed rats were still moderately stressed during the stress sessions. This piece of information may help explain why some of the unstressed rats that were treated with pharmacological agents appeared to exhibit greater levels of anxiety on the EPM than the unstressed rats treated with both vehicles. It is possible that these agents could intensify the lasting effects of a shorter and milder stressor, one that does not produce long-lasting effects in rats that have been stressed but not administered the drugs, on rats' behavior.

Why the Pharmacological Manipulations were, for the Most Part, Ineffective

There are several reasons why metyrapone and AF-DX 116 could have been ineffective in exacerbating the effects of stress on rats' behavior. For one, the stress procedure alone in this study led to a tremendous increase in rats' anxiety-like behaviors. On the EPM, rats that had been stressed and treated with both vehicles spent <10% of the trial in the open arms. With this low of a baseline in the stress condition, it is not surprising that the pharmacological agents could not exacerbate the effect. The floor effect created by the stressed rats treated with both vehicles rendered a greater enhancement of anxiety-like behavior on the EPM very unlikely.

The primary reason for using metyrapone in the present study was to prevent the increase in CORT levels in the stressed rats. However, as indicated above, metyrapone only *blunted* the CORT levels in stressed rats, as these levels were still elevated relative to unstressed controls. It is possible that a greater inhibition of the stress-induced increase in CORT is necessary to intensify the effects that stress has on rats' long-term behavior.

Thus, the use of a higher dose of metyrapone, or perhaps even a different pharmacological agent such as dexamethasone (which leads to greater suppression of CORT levels), would provide a better model of the low CORT levels that have been observed in traumatized individuals who later developed PTSD. Metyrapone also led to an unexpected *increase* in CORT levels in the unstressed rats. There is evidence to suggest that metyrapone actually delays the return of CORT to baseline levels (Rotllant & Armario, 2005). Thus, it is likely that the unstressed rats treated with metyrapone were stressed when they were brought to the lab (before they were injected with metyrapone), and the drug subsequently delayed these rats' CORT levels from returning to an absolute baseline level. Transportation to the lab and the injection procedure are both stressful events to the rats, and since these rats had been acclimated to the HR/BP machine the day before the first stress session, it is also possible that the lab reactivated this memory, leading to an even greater stress response in the rats.

Another reason why metyrapone may not have provided greater effects of stress on rats' long-term behavior is because of the alternative effects that it has on rats' physiology. For instance, this agent blunts the stress-induced increase in CORT by inhibiting the final step of CORT synthesis – that is, the conversion of 11-deoxycorticosterone to corticosterone. In doing so, metyrapone prevents the negative feedback of CORT onto areas of the brain (e.g., hippocampus, hypothalamus, pituitary) that house GRs and regulate production of the hormone. Without this negative feedback, the hypothalamus is not informed of the CORT status in the body and therefore keeps transmitting CRH to the pituitary, which releases ACTH, and so on down the cycle until

11-deoxycorticosterone is produced more and more. Ultimately, this means that metyrapone blocks the production of CORT but leads to an increased release of the precursors for the hormone's synthesis, (CRH, ACTH, and 11-deoxycorticosterone) at the same time. These are not the kind of physiological conditions that have been observed in traumatized humans shortly after enduring a traumatic experience. Thus, the release of these additional neuromodulators during the stress sessions could have very well influenced the long-term effects of stress on rats' behavior.

In addition to these claims, there is evidence that metyrapone itself can act as a pharmacological stressor and induce long-term dysregulation of the HPA axis. Rotllant, Ons, Carrasco, & Armario (2002) examined the effects of metyrapone (50, 100, and 200 mg/kg, s.c.) on baseline ACTH levels, plasma glucose levels, and fos-like immunoreactivity (FLI) throughout the brain in rats. The higher doses of metyrapone (100 and 200 mg/kg) led to significant elevations of ACTH and plasma glucose. While only the higher doses of metyrapone (100 and 200 mg/kg) led to FLI in brain regions such as the hypothalamic paraventricular nucleus, bed nucleus of the stria terminalis, and the lateral septum, even the lower dose of metyrapone (50 mg/kg) was capable of inducing significant FLI in the central amygdala (ACe) and paraventricular thalamic nucleus. The expression of c-fos in the ACe has been linked with stressful experiences and the induction of defense behaviors in rats (Beckett, Duxon, Aspley, & Marsden, 1997; Moller, Bing, & Heilig, 1994), suggesting that even at this dose, metyrapone may potentiate the stress response in rodents. Further work by Rotllant and colleagues (Rotllant & Armario, 2005) also demonstrated that a single dose of metyrapone

(200 mg/kg, s.c.) could induce long-term dysregulation of the HPA axis. Specifically, rats that were treated with this dose of metyrapone exhibited increased basal levels of CRH mRNA in the hypothalamic paraventricular nucleus and a reduction of GR mRNA in the dentate gyrus and CA1 region of the hippocampus 8 days after the drug's administration. These rats also displayed an exaggerated hormonal response to immobilization stress at this time point. Therefore, it would appear that just a single injection of metyrapone could have lasting effects on the HPA axis and the endocrinological response to stress.

In the present study, there was also evidence to suggest that metyrapone may reduce baseline HR and BP in rats and perhaps even blunt the stress-induced increase in cardiovascular activity. To my knowledge, this is the first evidence of a metyrapone-induced reduction of HR/BP in rats. Nevertheless, van den Buuse and colleagues (van den Buuse, van Acker, Fluttert, & de Kloet, 2002) found that adrenalectomized rats exhibited a reduced HR/BP response to novelty stress and that administering CORT could reverse these effects. There has also been some work in humans indicating that metyrapone prevents stress-induced increases in HR and BP. Broadley et al. (2005) found that individuals who received 1500 mg of metyrapone prior to mental stress did not display significant increases in HR and BP, as exhibited by placebo-treated controls. Preventing the stress-induced increase in cardiovascular activity would be counterproductive for the purposes of the present study, which provides another reason why metyrapone may not be the best pharmacological agent to model the low CORT levels during stress that have been observed in individuals who eventually develop PTSD.

I had also hypothesized that the administration of AF-DX 116 would lead to an exacerbation of the stress-induced increase in rats' HR and BP. However, despite the main effects of AF-DX 116 for HR and BP, the drug did not produce a greater increase in HR and BP than that which was produced by the stress alone. This finding may explain why AF-DX 116 did not exacerbate rats' response to stress.

Limitations of the Present Study

There were some limitations to the present study that must be considered. First, as mentioned above, the control rats in this experiment were not completely unstressed, as I would have hoped. However, given that HR/BP and CORT levels needed to be obtained from the controls to compare with the stressed rats, this situation seemed unavoidable. Perhaps separate groups of rats that did not undergo behavioral testing and were simply used for means of obtaining HR/BP and CORT levels could have been run, but this would have made it impossible to correlate the rats' physiological measures to their behavior weeks later. Moreover, if the behaviorally-tested, unstressed rats were not exposed to the HR/BP and CORT measurements, their experiences during the experiment would have been even more different from the stressed rats, making it difficult to infer the cause of any significant effects.

It is also possible that the rats in the present study were exposed to an excessive amount of behavioral testing. This could have led to one behavioral task having an influence on subsequent tasks. Theoretically, a valid animal model of PTSD should be able to show that stress has an effect on several measures of behavior in the same rats. Certainly, this seems to be the case in humans with the disorder, and my primary goal is

to create a model that can be applied to the human species. One could potentially run separate groups of rats for each behavioral test; however, this is simply an inefficient way to test the present hypotheses and, quite frankly, would take a considerable amount of time and money to accomplish. Nevertheless, when interpreting the results of the present study, especially those from the tasks that took place near the end of behavioral testing, it is important to keep these caveats in mind.

Chapter Three: Experiment Two

Does Stress Produce Dynamic Brain Changes that Increase Rats' Long-Term Sensitivity to Yohimbine?

The second experiment was designed to examine the effects of stress on rats' long-term sensitivity to yohimbine, an α_2 adrenergic receptor antagonist. This drug enhances neuronal firing in the LC, increases levels of NE, and tends to induce panic attacks in PTSD patients. Rats in the second experiment were again subjected to two stress sessions. However, given that HR/BP and CORT data were collected during the stress sessions in the first experiment, these assessments were avoided in the second experiment to prevent the control rats from being unnecessarily stressed. The behavioral testing paradigm was also slightly altered. Three weeks after the second stress session, all rats' sensitivity to yohimbine was examined by injecting them with yohimbine or vehicle prior to tests of general locomotor activity, anxiety, and startle. Since the stress alone produced the most robust effects in Experiment One, the only groups to be compared in Experiment Two were the stress and no stress groups.

Methods

Rats

Adult male Sprague-Dawley rats (225-250 g upon delivery) obtained from Charles River laboratories were used for the present experiment. The rats were housed two to a cage (standard Plexiglas – 46 x 25 x 21 cm), maintained on a 12-hr light-dark

cycle (lights on at 7:00 a.m.), and had access to food and water ad libitum. Upon arrival, all rats were afforded a habituation period of one week to acclimate to the housing room and cage changes before any experimental manipulations took place. All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Florida.

Design

The present study employed a 2 x 2 factorial design. The two manipulated factors were stress (stress, no stress) and yohimbine (yohimbine, vehicle). The sample sizes for each cell of the factorial are listed in Table 2.

Table 2

Samples Sizes for all Groups in Experiment 2

Condition	Sample size
Stress	
Yohimbine (1 mg/kg)	10
Vehicle	9
No Stress	
Yohimbine (1 mg/kg)	12
Vehicle	8

Stress Manipulations

After the one-week acclimation period, all rats were transported to the lab. Each rat was tail-marked with a permanent black marker and weighed. Then, the rats were

randomly assigned to the "stress" or "no stress" group. Rats in the "stress" group were restrained in plastic DecapiCones and placed in a perforated wedge-shaped Plexiglas enclosure. After 15 min, the stressed rats, restrained in the plastic DecapiCones and resting in the perforated, wedge-shaped Plexiglas enclosure, were placed in a metal cage with an adult female cat for 45 min in a room located adjacent to the rat housing rooms. Canned cat food was placed on top of the Plexiglas enclosure to direct cat activity towards the rats. After 45 min had elapsed, the rats were returned to the laboratory and then transported back to the housing room. After being weighed, the unstressed rats spent 1 hr (yoked to the stress procedure for the stressed rats) in their home cages in the laboratory, after which they were also returned to the housing room.

Stress Sessions

All of the manipulations during the first stress session were performed during the rats' light cycle, between 0700 and 1500 hrs. As in Experiment One, all rats that were stressed in the initial session were exposed to a second stress session 10 days later. The second stress session took place during the rats' dark cycle. During the second stress session, all rats were transported to the laboratory. The same manipulations that were performed prior to the first stress session were performed again. All manipulations during the second stress session took place between 1900 and 0200 hrs.

Randomized Housing

After the initial stress session, the stressed rats were exposed to unstable housing conditions until the commencement of behavioral testing. All stressed rats were still housed two per cage, but every day, the cage mate of each rat was changed. The

randomization of the stressed rats occurred within groups, so each rat was exposed to every other rat within its own group between 3 and 4 times prior to behavioral testing. No rat had the same cage mate on two consecutive days, and the randomization manipulations always took place between 0800 and 1200 hrs.

Handling and Weight

Before behavioral testing began, all rats were handled for three consecutive days, as per the methods in Experiment One. All rats were weighed prior to the first stress session, prior to the second stress session and on the last day of handling.

Behavioral Testing

Three weeks after the second stress session, all rats were transported to the laboratory and received i.p. injections of yohimbine (1 mg/kg) or vehicle. These doses of yohimbine were chosen because pilot work in our lab revealed that they produced a threshold level of anxiety-related behaviors in control animals. The drug was dissolved in distilled H_2O, brought to the appropriate volume with 0.9% saline, and then administered at a volume of 1 ml/kg. All behavioral testing took place during the light cycle between 0700 and 1500 hrs.

Behavioral Apparatus

Open field (see Figure 32). Thirty minutes after receiving injections of yohimbine or vehicle, all rats underwent open field testing, a test of general locomotor activity. Rats were placed in a large, translucent, plastic box (Hamilton-Kinder, San Diego, CA – 40 x 47 x 70 cm) with an open top in a light- and sound-attenuated room for 10 min. Rat's behavior was monitored by infrared photobeams connected to a computer program

(Motor Monitor) that analyzed the behavior. The program allowed for assessment of the rats' total distance traveled in each area of the open field (center and perimeter), total time spent in each area of the open field, rearing, ambulations, and entries into each area of the open field.

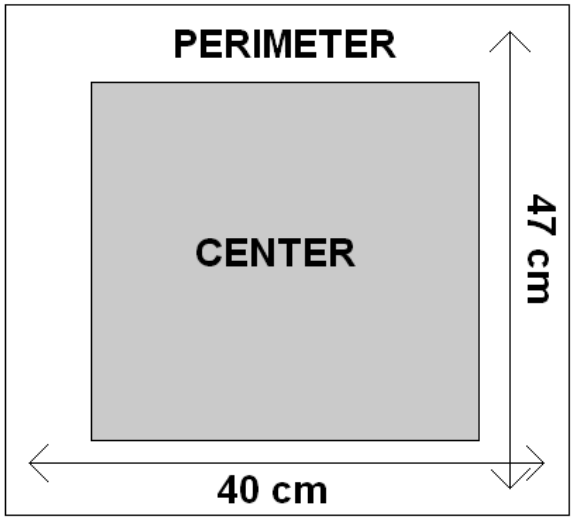

Figure 32. Schematic diagram of the open field apparatus.

Elevated plus maze (EPM). After all rats had been tested in the open field (approximately 45-60 min later), they were subjected to the EPM assessment. Testing conformed to the protocol used in Experiment One.

Startle response. After all rats have been tested on the EPM (approximately 45-60 min later), they were tested for their startle response. The rats were tested in same fashion as in Experiment One.

Blood samples. Twenty-four hrs after behavioral testing, all rats were transported to the laboratory and received i.p. injections of yohimbine (1 mg/kg) or vehicle. Thirty minutes later, three blood samples were collected from the rats at three separate time

points (0, 20, and 80 min). These three samples represented baseline, stress, and return-to-baseline samples, respectively, and the same procedures that were employed on the final day of Experiment One were employed here (with the exception of recording rats' HR/BP). The procedure was employed in Experiment Two to assess the rats' endocrinological response to yohimbine.

Statistical Analysis

Some data were analyzed through use of between-subjects analyses of variance (ANOVAs); mixed-model ANOVAs were employed when repeated measures variables were a part of the behavioral assessment being examined. In other cases, the behaviors were expressed as a percent change from the vehicle-injected animals to emphasize the stress-yohimbine interactions. Since it was hypothesized that yohimbine would produce an exaggerated behavioral response in the stressed rats, planned comparisons were conducted between the stressed, yohimbine-injected rats and the unstressed, yohimbine-injected rats in these instances.

Alpha was set at .05 for all analyses, and post-hoc comparisons were made through use of Bonferroni-corrected *t*-tests. Data points that were greater than three standard deviation units beyond the exclusive mean were considered outliers and removed from data analysis. Less than 1% of the data were outliers. All data are expressed as means ± SEM.

Open field. In all cases, the data were expressed as a percent change from the vehicle-injected animals to emphasize the stress-yohimbine interactions. Planned

comparisons were conducted between the stressed, yohimbine-injected rats and the unstressed, yohimbine injected rats.

Elevated plus maze (EPM). Some of the dependent measures acquired from the EPM were subjected to two-way ANOVAs, with Stress and Yohimbine serving as the between-subjects factors. In other cases, the behaviors were expressed as a percent change from the vehicle-injected animals to emphasize the stress-yohimbine interactions. In these instances, planned comparisons were conducted between the stressed, yohimbine-injected rats and the unstressed, yohimbine injected rats.

Startle response. For each rat, there were eight startle responses at each of three auditory intensities. These eight responses were averaged to create one data point per intensity per rat. For the reasons emphasized above, the data were analyzed using three separate two-way ANOVAs, with Stress and Yohimbine serving as the between-subjects factors at each auditory stimulus intensity.

Corticosterone. A mixed-model ANOVA was employed to analyze the CORT samples collected 24 hrs after behavioral testing. Stress and Yohimbine (vehicle, 1 mg/kg) served as the between-subjects factors, and time points (baseline, stress, return-to-baseline) served as the within-subjects factor.

Weight. Although each group of rats was ordered for the same weight range, an independent-samples *t*-test indicated that there were weight differences between the stressed and unstressed rats, $t(37) = 7.60$, $p < .001$. Nevertheless, both groups reached adult weight by the time of stress session one (see Table 3). In order to examine group differences in weight over the course of the experiment, the amount of weight that rats

gained between stress session one and each subsequent time point (i.e., stress session two and last day of handling) was analyzed using a mixed-model ANOVA. Since rats were injected with yohimbine on the day of behavioral testing, there was only one between-subjects factor for this analysis, which was stress. Time was the within-subjects factor (first stress session to second stress session, second stress session to last day of handling).

Results

Open Field

Ambulations (see Figure 33). An independent samples *t*-test indicated that there was no difference between the stressed and unstressed rats treated with vehicle in terms of ambulations in the open field, $t(18) = 0.40$, $p > .69$.

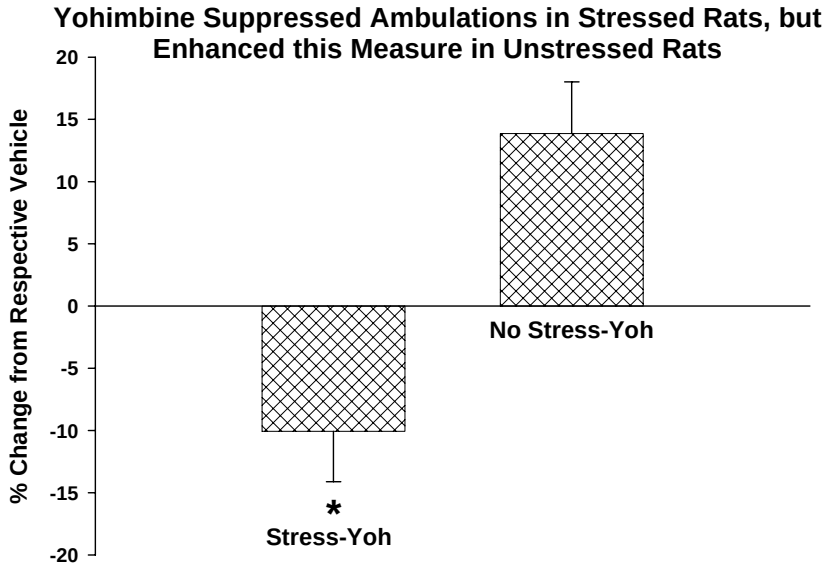

Figure 33. Percent change of ambulations (+ *SE*) in the open field in yohimbine-treated rats. Yohimbine suppressed the number of ambulations made by stressed rats, but enhanced this measure in the unstressed rats. * $p < .001$ compared to the unstressed rats treated with yohimbine.

The number of ambulations for all yohimbine-treated rats was converted into a percent change from vehicle score. A planned comparison was conducted on this data to compare the stressed, yohimbine-treated rats with the unstressed, yohimbine-treated rats. While yohimbine decreased the number of ambulations that stressed rats (-10.06 ± 4.06%) made in the open field, it led to an increase of this measure in the unstressed rats (13.86 ± 4.16%), $t(19) = -4.10$, $p < .001$.

Rearing (see Figure 34). An independent samples *t*-test indicated that there was no difference between the stressed and unstressed rats treated with vehicle in terms of rearing in the open field, $t(15) = 0.51$, $p > .62$.

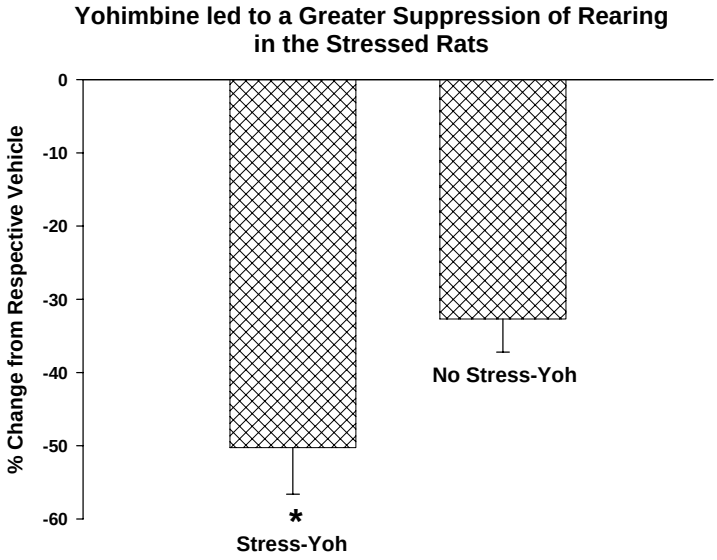

Figure 34. Percent change of rearing episodes (+ *SE*) in the open field in yohimbine-treated rats. Yohimbine led to a greater suppression of rearing in the stressed rats. * $p < .05$ compared to the unstressed rats treated with yohimbine.

The number of rearings for all yohimbine-treated rats was converted into a percent change from vehicle score. A planned comparison was conducted on this data to

compare the stressed, yohimbine-treated rats with the unstressed, yohimbine-treated rats. The stressed rats treated with yohimbine (-50.25 ± 6.37%) displayed a greater suppression of rearing in the open field than the unstressed rats treated with yohimbine (-32.70 ± 4.53%), $t(20) = -2.30$, $p < .05$.

Time spent in the perimeter (see Figure 35). An independent samples *t*-test indicated that there was no difference between the stressed and unstressed rats treated with vehicle in terms of the time that they spent in the perimeter of the open field, $t(15) = 1.38$, $p > .18$.

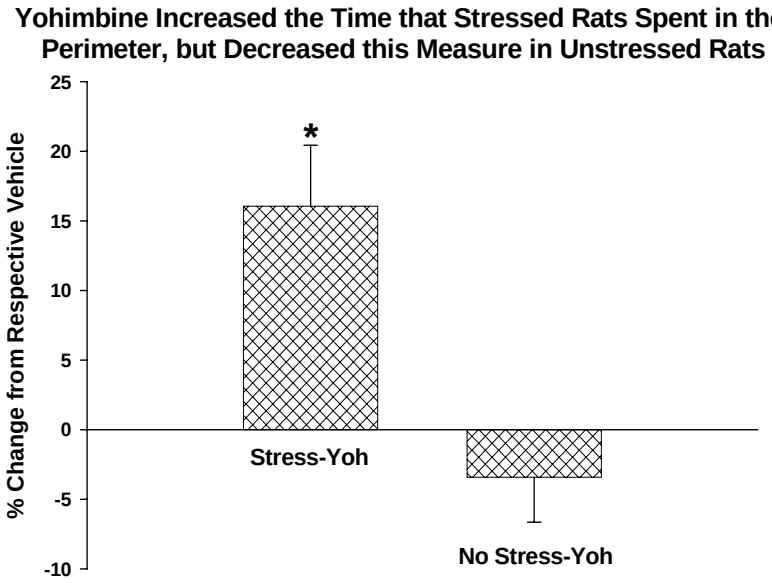

Figure 35. Percent change in time spent in the perimeter (+ *SE*) of the open field in yohimbine-treated rats. Yohimbine increased the amount of time that the stressed rats spent in the perimeter of the open field, but decreased this measure in the unstressed rats. * $p < .01$ compared to the unstressed rats treated with yohimbine.

The time spent in the perimeter for all yohimbine-treated rats was converted into a percent change from vehicle score. A planned comparison was conducted on this data to

compare the stressed, yohimbine-treated rats with the unstressed, yohimbine-treated rats. While yohimbine increased the amount of time that the stressed rats (16.06 ± 4.38%) spent in the perimeter of the open field, it led to a decrease of this measure in the unstressed rats (-3.42 ± 3.23%), $t(20) = 3.65$, $p < .01$.

Time spent in the center (see Figure 36). An independent samples *t*-test indicated that there was no difference between the stressed and unstressed rats treated with vehicle in terms of the time that they spent in the center of the open field, $t(15) = 1.39$, $p > .18$.

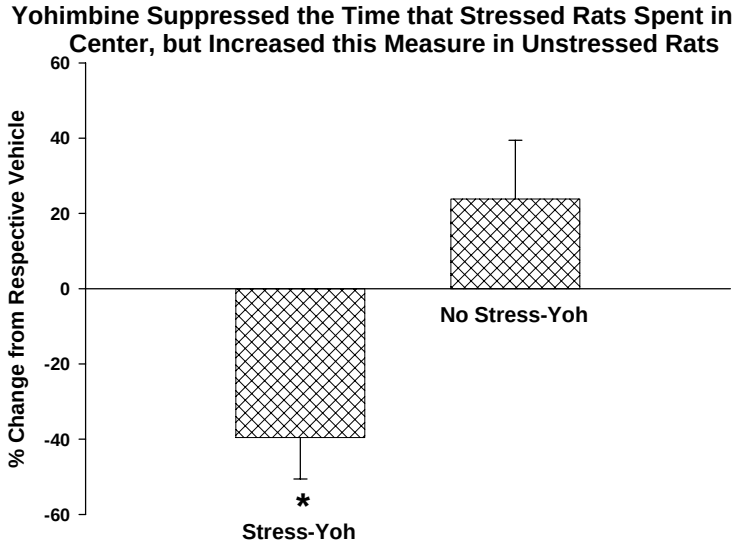

Figure 36. Percent change in time spent in the center (+ *SE*) of the open field in yohimbine-treated rats. Yohimbine suppressed the amount of time that the stressed rats spent in the center of the open field, but increased this measure in the unstressed rats. * $p < .01$ compared to the unstressed rats treated with yohimbine.

The time spent in the center for all yohimbine-treated rats was converted into a percent change from vehicle score. A planned comparison was conducted on this data to compare the stressed, yohimbine-treated rats with the unstressed, yohimbine-treated rats.

While yohimbine decreased the amount of time that the stressed rats (-39.54 ± 11.06%) spent in the center of the open field, it led to an increase of this measure in the unstressed rats (23.81 ± 15.64%), $t(19) = -3.25$, $p < .01$.

Distance in the open field (see Figure 37). An independent samples *t*-test indicated that there was no difference between the stressed and unstressed rats treated with vehicle in terms of the distance traveled in the open field, $t(13) = 1.43$, $p > .17$.

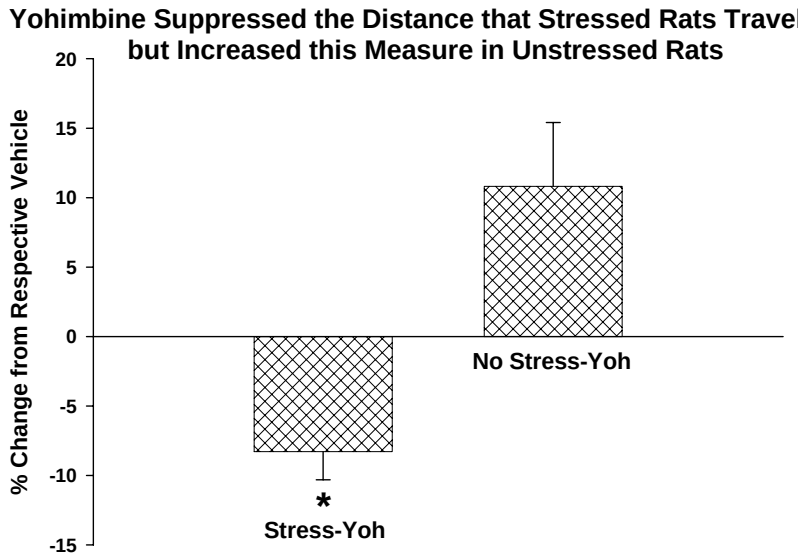

Figure 37. Percent change in distance traveled (+ *SE*) in the open field in yohimbine-treated rats. Yohimbine suppressed the distance that the stressed rats traveled in the open field, but increased this measure in the unstressed rats. * $p < .01$ compared to the unstressed rats treated with yohimbine.

The distance traveled in the open field for all yohimbine-treated rats was converted into a percent change from vehicle score. A planned comparison was conducted on this data to compare the stressed, yohimbine-treated rats with the unstressed, yohimbine-treated rats. While yohimbine decreased the distance traveled by

the stressed rats (-8.28 ± 2.03 %), it increased the distance traveled by the unstressed rats (10.82 ± 4.59 %), $t(18) = -3.53$, $p < .01$.

Fecal boli. The analysis of fecal boli deposited in the open field revealed no main effect of stress, $F(1,34) = 0.58$, $p > .45$, or yohimbine, $F(1,34) = 1.82$, $p > .18$, and the Stress x Yohimbine interaction was not significant, $F(1,34) = 0.43$, $p > .51$.

Elevated Plus Maze

Ambulations (see Figure 38). An independent samples *t*-test indicated that the stressed rats treated with vehicle (307.88 ± 22.08 ambulations) made fewer ambulations on the EPM than the unstressed rats treated with vehicle (425.50 ± 31.45 ambulations), $t(14) = 3.06$, $p < .01$.

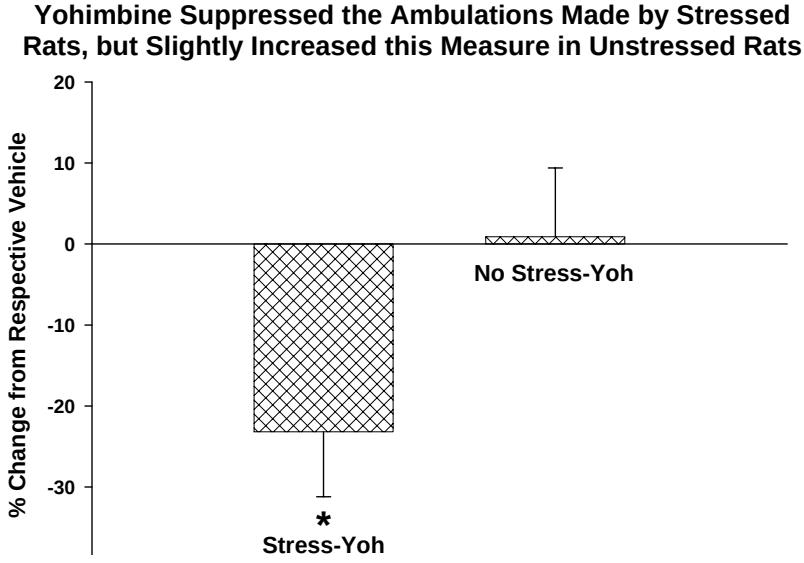

Figure 38. Percent change in ambulations (+ *SE*) on the elevated plus maze in yohimbine-treated rats. Yohimbine suppressed the number of ambulations made by the stressed rats, but slightly increased this measure in the unstressed rats. * $p = .05$ compared to the unstressed rats treated with yohimbine.

The number of ambulations for all yohimbine-treated rats was converted into a percent change from vehicle score. A planned comparison was conducted on this data to compare the stressed, yohimbine-treated rats with the unstressed, yohimbine-treated rats. While yohimbine decreased the number of ambulations made by the stressed rats (-23.18 ± 8.01 %), it increased the number of ambulations made by the unstressed rats (0.90 ± 8.49 %), $t(20) = -2.03$, $p = .05$.

Percent time spent in the open arms (see Figure 39). Time spent in the open arms of the EPM was converted into a percent of total time score. The analysis of these scores revealed a main effect of stress, $F(1,33) = 52.98$, $p < .001$, indicating that the stressed rats (2.94 ± 3.71 %) spent less percent time in the open arms than the unstressed rats (39.99 ± 3.49 %). There was also no main effect of yohimbine, $F(1,33) = 2.12$, $p > .15$, and the Stress x Yohimbine interaction was not significant, $F(1,33) = 1.82$, $p > .18$.

Percent time spent in the open arms, controlling for ambulations. Since the stressed rats treated with vehicle made fewer ambulations than the unstressed rats treated with vehicle, one could argue that the stressed rats spent less time in the open arms because they moved less. Therefore, a two-way ANCOVA was used to examine group differences in the percent of time spent in the open arms, with ambulations on the EPM serving as the covariate. The analysis revealed a main effect of stress, $F(1,33) = 12.92$, $p < .001$. There was no main effect of yohimbine, $F(1,33) = 3.91$, $p > .05$, and the Stress x Yohimbine interaction was not significant, $F(1,33) = 0.49$, $p > .49$.

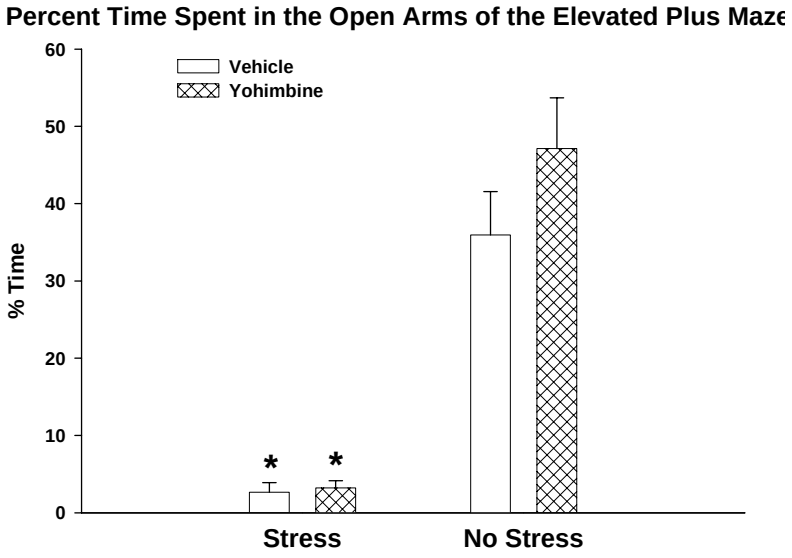

Figure 39. Percent time spent in the open arms (+ *SE*) of the elevated plus maze in Experiment 2. Stressed rats spent less time in the open arms than the unstressed rats. * $p < .001$ compared to respective unstressed groups.

Percent time spent in the closed arms (see Figure 40). Time spent in the closed arms of the EPM was converted into a percent of total time score. The analysis of these scores revealed a main effect of stress, $F(1,33) = 69.76$, $p < .001$, indicating that the stressed rats (93.22 ± 3.84 %) spent more percent time in the closed arms than the unstressed rats (49.23 ± 3.61 %). There was no main effect of yohimbine, $F(1,33) = 1.01$, $p > .32$, and the Stress x Yohimbine interaction was not significant, $F(1,33) = 1.17$, $p > .28$.

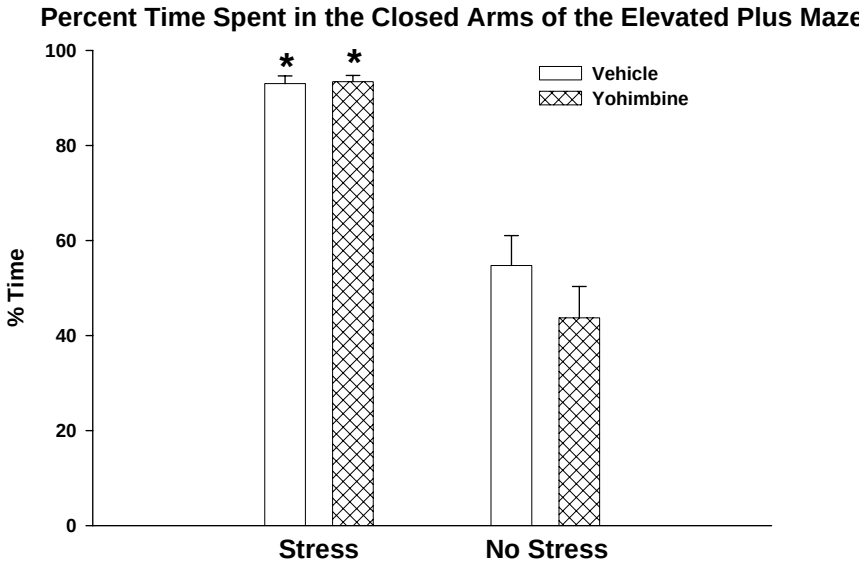

Figure 40. Percent time spent in the closed arms (+ *SE*) of the elevated plus maze in Experiment 2. Stressed rats spent more time in the closed arms than the unstressed rats. * $p < .001$ compared to respective unstressed groups.

Movement per unit time in the closed arms (see Figure 41). The distance that each rat traveled in the closed arms was divided by the amount of time it spent in the closed arms to produce a distance/time score. This score represented the distance that each rat traveled in the closed arms per the amount of time that it spent in the closed arms. The analysis of this data revealed a main effect of stress, $F(1,32) = 37.97$, $p < .001$, indicating that the stressed rats (7.46 ± 0.57 cm/sec) traveled less distance/time in the closed arms than the unstressed rats (12.38 ± 0.56 cm/sec). There was no main effect of yohimbine, $F(1,32) = 3.03$, $p > .09$, and the Stress and Yohimbine interaction was not significant, $F(1,32) = 0.73$, $p > .40$.

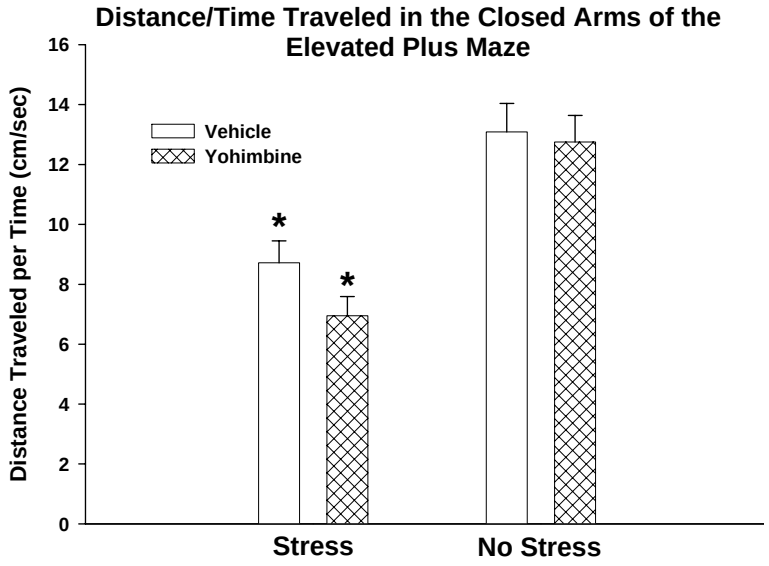

Figure 41. Distance/time traveled in the closed arms (+ *SE*) of the elevated plus maze in Experiment 2. Stressed rats traveled less distance/time in the closed arms than the unstressed rats. * $p < .001$ compared to respective unstressed groups.

Distance on the elevated plus maze (see Figure 42). An independent samples *t*-test indicated that the stressed rats treated with vehicle (5461.11 ± 430.84 cm) traveled less distance on the EPM than the unstressed rats treated with vehicle (7110.75 ± 355.84 cm), $t(15) = 2.91$, $p < .05$. The distance scores for all yohimbine-treated rats were converted into percent change from vehicle scores. A planned comparison was conducted on this data to compare the stressed, yohimbine-treated rats with the unstressed, yohimbine-treated rats. The stressed rats that were treated with yohimbine (-26.01 ± 6.74 %) exhibited a greater suppression of distance traveled on the EPM than the unstressed rats that were treated with yohimbine (-7.60 ± 4.05 %), $t(20) = -2.43$, $p < .05$.

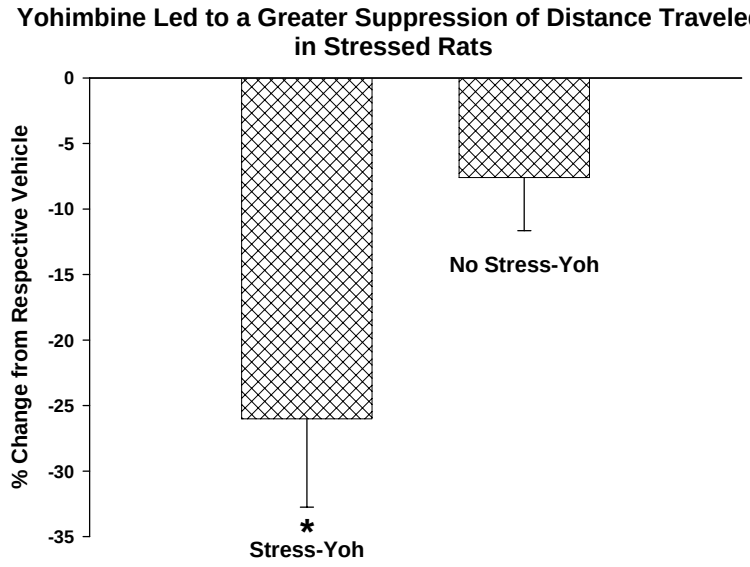

Figure 42. Percent change in distance traveled (+ *SE*) on the elevated plus maze in yohimbine-treated rats. Yohimbine led to a greater suppression of distance traveled on the elevated plus maze in the stressed rats. * $p < .05$ compared to the unstressed rats treated yohimbine.

Fecal boli. The analysis of fecal boli deposited on the EPM revealed a main effect of stress, $F(1,32) = 9.60$, $p < .01$, indicating that the stressed rats (0.72 ± 0.15 boli) defecated more on the EPM than the unstressed rats (0.07 ± 0.07 boli). There was no main effect of yohimbine, $F(1,32) = 1.24$, $p > .27$, and the Stress x Yohimbine interaction was not significant, $F(1,32) = 0.18$, $p > .67$.

Startle Response

90 dB auditory stimuli (see Figure 43). The analysis of startle responses to the 90 dB auditory stimuli revealed a main effect of stress, $F(1,32) = 39.76$, $p < .001$, indicating that the stressed rats (0.31 ± 0.02 Newtons) exhibited greater startle than the unstressed rats (0.16 ± 0.02 Newtons). There was no main effect of yohimbine, $F(1,32) =$

0.89, $p > .35$, and the Stress x Yohimbine interaction was not significant, $F(1,32) = 0.72$, $p > .40$.

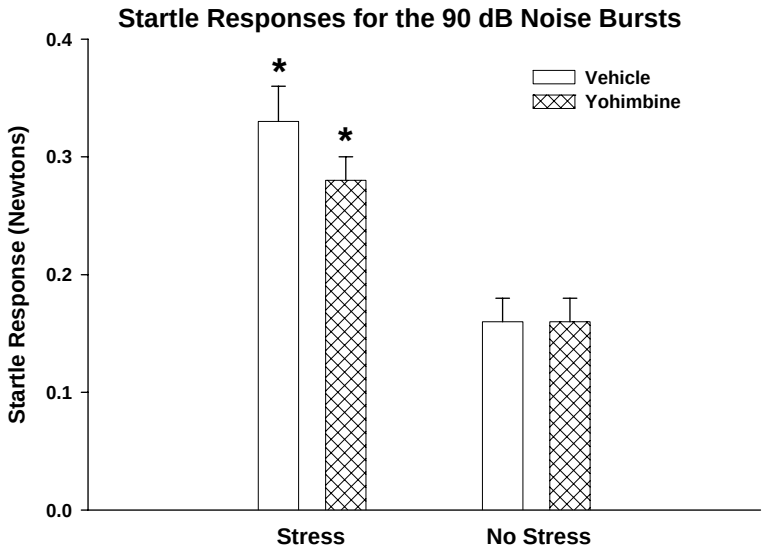

Figure 43. Startle responses (+ *SE*) for the 90 dB noise bursts in Experiment 2. Stressed rats exhibited greater startle responses than the unstressed rats. * $p < .001$ compared to respective unstressed groups.

100 dB auditory stimuli (see Figure 44). The analysis of startle responses to the 100 dB auditory stimuli revealed a main effect of stress, $F(1,33) = 32.43$, $p < .001$, indicating that the stressed rats (1.76 ± 0.12 Newtons) exhibited greater startle responses than the unstressed rats (0.82 ± 0.12 Newtons). There was no main effect of yohimbine, $F(1,33) = 0.01$, $p > .92$, and the Stress x Yohimbine interaction was not significant, $F(1,33) = 0.07$, $p > .78$.

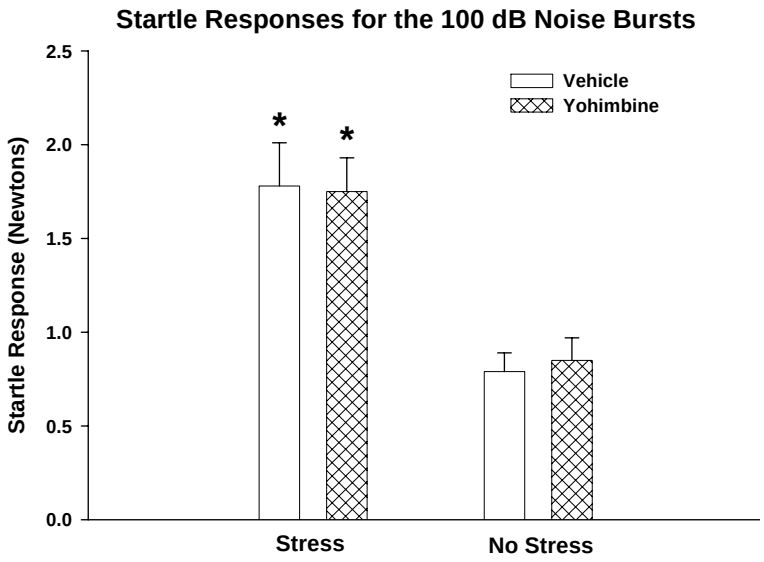

Figure 44. Startle responses (+ *SE*) for the 100 dB noise bursts in Experiment 2. Stressed rats exhibited greater startle responses than the unstressed rats. * $p < .001$ compared to respective unstressed groups.

110 dB auditory stimuli (see Figure 45). The analysis of startle responses to the 110 dB auditory stimuli revealed a main effect of stress, $F(1,33) = 11.98$, $p < .001$, indicating that the stressed rats (3.07 ± 0.19 Newtons) exhibited greater startle responses than the unstressed rats (2.15 ± 0.19 Newtons). There was no main effect of yohimbine, $F(1,33) = 2.72$, $p > .10$, and the Stress x Yohimbine interaction was not significant, $F(1,33) = 0.29$, $p > .59$.

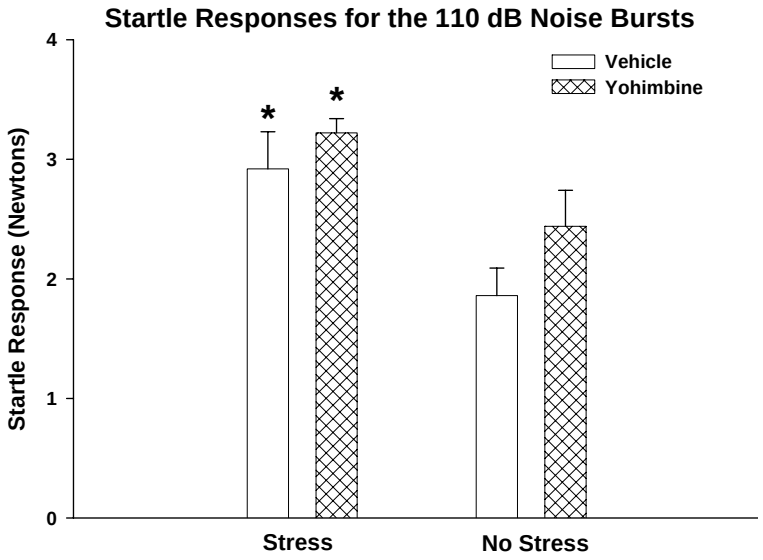

Figure 45. Startle responses (+ *SE*) for the 110 dB noise bursts in Experiment 2. Stressed rats exhibited greater startle responses than the unstressed rats. * $p < .001$ compared to respective unstressed groups.

Fecal boli. The analysis of fecal boli deposited in the startle apparatus revealed no main effects of stress, $F(1,35) = 0.24$, $p > .62$, or yohimbine, $F(1,35) = 0.87$, $p > .35$, and the Stress x Yohimbine interaction was not significant, $F(1,35) = 0.39$, $p > .53$.

Final Day's Corticosterone Levels (see Figure 46)

The within-subjects analysis revealed a main effect of time, $F(2,46) = 71.18$, $p < .001$. Post hoc analyses indicated that rats' CORT levels were greater after 20 min of restraint stress (31.59 ± 1.02 µg/dL) than at baseline (9.17 ± 1.16 µg/dL). These levels significantly declined 60 min later (16.71 ± 1.95 µg/dL), but were still elevated, relative to baseline levels (p's < .05). The Time x Stress interaction was significant, $F(2,46) = 10.65$, $p < .001$. The stressed rats exhibited greater CORT levels (baseline: 12.35 ± 1.29 µg/dL; stress: 38.35 ± 1.14 µg/dL) at baseline and after 20 min of restraint stress

than the unstressed rats (baseline: 5.99 ± 1.93 μg/dL; stress: 24.82 ± 1.70 μg/dL). However, the CORT levels of stressed (14.70 ± 2.17 μg/dL) and unstressed rats (18.72 ± 3.24 μg/dL) did not differ 60 min later (*p*'s < .05). The Time x Yohimbine interaction was significant, $F(2,46) = 3.82$, $p < .05$. Post hoc analyses indicated that yohimbine led to greater CORT levels at baseline and after 20 min of restraint stress. While the CORT levels of vehicle-treated rats (6.34 ± 2.47 μg/dL) returned to baseline levels 60 min later, those of yohimbine-treated animals (27.08 ± 3.01 μg/dL) remained elevated (*p*'s < .05). The Time x Stress x Yohimbine interaction was not significant, $F(2,46) = 1.03$, $p > .36$.

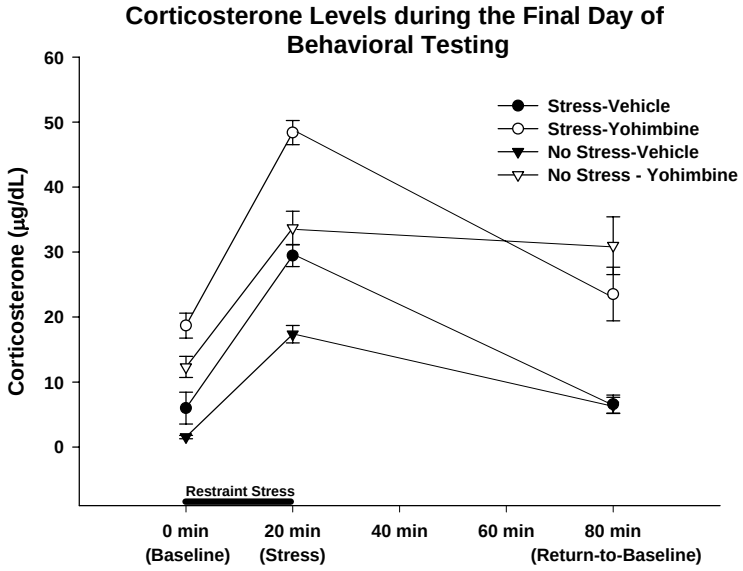

Figure 46. Corticosterone levels (± *SE*) during the final day of behavioral testing in Experiment 2. Stressed rats showed greater baseline levels and stress-induced elevations of corticosterone. Yohimbine also led to greater baseline levels and stress-induced elevations of corticosterone. Stressed rats treated with yohimbine demonstrated the greatest stress-induced elevation of serum corticosterone.

The between-subjects analysis revealed a main effect of stress, $F(1,23) = 8.29$, $p < .01$, indicating that the stressed rats (21.80 ± 1.02 μg/dL) had greater levels of CORT than the unstressed rats (16.51 ± 1.53 μg/dL). There was also a main effect of yohimbine, $F(1,23) = 82.72$, $p < .001$. Post hoc analyses indicated that the rats treated with yohimbine (27.51 ± 1.42 μg/dL) had greater CORT levels than the rats treated with vehicle (10.80 ± 1.17 μg/dL). The Stress x Yohimbine interaction was not significant, $F(1,23) = 0.00$, $p > .95$.

Weight (see Table 3 & Figure 47)

Table 3

Average Raw Weights for the Stressed Groups in Experiment 2

	Raw weights (g ± *SE*)		
Group	Stress session 1	Stress session 2	Last day of handling
Stress	300.63 (2.76)	345.61 (4.03)	430.00 (7.20)
No Stress	273.98 (2.19)	344.63 (4.89)	429.48 (7.72)

The within-subjects analysis revealed a main effect of time, $F(1,37) = 650.51$, $p < .001$, indicating that all of the rats gained a significant amount of weight over the course of the experiment. The Time x Stress interaction was not significant, $F(1,37) = 0.05$, $p > .94$. The between-subjects analysis revealed a main effect of stress, $F(1,37) = 18.91$, $p < .001$, indicating that the stressed rats (87.17 ± 4.27 g) gained less weight than the unstressed rats (113.08 ± 4.16 g).

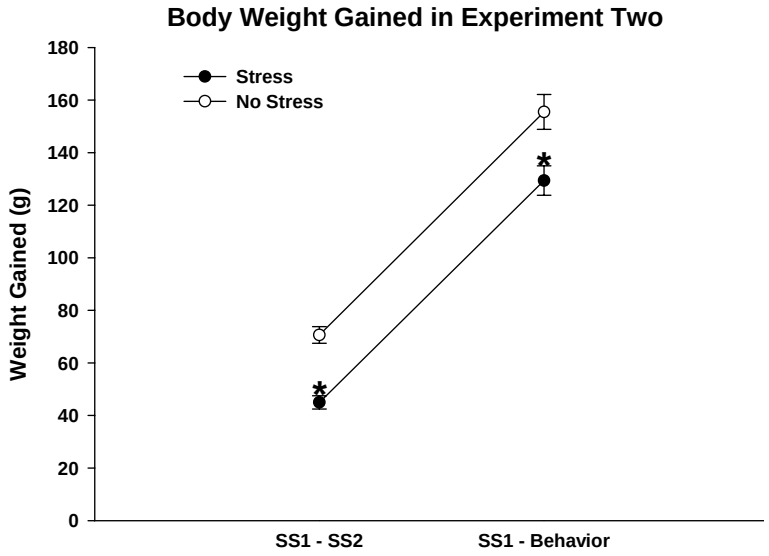

Figure 47. Body weight gained (± *SE*) throughout the course of Experiment 2. Stressed rats gained less weight. * $p < .001$ compared to unstressed rats.

Discussion

Major Findings and Significance

The most important finding of the current study is that the stressed rats exhibited an exaggerated response to yohimbine 3 weeks after the second stress session. This finding was predominantly evident for the first behavioral task examined (i.e., the open field) and provides support for the idea that the present stress paradigm has lasting effects on the noradrenergic system. The present study also replicated the stress effects found in Experiment One for the EPM and startle assessments. Specifically, the stressed rats exhibited greater levels of anxiety on the EPM and an exaggerated startle response, relative to the unstressed rats.

Enhanced Sensitivity to Yohimbine in the Open Field

The stressed rats displayed an enhanced sensitivity to yohimbine treatment, as measured by general locomotor activity in the open field. First, the stressed rats showed a greater suppression of rearing in the open field after yohimbine treatment, relative to the unstressed rats. In addition, yohimbine reduced the total distance that the stressed rats traveled in the open field, while it increased this measure in the unstressed rats. This finding is comparable to the differential effects that yohimbine had on the time that rats spent in the perimeter and center areas of the open field. The stressed rats that had been administered yohimbine spent more time in the perimeter and less time in the center of the open field, relative to vehicle-injected controls. This was in stark contrast to the unstressed rats, which spent less time in the perimeter and more time in the center of the open field after being treated with yohimbine. Upon placement in a novel environment, rats tend to avoid exploring the center area and spend more time in the perimeter. In fact, most of the rats in the present study spent approximately 70-80% of the trial in the perimeter of the open field. This type of behavior theoretically minimizes the rats' risk of being exposed to a predator-dominated, open area. When rats are anxious, this type of avoidant behavior is even more prevalent (Beck & Luine, 2002). Since the stressed rats displayed greater avoidance of the center area after being treated with yohimbine, it can be reasoned that the drug produced an anxiogenic response in these rats, a response that was not observed in the unstressed rats.

Elevated Plus Maze and the Magnitude of the Stress Effect

As in Experiment One, the stressed rats in the present study spent less time in the open arms of the EPM than the unstressed rats. The magnitude of this effect was again enough to generate a floor effect, which could not be further exacerbated by yohimbine treatment. The stressed rats that were treated with vehicle spent <5% of the trial in the open arms of the EPM. Clearly, it would very difficult, if not impossible, to intensify this effect. At any rate, it is important to emphasize the fact that these findings replicated the findings of Experiment One. Replication is always an important part of developing new paradigms and, in this case, provides further corroboration for the validity of the present stress paradigm.

Although yohimbine did not exacerbate the amount of time that the stressed rats spent in the open arms of the EPM, it did lead to a greater suppression of distance traveled on the plus maze in the stressed rats. In addition, the stressed rats that were treated with yohimbine made fewer ambulations than vehicle-injected controls, while the unstressed rats treated with yohimbine made more ambulations than vehicle-injected controls. Collectively, these findings indicate that yohimbine induced a greater suppression of locomotor activity in the stressed rats, relative to the unstressed rats.

Startle Response

Yohimbine did not lead to an exacerbation of startle in the stressed rats. This may be due to the fact that stress alone resulted in a significant enhancement of rats' startle, or it could be the case that the effects of yohimbine on startle are simply linear in nature, regardless of whether or not the rats are stressed. Furthermore, yohimbine alone did not

augment rats' startle responses. This finding lies in contrast to previous work that has reported yohimbine-induced enhancements of the acoustic startle response in rats (Kehne & Davis, 1985) and humans (Morgan, Southwick, Grillon, Davis, Krystal, & Charney, 1993). The inconsistency between previous work and the findings presented here is most likely attributable to the different doses of yohimbine that were employed in each case. The dose of yohimbine (1 mg/kg) employed in the present study is a very low, threshold dose that does not have significant effects on anxiety in unstressed rats (as evidenced by the data presented here). This low of a dose was employed because I was testing for a hyperresponsivity to the drug in the stressed rats; if the drug alone had too great of effects on controls, it would be difficult to detect an exaggerated response to the agent in stressed rats.

Weight as an Index of Stress

The effects of stress on body weight were much clearer in the present study than in Experiment One, given that the control rats were not stressed by HR/BP measurement and the collection of blood samples during the stress sessions. As shown in Figure 47, the stressed rats gained less weight from the first stress session to the second stress session and this effect was maintained to the commencement of behavioral testing. It would appear from the graph that it is the first stress session that leads to a reduction in weight gain, and this initial effect on the rats is maintained throughout the course of the experiment by the second stress session and randomized housing. It is clear from this study that the stress paradigm does lead to a significant reduction in weight gain, a

finding that is consistent with other research on chronic stress and supports the use of this paradigm as a model of PTSD in rats.

Effects of Stress and Yohimbine on Adrenal Activity

The stressed rats exhibited a greater stress-induced increase in CORT 3 weeks after the second stress session. This finding is consistent with the results of Experiment One, where the stressed rats exhibited greater increases in systolic BP and diastolic BP after acute restraint stress on the final day of behavioral testing. It is possible that the stressed rats in Experiment One did not demonstrate the same relative increase in CORT because the control rats themselves had been exposed to a moderate amount of stress throughout the course of the study. Indeed, the control rats from Experiment One (21.09 ± 2.19 μg/dL) appeared to exhibit slightly higher stress-induced increases in CORT on the final day of behavioral testing than the control rats from Experiment Two (17.37 ± 1.34 μg/dL). Ultimately, the finding of a greater stress-induced increase in CORT levels in the stressed rats is consistent with research in humans that have been diagnosed with PTSD. As mentioned above, these individuals often display significantly greater hormonal responses to acute stress than do control subjects. Elzinga et al. (2003) found that individuals with abuse-related PTSD exhibited greater cortisol levels than control subjects after exposure to a trauma-related script. The important point here is that the control subjects had also experienced severe childhood abuse, but they did not develop PTSD as a result of the trauma. Since stressed rats in the present study were immobilized during the stress sessions, it is possible that these rats demonstrated greater

increases in CORT after acute restraint stress 3 weeks later because it reminded them of the trauma that they had previously endured.

Yohimbine led to elevated baseline levels of CORT in both stressed and unstressed rats 30 min after its administration. This finding is consistent with previous work indicating that yohimbine increases HPA activity and anxiety-like behavior in both humans and rodents (Grunhaus, Tiongco, Zelnik, Flegel, Hollingsworth, & Smith, 1989; McDougle, Price, Heninger, Krystal, & Charney, 1995; Myers, Banihashemi, & Rinaman, 2005; Vythilingam et al., 2000). In addition, the stressed rats that had been treated with yohimbine appeared to show a greater stress-induced increase in CORT than the unstressed rats that had been treated with yohimbine. This is the first study to show such a response in stressed rats. Additionally, research on humans with PTSD has only shown an exaggerated autonomic response to yohimbine (Southwick et al., 1993), not an exaggerated hormonal response. Thus, the findings of the present study demand further investigation of the physiological response to yohimbine in individuals diagnosed with PTSD.

Limitations of the Present Study

As in Experiment One, there are some limitations and/or caveats to the findings of the present study that must be considered. Since all of the rats were tested on multiple behavioral assessments, it is again possible that some of the tasks interacted with one another. That is to say, one behavioral task could have affected some groups of rats differently than the way it affected others. Given that most of the evidence for an

exaggerated response to yohimbine was found for the open field assessment, which was the first task examined, this argument is very convincing.

In addition, it is possible that the effects of stress were so robust, that they prevented the detection of yohimbine effects in the stressed rats. This was particularly the case for the EPM, where stress alone led to a considerable suppression of open-arm exploration. Given that the stressed rats that were treated with vehicle spent approximately 2.66% of the trial in the open arms, it is not surprising that yohimbine did not exacerbate this effect. In sum, it is likely that this behavioral assessment did not retain the sensitivity to detect a heightened sensitivity to yohimbine in the stressed rats.

Chapter Four: General Discussion and Conclusions

Mechanisms Mediating the Long-Term Effects of Stress on Physiology and Behavior

Amygdala Plasticity

A considerable amount of work by Adamec and colleagues has suggested that NMDA-dependent plasticity in the amygdala is responsible for the long-term effects of predator stress on anxiety-like behavior in rats. Indeed, when the investigators treated rats with competitive NMDA receptor antagonists 30 min prior to cat exposure, the rats did not show heightened levels of anxiety or startle one week later (Adamec et al., 1999). Given the role of NMDA receptors in learning and memory, it would appear that these pharmacological agents prevent the memory of the traumatic experience from being adequately formed. Intuitively, this makes sense, since it is the powerful traumatic memories that ultimately lead to the disorder in humans. Adamec, Strasser, Blundell, Burton, & McKay (2006) provided further support for this argument when they found that the effects of predator stress on anxiety-like behaviors is dependent upon protein synthesis in the amygdala. Since memory consolidation has been shown to be dependent on protein synthesis (Lamprecht & LeDoux, 2004), this finding provides support for the idea that it is the memory of the cat exposure that leads to long-lasting effects on behavior.

Further work by Adamec and colleagues has examined the neural circuitry involved in the effects of predator stress on rats' behavior. Adamec, Blundell, and Collins

(2001) have shown that predator stress induces the potentiation of several important pathways to and from the amygdala. For instance, the right ventral angular bundle (VAB) input to the basolateral amygdala (BLA) has been linked with the anxiogenic effects of predator stress on behavior in the EPM (Adamec et al., 2001). This pathway is of an excitatory nature and extends from the hippocampus to the BLA. It supports NMDA-dependent plasticity and plays an important role in contextual fear conditioning (Maren, De Oca, & Fanselow, 1994; Maren & Fanselow, 1995). Another pathway that has been implicated in the effects of predator stress on anxiety-like behavior in rats is that which extends from the ACe to the lateral column of the periaqueductal gray (PAG) (Adamec et al., 2001). The ACe and PAG have both been associated with rodent defense and anxiety-like behaviors, and predator stress enhances activity in both areas. Theoretically, NMDA-mediated plasticity within these pathways plays a major role in the lasting effects of predator stress on anxiety-like behaviors in rodents.

Serotonin

Some PTSD patients have been effectively treated with a family of drugs known as the selective serotonin reuptake inhibitors (SSRIs), a finding that has drawn attention to the role of 5-hydroxytryptamine (5-HT; serotonin) in the progression of the disorder. Spivak and colleagues (Spivak, Vered, Graff, Blum, Mester, & Weizman, 1999) found significantly lower platelet-poor plasma concentrations of 5-HT in PTSD patients, while Fichtner and colleagues (Arora, Fichtner, O'Connor, & Crayton, 1993; Fichtner, Arora, O'Connor, and Crayton, 1994) detected a lower number of platelet 5-HT transporters in these individuals. Increased serotonergic activity is essential for a successful stress

response, and the findings of reduced 5-HT levels in PTSD patients suggest that 5-HT dysregulation may play a role in the pathophysiology of the disorder.

The 5-HT_{1A} receptors heavily innervate areas of the hippocampus (Richer, Hen, & Blier, 2002) and play a large role in mediating the stress response (Lopez, Liberzon, Vazquez, Young, & Watson, 1999). In addition, activation of the 5-HT_2 receptors, which primarily occupy cortical areas, leads to a significant increase in anxiety (McKittrick, Blanchard, Blanchard, McEwen, & Sakei, 1995). Investigators have observed elevated 5-HT_{1A} and 5-HT_{2A} receptor densities in the hippocampus and prefrontal cortex (PFC), respectively, of fear-sensitized rats (Kalynchuk et al., 2001). Harvey and colleagues (Harvey et al., 2003) examined the effects of the TDS stress model on rats' physiology and found that it led to an increase in 5-HT_{1A} receptor density and a reduction in ligand affinity for this receptor 7 days poststress. These investigators also found an increase in ligand affinity for the 5-HT_{2A} receptor in the PFC and a marginal increase in its density within the same area.

Research has indicated that the link between 5-HT and the stress response may involve the HPA axis (Cassano & D'Mello, 2001). For instance, activation of the 5-HT_{2A} receptors leads to HPA axis activation and a large increase in serum CORT levels (Heimrick-Leucke & Evans, 2002). In addition, CORT has been shown to downregulate the expression of 5-HT_{1A} receptors, while adrenalectomy results in an upregulation of these receptors (Neumaier, Sexton, Hamblin, & Beck, 2000). Accordingly, Harvey et al. (2003) found significantly lower baseline CORT levels in stressed rats 7 days poststress. These rats also displayed a significant increase in 5-HT_{1A} receptor density, as mentioned

above. These findings support the idea that reduced baseline levels of CORT, as a result of stress, leads to the modulation of 5-HT_{1A} receptor density in the hippocampus.

Noradrenergic Mechanisms

As mentioned above, individuals with PTSD tend to exhibit greater sympathetic tone at baseline. Research has found that these individuals have higher resting levels of EPI and NE and that they produce greater elevations of these agents in response to traumatic reminders. Previous work has also found that individuals with PTSD react adversely to the administration of yohimbine, an α_2 adrenergic receptor antagonist. Specifically, these individuals experience flashbacks and panic attacks after taking the drug, due to its effects on the noradrenergic system (Southwick et al., 1999b; Southwick et al., 1993). Investigators have observed analogous results in stressed primates. Specifically, Rosenblum and colleagues (Rosenblum, Coplan, Friedman, Bassoff, Gorman, & Andrews, 1994) found that macaques raised in a stressful environment displayed hyperresponsivity to yohimbine as adults. These investigators attributed the hyperresponsivity to an exaggerated suppression of locomotor activity in the stressed monkeys. Such a finding is comparable to the present set of experiments, where yohimbine produced a greater suppression of general activity, as evidenced by a greater suppression of rearing and total distance traveled in the open field and a greater suppression of ambulations and total distance traveled on the EPM, in the stressed rats.

Relevance of the Present Findings to Understanding PTSD in Humans

The present set of experiments provides a novel approach to modeling PTSD in rats, and a number of characteristics of this stress paradigm relate well to the symptoms

and general environment endured by humans diagnosed with the disorder. For instance, the present stress paradigm utilizes psychological stress of an ethologically relevant nature to produce its effects. Many other animal models (as described in the introduction) have failed to use purely psychological stress to produce behavioral responses characteristically observed in human PTSD patients. Instead, these models have employed physical stressors, such as electric shock or swim stress, to produce behavioral abnormalities in common with PTSD. Clearly, the use of an ethologically relevant stressor, such as predator stress, would be preferable as a valid animal model of PTSD.

Additionally, the present paradigm uses a stress-restress paradigm that can be related to the experiences of humans with PTSD. These individuals are exposed to an intense traumatic event, which is etched into the neural circuitry of their brain and produces the lasting consequences that have been described thus far. Individuals diagnosed with PTSD endure flashbacks and unavoidable memories of the initial trauma throughout most of their lives. The present model uses a second stressful experience to force rats to "relive" the initial event, a situation that is comparable to the experiences of human subjects with PTSD. This characteristic of the model, along with the presence of daily mild stress, is analogous to the stress that PTSD patients endure on a daily basis.

Behaviorally, the stressed rats in the present experiments exhibited robust enhancements of anxiety, startle, and a hyperresponsivity to yohimbine long after the initial trauma. The stressed rats showed a significant reduction in open-arm exploration in the EPM, a finding that is indicative of heightened anxiety in rats and that can be related to the anxiety often observed in humans with PTSD. For instance, individuals diagnosed

with PTSD tend to avoid public places because they fear having panic attacks. Consequentially, they become antisocial and, sometimes, even agoraphobic. Thus, the behavior of the stressed rats in the present studies can be related to the behavior that is often observed in PTSD patients.

The stressed rats also exhibited an exaggerated startle response during behavioral testing. Although the literature is not clear on whether or not baseline startle is elevated in PTSD patients, the individuals diagnosed with the disorder frequently report having heightened startle. Indeed, this symptom is often described as one of the most annoying characteristics of the disorder (Morgan, 1997).

Lastly, the stressed rats in the present study exhibited an exaggerated response to yohimbine. Park et al. (2001) found similar results in rats that had been exposed to a cat for 5 weeks. The present experiments provide the novel observation that only two exposures to a predator, along with daily unstable housing conditions, can produce analogous results. In addition, both of these experiments (Park et al, 2001 and the present study) appeared to find that yohimbine led to a greater suppression of locomotor activity in the stressed rats, which is consistent with the findings of Rosenblum et al. (1994), who found that yohimbine led to a greater suppression of locomotion in primates that had been raised in a stressful environment. The finding of hyperresponsivity to yohimbine in the stressed rats supports human research on PTSD and again provides further validation of the present stress paradigm as an animal model of the disorder.

Nevertheless, it must be noted that the present paradigm does not produce a condition in rats that is completely analogous to the span of behavioral symptoms

observed in human subjects with PTSD. It may be very difficult, if not impossible, to model every symptom that is observed in humans with the disorder. Even so, the current model is a step towards understanding how the behavioral symptoms that are common to PTSD (e.g., heightened anxiety, exaggerated startle, hyperresponsivity to yohimbine) are produced and what can be done to reverse these manifestations. It is clear that a valid animal model of PTSD would not only allow investigators to understand how the disorder progresses and better characterize its underlying neurobiological alterations, but also how to treat the disorder and potentially, with time, how to alleviate the disorder or even prevent it before it starts. The present experiments are only a small step to hopefully affording researchers these opportunities in the future.

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