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ABSTRACT

Title of Thesis: Interaction of Stress and Anxiogenic Drugs in Behaviors of Rats and Antagonism with Indomethacin

Jeffrey H. Cook, Doctor of Philosophy, 2001

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Panic disorder is a debilitating psychiatric condition characterized by the occurrence of discrete episodes of intense anxiety. Psychological and biological explanations for panic disorder have been offered, but there is no consensus regarding its etiology. Panic disorder patients are especially sensitive to the anxiety-provoking effects of certain pharmacological agents -- biological challenge agents (BCA). Psychological models propose that conditioning history or cognitions explain this sensitivity to BCAs. Biological models of panic disorder propose physiological dysregulations to explain sensitivity to BCAs.

This doctoral research examined the thesis that the greater sensitivity to BCAs in panic disorder patients is a result of stress-induced blood-brain barrier disruption. Several studies have reported that stress increases the permeability of the blood-brain barrier (BBB). Stress-induced blood-brain barrier disruption offers a parsimonious explanation for enhanced sensitivity to challenge agents -- as more of the agent enters the brain, stronger reactions occur.

Two experiments were conducted with 200 male Wistar rats. Experiment 1 examined the effects of 30 minutes of immobilization stress on behavioral responses

(elevated plus maze and open field test) to various dosages of two anxiogenic drugs (yohimbine, isoproterenol). Animals exposed to the stressor showed enhanced behavioral responses to both drugs. Experiment 2 examined the effects of pre-treatment with a drug (indomethacin), that antagonizes stress-induced blood-brain barrier disruption, followed by the two anxiogenic drugs on the same behavioral responses in different animals. Pre-treatment with indomethacin antagonized the stress-enhanced behavioral effects. These findings suggest that biologically-based theories regarding the etiology of panic disorder might be explained by effects of stress to disrupt the blood-brain barrier. These findings also suggest that reported differences in effects of anxiogenic drugs in animals and humans may be explained by baseline stress levels.

Interaction of Stress and Anxiogenic Drugs on Behaviors of Rats and Antagonism with Indomethacin

by

Jeffrey H. Cook

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INTRODUCTION

Since its recognition as a discrete psychiatric syndrome in 1980, panic disorder has been the focus of intense controversy in terms of etiology, treatment, and syndromal validity (McNally, 1994). Panic disorder has a lifetime prevalence rate of 3.8% (Katerndahl & Realini, 1993). The syndrome of panic disorder is characterized by the experience of recurrent, unexpected (spontaneous) panic attacks. These attacks are usually accompanied by either anticipatory anxiety or worries regarding the possibility of having another attack (Barlow, 1988; American Psychological Association [APA], 1994). Panic attacks may lead to the development of agoraphobic avoidance, in which individuals avoid situations where they have experienced panic attacks in the past.

The American Psychological Association's Diagnostic and Statistical Manual 4th edition (DSM-IV) characterizes panic attacks as discrete episodes of intense fear which develop suddenly and may occur with (situational) or without (spontaneous) clear environmental antecedents. Panic attacks are considered to be a possible component of syndromes because they occur across a range of anxiety and affective disorders (Barlow, 1988; APA, 1994). Panic attacks are characterized by physiological symptoms such as palpitations, trembling, sweating, dizziness, chest pain, chills/hot flashes, choking sensations, and nausea. Additionally, cognitive symptoms may be present, including depersonalization, derealization (a sense of loss of contact with external reality), fear of dying, and fear of losing control or going crazy. To meet DSM-IV criteria

for a panic attack there must be four of the above symptoms, and they must start abruptly and peak after 10 minutes (APA, 1994).

States of panic can be distinguished from anxiety states by the suddenness of onset, number and severity of symptoms and the presence of fears of dying, going crazy, or losing control (Argyle & Roth, 1989; APA, 1994). Panic typically has a rapid onset and short duration, whereas gradual onset and chronic duration characterize anxiety states. Additionally, panic states have a greater number of symptoms, and have more severe symptoms when compared to anxiety states (Argyle & Roth, 1989; APA, 1994). Panic and anxiety also can be differentiated in terms of their theoretical function. Panic serves a fight-or-flight function that is directed towards some immediate threat, and is arguably considered a state of extreme fear (Barlow, 1988). Anxiety is often defined as a state that is qualitatively different from panic. The function of anxiety is increased vigilance, which is directed towards some future (non-immediate) threat. Anxiety states lack the fight-or-flight and fear of dying/going crazy components of panic attacks (Barlow, 1988).

In addition to the distinction between anxiety and panic states, the terms panicogenic, panicolytic, anxiogenic, and panicolytic should be distinguished. Procedures that provoke anxiety are referred to as anxiogenic, and those provoking full-blown panic attacks are referred to as panicogenic. Procedures that reduce anxiety or prevent panic attacks are referred to as anxiolytic and panicolytic, respectively. Although often used synonymously in the literature, the terms stress and anxiety refer to different states. Anxiety is a negative emotional

state related to a dangerous and threatening stimulus. In contrast, stress reactions can occur following positive experiences (e.g., winning the lottery), in addition to negative experiences. Stress is, therefore, a much broader phenomenon, encompassing anxiety states and panic attacks.

Various categorization schemes for panic attacks have been proposed. Attacks without an identifiable trigger are called "unexpected" (APA, 1994), "spontaneous" (Klein, 1993), and "uncued/unexpected" (Barlow, 1988). Panic attacks for which a patient can identify a triggering stimulus have been termed situationally bound or situationally predisposed (APA, 1994; Klein, 1993). Under the DSM-IV's classification system, only external stimuli can trigger panic attacks. Other classification systems are broader, allowing the inclusion of panic attacks triggered by internal stimuli (Barlow, 1988; Craske, 1991). Two additional types of panic attacks are nocturnal panic (panic occurring during sleep), and relaxation-induced panic (occurring during relaxation). A full discussion of the relative strengths and weaknesses of these classification schemes can be found elsewhere (McNally, 1994).

Although there is agreement in terms of the DSM-IV for the symptoms of this disorder, the etiology of panic disorder remains the subject of intense controversy. A review of popular theories for the etiology of panic disorder is presented in this doctoral dissertation research proposal. Throughout this review, it is evident that studies using biological challenge agents play an integral role in the development and testing of etiological theories for panic. Additionally, this review makes the point that there is no consensus as to which explanation

best accounts for panic disorder, and that human studies have inherent limitations to address key questions regarding the etiology of panic disorder.

Following the review of etiological theories, a hypothesis is presented that offers a parsimonious explanation for the greater sensitivity panic disorder patients demonstrate compared to controls, across a range of biological challenge agents. Support for this hypothesis is drawn from pre-clinical and clinical investigations using anxiogenic agents.

An animal model to empirically test this new hypothesis for biological challenge agent super-sensitivity is presented. The doctoral research uses two animal models to empirically evaluate this hypothesis. Specifically, the experiments examine the role of stress in the response to anxiogenic drugs. The stress manipulation was conceptualized as a means to recreate the differences in baseline anxiety that occur in panic disorder and control subjects before biological challenge agent administration. The animal research allows control of biological function, cognitive variables (rats presumably do not catastrophically misinterpret bodily sensations), and conditioning history. Controlling variables such as cognition, conditioning history and biological dysregulation is of paramount importance to answer questions regarding why panic disorder patients show greater sensitivity to biological challenge agents.

Many different behavioral tasks have been used with animals in studies relevant to panic disorder. The current research used two different behavioral tasks (the elevated plus maze and open field test) to index anxiety in animal subjects. These two behavioral measures are the most commonly used in

studies of biological challenge agents, they are bidirectionally sensitive to changes in behavior that reflect anxiety, and they have proven to be reliable and sensitive in pilot studies. Because panic can be conceptualized as a state of high anxiety, animal models of anxiety are useful. The experiments examined the effects of acute stress on behavioral and biochemical responses to anxiogenic drugs in order to assess whether stress predisposes subjects to qualitative and quantitative differences in responses.

The specific aims of this research were to: 1) determine whether stress increases behavioral responses to anxiogenic agents that are used in biological challenge studies; 2) examine whether pharmacological antagonism of stress-induced disruption in the blood-brain-barrier attenuates the enhanced sensitivity to anxiogenic drugs.

As a background for the proposed research, **Section I** reviews the literature on etiological models for panic disorder, highlighting the central role that findings from biological challenge agent research have played in the development of these biological models. **Section II** presents a new hypothesis for increased sensitivity to various challenge agents seen in panic disorder, and reviews relevant evidence that support this hypothesis. **Section III** presents the rationales for each independent and dependent variables. **Section IV** presents the hypotheses, methods and data analytic strategy of this doctoral dissertation research project. **Section V** presents the results of the two experiments used in this research project and a discussion of the findings and their implications.

SECTION 1: ETIOLOGICAL MODELS OF PANIC DISORDER

Etiological theories for panic have traditionally been grouped into two domains, biological and psychological (McNally, 1994). The biological interpretation of the higher rates of panic attacks in panic disorder patients is that the challenge agent is triggering some biologically based dysfunction (either central or peripheral). The psychological interpretation is that panic disorder patients are prone to fear of the bodily sensations that challenge agents produce, and that these bodily sensations serve to trigger the panic attack (McNally, 1994). Of these two domains, there are more biologically based theories that have considered a variety of neurotransmitter systems and neuroanatomical structures in the pathogenesis of panic disorder.

Data from biological challenge studies are intimately related to both biological and psychological models. Biological challenge studies typically involve the administration of either a drug (e. g., sodium lactate, yohimbine, flumazenil) or manipulations of carbon dioxide (CO_2) via inhalation of carbon dioxide or hyperventilation. These manipulations elicit intense physical sensations when administered and cause panic attacks in panic disorder patients at much higher rates than in psychiatric or healthy controls (McNally, 1994).

Biological models assume that the agents administered in biological challenge procedures provoke panic attacks by exacerbating an underlying dysfunction in the neurobiological substrate affected by the agent. Many of the biological explanations were generated from experiments using biological

challenge paradigms (Nutt & Lawson, 1992). Various pharmacological agents have been used to induce panic attacks, including sodium lactate (Pitts & McClure, 1967), yohimbine (Charney, Heninger, & Breier, 1984), isoproterenol (Rainy et al., 1984), carbon dioxide (van den Hout, 1988), caffeine (Charney, Heninger, & Jatlow, 1985), cholecystokinin (Bradwejn, Kosztcki, & Shriqui, 1991), and pentagastrin (Abelson & Neese, 1990), flumazenil (Nutt, Glue, Lawson, & Wilson, 1990), ^{m}-CPP (Kahn, Wetzler, & van Praag, 1988), epinephrine (Veltman, van Zijdarveld, & van Dyck, 1996), and β-CCE (Dorow & Horowski, 1983).

The results of studies that have used biological challenge agents have produced many explanations that involve specific neurotransmitter systems or neuroanatomical structures as dysfunctional in panic disorder. Several of the more prominent biological modes are described in the next section. Then they are contrasted with two psychological theories of panic disorder, emphasizing the theories' ability to explain responses to biological challenge agents. Discussion of these various theories clarifies the utility of an animal model to address questions about panic disorder patients' greater sensitivity to challenge agents.

Biological Theories

Original theories for the onset of panic disorder were biologically based, largely because of the occurrence of spontaneous panic attacks (which suggest the occurrence of neurochemical events). Panic attacks resulting from the administration of biological challenge agents also were taken as evidence for a biochemical basis for panic disorder (Margraf & Ehlers, 1990).

Biological accounts for the cause of panic disorder implicate dysregulation in several neurotransmitter systems, such as the noradrenergic (Charney, Woods, Price, et al., 1990), serotonergic (Kahn, Asnis, & Wetzler, 1988), cholecystokinin (Bradwejn et al., 1992), and the benzodiazepine/ GABA (Nutt et al., 1990; Nutt & Lawson, 1992) systems. Besides central neurotransmitter systems, a variety of other systems have been proposed as dysfunctional in panic disorder, including hypersensitive peripheral β-adrenergic receptors (Rainey et al., 1984), hypersensitive CO_2 chemoreceptors (Gorman & Papp, 1990), and a hypersensitive "suffocation alarm" (Klein, 1993).

Of direct relevance to the present work is the fact that some drugs used to study panic disorder do not cross the blood-brain-barrier to an appreciable degree. The finding that such agents can provoke panic attacks in panic disorder patients has been used to support arguments that psychologically-based theories, and not biologically based theories, are better able to explain panic disorder.

Biological models as a group share conceptual flaws in their underlying premises. A problem with biological models of panic is that the disorder presents a relatively homogeneous behavioral profile, whereas these biological models propose a heterogeneous array of specific abnormalities. The marked diversity of neurotransmitter and neuroanatomical structures that have been proposed to be dysregulated in panic disorder is quite striking. In order to illustrate why so many systems have been proposed as dysfunctional in panic disorder, the

general pattern for the development of biological models for panic disorder is described as follows:

1. Administer a drug to both panic disorder patients and healthy controls.
2. Observe higher rates of panic attacks in panic disorder patients compared to healthy controls.
3. Propose a *post hoc* explanation to account for the higher sensitivity of panic disorder patients to the agent, implicating the biological substrate affected by the agent administered (if the substrate is known).
4. Conduct further studies to empirically test predictions drawn from the new explanation.

Studies that demonstrate different panic rates between panic disorder patients and healthy controls following administration of a drug usually have been the origin of biological theories of panic disorder. The authors of these studies then propose a hypothesis to account for the data, typically speculating that the biological substrate affected by the agent (if known) is dysregulated. The explanation implicates some physiological abnormality that panic disorder patients are proposed to have and the inference is drawn that this abnormality might be the underlying cause of panic disorder for, at least, a subset of panic disorder patients. Predictions drawn from the new hypothesis about other agents that should either provoke or prevent panic are then tested. Specific hypotheses for a biological basis of panic disorder have generally received little empirical support when such tests have been conducted. Administration of agents such as D-lactate (Nutt & Lawson, 1992) and low doses of isoproterenol (Nesse et al.,

1984) actually have provided evidence that directly contradicts their parent hypothesis.

The following section reviews biologically based models of panic disorder, first considering models that were derived from studies using agents with blood-brain-barrier penetration. Models derived from agents that do not penetrate the blood-brain-barrier are then reviewed.

Agents that are thought to cross the blood-brain-barrier at baseline

The majority of challenge agents used in panic provocation studies cross the blood-brain-barrier under normal conditions. The findings from studies of agents that do and not cross the blood-brain-barrier have provided evidence for both biological and psychological models of panic disorder. Etiological hypotheses that have been derived from biological challenge agent studies are presented below.

Noradrenergic Dysregulation

Noradrenergic dysregulation has been suggested as a possible mechanism for panic disorder (Charney et al., 1990). Redmond and Huang (1979) stimulated the locus coeruleus of primates by electrical and pharmacological (piperoxane and yohimbine administration) means. The sympathetic activation created by the locus coeruleus is regarded as a negative feedback system, wherein increases in norepinephrine trigger presynaptic inhibitory α_2 autoreceptors, which limit subsequent norepinephrine (NE) release.

Yohimbine is an α_2 -adrenergic antagonist that stimulates the locus coeruleus (Goldberg & Robertson, 1983). Yohimbine increases norepinephrine production from the locus coeruleus by inhibiting the feedback mechanism of the presynaptic α_2 adrenergic autoreceptor. The stump-tailed monkeys in Redmond and Huang's (1979) study had behavioral and physiological reactions that were nearly identical to those displayed when confronted with threatening stimuli, such as threats from other monkeys and humans. Results from studies involving yohimbine infusions along with animal work involving stimulation of the locus coeruleus have laid the foundation for the noradrenergic dysregulation theory (also know as the locus coeruleus model) of panic disorder (Charney, Woods, Krystal et al., 1992; Charney, Woods, Goodman, & Henninger, 1987). In its original formulation, the locus coeruleus model assumed that panic disorder is the result of abnormally high responsitivity in brain noradrenergic systems (Charney & Heninger, 1986).

The locus coeruleus model was reformulated from its earlier position that noradrenergic dysfunction is common to all patients by specifying that abnormality in noradrenergic systems may be characteristic of a distinct subgroup of patients (Charney & Heninger, 1986). Most biological models make the same assertion regarding dysfunction, namely that a subset of patients has a specific biological dysregulation. The α_2 adrenergic autoreceptor is the primary candidate for dysfunction in the reformulation of the locus coeruleus model. Charney, Woods, and Goodman (1987) reported a 54% incidence of panic in panic disorder patients following yohimbine administration, whereas only 5% of

normal controls panicked. The effects of yohimbine in triggering panic attacks do not occur in major depressive disorder, generalized anxiety disorder, obsessive-compulsive disorder, or schizophrenia (Charney, Woods, & Krystal, 1992). Because yohimbine does not trigger panic in these other disorders, its effects appear to be specific to panic disorder. Caffeine increases the rate of firing of the locus coeruleus in animals (Olpe, Jones, & Steinmann, 1983); therefore, the noradrenergic dysregulation theory might explain the greater sensitivity of panic disorder patients to caffeine.

The primary shortcoming of the noradrenergic dysregulation model is its predictive validity. The locus coeruleus model predicts that drugs that increase locus coeruleus firing (such as buspirone and carbamazepine) should be profoundly panicogenic (producing panic attacks). Contrary to this prediction, buspirone and carbamazepine actually have mild anxiolytic effects when given to panic disorder patients (Taylor, Eison, Riblet, & Van der Maelen, 1985; Cohn & Wilcox, 1986; Uhde et al., 1985). Furthermore, acute administration of mianserin, a drug that, among other effects, blocks α_2 adrenergic autoreceptors, fails to produce panic attacks, and even relieves anxiety in some patients (Klein, Rabkin, & Gorman, 1985). These criticisms should be qualified by the possible differences in the degree of locus coeruleus stimulation effected by carbamazepine, buspirone, and mianserin compared with yohimbine. Yohimbine may create greater NE output, or the locus coeruleus-mediated effects of these other drugs might be overshadowed by anxiolytic actions at other sites. The failure of clonidine as an effective treatment for panic attacks also argues against

the locus coeruleus model, because clonidine markedly decreases locus coeruleus firing, but it is ineffective in treating panic (Uhde et al., 1989).

Serotonergic Dysregulation

Based on pharmacological, biochemical, and behavioral evidence, serotonergic (5-HT) neurons have been implicated in anxiety disorders (Iverson, 1984). The two main lines of evidence implicating serotonin dysregulation in panic disorder are the efficacy of serotonergic drugs in the treatment of panic disorder and the panicogenic effects of directly and indirectly acting serotonin agonists.

Metachlorophenylpiperazine (^{m}-CPP) is a serotonin agonist that directly stimulates postsynaptic receptors (Maser & Woods, 1990). ^{m}-CPP has high affinity for 5-HT_{2c} and 5-HT_3 receptors (Kahn & Wetzler, 1991; Shen, Monsma, Metcalf, & Jose, 1993; Hoyer, 1988). Intravenous administration of 0.1 mg/kg ^{m}-CPP resulted in 45% of panic disorder patients versus 30% of controls experiencing a panic attack (Charney, Woods, Goodman, & Heninger, 1987). Oral ^{m}-CPP induced panic in 60% versus 0% of panic disorder patients and normal controls experiencing panic, respectively (Kahn, Wetzler, van Praag, & Asnis, 1988). Some studies have reported that ^{m}-CPP induces significantly higher degrees of cortisol response in panic disorder patients compared to controls (Klein, Zohar, Geraci, Murphy, & Uhde, 1991; Kahn, Asnis, Wetzler, & van Praag, 1988), although another study failed to replicate this relationship (Charney, Woods, Goodman, & Heninger, 1987).

The effects of indirect serotonin agonists also have been examined in panic disorder patients. Two studies have examined the effects of fenfluramine in panic disorder (Targum, 1991; Targum & Marshall, 1989). Targum (1991) reported that about two thirds of panic disorder subjects reacted with extreme anxiety to fenfluramine. Administration of serotonin precursors as a biological challenge agent also has been attempted. Infusions of tryptophan and 5-OH tryptophan failed to produce panic. In fact, 5-OH-tryptophan actually decreased anxiety slightly and produced sedation (Charney & Heninger, 1986; den Boer & Westenberg, 1990). Based on this pattern of biological challenge results, some investigators have concluded that at least a subset of panic disorder patients have hypersensitive post-synaptic 5-HT receptors (McNally, 1994).

The possibility that panic disorder is characterized by a dysregulation in brain serotonin is supported by the efficacy of serotonin specific reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) treat panic disorder (Pecknold, 1990). SSRIs and TCAs increase the synaptic availability of serotonin, eventually leading to down-regulation of the serotonin post-synaptic receptors (Hedaya, 1996). Panic disorder patients typically exhibit a biphasic response to SSRIs and TCAs, at first becoming more anxious, then gradually improving (Kahn & Westenberg, 1985). The initial worsening of anxiety with SSRI and TCA treatment is consistent with post-synaptic receptor hypersensitivity. This initial worsening is thought to be similar to that created by direct serotonin agonists such as ^{m}-CPP. SSRIs and TCAs initially produce anxiety because of a build-up in endogenous transmitter, rather that direct

stimulation of receptors by a drug. Treatment with TCAs and SSRIs increases serotonin in the synapse which, over a period of weeks, down-regulates serotonin receptors. Down-regulation of these receptors is thought to be responsible for the decreased anxiety.

The novel anxiolytic agent buspirone has some serotonin agonist properties, acting as an agonist at $5HT_{1A}$ receptors (Feldman, Meyer, & Quenzer, 1997). Buspirone treatment reportedly exacerbates panic disorder (Frazer & Lapierre, 1987), although it is effective in treatment of generalized anxiety disorder (Goa & Ward, 1986). Buspirone's aggravation of panic disorder is consistent with the post-synaptic receptor hypersensitivity hypothesis, because it has some agonist properties (Taylor & Moon, 1991). Buspirone produces many unpleasant side effects, such as nausea, insomnia, and dizziness, which may be especially difficult for panic disorder patients to tolerate (Frazer & Lapierre, 1987).

Benzodiazepine/GABA Receptor Dysfunction

Nutt, Glue, Lawson, and Wilson (1990) hypothesized that panic patients may be characterized by a dysfunction in the GABA/benzodiazepine receptor complex. The benzodiazepine site binds agonist, antagonist, and inverse agonists. It has been hypothesized that panic disorder patients may have high levels of an endogenous inverse agonist such as diazepam binding inhibitor (DBI). Nutt et al. (1990) administered flumazenil (a benzodiazepine antagonist) which occupies but does not activate the benzodiazepine site) to panic disorder

patients in an investigation of this possibility. Based on the elevated endogenous inverse agonist hypothesis, the effect of flumazenil was predicted to be anxiolytic in panic patients. Once administered, flumazenil produced panic attacks in 80% of panic disorder patients, while no controls panicked (Nutt et al., 1990). Another study reported that oral administration of flumazenil produced panic in 40% of panic disorder patients, whereas no normal controls panicked (Woods, Charney, Silver, Krystal, & Heninger, 1991). In this study, the 40% panic rate occurred with a low dose (200 mg) of flumazenil. However, no panic attacks were reported at a higher dose (600 mg), in either the patient or the control group.

The panicogenic effects of flumazenil have been hypothesized to result from the existence of an unidentified endogenous anxiolytic substance that is blocked to a greater degree in patients than in controls. While interesting as speculation, there is no evidence for this proposition. Another possible explanation for flumazenil's effects involves changes in the benzodiazepine/GABA receptor. Nutt et al. (1990) suggested that the "set point" in panic disorder patient's benzodiazepine receptors is "shifted" in the inverse agonist direction. With such a shift in receptor functioning, antagonists (such as flumazenil) would act like inverse agonists and agonists, such as diazepam, would have less of an effect. The ineffectiveness of low potency benzodiazepines, and the high doses of high potency benzodiazepines, such as alprazolam, required to treat panic disorder is consistent with Nutt et al.'s (1990) hypothesis. As the literature stands, no other studies are available to support this idea.

A newer study casts doubt on the ability of flumazenil to function as an effective panicogenic challenge agent. Strohle et al. (1998) administered flumazenil to panic disorder patients and found that no patient experienced a panic attack. Interestingly, in this same sample of patients, the rate of panic attacks to sodium lactate was 80%. Strohle et al. (1998) argued that methodological differences this study and the two earlier reports are insufficient to explain the differences in results. This failure to replicate, coupled with the lack of a dose-responsive relationship between flumazenil and panic attacks (Woods, Charney, Silver, Krystal, & Heninger, 1991) questions the dysregulation in the benzodiazepine system in panic disorder. In defense of altered benzodiazepine receptor functioning, evidence for sub-sensitivity to benzodiazepine agonists has been reported. Panic disorder patients have lower reductions in plasma catecholamines (a typical reaction to benzodiazepines) compared to healthy controls following benzodiazepine administration (Roy-Byrne et al., 1989). Whereas β-carbolines (benzodiazepine inverse agonists) has been reported to produce marked panic-like reactions in healthy controls (Dorow et al., 1983; Gentil, 1990), no study to date has reported panic disorder patient's reactivity to these compounds.

Caffeine and Panic

Caffeine is a xanthine derivative that is widely used as a psychotropic agent. Ingestion of low doses of caffeine increases alertness and decreases fatigue (Weiss & Laties, 1962). Although caffeine generally produces beneficial

effects at low doses, higher doses can induce insomnia, anxiety, tachycardia, and dyspnea (Greden, 1974). Panic disorder patients are more sensitive to the administration of caffeine than controls (Uhde, 1990). Uhde (1990) reported that 37.5% of panic disorder patients panicked in response to an oral dose of 480 mg/kg caffeine, while no controls panicked. Larger dosages (10 mg/kg) of caffeine produced panic in 71% of patients but in no control subjects (Charney, Henninger, & Jatlow, 1985). In some animal models (i.e., conflict models) caffeine has not been shown to be anxiogenic (Kuribara, Haraguchi, & Tadokoro, 1989).

The mechanism by which caffeine produces anxiety is unknown. Theories implicating inhibition of phosphodiesterase (Butcher & Sutherland, 1962), antagonism of adenosine receptors (Snyder & Sklar, 1984), and increased CNS catecholamine activity (Berkowitz, Tarver, & Spector, 1970) have been offered to account for caffeine-induced anxiety. The most likely explanation for caffeine's ability to trigger anxiety is its blockade of the adenosine receptor, because this effect occurs within the range of normally ingested doses (Shear, 1986; Snyder & Sklar, 1984). Inhibition of phosphodiesterase and increased CNS catecholamine activity occur only at doses that are outside the normally ingested range.

Cholecystokinin Dysregulation

Bradwejn et al. (1992) suggested that panic disorder is characterized by cholecystokinin (CCK) dysregulation. CCK, a peptide originally discovered in the gastrointestinal tract, is also found in significant concentrations in the central

nervous system (Dockray, 1976). CCK probably functions as a neuromodulator or neurotransmitter within the central nervous system (Cooper, Bloom, & Roth, 1996). Intravenous CCK-4 (a tetrapeptide) infusion produces anxiety and panic attacks in healthy volunteers (de Montigny, 1989) and panic disorder patients (Bradwejn, Kosztcki, & Shriqui, 1991). At a dose of 25μg, CCK-4 induced panic in 91% and 17% of panickers and controls, respectively. At 50μg, the infusion produced panic in 100% of patients and in 47% of the controls. Currently, it is not known whether CCK-4 crosses the blood-brain barrier (BBB) at baseline. However, no evidence against CCK-4's permeability to BBB exists, unlike other challenge agents (e.g., sodium lactate and isoproterenol). CCK-4 produces panic attacks in panic disorder patients and healthy controls, suggesting some BBB crossover (de Montigny, 1989; Bradwejn et al., 1992; Bradwejn, Kozynki, & Shriqui, 1991; Shlik et al., 1997).

CCK-4 is one of the few biological challenge agents that have been administered across a range of doses to both panic disorder patients and healthy controls. The combined results of several studies using CCK-4 as a challenge agent (de Montigny, 1989; Bradwejn et al., 1992; Bradwejn, Kozynki, & Shriqui, 1991; Shlik et al., 1997) are presented in Figure 1. The figure presents dose-response curves for both panic disorder patients and healthy controls to CCK-4. As can clearly be seen, both panic disorder patients and healthy controls panic in response to CCK-4 infusion. However, the panic disorder patients are more sensitive. Up to 70% of normal controls panic in response to high doses of CCK-4 (de Montigny, 1989). Greater sensitivity in panic disorder patients has been

repeatedly observed in past studies (Bradwejn et al., 1992; Bradwejn, Kozynki, & Shriqui, 1991). The somewhat parallel dose-response curves for both groups are noteworthy because it implies that panic disorder patients' reactions to CCK-4 are quantitatively and not qualitatively different from healthy control's reactions. This distinction is critically important in panic disorder because it suggests a similar mechanism of action between panic disorder patients and healthy controls with only a difference in sensitivity to the agent.

Carbon Dioxide Chemoreceptor Hypersensitivity

Gorman and Papp (1990) suggested that at least a subset of panic disorder patients are characterized by abnormally sensitive carbon dioxide (CO_2) chemoreceptors located in the medulla. These chemoreceptors are thought to monitor peripheral autonomic activity and to compare this activity with metabolic demand. Mismatches between metabolic demand and metabolic supply cause these receptors to stimulate the locus coeruleus, which then initiates a panic attack (Gorman & Papp, 1990). If these chemoreceptors are hypersensitive or some other brainstem regions in this system are malfunctioning, then the result is panic. Within Gorman and Papp's (1990) model, limbic structures such as the hippocampus and amygdala are hypothesized to be the sites where the tonic level of anticipatory anxiety observed in panic disorder patients originates. The panicogenic effects of challenge agents such as sodium lactate, carbon dioxide, hyperventilation, and sodium bicarbonate, in panic disorder patients have been explained in terms of the hypersensitive CO_2 chemoreceptor hypothesis.

The effects of carbon dioxide inhalation are obviously relevant to the carbon dioxide Chemoreceptor Hypersensitivity Theory. Inhalation of carbon dioxide has been repeatedly demonstrated to be a potent biological challenge agent (BCA), provoking panic in patients with panic disorder patients more often than controls. Doses of CO_2 between 5% and 35% reliably produce symptoms of panic (McNally, 1994). Many researchers have used a single breath of 35% CO_2 and 65% O_2 to elicit panic (van der Hout, 1988; Papp, Klein, & Gorman, 1993; Griez, et al., 1990; Griez, et al., 1987; van der Hout, 1985). Panic disorder patients generally experience panic attacks at a rate of about 70% in response to 35/65 % CO_2/O_2 (Papp, 1993). In perhaps the first use of CO_2 as a challenge agent, Gorman et al. (1984) reported that 5% CO_2 inhalation resulted in 58% of panic disorder patients having a panic attack, whereas only 25% of patients panicked while hyperventilating on room air.

A hypothesis related to the chemoreceptor hypersensitivity model is Klein's "suffocation alarm" hypothesis. According to Klein (1993), patients with panic disorder have a lowered threshold for an evolved "suffocation alarm." Rising serum levels of CO_2 and lactate normally associated with suffocation are the supposed triggers for the firing of this alarm. The abnormally lowered threshold for this alarm causes chronic hyperventilation, which is adaptive in that it lowers blood CO_2 levels.

A problem with the CO_2 chemoreceptor hypersensitivity and the suffocation alarm hypotheses is that hyperventilation also produces panic attacks. Hyperventilation produces respiratory alkalosis and hypocapnea

(reduced CO_2 tension in arterial blood), and inhalation of CO_2 produces acidosis and hypercampnea (increased CO_2 tension in arterial blood). Although they have opposing physiological mechanisms, both procedures are known to produce panic more often in panic disorder patients than in control groups (van der Hout, 1988). Another issue that both Gorman's and Klein's hypotheses has yet to address is that the level of CO_2 needed to trigger the suffocation alarm is not specified. Inhalation challenges containing up to 875 times the amount of CO_2 contained in normal room air have been used in BCA studies, but do not typically produce panic attacks in 100% of patients. Failure to produce panic attacks in all panic disorder patients suggests that some other mechanism might be involved in the genesis of panic following CO_2 inhalation (McNally, 1994). In addition to CO_2 manipulations, the hypersensitive CO_2 chemoreceptor hypothesis also is frequently used as a potential explanation for the panicogenic effects of sodium lactate infusions.

Most challenge agents used in panic disorder research cross the blood-brain-barrier under baseline (resting) conditions. Separate biological models have been advanced to account for the greater sensitivity panic disorder patients' show to these diverse agents that cross the blood-brain-barrier. No single biological model (to date) is able to explain the enhanced sensitivity to all agents, although some can encompass the effects of two or three agents.

Agents that are thought not to cross the blood-brain-barrier

One of the most intriguing aspects of the biological challenge agent literature is the finding that several of the challenge agents that provoke panic do not normally cross the blood-brain-barrier. Sodium lactate, sodium bicarbonate, epinephrine and isoproterenol do not normally cross from plasma into the brain. The finding that drugs without confirmed central effects can provoke panic attacks has been taken as evidence for both the cognitive and interoceptive conditioning models of panic disorder. Alternatively, biological models have been advanced to account for the panicogenic effects that do not require the involvement of psychological mechanisms, such as conditioning and cognition. The debate between biological and psychological models of panic disorder has become the centerpiece for the larger debate between biological psychiatry and psychological models of psychiatric disorders.

By far the most frequently employed, and controversial agent in terms of mechanism of action is sodium lactate. The following sections discuss research on sodium lactate and the other challenge agents that do not normally enter the CNS.

Sodium Lactate and Panic

Pitts and McClure (1967) first administered sodium lactate as a biological challenge agent in the late 1960s. In this classic study, 10 mg/kg of sodium lactate produced panic in 93% of anxiety neurotics (a diagnostic forerunner of panic disorder), but only 12% of normal controls. Overall, approximately 70% of

panic disorder patients responded to sodium lactate with panic, compared to few if any of the normal controls (Cowley & Arana, 1990). Explaining the mechanism by which sodium lactate produces panic in vulnerable individuals may provide clues to the etiology of the disorder. Data from challenge studies have prompted theories that largely focus on the peripheral effects of sodium lactate, especially because several studies indicate that lactate does not cross the blood-brain-barrier to an appreciable degree at normal pH levels (Gurdjian et al. 1944; Sacks, 1965; Posner & Plum, 1966; Friis, Paulson, & Hertz, 1979; Sandberg & Liebowitz, 1990).

Several hypotheses have been advanced to account for sodium lactate's mechanism of action. Perhaps the most popular biological mechanism used to explain sodium lactate's production of panic attacks is the hypersensitive CO_2 chemoreceptor model. The hypersensitive CO_2 chemoreceptor model was described earlier and relies on the metabolites of sodium lactate to explain the production of panic. Sodium lactate is metabolized into bicarbonate, which increases peripheral pH and plasma CO_2 (Gorman, 1989). Sodium bicarbonate infusions also produce panic attacks, although to a lesser degree than does sodium lactate (45% versus 59%, respectively) (Gorman, et al., 1989). Because both sodium lactate and sodium bicarbonate are metabolized into CO_2, hypersensitive chemoreceptors might account for the panicogenic nature of both of these agents in panic disorder patients.

Although the CO_2 chemoreceptor hypersensitivity hypothesis explanation of sodium lactate induced panic is intuitively appealing, evidence contrary to this

hypothesis has been reported. D-lactate (an isomer of L-lactate) is panicogenic, yet is not metabolized into CO_2 (Nutt & Lawson, 1992). Findings from studies using D-lactate argue against the CO_2 chemoreceptor hypersensitivity hypothesis as a viable explanation for panic disorder. In addition to the D-lactate finding, studies addressing the permeability of the blood-brain-barrier to sodium lactate have provided evidence against the CO_2 model, and suggest that in the dosages normally administered, sodium lactate does not cross the blood-brain-barrier (BBB).

Sodium lactate and the blood-brain-barrier

Perhaps the most controversial aspect regarding sodium lactate's mechanism of action in the production of panic is the status of the agent regarding permeability of the BBB. At normal physiological pH levels, lactate exists in the ionized form, and does not cross the BBB (Gurdjian et al., 1944; Sacks, 1965; Posner & Plum, 1966; Friis, Paulson, & Hertz, 1979; Sandberg & Liebowitz, 1990). At a pH level of 7.4, only one molecule of lactate acid per 28,000 is not ionized (Oldendorf et al., 1979). When in ionized form, lactate is more polar and, therefore, less permeable to the blood-brain-barrier. Changes in pH alter the permeability of lactic acid, with permeability increasing with decreases in pH, and lowering with increased pH (Oldendorf et al., 1979).

The relatively low K_m of BBB lactate transport approximates the normal lactic acid levels in plasma. Elevations of plasma lactic acid above normal (e.g., because of exercise) are thought not to be transferred to the CNS (Pardridge &

Oldendorf, 1977), suggesting that the brain is protected from elevations of lactate. As intense exercise can elevate plasma lactic acid levels to 10mM, this protective exclusion is probably of great functional value (Pardridge & Oldendorf, 1977).

The controversy regarding sodium lactate has prompted investigators to administer sodium lactate intravenously in animal models to determine if the brain lactate levels increase following administration. Dager et al. (1990) administered 1.0 M racemic sodium lactate to mechanically ventilated, anesthetized baboons. These investigators noted a significant increase in cerebrospinal fluid (CSF) lactate levels, and suggested that sodium lactate may directly affect the CNS to induce panic. Additionally, there was no rise in levels of CSF CO_2. Several methodological problems have been pointed out regarding this study (Coplan et al., 1992). The dosage selected is double the standard sodium lactate administered (0.5 M, 10 mg/kg), raising the possibility that some threshold for blood-brain-barrier permeability was exceeded. The fact that halothane anesthesia (which is known to increase the permeability to the BBB,) was used also raises the concern that the lactate elevations were an artifact of the procedure (Nemoto et al., 1978; Angel, Bounds, & Perry, 1972; Hannon et al., 1988). Additionally, the mechanical ventilation of the subjects may have inadvertently produced a state of hypoventilation, which would itself increase central lactate levels (secondary to hypoxia), independent of the infusion. This possibility cannot be addressed, because no control group or control procedure was included in Dager et al.'s (1990) study.

Coplan et al. (1992) administered sodium lactate to Bonnet macaques in an attempt to determine whether sodium lactate, administered in levels that trigger panic in humans, penetrates the blood-brain-barrier. This study included several improvements over Dager et al. (1990), including the use of a control infusion, anesthesia maintained with ketamine, and no mechanical ventilation. Additionally, Coplan et al. (1992) used a standard amount of sodium lactate (0.5M, 10 mg/kg). The levels of CSF lactate were unchanged following sodium lactate infusion; indeed, infusing 1.5M sodium lactate in one subject did not produce a detectable increase in CSF lactate levels. Contrary to some models of panic disorder, CO_2 levels in the CNS did not rise, despite high levels of lactate and bicarbonate in plasma. The absence of CSF elevations of CO_2 (both in Dager et al., 1990 and in Coplan et al., 1992) argues against central hypercarbia as a mechanism of action for sodium lactate.

Using Magnetic Resonance Spectroscopy (MRS) imaging capable of measuring lactate concentrations in living human brains, Dager et al. (1997) reported that brain lactate levels increased in panic disorder patients and controls following an infusion of sodium lactate. Patients have greater increases in lactate; however, lactate levels were unrelated to panic response. Another study using similar technology (proton echo-planar spectroscopic imaging) replicated the findings that sodium lactate infusion increased brain lactate levels in all subjects. Panic disorder patients had greater global brain lactate increases in response to lactate infusion (Dager et al., 1999). Rises in lactate were observed globally, indicating that there was no consistent neuroanatomical pattern of

lactate elevations-in patients or controls. Neither anxiety symptoms nor panic responses were related to lactate levels in this study or in previous work by Dager et al. (1997). Based on this pattern of findings, Dager et al. (1999) suggested that brain lactate levels are not related to symptom severity. The findings of these studies must be qualified by the fact that no placebo control was included and, therefore, the studies cannot address the possible effects of general arousal on brain lactate levels (Dager et al., 1999). Dager et al. (1999) cautioned that hyperventilation among all subjects "undoubtedly contributed to the brain lactate rise in response to lactate infusion" (Dager et al., 1999, p. 76). Even with methodological limitations taken into consideration, the findings of these studies are interesting in that the presence of lactate did not relate to anxiety symptoms, suggesting that the lactate ion may not be necessary for anxiety production. The greater rise in lactate levels suggests that patients may have received a greater amount of the compound into the CNS.

While there has been controversy for the past few decades regarding how sodium lactate produces panic attacks in vulnerable subjects, the debate has largely centered on lactate, and not on the sodium cation in sodium lactate. In a recent study, Peskind et al. (1998) reported that hypertonic sodium infusions and sodium lactate induced panic attacks at equivalent rates in panic disorder patients. This finding suggests that neither the presence of lactate nor its metabolites are necessary for inducing panic attacks. Both the L and D isomers of sodium lactate and sodium bicarbonate are all hypertonic solutions (as is hypertonic sodium chloride) and each produces high levels of sodium in the

bloodstream. Peskind et al. (1998) suggested that hypernatremia (high sodium levels in plasma) may be the operative factor in lactate and hypertonic saline-induced panic, possibly via a stimulatory effect on brainstem catecholaminergic systems.

The hypertonic (having a higher osmotic pressure than surrounding fluid) nature of sodium lactate, sodium chloride, sodium bicarbonate, and hypertonic saline also suggests another possible reason for the generation of panic attacks with these agents. Hypertonic solutions increase the permeability of the blood-brain-barrier to a variety of tracers and drugs (Neuwelt, 1989; Neuwelt, Johnson, Blank, Pagel, Maslen-McClure, McClure, & Wu, 1985; Brightman, Hori, Rapoport, Reese, & Wetergaard, 1973; Rapoport, Fredericks, Ohno, & Pettigrew, 1980; Neuwelt, Goldman, Dahlborg, et al., 1991; Rapoport & Robinson, 1986; Gumerlock & Neuwelt, 1990; Pappius, Savaki, Fieschi, Rapoport, & Sokoloff, 1978; Rapoport, 1988; Rapoport, Hori, & Klatxo, 1972). The proposed mechanism of action for osmotic opening of the BBB involves shrinkage of brain capillary endothelial cells, resulting in an opening of the tight junctions that compose the BBB (Rapoport, 1976). This shrinkage is a result of the hypertonic environment produced by hyperosmotic fluid infusion. This property of hypertonic solutions (inducing hyperosmolarity) may increase the permeability of substances (endogenous and exogenous) in the plasma.

If hypertonic solutions lower the blood-brain-barrier permeability threshold, then normally excluded circulating catecholamines (EPI, NE) or even excitatory amino acids may enter and stimulate regions of the CNS that mediate fear

responses. This possibility has not been addressed in the literature on panic disorder. The infusion of a solution that increases permeability to the BBB, coupled with the permeability changes effected by stress itself (see below) might allow circulating stress hormones to enter the CNS. This hypothesis provides a potential mechanism for L-lactate and D-lactate, sodium bicarbonate, and hypertonic saline-induced panic. While hypernatremia may indeed stimulate brainstem catecholaminergic systems, this hypothesis does not account for why only a portion of panic disorder patients panic with lactate (e.g., hypernatremia occurs in all subjects, patients and controls). Baseline stress produces elevations in plasma levels of catecholamines and serotonin which, in turn, may have play a role in the greater reactivity to panicogenic agents.

In summary, lactate-induced panic is not readily explainable by biological models. At normal pH, the vast majority of lactate is ionized and therefore impermeable. Studies which have sought to address whether sodium lactate in dosages administered to produce panic have contradicted the hypothesis that high levels of CO_2 are a viable explanation of panic, and have presented mixed evidence concerning blood-brain-barrier crossover.

Peripheral β-Adrenergic Receptor Hypersensitivity

Peripheral β-adrenergic receptor hypersensitivity has been considered as a possible mechanism for the etiology of panic disorder (Rainey et al., 1984). Several investigators (Frohlich, Tarazi, & Dustan, 1969; Easton & Sherman, 1976) have noted the similarity of symptoms of patients with β-adrenergic

hypersensitivity and panic patients. In a double blind study of isoproterenol (a β-adrenergic agonist) infusions in panic disorder patients and normal controls, 63% of panic disorder patients but only 11% of control subjects panicked (Freedman, Ianni, & Ettedgui, 1984). Rainey et al. (1984) compared the effects of isoproterenol to lactate infusions, reporting that isoproterenol and lactate-provoked panic attacks were both similar to naturally occurring panic attacks (as rated by subjects). However, isoproterenol-provoked panics were generally less severe than attacks provoked by lactate. Hypersensitive β-adrenergic receptors might explain the panicogenic effects of caffeine, because caffeine increases plasma epinephrine and norepinephrine in human subjects (Robertson, 1981). Norepinephrine and epinephrine have both been used to produce panic attacks, but neither is thought to cross the BBB at baseline (Pyke & Greenberg, 1986; Veltman, Zijdarveld, & Dyck, 1996). The finding of heightened sensitivity to these agents is also compatible with peripheral β-adrenergic hypersensitivity, because both are β-adrenergic agonists.

The β-adrenergic receptor hypersensitivity hypothesis of panic disorder was assessed by infusing low doses of isoproterenol to panic disorder patients and normal controls (Nesse, Cameron, Curtis, McCann, & Huber-Smith, 1984). The low doses of isoproterenol should have provoked stronger physiological reactions in the panic disorder patients if panic disorder patients' peripheral β-adrenergic receptors were hypersensitive. Contrary to prediction, the normal controls showed greater reactivity, implying that β-adrenergic receptors may in fact be down-regulated in panic disorder patients.

Because isoproterenol does not usually cross the blood-brain-barrier, its panicogenic effects have been offered as evidence for cognitive or interoceptive conditioning theories. These theories implicate either the psychological interpretation of, or the conditioned responses to peripheral symptoms of anxiety in the genesis of panic attacks. Taken together, down-regulation of peripheral beta-receptors and the lack of crossover into the central nervous system suggest that peripherally-based biologic dysregulation models cannot account for isoproterenol's effects. Norepinephrine and epinephrine's panicogenic effects in panic disorder patients warrant the same conclusion because epinephrine also does not enter the CNS (Pyke & Greenberg, 1986; Veltman, Zijdarveld, & Dyck, 1996).

Several challenge agents used in panic disorder research demonstrate little to no crossover of the blood-brain-barrier. Biological models are not able to account for the greater sensitivity panic disorder patients show to agents that do not cross the blood-brain barrier. The one theory positing a peripherally based biologic dysregulation (β-adrenergic receptor hypersensitivity hypothesis) has been contradicted by experimental evidence.

Summary and Critique of Biological Models for Panic Disorder

The introduction to this section noted several weaknesses in biologically based explanations for panic disorder. Reviewing the biological challenge agent literature and the biological models for panic reveals that agents that cross the

blood-brain-barrier can be explained by biological models, no one model can explain sensitivity to all challenge agents. Contrary to agents that do penetrate the blood-brain-barrier, those that do not demonstrate CNS crossover have not been adequately explained via biological models.

Besides the BBB issue, several other issues must be considered to evaluate the validity of biological models for panic disorder. These issues include: 1) panic disorder patients demonstrate a homogeneous behavioral profile; 2) the existence of "subsets" of patients with dysregulation; and 3) the assumption that any neurochemical dysregulations precede, rather than result from, the occurrence of panic attacks. These conceptual flaws argue against acceptance of a purely biological explanation for panic disorder. The following discussion considers each of these issues in depth.

It might be argued that while there are several biological hypotheses implicating dysregulation, a single biological dysregulation might eventually be found to underlie panic disorder. If this proves to be so, then the homogeneous behavioral profile of panic disorder would no longer be problematic. It is certainly possible that one of the biological models is correct, and that a panic disorder is characterized by dysregulation. However, a second conceptual flaw of biological models presented below argues against this possibility.

The supposition that "subsets" of panic disorder patients may have some biological abnormality is another conceptual weakness of biological models in general. The conception that "subsets" of patients may have the proposed abnormality is central to biological hypotheses because their explanations for the

results of biological challenge studies depend on this supposition. Before examining the evidence for these proposed subsets of panic disorder patients, the origin of the notion that there are "subsets of patients" with specific biological abnormalities should be clarified.

In the typical biological challenge study, not all of the panic disorder patients experience a panic attack in response to the challenge agent. For example, about 70% of panic disorder patients panic when given sodium lactate (Sandberg & Liebowitz, 1990). The fact that only some patients panic in response to biological challenge agents is the sole basis for the claim that within the larger population of panic disorder patients, a "subset" has the proposed abnormality. This finding suggests that those patients who do not panic do not have this abnormality, and healthy controls that do panic, by logical extension, also have the abnormality.

There seems to be an implicit acceptance of the validity of the idea that subgroups of panic disorder patients have at least one specific biological dysregulation (McNally, 1994). It is both premature and hazardous to accept this idea, because there is no convincing evidence for the concept. For example, it has not been reported that those subjects who panic in response to a challenge agent targeting a specific system remain constant over time. If the subset of patients or controls who panic in response to a specific challenge agent on one occasion is different from the subset who panic on later occasions, then the notion of subsets becomes less tenable. No systematic study of consistency of responses to challenge agents has been reported. Re-administration of a

challenge agent to the same group of subjects has been done in a few studies (Keck et al., 1993; Yeragani et al., 1988). These studies have typically involved adding a treatment medication to assess whether the medication can prevent panic upon re-administration with the challenge agent and do not address this broader issue of categorization.

If there are subsets of patients with specific abnormalities, then it would be worthwhile to examine whether panic disorder patients can be categorized based on their responses to multiple challenge agents. If patients with a consistent vulnerability to lactate also have vulnerability to yohimbine, but not to flumazenil, then this finding would be useful to integrate these various biological hypotheses. Such sub-typing of panic disorder patients might lead to findings of differential response to therapies and differing etiological factors. No systematic evaluation of multiple vulnerabilities has been reported. However, a few studies have examined the comparative panicogenic effects of two different challenge agents (e.g., Strolhe et al., 1998; Rainey et al., 1984; Ettedgui, 1984). Strolhe et al.'s (1998) study was the first to report that one group (sodium lactate responders) do not panic with a second agent (flumazenil). This one study has a small number of subjects (N=10), and the effect has yet to be replicated. If an aberrant biological vulnerability is actually the cause for a subset of panic disorder patients panicking, then the smaller subset of healthy controls who panic should also have the same biological vulnerability. This logical conclusion is typically not examined on theoretical or experimental grounds. If biological models are indeed correct, then studying this subset may be fruitful.

Finally, the issue of serial precedence should be considered. Within biological models, the panic attacks that patients experience are attributed to a pre-existing biological dysregulation. It is also conceivable that a history of experiencing panic attacks is actually the cause of a biological dysregulation, if such dysregulation does exist. The lack of prospective studies demonstrating that a biological dysregulation precedes the onset of panic disorder suggests that this possibility should not be dismissed. The plausibility of this possibility is supported by data from investigations of the learned helplessness effect. Studies of the physiological basis of the learned helplessness effect in animals suggest that the repeated experience of intense, uncontrollable and inescapable aversive events leads to actual changes in neurochemistry (Weiss, Stone, & Harrell, 1970). Panic attacks certainly are aversive and are often perceived as uncontrollable and unpredictable by patients (McNally, 1994). Therefore, rather than being a result of changes in neurotransmitter systems, panic attacks may be the cause of such changes.

Overall, the data supporting biological models of panic disorder are mixed. Some hypotheses have received support from studies using challenge procedures, only to later have predictions directly drawn from the hypothesis fail to support or contradict the hypothesis. Biological models of panic disorder have proposed an array of dysregulations to account for a disorder that has a homogeneous behavioral profile. These theories all rest on two unproven assumptions: 1) there are subsets of patients with specific (possibly multiple) vulnerabilities; and 2) dysregulations are causal, rather than consequential to the

onset of panic attacks. Given the tenuous status of biological explanations of panic disorder, it is possible that some other mechanism will account for the onset and maintenance of panic disorder.

Section II presents a hypothesis that might account for the heightened sensitivity of panic disorder patients to challenge agents. Before presenting this new hypothesis, two other influential accounts for panic disorder, both of which can be characterized as psychological explanations, are discussed.

Psychological Models

Cognitive Model

One of the most influential models for the etiology of panic disorder is Clark's (1986) cognitive model. This model views panic attacks as the result of catastrophic misinterpretation of bodily sensations associated with anxiety. The cognitive theory of panic disorder is often referred to as the catastrophic misattribution model. Within the cognitive model of panic disorder, bodily sensations or external stimuli can serve as triggers for a perceived threat. The patient then experiences apprehension regarding the perceived threat, which produces bodily sensations. It is the catastrophic misinterpretation of these bodily sensations that produces panic attacks (Clark, 1986, 1988). Contrary to the interoceptive conditioning model of panic disorder, bodily sensations are not triggers for a reflexive conditioned response of panic attacks. Instead, bodily sensations are one step in a positive feedback loop that culminates in a panic attack. For example, a patient climbing a set of stairs might notice their heart

beating faster than normal. The person perceives this rapid heartbeat as threatening and experiences apprehension. This apprehension produces bodily sensations of anxiety that are interpreted in a catastrophic manner (Clark, 1986, 1988). Examples of such cognitions would be: "I'm having a heart attack" or "I'm about to die." The cognitive model for the onset of panic attacks is presented schematically in Figure 2.

Clark (1988) argues that biological challenge agents provoke panic attacks by exacerbating a biological dysfunction. Instead, panic disorder patients are simply responding with catastrophic misinterpretations to sensations produced by these agents. All biological challenge agents produce bodily sensations that might trigger these catastrophic misinterpretations. As biological challenge agents produce sensations and act on biological substrates, determining the cause of laboratory-induced panic remains elusive.

Following biological challenges, panic disorder and healthy controls experience similar bodily sensations, yet only panic disorder patients respond with fears of dying or going crazy (Sanderson, 1988). This finding has been taken as support for cognitive theory. Additional support was provided by a study in which patients were given reassuring information regarding infusion of lactate. Reassuring information resulted in significantly lower panic rates in the group receiving reassurance (20%) compared to the group that received no reassurance (90%) (Clark, Salkovskis, & Anastasiades, 1990).

The cognitive model of panic proposes that once initiated, the panic sequence eventually culminates in a panic attack. There is a practical problem

with this vicious circle formulation; namely, that there is no clear reason why individual panics attacks, once initiated, should cease. Once a panic attack occurs, it should produce strong physical sensations associated with anxiety that should also be catastrophically misinterpreted. The vicious circle should continue to spiral, growing stronger and stronger. It is possible that a negative feedback system is operating, which terminates a panic attack after it has started. While this makes intuitive sense, cognitive and biological theories do not propose such a system. The biological theories also fail to address the reason why panic attacks end (Radomsky et al., 1998).

Contrary to the cognitive model of panic disorder, Aronson, Whitaker-Azmitia, and Caraseti (1989) reported that laboratory-induced panic attacks can occur in the absence of cognitive misinterpretations. Rachman (1988) also reported that for the patients in his sample, a large percentage of panic attacks are not accompanied by fearful cognitions. In another study, catastrophic misinterpretations were found to be a consequence of panic attacks rather than preceding the attacks (Wolpe & Rowan, 1988). In each of these studies, panic attacks were not preceded by catastrophic misinterpretations, arguing against the necessity for misattribution of symptoms for panic onset. The occurrence of nocturnal panic attacks, and panic attacks triggered by relaxation also provide evidence against a purely cognitive theory for panic. Nocturnal panic typically erupts during non-REM sleep and, therefore, is unlikely to be preceded by catastrophic misinterpretations (Craske & Freed, 1995; Craske & Barlow, 1989). Panic attacks triggered by relaxation occur fairly often (Cohen, Barlow, &

Blanchard, 1985), yet are difficult to reconcile with cognitive model of panic in that a perceived threat is difficult to identify.

In response to the criticism that some attacks are not preceded by catastrophic misinterpretations, Clark (1988) adjusted his model to include unconscious cognitions. Clark (1988) suggested that, in some cases, catastrophic misinterpretations occur so fast and automatically that panic disorder patients do not perceive them. This supposition has drawn criticism, because it is nearly impossible to test whether these supposedly unconscious catastrophic misinterpretations occur (McNally, 1994).

While the cognitive model of panic disorder has been highly influential and has inspired effective treatments, it has difficulties explaining many phenomena associated with panic disorder. The occurrence of panic attacks without fearful cognitions and the occurrence of nocturnal and spontaneous panic attacks (neither of which are preceded by catastrophic cognitions) compromise this model's validity.

Therefore, while the cognitive model has broad appeal, it cannot be regarded as a definitive explanation for panic disorder. Moreover, because some patients have panic attacks without catastrophic cognitions, the cognitive model cannot account for the higher sensitivity of panic disorder patients to biological challenge agents, without appealing to cognitions that occur outside of awareness.

Pavlovian interoceptive conditioning

The interoceptive conditioning conceptualization for the origin of panic disorder posits that conditioned fear to symptoms of bodily arousal cause panic attacks. In this formulation, the initial panic episode occurs as an unconditioned response as a result of a biological or psychological event such as hyperventilation or drug use (Goldstein & Chambless, 1978; Wolpe & Rowan, 1988). This initial panic attack typically occurs during periods of high stress and serves as an unconditioned response (UCR), becoming associated with the symptoms of bodily arousal that preceded the attack (Goldstein & Chambless, 1978; Wolpe & Rowan, 1988). These bodily arousal symptoms come to serve as conditioned stimuli (CS) for further panic attacks that occur as a conditioned reaction when exposure to internal sensations occurs. Examples of interoceptive cues that trigger panic include: higher heart rate, palpitations, rapid respirations, and shortness of breath (Acierno, Herson, & Van Hasslet, 1993). A schematic adaptation of the interoceptive-conditioning model of panic disorder is presented in Figure 3.

The interoceptive conditioning model is similar to the cognitive model of panic in many ways. Both models hold that the occurrence of bodily sensations triggers panic attacks and both view panic attacks following biological challenges as the result of reactions to challenge-induced bodily sensations. The interoceptive conditioning and cognitive models share the view that panic disorder patients do not have a biological dysregulation (Goldstein & Chambless, 1978; Wolpe & Rowan, 1988). In contrast to the cognitive model, the

interoceptive-conditioning model holds that cognitions do not play a causal role in panic disorder (Goldstein & Chambless, 1978; Wolpe & Rowan, 1988).

The interoceptive conditioning model of panic disorder has been criticized by researchers who claim that the conditioned stimulus and conditioned response are qualitatively identical (Reiss, 1988; McNally, 1990). According to these critics, the "low level of arousal" as a conditioned stimulus triggers the conditioned response of "increased arousal." Additionally, the first panic attack was cited by one critic (McNally, 1990) as serving as both the UCS and the UCR, a situation which requires the UCR to elicit itself (if the UCR is a panic attack). These criticisms, however, were, based on misunderstandings of the conditioning model of panic. Within the interoceptive conditioning model, panic attacks are both the unconditioned and the conditioned responses, but not the unconditioned stimulus (Goldstein & Chambless, 1978; Wolpe & Rowan, 1988). The UCS within the interoceptive conditioning model is the phenomenon of hyperventilation-induced dyspnea, which leads to panic (the UCR). Other stimuli besides dyspnea have been noted to function as a UCS and trigger the initial panic attack, such as cocaine, LSD, withdrawal from psychotropics, and various medical conditions (Acierno, Herson, & Van Hasslet, 1993).

Regardless of the cause for the initial panic episode, the conditioning model proposes that interoceptive stimuli present before this first panic attack may acquire the ability to elicit panic (Acierno, Herson, & Van Hasslet, 1993). The primary criticisms of the interoceptive conditioning model for panic have centered on the ambiguity of the UCS (Sanderson & Beck, 1989; McNally, 1990,

1994). Of relevance to this point, conditioning theorists have contended that exposure to an identifiable environmental UCS is neither necessary nor sufficient to initiate fear conditioning (Carter & Barlow, 1995; Forsyth & Eifert, 1996).

The "Learned Alarm" model of panic disorder is conceptually related to Goldstein and Chambless (1978) and Wolpe and Rowan's (1988) account for panic disorder (Barlow, 1988). The Learned Alarm model is presented in Figure 4. This model adds several variables to the classical conditioning account for panic onset, such as biological vulnerability, psychological vulnerability, and agoraphobic avoidance (Barlow, 1988). Within the Learned Alarm model of panic onset, the aversive event is the experience of a panic attack, which serves as the UCR. This panic attack (a false alarm within this theoretical framework) becomes associated with bodily sensations that preceded the attack. Later panic attacks (learned alarms) are triggered by exposure to bodily sensations. A fundamental difference between the interoceptive conditioning model and the learned alarm model is that the latter does not specify a UCS.

The interoceptive conditioning model contends that panic attacks following biological challenge agent administration are the result of conditioned fear of bodily sensations produced by biological challenge agents. Besides offering an account for biological challenge agent-induced panic attacks, the interoceptive-conditioning model may account for many other phenomena associated with panic disorder. Interoceptive conditioning has been used to account for the occurrence of spontaneous panic attacks and relaxation-induced panic attacks. The occurrence of spontaneous panic attacks has been cited as evidence

against a cognitive account of panic disorder (McNally, 1994). Spontaneous panics are distinguished by the absence of external cues or catastrophic thinking. These episodes, however, may be preceded by bodily sensations that occur inside or outside of awareness. The interoceptive-conditioning model can provide a potential explanation for so-called "spontaneous" panic attacks. According to the interoceptive conditioning model, spontaneous panic attacks are caused by the occurrence of bodily sensations that the patient is not aware of, triggering a conditioned response. Bodily sensations acting as conditioned stimuli also may be a viable explanation for the paradoxical occurrence of "relaxation-induced panic attacks." As relaxation is typically accompanied by bodily sensations (Cohen et al., 1985), patients who have a conditioned fear of interoceptive cues might experience attacks following relaxation.

A concept related to the other psychological models of panic is anxiety sensitivity. Anxiety sensitivity is conceptualized as a trait-like tendency to respond fearfully to anxiety symptoms (McNally, 1994). The key difference between anxiety sensitivity and the cognitive model is that patients do not have to misconstrue anxiety symptoms as something else (e.g., a heart attack) to have them be aversive. People with high anxiety sensitivity do not necessarily have to fear they are having a dreaded outcome, but, rather fear the symptoms can lead to a dreaded outcome. While there is a substantial literature on anxiety sensitivity as a factor in anxiety disorders, the present research does not fully address this topic because on a functional level, it is equivalent to one or the other of the other psychological models. For example, one might show a

tendency to be fearful of anxiety symptoms, and this tendency would be fully explainable in terms of the conditioning history of the person (interoceptive conditioning model). If this person also tended to have cognitions regarding anxiety symptoms that are not catastrophic in and of themselves (e.g., "I'm having a heart attack" vs. "anxiety can lead to heart attacks"), then this would still equate to having cognitions regarding bodily sensations, which spiral into a panic attack (cognitive model). In summary, anxiety sensitivity, like any other psychological trait, can be understood based on the learning history of the subject, and simply naming a behavior a "trait," whether it be anxiety sensitivity, or in general, adds nothing to the explanatory power of the concept.

Whether as conditioned fear cues, or as the subject of catastrophic misinterpretations, bodily sensations hold a central place in each of the psychological models for panic disorder. Studies using human subjects must account for the relative roles of conditioning history and catastrophic cognitions. The ability to control for differences in the interoceptive conditioning history of patients and controls has yet to be developed. Therefore, the possibility that subjects who are more sensitive to challenge agents have a conditioned fear to challenge-evoked sensations cannot be ruled out using human subjects. Additionally, the cognitive hypothesis of panic disorder posits the occurrence of unconscious, catastrophic cognitions. Based on the inability to control for conditioning history and catastrophic cognitions, determining the cause for greater reactivity to challenge agents using human studies alone seems unlikely.

Summary of etiological models

Both biological and psychological accounts for panic disorder have been advanced. For the past two decades, there has been controversy as to the cause of panic disorder. While the present review suggests that psychological models have the widest breath of explanatory power, no consensus exists in the field as to whether conditioning and cognitive models are more accurate to explain the origin and maintenance of panic disorder (McNally, 1994; Sanderson & Beck, 1989). The specific biological models of panic advanced cannot account for some findings that are inconsistent with their suppositions, and all rest on unsubstantiated claims that subsets of panic disorder patients are characterized by biological dysregulation. Additionally, biological models presuppose that these hypothetical dysregulations precede the onset of panic attacks--a supposition that has not been empirically studied. It may be that the experience of panic attacks leads to dysregulations across multiple neurotransmitter systems. Biological challenge agents act on some biological substrate, yet also cause bodily sensations. These bodily sensations may be catastrophically misinterpreted, triggering panic attacks. It also is possible that panic attacks following biological challenges are a classically conditioned response, being elicited by bodily sensations.

Therefore, the biological challenge literature provides a perplexing quandary: Is the greater sensitivity to biological challenge agents a result of psychological factors or biological dysregulation? Besides the biological and psychological explanations proposed to date, another

possibility is that a yet unexamined phenomenon might account for panic disorder patient's enhanced sensitivity to biological challenge agents. The following section presents a hypothesis that can potentially explain the greater sensitivity panic disorder patients have to the panicogenic effects of all biological challenge agents.

SECTION II: Blood-Brain Barrier Permeability Hypothesis for the Greater Sensitivity of Panic Disorder Patients to Biological Challenge Agents

Introduction

Currently, there is marked controversy regarding the greater sensitivity of panic disorder patients to biological challenge agents. Both biological and psychological models have been advanced to explain enhanced sensitivity to challenge agents. The present section presents a potential explanation for panic disorder patient's enhanced biological challenge agent sensitivity that does not rely on either biological dysregulation or psychological traits. This novel hypothesis integrates data from research on the blood-brain-barrier, animal and clinical findings on the actions of anxiogenic drugs.

Stated simply, the hypothesis is that the greater sensitivity of panickers that occurs in biological challenge agent studies may be the result of a transient increase in the permeability of the blood-brain-barrier (BBB). This increased permeability of BBB is hypothesized to be a result of differing levels of baseline anxiety between panickers and controls immediately prior to drug administration. The rationale for the hypothesis is based on literature (reviewed in detail below) that stress increases the permeability of the BBB to a variety of compounds. This stress-induced BBB permeability increase is conceptualized as a state variable, and not a trait. Patients with high basal anxiety/stress before drug administration would have lowered thresholds for drug entry. This increased

permeability would result in greater amounts of normally permeable compounds, and possibly allow the entry of normally impermeable compounds.

Even though they are administered the same dose of a compound, panic disorder patients who have a greater response to biological challenge agents may, in fact, be receiving a functionally higher dose because of greater entry of the drug into the central nervous system. Therefore, the hypothesis suggests that the leftward shift in the dose response curve seen in panic disorder patients may be a result of pharmacokinetic differences, rather than a result of biological dysregulation, catastrophic misattribution, or interoceptive conditioning. Additionally, this hypothesis may offer an explanation for the effects of agents that normally demonstrate poor permeability to the CNS.

The hypothesis makes several predictions regarding the panic disorder patients' reactions to biological challenge agents. For the hypothesis to be correct, these predictions would need to be borne out in the literature on biological challenge agent administration in panic disorder patients. These predictions are outlined below:

1) Compared to normal controls, panic disorder patients in BCA studies would necessarily have to have higher baseline anxiety.
2) Compared to other patients who do not panic, those patients who do panic would have to have higher baseline anxiety.
3) Indices of baseline anxiety would be statistically predictive of those who panic in biological challenge agent studies.

4) Decreasing baseline anxiety by any means (situational or pharmacological) before administration of a biological challenge agent would attenuate the rate of panic attacks.

A large literature (reviewed in detail below) does indeed suggest that baseline anxiety preceding biological challenge agent administration is higher in patients who experience panic attacks (predictions 1 & 2), and is even predictive of who will experience a drug-induced panic attack (prediction 3). Additionally, several environmental manipulations that lower baseline anxiety affect biological challenge agents (prediction 4).

It should be remembered that while a relationship between baseline anxiety level and response to biological challenge agents has been strongly supported by clinical studies, the mechanism of action for these relationships remain controversial. Moreover, the possibility that stress-induced permeability changes in the blood-brain-barrier may play a role in panic disorder has yet to be examined.

The current hypothesis is not limited to humans, with or without pathological states (panic disorder). It can be generalized to responses to anxiety or stress, suggesting that animals will display greater reactivity to anxiogenic drugs when tested under stress. Pre-clinical literature on the effects of classical "anxiogenic" drugs as well as panicogenic drugs (biological challenge agents) supports this hypothesis. Representative studies from animal models are reviewed in the next section. Because of the breadth of the pre-clinical literature on anxiogenic drugs, the research discussed below centers on studies

examining anxiogenic drug effects in non-human primates and the elevated plus maze. This literature suggests that responses to anxiogenic drugs is mediated by the context of administration, with stressful contexts generally producing greater anxiogenic reactions.

Although the literature on non-human animals' reactions to anxiogenic drugs is strongly suggestive that acute stress or anxiety enhances drug response, direct evaluation of the possibility has yet to be examined.

The following section includes a brief review of stress, the blood-brain-barrier, and research on the effects of stress on the blood-brain-barrier. Evidence of the role of baseline stress/anxiety levels on drug response from biological challenge studies is then presented. Pre-clinical research on anxiogenic drugs that support this pharmacokinetic explanation is also reviewed.

Stress

Modern conceptions of stress emphasize the interaction of the organism and its environment. The organism is seen as being confronted with various challenges from its external or internal environment that may or may not elicit a stress response. Stress produces an array of responses that include hormonal, cardiovascular, metabolic, and neural reactions. The following section presents only those aspects of the stress response that are directly related to the present research. Additionally, the relevant literature on mediators of stress responses also is presented.

Historical Understanding of Stress

Walter Cannon (1914) identified the fight-or-flight response that occurred in response to threatening or demanding situations and prepared the organism to confront the threat (fight) or escape the situation (flight) to ensure survival. This initial fight-or-flight response, which was characterized by activation of the sympathetic nervous system (increased heart rate, respiration and blood pressure), acted to mobilize the organism towards reducing the stress by activating the bodily systems necessary to respond. In the late 1930's, Hans Selye proposed a three-phase model for the stress response that he called the General Adaptation Syndrome (GAS). Phase one (alarm) prepares the individual to respond to the stressor. Phase two (resistance) allows the body to adapt to the stressor in an attempt to reestablish homeostasis. If the individual does not effectively deal with the stressor or if repeated activation occurs, then the

organism enters the third phase (exhaustion) in which symptoms of disease and disorder become manifested (Selye, 1976).

Hormonal aspects of the stress response

Exposure to both physiological and psychological stressors produces elevations in blood levels of norepinephrine (NE) and epinephrine (EPI). In humans, stressors such as taking examinations, loss of control, conflict, shock exposure, urban commuting and numerous others elevate NE and EPI blood levels (Frankenhaeuser, 1972; Frankenhaeuser, 1971; Patkai, 1971; Collins & Frankenhaeuser, 1978; Frankenhaeuser, 1977, 1978; Johansson, 1976; Singer, Lundberg, & Frankenhaeuser, 1978). In rodents, forced exposure to novel contexts, such as a new cage or a novel chamber, triggers the release of various stress hormones such as ACTH, norepinephrine, corticosterone and epinephrine (Dobrakovova & Jurcovicova, 1984; McCarthy, 1982; Weinstick, Razin, Schorer-Apelbaum, Men, & McCarthy, 1998; Kirby, Callahan, McCarthy, & Johnson, 1989).

Stress elevates plasma levels of serotonin (5HT) in rodents (Malyszko et al., 1994; Takada, et al., 1996; Malyszko Urano, Takada, & Takada, 1994; Sharma & Dey, 1986,1987; Oztas, 1995; Sharma, Navarro, & Dey, 1991, Sharma et al., 1996) and humans (Sauebier & von Mayersbach, 1976; Kalandarov, Frenkel, & Nekrasova, 1980) during anticipation of stress or following exposure to a stressor. The elevation of 5HT in plasma has been linked to increased permeability to the blood-brain-barrier in several stress

paradigms (Sharma & Dey, 1986; 1987; Sharma, Nyberg, Cervos-Navarro, & Dey, 1992; Sharma, Navarro, & Dey, 1991, Sharma et al., 1996).

Mediators of stress responses

Control: Heightened control over a stressor decreases the impact of the stressor. Compared to rats receiving the same amount of shock, rats with control over shock administration displayed attenuated stress responses (Weiss, 1968). The finding that stress with control produces fewer negative consequences is supported by findings from studies offering subjects a choice between self-administered and experimenter-administered shock. The shock schedule with an element of control is consistently preferred over externally-determined schedules (Pervin, 1963; Le Panto, Moroney, & Zenhausern, 1965; Staub, Tursky, & Schwartz, 1971).

Several studies have reported that perceptions of control in occupational settings can affect the stress levels of workers. Self-paced jobs (high control) were associated with lower stress, while machine paced (low control) were associated with higher stress levels. Importantly, both peripheral catecholamines and self-reported distress were associated with levels of control (Frankenhaeuser & Gardell, 1976; Frankenhaeuser & Johansson, 1982). Lack of control also has been associated with increased corticosterone release, which is another indicator of stress (Maier, Laudenslager, & Ryan, 1985).

Even the perception of control has powerful effects to mediate the response to stress. Glass and Singer (1972) reported that subjects given the

perception that they could predict and control noise stress had fewer negative aftereffects and adapted to the stressor faster than did subjects without perceived control. A similar effect was noted by Staub, Tursky, and Schwartz (1971) who gave subjects a perception of control over shocks. Subjects with perceived control reported less discomfort from the shocks.

Information: Information that allows subjects to appraise a stressor as less threatening can alter subject's reactions to stressors. Providing information that reduces the perception of threat decreases psychological and physiological reactions to exposure to threatening situations (Lazarus et al., 1965; Lazurus & Alfert, 1964, Nomikos et al., 1968). These studies indicate that altering the appraisal of a threat can decrease the stress evoked by exposure to the threatening event. Informational mediation of stress responses becomes directly relevant to understanding biological challenge agent studies that alter the level of information given to subjects. These studies, which are discussed in a later section, note reduced panic levels in subjects provided with reassuring information.

Social support: Social support is a major mediator of responses to stress. Social support has been defined as a feeling of belonging to a social network (Cobb, 1976). High levels of social support are associated with improved recovery from illness (Chen & Cobb, 1960) and surgery (Whitcher & Fisher, 1979). Additionally, social support improves coping responses to stressors including the loss of a parent, as well as job loss, hospitalization,

pregnancy, and threat of injury or death (Burch, 1972; Cohen & Wills, 1985; Kessler, Price, & Wortman, 1985).

A debate regarding whether social support improves stress-related outcomes by direct or indirect means has existed for years. Social support has been hypothesized to be directly beneficial to an individual (direct effects hypothesis), and to be beneficial only when individuals are under stress (buffering hypothesis). Research in the area of social support has provided evidence for both of these viewpoints (Cohen & Wills, 1985). Regardless of whether social support provides its stress reduction effects directly or by buffering the effects of stress, high social support is associated with lowered stress levels.

In summary, decreasing perceived level of control increases the level of stress in subjects. Providing information relevant to threatening events and having social support present decreases stress in subjects. The role of perceived control, providing information, and social support to alter baseline stress levels of subjects undergoing laboratory challenges will be noted in a subsequent section.

Blood-brain-barrier

History

In the late 19th century, the German microbiologist Paul Ehrlich conducted the first experiments of the blood-brain-barrier (Ehrlich, 1885). Using peripherally injected dyes, he found that nearly all bodily tissues were stained, except for the brain. In a later experiment, a student of Erilich's conducted an experiment where dye was injected into the cerebral spinal fluid, resulting in staining of the brain, but not the surrounding tissues (Goldmann, 1913). These early studies indicated that there was a physical barrier protecting the brain from insult.

Stern and Gauthier (1921) reported that some drugs given into the blood could not be detected in the CSF. Further, Stern and Gauthier (1921) reported that substances that entered the CSF affected CNS functioning, whereas those that did not enter had no effect on CNS function.

Brodie et al. (1960) first noted the vital importance of lipid solubility in the passage of drugs into the CSF. Reese et al. (1967) found that cerebral capillaries are the anatomical site of the blood-brain-barrier. Astrocyte foot processes surround the cerebral capillaries, and were believed to be the primary basis for the exclusion of substances from the brain. Brightman (1967) and others (Karnovsky, 1967; Reese & Karnovsky, 1967) found that, rather than the astrocyte foot processes, the anatomical basis of the blood-brain-barrier was primarily a result of "tight junctions."

Structure and Function

Structure: There are over 400 miles of brain capillaries in the human head (Pardridge, 1991). The vertebrate brain capillary has several special anatomical features, including the presence of tight junctions, minimal endothelial pinocytosis, and coverage of the great majority of the brain endothelium by astrocyte foot processes. The tight junctions are comprised of intercellular attachments between the capillary endothelial cells and are unique to the blood-brain-barrier. Tight junctions eliminate the pores that are present in the walls of capillaries perfusing peripheral tissues. The brain capillary endothelium also have low numbers of pinocytocic vesicles (Pardridge, 1991). The normal function of these vesicles is to transport substances across membranes. Astrocytes are a distinct type of glial cell that function to support neurons and form a part of the endothelial blood-brain-barrier (Schlossauer, 1993).

Some regions of the brain lack a blood-brain-barrier (e.g., have no tight junctions) allowing rapid exchange of solutes between the blood and brain. These areas, known as the circumventricular organs (CVO), are comprised of the median eminence, pineal gland, subfornical organ, area postrema, neurohypophysis (posterior pituitary gland), and the choroid plexus (Davson & Segal, 1996). These organs are not typical of the rest of the brain. In fact, these organs are separated from the rest of the brain by the presence of tight junctions, and substances that enter the "leaky" CVO's do not necessarily gain entry to the CNS as a whole (Davson & Segal, 1996).

Function: The blood-brain-barrier functions as a means of selectively restricting substances from entry into the brain. If the blood-brain-barrier completely blocked exchange of substances, then the result would be lethal, because nutrients and metabolic wastes could not be exchanged. For essential nutrients, specific membrane transport systems exist in high concentrations in endothelial cells and supply the CNS.

Besides the anatomical blood-brain-barrier, some substances are prevented from CNS entry via an enzymatic barrier. The enzymatic barrier metabolizes certain substances that gain entry into the endothelial cell. The blood-brain-barrier significantly impedes entry from blood to brain of virtually all molecules, except those that are small and lipophilic. The most important factors determining drug delivery from the blood to the CNS are lipid solubility, molecular mass, and charge (Brodie, Kurz & Schanker, 1960; Rapoport, Ohno, & Pettigrew, 1979; Levin, 1980).

Lipophilic substances, such as nicotine and diazepam, easily transverse the membrane and access the neurons of the CNS via passive diffusion. Once in the endothelial cell, less is known about what happens to the substance, but presumably it is able to leave the other side of the cell down a concentration gradient to reach the interior of the brain. Heavier substances tend to be excluded from CNS entry. The normal blood brain barrier prevents passage of water-soluble drugs with a molecular weight higher than 180 Da. Molecules that are polar or large have to use other routes, such as facilitated diffusion or active transport.

Stress and the blood-brain-barrier

The blood-brain-barrier is a dynamic regulatory barrier, rather than a passive static one (Ford, 1976). Various physiological and pathological conditions alter the permeability of the BBB. Stressors of various types produce alterations in the permeability of the blood-brain-barrier in different animals. The mechanism of action for stress-induced blood-brain barrier disruption is unknown. However, it has been proposed to result from a cascade of effects secondary to increased 5-HT. Stress increases prostaglandin levels in the plasma, which stimulate the synthesis of serotonin (Haubrich, Perez-Cruet & Reid, 1973). Serotonin itself stimulates additional prostaglandin synthesis and release, leading to increased cAMP formation (Baca & Palmer, 1978). Accumulation of cAMP in cerebral endothelial cells causes vasodilatation, resulting in increased transport of substances (Eakins, 1977; Joo, Rakonczay, & Wollemann, 1975)

Immobilization stress produces increased permeability of the blood-brain-barrier (Skultetyova, Tokarev, & Jezova, 1998; Sharma & Dey, 1986, Belova & Jonsson, 1982; Sharma & Dey, 1980). Belova and Jonsson (1982) exposed adult rats to 4 hours of immobilization stress and reported that there were marked increases over control levels in BBB permeability as indicated by extravasation of fluorescent dye. This increased amount of extravasation (passage from a vessel to a tissue) was noted in several brain regions.

Sharma and Dey (1986) reported increased permeability to two separate tracers following 8 hours of immobilization stress in 12 of 14 brain regions studied. The rats in this study were all young (9-10 weeks old). Other work by Sharma (Sharma, Westman, Navarro, Dey, & Nyberg, 1996; Sharma, Navarro, & Dey, 1991; Sharma & Dey, 1980; Sharma & Dey, 1986) has suggested that younger rats are more susceptible to stress induced blood-brain-barrier disruption. Sharma and Dey (1980) reported that 2-4 hours of immobilization increased permeability, but primarily in younger rats. Sharma suggested that this effect is mediated via a stress-induced increase in plasma 5-HT levels. Data demonstrating that blocking serotonin or effects secondary to higher serotonin levels eliminates restraint-induced blood-brain barrier disruption offers support to this suggestion.

Skultetyova, Tokarev, and Jezova (1998) reported that 30 minutes of restraint stress in adult rats resulted in increased permeability to endogenous albumin (a protein abundant in the blood, which normally does not cross into the CNS). Restraint induced significant permeability increases in the hippocampus, brainstem, and cerebellum. Dvorska et al. (1992) immobilized male rats for 60 minutes and found that, compared to controls, stressed rats exhibited greater blood-brain-barrier permeability to different radiolabeled peptides in ten of ten brain regions studied.

Another stress procedure is forced swimming (Essman, 1978; Porsolt, Bertin, Blavet, Daniel, & Jalfre, 1979; Sharma, Kretzschmar, Navarro, Ermisch, Ruhle, & Dey, 1992). Angel and Burkett (1966) reported that repeated exposure

to forced swimming in a water maze (12 daily sessions) produced BBB disruption in adult rats, allowing greater permeability of cocaine. Long & Holaday (1985) reported that BBB disruption following adrenalectomy is a result of the absence of cortisol, which seems to have a regulatory effect on BBB functioning

Friedman et al. (1996) reported that two 4-minute exposures to forced swim stress in adult male mice increased the permeability to pyridostigmine (an AChE inhibitor that normally does not penetrate the BBB). Stress exposure reduced the amount of pyridostigmine needed to inhibit central AChE activity to 1/100th the usual dose. Along with this dramatic shift in the sensitivity to pyridostigmine, stress exposure also increased the entry of a dye tracer into the brains of stressed mice, indicated by marked staining in stressed, but not into non-stressed mice brains. The authors concluded "compounds considered to be limited to the periphery may become centrally active under stress conditions" (Friedman, Kaufer, Shemer, Hendler, Soreq, & Tur-Kaspa, 1996, p. 1384). Two recent studies have failed to replicate the heightened permeability to pyridostigmine (Lallement et al., 1998; Sinton, Fitch, Petty, & Haley, 2000). These failures to replicate might be explained in terms of species or methodological differences between the studies.

Sharma, Navarro, and Dey (1991) found that young rats subjected to 30 minutes of forced swim stress exhibited marked increases in permeability in either 5 or 8 brain regions, depending upon the tracer used. The increase in permeability correlated with rises in plasma 5-HT. Another study using the same paradigm found essentially the same effect but, in this study, adult rats were also

used, and did not have stress-induced blood-brain-barrier disruption (Sharma et al., 1996). Heat stress exposure also alters the blood-brain-barrier (Sharma & Dey, 1986, 1987; Sharma, Nyberg, Cervos-Navarro, & Dey, 1992; Oztas, 1995).

Besides behavioral stressors, infusion of a hypertonic fluid into the bloodstream, resulting in hyperosmolarity, produces transient changes in the BBB to various tracers (Neuwelt, 1989; Neuwelt, Johnson, Blank, Pagel, Maslen-McClure, McClure, & Wu, 1985; Hirano, Kawanami, & Llena, 1994; Mackie, De Pasquale, & Cserr, 1986; Gumerlock & Neuwelt, 1990; Pappius, Savaki, Fieschi, Rapoport, & Sokoloff, 1978; Rapoport, 1988; Rapoport, Hori, & Klatxo, 1972). Acute hypertension also opens the blood-brain-barrier (Bolwing, 1989; Johansson, 1981; Ozta &, Sandalci, 1984; Oztas, Sandalci, Eren, & Gokhan, 1984; Byrom, 1954; Feigin & Popoff, 1962; Giacomelli, Wiener, & Spiro, 1970; Haggendal & Johansson, 1972; Hara, 1966; Johansson, Li, Olsson, et al., 1970). Anoxia and hypercapnia increase blood-brain-barrier permeability (Hardebo, 1981; Johansson, 1976; Johansson & Nilsson, 1977). Seizures (Johansson & Nillson, 1977), as well as electric shock (Skinhoj, 1966) have also produced BBB disruption. Exposure to aluminum chloride increases the permeability of the BBB (Kim, Lee, Wisniewski, 1986; Leblonde & Allian, 1980).

Pre-treating animals with certain drugs can block the stress-induced increases in BBB permeability. Indomethacin (a prostaglandin synthesis inhibitor), parachlorophenylalanine (a serotonin synthesis inhibitor), cyproheptadine (a serotonin receptor antagonist), diazepam (benzodiazepine agonist), and vinblastine (vesicular transport blocker) prevent the increase in

BBB permeability to tracers following such stressors as immobilization, heat stress, and forced swim (Sharma & Dey, 1984; 1986; 1987; Sharma, Nyberg, Cervos-Navarro, & Dey, 1992; Sharma et al., 1996).

Summary

The general finding of stress-induced increases in permeability is a relatively well-established phenomenon. Although not all studies have been able to confirm the effect, the majority of studies to date indicate that stress can disrupt the blood-brain barrier under the right parameters. The variability in brain regions that increase in permeability following stress may be explained by the use of different stress-induction paradigms, different species/strains, and different procedures for measuring blood-brain-barrier disruption. Younger animals seem to be more susceptible to stress-induced BBB disruption. This finding is interesting from the perspective of psychopathology, offering a possible physiological mechanism for the greater sensitivity of children to stress. Although younger animals are more sensitive to the effects of stress-induced disruption, the effect also has been observed in adult animals, using both forced swim and immobilization stressors (Skultetyova, Tokarev, & Jezova, 1998; Friedman et al., 1996; Dvorska et al., 1992; Angel & Burkett, 1966).

The implications of this phenomenon for the development and maintenance of anxiety disorders are unknown. The possibility that stress-induced BBB changes in permeability may play a role in the enhanced sensitivity of panic disorder patients to challenge agents has yet to be examined. If

responses to challenge agents are found to be enhanced by stress, then the necessity for invoking biological dysregulation, conditioning history or catastrophic cognitions to explain the enhanced sensitivity is brought into question. Moreover, the onset of the first panic attack typically occurs during periods of high stress, raising the possibility that stress-induced permeability changes can be involved in the etiology of anxiety disorders. The field is currently dominated by the models of panic disorder that have been presented, and the results of the present research could suggest that radical shifts in research direction be undertaken.

Evidence for the hypothesis that stress induced blood-brain barrier disruption could account for heightened sensitivity to anxiogenic drugs:

Biological Challenge Agent studies

Data from biological challenge agent studies provide a wealth of evidence for a role of stress to mediate the effects of anxiogenic drugs. Based on anecdotal observations, researchers commonly stated they can tell who will panic based on the subjects' level of anxiety upon walking into the challenge situation. Empirical data from biological challenge agent studies also support the idea that there is a strong association between baseline levels of anxiety and drug response. Indices of baseline anxiety are higher for patients in studies employing such diverse challenge agents as yohimbine (Gurguis & Uhde, 1990), CO_2 (Perna, Battaglia, Garberi, Arancio, Bertani & Bellodi, 1993), ^{m}-CPP (Charney, Woods, Goodman, & Heninger, 1987), and caffeine (Charney,

Heninger, & Jatlow, 1985). The majority of reports linking baseline anxiety to panic have used sodium lactate as the challenge agent. Therefore, the present review focuses on sodium lactate studies.

When discussing differences between groups at baseline, differences are usually reported as group differences between all patients and all controls. This practice is problematic because panic responses in the patient group range can be anywhere between 0-100%. Because more than half of the patients do not panic in some studies, real relationships between baseline variables and panic responses can be obscured. Even with the group differences attenuated by patients who do not panic, many studies have reported significant baseline differences in anxiety indices between patients and controls.

Several studies have examined subjects in either three or four groups, patients who panic (PP), patients who do not panic (PNP), controls who do not panic (CNP), and rarely, controls who panic (CP). Table 1 presents this distinction. Because of the low numbers of controls panicking, the usual reports that do examine these subgroups compare differences in only PP, PNP, and controls C. Although some studies have made this important distinction between PP/PNP, the majority report differences between patients and controls as homogeneous groups.

The following discussion delineates findings of sodium lactate studies into four sections. Section one describes reports indicating baseline differences between patients and controls. Section two presents reports where baseline differences are examined between PNP, PP, and controls C. Section three

discusses studies that used regression analysis to show that baseline anxiety indices are predictive of anxiogenic drug response. Section four presents data from studies that have sought to manipulate the level of baseline anxiety directly and demonstrated altered responses to challenge agents.

Section 1) Baseline differences between patients and controls in lactate studies

Several studies have reported group differences between patients and controls on baseline heart rate (HR). Eight studies reported that patients had significantly higher HRs than controls (Cowley et al., 1984; Freedman at al., 1984; Liebowitz et al., 1984; 1985; Nesse et al., 1984; Rainey, et al., 1984; Hollander, Liebowitz, Cohen, Gorman, Papp, 1989; Aronson et al., 1989; Yeragani & Balon, 1989). Three other studies reported non-significant differences between groups (Cowley et al., 1987; Gaffney et al., 1988; Yeragani et al., 1987). Baseline systolic blood pressure has been reported as significantly higher in patients compared to controls in three out of five studies (Cowley, Hyde, Dager, et al., 1987; Gorman, Fyer, Goetz, et al., 1988; Yergani, Balon, & Pohl, 1989; Gaffney, Fenton, Cane, et al., 1988; Hollander, Liebowitz, Cohen, Gorman, Fyer, Papp et al., 1989). Baseline diastolic blood pressure have been reported to be significantly higher in patients in five studies (Cowley, Hyde, Dager, et al., 1987; Gorman, Fyer, Goetz et al., 1988; Liebowitz, Gorman, Fyer, et al., 1985; Yeragani, Balon, & Pohl, 1989; Hollander, Liebowitz, Cohen, Gorman, Fyer, &

Papp, 1989). One study, however, reported a non-significant difference between groups (Gaffney, Fenton, Cane, et al., 1988).

Baseline differences in levels of stress hormones have been variable across studies. Plasma epinephrine was reported to be higher in five studies (Wyatt, Portnoy, Kupfer, et al., 1971; Mathew, Ho, Kralik, et al., 1980; Appleby, Klein, Sachar et al., 1981; Nesse, Cameron, Curtis, et al., 1984; Villacres, Hollifeld, Katon, et al., 1987). Seven other reports have not found significant differences in plasma EPI (Mathews, Ho, Francis, et al., 1982; Cameron, Smith, Hollingsworth, et al., 1984; Ballenger, Peterson, Laraia, et al., 1984; Liebowitz, Gorman, Fyer, et al., 1985; Carr, Sheehan, Surman, et al., 1986; Gaffney, Fenton, Cane, et al., 1988; Hollander, Liebowitz, Cohen, Gorman, Fyer, Laszlo, et al., 1989). Plasma norepinephrine has been reported to be significantly higher in patients by several investigators (Ballenger, Peterson, Laraia, et al., 1984; Mathew, Ho, Kralik et al., 1980; Villacres, Hollifeld, Katon, et al., 1987). Six other studies did not report significantly different levels of NE in their samples (Appleby, Klein, Sachar, et al., 1981; Cameron, Smith, Hollingsworth, et al., 1984; Nesse, Cameron, Curtis, et al., 1984; Liebowitz, Gorman, Fyer, et al., 1985; Carr, Sheehan, Surman, et al., 1986; Gaffney, Fenton, Cane, et al., 1988).

Similar to EPI and NE, baseline cortisol differences between groups have been variable. Some reports found that patients as a group have significantly higher plasma cortisol (Nesse, Curtis, Thyer, et al., 1985; Hollander, Liebowitz, Cohen, Gorman, Fyer, Papp, et al., 1989; Coplan, Goetz, Klein, Laszlo, Papp, Fyer, et al., 1998). Others have not found significant differences (Appleby, Klien,

Sachar, et al., 1981; Liebowitz, Gorman, Fyer, et al., 1985; Carr, Sheehan, Surman, et al., 1986; Levin, Doran, Liebowitz, et al., 1987) between patients and controls.

Lowered plasma CO_2 pressure is an index of hyperventilation. Four studies have reported lower plasma CO_2 pressure in patients compared to controls (Gorman, Cohen, Liebowitz, et al., 1986; Gorman, Fyer, Goetz, et al., 1988; Liebowitz, Gorman, Fyer, et al., 1985; Liebowitz, Gorman, Fyer, Levitt, Dillon, Levy, et al., 1985; Papp, Martinez, Klein, Ross, Liebowitz, Fyer, et al., 1989; Coplan, Goetz, Klein, Papp, Fyer, et al., 1998).

Baseline self-reported anxiety has been reported to be significantly higher in all studies which report this measure (Coplan, Goetz, Klein, et al., 1998; Aronson, Caraseti, McBane, & Whitaker-Azmitia, 1989; Hollander, Liebowitz, et al., 1989; Aronson, Whitaker-Azmitia, & Caraseti, 1989; Yeragani, Balon, & Pohl, 1989; Cowley, Hyde, Dager, & Dunner, 1987; Liebowitz, Fyer, Gorman, et al., 1984; Hoehn-Saric, McLeod, & Zimmerli, 1991; Den Boer, Herman, Westenberg, Klompmakers, & van Lint, 1989; Balon, Pohl, Yeragani, Rainey, & Weinberg, 1988; Goetz, Klein, & Gorman, 1996).

Section 2) Baseline differences between patient panickers vs. patient non-panickers vs. controls in lactate studies

Several investigators have examined subgroups of patients and controls based on who panics and who does not. Examinations of baseline differences in anxiety indices generally support the hypothesis that higher baseline anxiety

predisposes to having a panic attack during a biological challenge. Several studies that examined patient panickers (PP), patient non-panickers (PNP), and controls (C) as groups are summarized below. Considering patients in terms of PP and PNP represents a key distinction from the perspective of the proposed research. If patients are not stressed at baseline (e.g., low baseline anxiety), then they would be predicted to have panic rates comparable to controls. In contrast, patients who are stressed at baseline (e.g., high baseline anxiety) would be predicted to respond to challenge agents with high panic rates.

Hollander et al. (1989) reported that although no significant increase in cortisol followed lactate infusion, those patients who did panic (PP) had higher baseline cortisol levels. Patients in this study also had higher baseline self-reported anxiety. Hollander, Liebowitz, Cohen, et al. (1989) reported that PP had higher DBP compared to PNP and controls. No group differences were observed in baseline plasma EPI or in SBP. Den Boer et al. (1989) suggested that higher baseline arousal makes subjects more "vulnerable" to challenge-induced panic attacks. As with other studies, Den Boer et al. found that PP had higher baseline anxiety scores compared to NP and C. Additionally, pre-lactate MHPG was elevated in PP compared to PNP and C. Papp, Martinez, Klein et al. (1989) examined pre-lactate levels of blood gases and reported that PP had significantly lower PCO_2 and higher plasma PH compared to PNP and C.

Another study using lactate noted that about 60% of panic disorder patients panicked following infusion, while only one in 44 controls panicked (Coplan, Goetz, et al., 1998). Baseline plasma cortisol was significantly elevated

in the PP, while the PNP and C were indistinguishable on this measure. Similar to cortisol, the baseline PCO_2 levels of the PNP and Cs groups were indistinguishable, while the PP had significantly lower baseline PCO_2 (indicating hyperventilation). The groups also differed in self-reported fearfulness and dyspnea before infusions, with PP having significantly higher fear and dyspnea than PNP and Cs. Diastolic blood pressure was elevated in the PP compared to PNP and Cs.

Section 3) Regression analyses of baseline anxiety measures in lactate studies

Goetz, Klein, and Gorman (1996) reported that self-reports of feeling "afraid in general" and "feeling confused" significantly predicted panic response to sodium lactate, using logistic regression. Coplan, Goetz, et al. (1998) reported that a logistic regression analyses indicated that baseline levels of anxiety indices predicted panic following lactate. High levels of self-reported anxiety, high baseline cortisol levels, and hyperventilation were each significantly predictive of panic when infused with lactate. Yeragani et al. (1988) reported that baseline self-reports of feeling "afraid of going crazy," "feeling paralyzed," and feeling "unsteady" each significantly predicted panic attacks following sodium lactate.

Section 4) Effects of manipulations of baseline anxiety in lactate studies

Four studies have indicated that laboratory-induced panic attacks can be mediated by situational variables (Clark et al., 1990; Carter, Hollon, Carson, & Shelton, 1995; Rapee, Mattick, & Murrell, 1986; Sanderson et al., 1989). Clark, Salkovskis, and Anastasiades (1990) gave reassuring information to one group of panic disorder patients, and no reassuring information (standard treatment) before an infusion of sodium lactate. Reassuring information resulted in significantly lower panic rates in the group receiving reassurance (30%) compared to the group that received no reassurance (90%). This finding was interpreted as evidence that catastrophic misattributions of bodily sensations are causally related to panic following lactate. The finding also is consistent with the simpler hypothesis that lowering baseline anxiety before an infusion lowers the likelihood of panicking. Rapee, Mattick, and Murrell (1986) provided detailed information regarding the physiological effects of CO_2 to one group of panic patients, and minimal information to another panic patient group. Rapee, Mattick, and Murrell (1986) reported lower panic rates in the group receiving the detailed information. Assuming that having detailed reassuring information regarding the safety of a drug results in decreased stress, these two studies are consistent with the current hypothesis.

Carter, Hollon, Carson, and Shelton (1995) assessed the effects having a "safe person" nearby when patients undergo CO_2 administration. Carter et al. (1995) reported that when CO_2 was administered, patients without a safe person present experienced greater anxiety compared to patients with a safe person

present. Baseline anxiety measures were assessed before group assignment, and the levels did not differ between the two groups. Unfortunately, further measures of anxiety did not occur until after the CO_2 administration. Therefore, no data are available to assess whether there was a decline in baseline anxiety because of the presence of a safe person. The present analysis would suggest that those subjects allowed to have a safe person present would have reduced baseline anxiety. Another unanswered issue with Carter et al.'s study is whether the safe person's presence shifted patients' attention away from interoceptive stimuli and towards exteroceptive stimuli. Related to this point is Baum's (1976) report that rats with a history of shocks in a particular context displayed marked behavioral reactivity when re-exposed to this context. Interestingly, testing such rats with other rats with no history of shocks markedly attenuated this behavioral reactivity. Baum's (1976) findings suggest that some part of the decreased reactivity of the "safe person" group may have resulted from the presence of another salient stimuli, independent of changes in cognitions and or conditioning history of the subjects.

In an often cited study, Sanderson et al. (1989) gave patients about to receive an inhalation of CO_2 an "illusion of control," and noted that those patients with the illusion of control panicked at a significantly lower rate (20%), compared to those patients with no such information (80%). Similar to other studies of this kind, the results are often cited as evidence for cognitive mediation of laboratory-induced panic. The results also are consistent with the proposed hypothesis. Because uncontrollability is known to be stressful (see discussion under stress

subsection), increasing one groups' sense of control is also consistent with creating a difference in baseline stress levels. As noted earlier, a perception of control, even in the absence of any real control, can decrease the stress level of subjects. The current hypothesis suggests that lowering the stress level of subjects who were anxious at baseline, rather than reducing the frequency of catastrophic cognitions, was the operative factor in reducing CO_2 induced panic attacks.

Summary of clinical evidence for the role of baseline stress levels

In summary, many studies suggest that high baseline anxiety is a predisposing factor towards panic in biological challenge agent studies. Comparisons of patient versus control groups leads to mixed results, with some anxiety indices higher in patients. Comparisons of patients who panic, those who do not, and controls generally provides evidence for baseline anxiety in anxiogenic response. Even stronger evidence comes from studies that have reported that stress level at baseline actually predicts who will and who will not panic.

A correlate of the hypothesis is that decreasing the level of baseline stress/anxiety in subjects should decrease the likelihood of panic attacks. Investigations that manipulated the information (reassurance) or the subjects' perceptions of control reported lower rates of panic following challenges in subjects who were in the stress-reducing condition. This effect may result from a decline in catastrophic cognitions. Alternatively, the effect could be the result of

a decrease in bodily sensations, in accord with the conditioning theory of panic. Each of the manipulations in these studies (perceived control, social support, and information) decreased stress levels and, therefore, the results of these studies are congruent with the present analysis. The operative factor in decreasing the rates of panic attacks in these studies is decreased stress levels, and not decreased occurrence of catastrophic cognitions or conditioned responses to bodily sensations.

The cited studies strongly suggest that baseline anxiety affects behavioral responses to biological challenge agents in panic disorder patients. The limitations of human studies prohibit many manipulations that might identify the underlying mechanism of action through which baseline stress may operate.

An initial review of the findings might suggest that elevated baseline anxiety simply place anxious subjects closer to a threshold of panic attacks, and once biological challenge agents are administered, then those subjects more anxious at baseline surpass that threshold more often. If this were so, then the role of baseline anxiety in biological challenge agent response would be uninteresting. The present analysis suggests that higher basal stress levels are not simply a means of bringing subjects closer to a threshold, but are an operative factor in producing panic attacks. Moreover, the present hypothesis offers an explanation for the mechanism wherein baseline anxiety exerts its effects on challenge response. Evidence for this viewpoint can be drawn from several aspects of studies using BCAs. Certain challenge agents seem to produce anxiogenic responses in all subjects, with panic disorder patients simply

demonstrating greater sensitivity. More importantly, other challenge agents seem to evoke a qualitatively different state in responders and non-responders. This qualitative difference argues against a purely threshold-enhancement explanation for the role of baseline stress.

Another factor mitigating against the view that heightened sensitivity in subjects with high baseline anxiety is simply a threshold effect is that challenge-induced panic differences still exist between groups even after controlling for baseline anxiety levels. The present hypothesis argues that panic produced via biological challenges can be explained by an interaction between baseline stress levels and drug administration.

The available clinical data strongly implicate baseline stress levels of subjects in the production of panic attacks following challenge agent administration. The mechanism by which baseline anxiety exerts its role in panicogenic drug response is unknown. A possible source of insight into this mechanism lies in studies of species other than humans. It is possible that baseline stress levels in non-human animals also mediate the response to anxiogenic drugs. If this were so, then it would suggest that the increased sensitivity that occurs in subjects with heightened basal stress is not necessarily the result of catastrophic cognitions, multiple biological dysregulations, or interoceptive conditioning. The following section presents literature from pre-clinical research on anxiogenic drug responses in non-human animals.

Animal studies

Anxiogenic agents in primate studies

The hypothesis stated above suggests that the level of stress present during testing with a putative anxiogenic agent may influence behavioral effects of the drug. One hypothesized mechanism of action is stress-induced blood-brain-barrier disruption. A number of studies administering panicogenic agents to non-human primates are consistent with the novel hypothesis regarding biological challenge agent responses in panic disorder patients and healthy controls. Many of these reports center on biological challenge agents administered to non-human primates, but some studies involve other anxiogenic agents.

Classical "anxiogenic" agents

A series of reports by Kalin and colleagues has demonstrated differential effects of corticotrophin-releasing hormone (CRF) on behavioral and physiological variables, based on experimental context. Kalin et al. (1983) reported that intravenous CRF resulted in increased anxiety-like responses (struggling and exploratory behaviors) in chair-restrained rhesus monkeys. When tested in their familiar home cages, the same subjects reacted differently. In unrestrained monkeys, CRF produced increased amounts of vocalizations, self-directed behaviors, and lying-down behaviors. These unrestrained monkeys displayed significant decreases in exploratory, grooming, threatening, and huddling behaviors. Two subsequent studies using intraventracular administration of CRF to rhesus monkeys reported similar findings. Kalin et al.

(1983) and Kalin et al. (1985) reported increased behavioral arousal in chair-restrained subjects, but behavioral inhibition (lying down) in the same subjects when tested in their home cages. Interestingly, a similar effect was found in rats tested in novel and familiar environments. CRF administration produced decreases in locomotion in a novel open field environment (consistent with an anxiogenic interpretation), but increased locomotion in a familiar cage environment (Sutton et al., 1982). Novelty is known to be a stressful event (Dobrakovova & Jurcovicova, 1984; McCarthy, 1982; Weinstick, Razin, Schorer-Apelbaum, Men, & McCarthy, 1998; Kirby, Callahan, McCarthy, & Johnson, 1989). This series of CRF studies indicates that behavioral responses to anxiogenic drugs may be affected by exposure to novel (stressful) contexts. The direction of the effect of stress on CRF response is in the predicted direction (augmented by stress).

A second series of reports using β-CCE also provides data relevant to the current hypothesis. β-CCE administration to chair-restrained primates produced anxiety-like (struggling, immobilization, defecation, distress vocalization) symptoms (Ninan et al., 1982; Crawley et al., 1985; Skolnick et al., 1994), consistent with the present hypothesis.

Contrary to the hypothesis, Lagarde et al. (1990) reported that β-CCE had similar effects in chair restrained and unrestrained rhesus monkeys. This finding is problematic for the present hypothesis, in that the presumably more stressed chair-restrained subjects reacted in a similar fashion as the unrestrained subjects. Lagarde et al.'s (1990) findings must be qualified by mention of

methodological problems in the study. The rhesus monkeys in Lagarde et al.'s study were tested under non-standard conditions, which could easily be argued to affect the stress level of the subjects. Chair-restrained animals were tested in pairs, in immediate proximity to one another. Additionally, animals were so well acclimatized to the chair that the predominant responses to being placed in the chair were described by the investigators as "sleep and calm." Indeed, the restraint chairs used in this study were "specially designed" to allow animals to sleep. A well-acclimatized stimulus context where the subject's predominant response is to fall asleep would not be described as a stressful environment. Additionally, testing subjects in pairs attenuates the effects of anxiogenic manipulations (Baum, 1976), offering another possible explanation for the attenuated anxiogenic effects of β-CCE in restrained animals. Each of these factors could decrease the stressfulness of being in chair-restraint. Complicating interpretation of Lagarde's findings even further, the unrestrained primates were tested individually, while chair-restrained subjects were tested in pairs.

In summary, the majority of studies that have administered β-CCE to chair-restrained primates report marked anxiety-like responses, consistent with the present hypothesis, assuming that chair-restraint is a more stressful context compared with no restraint conditions. One study suggests that chair restrained and unrestrained subjects react similarly to β-CCE; however, this study had several methodological problems.

Two other studies reported β-CCE's effects on unrestrained primates when the subjects were tested in their social groups. Valucci et al. (1986)

reported that β-CCE increased levels of aggressive (not anxious) behaviors and vigilance in dominant talapoin monkeys, but only increased vigilance in subordinate monkeys. Another study using rhesus monkeys also reported increases in aggressive, rather than anxious, responses in group-tested unrestrained monkeys (Insel et al., 1988). These reports of β-CCE producing aggressive behaviors rather than anxious behaviors stand in contrast to prior reports of dose-responsive anxiogenic effects in individually tested chair-restrained subjects (Ninan et al., 1982; Crawley et al., 1985).

Therefore, there can be a qualitatively different response to the same drug in chair-restrained and unrestrained subjects. Similar to the data from CRF studies, these reports suggest that the context of administration (stressful vs. no stress) can influence drug response to anxiogenic agents in a quantitative and qualitative manner.

Biological challenge (panicogenic) agents

Many investigators have used biological challenge agents in non-human primates to model panic disorder. In general, these studies' findings support the proposed hypothesis.

Freedman, Sunderland, and Rosenblum (1987) administered sodium lactate to bonnet macaques. The experimental reproduction of "panic-anxiety" was accomplished by administering sodium lactate to eight macaque subjects via subcutaneous injection. The subjects had to be captured in a restraint cage for 10 minutes to administer the drug. The behavioral reactions of the macaques to

the lactate or vehicle injection was observed. Friedman, Sunderland, & Rosenblum, (1988) reported that 63% of the macaques responded with greater affective response to lactate verses vehicle trials (e.g., a panic-like reaction as defined by the authors).

Another study employing the same species provided a replication of the anxiogenic effects of sodium lactate (Sunderland et al., 1989). This study also used a 10-minute restraint period to administer the subcutaneous sodium lactate, a procedure that would be expected to be a marked stressor. Testing also was conducted in a novel test pen (a stressful context). In the Friedman, Sunderland, & Rosenblum, (1988) study, baseline levels of distress were "strongly predictive" of response to the drug, correlating with reactions to different drug doses in the order of (.61)—(<.90). Sunderland et al., (1989) did not report baseline levels of stress in their subjects.

Rosenblum, Coplan, Friedman, and Bassoff (1991) administered oral yohimbine to unrestrained bonnet macaques. In this study, there was no clear dose-response relationship between yohimbine and a behavioral scoring index. The subjects alternated between periods of activation (e.g., startle, freezing, frenzied pacing) and enervation (e.g., lying down, leaning against the wall, sighing). Although there is no universally accepted taxonomy of what constitutes a panic like reaction in primates, neither the activation nor the enervation behavioral patterns was deemed similar to panic-like anxiety by the investigators. It is useful to note that the failure to produce anxiogenic effects was observed in unrestrained primates, and that the periods of enervation (e.g., lying-down) were

similar to those reported by Kalin et al. (1983; 1985) in unrestrained primates following administration of a different "anxiogenic" drug, CRF.

Other work in primates has involved intravenous infusions of yohimbine and resulted in a dose-responsive increase in panic-like responses (Redmond & Huang, 1979; Charney, unpublished observations, cited in Rosenblum et al., 1991). The results are consistent with the current hypothesis in that these subjects were chair-restrained, which would constitute a more stressful context. The different route of administration across studies makes direct comparison of results difficult.

Other studies of panicogenic drug effects in primates have been conducted with pentagastrin and CCK-4 (Rupnick, Schaffer, Siegel, & Iverson, 1993; Erwin, Palmour, & Bradwejn, 1991). Erwin, Palmour, and Bradwejn (1991) administered CCK-4 to African green monkeys. The anxiogenic effects of CCK-4 were more apparent in those monkeys that were "nervous" before testing. Indeed, animals that were nervous at baseline showed restlessness at low doses, and marked anxiety-like (frozen immobility, self-clasping, tremors) symptoms at high doses (Erwin, Palmour, & Bradwejn, 1991). Subjects that were "calm" at baseline showed no behavioral effects at low doses, and at high doses showed only subtle changes in vigilance, pacing and threat behaviors. Animals in this study were housed in a naturalistic setting at baseline and, therefore, being brought into a laboratory constituted a novel and, presumably, stressful environment.

A subsequent study used rhesus monkeys that were acclimatized to laboratory settings. Rupnick, Schaffer, Siegel, and Iverson (1993) administered pentagastrin and CCK-4, which has been reported to possess panicogenic properties in man (Abelson & Neese, 1990) to rhesus monkeys. Pentagastrin and CCK-4 failed to elicit behavioral or cardiovascular changes in the monkeys. It is interesting to note how the monkeys received the drug infusions:

"Before, during and for the first 5 minutes after the injection of pentagastrin or water, monkeys sat calmly in the lap of the handler. At no time did the animals appear agitated or distressed as a result of drug treatment." (Rupnick, Schaffer, Siegel, & Iverson, 1993, p. 117)

This description emphasizes how calm and relaxed animals were prior to administration of the panicogenic agent. The authors concluded that panic-like effects may not be demonstrable utilizing pentagastrin or CCK-4 as challenge agents with rhesus monkeys. The present hypothesis argues that the difference in baseline arousal, rather than species difference, is probably the key variable to explain the differences between studies.

While several studies support the hypothesis, one study using BCAs in primates was found that does not offer support. Carey et al. (1992) administered caffeine and yohimbine (both BCAs) along with several other anxiogenic agents (pentylenetetrazol, FG-7142, amphetamine) to marmosets in "low" and "high anxiety" settings. The high-anxiety condition consisted of a human experimenter standing in front of the cage watching the subjects, and a history of aversive handling by the experimenter. The low-anxiety condition consisted of video

recording subjects in their home cages. Anxiogenic agents (yohimbine, caffeine, pentylenetetrazol, FG-7142, and one dose of amphetamine) produced anxiogenic profiles in the "low-anxiety" condition, but not in the "high-anxiety" condition. Anxious behaviors were defined as increased time spent in the nest box, less time spent at the front of the cage, and decreased locomotion. Aside from possible species differences (which are unlikely to be the causal agent), several aspects of the study may explain the failure to observe an anxiogenic effect of the drugs in the "high-anxiety" condition. The behavioral samplings for high and low anxiety conditions were different in amount and content. The behavioral scoring was based on a 15-minute observation period for the low-anxiety group, while the high-anxiety group was observed for only 2 minutes. Other studies of anxiogenic agents in primates have used at a minimum observation times of 15 minutes, with higher observation periods continuing well over one hour post drug onset (Skolnick & Paul, 1982; Ninan, Insel, Cohen, Skolnick, & Paul, 1982; Crawley et al., 1985; Skolnick et al., 1984; Rosenblum et al., 1991; Lagarde et al., 1990; Sunderland, Friedman, & Rosenblum, 1989; Velucci, Herbert, & Keverne, 1986). Additionally, different sets of behavioral criteria for an anxiety response were used between groups. Based on the questionable data sampling strategy and the different handling history of the groups, this one discrepant study's findings should be regarded with caution.

In summary, several studies have administered anxiogenic agents to non-human primates and provided findings consistent with the current hypothesis. One study has administered two BCAs and reported no effect under

high anxiety, but anxiogenic effects under low anxiety conditions (Carey et al., 1992). This study's findings are likely confounded by the inadequate data collection and differences between groups (alternate scoring taxonomies and alternate handling procedures). The majority of the evidence from primate studies is consistent with the supposition that higher baseline stress influences behavioral response to anxiogenic agents. Only indirect support for the hypothesis can be drawn from the studies cited, because none of the cited investigations directly manipulated the stress levels of the subjects. Additionally, no direct data is available from these studies on the effects of stress on permeability to the blood-brain-barrier.

While primate studies have provided relevant data regarding anxiety, the majority of research on anxiogenic drugs uses rodents as subjects. Examination of the wider literature available on the effects of stress and the actions of anxiogenic drugs in rodent anxiety models provides even more evidence for a possible relationship between basal stress level and anxiogenic drug response. Results from studies using anxiogenic drugs in one of the most frequently used animal models of anxiety (the elevated plus maze) are presented below.

The Elevated Plus Maze

The effects of several biological challenge agents (yohimbine, flumazenil, sodium lactate, isoproterenol, CCK-4, ^{m}-CPP, and caffeine) have been assessed using the elevated plus maze. Besides the BCAs, several other anxiogenic drugs have been administered in the EPM paradigm (picrotoxin, PTZ,

^{m}-CPP, FG 7142). The following discussion of BCAs in the EPM will first present findings from studies that have examined agents which do not cross the BBB at baseline (sodium lactate, isoproterenol) and then those which do cross the BBB at baseline (yohimbine, flumazenil, ^{m}-CPP, caffeine, CCK-4).

Of the four BCAs which do not cross the BBB under baseline conditions (sodium lactate, sodium bicarbonate, epinephrine, and isoproterenol), only two have been assessed in the EPM: sodium lactate and isoproterenol. These two agents also have been used in human research. Johnston & File, (1988) studied rats and Rodgers, Cole, Aboualfa, and Stephenson (1995) studied mice. Both studies assessed multiple doses of sodium lactate and isoproterenol, but neither of these studies reported an anxiogenic effect for sodium lactate. Only File and Johnston (Johnston & File, 1988) reported a mild anxiogenic effect for isoproterenol, at the highest dose (0.6) tested. The Rodger's laboratory tests subjects after giving them an "adaptive acclimation period" which would be expected to decrease the baseline stress/anxiety of the subjects.

The findings of no effect for sodium lactate and isoproterenol in the EPM merit discussion. From the perspective of the current hypothesis, sodium lactate would not be expected to produce anxiety if administered intraperitoneally to unstressed subjects (as both studies to date have done). The present hypothesis suggests that sodium lactate: either 1) acts to open the blood-brain-barrier and directly triggers anxiety reactions or 2) opens the blood-brain-barrier and allows peripheral catecholamines to cross the blood-brain-barrier and trigger anxiety. Administering sodium lactate intraperitoneally would be expected to

result in far less of the substance reaching the bloodstream. Isoproterenol administered to unstressed animals (with no BBB disruption) also would be predicted to have minimal effects on anxiety.

Of the biological challenge agents that normally cross the BBB at baseline, yohimbine and flumazenil have been most often used in the EPM. Yohimbine has repeatedly produced an anxiogenic profile in the EPM (Pellow, Chopin, File, & Briley, 1985; Mangiafico, Cassetti, & Farrari, 1989; Pellow, Johnston, & File, 1987; Johnston & File, 1988; Bhattacharya, Satyan, & Chakrabarti, 1997; Johnston & File, 1989; File & Johnston, 1987; Wada & Fakuda, 1991). Yohimbine's ability to reliably produce anxiety in the EPM has make it a reference compound for anxiogenic effects. Contrary to these series of findings, one study has paradoxically reported an anxiolytic effect in the EPM (Cole, Burroughs, Laverty, Sherrif, Sparham, & Rodgers, 1995). Cole and Rodgers (Cole, Burroughs, Laverty, Sherrif, Sparham, & Rodgers, 1995) reported that yohimbine produced a clear anxiolytic profile in three different strains of mice when tested in the EPM, across a behaviorally active dose-response range.

The unexpected anxiolytic effects could be a result of inter-species differences, but, the general pattern of findings in the EPM suggests that both rats and mice react similarly to anxiogenic such agents as caffeine (Lister, 1987; Pellow, Chopin, File, & Briley, 1985), m-CPP (Handley, McBlane, Critchley, & Njung'e, 1993; Benjamin, Lal, & Meyerson, 1990) and CCK-4 (Lang, Harro, Bourin, & Bradwejn, 1994; Harro & Vassar, 1991). Another possibility for this result is that some aspect of the testing conditions altered the behavioral reaction

to the drug. Table 2 presents EPM studies that have used yohimbine. The finding of anxiolytic action in yohimbine is quite striking, considering the number of studies demonstrating clear-cut anxiogenic behavioral profiles. It should be noted that the aberrant study used an atypical pre-test manipulation. The study provided subjects with a <1 hour pretest acclimatization period to the testing room. A long adaptation period would be expected to decrease the level of stress that experimental animals experience, because transferring animals between rooms produces a stress response (Dobrakovova & Jurcovicova, 1984; McCarthy, 1982; Weinstick, Razin, Schorer-Apelbaum, Men, & McCarthy, 1998; Kirby, Callahan, McCarthy, & Johnson, 1989). Decreased stress in the study's subjects might be a crucial factor in explaining the failure to demonstrate an anxiogenic effect. Another atypical aspect of this study's methodology is the fact that testing was conducted under red light. The effect of lighting level in the EPM is controversial. Some investigations have reported that lighting changes have no effect (Falter et al., 1992; Pellow et al., 1985) on plus maze behavior. Other studies have reported that lighting level can change subject's behavior (Benjamin et al., 1990; Lee & Rodgers, 1990; Morato & Castrechini, 1989), even to the point of reversing 8-OH-DPAT's drug effect from anxiogenic to anxiolytic depending on lighting level (Handley & McBlane, 1993). The relative roles of the red lighting and the acclimatization period, if any, in the atypical drug effect for yohimbine are unknown.

To date no systematic evaluation of how stressors influence anxiogenic drug response in the EPM has been reported.

Of all the challenge agents used to provoke panic attacks in biological challenge studies, flumazenil is one of the most intriguing. Flumazenil has an anxiogenic agent in certain behavioral paradigms (Da Cunha, Wolfman, Levi de Stein, Ruschel, Izquierdo & Medina, 1992; Pokk & Zharkovsky, 1998). In the EPM, flumazenil is generally given as a means of testing GABA involvement—e.g., antagonizing agonists or inverse agonists. Of the EPM studies that have used flumazenil, relatively few included a group receiving flumazenil alone. For the majority of these studies (see Table 2), flumazenil had no significant effect (Benjamin, Lal, & Meyerson, 1990; Pellow & File, 1986; File, Johnston, & Baldwin, 1988; Chopin & Briley, 1993; Kulkarni & Sharma, 1993). Studies that have reported an anxiogenic effect for systemically administered flumazenil suggest a role of baseline anxiety in modulating the behavioral response to the drug. Lee and Rodgers (1991) found that DBA/2 mice respond with anxiogenic profile in the EPM when given flumazenil. In this study, Rodgers did not include the 1-2 hour acclimatization period, and the DBA/2 strain of mouse is reported to be quite anxious at baseline.

Direct infusion of flumazenil into fear-relevant brain centers such as the amygdala (DeCunha, Wolfman, et al., 1992), but not the dorsal periaqueductal gray (Russo, Guimaraes, et al., 1992), results in an anxiogenic profile in the EPM. These findings are consistent with the present hypothesis, in that the drug may produce anxiety via entry and activation of specific brain regions.

File and Hitchcott (1990) reported that inter-batch differences in baseline anxiety were related to behavioral reactivity to flumazenil in rats. File and

Hitchcott examined batches (groups of animals shipped at different times) of rats and determined that some batches had higher exploration of the EPM than others. Rats from low rates of exploration batches were characterized as the "high anxiety" group, and rats from the batch with high exploration were termed the "low anxiety" group. When given flumazenil, "high anxiety" rats showed an anxiolytic effect, and "low anxiety" rats displayed an anxiogenic profile. This finding seems directly contrary to the current hypothesis; however, it must be emphasized that the subjects were not differentially stressed once in the laboratory. The higher and lower levels of exploration between batches might be conceptualized as trait anxiety, whereas the current hypothesis concerns state anxiety immediately prior to BCA administration.

More relevant to the hypothesis is whether anxiety or stress immediately before testing can alter the behavioral response to flumazenil. Pokk and Zharkovsky (1997) reported that mice stressed by exposure to 24 hours of small platform stress (SPS) reacted with a clear anxiogenic profile to flumazenil. In this study, control rats (not exposed to SPS) had no anxiogenic reaction to flumazenil, except at an exceptionally high dose. This finding is consistent with stress inducing a leftward shift in the dose-response curve to flumazenil. Flumazenil following stress was more anxiogenic than two separate benzodiazepine inverse agonists were. Low doses of flumazenil produced no effect in the controls, but marked anxiogenic reactions in the stressed rats. One can speculate that SPS may have: 1) increased the permeability of the blood-brain barrier to flumazenil; 2) interacted with CA crossing over the BBB; 3)

changed the "tone" of the benzodiazepine receptors, or some combination of these mechanisms.

Metachlorophenylpiperazine (^{m}-CPP) is a serotonin agonist that provokes panic attacks in panic disorder patients at higher rates than controls. ^{m}-CPP has been tested in the EPM in four independent studies (Gibson, Barnfield, & Curzon, 1994; Rodgers, Cole, Cobain, Daly, Doran, Eells, & Wallis, 1992; Handley, McBlane, Critchley & Njung'e, 1993; Benjamin, Lal, & Meyerson, 1990). All four studies have reported an anxiogenic effect of ^{m}-CPP, using both mice and rats as subjects. In one of these studies (Rodgers, Cole, Cobain, Daly, Doran, Eells, & Wallis, 1992), ^{m}-CPP produced a weak anxiogenic profile on traditional indices (% time and entries), but produced a strong anxiogenic effect on indices that incorporate behaviors such as rearing (ethoscoring). In the one study reporting weak anxiogenic effects on traditional measures, the investigators allowed at least 2 hours of adaptation to the testing room. From the perspective of the present research, the attenuation of ^{m}-CPP's anxiogenic effect may reflect subjects being in a calmer state at the point of testing.

EPM studies evaluating caffeine have consistently reported that it produces an anxiogenic profile (Lister, 1987; Pellow, Chopin, File, & Briley, 1985; Bhattacharya, Satyan, & Chakrabarti, 1997; Baldwin, Johnston, & File, 1989). Table 2 lists the EPM studies of caffeine. Baldwin, Johnston, and File, (1989) reported anxiogenic effects of caffeine at one of the doses tested. Caffeine's anxiogenic effects were independent of the strains and species tested to date. Caffeine is known to cross the BBB and provokes anxiety in healthy controls and

panic disorder patients (Uhde, 1990; Charney, 1985). Panic disorder patients show greater reactivity to caffeine (Uhde, 1990).

Several studies have assessed CCK-4 and the related peptide CCK-8 in the EPM (Harro & Vassar, 1991; Rex, Fink & Marsden, 1994; Belcheva, Belcheva, Petkov & Petkov, 1994, Vasar, Lang, Harro, Bourin & Bradwejn, 1994). As with ^{m}-CPP, caffeine, and yohimbine, CCK-4/CCK-8 produces an anxiogenic profile in the EPM. One study failed to find an anxiogenic effect in mice (Johnson & Rodgers, 1996). This lack of effect was observed across a range of doses that are known to be behaviorally active. This one study stands in contrast to the five studies that reported anxiogenic effects using mice (Vasar, Lang, Harro, Bourin & Bradwejn, 1994), rats (Harro & Vasar, 1991; Becheva, Becheva, Petkov & Petkov, 1994; Ladurelle, Roques & Dauge, 1991), and guinea pigs (Rex, Fink & Marsden, 1994). Of note from the current position that stress is related to anxiogenic drug response, the Johnson and Rodgers (1996) study included a long adaptation time and tested subjects under red lighting.

The same pattern observed in yohimbine, caffeine, and ^{m}-CPP also was observed with CCK. Testing in non-standard conditions (allowing rodents an acclimatization period to the testing room; using dim red light) is associated with attenuated (Rodgers, Cole, Cobain, Daly, Doran, Eells & Wallis, 1992), nullified (Johnson & Rodgers, 1996), or even reversed (Cole, Burroughs, Laverty, Sherrif, Sparham & Rodgers, 1995) anxiogenic effects of the drug in question. Such non-standard testing conditions arguably decreased the stress level of subjects, suggesting the hypothesis that

reactions to anxiogenic drugs are dependent to some degree on the baseline stress of the subject.

Overall Summary:

The role of higher baseline anxiety in challenge agent response is well documented in humans. However, there is no current explanation of the mechanism by which higher basal stress predisposes towards panic. A nearly insurmountable problem in interpretation of biological challenge agent studies in panic disorder lies in controlling variables that the subjects bring into the testing situation. The conditioning history of the subjects, the occurrence of catastrophic cognitions (conscious as well as unconscious), and the possibility of one or more biological dysregulations have been postulated to play a primary role in the response to challenge agents.

Different theories have polarized around biological or psychological explanations of the heightened sensitivity to challenge agents, with no single explanation emerging as dominant. Psychological models rely on either a history of interoceptive conditioning or catastrophic cognitions about the occurrence of bodily sensations to explain the higher sensitivity panic disorder patients have to challenge agents. According to psychological models, the conditioning history and cognitive activities of human subjects produce heightened sensitivity. The inability to fully control for these variables in human studies is exemplified in the contention that catastrophic cognitions (e.g., I am dying) can occur unconsciously and produce panic attacks.

Evidence from pre-clinical research suggests that the levels of stress before testing, not psychological factors, mediate responses to biological challenge agents. Factors such as interoceptive conditioning and catastrophic cognition that are central to psychological models of panic disorder cannot account for the role of baseline stress in the rodent and primate studies reviewed earlier.

The evidence, while suggestive, is indirect, and no studies have examined the role of acute stress induction in the response to biological challenge agents. A few studies, however, have given a biological challenge agent in different contexts, and noted differing responses based on the stress level of the subjects. An early study on the effects of sympathetic arousal on fear responses offers support for the present hypothesis. Singer (1963) administered epinephrine to rats in both stressful and non-stressful contexts. Singer (1963) reported that epinephrine produced no effect in the non-stress condition, but increased indices of fright in the stressful condition (a significant stress x drug interaction). While this study did not stress the rats prior to drug administration, the immediate context itself was stressful, and therefore the stress x drug interaction is consistent with the present hypothesis. Caffeine also has to have a qualitatively different effect when given in a novel (stressful) context, compared to familiar (non-stressful) contexts (Britton & Indyk 1990). Britton and Indyk (1990) reported that caffeine dose-dependently increased locomotion in home cages, but dose-dependently decreased locomotion in the open field test, consistent with an anxiogenic effect.

Enhanced sensitivity to challenge agents following exposure to acute stress would suggest that at least a portion of the enhanced sensitivity that occurs in panic disorder patients might be a result of stress at the time of testing. Testing this hypothesis in an animal model would allow the controlled elimination of each of the proposed causal factors for enhanced sensitivity. Indeed, the use of an animal model is the only means to exert complete experimental control over such factors as cognition and conditioning history.

Section III: Description and Rationale for Selection of Animal Models of Anxiety and Stress Manipulations

Animal Models of Anxiety

Overview

Animal models avoid many of the potential confounds of human studies, including life history, behavior, biochemical states, and wide genetic variability (Telner, 1984). Advantages to animal modeling in psychopathology include the ethical acceptability of physiological, pharmacological, and chromosomal manipulations (e.g., knock-out/transgenic strains) that are not possible with human subjects (Suomi, 1989). McKinney and Bunney (1969) recommended that an animal model resemble a psychiatric condition in terms of etiology, biochemistry, symptomatology, and treatment.

The primary criterion by which an animal model of psychopathology is judged is its validity. Evaluations of the validity of animal models typically follow three lines: face validity, predictive validity, and construct validity (Willner et al., 1992; Willner, Muscat, & Papp, 1992; Harris, 1989). Predictive validity refers to the model's sensitivity to drug challenges. To establish predictive validity, drugs or behavioral manipulations that alleviate a condition in humans should have a parallel effect in the model. Likewise, manipulations that exacerbate the condition in humans should have an opposite effect on the animal model. Face validity refers to the phenomenological similarities between the model and some aspect of the disorder. Construct validity refers to the degree that the cause of

behavioral change in the animal is sufficient to cause a similar response in humans (Triet, 1985; Sanger, 1991). Models can have different levels of each type of validity. For example, an animal model may have little face or construct validity, but possess high predictive validity. Such a model would be quite useful from a practical standpoint, as a means of screening new medications. However, it does not allow one to answer the questions regarding the etiology of a disorder. Etiology is addressed by the construct or face validity of the model. Animal models usually possess face validity for a specific facet of a disorder, such as a symptom or a cluster of symptoms. It is unlikely that any one animal model will be able to adequately encompass all the behavioral features associated with a psychiatric disorder.

Elevated Plus Maze-Behavioral Pharmacology

The elevated plus maze is perhaps the most popular animal model of anxiety, being used by over 100 research laboratories around the world (Hogg, 1996). Whether constructed for mice, rats, or other rodent species, the EPM is generally manufactured to have four arms radiating out from a central square platform. It is a plus-shaped (also referred to as an x shaped) platform, and is generally elevated 50-70 cm above the floor. Two of the four arms have side walls (usually made to a height of 50 cm), while the remaining two arms have either no walls or only low ledges. These two types of arms (enclosed and non-enclosed) are placed on opposing sides of the central platform, and are generally referred to as closed and open arms respectively. Figure 5 presents the EPM

used in the present research. Animals are placed in the center of the maze and allowed to explore the maze for 5 minutes.

There are many factors making the EPM popular, including the fact that the test does not require animals to be trained, eliminating the need to use food/water deprivation, shock, or other aversive stimuli. The test is rapid, with each animal being tested once, and test sessions are usually only 5 minutes in duration. A variety of species have been used in the EPM, including rats (Pellow, Chopin, File, & Briley, 1985), mice (Lister, 1987), guinea pigs (Rex, Fink, & Marsden, 1994), wild voles (Hendrie, Eilam, & Weiss, 1994), and Syrian hamsters (Yannielli, Kanterewicz, & Cardinalli, 1996). A principle advantage of the EPM is that the test is bi-directionally sensitive to anxiety manipulations, in that it can detect anxiolytic agents as well as anxiogenic agents. Many variations in EPM responses have been attributed to inter-laboratory differences in procedures (Rodgers, Cao, Dalvi, & Holmes, 1997). Some notable sources of variability in drug effects produced by this model include: prior handling/injection experience, species/age/strain/gender, prior maze experience, lighting level, and the presence of experimenter in the room (Hogg, 1996; Rodgers, Cao, Dalvi, & Holmes, 1997).

The two primary indices of anxiety in the EPM are the amount of time spent on the open arms, expressed as a ratio of the total, and the number of entries into open arms, also expressed as a ratio of the total. Anxiolytic agents tend to increase the exploration of open arms, and anxiogenic agents tend to decrease open arm exploration (% time and % entries). Conceptually, the effects

of drugs on the behavior of animals in the EPM can be understood by referring to Figure 6. The % time or % entries measure may be increased, decreased, or unaffected by a drug. If the drug increases % time and % entries, then it is said to have an anxiolytic effect, and conversely, if %time and %entries decrease, then the effect is said to be anxiogenic. The basal level of exploration % time or % entries determines whether an effect occurs. For example, if a species has low open arm exploration at baseline, then the likelihood of detecting an anxiogenic compound is quite low (floor effect), while the likelihood of detecting anxiolytics is relatively high. The converse also is true.

Triet, Menard, and Royan (1995) found that the lack of thigmotactic cues (absence of walls), rather than the elevation of the maze, is the aspect that makes the open arms aversive. Exposure to a holeboard or to an open field before testing with the EPM has been suggested as a means of increasing exploration on the maze (Pellow et al., 1985; Lister et al., 1987). Anecdotally, it is believed that the exposure to such a test prior to plus-maze testing enhances sensitivity of the test, perhaps because the stress of open-field or novelty exposure (Hogg, 1998; Rodgers & Dalvi, 1997; Handley & McBlane, 1993). Based on a review of EPM studies, a recent reviewer concluded that:

> *"it would appear that sensitivity to potential anxiolytics is enhanced by stressing animals prior to testing (e.g., by moving from holding to test room) or by using more aversive test conditions (e.g., high light)..." (Rodgers, Cao, Dalvi, & Holmes, 1997, p. 293)*

Clearly, stress plays a role in rodents' responses to anxiolytics in the plus maze. However, the role of stress in responses to anxiogenic drugs is unknown. Moreover, the mechanism by which baseline stress acts is unknown. The present hypothesis suggests that changes in the BBB may play a role in the response to drugs in animal models.

Behavioral Pharmacology of the Open Field Test (OFT)

Another frequently used screening tool in behavioral pharmacology is the open field test. This test consists of exposing animals to an arena and allowing them to explore the space freely for a set amount of time. There is marked variation in how the test is conducted, and in how the results are interpreted. Animal behaviors in the OFT have been used as measures of general locomotion, exploration, "emotionality," and anxiety. The test can last from a few minutes to several hours, depending on the construct under examination (e.g., tests of locomotion typically are longer-hours, while tests of anxiety are typically short-minutes).

In studies examining the effects of anxiety-modulating drugs, several variables are typically reported. In the OFT, decreased ambulation and decreased rearing are interpreted as anxiety-like reactions. Increases in defecation and immobility time are indicative of an anxiety-like reaction (Royce, 1977; Bhattacharya, Bhattacharya, & Ghosal, 1998). The conceptualization of locomotion levels as a measure of fear in the OFT is supported by the finding that mice decreased locomotion if tested following attack by another mouse

(Kvist, 1986). Another measure of anxiety is the time spent in the center versus the periphery of the open field. Increased center time spent in the OFT has been interpreted as indicating decreased anxiety, and decreased center time is interpreted to reflect increased anxiety (Lee, Tsai, & Chai, 1986).

Benzodiazepine agonists increase ambulation and rearing (Novas & Wolfman, 1988), while benzodiazepine inverse agonists decrease these measures (Novas & Wolfman, 1988). Biological challenge agents have not been extensively evaluated in the OFT (see Table 3). A literature search revealed no reports on the effects of sodium lactate, sodium bicarbonate, and isoproterenol are available. Yohimbine, however, has frequently been used in the OFT, generally producing an anxiogenic profile. Six studies report decreases in ambulation or rearing, consistent with anxiogenic effects (Ferrari, Tartoni, Monti, & Mangiafico, 1989; Florio, Sakate, & Palermo-Neto, 1993; Bhattacharya, Mohan, & Sen, 1997; Bhattacharya, 1995; Bhattacharya, Bhattacharya, & Ghosal, 1998; Bhattacharya, Satyan, & Charkrabarti, 1997; Koechling, Smith, & Amit, 1990). Contrary to these positive effects, Verleye and Bernet (1987) reported that intra-hippocampal infusions of yohimbine had no significant effect in the behavior of rats in the OFT. This negative finding may reflect the route and dosage used in this study.

Other biological challenge agents have been assessed in the OFT. Flumazenil has been reported to have no effect (Nazar & Plaznik, 1997; Lopez, Miller, Greenblatt, Paul & Shader, 1988). No studies have examined the effects of stress on the OFT responses to flumazenil. ^{m}-CPP reportedly produces an

anxiogenic profile in the OFT (Meert, Melis & Clincke, 1997). CCK-type compounds (CCK-4 and CCK-8) have been reported to produce mixed effects in the OFT (1993). CCK-4, which is used in challenge studies in man, produced increased ambulation (consistent with an anxiolytic effect) in two studies where it was administered directly into the CNS (intraventricular and nucleus accumbens) (Hsiao, Katsuura, & Itoh, 1984; Katsuura, Itoh, & Hsiao, 1985). Another study, however, reported that CCK-4 administered subcutaneously had no effect on OFT behaviors (Hadjiivanova, Kehayov, Petkov, Amblard, & Martinez, 1995). In contrast to the increased ambulation produced by CCK-4 in some studies, CCK-8 produced decreased ambulation in another study (Katsuura, Itoh, & Hsiao, 1984). The reason for these differences in responses to these closely related peptides remains unknown.

Caffeine has been reported to produce increased ambulation (Britton & Indyk 1990; Meliska & Loke, 1984), have no effect (Molinego & Orssetti, 1995), and to have an anxiogenic effect in the OFT (Britton & Indyk 1990). Most interesting from the present perspective is the findings of Britton and Indyk (1990). Britton and Indyk (1990) administered caffeine to animals in the novel open field environment as well as in their familiar home cage environments. In rats tested in the open field, caffeine produced dose-dependent decreases in activity (anxiogenic effect). Rats tested in their familiar home cages had dose-dependent increases in activity. That this same effect was found in responses to CRF (Britton & Indyk 1990; Sutton et al., 1982), and panic disorder patients show enhanced sensitivity to caffeine.

Stress manipulation: Immobilization

Immobilization or restraint is a one of the most widely used stress manipulations. Restraint is non-painful, but it elicits stress responses in rodents. Forced restraint produces elevations in stress hormones, including ACTH, beta-endorphin, prolactin, corticosterone, serotonin, and others (Kant et al., 1983; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Acri, 1994; Shaham, Alvares, Nespor, & Grunberg, 1992; Sharma, Westman, Navarro, Dey & Nyberg, 1996; Sharma, Navarro & Dey, 1991; Sharma & Dey, 1980; Sharma & Dey, 1986). Exposure to varying amounts of immobilization stress produces disruption in the BBB to endogenous and exogenous tracers, as well as enhanced drug effects (Sharma, Westman, Navarro, Dey, & Nyberg, 1996; Sharma, Navarro & Dey, 1991; Sharma & Dey, 1980; Sharma & Dey, 1986; Skultetyova, Tokarev, & Jezova, 1998; Dvorska et al., 1992).

Restraint and the EPM

The effects of restraint stress on the EPM have been assessed in several studies, the majority of which have not tested subjects immediately after the restraint period. Martinenga, Calvo, Volosin, and Molina (1997) exposed rats to 15 minutes of restraint and tested them 24 hours later on the EPM, reporting that the restraint manipulation produced an anxiogenic profile. Similar results have been reported for 2 hours of restraint (Padovan, Del-Bel, & Guimaraes 1996; Guimaraes, Del-Bel, Padovan, Mendonca, & Titze de Almeida 1993; Mendonca & Guimaraes 1998). In each of these studies, the restraint to testing delay was

24 hours. Chaouloff, Baudrie, and Coupry (1994) reported that a 3-hour restraint period produced a partial anxiogenic profile (decreased entries, but not time) when tested 2 hours after restraint.

Only a few studies have examined the effect of restraint on EPM responses immediately following restraint. Falter, Gower, and Gobert (1992) reported that 10 and 20 minutes did not produce anxiogenic profiles; indeed, the restraint actually increased open arm exploration. McBlane and Handley (1994) reported that 15 minutes of restraint had no effect on EPM indices, while one hour of restraint produced a significant decrease in open arm exploration (anxiogenic). In summary, testing from 2 to 24 hours after restraint generally produces an anxiogenic profile, while the effects of restraint immediately following restraint have been variable. These studies are summarized in Table 4.

EPM studies using restraint typically do not test the effects of restraint on drug response, with a notable exception being McBlane and Handley's (1994) examination of restraint and 8-OH-DPAT. The usual pattern in these studies is to either test no drugs at all (Martijena, Calvo, Volosin, & Molina, 1997; Padovan, Del-Bel, & Guimaraes 1996), or test the ability of a drug to attenuate the effects of restraint (Chaouloff, Baudrie, & Coupry 1994). To date no reports are available on the interaction of restraint stress and anxiogenic drugs in the EPM.

Restraint and the Open Field Test

Compared with the elevated plus maze, less research is available on the effects of restraint in the open field test. In the open field test, several studies

have examined the effects of restraint, with the majority of studies reporting effects of restraint following a 24-hour delay. Kennett et al. (1985, 1986, 1987) and others (Carli et al., 1989) have administered either 1 or 2 hours of restraint to rodents and demonstrated anxiogenic effects 24 hours later. Testing rodents in an open field immediately following exposure to restraint for 2 hours decreases locomotor activity (Zafar, Pare, & Tejani-Butt, 1997). Contrary to this finding, Lee, Tsai, and Chai, (1986) tested mice immediately following 1 hour of restraint, and reported an increase in locomotor activity (Lee, Tsai, & Chai, 1986). A literature search revealed no study examining the effects of acute restraint stress exposure on the response to anxiogenic drugs in the open field paradigm.

Section IV: Hypotheses, methods, and data analytic strategy

HYPOTHESES

This doctoral dissertation work consists of two separate experiments. Experiment 1 examines effects of acute stress exposure on behavioral responses to anxiogenic drugs. Broadly, the goal of the experiment was to examine the effects of stress on performance on two measures of anxiety in rats. Experiment 2 assessed whether the effects of stress on anxiogenic drugs can be antagonized via a drug that blocks stress-induced BBB disruption. More specifically, the goals were to:

- determine the extent that responses to anxiogenic drugs which normally do not gain entry into the CNS depend on baseline stress,
- examine whether stress can enhance sensitivity to a drug that crosses the BBB at baseline,
- examine whether indomethacin can antagonize the effects of stress on drug response.

Experiment 1, specific hypotheses were:

1) Yohimbine, a drug that **does** cross the blood brain barrier at baseline **will** produce a dose-responsive increase in anxiety in the elevated-plus-maze and open field test in non-stressed rats.

Rationale: This hypothesis was based on yohimbine's status as a freely permeable compound, and the numerous studies that support yohimbine's anxiogenic properties in animal models of anxiety. Additionally, pilot work in preparation for this project suggested that yohimbine produces dose-dependent increases in anxiety.

2) Yohimbine **will** show a heightened effect in stressed animals (stress x drug interaction—shifting the dose-response curve to the left).

Rationale: There have been several studies which indirectly suggest that compounds which normally enter the CNS can produce greater effects in stressed animals. Pilot work indicated trends consistent with this hypothesis.

3) Isoproterenol (a drug that **does not** cross the blood brain barrier at baseline) **will not** produce a dose-responsive increase in anxiety in the EPM, and OF in non-stressed rats.

Rationale: This hypothesis was based on the poor permeability status of isoproterenol. Additionally, several studies have examined isoproterenol in the elevated plus maze, and failed to find anxiogenic effects, and the present hypothesis suggested that this effect would be replicated in the non-stressed animals. Moreover, pilot work suggested that isoproterenol generally does not produce increases in anxiety in the animal models studied.

4) Isoproterenol **will** show a dose-responsive anxiogenic effect in stressed animals (stress x drug interaction).

Rationale: This hypothesis is based on the clinical and pre-clinical literature which suggested that subjects who are stressed at baseline show vulnerability to sodium lactate and other challenge agents. Additional support was drawn from reports in primates that sodium lactate exerts an anxiogenic effect in stressed animals. Pilot work indicated an anxiogenic effect for isoproterenol following stress exposure.

5) The stress manipulation (restraint) **will** have little if any effect on anxiety indices.

Rationale: The literature suggested that 10 and 20 minutes of restraint either increased or has no effect on open arm exploration in the EPM (Falter, Gower, & Gobert, 1992; McBlane & Handley, 1994). One hour of restraint produces significant decreases in open arm exploration in the EPM (McBlane & Handley, 1994). Pilot data revealed that 30 minutes of restraint did not affect % time or % entries in the EPM. In the OFT, restraint moderately reduced horizontal activity but did not affect other behavioral indices.

Experiment 2 was contingent on finding stress x drug interactions in Experiment 1. Because several stress by drug interactions were observed in Experiment 1, the second experiment was conducted, using one dose of isoproterenol and two doses of yohimbine. The goal of Experiment 2 was to assess (indirectly) the extent to which enhanced sensitivity to anxiogenic drugs is a result of BBB disruption.

Experiment 2, specific hypotheses:

1. Indomethacin pre-treatment will antagonize the stress x drug interactions found in Experiment 1.

Rationale: This hypothesis was based on reports that indomethacin blocks stress-induced BBB permeability (Sharma, Westman, Navarro, Dey, & Nyberg, 1996; Sharma & Dey, 1981; 1984; 1986; 1987) and was based on the hypotheses of Experiment 1.

METHODS

Overview

The experiments in this research project examined the role of stress in behavioral responses to anxiogenic agents (isoproterenol and yohimbine) in rats. The experiments were conducted in serial order, with the performance of Experiment 2 contingent on finding drug X stress interactions in Experiment 1. Within each experiment, all subjects were evaluated using two different indices of anxiety in animals, each of which included several measures. The stressor in the experiments was conceptualized as a means of recreating the high baseline anxiety some panic disorder patients exhibit before biological challenge agent administration. According to the present hypothesis, this higher baseline stress places these patients at greater risk for panicking during challenges, because of pharmacokenetic changes. The particular non-painful stressor (i.e., restraint) has repeatedly been found to increase permeability to the BBB in animals. The experimenter was not blind to animals' condition during testing, but, was blind to condition during scoring the video-taped EPM data. Data from the OFT was scored via computer.

Experiment 2 was an attempt to pharmacologically antagonize (with indomethacin) the stress X drug interaction findings that occurred in Experiment 1. Indomethacin has been reported to prevent stress-induced BBB disruption (Sharma, Westman, Navarro, Dey, & Nyberg, 1996; Sharma & Dey, 1981; 1984; 1986; 1987). A timeline for these experiments appears in Table 5.

Experimental design and sample size

This research was conducted as two separate experiments. Experiment 1 was a 2 (yohimbine or isoproterenol) X 3 (vehicle, dose 1, or dose 2) X 2 (no stress or immobilization) factorial design, with 10 subjects per cell. Experiment 2 included one dosage for those drugs that reveal a stress x drug interaction on either of the behavioral indices of anxiety and control groups with 10 subjects per cell. The sample size per cell was determined in two ways: (1) using an approximation of the n used in published reports using the same behavioral measures and the same drugs (Cole, Burroughs, et al., 1995; Johnston & File, 1989; Rodgers, Cole, Aboualfa, & Stephenson, 1995; Bhattacharya, 1985), and (2) a power analysis based on pilot work performed in preparation for this research.

The power analyses were conducted using procedures of Keppel (1991), Keppel, Saufley, and Tokunaga (1992), and Cohen (1988). Using phi and the appropriate power function in Table A-6 (Keppel et al., 1992, p. 539), power was determined. The same formulas were then used to calculate the n per cell necessary to achieve power of 0.80 as recommended by Cohen (1988). For those significant effects evident in pilot data, this analysis revealed that a sample size of 10 per cell was adequate to replicate these effects at a level of power of 0.80 or better.

It was hypothesized that there would be a statistically significant drug X stress interaction for each of the two anxiogenic drugs studied. If evident, this interaction would offer evidence that the response to anxiogenic agents depends

in part on the basal level of stress. If a significant drug X stress interaction occurred in the drug that does not cross the BBB at baseline, then it would suggest that there might be a central mechanism of action for its anxiogenic properties. The agent that crosses the BBB at baseline (yohimbine) was expected to show greater effects with stress due to its higher permeability.

Experiment 2 was an assessment of whether the effects of stress plus anxiogenic drugs could be antagonized via a drug (indomethacin) that blocks stress-induced BBB disruption. The performance of Experiment 2 and the type and dosage of drugs to be used in Experiment 2 were contingent on the findings of Experiment 1. Experiment 2 examined whether pretreatment with indomethacin (which blocks stress-induced BBB disruption) antagonizes the stress-enhanced sensitivity to anxiogenic drugs used in Experiment 1.

Both yohimbine and isoproterenol demonstrated interactions with stress, with both doses of yohimbine producing greater effects when combined with stress. For isoproterenol, only the high dose of isoproterenol interacted significantly with stress. Therefore, both doses of yohimbine and the high dose of isoproterenol were tested in Experiment 2.

Subjects

Subjects for Experiment 1 were 120 male Wistar rats (Charles river), weighing approximately 180-220g at the beginning of the study. Wistar rats were selected based on their frequency of use in previous studies using the dependent variables. Subjects were housed in groups of two, in standard polycarbonate

shoebox cages (42 x 20.5 x 20 cm) on hardwood chip bedding (Pine-dri). An adaptation period of at least 7 days was allowed before beginning experiments. Rats had continuous access to rodent chow (Haran Teklad 4% Mouse/Rat diet) and water throughout the experiment, except during experimental sessions. Rooms were maintained on a twelve-hour light-dark cycle, with onset at 0600 hours. Temperature and relative humidity in the housing and testing rooms were maintained at 23^0 C and 50%, respectively. Experiment 2 used 80 male Wistar rats as subjects, with identical housing conditions as the rats in Experiment 1.

Independent variables

Drugs

All drugs were adjusted to a neutral pH using NaOH before administration. Drugs requiring dilution in vehicle were mixed into sterile de-ionized water. Vehicle injections consisted of sterile, de-ionized water. The experimenter was not blind to condition during drug administration.

Yohimbine

Yohimbine is an α_2-adrenergic antagonist. Yohimbine was administered in two dosages, 1.0 and 2.5 mg/kg, ip. Yohimbine was administered 30 minutes before testing (for animals in the stress groups, this requires administration immediately before placement in restrainers). The dosage range and timing of administration were based on previous studies using the EPM and OFT (Bhattacharya, 1985; Bhattacharya, Bhattacharya, & Ghosal, 1998; Bhattacharya, Mohan, & Sen, 1997; Florio, Sakate, & Palermo-Neto, 1993;

Johnston & File, 1988; Mangiafico, Cassetti, & Farrari, 1989; Pellow, Chopin, File, & Briley, 1985; Pellow, Johnston, & File, 1987).

Isoproterenol

Isoproterenol is a non-selective beta-adrenergic agonist. Isoproterenol was administered in two dosages, 0.4 and 0.8 mg/kg, ip. Isoproterenol was administered 30 minutes before testing (for animals in the stress groups, this requires administration immediately before placement in restrainers). The dosage range and timing of administration were based on previous studies using the elevated plus maze (Johnston & File, 1988; Rodgers, Cole, Aboualfa, & Stephenson, 1995).

Indomethacin

Indomethacin is a prostaglandin synthesis inhibitor. Indomethacin was administered (10 mg/kg ip) to all rats in Experiment 2, 60 minutes prior to testing. Rats in the stress groups, therefore, received the indomethacin 30 minutes before the immobilization session (allowing for onset). Rats in the control groups received indomethacin at the same time (i.e., 60 minutes before testing), but were not immobilized. The dosage range and timing of administration were based on previous studies using indomethacin to antagonize BBB disruption (Sharma & Dey, 1981; 1984; 1986; 1987; Sharma et al., 1996).

Stressor

Immobilization

Apparatus & Procedure

Rats in the restraint condition were placed in commercially available finger-like restraint devices (Centrap Cage, Fisher Scientific). Once a rat was placed into the apparatus, the restraining fingers were tightened to hold subjects securely, but not pinch or cause apparent pain. The investigator checked restrained animals every 5 minutes during immobilization. This procedure reliably produces increases in stress hormones (Acri, 1994; Kant et al., 1983; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Shaham, Alvares, Nespor, & Grunberg, 1992; Sharma & Dey, 1980; Sharma & Dey, 1986; Sharma, Navarro, & Dey, 1991; Sharma, Westman, Navarro, Dey, & Nyberg, 1996).

Dependent Variables

Elevated Plus Maze (EPM)

Apparatus:

The EPM was built based on the apparatus of Pellow et al. (1985). The EPM consisted of a square central platform (10 x 10 cm), with four arms (45 x 10 cm) radiating out from the center platform. This plus-shaped platform was elevated to a height of 50 cm above the floor. The center platform and arms were all made of smooth plywood, painted black. Two of the four arms were open and two arms are closed. Open and closed arms were placed on opposing sides of the center platform. Both the closed arm walls (45 x 50 x .5 cm) and

open arms walls (45 x 3 x .5 cm) were constructed from clear Plexiglas. The open arms were equipped with these short walls to increase the detection of anxiogenic drug effects (Cole, Hillmann, Seidelmann, Klewer & Jones, 1995; Fernandez & File, 1996; Jones & Cole, 1994). The only illumination in the room came from a single 60-watt light bulb aimed at the ceiling directly above the maze.

Procedure:

Rats were individually placed in the center of the maze facing a closed arm, and allowed to explore the maze for 5 minutes. Behaviors during the 5-minute session were monitored via a closed circuit TV camera, and video-recorded for later scoring. Behaviors were scored for time spent in the open/closed arms, number of entries into open and closed arms, number of entries, time spent in, and the latency to enter the end sections of the open arms. Entries and time spent in the back section of the closed arms also was scored. Time spent head-dipping and immobility time were also scored. Additionally, the total number of arm entries, the time spent in the center square, and entries and time spent in the back section of the closed arms were scored. The maze floor and walls was wiped clean with a wet (90% water and 10% isopropyl alcohol) cloth following each session. Table 6 presents the variables and the interpretation of direction of changes for the EPM.

Open Field Test (OFT)

Apparatus

The open field test consisted of a 16 in x 16 in x 12 in Plexiglas box. Rats were placed into the center of the open field and allowed to move around the arena for a 10-minute period. The open field was situated inside an Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM (16 TAO)). Two arrays of photocells measured horizontal locomotor activity. The first array spans side-to-side and had 16 pairs of photocells, located on a plane 2 cm from the floor of the arena, and spaced 2.5-cm apart from each other. The second array is configured identically, with the exception that it runs front-to-back. Vertical activity is measured via a third photocell array, located on a plane 10.5 cm above the arena floor. Data were collected and transmitted to a computer via an Omnitech Model DCM-I-BBU analyzer. The data files were processed through a software program (VersaMax, 2000).

Procedure

After the rat completed the EPM, it was placed into the center of the open field, and assessed in the OFT for a 10-minute period. Behaviors that were recorded include: movement in the center, immobility time, rearing, and total locomotion. The entire chamber was wiped clean with a wet cloth (90% water and 10% isopropyl alcohol) following each session. Following completion of the OFT, rats were immediately transferred to an adjacent room and sacrificed.

Biological sample collection

In the sample collection room, animals were anesthetized with 5% Isoflurane until unconscious (approximately 20 seconds), using a rodent nose cone with a scavenging system. Anesthesia was maintained while the chest cavity was surgically opened and the heart exposed. The right atrium was punctured surgically, allowing blood to escape the heart. Blood samples were collected via a pipette from the chest cavity, where blood pooled next to the atrium. Blood collection was completed prior to beginning saline infusion. The animals were perfused using normal saline for a period of 4-6 minutes. Following the perfusion procedure, animals were decapitated and the brains carefully removed and placed into crushed dry ice. Brains were stored at – 80 degrees C.

Corticosterone Extraction Process

Corticosterone was extracted from brain tissue following the methodology of McEwen, Stephenson, and Krey (1980). Briefly, the forebrain of the left hemispheres was separated from the cerebellum and brainstem with a saggital cut. Forebrains were placed in glass tubes with 10 ml of Dichloromethane (DCM) and homogenized using a Brinkman Instruments Poyltron homogenizer (Westbury, NY). The tubes were centrifuged at 1000 x g at 0^0 C for 10 minutes. The DCM was decanted and 1 mL of 0.1 N NaOH was added to the mixture, which was then vortexed for 3 seconds. Next, 1 mL of 0.1 N Acetic acid was added and the mixture was vortexed for another 3 seconds. The mixture was then centrifuged at 1000 x g at 0^0 C for 10 minutes. The DCM layer was

transferred to a 16 x 100 mm glass tube with a Drummond Pipet-aid loaded with a 5 ¾ in. pasture pipette. This layer was evaporated to dryness for 2-3 nights in a ventilation hood. The extract was re-dissolved into 500 microliters of ethanol in preparation for the assay. The extract was covered and sonicated until fully dissolved. Assay samples were pipetted off from the ethanol.

Corticosterone Measurement

Total serum corticosterone was measured by a double-antibody radioimmunoassay (RIA) kit using ^{125}I-labled corticosterone (ICN Biomedicals, Costa Mesa, CA). A limited amount of specific antibody (rabbit anti-corticosterone antiserum) is reacted with a fixed quantity of ^{125}I-labled corticosterone. The concentration of unlabeled corticosterone in samples increased as a function of the decreasing percentage of bound radioisotope-labeled corticosterone. The second antibody (goat anti-rabbit gamma globulin) precipitates antibody bound to antigen. The quantity of endogenous corticosterone was determined by measuring the radioactivity of the precipitate with known standards from the same assay in a gamma counter and converting DPM into concentrations. All samples and standards were run in duplicate. This measure was included to verify that the stress manipulation was effective.

DATA ANALYSIS

The goals of data analysis for Experiment 1 were to determine: whether drug exposure altered indices of anxiety (dependent measures); whether stress exposure altered each dependent measure; whether there was a significant stress X drug interaction in altering the dependent measures.

The goals of data analysis for Experiment 2 were to determine: whether the stress X drug interaction effect hypothesized in Experiment 1 was antagonized by pretreatment with an anti-inflammatory compound that prevents stress-induced BBB disruption; whether the stress X drug interaction replicated in the control conditions of this experiment.

Dependent measures of anxiety from the EPM and OFT were analyzed using multivariate analysis of variance (MANOVA), because these variables are conceptually related to one another. In order to minimize type 1 error, only variables that were significant in the MANOVA were pursued for further analysis. Data were examined in scatterplots for normal distribution and evaluated using Levene's test of homogeneity of variance. Whenever data from the animal models of anxiety failed to meet criteria for parametric tests (i.e., normal distribution, homogeneity of variance), and were not correctable using logarithmic transformations, they were analyzed using nonparametric ANOVAs (Kruskal-Wallis test). Data from corticosterone measures were analyzed using one-way ANOVAs, with stress condition as the between-subjects factor. All tests were two-tailed. Where appropriate, Dunnett's test was used to determine differences between the treatment and control groups.

Several strategies were used to minimize the probability of Type I error. First, global analyses incorporating all factors (Drug, Dosage, Stress) were used to guide internal analyses. Therefore, analysis of subgroups was pursued only if overall analyses revealed main effects or interactions. Second, conservative analytic approaches were used whenever appropriate (i.e., MANOVA rather than ANOVA). Third, although the hypotheses in this project are almost all directional in nature, the statistical tests were all two-tailed.

SECTION V: RESULTS AND DISCUSSION

RESULTS OF EXPERIMENT ONE

Elevated Plus Maze

The Elevated Plus Maze provides multiple indices of anxiety, including % time in the open arms, % time in the closed arms, number of entries and time spent in the end sections of the open arms, latency to enter the end sections of the open arms, entries and time spent in the back section of the closed arms, time spent head dipping, and time spent immobile (Hogg, 1996; Setem, et al., 1999; Rodgers & Dalvi, 1997; Rodgers, Cao, Dalvi, & Holmes, 1997). Total arm entries and entries into the closed sections are thought to reflect general locomotor activity. The % time in the center is believed to be a measure of decision-making (Hogg, 1996; Rodgers & Dalvi, 1997). In the present experiment, these indices were used to examine whether stress would produce an interaction with the anxiogenic drugs.

A MANOVA incorporating the effects of both doses of Yohimbine and Isoproterenol with the effects of stress was run. Variables included in the analyses included % time in the open arms, % entries into the open arms, % time in the closed arms, frequency of exploring end sections of open arms, time spent in end sections of open arms, and latency to enter open arm end sections. Additionally, time spent in the back of closed arms, entries into the back of the closed arms, time spent immobile, time spent head dipping, total entries, and entries into the closed sections were examined (the overall F was significant [$F(117,848) = 2.22$, $p < 0.001$], indicating that there was a significant difference

among groups). Of the 13 dependent variables included, 11 were significant (at least < .01), and time head dipping showed a trend (p= 0.09). Entries into the open arms failed to reach significance, so this variable was not pursued in further analyses. Individual EPM variables were examined using a two-way ANOVA with stress and drug conditions as the between-subjects factors.

Yohimbine (1.25 & 2.5 mg/kg)

Indices of Anxiety

Increases in anxiety in EPM indices are reflected by variables either increasing or decreasing. *Decreases* in the % time in the open arms; frequency of end section exploration, time spent exploring end sections, and time spent head-dipping are interpreted as heightened anxiety. *Increases* in % time in the closed arms; latency to enter the end sections of the open arms, frequency of entry into the back of the closed arms, time spent in the back of the closed arms, and time spent immobile also reflect increased anxiety.

Significant main effects and interactions for the EPM variables are reported below. The principal question of this experiment was whether or not the interaction of stress and the drug would be significant. In that respect, the interaction effects are therefore primary, and the main effects of stress and drug are secondary.

Data are presented schematically for % time in open arms (Figure I-1), % time in closed arms (Figure I-2), entries into end sections (Figure I-3), time spent in end exploration (Figure I-4), latency to enter ends (Figure I-5), entries into

back of closed (Figure I-6), time spent in back of closed (Figure I-7), time spent head dipping (Figure I-8), and time spent immobile (Figure I-9).

Interaction Effects

The ANOVA results for the interaction of Stress and Yohimbine are summarized in Table 9. The interaction of stress and yohimbine significantly *decreased* % time in the open arms [F (1, 78) = 5.55, p < .01], time spent in end exploration [F (1, 75) = 3.99, p < .05], frequency of end exploration [F (1, 77) = 9.02, p < .001]. Stress x drug interactions also significantly increased % time in the closed arms [F (1, 78) = 5.55, p < .01]; latency to explore end sections [F (1, 71) = 5.24, p < .01], and entries into the back of the closed arms [F (1, 79) = 4.50, p < .05]. The direction of each of the interaction effects was consistent with an anxiogenic effect for stress interacting with yohimbine.

The main effects for drug and stress presented below should be qualified by noting that stress had very little effect on its own, and for many variables, drug had little effect on its own as well. Examination of graphed data from the variables (Figures I-1 to I-9) shows that the majority of the statistical main effect observed for stress and drug is the result of the interaction effect.

Drug Effects

Main effects for Yohimbine in the ANOVA results are summarized in Table 7. Yohimbine administration significantly *decreased* % time in the open arms [F (2, 78) = 6.70, p < .01], frequency of end exploration [F (2, 77) = 9.42, p < .001], time spent in end exploration [F (2, 75) = 6.38, p < .005], and time spent head dipping [F (2, 62) = 7.06, p < .01]. Yohimbine significantly *increased* % time in

the closed arms [F (2, 78) = 11.88, p < .001], and entries into the back of the closed arms [F (2, 79) = 13.76, p < .001]. A trend towards a significant main effect for Yohimbine was observed in latency to explore end sections of the open arms [F (2, 71) = 2.88, p < .06], such that Yohimbine increased the latency period. The direction of each of these main effects is consistent with an anxiogenic effect for yohimbine (i.e., variables either increased or decreased in the predicted direction), however, this anxiogenic effect must be qualified by the presence of a significant interaction.

Stress Effects

Table 8 summarizes the main effects of Stress. The 30-minute restraint stressor significant *decreased* % time in the open arms [F (1, 78) = 10.55, p < .01]; frequency of end exploration [F (1, 77) = 24.25, p < .001], and time spent in end exploration [F (1, 75) = 15.55, p < .001]. Stress significantly *increased* % time in the closed arms [F (1, 78) = 13.04, p < .01], and latency to explore end sections [F (1, 71) = 13.43, p < .001]. The direction of each of these main effects is consistent with an anxiogenic effect for stress (i.e., variables either increased or decreased in the predicted direction), however, this anxiogenic effect must be qualified by the presence of a significant interaction.

Post hoc analysis revealed that only the two groups receiving stress and drug were significantly different from non-stressed control animals for the following variables: % time in the open arms, % time in the closed arms, frequency of end exploration, time spent in end exploration, and time spent

immobile on the maze. For entries into the back of the closed and time spent head dipping, *post hoc* analysis revealed that only the non-stressed high dose yohimbine group was significantly different from controls. Only the low dose yohimbine and stress group significantly differed from controls for latency to enter open end sections and time in the back of the closed.

Data for two variables did not meet criteria for homogeneity of variance, and was not able to be corrected with logarithmic transformations, therefore a non-parametric Kruskal-Wallis (K-W) test was used. For time spent immobile on the maze, K-W revealed a significant difference between the groups [H (5) = 28.79, $p < .001$]. For time spent in the back of the closed arms, the K-W test revealed a significant difference between the groups [H (5) = 15.69, $p < .01$]. Although non-parametrics cannot yield interaction effects, the pattern of group differences was consistent with the hypothesis that stress and yohimbine had an interactive effect in both of these variables. K-W tests statistics are not included in the ANOVA tables, as they do not yield main or interaction effects.

Other Indices: Locomotion and Decision making

Locomotion

The two variables that are thought to reflect locomotor changes in the EPM are the total number of entries and the number of entries into closed arms (Hogg, 1996; Rodgers & Dalvi, 1997; Rodgers, Cao, Dalvi, & Holmes, 1997).

A significant main effect was observed for yohimbine in reducing total entries [F (2, 77) = 6.50, $p < .005$] and closed arm entries [F (1, 73) = 5.00, $p <$

.01]. ANOVA revealed a significant main effect of stress on total entries [F (1, 77) = 29.32, p < .001], and closed arm entries [F (1, 73) = 19.15, p < .001]. Significant stress by drug interactions were also observed, wherein both total entries [F (1, 77) = 6.97, p < .005] and closed arm entries [F (2, 73) = 3.97, p < .05] were reduced. Figure I-10 presents the total entries, and Figure I-11 presents the closed arm entries for control and yohimbine animals.

Decision making

The amount of time an animal spends in the center square of the plus maze reflects decision-making processes. ANOVA revealed a significant main effect of stress on % time spent in the center [F (1, 77) = 8.54, p < .01]. A significant main effect was observed for drug [F (2, 77) = 11.57, p < .001]. There was no significant interaction of stress X drug for % time spent in the center [F (1, 77) = 1.34, p = NS]. Figure I-12 presents the % time spent in the center for control and yohimbine animals.

Isoproterenol (0.4 & 0.8 mg/kg)

Indices of Anxiety

Significant main effects and interactions for the EPM variables are reported below. The principal question of this experiment was whether or not the interaction of stress and the drug would be significant. Therefore, the interaction effects are primary, and the main effects of stress and drug are secondary.

Data are presented schematically for % time in open arms (Figure I-13), % time in closed arms (Figure I-14), entries into end sections (Figure I-15), time

spent in end exploration (Figure I-16), latency to enter ends (Figure I-17), entries into back of closed (Figure I-18), time spent in back of closed (Figure I-19), time spent head dipping (Figure I-20), and time spent immobile (Figure I-21).

Interaction Effects

The ANOVA results for the interaction of Stress and Isoproterenol are summarized in Table 12. Contrary to the weak or non-existent effects of stress and isoproterenol alone, the interaction of these two factors produced several interaction effects. The interaction of stress and isoproterenol significantly *decreased* time spent in end exploration [F (1, 80) = 2.60, $p < .05$], frequency of end exploration [F (1, 79) = 3.96, $p < .05$], and showed a trend at reducing time spent head dipping [F (1, 79)= 2.62, $p < .07$]. There was a strong interaction effect for *increasing* entries into the back of the closed arms [F (1, 63) = 3.35, $p < .05$], and trends towards significant effects for time spent in the back of the closed arms [F (1, 63) = 2.43, $p = .09$], and latency to explore end sections [F (1, 76) = 2.61, $p = .08$]. The direction of each of these interaction effects and trends was consistent with an anxiogenic effect for the combined effects of stress and isoproterenol (i.e., variables either increased or decreased in the predicted direction). This finding is consistent with hypothesis 4.

The main effects for drug and stress presented below should be qualified by noting that stress had very little effect on its own, and for many variables, drug had little effect on its own as well. Examination of graphed data from the variables (Figures I-13 to I-21) shows that the majority of the statistical main effect observed for stress and drug is because of the interaction effect.

Drug Effects

Main effects for isoproterenol (ANOVA results) are summarized in Table 10. Isoproterenol administration did not significantly increase any EPM variable. A trend towards a significant main effect for isoproterenol was observed in isoproterenol reducing entries into the back of the closed arms [F (2, 63) = 2.67, p = .06]. While one variable did show a trend-overall, it must be concluded that isoproterenol alone was not anxiogenic. This finding replicates findings from two other laboratories (Johnston & File, 1988; Rodgers, Cole, Aboulafa, & Stephenson, 1995). A lack of effect for drug alone is also consistent with hypothesis 3.

Stress Effects

Table 11 summarizes the main effects of Stress. The 30-minute restraint stressor significant *increased* latency to explore end sections [F (1, 76) = 5.92, p < .01], and showed a trend towards *decreasing* time spent head dipping [F (1, 79) = 3.06, p = .08]. These two variables indicate a very weak effect of stress in affecting behavior in the plus maze.

Post hoc analysis did not detect significant differences between groups for the majority of variables. However, for time spent immobile, the high dose isoproterenol group receiving stress was significantly different from the control group. Additionally, entries into the back of the closed arms showed a trend towards the high dose + stress group being significantly different from the control group.

Data for two variables did not meet criteria for homogeneity of variance, and was not corrected with logarithmic transformations, therefore a non-parametric Kruskal-Wallis (K-W) test was used. For time spent immobile on the maze, there was a significant difference between the groups [H (5) = 15.35, p < .01]. For % time spent in the closed arms, there were no significant differences between groups [H (5) = 4.00, p = NS]. Although non-parametrics cannot yield interaction effects, the pattern of group differences in time spent immobile was consistent with the hypothesis that stress and isoproterenol had an interactive effect. K-W test statistics are not included in the ANOVA tables, as they do not yield main or interaction effects.

Other Indices: Locomotion and % time in center (decision making)

Besides indices of anxiety, the EPM yields measures of locomotion and decision-making.

Locomotion

The two variables that reflect locomotor changes in the EPM are the total number of entries and the number of entries into closed arms (Hogg, 1996; Rodgers & Dalvi, 1997; Rodgers, Cao, Dalvi, & Holmes, 1997). No significant main effects were observed for isoproterenol in altering total entries or closed arm entries. ANOVA revealed a significant main effect of stress on total entries [F (1, 73) = 6.95, p < .05]. Stress had no significant effect on closed arm entries [F (1, 80) = 1.80, p = NS]. No significant stress by drug interactions were

observed. Figure I-22 presents the total entries, and Figure I-23 presents the closed arm entries for control and isoproterenol animals.

Decision making

The amount of time an animal spends in the center square of the plus maze is thought to reflect decision-making processes (Hogg, 1996; Rodgers & Dalvi, 1997, Rodgers, Cao, Dalvi, & Holmes, 1997). ANOVA revealed no significant main effect of stress or drug on % time spent in the center. Additionally, there was no significant interaction of stress X drug for % time spent in the center. Figure I-24 presents the % time spent in the center for control and isoproterenol animals.

Open Field Test

A MANOVA incorporating the effects of both doses of yohimbine and isoproterenol with the effects of stress was run. The overall F for was significant (F (36,418) = 4.86, $p < 0.001$), indicating that there was a significant difference among groups. Variables included in the analyses included locomotion, rearing, immobility time and time spent in the center. All four dependent variables were significant.

Yohimbine (1.25 & 2.5 mg/kg)

Table 13 presents the ANOVA results for Yohimbine in the OFT. Data are presented schematically for locomotion (Figure I-25), rearing (Figure I-26), immobility (Figure I-27), and center time (Figure I-28).

Interaction effects

There was a significant interaction of stress X drug for rearing [F (1, 78) = 3.44, p = <0.5], wherein stress and drug reduced the rearing time.

Stress effects

There was a significant main effect of stress to *decrease* locomotion [F (1, 78) = 6.60, p = <0.05], rearing [F (1, 78) = 14.84, p = <0.001], and *increase* immobility time [F (1, 76) = 9.00, p = < 0.005].

Drug effects

There was a significant main effect was observed for drug in reducing locomotion [F (2, 78) = 6.36, p < .005] and rearing [F (2, 78) = 46.66, p < .001]. A trend towards a significant main effect was observed for yohimbine reducing center time [F (2, 76) = 2.67, p < .07].

Post Hoc Analysis revealed that for locomotion and immobility time, only the high dose stressed animals were significantly different from controls. For rearing, both the high dose animals (stressed and non-stressed) and the low dose stressed groups were significantly different from controls. For time in the center, only the high dose animals (stressed and non-stressed) were significantly different from controls.

Isoproterenol (0.4 & 0.8 mg/kg)

Table 14 presents the ANOVA results for the OFT. Data are presented schematically for locomotion (Figure I-29), rearing (Figure I-30), immobility (Figure I-31), and center time (Figure I-32).

Interaction effects

There was a significant interaction of stress X drug for decreasing Center time [F (1, 78) = 3.70, p = < .05]. There was a trend towards a significant interaction of stress X drug for decreasing locomotion [F (1, 78) = 2.77, p = < .06]. Another trend towards a significant interaction of stress X drug was observed in increasing immobility time [F (1, 77) = 2.38, p = < .09].

Stress effects

There were no significant main effects of stress on any OFT variable

Drug effects

There was a significant main effect for drug to reduce locomotion [F (2, 78) = 4.24, p < .05] and rearing [F (2, 74) = 7.56, p < .005]. Isoproterenol increased immobility time [F (2, 77) = 4.88, p < .05].

Post hoc analysis revealed that the low-dose non-stressed group was significantly different from control animals for locomotion, rearing, and immobility time.

Corticosterone Data

Data are presented schematically for blood and brain corticosterone levels (Figure I-33). Corticosterone RIA results were submitted to a one-way ANOVA

with stress condition as the between-groups factor. Stressed animals had a higher blood level of corticosterone, however, this was only a trend towards significance based on a two-tailed test [F (1, 178) = 3.26, p = < .072]. Contrary to expectation, stressed animals displayed lower levels of corticosterone in brain tissue [F (1, 178) = 7.03, p = < .01]. The finding of lower, rather than higher, brain levels of corticosterone of stressed rats was unexpected. Several possible reasons for this finding are presented in the discussion.

EXPERIMENT 1: ASSESSMENT OF STUDY HYPOTHESES

The present experiments were designed to answer two Major Hypotheses, each addressed in separate experiments. Major Hypothesis 1 was the broad prediction that acute stress exposure would heighten the behavioral effects of anxiogenic drugs. Experiment 1 was framed to answer this broad hypothesis, and contained five minor hypotheses that supported the broader hypothesis. Minor Hypotheses 1 and 2 addressed the effects of yohimbine in non-stressed and stressed rats, respectively. Minor Hypotheses 2 and 3 addressed the effects of isoproterenol in non-stressed and stressed rats respectively. Minor hypothesis 5 addresses the effects of stress on anxiety indices.

Major hypothesis 1

This hypothesis was **confirmed**. The pattern of results from Experiment 1 (see minor hypotheses 1-5) indicates that there was an interactive effect of stress with the drugs administered. The combination of stress and anxiogenic drugs effectively shifted the dose-response curve in the predicted leftward direction.

Minor hypothesis 1

The hypothesis that yohimbine, which easily passes the BBB, will produce dose-response anxiety effects in non-stressed rats in EPM and OFT indices was **confirmed**. The lower dose of yohimbine produced little anxiogenic effect in non-stressed animals, while the higher dose produced anxiogenic effects.

Minor hypothesis 2

The hypothesis that yohimbine will produce heightened dose-responsive anxiety effects in stressed rats in EPM and OFT indices was **partially confirmed**. In the EPM, the predicted shift in the effects of yohimbine was observed. In contrast to the non-stressed rats, the lower dose of yohimbine produced a strong anxiogenic effect in stressed animals. In the OFT, however, no stress by drug interactions were observed.

Minor hypothesis 3

The hypothesis that isoproterenol, which does not pass the BBB at baseline, will not produce dose-response anxiety effects in non-stressed rats in EPM and OFT indices was **partially confirmed**. The lower dose of isoproterenol produced virtually no anxiogenic effects in non-stressed animals in the EPM, while the higher dose produced only mild anxiogenic effects. While results from the EPM were as predicted, OFT indices revealed an unpredicted effect of the low dose of isoproterenol, indicating that the low dose produced locomotor and other deficits in the OFT. The pattern of results for the OFT did not conform to predictions

Minor hypothesis 4

The hypothesis that isoproterenol will produce heightened dose-response anxiety effects in stressed rats in EPM indices was **confirmed**. In the EPM, the predicted shift in the effects of isoproterenol was observed. In contrast to the non-stressed rats, the high dose of isoproterenol produced a moderate

anxiogenic effect in stressed animals. Although the two traditional measures (% time and entries into the open arms) failed to show this effect, ethological measures (e.g., immobility time) clearly demonstrated stress by drug interactions. The hypothesis was **confirmed** in the OFT, however, there were stress by drug interactions which did not conform to predictions.

Minor hypothesis 5

The hypothesis that stress will produce little effect on anxiety indices in EPM and OFT indices was **partially confirmed**. For the majority of indices in the EPM and OFT, the effects of stress were minimal or anxiolytic.

DISCUSSION OF EXPERIMENT 1

Experiment 1 was designed to test the hypothesis that stress would produce a shift in the dose-response curve for anxiogenic drugs. While a strong profile for stress x drug interactions emerged for yohimbine, isoproterenol demonstrated only a moderate stress by drug interaction profile. Yohimbine is the more permeable of the two compounds tested in this analysis and, consistent with minor hypotheses 1 and 2, it showed anxiogenic effects alone and heightened effects with stress. This finding is consistent with a leftward shift in the dose response curve to yohimbine. How a brief and mild stress exposure effected this shift is unknown. Some evidence is consistent with the hypothesis that stress induced a disruption in the blood-brain-barrier (BBB).

Isoproterenol is much less permeable compared to yohimbine. Consistent with lower baseline permeability, it showed no effects on anxiogenic measures in the elevated plus maze (EPM). Indeed, neither low dose nor high doses of isoproterenol showed anxiogenic effects on most measures in non-stressed animals. Contrasted to this lack of effect in terms of EPM variables for isoproterenol in non-stressed animals, stress by drug interactions were demonstrated for several EPM variables, each in the predicted direction. This finding is consistent with the hypothesis that stress lowered the threshold for permeability to isoproterenol, allowing more drug to enter the CNS. Although this explanation is consistent with the data, the current experimental design did not directly evaluate the levels of drug entering the CNS.

The hypothesized interactions of stress and drug were observed in the EPM but not in the open field test (OFT). The reason for the failure of the open field test to reveal the same type of interaction is unclear. Perhaps, the two tests may measure different aspects of anxiety. As noted by Sanger, (1991) different tests of anxiety often tap into different facets of anxiety. Alternatively, the use of the open field test in immediate succession to the elevated plus maze might have altered the results obtained with the open field test. Rodgers and Shepard (1993) reported that exposure to the EPM resulted in an anxiolytic effect in rodents upon subsequent testing in a light dark transition test, and eliminated expected drug effects. A third possibility for the differences in results between the two tests is a time of testing effect. The interaction of stress and drug may be detectable only within a short time frame. Because the rats were tested in the EPM for 5 minutes, and then a period of several seconds went by before transferring to the OFT, the effect could have been evident only in the first few minutes, and was not able to be detected in the OFT. The reason for the effects being evident only in the EPM is unknown, and should be evaluated in future research.

Surprisingly, an unpredicted pattern of reactions emerged in the OFT with isoproterenol administration. The low dose non-stressed animals emerged as having the lowest scores on locomotion, center time and rearing, and the highest scores on immobility time. It is unknown why this effect of low dose isoproterenol occurred. It is interesting to note that animals given the same low dose in the stressed group responded in the same manner as those given the higher dose.

Only animals in the low dose non-stressed group demonstrated the marked reductions in activity levels. This finding, although unpredicted and not explainable, highlights that the stress manipulation altered the response to the drug. The finding is also consistent with a leftward shift in the dose response curve, where the stressed low dose group behaviorally looked identical to the high dose non-stressed group.

A curious finding was that the low dose of yohimbine administered under stress conditions produced a stronger behavioral response than the high dose in non-stressed conditions. This finding is compatible with a shift in the dose-response curve as a result of stress. The behavioral response to the low dose of yohimbine in stressed animals exceeded that of both high dose stressed and non-stressed rats for a few variables in this study. The reason for this finding is unclear, and should be examined in future studies.

Blood levels of corticosterone were examined as a means to validate the effects of the stressor. The corticosterone levels in the stressed rats' blood were comparable to values reported in other restraint stress studies using the same equipment (Acri, 1992; Faraday, 2000). The non-stressed rats' corticosterone blood levels were higher than reported in previous studies. This difference in corticosterone blood levels between the non-stressed subjects in the present study and previous reports might be explained by different procedures. Specifically, nonstressed subjects in previous studies remained in their home cages until the time of sacrifice and blood collection. Nonstressed animals in the current studies received injections, were transported between treatment rooms,

and they were exposed to behavioral testing. Each of these procedures was a necessary part of the experimental protocol. It is likely, however, that the control animals in the present study were somewhat stressed by the procedures. The accumulation of these minor stressors is consistent with the corticosterone levels in the plasma of non-stressed rats. Although the stressed rats were not significantly higher (using a two-tailed test) in plasma corticosterone levels, a one-tailed test would have easily detected a significant difference in the hypothesized direction. Therefore, the stressor was validated, but the differences between stressed and nonstressed rats were partially obscured because of the stress of testing the animals.

The ratio of corticosterone in the blood and brain was investigated as a possible means of examining blood-brain barrier disruption. Results of these analyses revealed that corticosterone levels were not higher in the brains of the stressed rats, despite stressed rats having higher plasma levels. This finding suggests that despite the strong behavioral evidence that animals were more sensitive to the drugs given, less corticosterone entered the brains of the stressed rats. This finding might be construed as evidence against blood-brain barrier disruption, or it might not. Because corticosterone freely passes through the blood-brain barrier, and other endogenous tracers (e.g., albumin) normally do not cross the blood-brain barrier, the suitability of corticosterone as an index of blood-brain barrier disruption is questionable. Several possible explanations for the slightly lower ratios of corticosterone in the stressed rats' brains can be offered. One explanation, based on a general finding in blood-brain barrier

research, is that substances tend to follow an "easy in, easy out" rule. Substances that easily enter the CNS usually exit the CNS rapidly as well. Conversely, substances that do not readily enter the CNS do not freely leave the CNS once in. Because corticosterone readily passes the blood-brain barrier, it may have flowed out of the brain more readily under stress. This possibility would rely on corticosterone not being substantially sequestered in the brain, which is well-established (Pardridge, Moeller, Mietus, & Oldendorf, 1980). Another possibility is that some aspect of the stress manipulation altered the binding affinity of corticosterone to corticosterone binding globulin (CBG). If the rats undergoing the 30-minute stressor hyperventilated, a result would be hypocapnia and alkalosis (Guyton & Hall, 1996). Alkalosis is known to increase the binding of corticosterone to CBG (Westphal, 1971), and corticosterone bound to CBG is unable to traverse the BBB (Pardridge & Mietus, 1979). Heightened binding of corticosterone to CBG is consistent with the pattern of results, in that less corticosterone would have been available for transfer across the BBB. Based on the present analysis, it is unclear why stressed rats had lower levels of brain corticosterone. Regardless of the brain ratios of corticosterone, the behavioral data clearly suggest that stress enhanced the effects of drugs.

RESULTS OF EXPERIMENT TWO

Experiment 1 revealed a leftward shift in the dose-response curve to two different anxiogenic agents, wherein stress heightened sensitivity to the agents. This shift is essentially the same as that displayed in Figure 1. The stressed rats showed a stronger reaction to the drug, just as panic disorder patients show to biological challenge agents. To isolate the mechanism by which stress affected this shift, Experiment 2 was conducted. If stress was inducing disruption in the blood-brain barrier, then pretreatment with an agent that has previously been shown to block stress-induced blood-brain barrier disruption should antagonize the heightened effects of stress. The indomethacin pretreatment was, therefore, anticipated to pharmacologically antagonize the blood-brain-barrier disruption created by restraint stress (Sharma & Dey, 1981; 1984; 1986; 1987; Sharma et al., 1996).

Each variable examined in Experiment 1 was again examined in Experiment 2. Based on the data from Experiment 1, two does of yohimbine were retested with indomethacin pretreatment. Because of the lack of effect for the low dose of isoproterenol, only the high dose of this agent was retested with indomethacin pretreatment. Experiment 2's design was identical to Experiment 1, with the exception of adding a treatment to block blood-brain barrier disruption, and elimination of the low dose of isoproterenol.

As with Experiment 1, data were analyzed using ANOVA, except when the requirements for ANOVA could not be met, and non-parametric analysis were

then *post hoc* analyses utilized Dunnett's T, where all groups are compared to a control group (non-stressed vehicle with pretreatment).

Elevated Plus Maze

Yohimbine (1.0 & 2.5 mg/kg) & Indomethacin (10 mg/kg) Pretreatment

Indices of Anxiety

Increases in anxiety in EPM indices are reflected by variables either increasing or decreasing. *Decreases* in the % time in the open arms; frequency of end section exploration, time spent exploring end sections, and time spent head-dipping are interpreted as heightened anxiety. *Increases* in % time in the closed arms; latency to enter the end sections of the open arms, frequency of entry into the back of the closed arms, time spent in the back of the closed arms, and time spent immobile also reflect increased anxiety.

Significant main effects and interactions for the EPM variables are reported below. The principal question of this experiment was whether or not the interactions of stress and drug observed in Experiment 1 would be blocked by indomethacin. In that respect, the interaction effects are therefore primary, and the main effects of stress and drug are secondary.

Data are presented schematically for % time in open arms (Figure II-1), % time in closed arms (Figure II-2), entries into end sections (Figure II-3), time spent in end exploration (Figure II-4), latency to enter ends (Figure II-5), entries into back of closed (Figure II-6), time spent in back of closed (Figure II-7), time spent head dipping (Figure II-8), and time spent immobile (Figure II-9).

Interaction Effects

The ANOVA results for the interaction of stress and yohimbine are summarized in Table 17. The interaction of stress and yohimbine did not significantly alter any EPM variable. This result is consistent with hypothesis 1 of Experiment 2, and with the expected effect that indomethacin antagonized the stress-induced blood brain barrier disruption.

The main effects for drug and stress presented below should be qualified by noting that stress had very little effect on its own, and for many variables, drug had little effect on its own as well. Examination of graphed data from the variables (Figures 40-48) shows that the majority of the statistical main effect observed for stress and drug is the result of the interaction effect.

Drug Effects

Table 15 summarizes the main effects of yohimbine. Yohimbine administration significantly *decreased* Frequency of end exploration [F (2, 58) = 8.95, $p < .001$] and time spent in end exploration [F (2, 57) = 8.18, $p < .005$]. Yohimbine significantly *increased* % time in the closed arms [F (2, 60) = 16.16, $p < .001$], time spent immobile [F (2, 57) = 32.56, $p < .001$], entries into the back of the closed arms [F (2, 60) = 20.51, $p < .001$], time spent in the back of the closed arms, [F (2, 51) = 18.06, $p < .001$] and latency to explore end sections of the open arms [F (2, 58) = 3.85, $p < .05$]. The direction of each of these main effects is consistent with an anxiogenic effect for yohimbine (i.e., variables either increased or decreased in the predicted direction).

Stress Effects

Table 16 summarizes the main effects of stress. The 30-minute restraint stressor did not significantly alter any EPM variable. However, stress did produce a trend towards *increasing*

time spent immobile [F (1, 57) = 2.82, p < .09]. The direction of this trend is consistent with an anxiogenic effect for stress.

Data for two variables did not meet criteria for homogeneity of variance, and was not corrected with logarithmic transformations, therefore a non-parametric Kruskal-Wallis (K-W) test was used. For % time spent in the open arms, there was a significant difference between the groups [H (5) = 16.50, p < .01]. For time spent head-dipping, there was no significant difference between the groups [H (5) = 7.82, p = NS]. While non-parametrics cannot yield interaction effects, the pattern of group differences was consistent with the hypothesis that indomethacin blocked the interactive effects of stress and yohimbine. K-W test statistics are not included in the ANOVA tables, as they do not yield main or interaction effects.

Other Indices: Locomotion and Decision making

Locomotion

The two variables that reflect locomotor changes in the EPM are the total number of entries and the number of entries into closed arms (Hogg, 1996; Rodgers & Dalvi, 1997; Rodgers, Cao, Dalvi, & Holmes, 1997).

Yohimbine reduced total entries [F (2, 59) = 8.13, p < .005] but not closed arm entries [F (2, 40) = 0.32, p = NS]. There were no significant main effect of

stress on total entries or closed arm entries, and no significant stress by drug interactions were observed. Figure II-10 presents the total entries, and Figure II-11 presents the closed arm entries for control and yohimbine animals.

Decision making

The amount of time an animal spends in the center square of the plus maze reflects decision-making processes. There were no significant main effects of stress or drug on % time spent in the center, and no interaction was noted for % time in the center. Figure II-12 presents the % time spent in the center for control and yohimbine animals.

Isoproterenol (0.8 mg/kg) & Indomethacin (10 mg/kg) Pretreatment

Indices of Anxiety

Significant main effects and interactions for the EPM variables are reported below. The principal question of this experiment was whether or not the interactions of stress and drug observed in Experiment 1 would be blocked by indomethacin. In that respect, the interaction effects are therefore primary, and the main effects of stress and drug are secondary.

Data are presented schematically for % time in open arms (Figure II-13), % time in closed arms (Figure II-14), entries into end sections (Figure II-15), time spent in end exploration (Figure II-16), latency to enter ends (Figure II-17), entries into back of closed (Figure II-18), time spent in back of closed (Figure II-19), time spent head dipping (Figure II-20), and time spent immobile (Figure II-21).

Interaction Effects

The ANOVA results for the interaction of stress and Isoproterenol are summarized in Table 20. The interaction of stress and drug produced no significant changes in EPM variables, and no trends towards significance were observed. The lack of an interaction effects is striking, considering that six variables had either a significant interaction or a trend towards significance in Experiment 1. The lack of interactions suggests that the indomethacin acted to antagonize stresses effects on increasing the sensitivity to anxiogenic agents. This finding is consistent with hypothesis 1 of Experiment 2.

The main effects for drug and stress presented below should be qualified by noting that stress had very little effect on its own, and for many variables, drug had little effect on its own as well. Examination of graphed data from the variables (Figures 52-60) shows that the majority of the statistical main effect observed for stress and drug is the result of the interaction effect.

Drug Effects

Main effects for isoproterenol in the ANOVA results are summarized in Table 18. Isoproterenol administration did not significantly increase any EPM variable normally associated with anxiety. Two trends towards a significant main effect were observed with isoproterenol increasing latency to enter end sections of the open arms [F (2, 38) = 3.08, p = .08] and time spent in the back of the closed arms [F (2, 40) = 3.62, p = .06]. While two variables did show a trend, overall, it must be concluded that isoproterenol alone was not anxiogenic. A lack of effect for drug alone is consistent with hypothesis 1 of Experiment 2.

Stress Effects

Table 19 summarizes the main effects of stress. The 30-minute restraint stressor did not significantly alter any EPM variable. Stress did show a trend towards *increasing* latency to explore end sections [F (1, 38) = 3.35, p = .07]. This lack of effect is consistent with stress having a negligible effect on the animals' behavior in the elevated plus maze (hypothesis 5).

Other Indices: Locomotion and Decision making

Locomotion

The two variables that are thought to reflect locomotor changes in the EPM are the total number of entries and the number of entries into closed arms. No significant main effects were observed for isoproterenol to alter total entries or closed arm entries. There was a significant main effect to decrease closed arm entries [F (1, 40) = 5.11, p < .05]. Stress had no significant effect on total entries, and no significant stress by drug interactions were observed. Figure II-22 presents the total entries, and Figure II-23 presents the closed arm entries for control and isoproterenol animals.

Decision making

There was no significant main effect of stress on % time spent in the center. Isoproterenol significantly increased % time spent in the center [F (2, 39) = 6.10, p < .05]. Additionally, there was no significant interaction of stress X drug for % time spent in the center. Figure II-24 presents the % time spent in the center for control and isoproterenol animals.

Open Field Test

Yohimbine (1.0 & 2.5 mg/kg) & Indomethacin (10 mg/kg) Pretreatment

Table 21 presents the ANOVA results for yohimbine in the OFT. Data are presented schematically for locomotion (Figure II-25), rearing (Figure II-26), immobility (Figure II-27), and center time (Figure II-28).

Interaction effects

There were no significant interactions of stress X drug for OFT variables.

Stress effects

ANOVA revealed no significant main effects of stress OFT variables.

Drug effects

There was a significant main effect for drug to reduce rearing [F (2, 58) = 48.02, $p < .001$], and increase immobility time [F (2, 58) = 13.06, $p < .001$]. Both of these effects are consistent with yohimbine exerting an anxiogenic effect.

Data for two variables did not meet criteria for homogeneity of variance, and was not corrected with logarithmic transformations, therefore a non-parametric Kruskal-Wallis (K-W) test was used. For locomotion, there was a significant difference between the groups [H (5) = 22.50, $p = < .001$]. For center time, there was no significant difference between the groups [H (5) = 33.10, $p = < .001$]. While non-parametrics cannot yield interaction effects, the pattern of group differences is consistent with the hypothesis that indomethacin blocked the interactive effects of stress and yohimbine. K-W test statistics are not included in the ANOVA tables, as they do not yield main or interaction effects.

Isoproterenol (0.8 mg/kg) & Indomethacin (10 mg/kg) Pretreatment

Table 22 presents the ANOVA results for the OFT. Data are presented schematically for locomotion (Figure II-29), rearing (Figure II-30), immobility (Figure II-31), and center time (Figure II-32).

Interaction effects

There were no significant interactions of stress X drug in the OFT. The blockade of stress by drug interactions by pretreatment with indomethacin is consistent with hypothesis 1 of Experiment 2.

Stress effects

ANOVA revealed no significant main effects of stress on any OFT variable

Drug effects

There was a significant main effect for isoproterenol, wherein drug decreased center time [F (2, 58) = 3.85, $p < .05$] and a trend to decrease rearing [F (2, 38) = 3.10, $p = .08$]. These effects are consistent with an anxiogenic effect for isoproterenol in the OFT.

EXPERIMENT 2: ASSESSMENT OF STUDY HYPOTHESES

Major Hypothesis 2 was a prediction based on previous research that the effects of stress on blood-brain-barrier (BBB) disruption can be pharmacologically antagonized. Specifically, because indomethacin can block the increased flow of various tracers into the CNS (Sharma & Dey, 1981, 1984, 1986, 1987; Sharma et al., 1996), it was hypothesized to antagonize the stress by drug interactions that were observed in Experiment 1.

Major Hypothesis 2

This hypothesis was **confirmed**. The pattern of results from Experiment 2 clearly indicates that the stress by drug interactions observed in Experiment 1 were blocked. That these interactions were pharmacologically antagonized by a drug that has no effect on anxiety suggested that the drug was acting at the level of the blood-brain-barrier. Although the present study does not prove this point, it does strongly implicate involvement of the blood-brain-barrier in the response to anxiogenic challenge agents.

DISCUSSION OF EXPERIMENT 2

As predicted in Hypothesis 1, pretreatment with indomethacin 10 mg/kg blocked the stress by drug interactions of Experiment 1. As reviewed in the results section, each stress by drug interaction was attenuated upon testing with pretreatment. The finding that the stress by drug interactions were blocked in a uniform manner suggests that this a potent manipulation. Indeed, the same dose of indomethacin has been used to antagonize the marked blood-brain barrier disruption after several hours of stress (Sharma & Dey, 1981, 1984, 1986, 1987; Sharma et al., 1996).

It is important to reiterate that indomethacin has no reported anxiolytic properties. There is no evidence to suggest that it has these properties, and within the present research, indomethacin administered to control rats did not affect the response to the elevated plus maze. The lack of anxiolytic properties is the primary reason that indomethacin was selected rather than other drugs that block stress-induced blood-brain barrier disruption (SIBBBD). Ketanserin and cyproheptadine and p-chlorophenylalanine each block SIBBBD, but they also affect serotonergic functioning and, therefore, may affect anxiety levels. Diazepam also antagonizes SIBBBD, but it affects anxiety as well. Indomethacin seemed to be an ideal agent to isolate the effect of antagonizing BBB disruption, because it minimized the likelihood that the antagonism resulted from any anxiolytic effect of the drug.

The mechanism of action for indomethacin's blocking blood-brain barrier disruption is unknown. It has been proposed to result from blocking a cascade of

effects secondary to increased 5-HT. Stress increases prostaglandin levels in plasma, which stimulates the synthesis of serotonin (Haubrich, Perez-Cruet & Reid, 1973). Serotonin itself stimulates additional prostaglandin synthesis and release, leading to increased cAMP formation (Baca & Palmer, 1978). Accumulation of cAMP in cerebral endothelial cells causes vasodilatation, resulting in increased transport of substances (Eakins, 1977; Joo, Rakonczay & Wollemann, 1975). Indomethacin interferes with this cascade by inhibiting the increase in prostaglandins.

It is critical to emphasize several points regarding the role of stress in producing the interactions, and in indomethacin to block these interactions. Although the stressor produced the expected shifts in dose-response curves, this finding does not prove involvement of the blood-brain-barrier. Although indomethacin produced the predicted effects in blocking the heightened sensitivity to anxiogenic drugs, this finding is consistent with, but does not prove, that blood-brain-barrier disruption was the operative factor in the heightened sensitivity. In order to confirm blood-brain-barrier disruption, additional studies employing techniques such as autoradiography or mass spectroscopy to measure drug levels in the brain must be conducted.

GENERAL DISCUSSION

Experiments 1 and 2 examined the effects of stress on anxiogenic drugs in the indices of anxiety and examined a possible mechanism for this shift in the dose-response curves. The aim of these experiments was to explain the greater sensitivity of panic disorder patients to anxiogenic drugs. In Experiment 1, the interactive effects of stress and two anxiogenic drugs was examined on behavioral indices of anxiety. The two drugs in this study were selected because panic disorder patients show greater sensitivity to both (Charney et al., 1990). Yohimbine has been shown to be anxiogenic in normal controls as well as patients, while isoproterenol generally does not provoke anxiety in normal controls (Rainey et al., 1984). Yohimbine traverses the blood-brain barrier, while it is thought that isoproterenol does not cross the blood-brain barrier at baseline. Experiment 2 was an attempt to pharmacologically antagonize the effects of stress on the blood-brain barrier. Previous studies have demonstrated that indomethacin, as well as other drugs (Sharma & Dey, 1981, 1984, 1986, 1987; Sharma et al., 1996), can block stress-induced increases in permeability to a variety of tracers. The present research is the first application of indomethacin to block blood-brain barrier disruption as measured via drug effects on behavioral indices.

Results of Experiment 1 supported the hypothesis that stress would produce a leftward shift in the dose response curve to the drugs in this study. While yohimbine demonstrated a stronger effect compared to isoproterenol, this finding is not altogether unexpected. Isoproterenol has much lower blood-brain

barrier permeability compared to yohimbine and therefore, would be expected to produce lesser effects given a comparable stressor. An unexpected finding of Experiment 1 was the lack of significant stress by drug interactions in the open field test. Further research in this area should examine specific experimental parameters that determine whether stress by drug interaction effects can be produced in different animal models of anxiety. It is possible that the open field test can detect stress by drug interactions, especially if it is not given following another behavioral measure as it was in the present studies. Testing various combinations of other animal models of anxiety (i.e., the light-dark transition test, conditioned suppression), and other stressors (forced swim, electric shock) should be undertaken in order to determine which stressors enhance responses to anxiogenic drugs in animal models.

One interpretation of heightened sensitivity to anxiogenic drugs in panic disorder patients might be that elevated baseline stress/anxiety simply places anxious subjects closer to a threshold of panic attacks, and once biological challenge agents are administered, then those subjects more anxious at baseline surpass that threshold more often. This explanation does not fit the current data, which indicate that rather than baseline stress level simply having an additive effect, the effect was interactive. For many variables in this study, the individual effects of stress and drug were either neutral or opposite of the effects of their interaction. For example, in the elevated plus maze, stress alone decreased time spent in the back sections of the closed arms, as did the low dose of yohimbine, but when the same two treatments were combined, the effect was in the opposite

direction (see Figure I-6). Therefore, individually, the effects of stress and the low dose of yohimbine were anxiolytic, however, combining the two conditions generated an anxiogenic effect.

This research extends prior studies (Singer, 1963; Britton & Indyk, 1990; Pokk & Zharkovsky, 1997) indicating that drug responses are altered in stressful circumstances. The present research adds two additional drugs to the list of drugs that show enhanced behavioral effects when testing during or following stress. Singer (1963) and Britton and Indyk's (1990) studies with adrenalin and caffeine, respectively, produced different effects in novel or stressful testing conditions. Pokk and Zharkovsky (1997) reported that flumazenil could produce an anxiogenic effect if administered following a 24 hours exposure to small platform stress. The basic aims of these studies was radically different (e.g., effects of arousal on emotion). However, their results strongly support the hypothesis that stress can enhance drug effects. Additionally, the present research proposes that the observed shift in sensitivity may be due to stress-induced disruption in the blood-brain barrier. This fundamental hypothesis has various implications for other pharmacological and psychiatric research in human and non-human animals.

Limitations/future research

Ideally, the effects of stress across the full dose response curves of both drugs would have been assessed. Practical restraints forced the selection of only two dosages of each drug. Future studies should address the pattern of

stress by drug interactions that occur across the full dose range. The finding that low dose isoproterenol did not have an interactive effect with stress on the EPM indices suggests that the there are variations in the ability to demonstrate stress by drug interactions at some dosages. Experiment 1 only assessed two anxiogenic drugs, and they are only two of more than fifteen agents that provoke panic attacks in humans. Besides these other agents in the human literature, literally dozens of other agents have anxiogenic activity in pre-clinical research on anxiety. The relevance of the present findings should be assessed across other agents acting across various receptor sites.

The use of only one type of stressor is a limitation of the current experiments. A vast array of stressors have been used and validated in the rodent experimentation literature, and many of these stressors may produce stress by drug interactions. The present results should be replicated with other stressors.

A key improvement for future studies would be the addition of a more specific and quantitative method for determining blood-brain barrier disruption and the levels of drug in the brains of rats. The present research project examined an indirect index of blood-brain barrier disruption in the form of a pharmacological antagonism of BBB disruption. Additionally, an attempt to quantify disruption using corticosterone levels in the brains and plasma of rats was undertaken. While the pharmacological antagonism manipulation resulted in the predicted behavioral results, data from corticosterone in blood and brains were unexpected. Pharmacological antagonism is an effective behavioral

pharmacology technique, however, as used in the present study, it is an indirect method.

More specific techniques are available that would allow direct quantification of not only drug entry, but also measures of blood-brain barrier disruption. Autoradiography would allow precise measure of drug levels in the brain, and the additional information of regional differences in individual rats. This direct measure would allow definitive statements to be made regarding stress-induced changes in pharmacokinetics. An additional measure that would complement autoradiography would be to determine endogenous tracer levels (e.g., albumin, IGG) in the brains of stressed and non-stressed rats, which would provide an index of global and even regional blood-brain barrier disruption.

Implications for future research

The implications of the current research for non-human animal studies suggest that greater attention be devoted to the effects of stress on drug effects. The possibility of stress by drug interactions presents a potential confounding variable to tests of anxiety, and is a possible means of examining and understanding variability in the behavioral responses to anxiogenic drugs. The literature on the EPM and primate studies is replete with examples of variability in drug response with anxiogenic agents. The same literature clearly suggests that stress predisposes to heightened sensitivity to anxiogenic agents. Stress induced blood-brain barrier disruption offers a parsimonious explanation for this variability, and presents a testable hypothesis to account for such variability.

Stress of handling, housing conditions, and procedural differences may be the root causes of inter-and intra-laboratory differences in drug responses.

The present research suggests that stress may effect a pharmacokinetic difference in subjects. Tied to this understanding of stress and the blood-brain barrier, the findings suggest caution in dismissing an animal model as a tool for studying a disorder, based on results using non-stressed animals. The EPM for example has widely been regarded as a model of generalized anxiety, but specifically not a model for panic disorder (File, 1990). The present research challenges this position, in that the EPM proved to be quite sensitive to detecting anxiogenic drug effects in stressed and non-stressed animals in the predicted pattern. In non-stressed subjects, isoproterenol produced no effects on its own, analogous to the reactions of human normal controls. When administered to stressed animals, isoproterenol produced anxiety in a subset of subjects, suggesting a closer model of panic disorder patients' reactions than has been found to date in animal research on panic disorder.

In terms of implications for humans, the findings of these studies speak specifically to the BCA literature on panic disorder. As reviewed earlier, there are a number of theories that attempt to explain panic disorder patients' heightened sensitivity to challenge agents in terms of some underlying neurochemical/ neurobiological defect. The present findings argue that another, more parsimonious explanation may be that those subjects who are stressed (patients) may be receiving more drug, and this effect might account for the leftward shift in sensitivity.

Psychological theories posit catastrophic cognitions and conditioning to bodily sensations may be rendered unnecessary to explaining heightened sensitivity to BCA's. While these phenomena might indeed exist, their causal status in creating the shift is drawn into question if the effect can be traced to stress-induced blood-brain barrier disruption. Psychological models might explain the greater baseline anxiety seen in subsets of panic disorder subjects immediately prior to entering a BCA study. Biological theories, in contrast, have focused on supposed biological defects to explain the differential sensitivities, and do not predict heightened anxiety to such cues. The results of the present studies are particularly relevant to biological theories of panic disorder. The biological challenge paradigm is a widely used tool in the search for underlying, biologically-based dysregulations in psychiatric conditions such as panic disorder. Biological theories of panic disorder predominately rest on two main pillars, 1) positive psychopharmacological treatment response, and 2) the enhanced sensitivity to biological challenge agents. Finding that baseline anxiety at the time of testing is the actual cause of the greater sensitivity to challenge agents in humans would be highly damaging to theories that depend heavily on such findings as evidence for a biologic basis to the condition in question.

Several recommendations for future studies using humans can be made based on the current research. First, a line of studies administering various challenge agents to normal controls under stressful and non-stressful conditions should be pursued. These studies should yield the finding that stress at the time of testing greatly increases panic rates among controls. A second line of studies

should administer challenge agents only once all patients and controls are at the same baseline level of anxiety. Based on the current hypothesis, under these conditions, the group differences between patients and controls would be markedly attenuated, if not abolished. A third line of research should examine whether the findings generalize to all drugs. In addition, indomethacin could be administered as a pretreatment to various biologic challenge agents to attempt to replicate the findings of the present studies.

Should the results of future studies on stress-induced blood-brain barrier disruption find that this phenomenon occurs in humans, conceptualization of a variety of clinical conditions might be altered. In general, there seems to be a general tendency to inherit an anxiety disorder. However, no one is sure at which level this genetic liability is expressed. One intriguing possibility is a lowered threshold for stress-induced blood-brain barrier disruption. This idea suggests that normally excluded or limited substances would have greater access to certain fear-relevant brain structures during stressful situations. One salient example of a disorder where this idea offers an intriguing perspective is post-traumatic stress disorder (PTSD). Damage to the hippocampus in PTSD patients is a replicated finding (Bremner, 1999). Stress-induced blood-brain barrier disruption would suggest that the damage might be caused by repeated exposure to greater than normal levels of corticosteriods and neurotransmitters.

Summary

In summary, the current research assessed the hypothesis that baseline stress levels are a potential cause of greater sensitivity to anxiogenic agents, and examined a possible physiologic mechanism of action for the effect of stress on drug response. Results of Experiment 1 suggest that stress can produce a leftward shift in the dose response cure for anxiogenic drugs, including a drug that normally does not traverse the blood-brain barrier. The results of Experiment 2 suggest that pretreatment with indomethacin can antagonize the interactive effects of stress and drug, implying the involvement of the stress-induced blood-brain barrier disruption. Because results of biological challenge agent studies are a cornerstone of theories regarding panic disorder and other psychiatric conditions, these data suggest that biologically based theories dependent on heightened sensitivity to drugs must be reevaluated. In addition, these data suggest that variability in drug responses in animal models of anxiety may be due to baseline stress interacting with the drug, producing confounded data. Therefore, the data suggest that in human and non-human research, if stress is controlled as a variable, clearer and more accurate data on drug mechanism and possible biological dysregulations would result.

SECTION VI: TABLES, FIGURES AND REFERENCES

TABLES

Table 1. Distinction between subject groups in biological challenge agent studies.

		Panic?	
		Yes	No
Group?	Patient	Patient panickers (PP)	Patient non-panickers (PNP)
	Control	Control panickers (CP)	Control non-panickers (CNP)

Table 2. Elevated plus maze studies that have used biological challenge agents

DRUG	DOSES mg/kg	COMMENT	RESULTS	SP	STRN	GN	REFERENCES
S.Lact	60, 120		No effect	R	List	M	Johnston & File, 1988
S.Lact	32, 65 131,261	>1 HR adaptation time	No effect	Mo	DBA/2	M	Rodgers, Cole, Aboualfa, et al., 1995
Isopro.	0.1, 0.2, 0.4, 0.6		Partial anxiogenic	R	List	M	Johnston & File, 1988
Isopro.	0.125,0.25 , 0.5, 1.0	>1 HR adaptation time	No effect	Mo	DBA/2	M	Rodgers, Cole, Aboualfa, et al., 1995
Caffe	15, 30, 60		Anxiogenic	Mo	NIH s	M	Lister, 1987
Caffe	15, 30		Anxiogenic	R	List	M	Pellow, Chopin, File, Briley, 1985
Caffe	10, 25, 50		Anxiogenic	R	Wist	M	Bhattacharya, Satyan, et al., 1997
Caffe	40		Anxiogenic	R	List	M	Baldwin, Johnston, File, 1989
Caffe	15, 30, 60		Anxiogenic	Mo	ICR	M	Jain, Kemp, Adeycomo, Buchanan, Moore, 1995
mCPP	0.25, 1.0, 5.0		Anxiogenic	R	List	M	Handley, McBlane, Crichley, Njunge, 1993
mCPP	1.56, 2.21, 3.125		Anxiogenic	Mo	NIH s	M	Benjamin, Lal, Meyerson, 1990
mCPP	0.5, 2, 4	>2 HR adaptation time	Weak anxiogenic	R	DBA/2	M	Rodgers, Cole, Cobain, Daly, Doran, Eells, Wallis, 1992
mCPP	0.125, 0.25, 0.5, 1.0		Anxiogenic	Mo	S D	M	Gibson, Boufield, Caryon, 1994
CCK-4	12, 25, 50, 100	>1 HR adaptation time, red light	No effect	Mo	DBA/2	M	Johnson, Rodgers, 1996
CCK-4	10μg		Anxiogenic	GP	BFA	F	Rex, Fink, Marsden, 1994
CCK-4	1,10,25,50 ,100μg sc		Anxiogenic	R		B	Harro, Vasar, 1991
CCK-4	2, 10, 50μg		Anxiogenic	R	Wist	M	Rex, Barth, Virgil, et al., 1991
CCK-4	10μg		Anxiogenic	GP	BFA	F	Rex, Fink, 1998
CCK-8	0.05,0.2,1, 2.5,5,10 sc		Anxiogenic	Mo		F	Vasar, Lang, Harro, Bourin, Bradwejn, 1994
CCK-8	0.01, 0.1, 0.5, 1μg (AMY)		Anxiogenic	R	Wist	M	Belcheva, Belcheva, Petkov, Petkov, 1994
Cck-8	25 pmol (NA)	Only in rats exposed to holeboard	Anxiogenic	R	Wist	M	Ladurelle, Rogues, Dauge, 1997

Note: Yoh-yohimbine, Flum-flumazenil, S Lact-sodium lactate, Isopro-isoproterenol, Caffe-caffeine, SPC-species, STN-strain, GN-gender, R-rat, Mo-mouse, Wist-wistar, SD-sprauge-dawley, SW-swiss webster, List-lister, NIH s-NIH swiss, AMY-amygdala, dPAG-dorsal periaqueductal gray, NA-nucleus accumbens.

Table 2 (cont). Elevated plus maze studies that have used biological challenge agents.

Yoh	1.25, 2.5		Anxiogenic	R	List	M	Pellow, Chopin, File, Briley, 1985
Yoh	2.5		Anxiogenic	R	List	M	Pellow, Johnston, File, 1987
Yoh	2.5, 5.0		Anxiogenic	Mo	Wist	M	Bhattacharya, Mitra, 1992
Yoh	2.0		Anxiogenic	R	Wist	M	Bhattacharya, Satyan, Chakrabata, 1997
Yoh	2.5, 4.0		Anxiogenic	R	List	M	Johnston, File, 1989
Yoh	1.25, 2.5, 5.0		Anxiogenic	R	Wist	M	Cole, Hillmann, Seidelmann, Klewer, Jones, 1995
Yoh	2.5		Anxiogenic	R	List	M	File, Johnston, 1987
Yoh	1.5, 3.0		Anxiogenic	R	Wist	M	Wada, Fukuda, 1991
Yoh	1.5, 2.5		Anxiogenic	R	List	M	Stanford, Baldwin, File, 1989
Yoh	2.5		Anxiogenic	R	Albn	M	Muthal, Chopde, 1994
Yoh	2.5		Anxiogenic	R	List	M	Baldwin, Johnston, File, 1989
Yoh	1.25, 2.5, 5.0		Anxiogenic	R	List	M	Handley, Mithani, 1984
Yoh	0.5, 1.0, 2.0, 4.0	<1Hour acclimatization Red light	Anxiolytic	Mo	DBA/T 1/BAL	M	Cole, Burroughs, Laverty, Sherriff, Spashoni, Rodgers, 1995
Flum	10		No effect	Mo	NIH s	M	Benjamin, Lal, Meyerson, 1990
Flum	80 nmol ic (dPAG)	Directly into CNS	No effect				Russo, Guimaraes, et al., 1992
Flum	0.5µl i.c.-AMY	Directly into CNS	Anxiogenic	R	Wist	M	DaCunha, Wolfman, et al, 1992
Flum	4		No effect	Mo	Balb, LAKA		Kulkerri, Sharma, 1993
Flum	10, 20		No effect	R	List	M	Pellow, File, 1986
Flum	4		No effect	R			File, Baldwin, 1988
Flum	4		No effect	R	S D	M	Chopin, Briley, 1993
Flum	4	High baseline anxiety	Anxiolytic	R	List	M	File, Hitchcott, 1990
Flum	4	Low baseline anxiety	Anxiogenic	R	List	M	File, Hitchcott, 1990
Flum	5, 10, 20	Strain very anxious at baseline.	Weak anxiogenic	Mo	DBA/2	M	Lee, Rodgers, 1991
Flum	2, 10	24 hr platform stress	Anxiogenic	Mo	NMRI	M	Pokk, Zharkovsky, 1997

Note: Yoh-yohimbine, Flum-flumazenil, S Lact-sodium lactate, Isopro-isoproterenol, Caffe-caffeine, SPC-species, STN-strain, GN-gender, R-rat, Mo-mouse, Wist-wistar, SD-sprauge-dawley, SW-swiss webster, List-lister, NIH s-NIH swiss, AMY-amygdala, dPAG-dorsal periaqueductal gray, NA-nucleus accumbens.

Table 3. Open field test studies that have used biological challenge agents.

DRUG	DOSES mg/kg	COMMENT	RESULTS	SPC	STRN	GN	REFERENCES
Yoh	1, 5, 10		Anxiogenic	R	Wist	M	Ferrari,Tartoni, Monti & Mangiafico, 1989
Yoh	10		Anxiogenic	R	Wist	M	Florio, Sakate & Palermo-Neto, 1993
Yoh	2		Anxiogenic	R		M	Bhattacharya, Mohan & Sen, 1997
Yoh	2		Anxiogenic	R		M	Bhattacharya, 1985
Yoh	2		Anxiogenic	R		M	Bhattacharya, Bhattacharya & Ghosal, 1998
Yoh	2		Anxiogenic	R		M	Bhattacharya, Satyan & Charkrabarti, 1997
Yoh	0.0125, .025, 1, 4, 8		Anxiogenic	Mo	S W	M	Koechling, Smith & Zalman, 1990
Yoh	90 n moles		No effect	R	S D	M	Verleye & Bernet, 1987
Flum	2		No effect	Mo	CD1	M	Lopez, Miller, Greenblatt, Paul & Shader, 1988
Flum	0.1-5.0		No effect	R	Wist	M	Nazar & Plaznik, 1997
MCPP			Anxiogenic	R			Meert, Melis & Clincke, 1997
CCK-4	10, 50 s.c.		No effect	R	Wist	M	Hadjiivanova, Kehayov, Petkov, Amblard & Martinez, 1995
CCK-4	1, 2, 5, 10 ug ICV		Anxiolytic	R	Wist	M	Hsiao, Katsuura & Itoh, 1984
CCK-4	.5, 1, 2.5 IC-NA		Anxiolytic	R	Wist	M	Katsuura, Itoh & Hsiao, 1984
CCK-8	1, 2, 5, 10,25, 50, 100, 250, 500		Anxiogenic	R	Wist	M	Katsuura, Itoh & Hsiao, 1984
Caffe	5, 15, 45		Increased Activity	R	S D	M	Meliska & Loke, 1984
Caffe	15 s.c.		No effect	R	Albin	M	Molinengo, Orsetti, Pastorello, Scordo & Ghi, 1995
Caffe	10, 25, 50		Anxiogenic	R		M	Bhattacharya, Satyan & Charkrabarti, 1997
Caffe	2.5, 5, 10	Home cage	Increased Activity	R	S D	M	Britton & Indyk, 1990
Caffe	2.5, 5, 10	Open field	Anxiogenic-decreased activity	R	S D	M	Britton & Indyk, 1990

Note: Yoh-yohimbine, Flum-flumazenil, S Lact-sodium lactate, Isopro-isoproterenol, Caffe-caffeine, SPC-species, STN-strain, GN-gender, R-rat, Mo-mouse, Wist-wistar, SD-sprauge-dawley, SW-swiss webster, List-lister, NIH s-NIH swiss, AMY-amygdala, dPAG-dorsal periaqueductal gray, NA-nucleus accumbens.

Table 4. EPM and restraint

Duration of Restraint	Delay to Testing	Effects	Cite
15 min	24hours	Anxiogenic	Martijena, Calvo, Volosin, & Molina (1997)
2 hours	24hours	Anxiogenic	Padovan, Del-Bel, &Guimaraes (1996)
2 hours	24hours	Anxiogenic	Guimaraes, Del-Bel, Padovan, Mendonca, & Titze de Almeida (1993)
2 hours	24hours	Anxiogenic	Mendonca & Guimaraes (1998)
3 hours	2 hours	Anxiogenic	Chaouloff, Baudrie, & Coupry (1994)
15 min, 1 hour 1 hour	None; None; 24 hours	None Anxiogenic Anxiogenic	McBlane & Handley (1994)
10, 20 minutes	None	None	Falter, Gower, &Gobert (1992)

Table 5. Timetable for experimental procedures

		Experiment 1		Experiment 2	
		Stressed	Non-stressed	Stressed	Non-stressed
Day 1		Arrive at LAM	Arrive at LAM	Arrive at LAM	Arrive at LAM
Day 2-8		Gentle	Gentle	Gentle	Gentle
Day 9	30 min before restraint			Pretreat with indomethacin	Pretreat with indomethacin
	Immediately before restraint	Inject drug or vehicle	Inject drug or vehicle	Inject drug or vehicle	Inject drug or vehicle
	30 min before testing	Place in restraint	Remain in home cage	Place in restraint	Remain in home cage
	5 minute test	Testing on EPM	Testing on EPM	Testing on EPM	Testing on EPM
	10 minute test	Testing in OFT	Testing in OFT	Testing in OFT	Testing in OFT
	15-20 minutes after restraint	Sacrifice/blood sampling	Sacrifice/bloo d sampling	Sacrifice/blood sampling	Sacrifice/bloo d sampling

Table 6. Variables in the elevated plus maze

Interpreting variables in the EPM		
Scoring Variable:	**Anxiogenic effect**	**Anxiolytic effect**
% time in open arms	Decreases	Increase
% entries in open arms	Decreases	Increase
Total entries [a]	No effect/ decrease	No effect
Immobility time	Increase	Decrease
Time spent head dipping	Decrease	Increase
Closed arm entries [a]	No effect/ decrease	No effect
% time in closed arms	Increase	Decrease
% time in center section [b]	No effect	No effect
Time spent in open arm end exploration	Decrease	Increase
Entries into end sections of open arms	Increase	Decrease
Time in back of closed arms	Increase	Decrease
Entries into back of closed arms	Increase	Decrease
Latency to enter ends of open arms	Increase	Decrease

a. measure of locomotor changes
b. measure of decision making

Table 7. ANOVA results in the EPM for Yohimbine-Main effects for Stress

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms	↓	Anxiogenic	1, 78	10.55	<0.01
Time Spent in End Exploration	↓	Anxiogenic	1, 75	15.55	<0.001
Frequency of End Exploration	↓	Anxiogenic	1, 77	24.25	<0.001
Time Spent Head-dipping †			1, 62	0.18	NS
Latency to Explore End Sections	↑	Anxiogenic	1, 71	13.43	<0.001
Percent Time in the Closed Arms	↑	Anxiogenic	1, 78	13.04	<0.01
Entries into Back of Closed Arms	↑	Anxiogenic	1, 79	0.01	NS
Time Spent in Back of Closed Arms			*	*	*
Time Spent Immobile on Maze			*	*	*
Locomotion and Decision-Making Indices					
Total Entries	↓	Decreased	1, 77	29.32	<0.001
Entries into Closed Arms	↓	Decreased	1, 73	19.15	<0.001
Percent Time in the Center	↓	Decreased	1, 77	8.54	<0.01

† Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 8. ANOVA results in the EPM for Yohimbine -Main effects for Drug

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms	↓	Anxiogenic	2, 78	6.70	<0.01
Time Spent in End Exploration	↓	Anxiogenic	2, 75	6.38	<0.005
Frequency of End Exploration	↓	Anxiogenic	2, 77	9.42	<0.001
Time Spent Head-dipping †	↓	Anxiogenic	2, 62	7.06	<0.01
Latency to Explore End Sections	↑	Anxiogenic	2, 71	2.88	<0.06
Percent Time in the Closed Arms	↑	Anxiogenic	2, 78	11.88	<0.001
Entries into Back of Closed Arms	↑	Anxiogenic	2, 79	13.76	<0.001
Time Spent in Back of Closed Arms			*	*	*
Time Spent Immobile on Maze			*	*	*
Locomotion and Decision-Making Indices					
Total Entries	↓	Decreased	2, 77	6.50	<0.005
Entries into Closed Arms	↓	Decreased	2, 73	5.00	<0.01
Percent Time in the Center	↓	Decreased	2, 77	11.57	<0.001

† Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 9. ANOVA results in the EPM for Yohimbine-Stress X Yohimbine Interaction

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms	↓	Anxiogenic	1, 78	5.55	<0.01
Time Spent in End Exploration	↓	Anxiogenic	1, 75	3.99	<0.05
Frequency of End Exploration	↓	Anxiogenic	1, 77	9.02	<0.001
Time Spent Head-dipping [1]			1, 62	0.91	NS
Latency to Explore End Sections	↑	Anxiogenic	1, 71	5.24	<0.01
Percent Time in the Closed Arms	↑	Anxiogenic	1, 78	5.55	<0.01
Entries into Back of Closed Arms	↑	Anxiogenic	1, 79	4.50	<0.05
Time Spent in Back of Closed Arms			*	*	*
Time Spent Immobile on Maze			*	*	*
Locomotion and Decision-Making Indices					
Total Entries	↓	Decreased	1, 77	6.79	<0.005
Entries into Closed Arms	↓	Decreased	1, 73	3.97	<0.05
Percent Time in the Center			1, 77	1.34	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 10. ANOVA results in the EPM for Isoproterenol-Main effects for Stress

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			1, 80	1.42	NS
Time Spent in End Exploration[1]			1, 80	0.56	NS
Frequency of End Exploration[1]			1, 79	2.37	NS
Time Spent Head-dipping	↓	Anxiogenic	1, 79	3.06	<0.08
Latency to Explore End Sections	↑	Anxiogenic	1, 76	5.92	<0.01
Percent Time in the Closed Arms			*	*	*
Entries into Back of Closed Arms[1]			1, 63	0.18	NS
Time Spent in Back of Closed Arms[1]			1, 63	0.01	NS
Time Spent Immobile on Maze			*	*	*
Locomotion and Decision-Making Indices					
Total Entries	↓	Decreased	1, 73	6.95	<0.05
Entries into Closed Arms			1, 80	1.80	NS
Percent Time in the Center			1, 80	0.00	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 11. ANOVA results in the EPM for Isoproterenol -Main effects for Drug

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			2, 80	0.18	NS
Time Spent in End Exploration[1]			2, 80	1.77	NS
Frequency of End Exploration[1]			2, 79	1.24	NS
Time Spent Head-dipping			2, 79	0.90	NS
Latency to Explore End Sections			2, 76	0.42	NS
Percent Time in the Closed Arms			*	*	*
Entries into Back of Closed Arms[1]	↑	Anxiogenic	2, 63	2.67	<0.06
Time Spent in Back of Closed Arms[1]			2, 63	0.70	NS
Time Spent Immobile on Maze			*	*	*
Locomotion and Decision-Making Indices					
Total Entries			2, 73	0.85	NS
Entries into Closed Arms			2, 80	0.21	NS
Percent Time in the Center			2, 80	1.94	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 12. ANOVA results in the EPM for Isoproterenol -Stress X Isoproterenol Interaction

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			1, 80	1.44	NS
Time Spent in End Exploration[1]	↓	Anxiogenic	1, 80	2.60	<0.05
Frequency of End Exploration[1]	↓	Anxiogenic	1, 79	3.96	<0.05
Time Spent Head-dipping	↓	Anxiogenic	1, 79	2.62	<0.07
Latency to Explore End Sections	↑	Anxiogenic	1, 76	2.61	<0.08
Percent Time in the Closed Arms			*	*	*
Entries into Back of Closed Arms[1]	↑	Anxiogenic	1, 63	3.35	<0.05
Time Spent in Back of Closed Arms[1]	↑	Anxiogenic	1, 63	2.43	<0.09
Time Spent Immobile on Maze			*	*	*
Locomotion and Decision-Making Indices					
Total Entries			1, 73	0.90	NS
Entries into Closed Arms			1, 80	0.54	NS
Percent Time in the Center			1, 80	0.26	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 13. ANOVA results in the EPM for Yohimbine with Indomethacin Pretreatment-Main effects for Stress

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			*	*	*
Time Spent in End Exploration[1]			1, 57	0.43	NS
Frequency of End Exploration			1, 58	0.19	NS
Time Spent Head-dipping			*	*	*
Latency to Explore End Sections			1, 58	0.14	NS
Percent Time in the Closed Arms[1]			1, 60	0.19	NS
Entries into Back of Closed Arms			1, 60	0.05	NS
Time Spent in Back of Closed Arms[1]			1, 51	0.07	NS
Time Spent Immobile on Maze	↑	Anxiogenic	1, 57	2.82	<0.09
Locomotion and Decision-Making Indices					
Total Entries			1, 59	1.48	NS
Entries into Closed Arms			1, 60	0.76	NS
Percent Time in the Center	↓	Decreased	1, 58	0.38	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 14. ANOVA results in the EPM for Yohimbine with Indomethacin Pretreatment-Main effects for Drug

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			*	*	*
Time Spent in End Exploration[1]	↓	Anxiogenic	2, 57	8.18	<0.005
Frequency of End Exploration	↓	Anxiogenic	2, 58	8.95	<0.001
Time Spent Head-dipping			*	*	*
Latency to Explore End Sections	↑	Anxiogenic	2, 58	3.85	<0.05
Percent Time in the Closed Arms[1]	↑	Anxiogenic	2, 60	16.16	< 0.001
Entries into Back of Closed Arms	↑	Anxiogenic	2, 60	20.51	<0.001
Time Spent in Back of Closed Arms[1]	↑	Anxiogenic	2, 51	18.06	<0.001
Time Spent Immobile on Maze	↑	Anxiogenic	2, 57	32.56	<0.001
Locomotion and Decision-Making Indices					
Total Entries	↓	Decreased	2, 59	8.13	<0.005
Entries into Closed Arms			2, 60	1.83	NS
Percent Time in the Center	↓	Decreased	2, 58	3.85	<0.05

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 15. ANOVA results in the EPM for Yohimbine with Indomethacin Pretreatment-Stress X Yohimbine Interaction

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			*	*	*
Time Spent in End Exploration[1]			1, 57	0.03	NS
Frequency of End Exploration			1, 58	0.018	NS
Time Spent Head-dipping			*	*	*
Latency to Explore End Sections			1, 58	0.14	NS
Percent Time in the Closed Arms[1]			1, 60	0.52	NS
Entries into Back of Closed Arms			1, 60	0.22	NS
Time Spent in Back of Closed Arms[1]			1, 51	0.18	NS
Time Spent Immobile on Maze			1, 57	0.9	NS
Locomotion and Decision-Making Indices					
Total Entries			1, 59	1.22	NS
Entries into Closed Arms			1, 60	1.71	NS
Percent Time in the Center			*1, 58*	*0.68*	*NS*

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.

* Data were analyzed using Non-parametric K-W test.

Table 16. ANOVA results in the EPM for Isoproterenol with Indomethacin Pretreatment-Main effects for Stress

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			1, 40	0.34	NS
Time Spent in End Exploration			1, 40	0.63	NS
Frequency of End Exploration			1, 37	0.00	NS
Time Spent Head-dipping[1]			1, 33	0.73	NS
Latency to Explore End Sections	↑	Anxiogenic	1, 38	3.35	<0.07
Percent Time in the Closed Arms			1, 39	0.04	NS
Entries into Back of Closed Arms			1, 40	0.72	NS
Time Spent in Back of Closed Arms[1]			1, 40	0.11	NS
Time Spent Immobile on Maze			1, 40	0.43	NS
Locomotion and Decision-Making Indices					
Total Entries			1, 37	1.84	NS
Entries into Closed Arms	↓	Decreased	1, 40	5.11	<0.05
Percent Time in the Center			1, 39	0.04	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.

* Data were analyzed using Non-parametric K-W test.

Table 17. ANOVA results in the EPM for Isoproterenol with Indomethacin Pretreatment-Main effects for Drug

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			1, 40	0.20	NS
Time Spent in End Exploration			1, 40	0.06	NS
Frequency of End Exploration			1, 37	0.02	NS
Time Spent Head-dipping[1]			1, 33	1.22	NS
Latency to Explore End Sections	↑	Anxiogenic	1, 38	3.08	<0.08
Percent Time in the Closed Arms			1, 39	0.13	NS
Entries into Back of Closed Arms			1, 40	1.34	NS
Time Spent in Back of Closed Arms[1]	↑	Anxiogenic	1, 40	3.62	<0.06
Time Spent Immobile on Maze			1, 40	0.13	NS
Locomotion and Decision-Making Indices					
Total Entries			1, 37	0.09	NS
Entries into Closed Arms			1, 40	0.32	NS
Percent Time in the Center	↓	Decreased	1, 39	6.10	<0.05

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 18. ANOVA results in the EPM for Isoproterenol with Indomethacin Pretreatment-Stress X Isoproterenol Interaction

Variable Name	Effect	Interpretation	df	F Value	P value
Anxiety Indices					
Percent Time in the Open Arms			1, 40	0.22	NS
Time Spent in End Exploration			1, 40	0.89	NS
Frequency of End Exploration			1, 37	0.10	NS
Time Spent Head-dipping[1]			1, 33	0.18	NS
Latency to Explore End Sections			1, 38	0.02	NS
Percent Time in the Closed Arms			1, 39	0.80	NS
Entries into Back of Closed Arms			1, 40	0.15	NS
Time Spent in Back of Closed Arms[1]			1, 40	0.67	NS
Time Spent Immobile on Maze			1, 40	0.22	NS
Locomotion and Decision-Making Indices					
Total Entries			1, 37	1.96	NS
Entries into Closed Arms			1, 40	0.49	NS
Percent Time in the Center			1, 39	0.53	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
*Data were analyzed using Non-parametric K-W test.

Table 19. ANOVA results in the OFT for Yohimbine

Variable Name	Effect	Interpretation	df	F Value	P value
Stress					
Locomotion	↓	Anxiogenic	1, 78	6.60	<0.05
Rearing	↓	Anxiogenic	1, 78	14.84	<0.001
Time spent immobile	↓	Anxiogenic	1, 76	9.00	<0.005
Time spent in center of field			1, 76	1.53	NS
Drug					
Locomotion	↓	Anxiogenic	2, 78	6.36	<0.005
Rearing	↓	Anxiogenic	2, 78	46.66	<0.001
Time spent immobile	↓	Anxiogenic	2, 76	2.67	<0.07
Time spent in center of field	↓	Anxiogenic	2, 76	4.90	<0.05
Stress X Drug Interaction					
Locomotion			1, 78	1.06	NS
Rearing	↓	Anxiogenic	1, 78	3.44	<0.05
Time spent immobile			1, 76	0.98	NS
Time spent in center of field			1, 76	0.71	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 20. ANOVA results in the OFT for Isoproterenol

Variable Name	Effect	Interpretation	df	F Value	P value
Stress					
Locomotion			1, 78	0.82	NS
Rearing			1, 74	0.00	NS
Time spent immobile			1, 77	0.59	NS
Time spent in center of field			1, 78	0.02	NS
Drug					
Locomotion	↓	Anxiogenic	1, 78	4.24	<0.05
Rearing	↓	Anxiogenic	1, 78	7.56	<0.005
Time spent immobile	↓	Anxiogenic	1, 77	4.88	<0.05
Time spent in center of field			1, 78	1.11	NS
Stress X Drug Interaction					
Locomotion	↓	Anxiogenic	1, 78	2.27	<0.06
Rearing			1, 74	0.34	NS
Time spent immobile	↓	Anxiogenic	1, 77	2.38	<0.09
Time spent in center of field	↓	Anxiogenic	1, 78	3.70	<0.05

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 21. ANOVA results in the OFT for Yohimbine with Indomethacin Pretreatment

Variable Name	Effect	Interpretation	df	F Value	P value
Stress					
Locomotion			*	*	*
Rearing[1]			1, 58	2.33	NS
Time spent immobile			1, 58	0.53	NS
Time spent in center of field			*	*	*
Drug					
Locomotion			*	*	*
Rearing[1]	↓	Anxiogenic	2, 58	48.02	<0.001
Time spent immobile	↓	Anxiogenic	1, 58	13.06	<0.001
Time spent in center of field			*	*	*
Stress X Drug Interaction					
Locomotion			*	*	*
Rearing[1]			1, 58	0.66	NS
Time spent immobile			1, 58	0.40	NS
Time spent in center of field			*	*	*

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

Table 22. ANOVA results in the OFT for Isoproterenol with Indomethacin Pretreatment

Variable Name	Effect	Interpretation	df	F Value	P value
Stress					
Locomotion			1, 38	0.48	NS
Rearing			1, 38	0.69	NS
Time spent immobile			1, 38	0.43	NS
Time spent in center of field			1, 38	0.69	NS
Drug					
Locomotion			2, 38	0.90	NS
Rearing	↓	Anxiogenic	2, 38	3.10	<0.08
Time spent immobile			2, 38	0.00	NS
Time spent in center of field			1, 38	0.69	NS
Stress X Drug Interaction					
Locomotion			1, 38	0.00	NS
Rearing			1, 38	0.02	NS
Time spent immobile			1, 38	0.16	NS
Time spent in center of field			1, 38	0.69	NS

[1] Data were logarithmically transformed to meet homogeneity of variance requirements.
* Data were analyzed using Non-parametric K-W test.

FIGURES

Figure 1. Panic rates for panic disorder patients and healthy controls following different doses of CCK-4

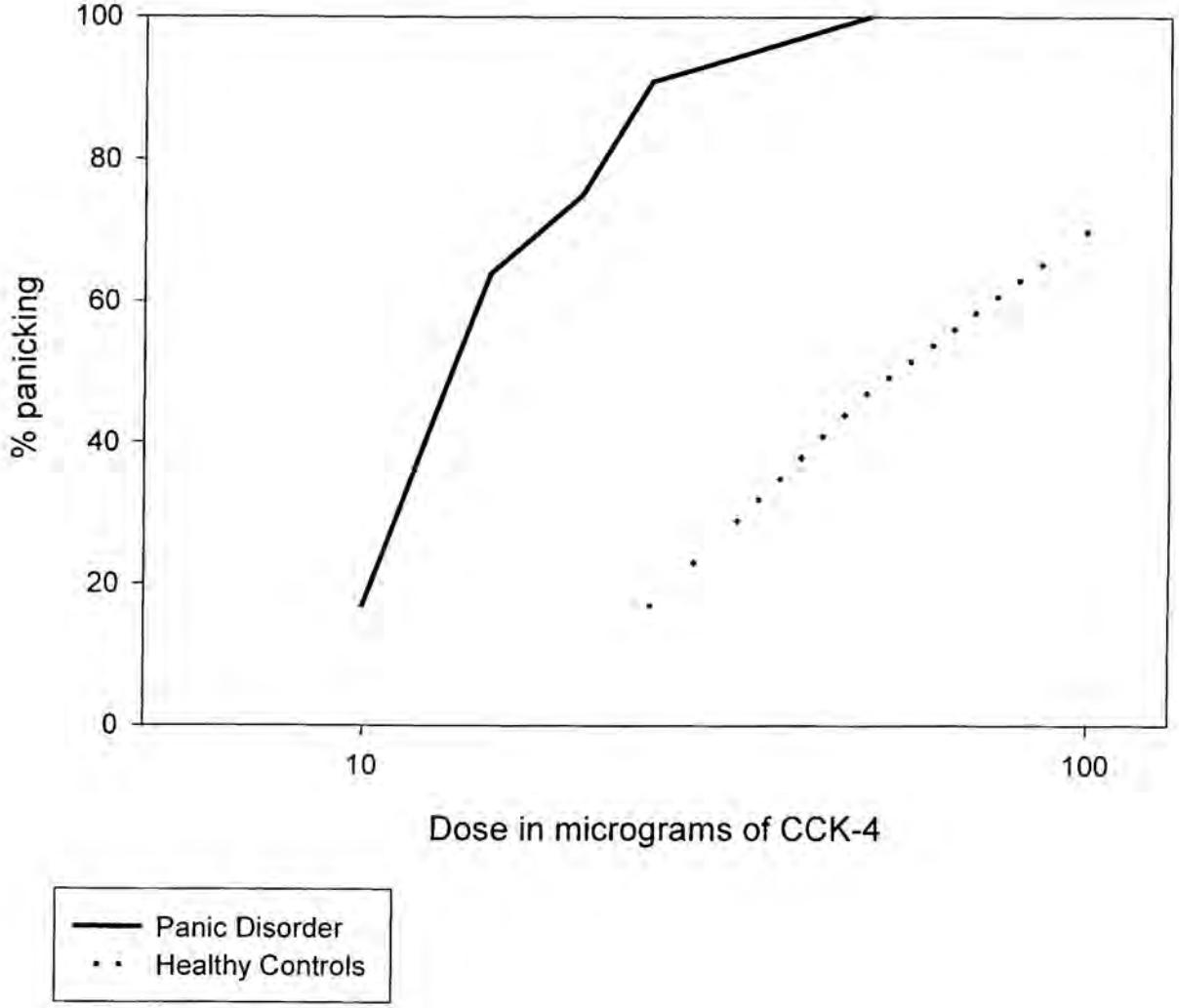


Figure 2: A cognitive model of panic attacks. Adapted from Clark (1986).

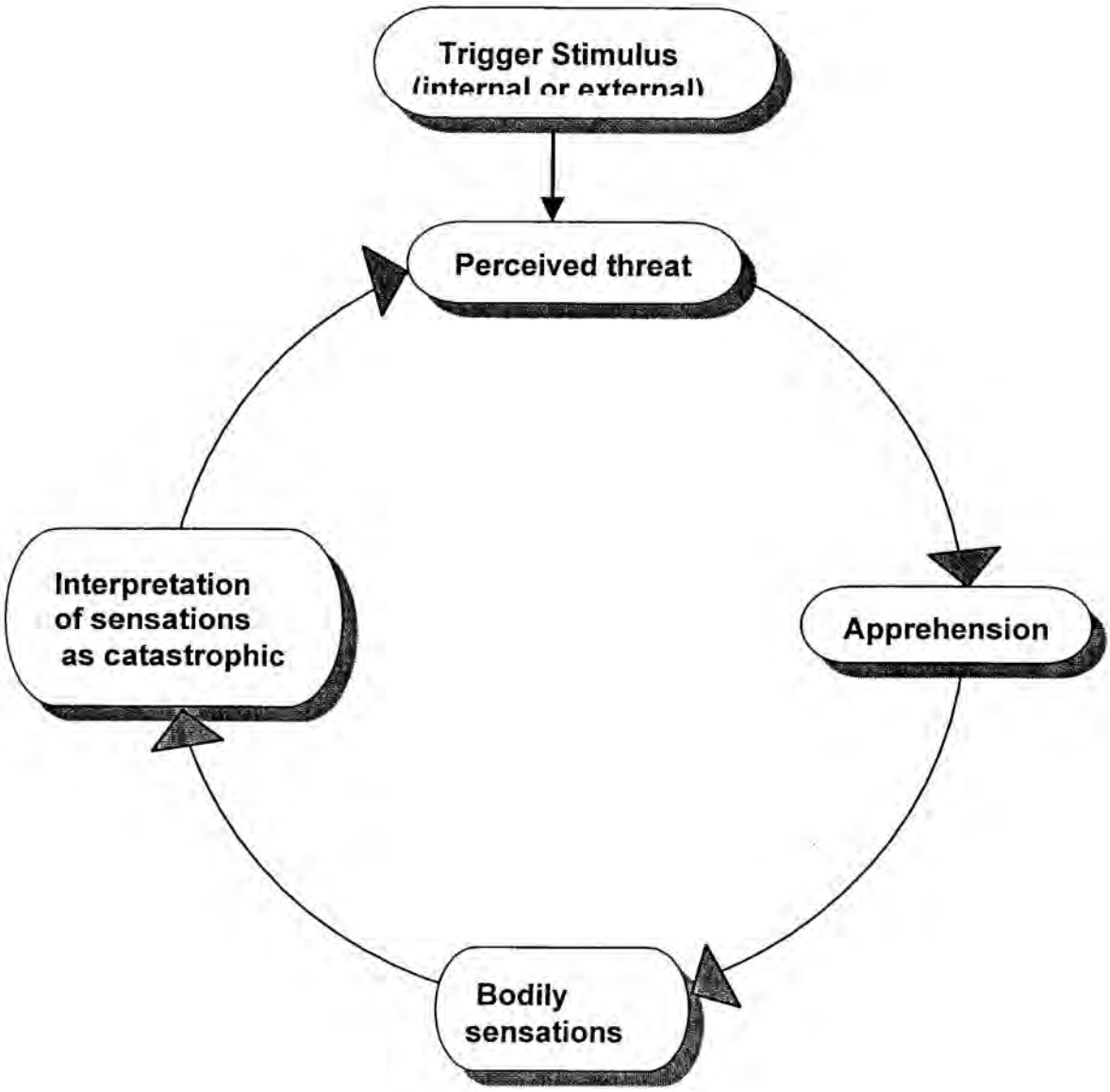


Figure 3: Classical Conditioning model for panic disorder.

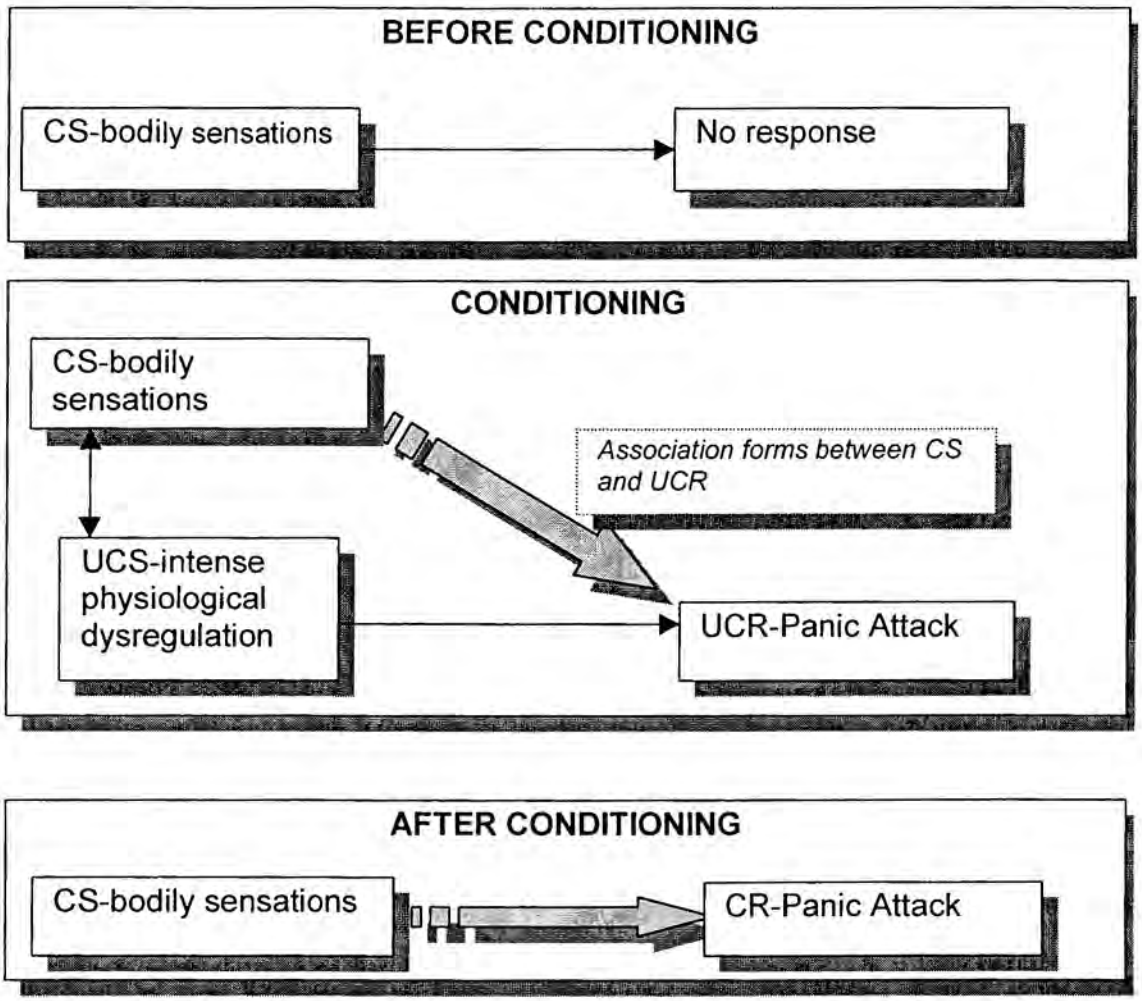


Figure 4: The Learned Alarm model for the development of panic disorder. Adapted from Barlow (1988).

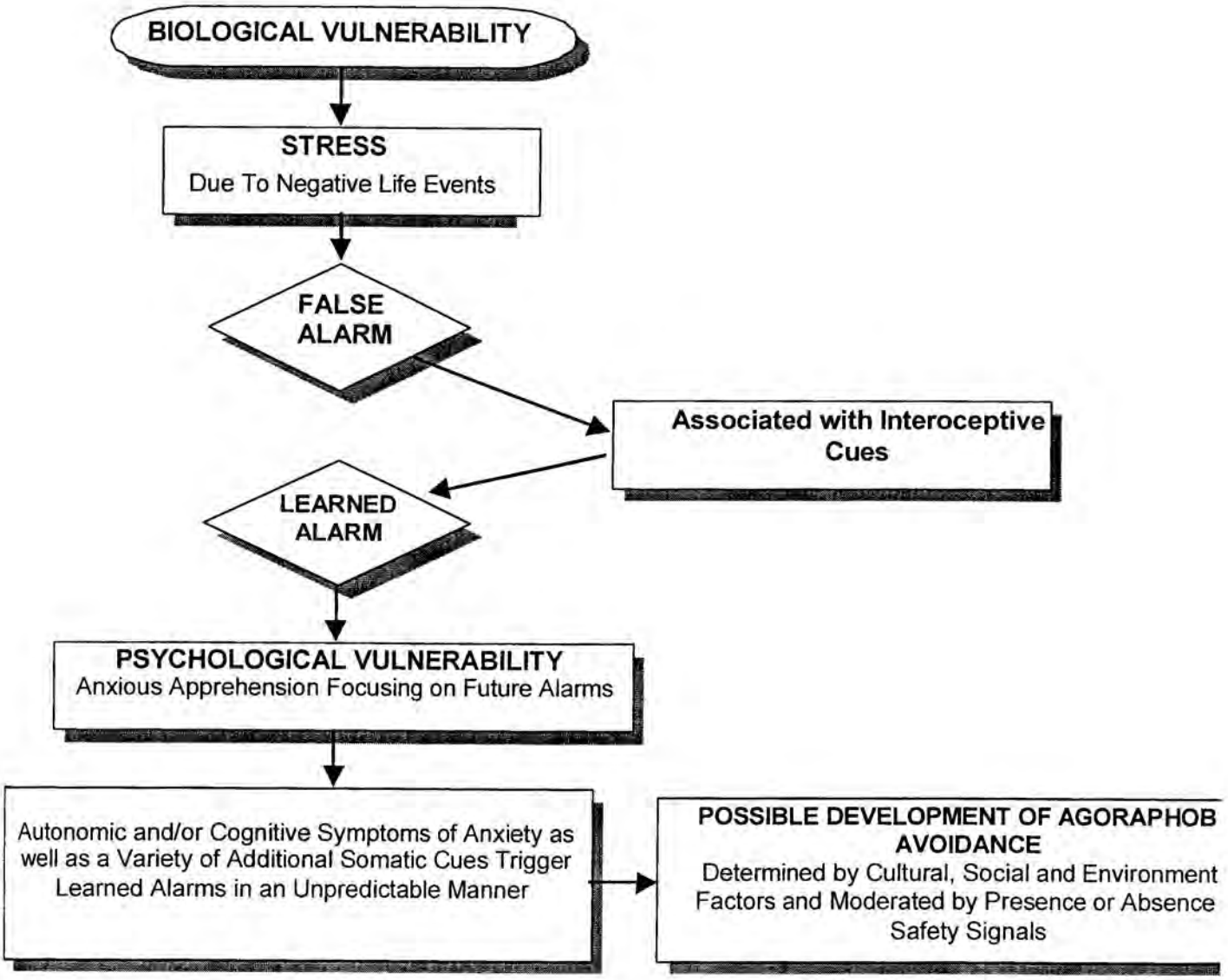


Figure 5. Picture of Elevated Plus Maze

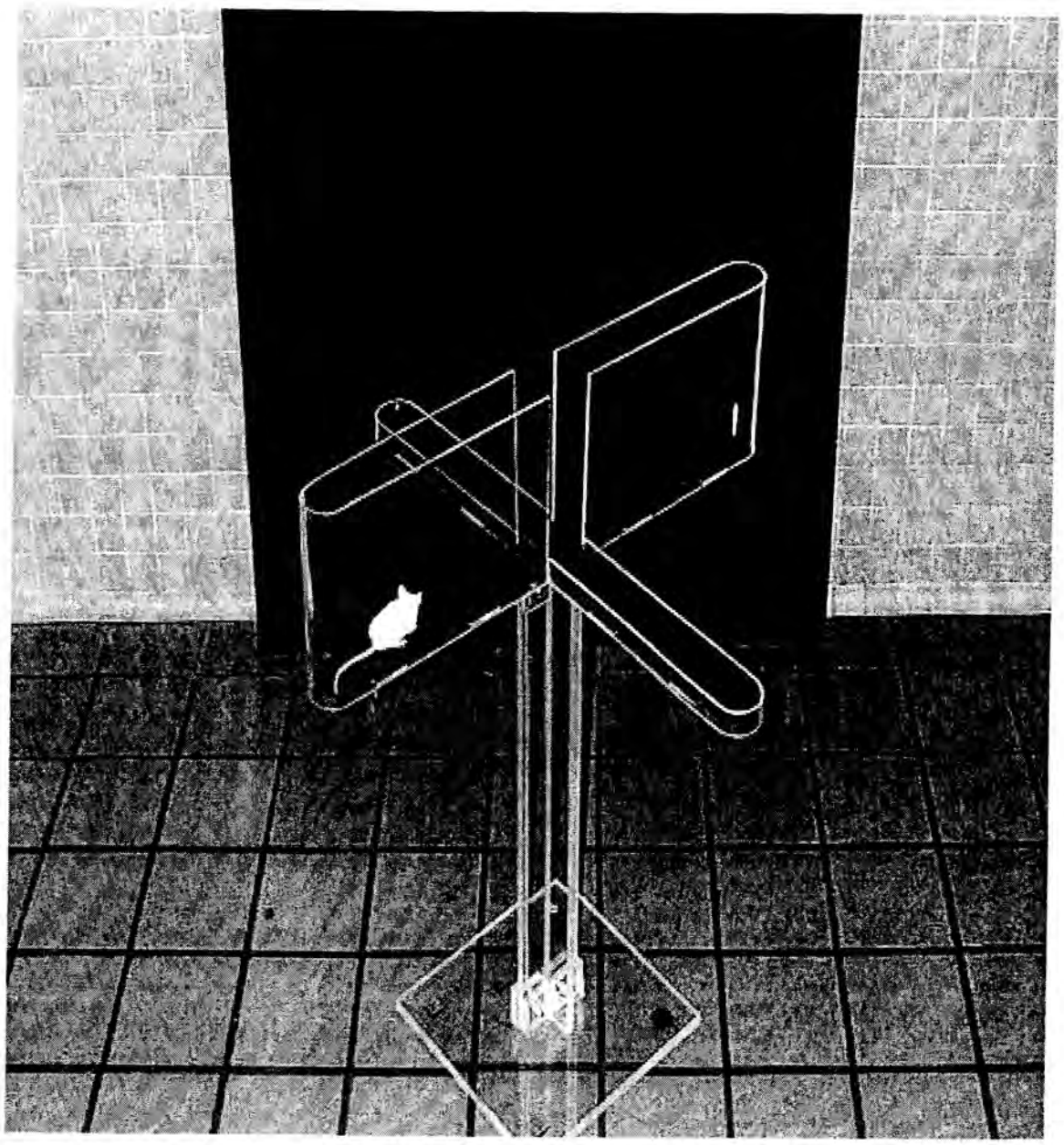
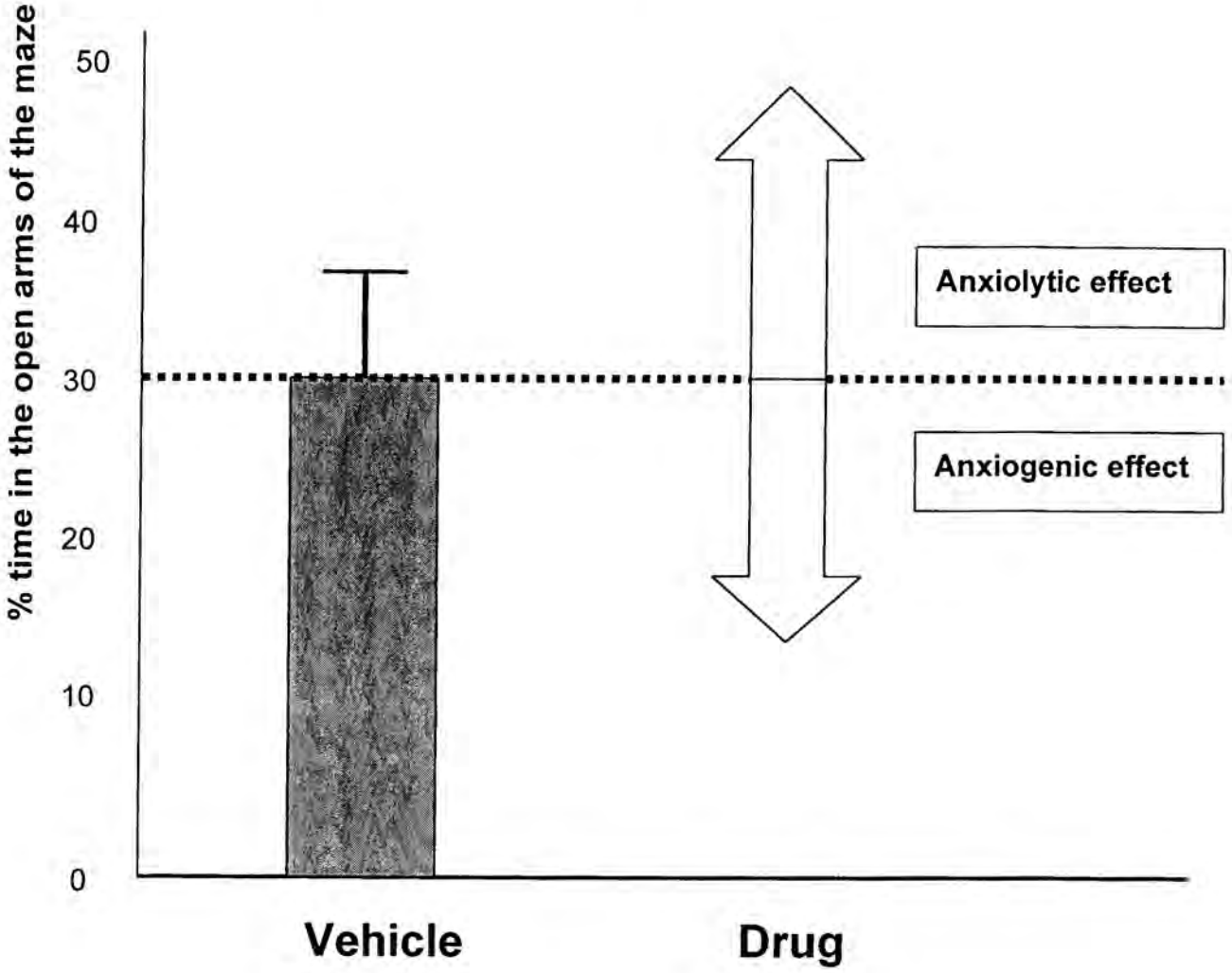


Figure 6. Drugs effects in the Elevated Plus Maze.



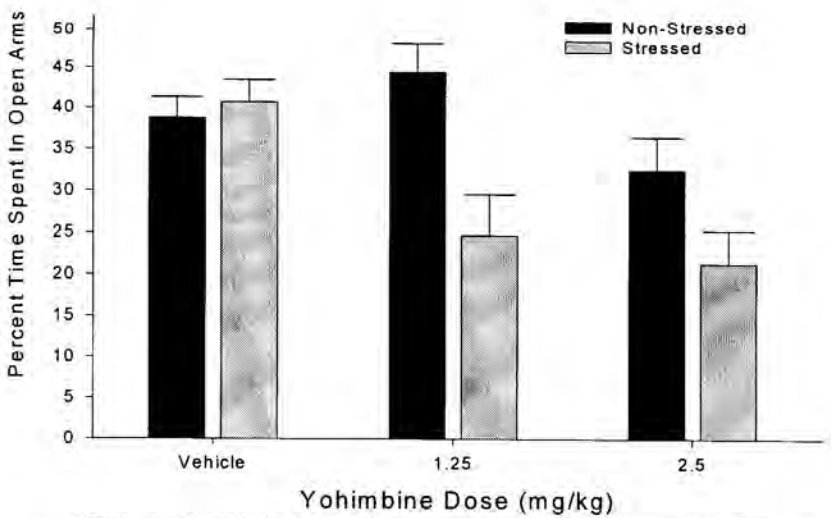


Figure I-1. Percent time spent in the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

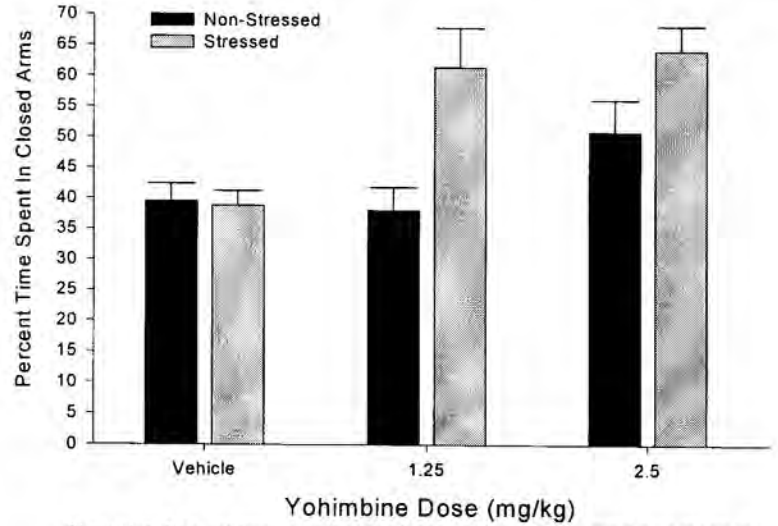


Figure I-2. Percent time spent in the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

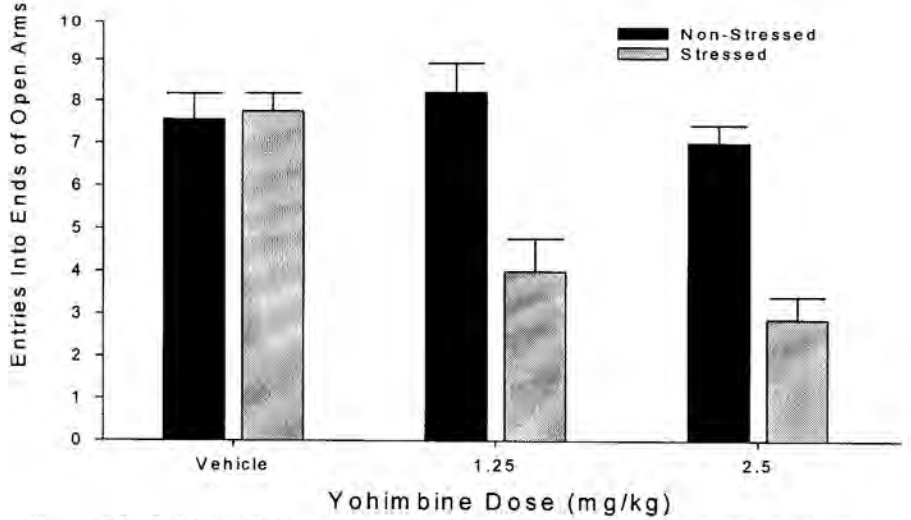


Figure I-3. Entries into the ends of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

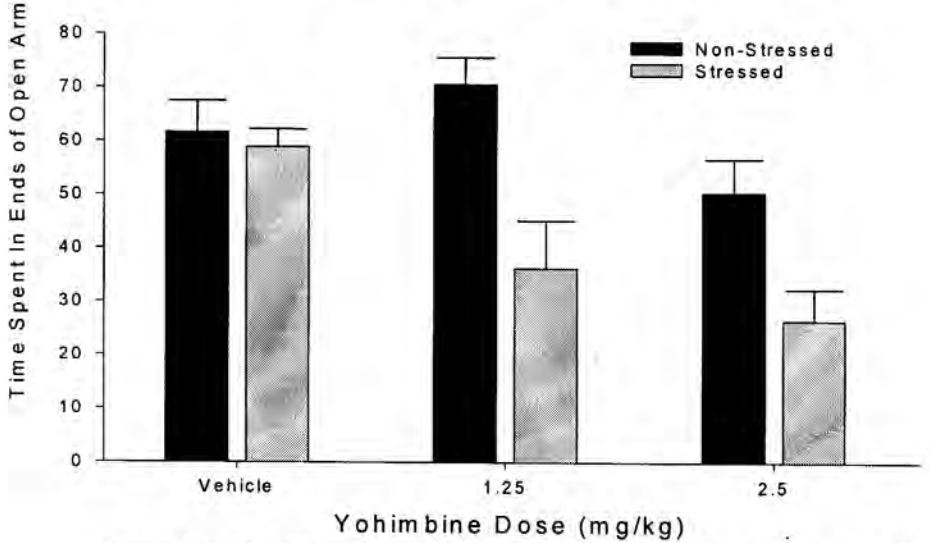


Figure I-4. Time spent in the ends of the open arms of the elevated plus maze i stressed and non-stressed rats following treatment with vehicle or yohimbine.

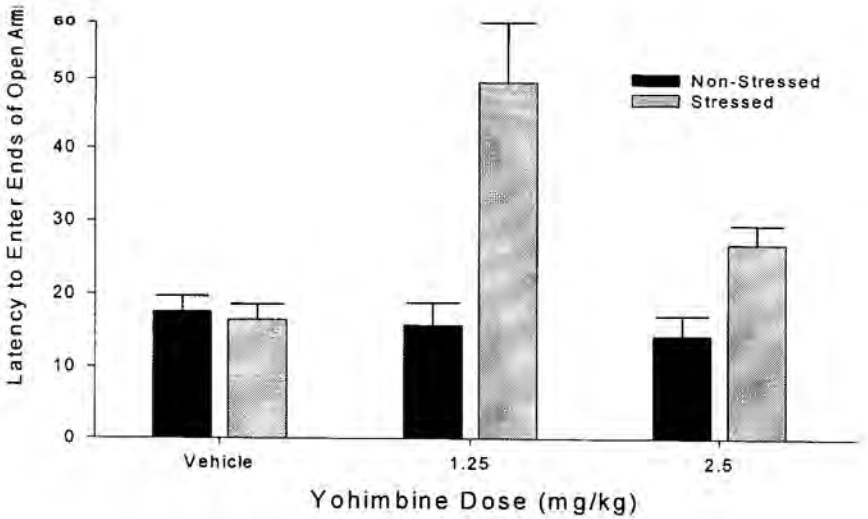


Figure I-5. Latency to enter the end sections of the open arms of the elevated plus ma stressed and non-stressed rats following treatment with vehicle or yohimbine.

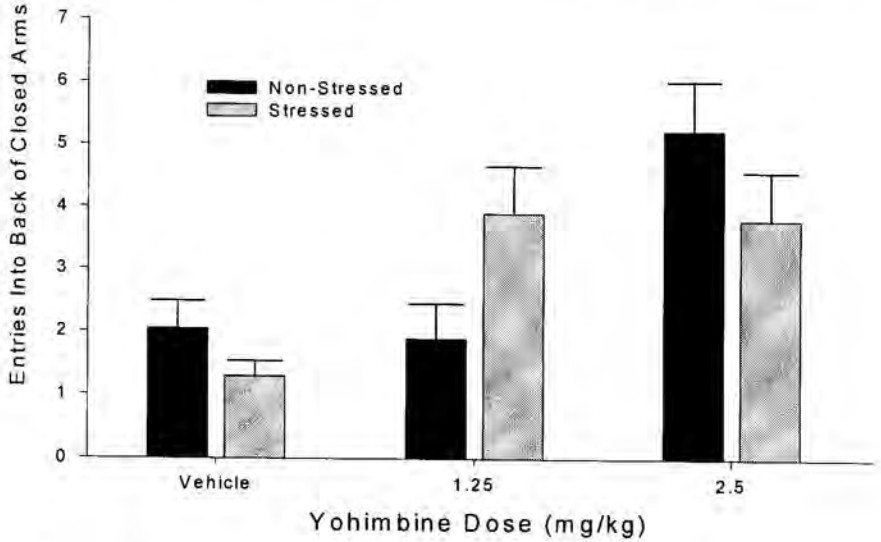


Figure I-6. Entires into the back section of the closed arms of the elevated plus maze stressed and non-stressed rats following treatment with vehicle or yohimbine.

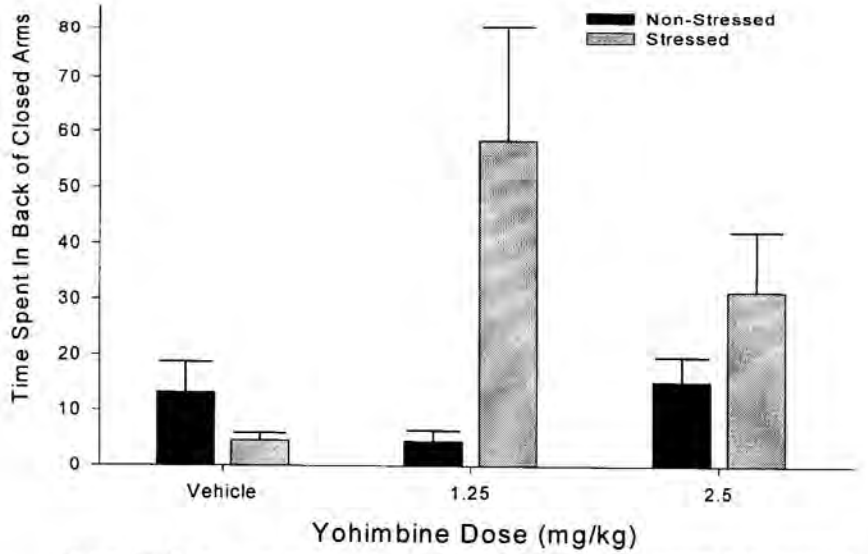


Figure I-7. Time spent in the back of the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

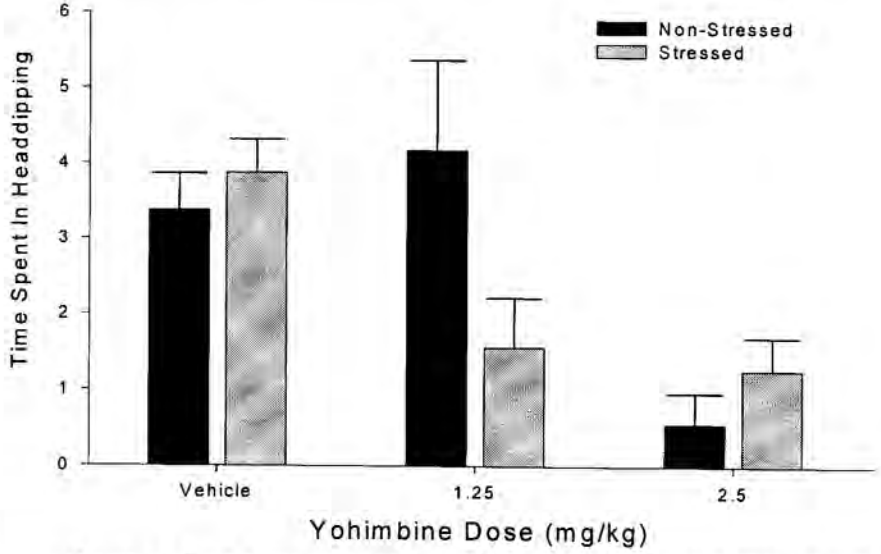


Figure I-8. Time spent headdipping in the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

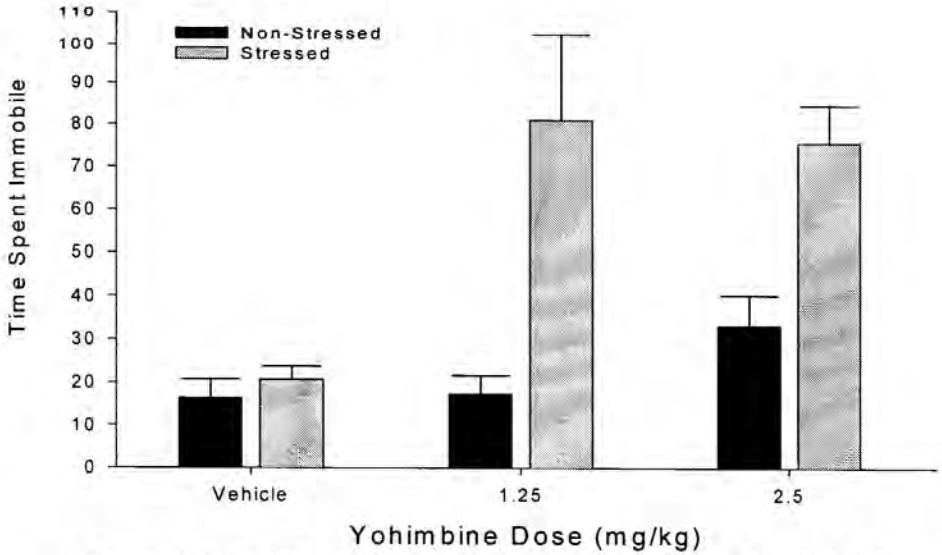


Figure I-9. Time spent immobile on the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

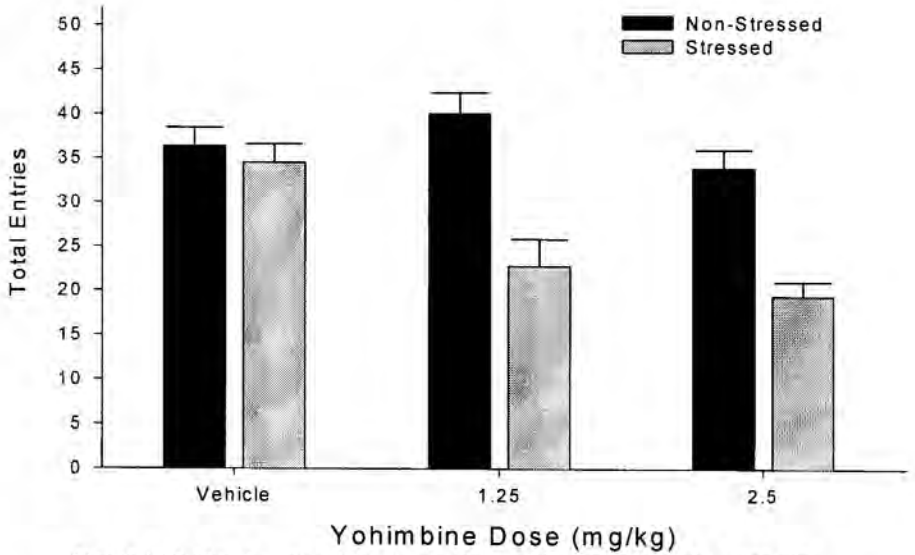


Figure I-10. Total entries into the sections of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

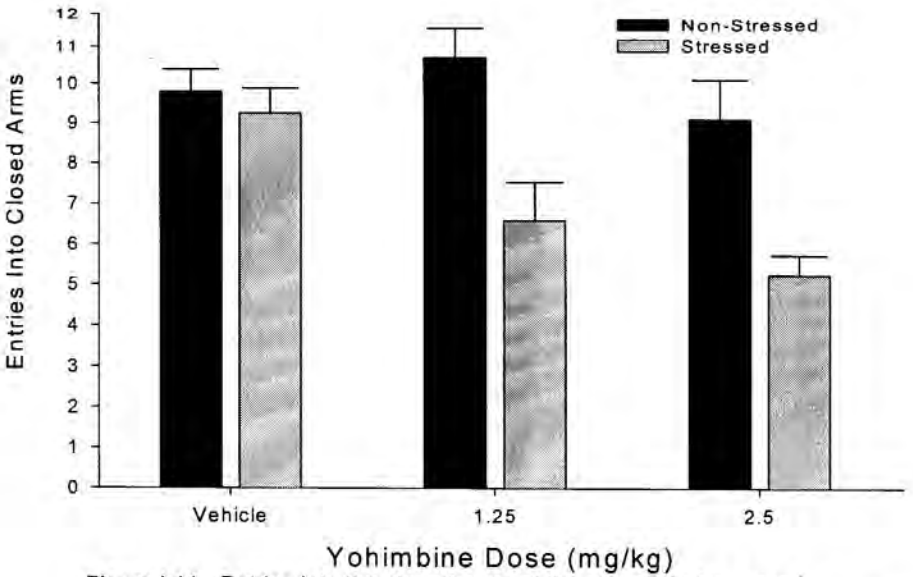


Figure I-11. Entries into the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

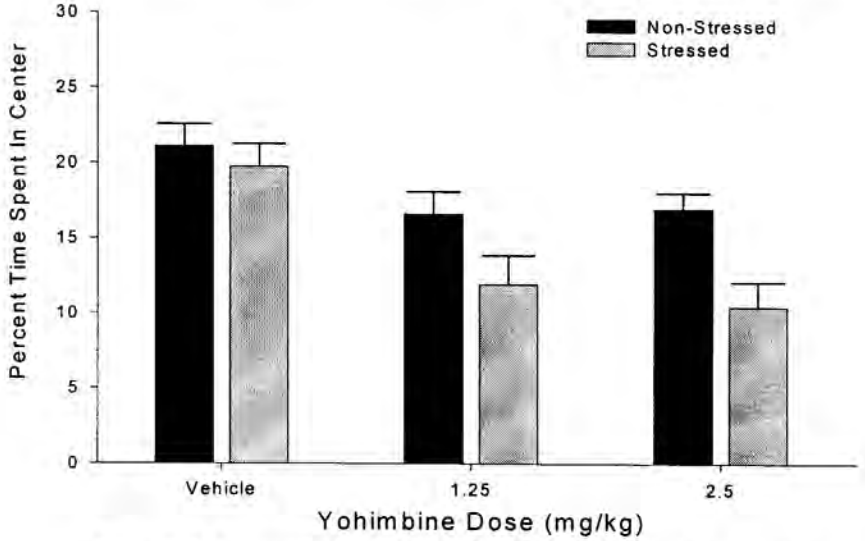


Figure I-12. Percent Time spent in the center of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine.

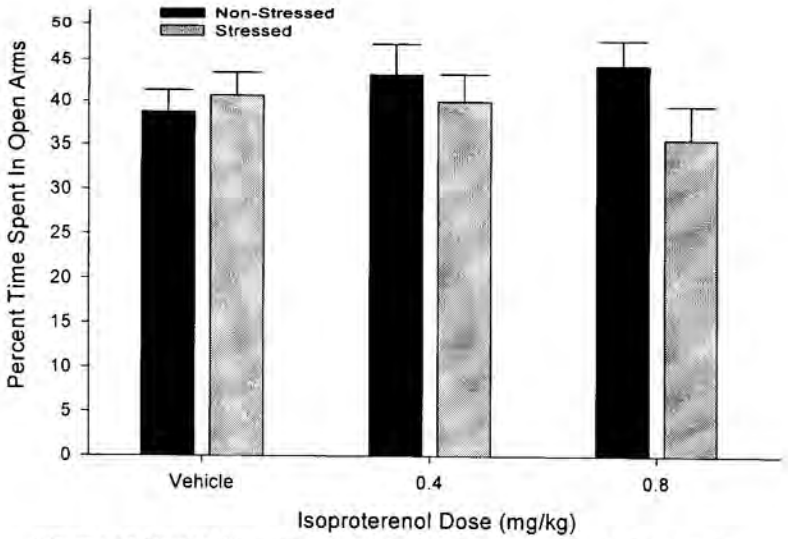


Figure I-13. Percent time spent in the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

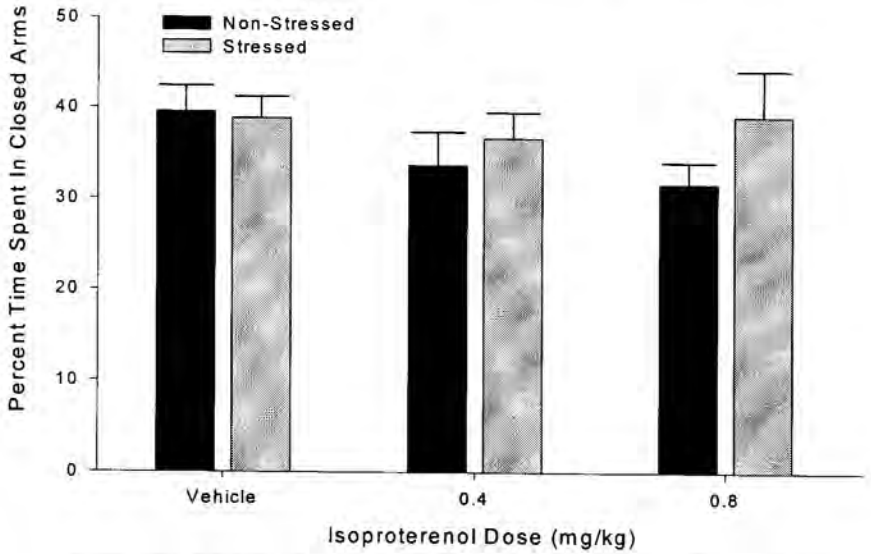


Figure I-14. Percent time spent in the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

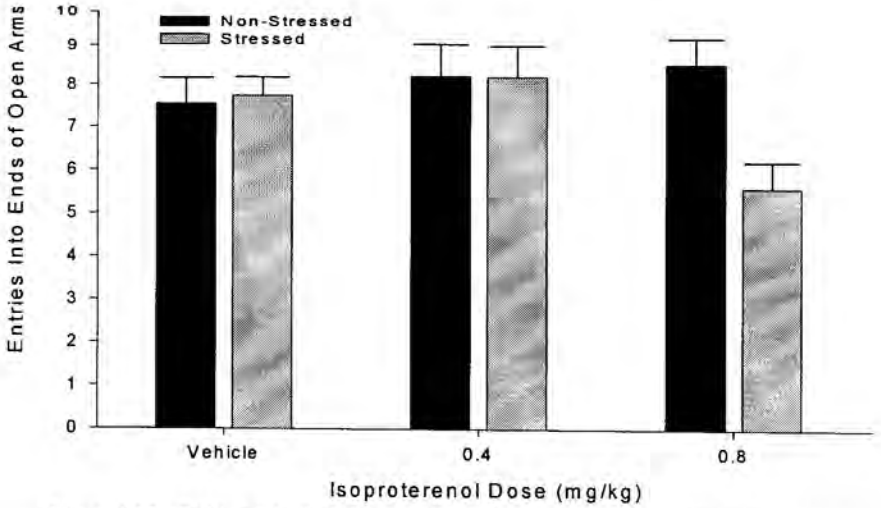


Figure I-15. Entries into the ends of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

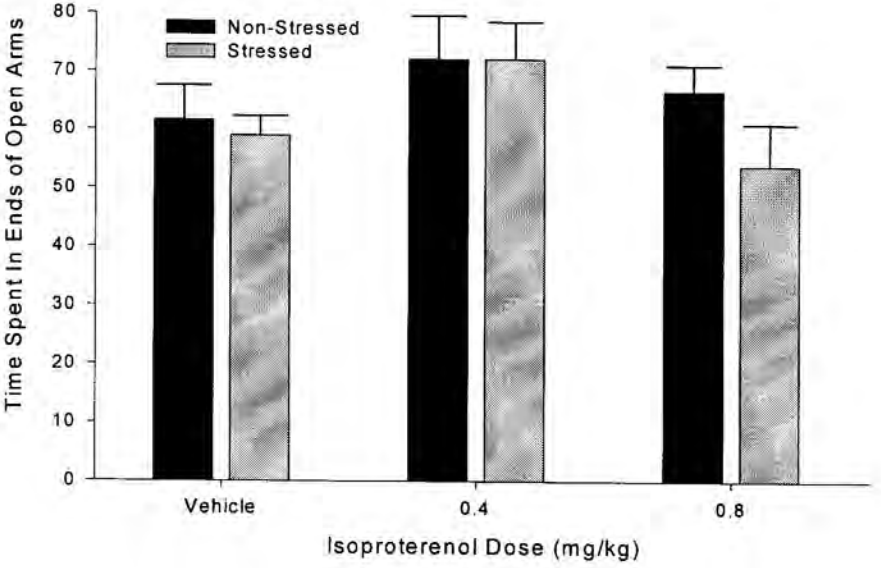


Figure I-16. Time spent in the ends of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

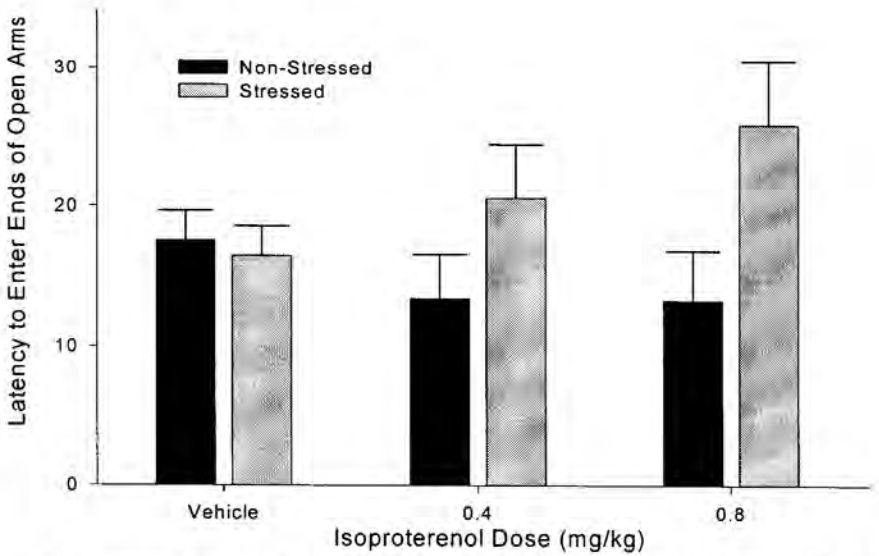


Figure I-17. Latency to enter the end sections of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

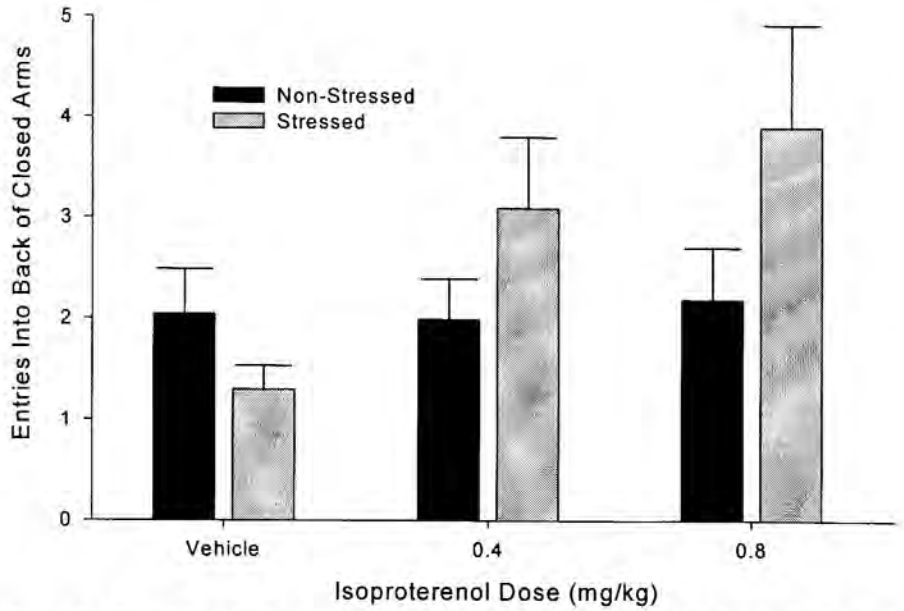


Figure I-18. Entires into the back section of the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

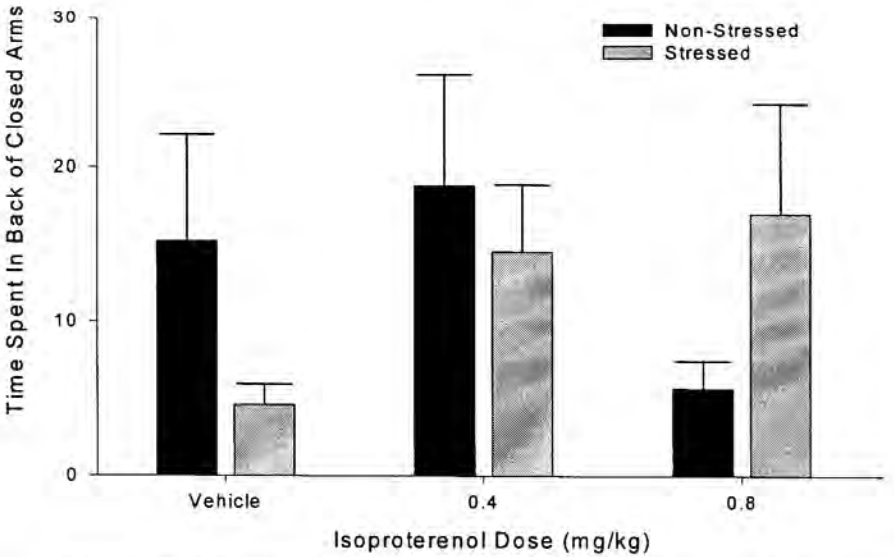


Figure I-19. Time spent in the back of the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

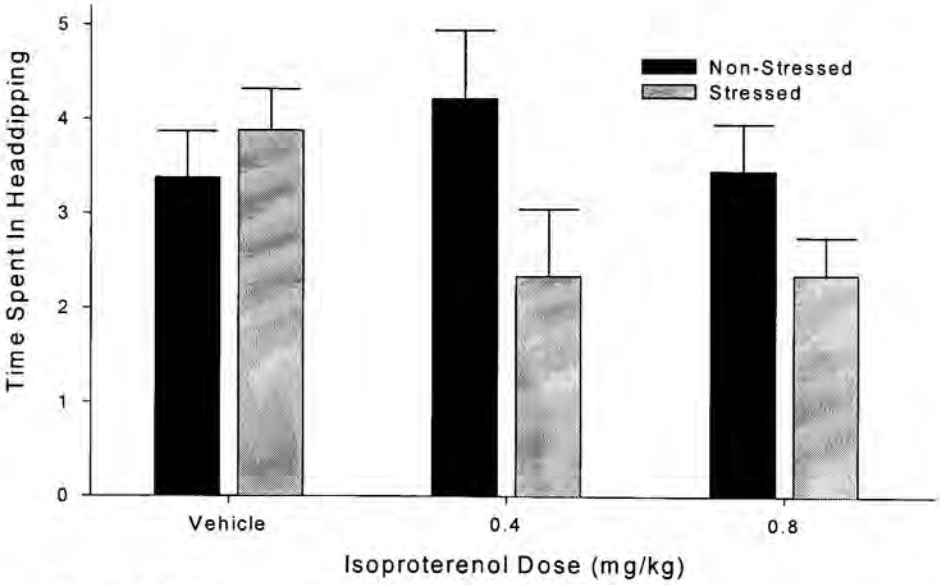


Figure I-20. Time spent headdipping in the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

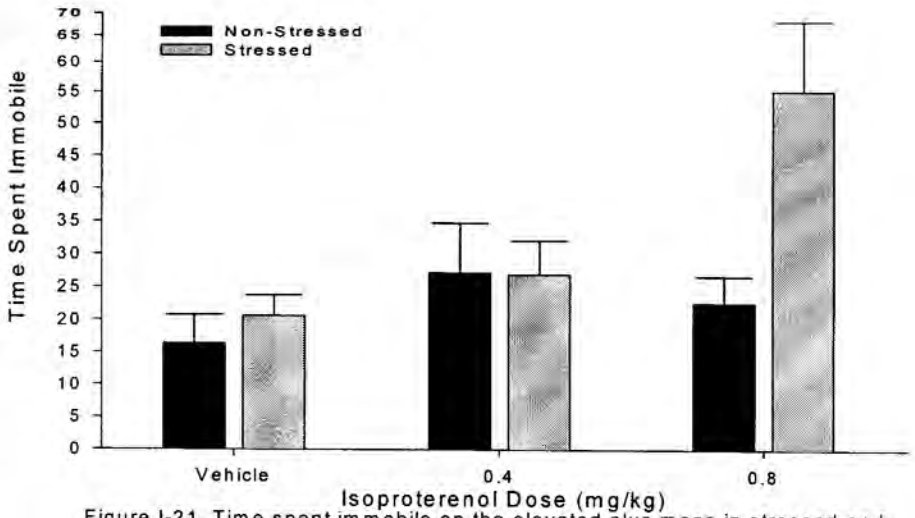


Figure I-21. Time spent immobile on the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

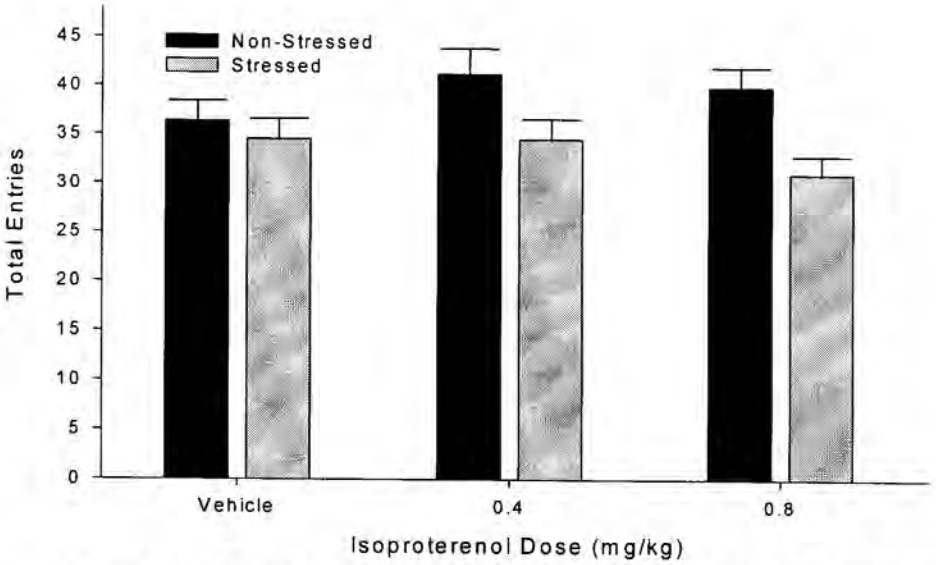


Figure I-22. Total entries into the arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

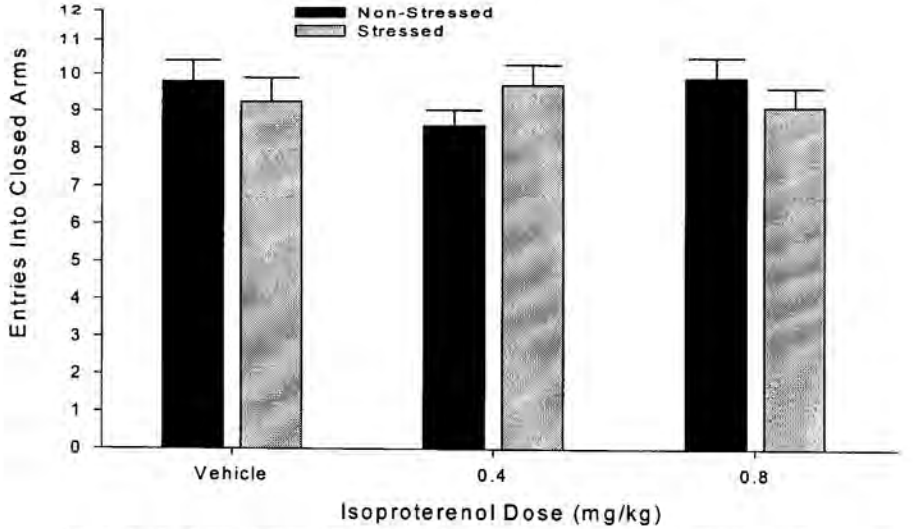


Figure I-23. Entries into the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

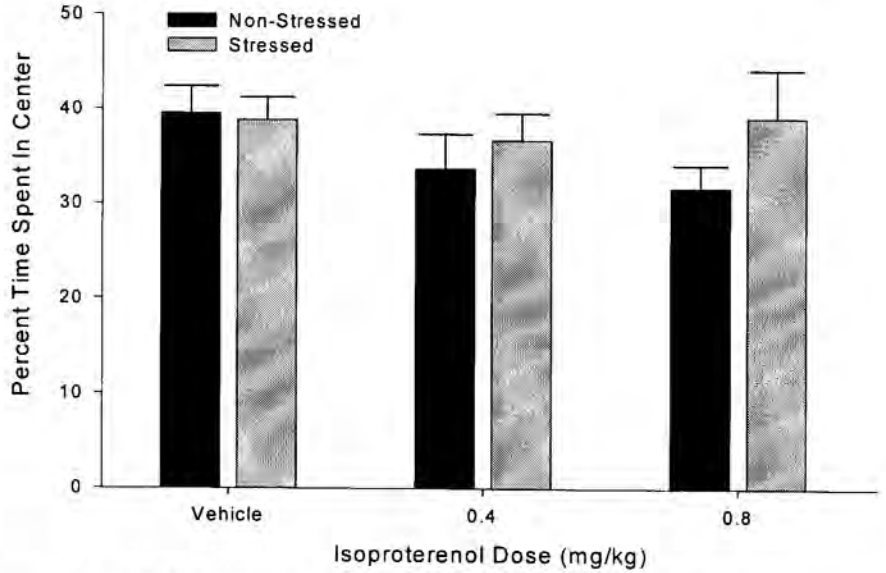


Figure I-24. Percent time spent in the center of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

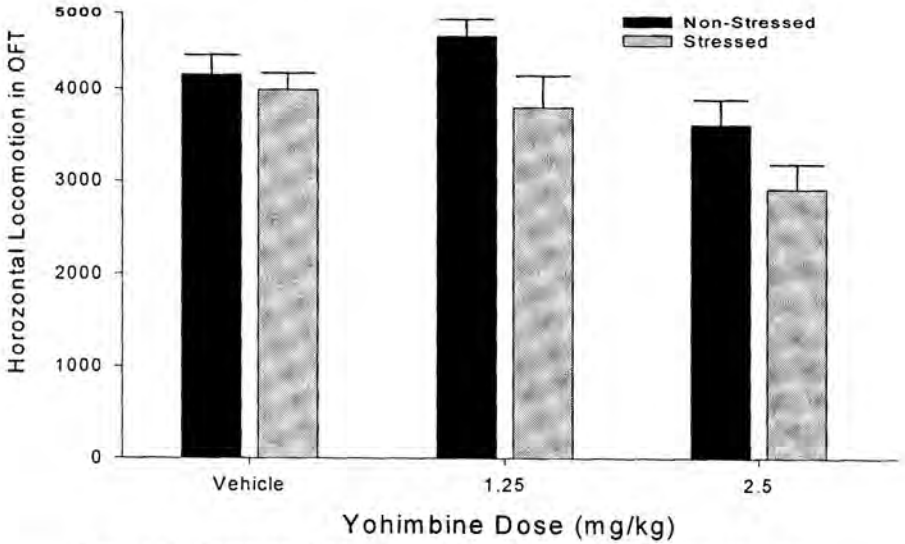


Figure I-25. Horozontal beam breaks (locomotion) in the open field test in stressed and non-stressed rats following treatment with vehicle or yohimbine.

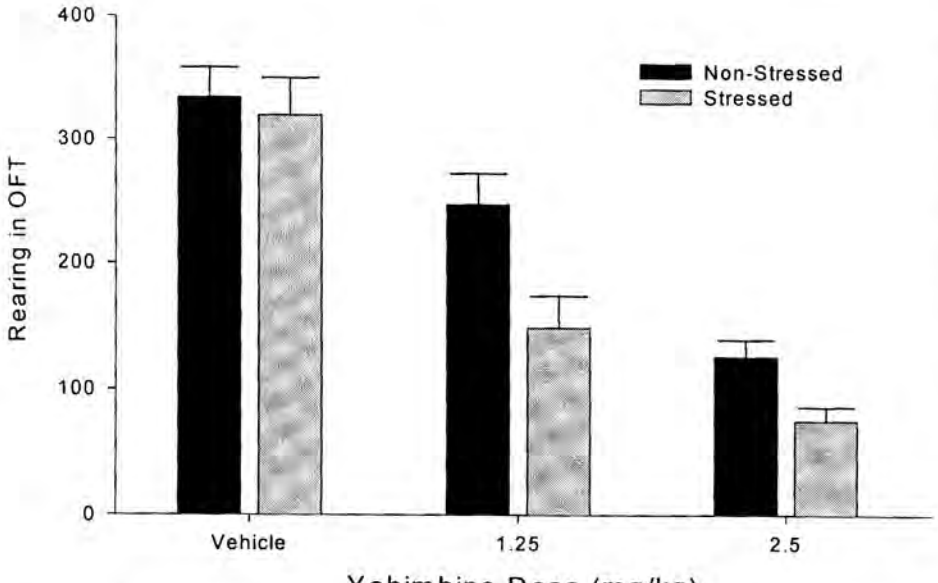


Figure I-26. Rearing (vertical beam breaks) in the open field test in stressed and non-stressed rats following treatment with vehicle or yohimbine.

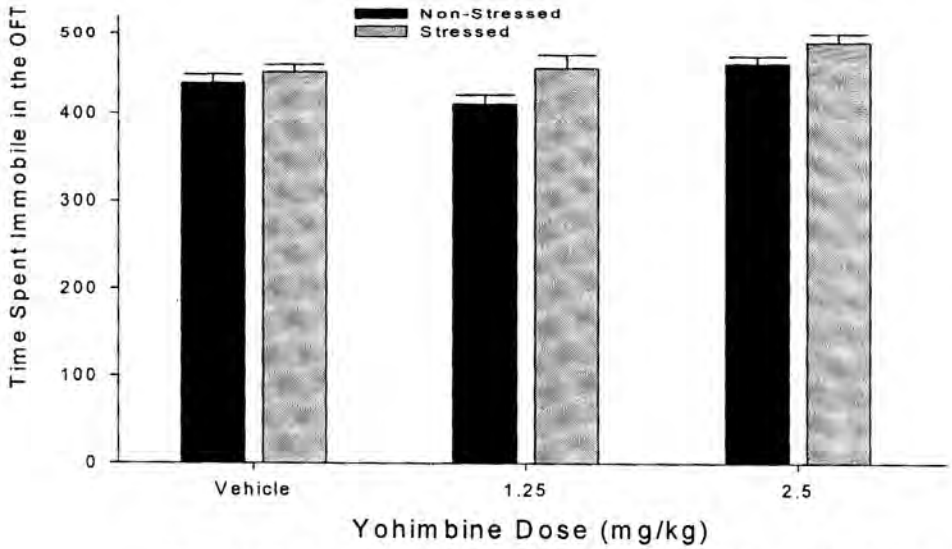


Figure I-27. Time spent immoble in the open field test in stressed and non-stressed rats following treatment with vehicle or yohimbine.

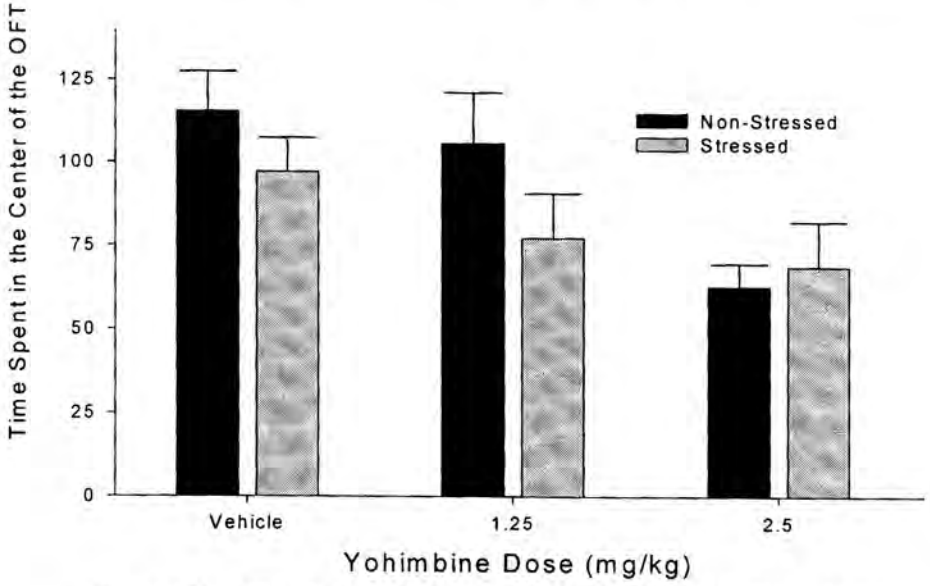


Figure I-28. Time spent in the center of the open field test in stressed and non-stressed rats following treatment with vehicle or yohimbine.

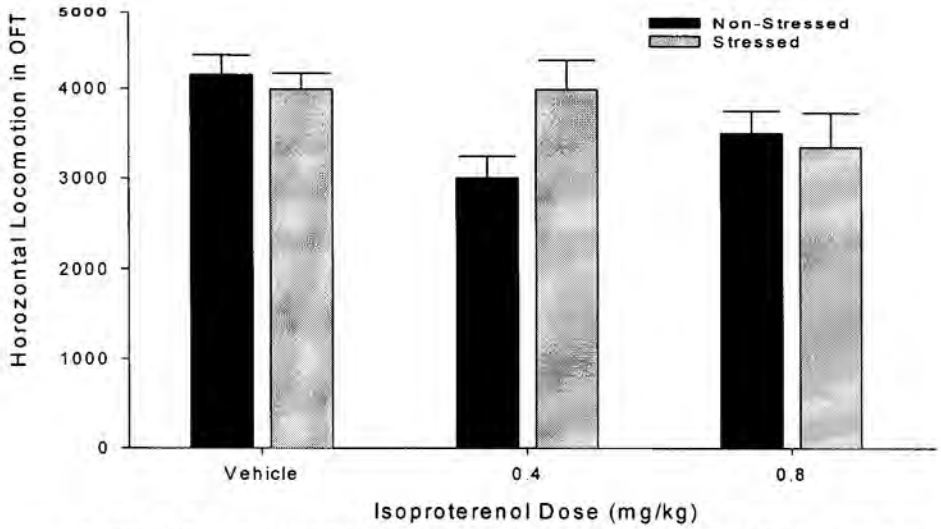


Figure I-29. Horizontal motion (locomotion) in the open field test in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

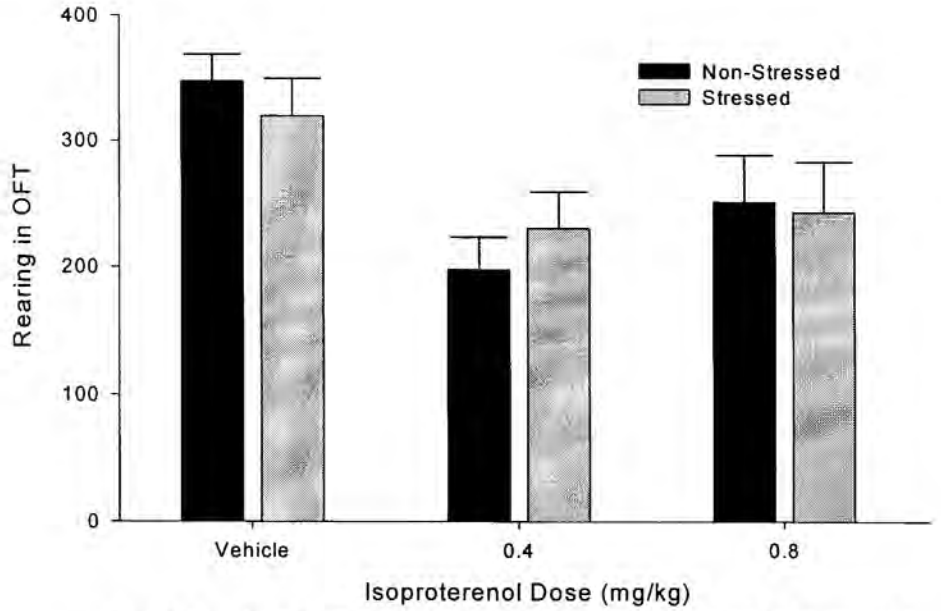


Figure I-30. Rearing time in the open field test in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

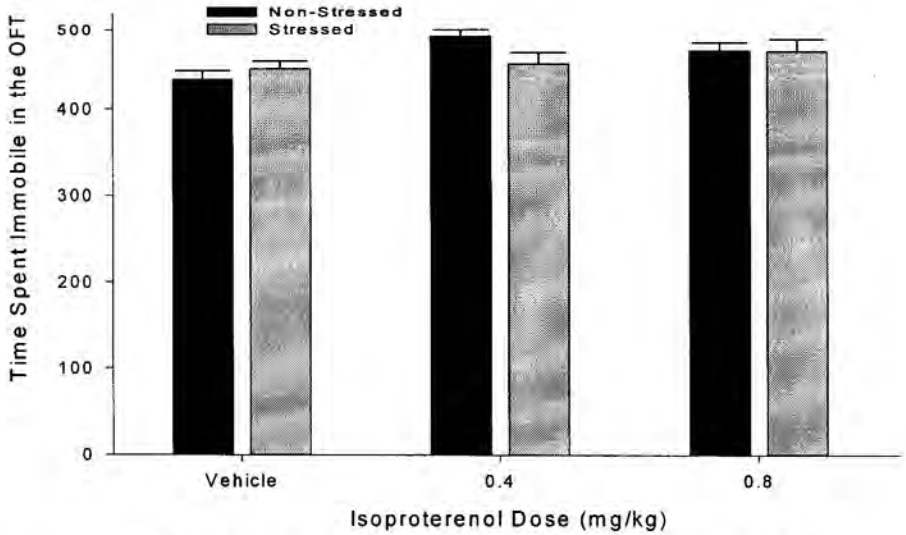


Figure I-31. Time spent immoble in the open field test in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

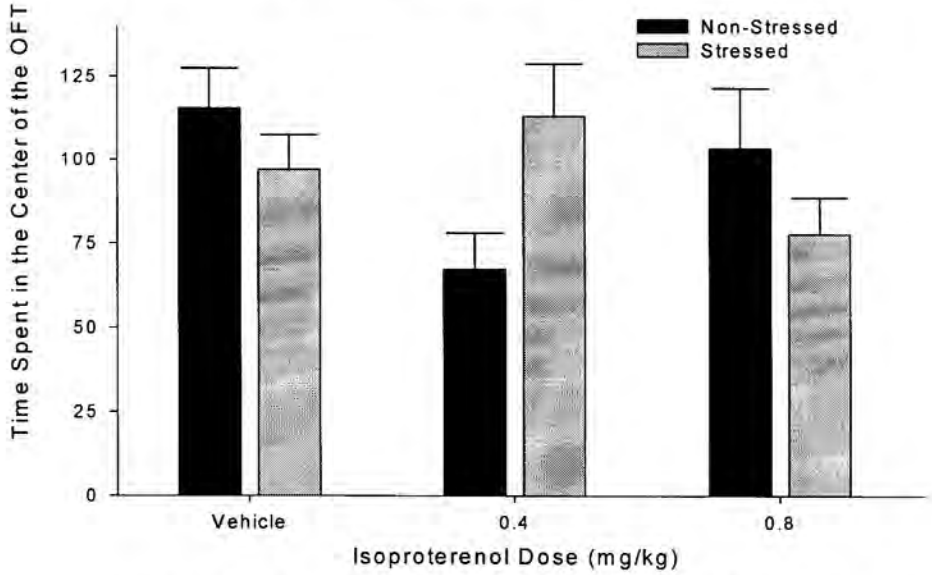


Figure I-32. Time spent in the center of the open field test in stressed and non-stressed rats following treatment with vehicle or isoproterenol.

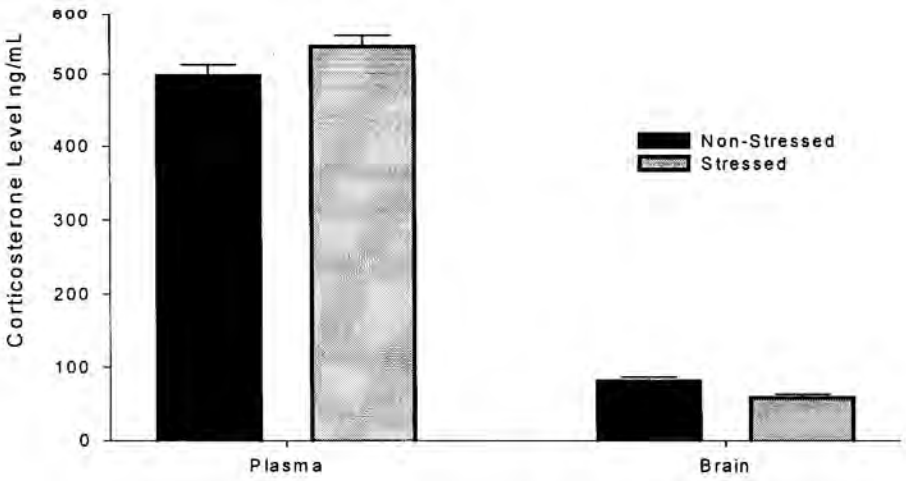


Figure I-33. Levels of corticosterone in plasma and brain samples in stressed and non-stressed rats.

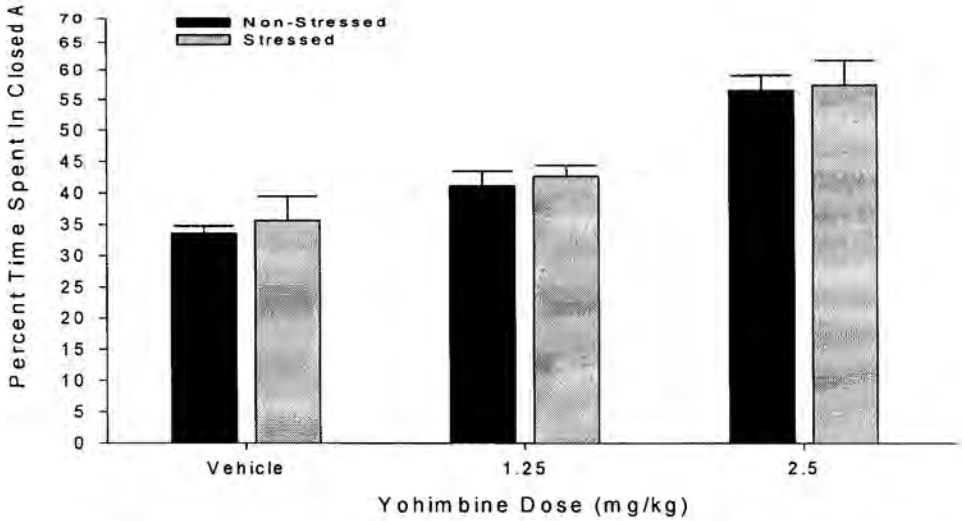


Figure II-2. Percent time spent in the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

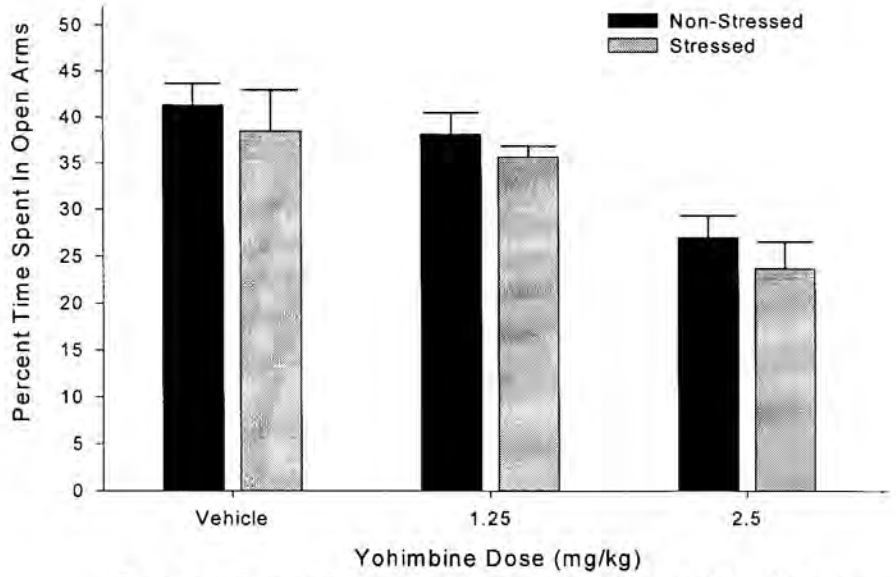


Figure II-1. Percent time pent in the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

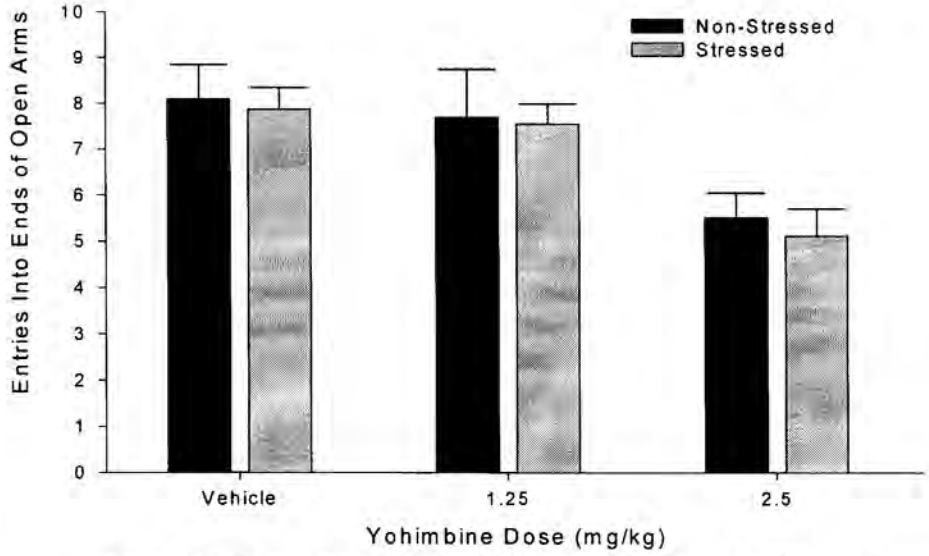


Figure II-3. Entries into the ends of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

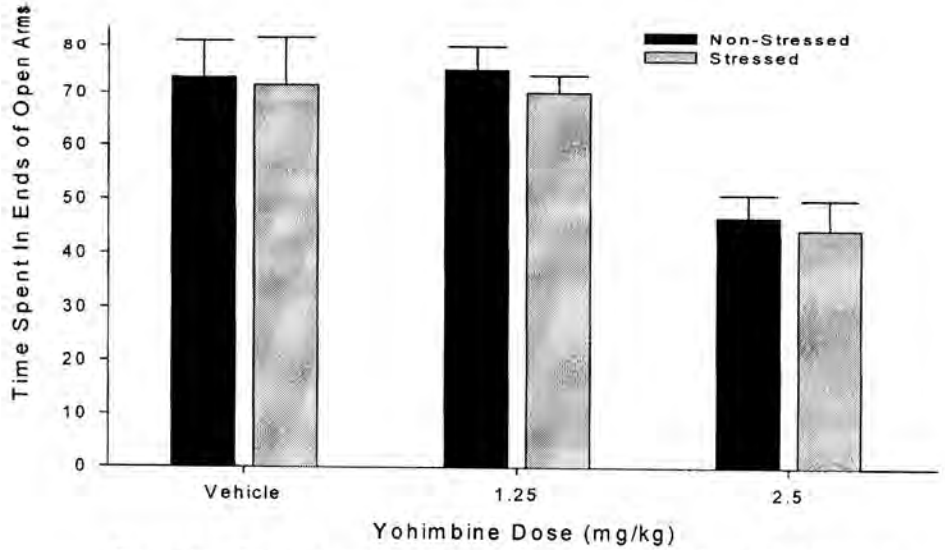


Figure II-4. Time spent in the ends of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

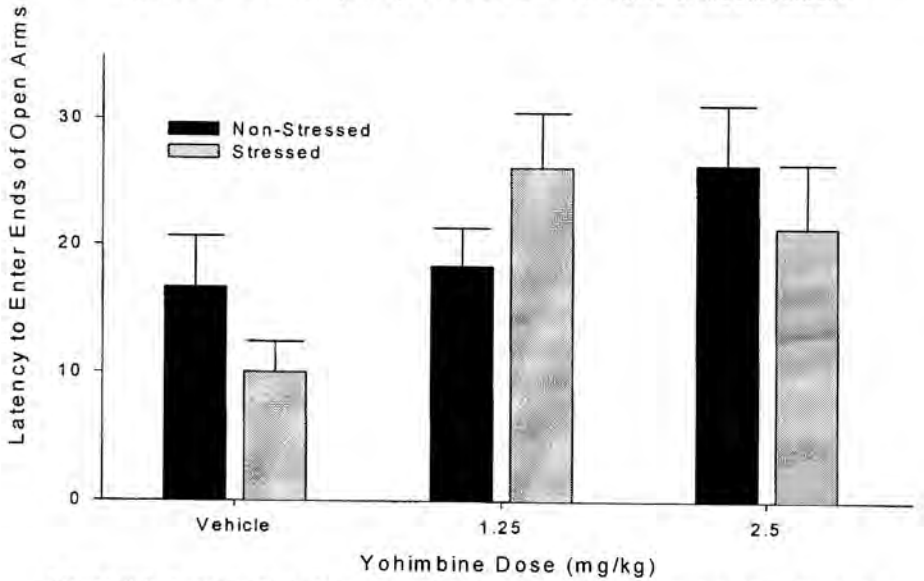


Figure II-5. Latency to enter the end sections of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

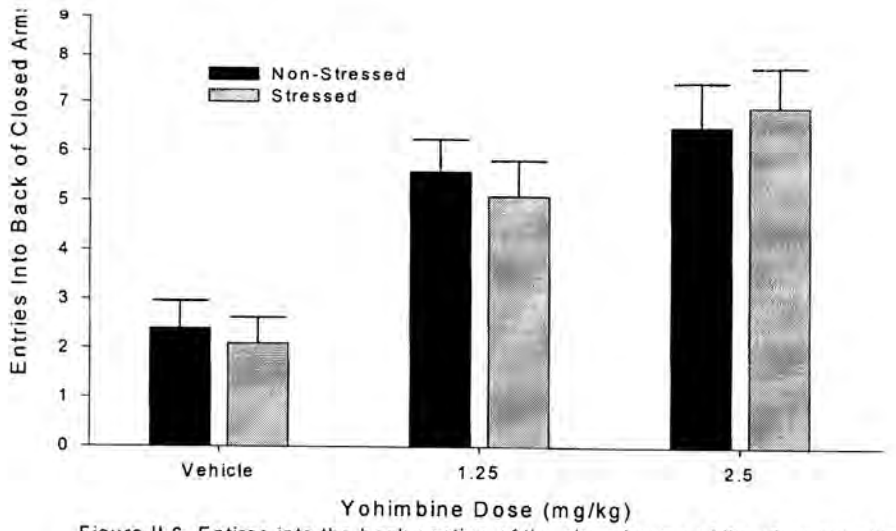


Figure II-6. Entires into the back section of the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

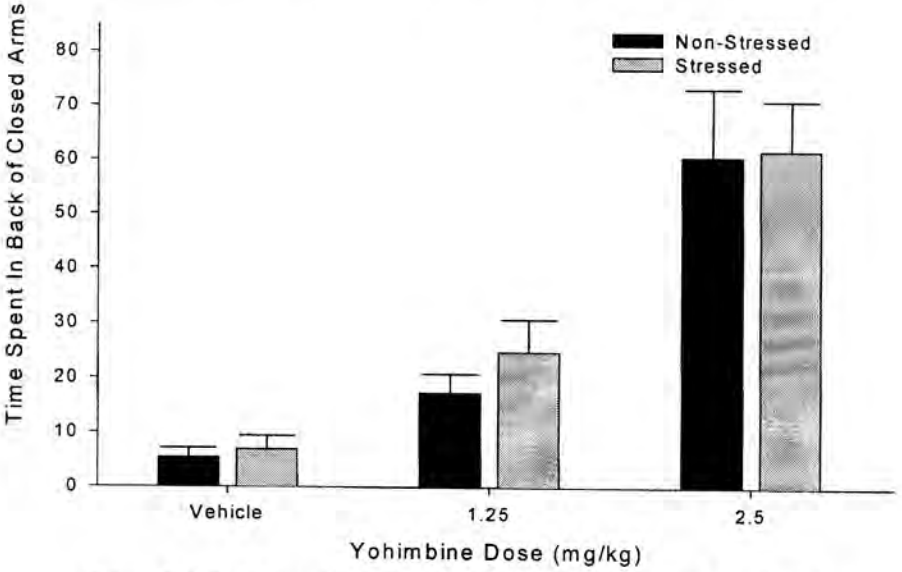


Figure II-7. Time spent in the back of the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

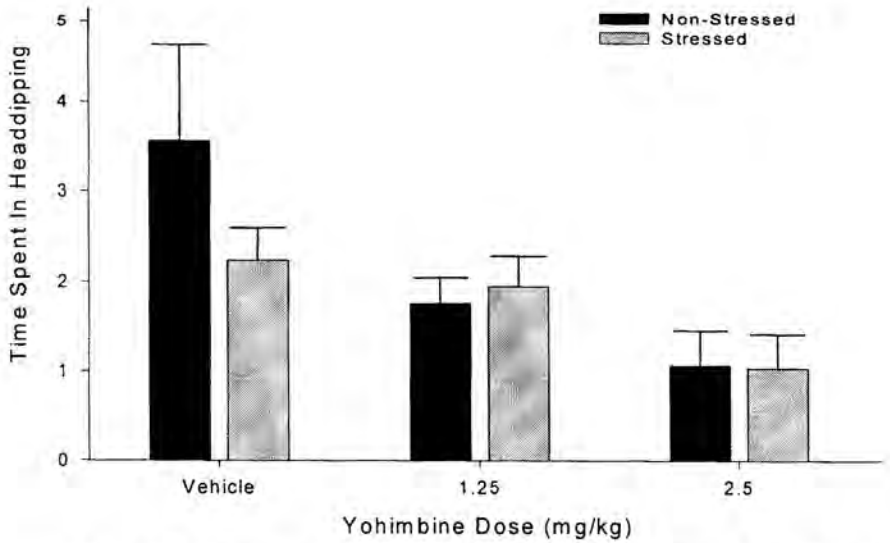


Figure II-8. Time spent headdipping in the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

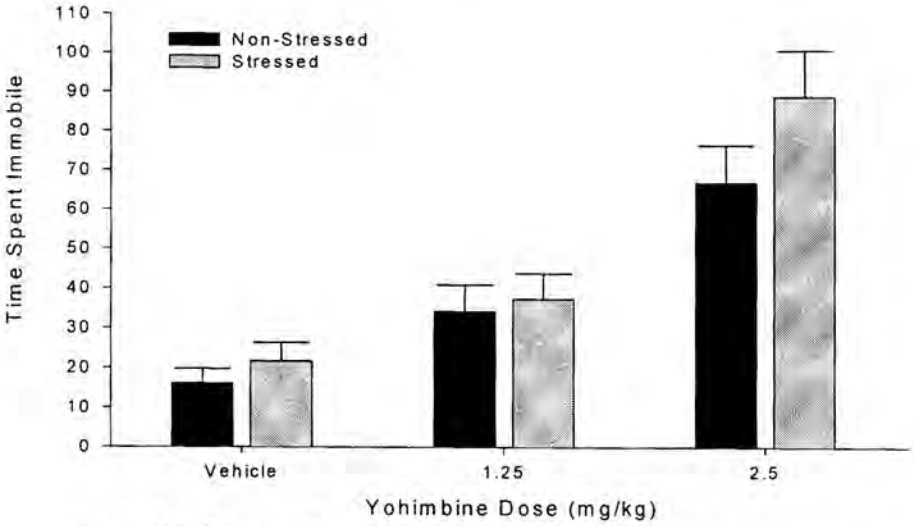


Figure II-9. Time spent immobile on the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

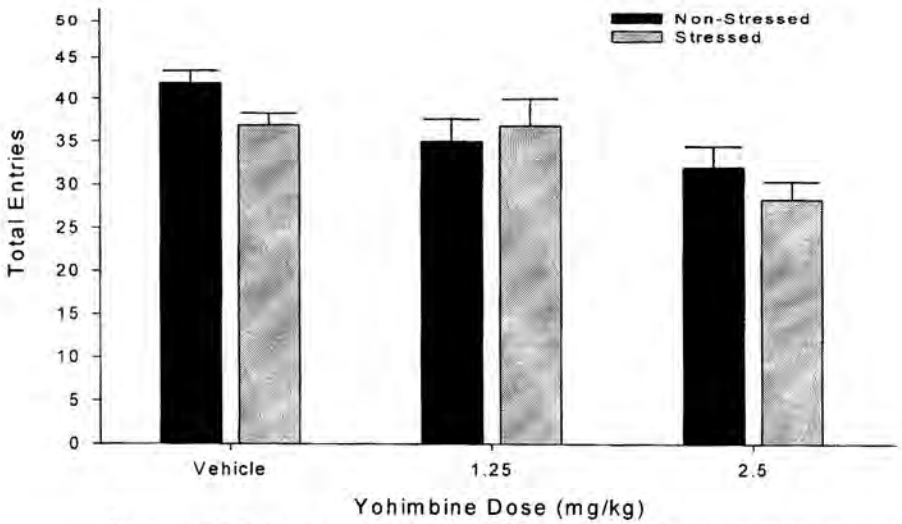


Figure II-10. Total entries into the sections of the elevated plus maze in stresse and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

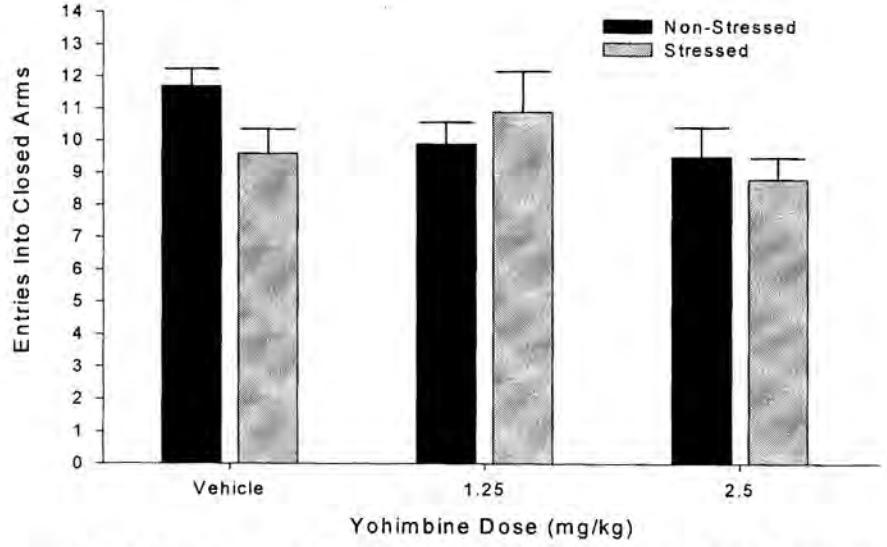


Figure II-11. Entries into the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

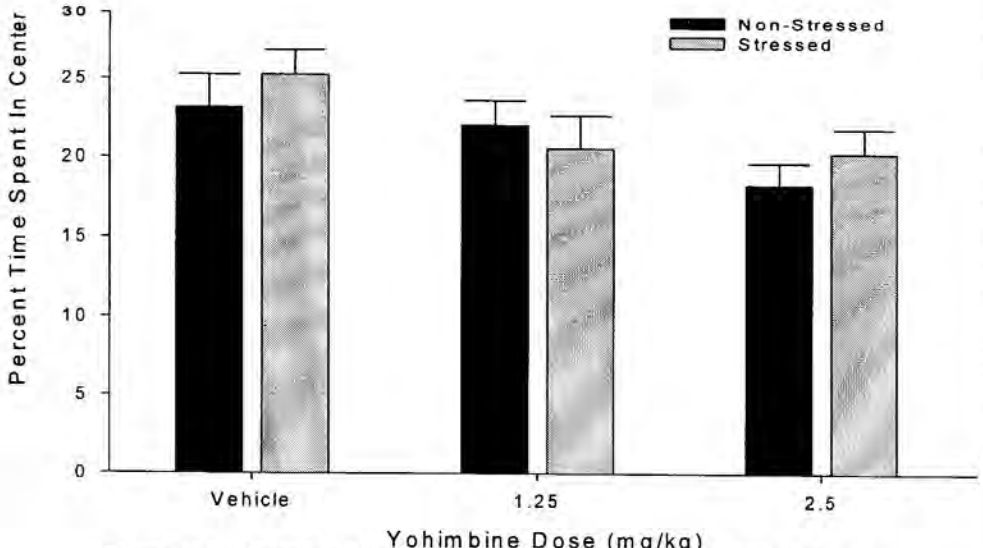


Figure II-12. Percent Time spent in the center of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

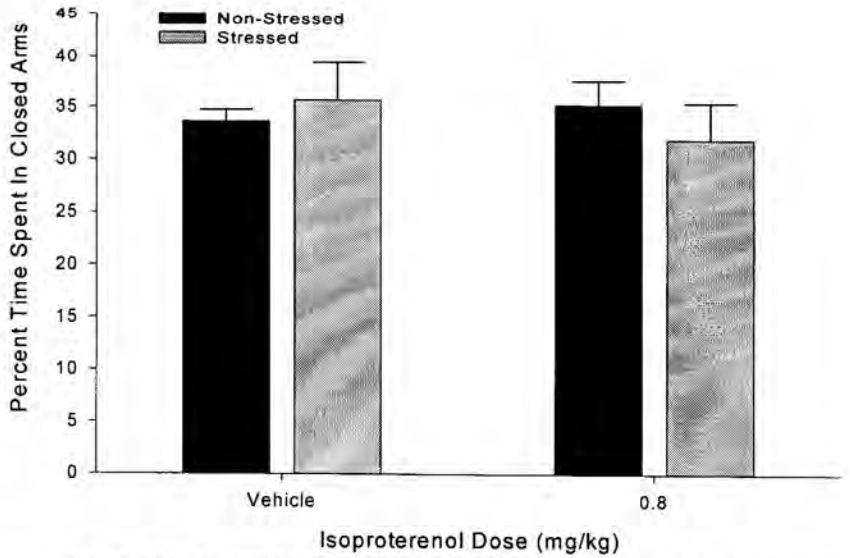


Figure II-14. Percent time spent in the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

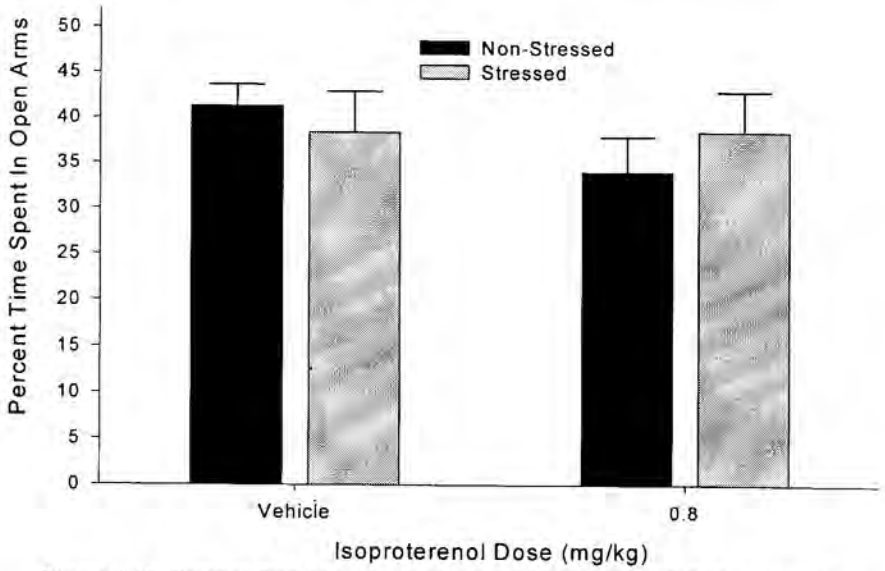


Figure II-13. Percent time spent in the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

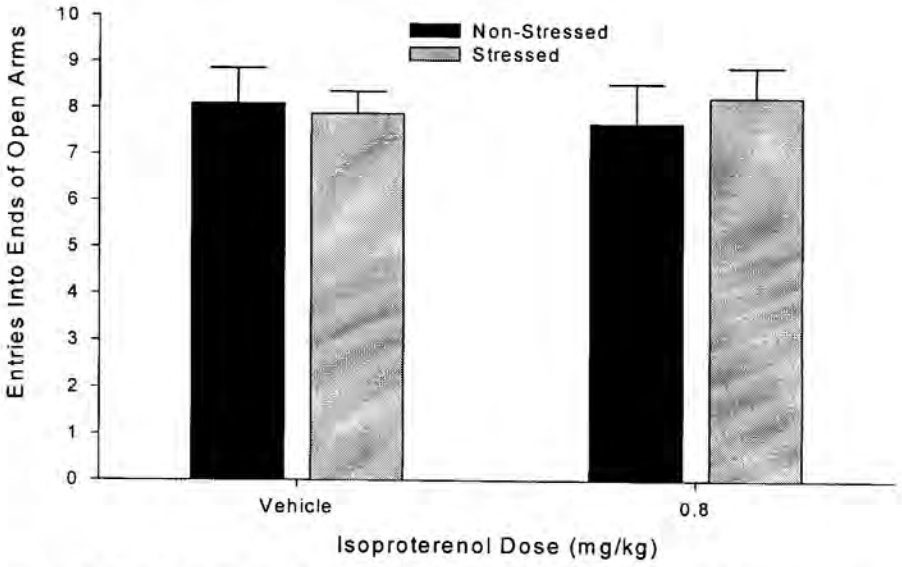


Figure II-15. Entries into the ends of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

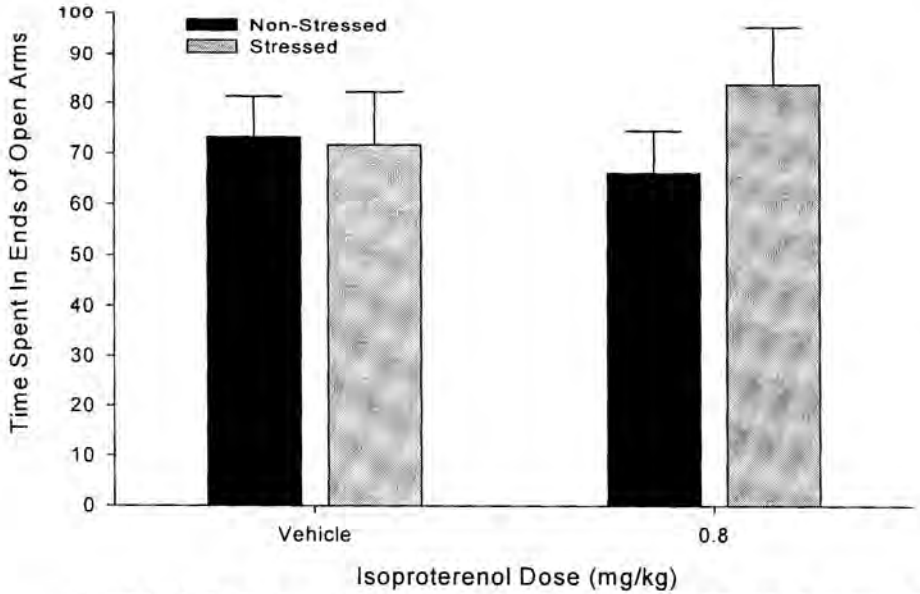


Figure II-16. Time spent in the ends of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

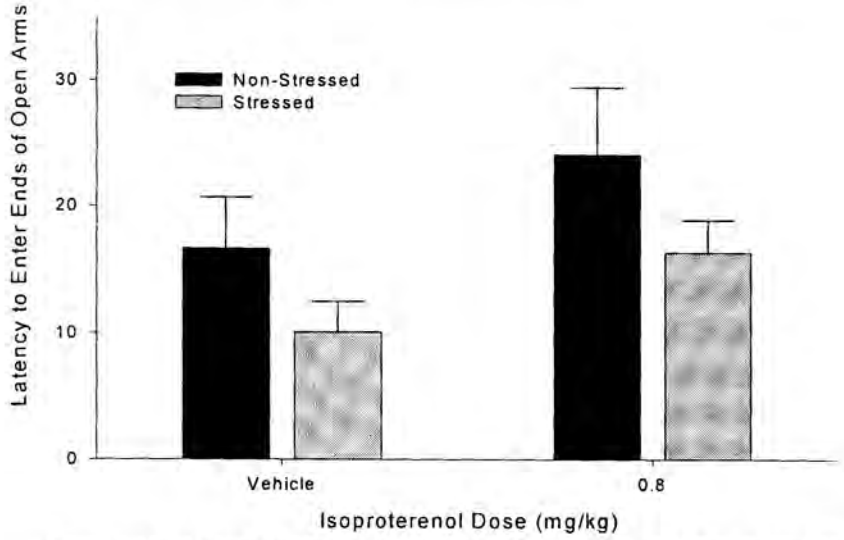


Figure II-17. Latency to enter the end sections of the open arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

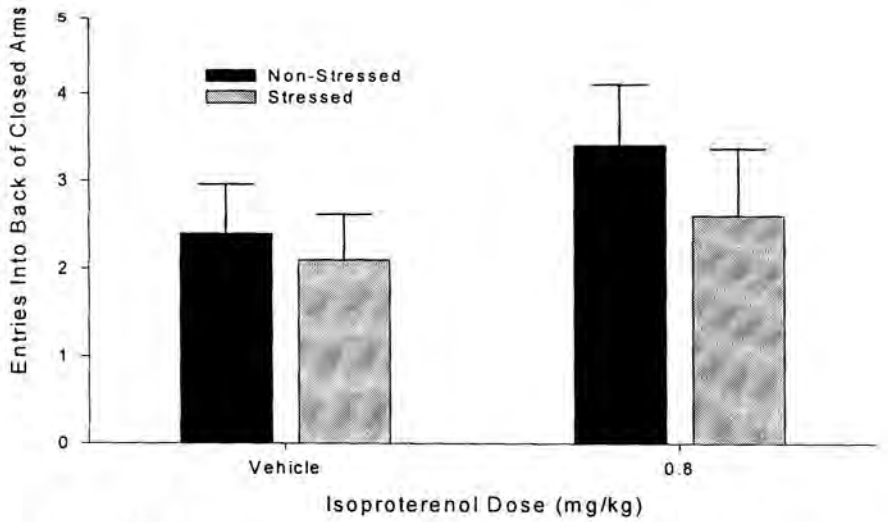


Figure II-18. Entires into the back section of the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

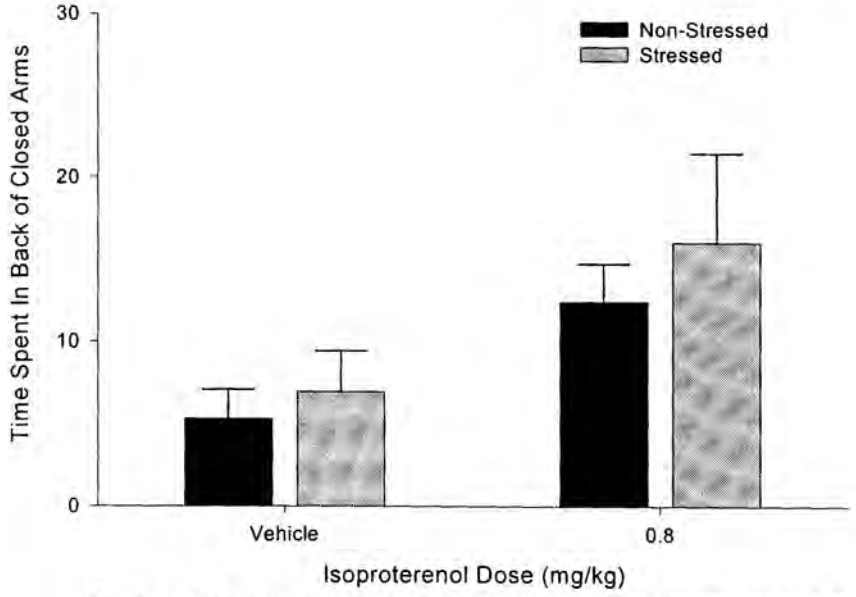


Figure II-19. Time spent in the back of the closed arms of the elevated plus maze stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

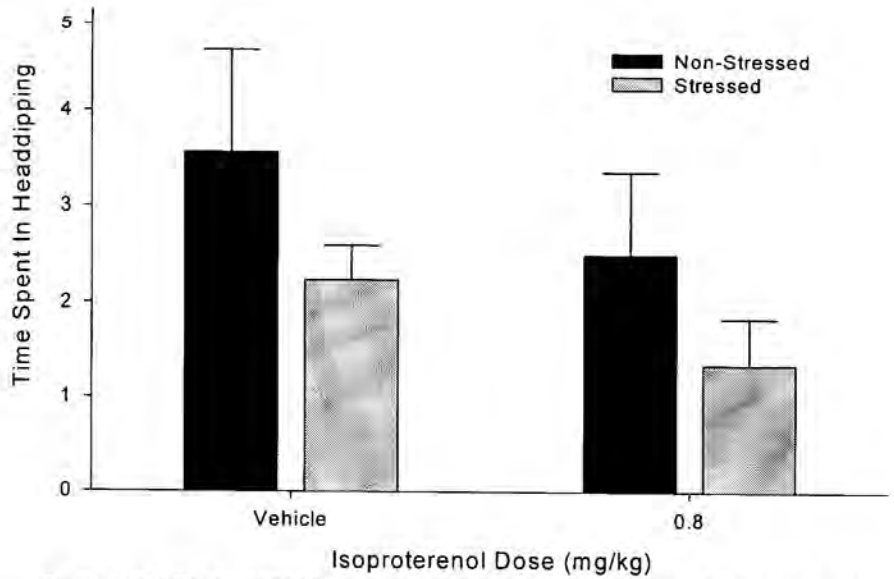


Figure II-20. Time spent headdipping in the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

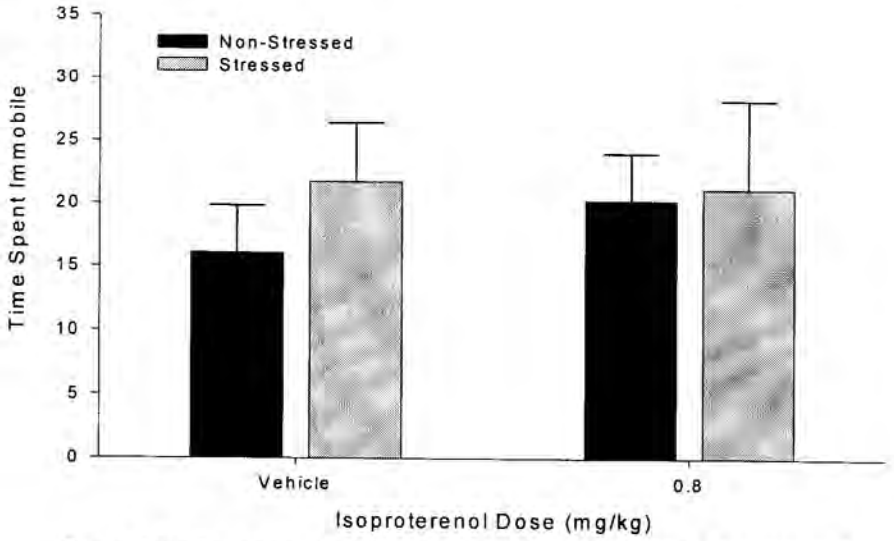


Figure II-21. Time spent immobile on the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

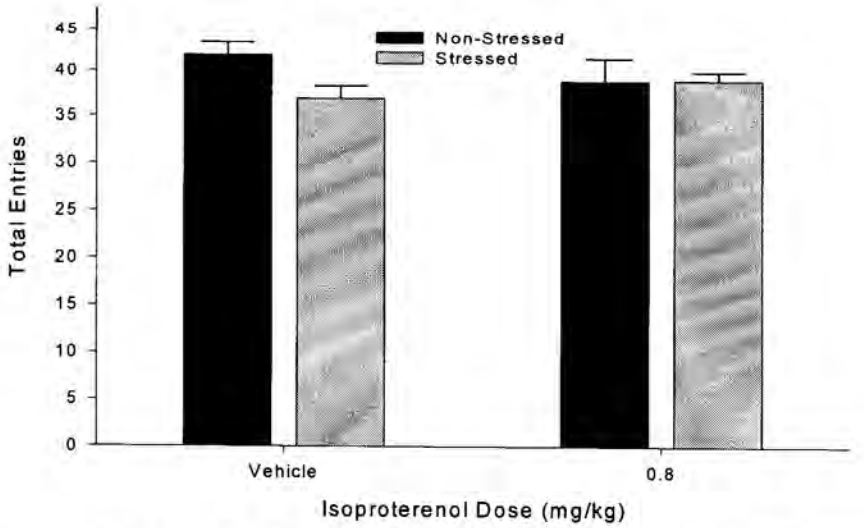


Figure II-22. Total entries into the sections of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

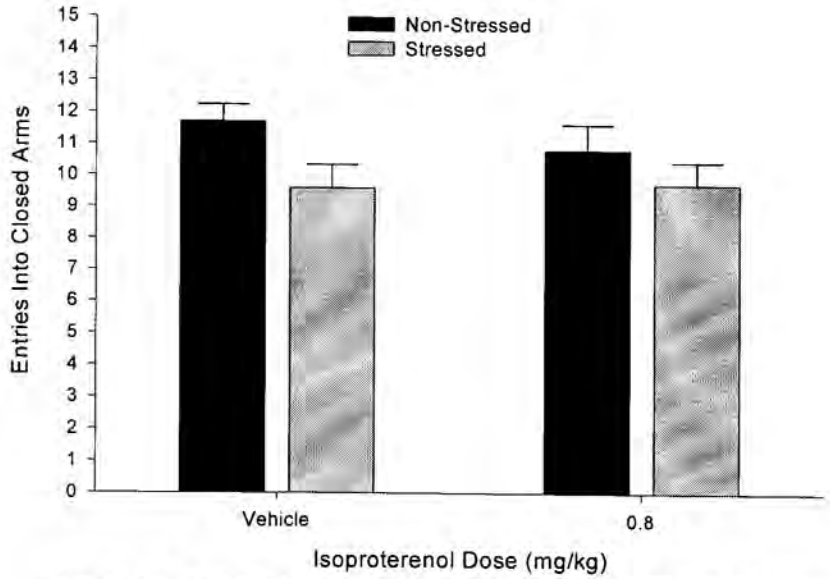


Figure II-23. Entries into the closed arms of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

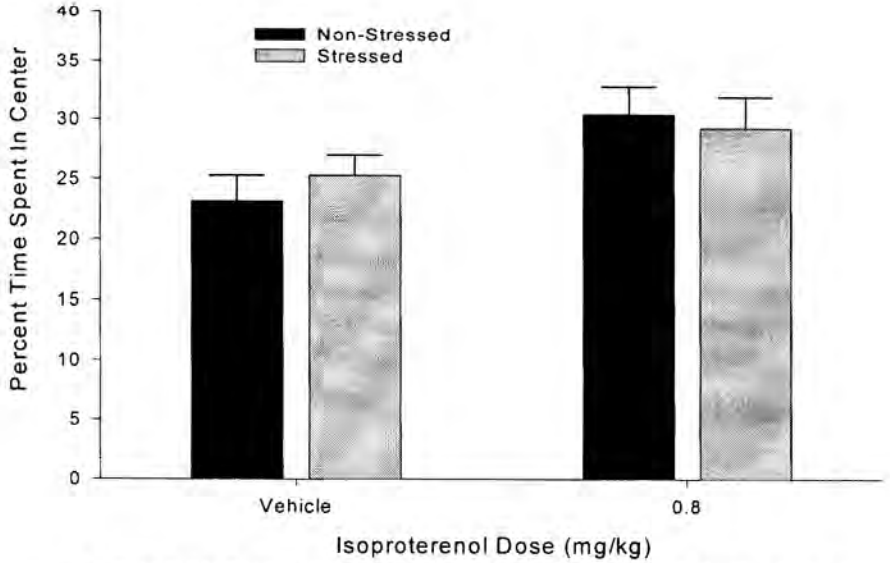


Figure II-24. Percent time spent in the center of the elevated plus maze in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

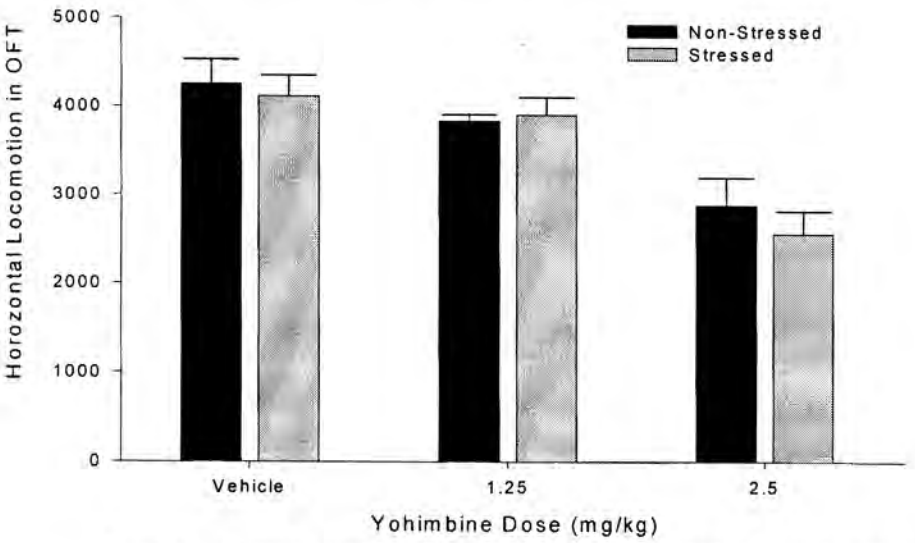


Figure II-25. Horizontal beam breaks (locomotion) in the open field test in stresse and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

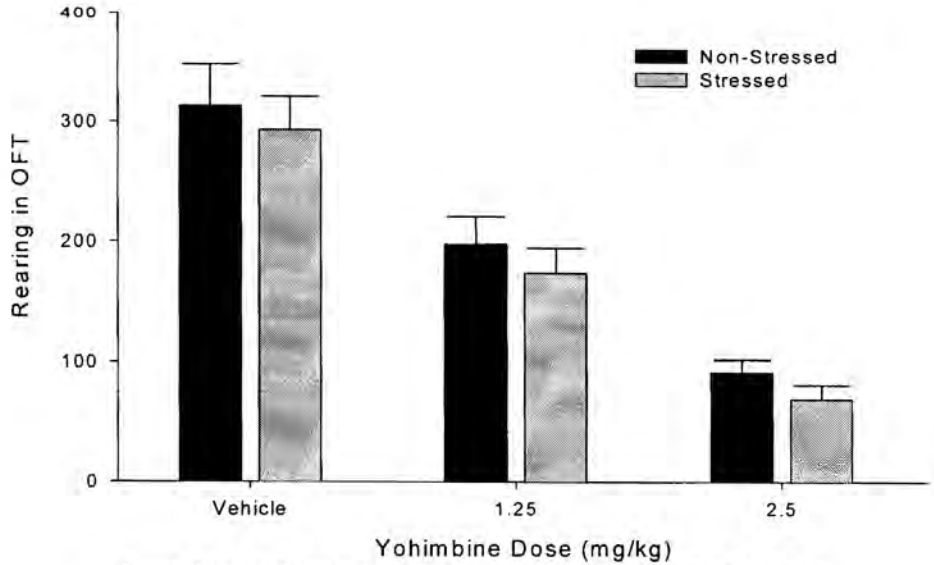


Figure II-26. Rearing (vertical beam breaks) in the open field test in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

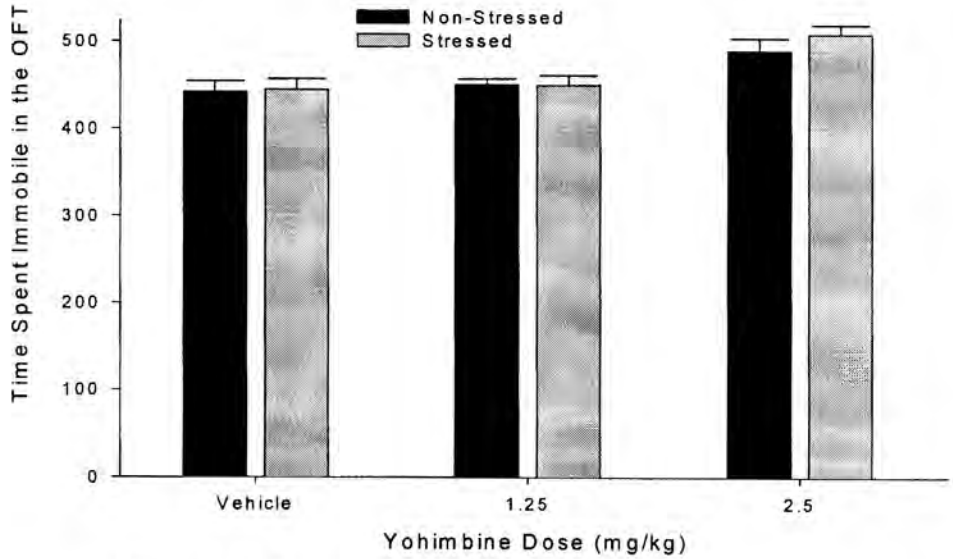


Figure II-27. Time spent immoble in the open field test in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

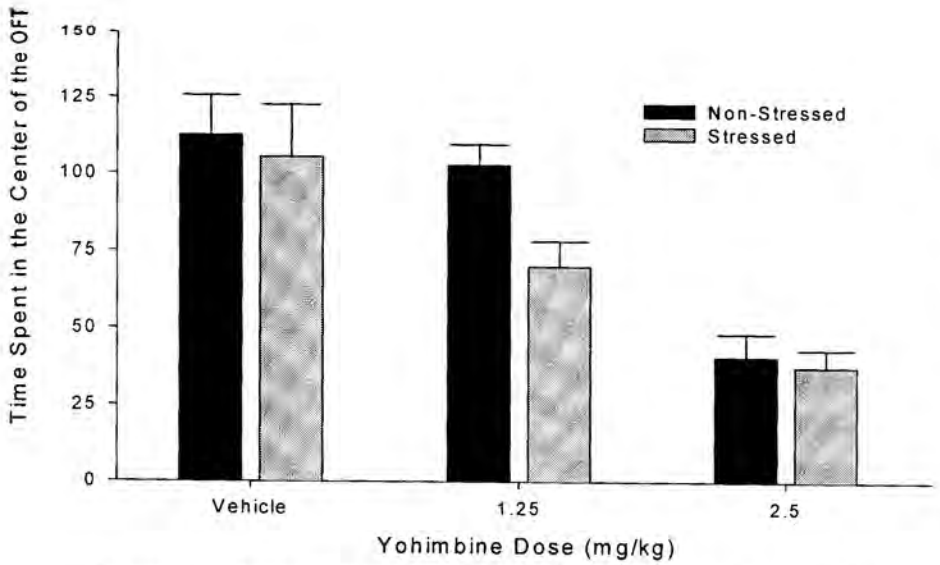


Figure II-28. Time spent in the center of the open field test in stressed and non-stressed rats following treatment with vehicle or yohimbine. All subjects pretreated with 10 mg/kg of indomethacin.

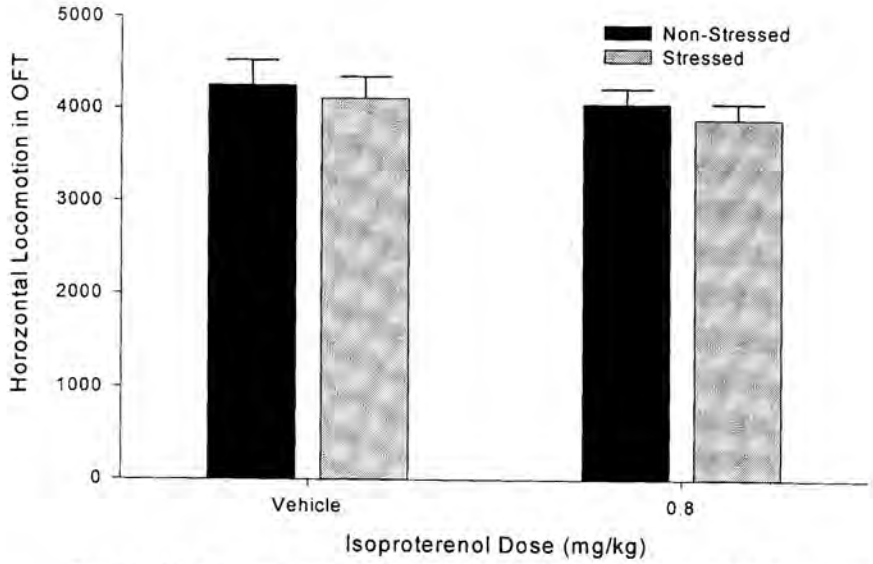


Figure II-29. Horizontal beam breaks (locomotion) in the open field test in stressed an non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

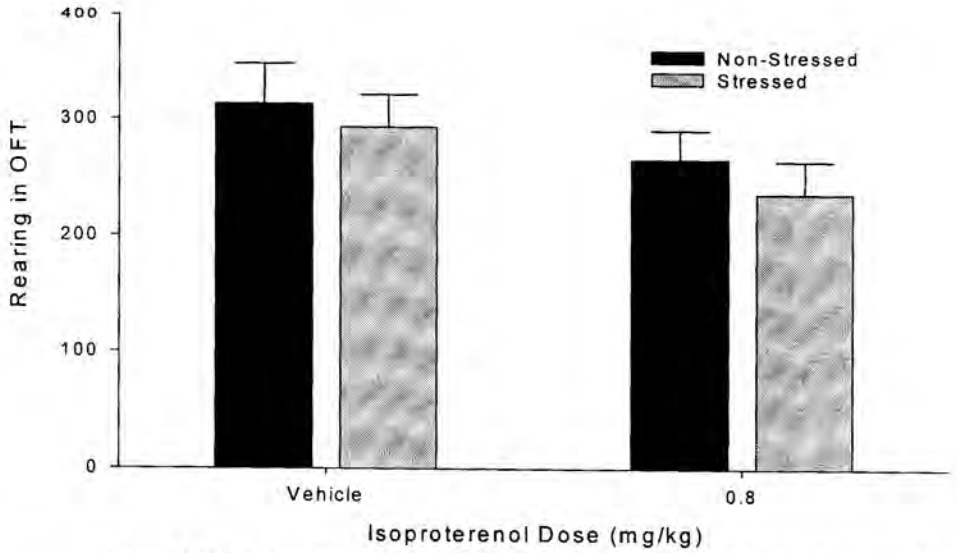


Figure II-30. Rearing time in the open field test in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

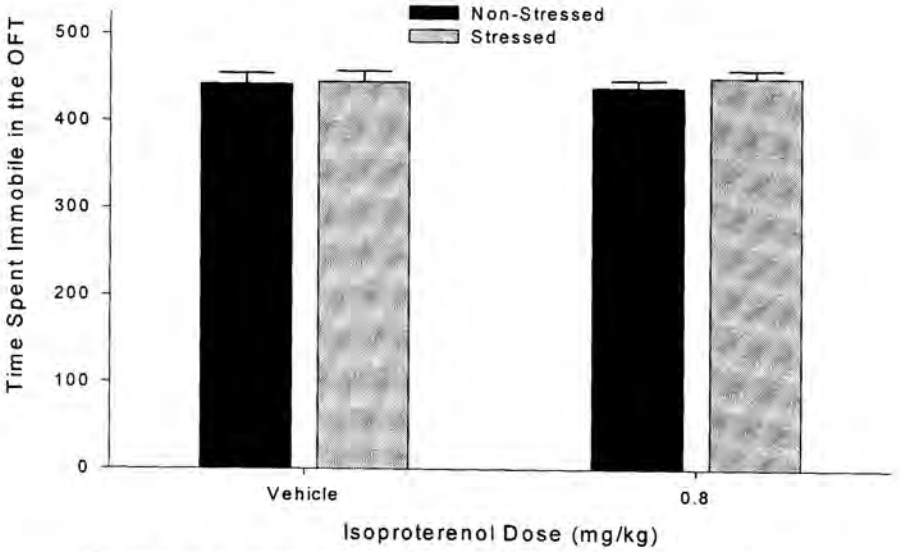


Figure II-31. Time spent immoble in the open field test in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

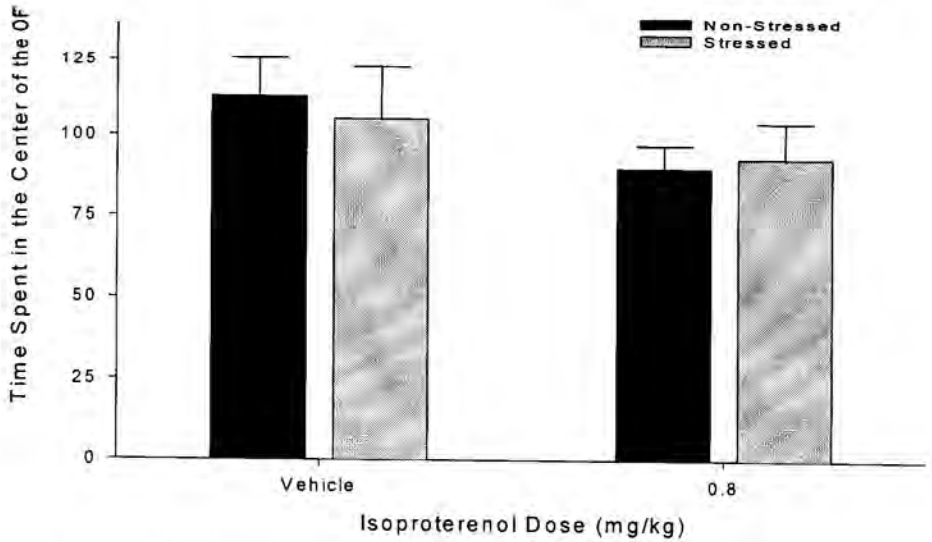


Figure II-32. Time spent in the center of the open field test in stressed and non-stressed rats following treatment with vehicle or isoproterenol. All subjects pretreated with 10 mg/kg of indomethacin.

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