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The Antidepressant Drug Tianeptine Blocks Working Memory Errors: Pharmacological and Endocrine Manipulations of Stress-Induced Amnesia in Rats

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by

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Dedication

My dissertation is dedicated to the memory of my grandmother Lillie Campbell and my grandfather Lloyd Van Winkle.

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ABSTRACT

Stress has been shown to influence learning and memory in humans and rats (Diamond et al, 1996; Diamond et al, 1999; Krugers et al, 1997; Kirschbaum et al, 1996; Lupien et al, 1997). The hippocampus and is an area of the brain involved in memory function in humans and rats (Kirschbaum et al, 1996; Lupien et al, 1997) and is highly susceptible to stress (Diamond et al, 1990). Research has indicated that a number of stressors such as exposure to a predator (Diamond et al, 1999) can lead to stress effects. Recently efforts have been made to counteract the effects of stress on brain function and related behavioral performance. The antidepressant drug tianeptine has been used in this setting. Little is known about tianeptine's role in blocking stress effects on behavior and memory performance with regard to interactions with stress hormones, such as corticosterone. Here a set of experiments delineates the role of corticosterone and its link to stress effects on memory as well as an investigation into the actions of tianeptine and ADX in the blockade of stress effects on memory. First, I examined the effects of tianeptine on multi-day RAWM working memory training and a novel one-day learning and memory training task. Second, the effects of propranolol, an anti-anxiety medication, were tested with regard to the alleviation of stress effects on memory, allowing for a comparison between two anti-anxiety drugs, tianeptine and propranolol. Third, adrenalectomy (ADX) and the resultant depletion of adrenal hormones were examined in connection with learning and memory in the one-day learning task. Fourth, the effects

and interactions of tianeptine and ADX were examined to see if tianeptine can exert its effects in the absence of adrenal hormones. Tianeptine blocked stress-induced memory errors in two different tasks and under ADX conditions. All effects were independent of corticosterone levels. In contrast, propranolol was ineffective in blocking stress-induced memory changes. The current data may prove useful in the development of antidepressant drugs and further the study of the mechanisms by which stress affects memory.

Chapter One

Introduction

Hippocampus, Neuronal Atrophy and Memory

Stress has been shown to influence learning and memory in humans and rats (Diamond et al, 1996; Diamond et al, 1999; Krugers et al, 1997; Kirschbaum et al, 1996; Lupien et al, 1997). The hippocampus is an area of the brain that is highly susceptible to stress (Diamond et al, 1990; Diamond et al, 1994; Diamond et al 1996; Joels et al, 2001; Kim et al, 1996). The hippocampus is involved with declarative memory in humans (Kirschbaum et al, 1996; Lupien et al, 1997) and spatial and working memory in rats (de Quervain et al, 1998; Diamond et al, 1996). Research has indicated that a number of stressors including restraint (Kuroda et al, 1998; Magarinos et al, 1995), exposure to a novel environment (Diamond et al, 1994) and exposure to a predator such as a cat (Diamond et al, 1999) can lead to behavioral and physiological stress effects.

Chronic stress or chronically elevated levels of stress hormones such as corticosterone in rats or cortisol in humans can lead to neural atrophy and cell death in the hippocampus (Luine et al, 1993; Arbel et al, 1994; Magarinos et al, 1995; Conrad et al, 1999; Uno et al, 1989). Luine et al (1993) found that 21 days of corticosterone treatment or 21 days of 6-hour restraint stress per day in rats caused atrophy of apical dendrites of pyramidal neurons in the CA3 region of the hippocampus. Furthermore, the atrophy was restricted to the apical dendrites of the CA3c pyramidal cell population in the hippocampus. No atrophy was present in other hippocampal sub-regions such as dentate gyrus, the CA1 and CA2 pyramidal cells or the basal dendritic tree of the CA3 pyramidal cell population. Although the atrophy was restricted to a specific sub-region of

hippocampal cells, Luine et al (1995) showed that chronic stress or corticosterone treatment and the subsequent dendritic atrophy was associated with impairment of initial learning of a hippocampal-dependent radial arm spatial learning task. Work by Magarinos et al (1995) again showed that 21 days of stress or corticosterone application resulted in apical dendritic atrophy of CA3c pyramidal cells in the hippocampus. Magarinos et al (1995) reported that rats treated with the steroid synthesis blocker cyanoketone showed an impaired secretion of corticosterone in response to stress while maintaining basal corticosterone levels. Also, cyanoketone treated rats exhibited no apical dendritic atrophy suggesting that the atrophy was, in part, caused by the actions of stress levels of corticosterone on the CA3c hippocampal excitatory amino acid (EAA) receptor sites. Two EAA receptors found plentifully in the hippocampus are the N-methyl-D-aspartate (NMDA) receptor and the AMPA receptor. Magarinos et al (1995) also showed that application of CGP 43487, a competitive NMDA receptor antagonist, blocked stress-induced dendritic atrophy, while the AMPA receptor antagonist NBQX did not block the atrophy. This suggests that the occurrence of dendritic atrophy is specific to an NMDA receptor mechanism in the CA3c region. Similar research with tree shrews has suggested that a chronic psychosocial subordination stressor, as opposed to a restraint stressor, causes CA3 dendrites to atrophy (Magarinos et al, 1996). Subsequent work indicates that dendritic atrophy induced by stress or corticosterone administration could be prevented by phenytoin (Dilantin), an anti-epileptic drug (Watanabe et al, 1992c). The fact that phenytoin is an anti-epileptic drug and, in effect, reduces the release of the excitatory amino acid (EAA) glutamate, suggests that the mechanism of the dendritic atrophy is based upon the release of excitatory amino acids, and the EAA's

actions on the corresponding receptor. In effect, the receptor, due to the increase in glutamate release becomes overexcited, a condition that can lead to neuronal atrophy or cell death. As stated above, the presence of large numbers of NMDA receptors makes the hippocampus extremely vulnerable to the effects of EAAs such as glutamate when they are released in increased quantities. In fact, in concert with the glutamate action theory is the idea that glutamate is released in the hippocampus during stress (Joels and DeKloet, 1993; Reineld et al, 1984), thus linking the stress release of glutamate to the EAA mechanism of dendritic atrophy. Other research has found that the antidepressant drug tianeptine blocks the effects of stress on hippocampal cell morphology (Watanabe et al, 1992b). Watanabe et al (1992b) found that 21 days of restraint stress or corticosterone treatment led to dendritic atrophy in the CA3c region of the hippocampus. Watanabe et al (1992b) also reported that daily treatment with the drug tianeptine (15 mg/kg) given concurrently with the chronic stressor prevented the apical dendrite atrophy. Give the evidence that tianeptine has effects on the serotonin system and the NMDA receptor system, Watanabe et al (1992b) and others (Kole et al, 2002) suggests that hippocampal atrophy processes may be influenced by serotonin as well as corticosterone or glutamatergic processes. Other recent research (Conrad et al, 1999) also found that tianeptine treatment (10 mg/kg) given during the three-week chronic restraint stress regimen prevented CA3 atrophy. Conrad et al (1999) also found that 10 days after the cessation of the stressor the dendritic atrophy reversed to pre-stress levels, indicating that the chronic stressor did not produce permanent cell damage. Conrad et al (1999) also reported that there was a preservation of hippocampal-dependent behaviors (freezing to

context) in both tianeptine injected and non-injected rats, suggesting that chronic restraint stress does not effect tasks specific to fear conditioning.

Stress, Corticosterone, and Synaptic Plasticity

Stress or corticosterone treatment has been found to inhibit the induction of long-term potentiation (LTP), a form of synaptic plasticity found among hippocampal cell groups (Foy et al, 1987; Shors et al, 1989, Kim et al, 2002). Long-term potentiation is an enhancement in synaptic efficacy following electrical stimulation of an afferent pathway and is considered a physiological model of memory formation. Foy et al (1987) stressed rats by giving them one tail shock a minute for thirty minutes. After the tail shocks were implemented, the rats' hippocampi were removed and the hippocampal sections were tested for the occurrence of long-term potentiation. Foy et al (1987) found that the stressful event given *in vivo* blocked the occurrence of long-term potentiation *in vitro*. Similar research by Shors et al (1989) also found that inescapable shock blocked hippocampal LTP. A group of rats was trained to escape low-intensity shock in a shuttle-box test, while another group of yoked controls could not escape but was exposed to the same amount and regime of shock. After 1 week of training, long-term potentiation (LTP) was measured *in vitro* in hippocampal slices. Exposure to uncontrollable shock massively impaired LTP relative to exposure to the same amount of controllable shock.

Extending the idea of stress blocking LTP, Diamond et al (1992) found that the magnitude of LTP was related to the stress hormone corticosterone in an inverted-U manner. That is, at low and high stress levels of corticosterone, LTP was reduced, and there was induction of LTP at an optimal moderate level of corticosterone. Diamond et al (1994) also found that when rats were exposed to an unfamiliar environment, primed-

burst potentiation (PBP), a physiologically relevant form of LTP, was inhibited in the hippocampus. In a more recent study (Mesches et al, 1999), PBP was blocked in hippocampal slices obtained from rats exposed to a cat. The study performed by Mesches et al (1999) evaluated the effects of acute psychological stress (cat exposure) in adult male rats on synaptic plasticity assessed *in vitro* in hippocampal slices. Two physiological models of memory were studied in CA1 in each recording session: first, primed burst potentiation (PBP), a low-threshold form of plasticity produced by a total of five physiologically patterned pulses; and second, long-term potentiation (LTP), a supra-threshold form of plasticity produced by a train of 100 pulses. Three groups of rats were studied: (1) undisturbed rats in their home cage (home cage); (2) rats placed in a chamber for 75 min (chamber); and (3) rats placed in a chamber for 75 min in close proximity to a cat (chamber/stress). At the end of the chamber exposure period, blood samples were obtained, and the hippocampus was prepared for *in vitro* recordings. Only the chamber/stress group had elevated stress levels of corticosterone. The major finding was that PBP, but not LTP, was blocked in the chamber/stress group. Thus, the psychological stress experienced by the rats in response to cat exposure resulted in an inhibition of hippocampal plasticity.

It may be deduced that since stress or high levels of corticosterone inhibit hippocampal synaptic plasticity that stress may also interfere with performance on behavioral tasks dependent on the hippocampus. In fact, stress does exert effects on behavioral measures of learning and memory, especially those testing hippocampal-dependent memory types such as spatial and working memory (de Quervain, et al, 1998; Diamond et al, 1996; Diamond et al, 1998; Diamond et al, 1999; Krugers et al, 1997;

Luine et al, 1994). Diamond et al (1996) showed that rats placed in a novel environment showed impairments on working memory function but not reference memory function. Reference memory function is hippocampal-independent and can be defined as the long-term memory of events that do not change from day to day, or trial to trial. The Diamond et al (1996) work suggests that stress can inhibit working memory (hippocampal-dependent) memory function while leaving reference memory (hippocampal-independent) memory intact. Furthermore, this finding suggests that stress impairs hippocampal functioning, as measured by behavioral testing while also impairing physiological measures of memory function, such as PBP and LTP.

Current Stress and Working Memory Research

More recent behavioral data also support the idea that stress impairs hippocampal-type memory. Diamond et al (1999) showed that rats exposed to a cat exhibited errors on a radial arm water maze (RAWM) task. Rats were trained to locate a hidden submerged platform in one of six arms in the RAWM. The rats were given 4 trials per day to learn where the submerged platform was located. The platform was in the same arm for every trial on that day while the platform location was different across days. Changing the platform location required the rat to learn a new location each day constituting the use of working memory. After the fourth trial, rats were stressed 30 minutes with the cat after learning the platform location for a particular day. After the 30 minutes, the rats were given a retention trial to evaluate if they had remembered the platform location. Rats that were stressed committed significantly more errors in the RAWM. That is, they entered arms not containing the platform more often than rats that were not stressed. As the

RAWM is a working memory task, it is implied that the stress interfered with hippocampal functioning.

More recently, work from Diamond's group has suggested that rats that have artificially elevated levels of corticosterone (CORT) are not impaired on working memory tasks (Park, et al, 2001). Rats were trained on a spatial working memory task (the radial arm water maze) and then were given a retention trial 30 minutes later. The object was to find the arm containing the hidden platform on that day in the retention trial. As before, rats that were not exposed to the cat had good memory and rats that were exposed to the cat exhibited memory impairment, and elevated levels of endogenous CORT. However, rats that were injected with stress levels of CORT, but were not placed with the cat, did not show memory impairment. Since these rats were not stressed, this would suggest that elevated levels of CORT, alone, are not sufficient to cause spatial working memory errors. In related work, Woodson et al (2003) found that rats that were stressed with a cat and those that were given access to an estrous female both exhibited high levels of CORT. But whereas the cat exposed rats showed an increase in errors the rats given access to the female did not show an increase in error rate. The findings of Woodson et al (2003) are consistent with the Park et al (2001) findings because certain groups of rats in both studies exhibited high levels of CORT while not showing spatial memory impairments. These data again suggest that high levels of CORT alone are not sufficient to produce memory impairment. Fear provoking stimuli such as the cat, which are known to activate the amygdala, may interact with corticosterone to impair hippocampal-dependent memory. Based on these findings, the theory that stress effects

on learning and memory are dependent on high corticosterone levels needs to be examined further.

Tianeptine, Serotonin, NMDA and Memory

In recent years efforts have been made to counteract the effects of stress on brain function and related behavioral performance. A drug known as tianeptine 7-[(chloro-6,11-dihydro-5,5-dioxo-6-methyldibenzo[c,f][1,2] thiazepin-11-y1 amino] heptanoic acid, sodium salt, has been developed and used in this setting. Originally developed as an antidepressant medication (Labrid, 1992), tianeptine has been shown to have varied effects regarding the physiology, neurochemistry and behavioral measures associated with learning and memory. Neurochemically, tianeptine has been shown to reduce extracellular serotonin (5-HT) levels by increasing 5-HT reuptake in the rat brain and in rat and human platelets ex vivo (Mennini et al, 1987, Mocaer et al, 1988, de Simoni et al, 1992). Extensive research has since focused on the connection between the decrease in extracellular 5-HT and tianeptine's ability to block effects of stress on learning and memory (Conrad et al, 1996; Luine et al, 1994). The theory that tianeptine has its effects via a decrease in 5-HT comes from the suggestion that stress increases 5-HT levels in the brain (Matsuo et al, 1996; Yoshioka et al, 1995), and that 5-HT has been associated with blockage of primed-burst potentiation (Corradetti et al, 1992). Thus a threefold hypothesis linking stress, 5-HT, and memory can be formed. That is, stress increases 5-HT levels, 5-HT has been suggested to impair learning and memory functioning, and tianeptine, which lowers extracellular 5-HT levels, has been shown to block stress effects on memory and LTP (Shakesby et al, 2002).

Although a large amount of research has been conducted linking memory impairment to serotonin and alleviation of stress effects on memory by tianeptine, tianeptine has recently been shown to exhibit effects on the NMDA receptor (Kole et al, 2002). NMDA receptors became more excitatory when tianeptine was applied *in vitro*. Tianeptine increased excitatory post-synaptic currents (EPSCs) in the hippocampal slices, indicating that tianeptine application is effective in making the NMDA receptor site more efficacious. The Kole et al (2002) study suggests that besides having 5-HT effects, tianeptine may also have effects linked directly to NMDA activation and enhancement. The NMDA receptor plays an important role in the generation of LTP, and NMDA antagonism has been shown to cause disturbances in learning and memory performance (Castellano et al, 2001; Kawabe et al, 1998). This is an important note considering recent research (Shakesby et al, 2002) found that tianeptine blocked the stress induced inhibition of LTP. Thus, recent data theorizes that the NMDA receptor may play an important role in tianeptine's ability to block stress effects on synaptic plasticity and memory function.

The Beta-Adrenergic System and Propranolol

In contrast to the NMDA receptor role in memory function, a second mechanism is involved in the emotional modulation of learning and memory: the beta-adrenergic system. Enhanced memory associated with arousing emotional experiences involves activation of the beta-adrenergic system (Cahill et al, 1995; Cahill and McGaugh, 1996a, 1996b; McGaugh and Cahill, 1997; McGaugh, 2000). A large amount of research has suggested that the amygdala, and more specifically the basolateral amygdala (BLA), is central to the beta-adrenergic memory system (Hamann et al, 1999; Canli et al, 2000). The noradrenergic system, especially within the amygdala, is a central mechanism in the

modulation of memory consolidation. Current research indicates that the adrenal hormone epinephrine and the stress hormone corticosterone act in interactive ways to affect memory consolidation (McGaugh et al, 2000; Roozendaal, 2000).

Epinephrine is also involved in the modulation of memory processes (Gold et al, 2001; Gamaro et al, 1997). Gold et al (1975) first found that systemic injections of epinephrine after inhibitory avoidance training enhanced long-term retention of the task. Subsequent studies have shown that epinephrine activates beta-adrenoceptors on vagal afferents terminating in the nucleus of the solitary tract (NTS). Memory enhancement is also achieved by electrical stimulation of the ascending vagus nerve, similar to that seen with epinephrine injection (Clark et al, 1998). The NTS innervates the amygdala via noradrenergic projections. The amygdala innervates several forebrain structures including the hippocampus via the locus coeruleus (van Bockstaele et al, 1998). Inhibition of the NTS with lidocaine blocked the memory enhancing effects of epinephrine (Williams et al, 1993), while the beta-adrenergic agonist clenbuterol infused into the NTS induced memory enhancement (Williams et al, 2000). The NTS-amygdala-hippocampal pathway is thus seen as fundamental to the enhancement of memory consolidation.

Glucocorticoids (corticosterone in rats, cortisol in humans) also enhance long-term memory consolidation, similar to that seen with epinephrine (Roozendaal, 2000; deKloet et al, 1999). Blockade of corticosterone synthesis with the synthesis inhibitor metyrapone prevents enhancement of retention on the inhibitory avoidance (IA) task by epinephrine. The idea that metyrapone can block epinephrine's ability to enhancement retention on the IA task indicates that there is an adrenergic-glucocorticoid interaction influencing memory consolidation.

Previous research indicating epinephrine and glucocorticoids enhance memory consolidation used the inhibitory avoidance task (Liang et al, 1986, Roozendaal, 2000). Even though the IA task is stressful, an enhancing effect was seen upon hormone administration. The memory being enhanced was that of the stressful experience itself, which is the context in which the shock that was to be avoided was given. Subsequent epinephrine or glucocorticoid administration sought to strengthen the memory of the stressful experience. Further research has indicated that the beta-adrenergic antagonist propranolol impairs the enhancement of memory for emotional experiences in rats (Roozendaal et al, 1999). Memory consolidation of emotional experiences has also been shown to be impaired by propranolol in humans (Cahill et al, 1994; van Stegeren et al, 1998). That is, the memory of the stressful experience was not as strong after administration of the antagonist. In the present experiments predator (cat) exposure is the source of stress. In theory, application of epinephrine or glucocorticoids such as CORT would enhance the memory of the cat and inhibit the memory of events peripheral to the stressful experience; in the case of the current experiments the peripheral memory event would be the memory of the platform location (see Methods below). Based on the findings of previous research (Roozendaal et al, 1999), the beta-blocker propranolol can be given in an effort to reduce or eliminate the enhancement of the memory of the cat exposure. The reduction of the memory for the cat would be beneficial for the memory of the platform location. Thus, propranolol would be expected to preserve the platform memory by inhibiting the memory of the cat.

Adrenalectomy and Memory

When discussing the idea of stress it is important to note that stress has two separate components: the physical and the psychological. The current set of experiments has focused on both of these components. The exposure of the rat to the cat predator is both a psychological and physical stressor. The physical component of a stressful event was further investigated in the current experiment by examining the levels of the stress hormone corticosterone in the blood stream. The physical component of the stress reaction focuses, in part, on the activation of the endocrine system. Upon the introduction of a stressor the hypothalamic-pituitary-adrenal (HPA) axis is activated. A neurochemical cascade occurs within the HPA-axis eventually leading to the release of epinephrine, norepinephrine and corticosterone into the bloodstream by the adrenal glands. The chemical cascade begins with the release of corticotropin-releasing hormone (CRH) by the hypothalamus. The release of CRH then causes the pituitary gland to release adrenocorticotrophic hormone (ACTH), which in turn triggers the adrenal glands to release corticosterone.

The adrenal gland is considered the major gland in reacting to stress. The adrenal gland is made up of two interacting bodies; the adrenal medulla (central adrenal) and the adrenal cortex (outer adrenal). The adrenal medulla is controlled by the sympathetic division of the autonomic nervous system which is the division activated under conditions of sudden stress. Motor axons from the autonomic nervous system synapse upon specialized cells in the adrenal medulla called chromaffin cells. It is the chromaffin cells that are responsible for the release of epinephrine and norepinephrine into the bloodstream shortly after the stressful event has occurred. It is the release of epinephrine

and norepinephrine that leads to physical manifestations of stress like increased heart rate, increased blood pressure and metabolic changes to name a few. While the adrenal medulla is integral to the release of epinephrine and norepinephrine, the endocrine gland cells of the adrenal cortex are responsible for the release of the stress hormone corticosterone and the regulatory hormone aldosterone by the adrenal glands. Aldosterone is important for the regulation of sodium, potassium and chloride within the body. The increase of the adrenal neurochemicals and hormones under conditions of acute stress are highly adaptive and return to basal levels shortly after the event. For example, corticosterone levels in the blood have been shown to peak 20-30 minutes post-stress. Chronic release of these substances under conditions of chronic stress becomes maladaptive and injurious. The current experiments incorporated acute stress situations (exposure to the cat for thirty minutes) in all instances.

The removal of the adrenal glands eliminates the production of corticosterone, epinephrine, norepinephrine and aldosterone. A large body of research has focused on the effects of adrenalectomy (ADX), that is, the removal of the adrenal glands on behavior and the brain. One line of research has focused on ADX and its effects on hippocampal morphology. Granule cells within the dentate gyrus of the hippocampus have a high rate of turnover and new cells are continuously formed (Gage et al, 1998; Gould and Cameron, 1996; Gould and McEwen, 1993). Previous research found that adrenalectomized rats exhibited selective loss of granule cells in the dentate gyrus of the hippocampus (Sloviter et al, 1989; Conrad et al, 1993), which suggests that ADX accelerates both the neurogenesis and apoptosis of granule cells (Cameron and Gould, 1994; Gould et al, 1990; Hornsby et al, 1996; Hu et al, 1997; Jaarsma et al, 1992;

Sapolsky et al, 1991). In addition, the replenishing of granule cells occurs with a longer delay than under non-ADX conditions (Cameron and Gould, 1996). The accelerated granule cell turnover caused by ADX is prevented by corticosterone replacement at low doses (Reul and DeKloet, 1985). Degeneration of granule cells is evident as early as two to three days after ADX surgery (Gould et al, 1990; Jaarsma et al, 1992; Hu et al, 1997). Adrenal steroids such as corticosterone control the rate of cell turnover in the dentate gyrus and may also control behavioral measures associated with hippocampal activity such as spatial memory tasks. Vaher et al (1994) showed that rats that had been adrenalectomized exhibited neuronal degeneration and cell loss in the dentate gyrus as well as deficits in memory performance on a spatially oriented eight-arm radial maze. Vaher et al (1994) found that corticosterone levels were lower in ADX rats than in sham control animals, and that corticosterone levels were negatively correlated with maze performance. That is, the rats with lower corticosterone levels (the ADX rats) made a greater number of errors on the memory test. The idea that lower corticosterone levels are associated with a deficit in memory performance suggests that adrenal hormones play an important role in the physical maintenance of hippocampal cells, most notably dentate granule cells, and also an important role in the performance of hippocampal-dependent spatial memory tasks.

Adrenalectomy has also been found to have detrimental effects on measures of electrophysiology within the hippocampus (Stienstra et al, 1997; Stienstra et al, 2000; Joels et al, 2001). Stienstra et al (1997) found that three days after adrenalectomy orthodromic field responses, an electrophysiological measure of synaptic plasticity in the dentate gyrus were reduced in amplitude *in vitro*. While the adrenalectomized rats

showed a marked decrease in field responses, sham-operated controls exhibited normal synaptic plasticity. Stienstra et al (1997) also showed that adrenalectomized rats that were treated with corticosterone during the three days post-ADX did not exhibit a reduction in cell signal amplitude. Stienstra et al's (1997) results indicate that measures of hippocampal electrophysiology, in this case the dentate gyrus, are disrupted *in vitro* by adrenalectomy three days prior. Stienstra and Joels (2000) found that post-synaptic potentials in dentate gyrus cells were blocked by ADX, and that treatment with corticosterone *in vitro* increased the EPSP slopes 2.5-3 hours after treatment in ADX rats. These findings indicate that delayed corticosterone effects in vitro are sufficient to normalize synaptic transmission in the dentate gyrus of ADX rats, even in the presence of apoptotic cells three days after ADX. Thus, the impairment of synaptic transmission after ADX may not be due to cell loss but rather due to a reduction of adrenal steroids and its actions on the NMDA receptor.

The aforementioned research has indicated that corticosterone treatment prevents cell loss in the hippocampus due to ADX (Reul and DeKloet, 1985) and that corticosterone treatment *in vitro* reverses the deleterious effects of ADX on synaptic transmission (Stienstra and Joels, 2000). In the current experiment rats received a low dose of corticosterone replacement after ADX, thus preventing rapid cell loss within the hippocampus. The corticosterone manipulation after ADX allows for the study of the effects of the elimination of adrenal steroids on memory while keeping the hippocampus intact. Thus, the current experiment studied the effect of ADX, per se, on memory performance rather than ADX-induced hippocampal damage. While previous research has shown that ADX leads to spatial memory impairment, the current study hypothesized

that spatial memory will be unaffected by ADX due to our use of corticosterone replacement. Also, based on pilot data which showed that tianeptine was effective in blocking a stress effect in the presence of stress levels of corticosterone, we hypothesized that stress would still cause impairment in the presence of ADX.

Current Experiments

Although a large amount of research exists, little is known about tianeptine's role in blocking stress effects on behavior and memory performance with regard to interactions with stress hormones, such as corticosterone. What was needed was a set of experiments to delineate the role of corticosterone and its link to stress effects on memory as well as an investigation into the actions of tianeptine and ADX in the blockade of stress effects on learning and memory. What follows is an examination of the effects of stress on memory in general, and how tianeptine, propranolol and adrenalectomy interact with stress and memory. First, the current series examined the effects of tianeptine on standard multi-day RAWM working memory training. Also, tianeptine's effects on a novel one-day learning and memory training task were examined. Specifically, the following experiments were conducted to determine if tianeptine could block stress effects on memory in each of the two types of memory testing, bringing the level of prior experience in the maze for the rats into consideration. Second, the effect of propranolol was tested with regard to the alleviation of stress effects on memory, allowing for a direct comparison between tianeptine and propranolol in their memory modulating capacity. It was hypothesized that propranolol would block the enhanced memory of the cat stress experience and reduce the cat stress effects on the peripheral (non-stress) memory of the platform location. Third, adrenalectomy (ADX) and the resultant depletion of adrenal

hormones were examined in connection with learning and memory in the one-day learning task, allowing the further study of the role of corticosterone on learning and memory. Fourth, the effects and interactions of tianeptine and ADX were examined to see if tianeptine can exert its effects in the absence of adrenal hormones.

Chapter Two

Experiment One: Tianeptine blocks stress-induced memory errors on the criterion-based multi-day working memory task

Method

Rats and Handling

Fifty-six male Sprague-Dawley rats (175-200 grams) were given a two-week habituation period within the vivarium upon arrival. Rats were housed two to a cage in standard Plexiglas cages and given food and water *ad libitum*. All rats were handled on Days 10, 12 and 14 of the two-week habituation period. During the handling procedure rats were taken in their cages, four cages at a time, to the behavioral testing room and placed on a table along one wall. Rats were then gently handled one at a time for approximately 2 minutes each. On Day 14 of the habituation period rats were handled in addition to receiving numerical tail markings with a permanent marker. At the end of each handling session rats were taken back to the vivarium. All handling sessions were given in the morning, the same time as subsequent behavioral testing.

Rats were run in the radial six-arm water maze (RAWM) using a criterion-based working memory task (as previously described, Diamond et al, 1999). The RAWM was a black galvanized round tank (168 cm diameter, 56 cm height, 43 cm deep) filled with clear water (23º-24º C). The tank was divided into six arms radiating from a central area using 6 V-shaped stainless steel walls (54 cm height, 56 cm length). A plastic platform (12 cm diameter) was hidden from view 1 cm below the surface of the water at the end of one of the arms.

Rats were trained to find the submerged platform placed at the end of one of the six arms. Rats were given four acquisition trials per day in which they started in one of the non-goal arms. The platform remained in the same arm within each day, but the platform location was changed between days, giving the rats a new location to learn each day. After the four acquisition trials the rats were given a 30-minute period in the home cage. After the 30 minutes the rats were given a retention probe trial to the platform. All rats were run until they met the criterion of no more than one error on the retention trial over three consecutive training days. Once a rat met the criterion it was placed into one of four groups: Home Cage/Saline (n=15), Home Cage/Tianeptine (n=14), Stress/Saline (n=15), Stress/Tianeptine (n=12). After meeting the criterion and placed into a manipulation group, rats were run for two days post-criterion using the same within-day training regimen. During these two post-criterion days drug manipulations and stress manipulations were performed. After the second post-criterion day blood samples were taken within two minutes after the retention trial. Blood samples were centrifuged and the plasma collected. The plasma samples were then assayed for levels of corticosterone.

Drug

Tianeptine (10 mg/kg) or saline-vehicle (0.9 % NaCl in distilled water) was administered 30 minutes before the first acquisition trial on the two post-criterion days. All injections were given interperitoneally (IP).

Stress

During the 30-minute manipulation period on the two post-criterion days, rats were either 1) put back into the home cage for 30 minutes, or 2) placed in a small Plexiglas box and transported to a separate room containing a larger sound attenuating

chamber box (750 cm x 570 cm x 580 cm). The rats were placed in the large chamber containing the cat for 20 minutes and then placed into the home cage for 10 minutes. While in the chamber with the cat the rats were housed in the small Plexiglas boxes with small holes in the top, allowing them to see and smell the cat but not allowing the cat access to the rat. After the 30-minute period the rats were given the retention trial.

Statistical Procedures

A repeated-measures ANOVA was performed on the mean errors on all groups by trial for the two days post-criterion combined. A one-way ANOVA was performed on the means for all groups on the retention trial. A one-way ANOVA was also performed on the means for the corticosterone assays.

Results: Within Trials Analysis

Means and standard errors of the mean were obtained for all four groups (HC/ Vehicle, HC-TIA, Stress/Vehicle, Stress/TIA) by trial (T1-RT). A within trial test of linearity showed that there was a significant linear trend for all groups combined by trial ($F [1, 51] = 103.737$, $p < 0.0001$), indicating significant acquisition performance across the four learning trials (T1-T4) (see Figure One).

A repeated-measures ANOVA indicated that there was a significant TRIALS effect for all groups combined ($F [4, 204] = 43.932$, p 0.0001). All other within subject effects and interactions were not significant. The repeated measures ANOVA also found that there was a significant between subjects STRESS main effect among the groups ($F [1, 51) = 5.412$, $p < 0.024$). All other between subject effects and interactions by trial were not significant (see Figure One).

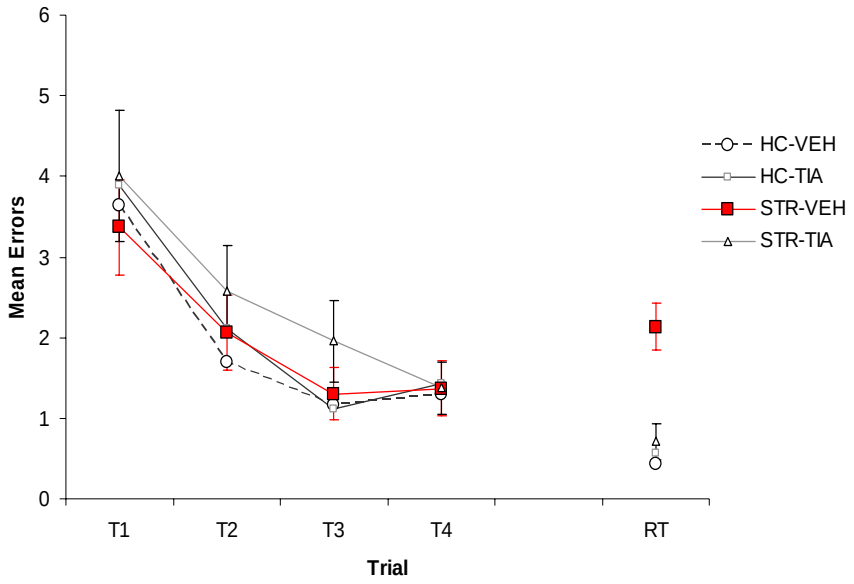

Figure One: Acquisition Curve and Retention Trial Performance for All Groups by Trial: Tianeptine and Criterion-Based Task.

Results: Retention Trial Analysis

Mean errors for the two-day post-criterion days combined are as follows: Home Cage/Vehicle = 0.433 ± 0.118, Home Cage/TIA = 0.578 ± 0.198, Stress/Vehicle = 2.133 ± 0.291, Stress/Tianeptine = 0.708 ± 0.224. Again, all variances are calculated as Standard Errors of the Mean (SEM).

There was a significant STRESS main effect ($F [1, 52] = 22.704$, $p < 0.0001$) with stress animals making significantly more errors than control animals overall. There was also a significant DRUG main effect ($F [1, 52] = 11.144$, $p < 0.002$) indicating that Vehicle groups made significantly more errors than the tianeptine groups overall. There was also a STRESS x DRUG interaction effect ($F [1, 52] = 16.440$, $p < .0001$) showing

that the Stress/Vehicle group made significantly more errors than the other groups. The Stress/Tianeptine group was significantly different from the Stress/Vehicle group, but not significantly different from the two control groups (see Figure Two).

These data indicate that stress significantly impaired spatial learning in the Stress/Vehicle group on the task compared to both home cage groups. Cat exposure led to an increase in errors on the retention trial in the Stress/Vehicle group. The data also indicate that tianeptine was effective in blocking the stress-induced memory deficit. That is, the mean error rate was significantly reduced in the Stress/Tianeptine group compared to the Stress/Vehicle group. Also, tianeptine injection did not increase error rates in the Home Cage/Tianeptine group, eliminating the idea that IP injection alone could increase stress effects on spatial memory.

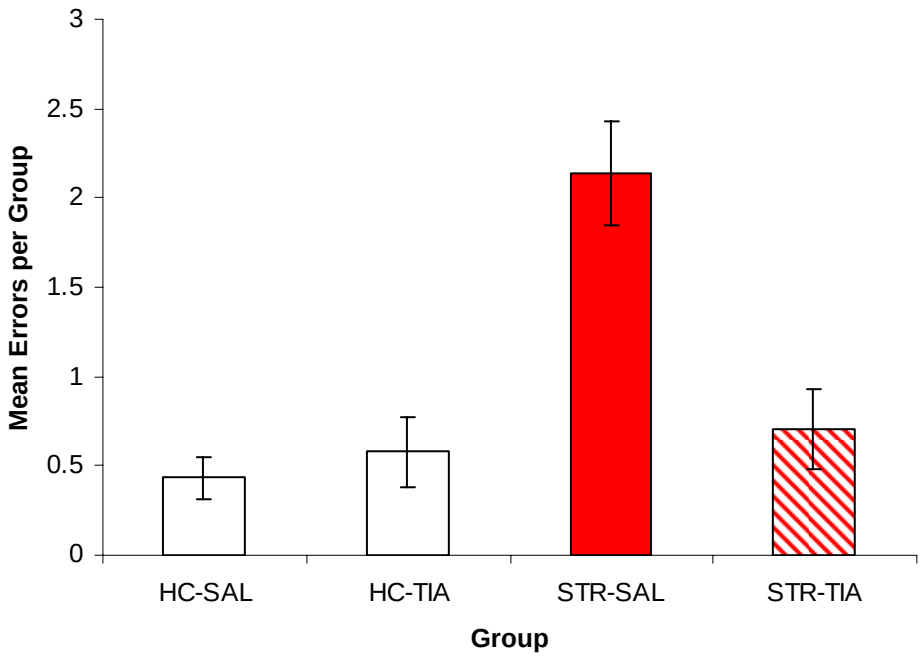

Figure Two: Mean Errors per Group on the Retention Trial: Tianeptine and Criterion-Based Task.

Results: Plasma CORT Analysis for RAWM Criterion Testing

The mean corticosterone values (µg/dl) obtained from plasma samples are as follows: Home Cage/Vehicle = 11.880 ± 1.351, Home Cage/TIA = 8.773 ± 1.591, Stress/Vehicle = 41.093 ± 3.044, Stress/TIA = 39.623 ± 2.868.

There was a significant STRESS main effect for corticosterone levels ($F [3, 25] = 47.928$, $p < .001$). (see Figure Three). All other effects were not significant. Data indicate that both stress groups had significantly higher CORT levels than those of the home cage groups independent of drug manipulation. Tianeptine alone did not raise CORT levels in the HC-TIA group nor did tianeptine lower CORT levels significantly in the STR-TIA group.

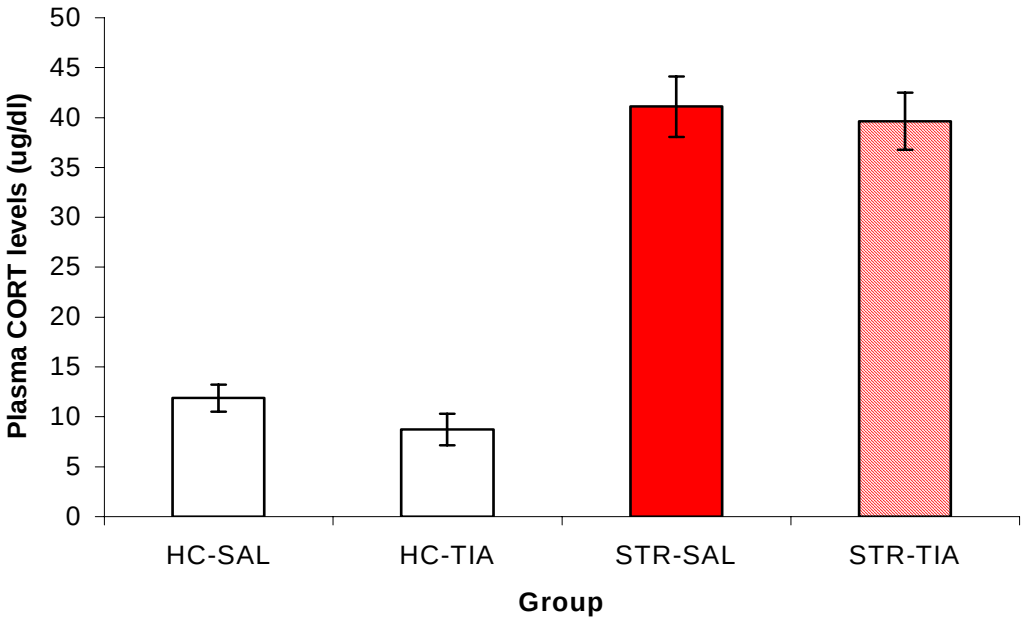

Figure Three: Corticosterone Levels per Group: Tianeptine and Criterion-Based Task.

Chapter Three

Experiment Two: Tianeptine does not block stress-induced memory errors when given *after* the stressor on the multi-day RAWM task

Method

Rats and Handling

Thirty-two male Sprague-Dawley Rats, (175-200 grams) were given a two-week habituation period within the vivarium upon arrival. Rats were housed two to a cage in standard Plexiglas cages and given food and water *ad libitum*. All rats were handled on Days 10, 12 and 14 of the two-week habituation period. During the handling procedure rats were taken in their cages, four cages at a time, to the behavioral testing room and placed on a table along one wall. Rats were then gently handled one at a time for approximately 2 minutes each. On Day 14 of the habituation period rats were handled in addition to receiving numerical tail markings with a permanent marker. At the end of each handling session rats were taken back to the vivarium. All handling sessions were given in the morning, the same time as subsequent behavioral testing.

Rats were run in the same radial six-arm water maze (RAWM) as in Experiment One using the same criterion-based working memory task (as previously described, Diamond et al, 1999). The RAWM was a black galvanized round tank (168 cm diameter, 56 cm height, 43 cm deep) filled with clear water (23º-24º C). The tank was divided into six arms radiating from a central area by using 6 V-shaped stainless steel walls (54 cm height, 56 cm length) (Figure 1). A plastic platform (12 cm diameter) was hidden from view 1 cm below the surface of the water at the end of one of the arms.

Rats were trained to find the hidden platform placed at the end of one of the six arms. Rats were given four acquisition trials per day in which they started in one of the non-goal arms. The platform remained in the same arm within each day, but the platform location was changed between days, giving the rats a new location to learn each day. After the four acquisition trials the rats were given a 90-minute period in the home cage. After the 90 minutes the rats were given a retention probe trial to the platform. All rats were run until they met the criterion of no more than one retention error over three consecutive training days. Once a rat met the criterion it was placed into one of four groups: Home Cage/Vehicle (n=6), Home Cage/Tianeptine (n=6), Stress/Vehicle (n=7), Stress/Tianeptine (n=7). After meeting the criterion and placed into a manipulation group, rats were run for two days post-criterion to the platform. During these two post-criterion days drug manipulations and stress manipulations were performed.

Drug

Tianeptine (10 mg/kg) or saline vehicle (0.9 % NaCl in distilled water) was administered immediately after the rats were taken from the cat or 30 minutes after they were placed back into the home cage, depending on group. The rats were then placed in the home cage for sixty minutes to simulate the time course of the drug in Experiment One. After the sixty minutes in the home cage, the rats ran the retention trial.

Stress

During the 90-minute manipulation period on the two post-criterion days, rats were either put back into the home cage for 90 minutes or placed in a large box containing a cat for 30 minutes and then placed into the home cage for 60 minutes. While in the cat box the rats were housed in small Plexiglas boxes, allowing them to see and

smell the cat but not allowing the cat access to the rat. After the 90-minute period the rats were given the retention trial.

Statistical Procedures

A repeated measures ANOVA was performed to analyze the significant differences among all groups by trial. A one-way ANOVA was also performed on the mean errors on the retention trial for the two days post-criterion.

Results: Within Trials Analysis

Means and standard errors of the mean (SEM) were calculated for all groups by trial. A test of linearity among the groups by trials indicated that there was a significant linear trend across the trials ($F [1, 22) = 89.306$, $p < 0.0001$) (see Figure Four). The significant linear trend among the groups indicated that there was a significant acquisition of the task or reduction in errors across the learning trials (T1-T4). The repeated measures ANOVA revealed that there was a significant within-subject TRIALS effect ($F [4, 88] = 42.001$, $p < 0.0001$). There was also a significant TRIALS x STRESS interaction ($F [4, 88] = 4.462$, $p < 0.002$). The between-subjects analysis indicated that there was a significant STRESS effect among all groups combined across trials ($F [1,22] = 5.573$, $p < 0.028$). The DRUG main effect and the STRESS x DRUG interaction was non-significant (see Figure Four).

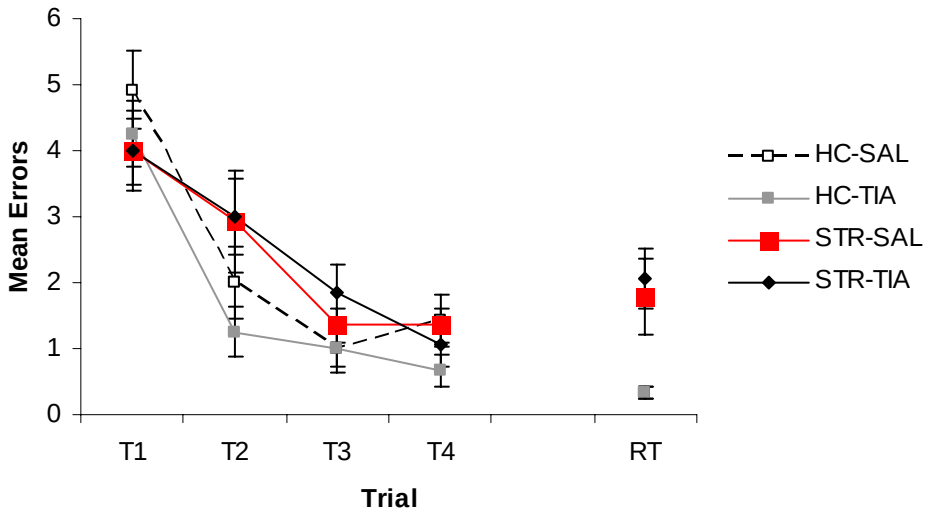

Figure Four: Acquisition Curves and Retention Performance for All Groups by Trials: Tianeptine Given After Stress.

Results: Retention Trial Analysis

The mean number of errors on the retention trial by group was as follows: Home Cage/Vehicle = 0.33 ± 0.105; Home Cage/TIA = 0.333 ± 0.105; Stress/Vehicle = 1.786 ± 0.565; Stress/TIA = 2.071 ± 0.456 (see Figure Five).

A one-way ANOVA was performed on the retention trial errors. The ANOVA indicated that there was a significant STRESS main effect ($F [1, 22] = 18.639$, $p < .0001$) between groups. The ANOVA also showed that there was no significant main effect of DRUG ($F [1, 22] = .279$, $p < .602$) and no significant STRESS x DRUG interaction ($F [1,22] = .279$, $P < .602$).

Stress impaired spatial learning as evidenced by an increase in errors compared to home cage groups. A significant stress effect was found in both the STR-SAL and STR-TIA groups compared to home cage groups even after a 60 minute delay between the

stress exposure and the retention trial. The increase in the delay period, extended to keep the time course of tianeptine consistent among experiments, did not hinder the stress exposure's ability to create a stress-induced memory deficit. Finally, data indicate that tianeptine given after the stressor did not significantly reduce the stress-induced memory impairment.

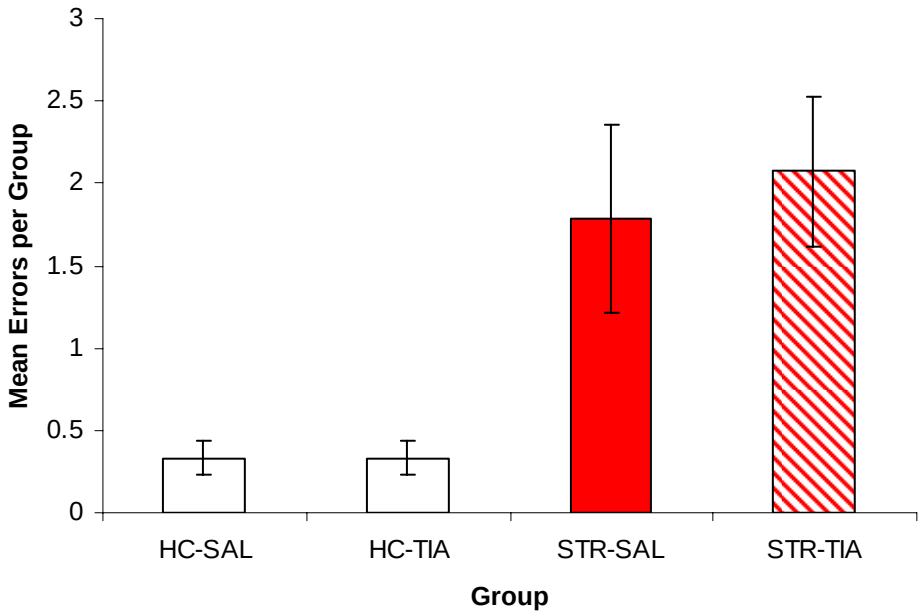

Figure Five: Mean Errors by Group for Retention Trial When Tianeptine is Given after Cat Exposure: Tianeptine Given After Stress.

Chapter Four

Experiment Three: Tianeptine reduces stress-induced memory errors on the novel One-Day Learning (ODL) task

Method

Rats and Handling

Thirty-two male Sprague-Dawley Rats, (175-200 grams) were given a two-week habituation period within the vivarium upon arrival. Rats were housed two to a cage in standard Plexiglas cages and given food and water *ad libitum*. All rats were handled on Days 10, 12 and 14 of the two-week habituation period. During the handling procedure rats were taken in their cages, four cages at a time, to the behavioral testing room and placed on a table along one wall. Rats were then gently handled one at a time for approximately 2 minutes each. On Day 14 of the habituation period rats were handled in addition to receiving numerical tail markings with a permanent marker. At the end of each handling session rats were taken back to the vivarium. All handling sessions were given in the morning, the same time as subsequent behavioral testing. The rats were assigned to the following groups after handling: Home Cage/Vehicle (n=8); Home Cage/Tianeptine (n=8); Stress/Vehicle (n=8); Stress/Tianeptine (n=8).

Rats were run in the radial six-arm water maze (RAWM) using a one-day learning task. The RAWM was a black galvanized round tank (168 cm diameter, 56 cm height, 43 cm deep) filled with clear water (23º-24º C). The tank was divided into six arms radiating from a central area by using 6 V-shaped stainless steel walls (54 cm height, 56 cm length). A plastic platform (12 cm diameter) was hidden from view 1 cm below the surface of the water at the end of one of the arms.

Each rat was run only one day, with each day containing an initial acquisition of twelve trials in succession. The twelve trial acquisition sequence was then followed by a thirty-minute manipulation period in which the rat was either returned to the home cage or given cat exposure. After the thirty-minute manipulation period the rats were given a single retention trial. After the retention trial tail blood was taken from each rat and frozen for future analysis of plasma corticosterone levels.

Drug

Drug and saline vehicle injections were given thirty minutes prior to Trial One. Both drug (Tianeptine, 10 mg/kg) and vehicle (0.9 % NaCl in distilled water) were administered interperitoneally.

Statistical Procedures

A repeated measures ANOVA was used to analyze the significance among all groups by blocks of two trials (6 blocks of 2 trials + RT = 13 total trials). A one-way ANOVA was performed on the mean errors per group on the retention trial (Trial 13). A one-way ANOVA was also performed on the mean results per group for plasma corticosterone levels.

Results: Within Trials Analysis

Means and standard errors of the mean (SEM) were calculated for all groups by block of trials. A test of linearity among the groups revealed that there was a significant linear trend among all groups ($F [1, 28] = 58.474$, $p < 0.0001$) (see Figure Six). The test for linearity also showed a significant BLOCKS x STRESS linear trend ($F [1, 28] = 10.208$, $p < 0.003$). A significant linear trend was also found for the BLOCKS x DRUG interaction ($F [1, 28] = 4.839$, $p < 0.036$.

The repeated measures ANOVA revealed that there was a significant BLOCKS effect ($F [6,168] = 20.636$, $p < 0.0001$). There was also a BLOCKS x STRESS interaction effect shown by the ANOVA ($F [6, 168] = 3.862$, $p < 0.001$). A significant BLOCKS x DRUG effect was also revealed ($F [6, 168] = 2.902$, $P < 0.01$). The repeated measures ANOVA also indicated a significant BLOCKS x STRESS x DRUG interaction ($F [6, 168] = 2.377$, $p < 0.031$). The between subjects component of the ANOVA showed a significant STRESS x DRUG interaction ($F [1, 28] = 12.501$, $p < 0.001$). The two main effects, STRESS and DRUG were not significant (see Figure Six).

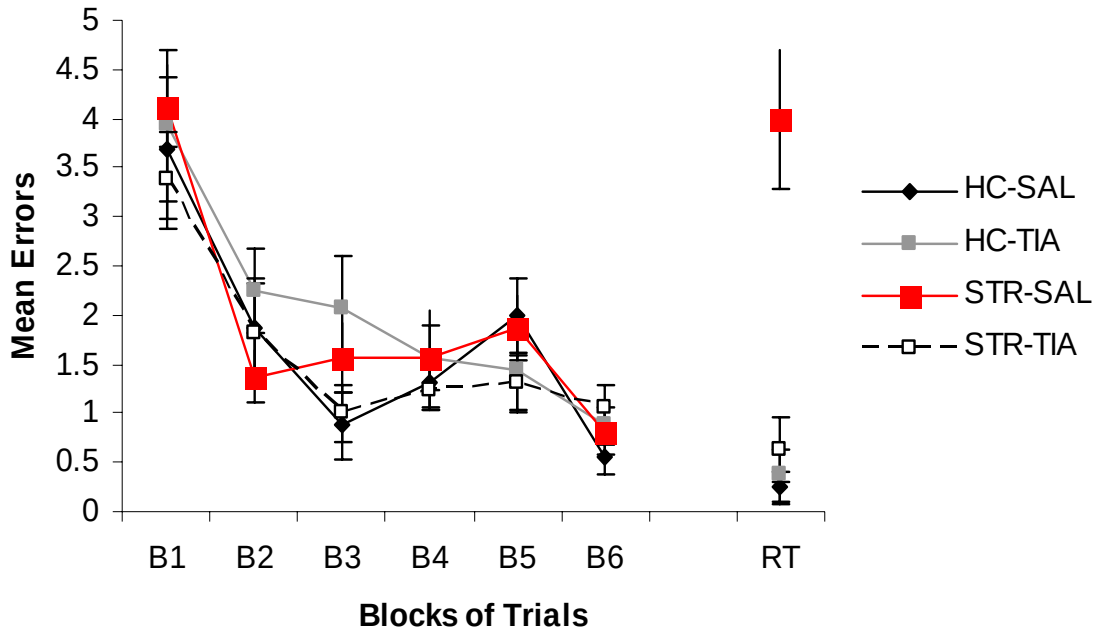

Figure Six: Acquisition Curves and Retention Performance for all Groups by Blocks of Trials: Tianeptine and One-Day Learning Task.

Results: Retention Trial Analysis

Means for each group were as follows: Home Cage/Vehicle = 0.25 ± 0.164; Home Cage/Tianeptine = 0.375 ± 0.263; Stress/Vehicle = 4.00 ± 0.707; Stress/Tianeptine = 0.625 ± 0.324 (see Figure Seven).

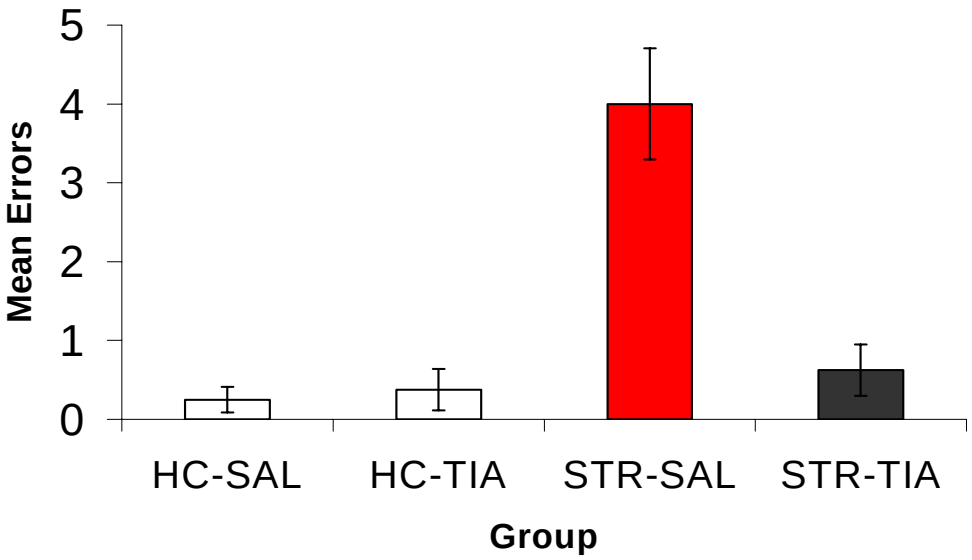

Figure Seven: Mean Errors per Group on Retention Trial: Tianeptine and One-Day Learning Task.

The ANOVA indicated that there was a significant STRESS effect ($F [1, 28] = 22.828$, $p < .0001$). The ANOVA also showed that there was a significant DRUG effect ($F [1, 28] = 15.070$, $p < 0.001$). Lastly, the ANOVA indicated that there was a significant STRESS x DRUG effect ($F [1, 28] = 17.478$, $p < .0001$).

The data show that there was a stress-induced memory impairment in the STR-SAL group, and this impairment was blocked by tianeptine treatment (STR-TIA). The Experiment Three data are analogous to the Experiment One data in that tianeptine was effective in blocking the memory deficit due to cat exposure.

Results: Plasma CORT Analysis for One-Day Learning Paradigm

Means were calculated and a one-way ANOVA was performed on plasma corticosterone levels for all groups. The means were as follows: Home Cage/Vehicle =

13.269 ± 1.16; Home Cage/TIA = 18.94 ± 2.70; Stress/Vehicle = 54.174 ± 3.20; Stress/TIA = 55.297 ± 3.27 (see Figure Eight).

The ANOVA revealed a significant STRESS effect ($F [1, 36] = 202.103$, $p < 0.0001$). The DRUG main effect and the STRESS x DRUG interaction were not significant ($p < 0.219$ and 0.408, respectively). As in Experiment One, Experiment Three CORT data indicate that CORT levels were significantly elevated in both stress groups compared to home cage groups. Again, tianeptine injection did not independently raise CORT levels nor did they reduce CORT levels in the STR-TIA group.

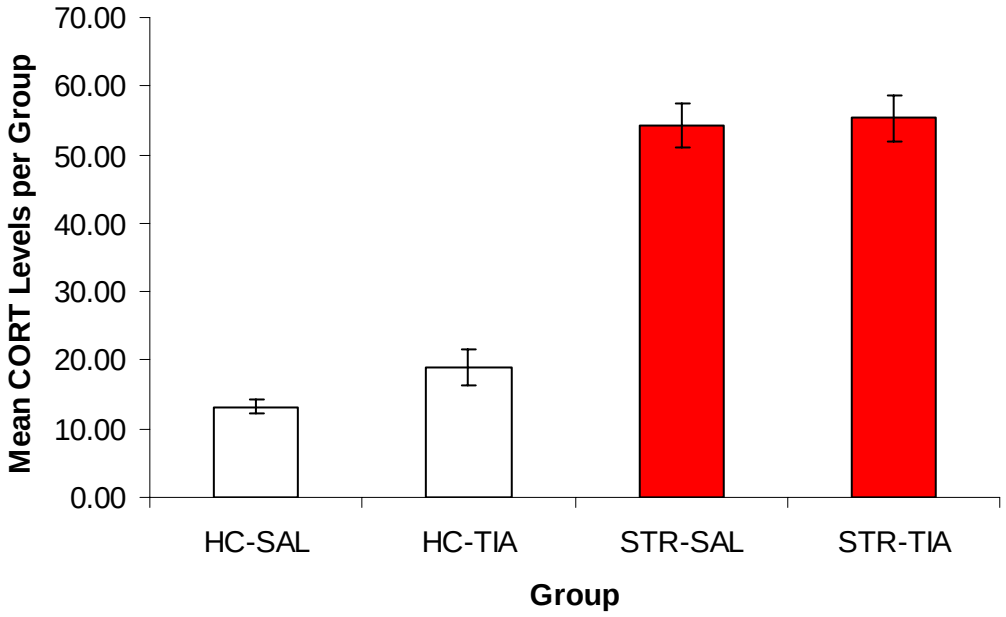

Figure Eight: Mean Plasma CORT Levels by Group for One-Day Learning Paradigm: Tianeptine and One-Day Learning Task.

Chapter Five

Experiment Four: Propranolol does not block stress-induced memory errors on the One-Day Learning Task

Method

Rats and Handling

Fifty-six male Sprague-Dawley Rats, (175-200 grams) were given a two-week habituation period within the vivarium upon arrival. Rats were housed two to a cage in standard Plexiglas cages and given food and water *ad libitum*. All rats were handled on Days 10, 12 and 14 of the two-week habituation period. During the handling procedure rats were taken in their cages, four cages at a time, to the behavioral testing room and placed on a table along one wall. Rats were then gently handled one at a time for approximately 2 minutes each. On Day 14 of the habituation period rats were handled in addition to receiving numerical tail markings with a permanent marker. At the end of each handling session rats were taken back to the vivarium. All handling sessions were given in the morning, the same time as subsequent behavioral testing. The rats were assigned to the following groups after handling: Home Cage/Vehicle (n=8); Home Cage/5 mg PROP (n=8); Stress/Vehicle (n=8); Stress/5 mg PROP (n=8), Home Cage/10 mg PROP (n = 8); Stress/10 mg PROP (n = 8).

Rats were run in the radial six-arm water maze (RAWM) using the one-day learning working memory task (as previously described). To review, the RAWM was a black galvanized round tank (168 cm diameter, 56 cm height, 43 cm deep) filled with clear water (23º-24º C). The tank was divided into six arms radiating from a central area by using 6 V-shaped stainless steel walls (54 cm height, 56 cm length). A plastic platform

(12 cm diameter) was hidden from view 1 cm below the surface of the water at the end of one of the arms.

Each rat was run only one day, with each day containing an initial acquisition of twelve trials in succession. The twelve trial acquisition sequence was then followed by a thirty-minute manipulation period in which the rat was either returned to the home cage or given cat exposure. After the thirty-minute manipulation period the rats were given a single retention trial. After the retention trial tail blood was taken from each rat within two minutes of the retention trial and frozen for future analysis of plasma corticosterone levels.

Drug

Drug and vehicle injections were given thirty minutes prior to Trial One (T1). Both drug (Propranolol, 5mg/kg and 10 mg/kg) and vehicle (0.9 % NaCl in distilled water) were administered interperitoneally.

Statistical Procedures

A repeated measures ANOVA was performed on all groups by six blocks of two trials. An ANOVA was performed on the mean errors per group on the retention trial (Trial 13). As well, an ANOVA was also performed on the mean results per group for plasma corticosterone levels. Each of the ANOVA procedures was performed individually on the 5 and 10 mg/kg PROP groups. Independent t-tests were then performed to compare the means across the two STRESS, and STRESS x DRUG groups for each dose, to preserve statistical power.

Results: Within Subject Analysis: 5 mg/kg Propranolol Dose

Means and standard errors of the mean were calculated for all groups by blocks of trials. The repeated measures ANOVA showed that there was a significant BLOCKS effect for all groups combined ($F [6, 168) = 6.623$, $p < 0.0001$). There was also a significant BLOCKS x STRESS interaction ($F [6, 168] = 5.373$, $p < 0.0001$). A test of linearity indicated that there was a significant linear trend among the groups on all acquisition blocks ($F [1, 28) = 11.765$, $p < 0.002$). The test of linearity also indicated a significant linear trend for the BLOCKS x STRESS interaction ($F [1, 28) = 4.235$, $p < 0.049$). The between subjects analysis indicated that there was a significant DRUG main effect among groups by blocks of trials ($F [1, 28] = 10.358$, $p < 0.003$) (see Figure Nine).

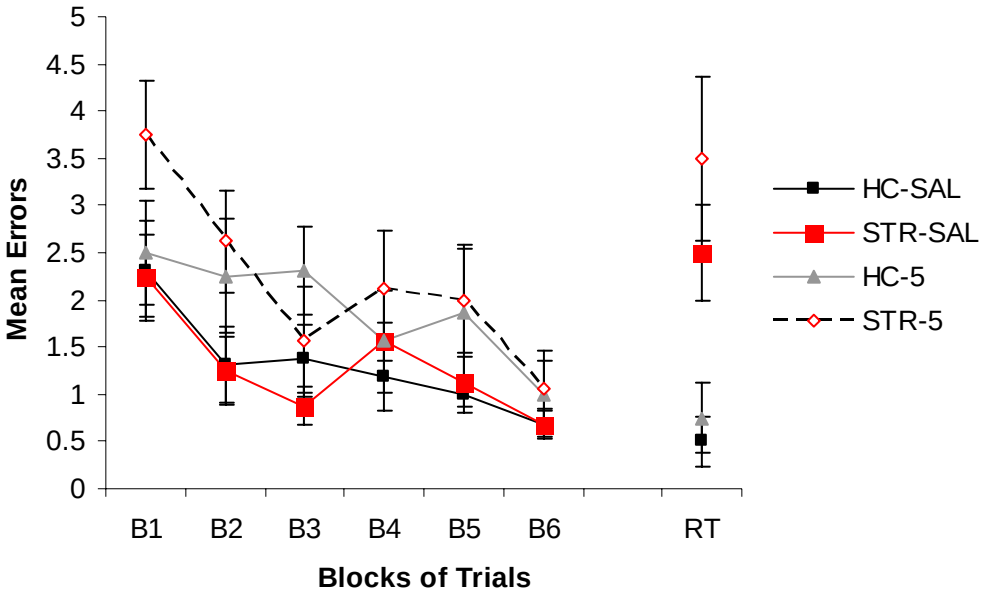

Figure Nine: Acquisition Curves and Retention Performance for all Groups by Blocks of Trials: 5 mg/kg Dose of Propranolol and One-Day Learning Task.

Results: Retention Trial Analysis: 5 mg/kg Propranolol Dose

The between subjects ANOVA performed on retention trial means indicated that there was a significant STRESS main effect ($F [1, 44] = 30.188$, $p < 0.0001$). The ANOVA revealed that both the DRUG and STRESS x DRUG interaction were not significant, suggesting the ineffectiveness of the 5 mg/kg dose of propranolol (PROP) on stress-induced memory changes (see Figure Ten). The data showed that rats given the 5 mg/kg dose of PROP and were placed in the home cage during the delay period did not exhibit more spatial memory errors on the retention trial. Also, the 5 mg/kg dose of PROP was not effective in blocking the stress effect on spatial memory retention performance.

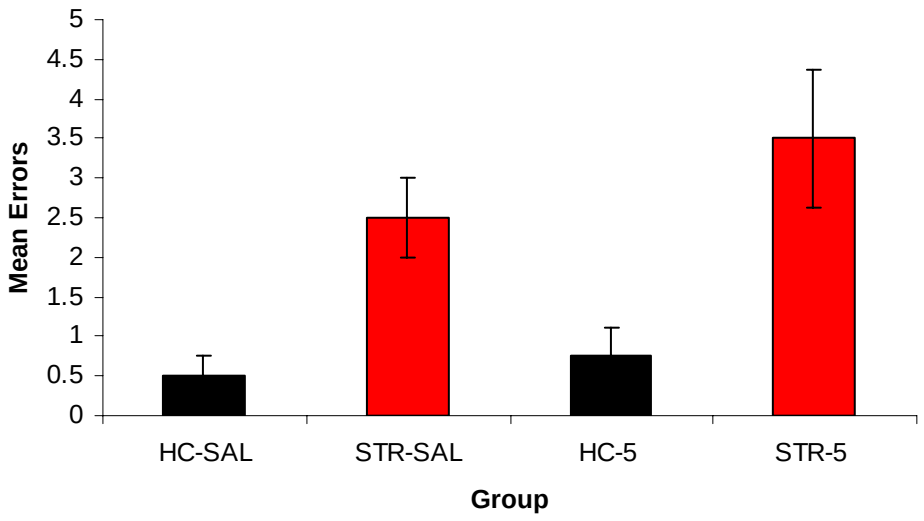

Figure Ten: Errors on Retention Trial for all Groups: 5 mg/kg Dose of Propranolol and One-Day Learning Task.

Results: Within Subject Analysis: 10 mg/kg Propranolol Dose

Means and SEMs for all groups by blocks of two trials were calculated. The repeated measure ANOVA showed that there was significant BLOCKS effect ($F [6, 168) = 6.817$, $p < 0.0001$). There was also a significant BLOCKS x STRESS interaction ($F [6, 168] = 5.317$, $p < 0.0001$). A test of linearity indicated that there was a significant linear trend for the BLOCKS effect ($F [1, 28] = 10.125$, $p < 0.004$). There was also a significant BLOCKS x STRESS linear trend ($F [1, 28] = 6.596$, $p < 0.016$). The between subjects analysis revealed that there was a significant STRESS main effect ($F [1, 28] = 10.248$, $p < 0.003$). There was also a significant DRUG effect ($F [1, 28] = 27.179$, $p < 0.0001$). The STRESS x DRUG interaction was not significant (see Figure Eleven). All groups exhibited a significant learning curve over the six blocks of two trials.

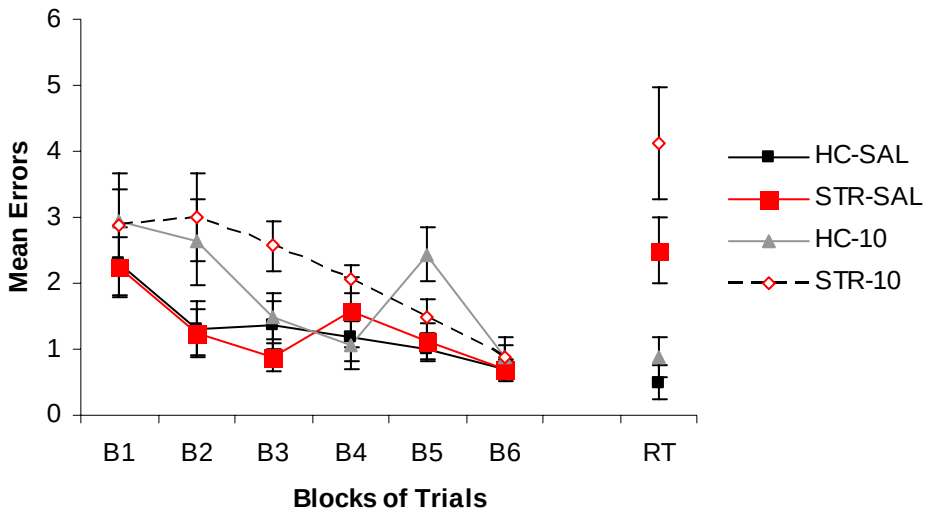

Figure Eleven: Acquisition Curves and Retention Performance for all Groups by Blocks of Trials: 10 mg/kg Dose of Propranolol and One-Day Learning.

Results: Retention Trial Analysis: 10 mg/kg Propranolol Dose

The between subjects ANOVA performed on the retention trial means indicated that there was a significant STRESS main effect ($F [1, 44] = 30.188$, $p < 0.0001$). The ANOVA showed that the DRUG and STRESS x DRUG interaction was not significant (see Figure Twelve). As with the 5 mg/kg dose of PROP, the 10 mg/kg dose of PROP did not significantly raise error rates in the HC-10 mg/kg PROP group. Also, the 10 mg/kg dose of PROP was ineffective in reducing retention errors in the STR-PROP group.

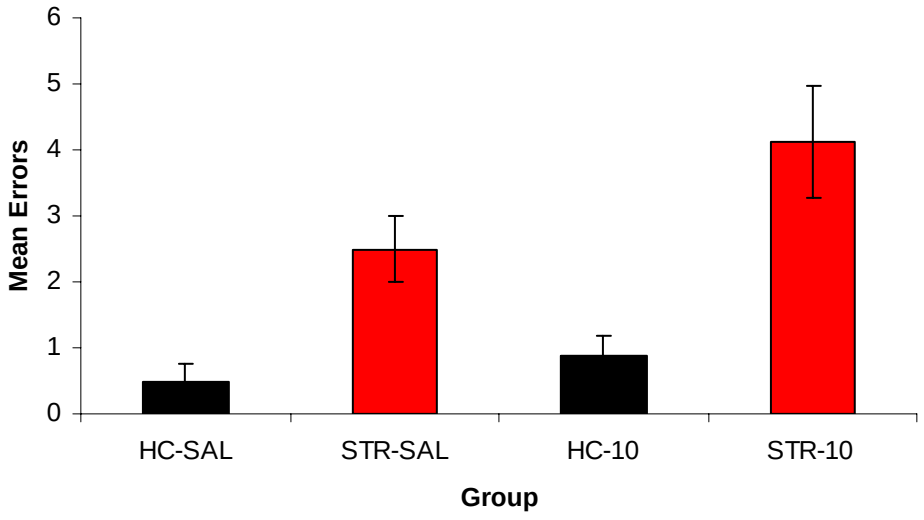

Figure Twelve: Retention Errors for all Groups: 10 mg/kg Dose of Propranolol and One-Day Learning Task.

Independent T-test Analysis Between 5 mg and 10 mg Propranolol Doses

An independent two-tailed t-test revealed that there was no significant difference between the STRESS-5 mg/kg PROP group and the STRESS-10 mg/kg group ($t = 0.615$). An independent t-test also showed that there was no difference between the HC-5 mg/kg PROP group and the HC-10 mg/kg PROP group ($t = 0.794$). The independent t-

test shows that there was no difference among the groups when comparing the groups across dosage.

Results: Plasma CORT Analysis for 5 mg/kg Propranolol Dose

Means were calculated and a one-way ANOVA was performed among groups. The following are the means and standard errors of the mean for all groups: Home Cage/Vehicle = 13.26 ± 1.16; Home Cage/PROP = 62.595 ± 4.041; Stress/Vehicle = 55.174 ± 3.27; Stress/PROP = 60.393 ± 3.036 (see Figure Thirteen).

The ANOVA showed that there was a significant STRESS main effect ($F [1, 32] = 43.123$, $p < 0.0001$). There was also a significant DRUG main effect ($F [1, 32] = 88.821$, $p < 0.0001$). The STRESS x DRUG interaction was also significant ($F [1, 32] = 53.494$, $p < 0.0001$). The 5 mg/kg dose of PROP significantly raise CORT levels in rats compared to the HC-SAL group. The 5 mg/kg dose of PROP, however, did not significantly lower high stress levels of CORT in the STR-PROP group.

Results: Plasma CORT Analysis for 10 mg/kg Propranolol Dose

Means were calculated and a one-way ANOVA performed on data for all groups. The following are the means for all groups: Home Cage/Saline = 13.26 ± 1.16; Home Cage/PROP = 60.951 ± 3.985; Stress/Saline = 55.174 ± 3.27; Stress/PROP = 84.095 ± 10.56 (see Figure Thirteen).

An ANOVA indicated that there was a significant STRESS main effect between groups ($F [1, 29] = 54.473$, $p < 0.0001$). There was also a significant DRUG main effect ($F [1, 29] = 79.966$, $p < 0.0001$). The STRESS x DRUG interaction was also significant ($F [1, 29] = 4.189$, $p < 0.05$). The 10 mg/kg dose of PROP significantly raise CORT

levels in rats compared to the HC-SAL group. The 10 mg/kg dose of PROP, however, did not significantly lower high stress levels of CORT in the STR-PROP group.

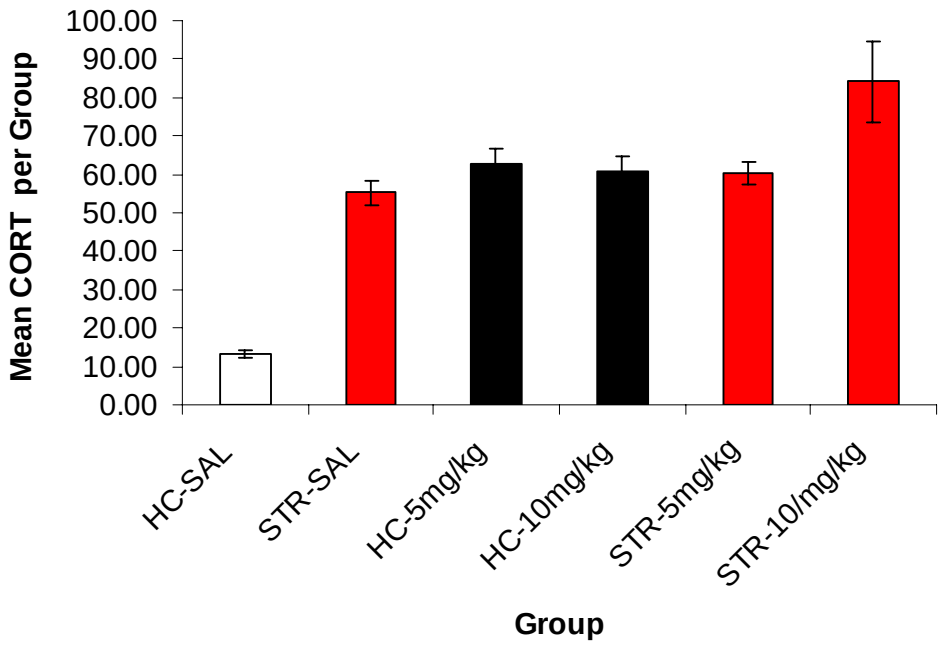

Figure Thirteen: Mean Plasma CORT Levels for All Groups: 5 mg/kg and 10 mg/kg Dose of Propranolol and One-Day Learning Task.

Results: Independent T-Test Between Stress Groups

An independent t-test was performed to analyze the difference between the Stress/5 mg/kg PROP and Stress/10 mg/kg group. The t-test was not significant (t = 0.02). This indicated that the 10 mg/kg dose did not lead to significantly more errors in the stress groups than did the 5 mg/kg dose.

In summary, Experiment Four found that propranolol was not effective in blocking stress-induced memory errors in the RAWM. All three stress groups, Stress/Saline, Stress-5 mg/kg of propranolol and Stress- 10 mg/kg of propranolol

exhibited statistically significant elevations in mean error rate compared to Home Cage groups. Also there was no statistically significant difference between the 5 mg/kg and the 10 mg/kg dosages of propranolol and its effects on memory. Both doses of propranolol did raise CORT levels in both Home Cage/Propranolol groups, but error rates in both groups remained at control levels.

Chapter Six

Experiment Five: Adrenalectomy, Stress and Memory: Effects of adrenal steroids on stress-induced memory changes

Method

Rats and Handling Procedure

Seventy-one rats (Harlan Laboratories) were used in this experiment. The rats per group were as follows: Sham/Vehicle (n = 12), ADX/Vehicle (n = 12), Sham/Stress (n = 9), ADX/Stress (n = 10), Sham/TIA (n = 6), ADX/TIA (n = 6), Sham/Stress/ TIA (n = 8), ADX/Stress/TIA (n = 8). Rats were approximately 250 grams upon arrival and had an approximate weight of 350 grams at the beginning of surgery and testing. All rats were housed two to a cage in standard Plexiglas rat cages with wire tops. Rats were given a habituation period of two weeks. During the two-week habituation period all rats were given water and standard rat chow *ad libitum*. Also during the two-week habituation period the rats received the handling regime as described earlier.

Adrenalectomy (ADX) Procedures

The day after the two-week habituation period had ended all rats were taken from the vivarium in their home cage by cart to the surgery room. Rats were given one hour to acclimate to the surgery room surroundings. During the one-hour pre-surgery period all rats were weighed in a standard electronic scale and placed back into the home cage. Separate cages were used on a far table to house the rats post-surgery.

At the beginning of the surgical procedure individual rats were placed upon the surgery table and administered the anesthetic halothane with an oxygen mixture via a nose cone. During the surgery the rats were given continuous maintenance applications of

the anesthesia through the nose cone. Upon proper anesthesia the rat was then shaven bilaterally posterior to the rig cage, an area directly above the visceral location of the adrenal gland/kidneys. After shaving the rat was swabbed with an antiseptic solution and placed on a heating pad on the surgery table. A one-inch incision was then made through the skin and muscle. After the incision the adrenal gland was carefully located and removed using a circle-tipped forceps and small surgical scissors. The rat was then swabbed internally and monitored for excess bleeding. After any possible bleeding had stopped the muscle and skin was sutured using Ethicon Coated vicryl 18-inch suture thread (muscle) and silk braided 18-inch suture thread (skin). After one side was complete the rat was then carefully turned and the procedure was repeated on the remaining side. All surgeries were done left side first on all rats for continuity. After the final suturing rats were treated with an antiseptic solution and placed in a holding cage for post-surgery evaluation. Respiration and heartbeat were monitored for all rats. Sham-control rats received all procedures consistent with adrenalectomy except for the removal of the glands. When rats were successfully recovered they were returned to their original home cage. After all rats were done they were returned to the vivarium and were closely monitored for regular home-cage activities such as eating, drinking and grooming.

Corticosterone and Sodium Replacement Procedures

Sham-control rats were given normal drinking water upon returning to the vivarium after surgery. ADX rats were given a special saline water solution to compensate for the loss of corticosterone (CORT) and aldosterone. Corticosterone (100 mg) was dissolved in 8 ml of ethyl alcohol (EtOH) and then added to four liters of saline

solution (20 g of NaCl per 4 liters of distilled water with CORT replacement) All ADX rats remained on the CORT replacement saline solution for the entirety of the study.

Behavioral Testing Regime

Rats were given seven days to recover from ADX surgery. After the seven day period rats were run using the One-Day Learning (ODL) radial arm water maze (RAWM) task. The exact procedures for the ODL task were stated previously. In short all rats were given twelve trials (six visible, six hidden/submerged) to find the escape platform. After the twelve acquisition trials were given the rats were either placed back in the home cage or with the cat for thirty minutes depending on group. After the thirty minutes rats were given a one-trial retention test to the previous platform location. The thirteen total trials for each individual rat were consistent, but the platform location was randomized among the rats so no two consecutive rats went to the same platform location.

Blood Sampling and Preparation

After the final retention trial the rat was quickly taken to an adjacent room for blood sampling. Each rat was placed in a wire restraint after each rats tail was soaked in warm water for thirty seconds to aid blood flow from the tail. After the tail soaking a 1-mm cut was made at the end of the tail. The tail was then gently massaged from base to tip to take the blood. Blood was collected into plastic centrifuge tubes and placed under a blanket out of the light and allowed to settle. After all blood samples were taken the blood was centrifuged and the resulting cleared plasma was pipetted into a new plastic tube. All blood samples were then placed into a deep freezer and frozen at –70 degrees centigrade and awaited radioimmunoassay (RIA) manipulation.

Statistical Procedures

Means and standards errors of the mean (SEM) were calculated for all groups on all individual trials. A univariate ANOVA was performed on blocks of two trials for the twelve acquisition trials (six blocks of two trials). An ANOVA was also performed on the means of the one-trial retention test (Trial 13). Means and SEMs were also calculated for serum CORT levels, and an ANOVA was performed on serum CORT levels for differences among the various groups.

Results: Within Blocks Analysis

The following table contains the means and standard errors of the mean for all groups by blocks of two trials (see Table One).

	ADX-SAL		SHAM-SAL		ADX-STR		SHAM-STR	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
B1	2.667	0.386	2	0.511	3.8	0.309	2.611	0.415
B2	1.625	0.283	2	0.364	2.25	0.454	1.5	0.363
B3	1.291	0.339	1.542	0.217	1.3	0.249	1.278	0.237
B4	0.917	0.192	0.875	0.262	0.95	0.240	0.778	0.169
B5	0.833	0.112	0.958	0.199	0.5	0.129	0.889	0.320
B6	0.375	0.175	0.458	0.114	0.75	0.200	0.389	0.111
RT	0.25	0.179	0.333	0.188	3.5	0.619	2.444	0.669

	ADX-TIA		SHAM-TIA		ADX-STR-TIA		SHAM-STR-TIA	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
B1	2.667	0.615	3.583	0.5833	2.688	0.582	3.125	0.440
B2	2.083	0.810	1.667	0.279	2.063	0.678	2.563	0.417
B3	2.5	0.695	1.667	0.667	0.875	0.263	1.625	0.245
B4	1.25	0.309	1.333	0.210	0.75	0.25	1.125	0.363
B5	1.083	0.238	0.833	0.210	0.688	0.230	1.125	0.295
B6	0.917	0.300	0.833	0.307	0.438	0.147	0.563	0.290
RT	0.667	0.422	0.667	0.333	0.625	0.375	0.875	0.295

Table One: Means and Standard Errors of the Mean for all Groups by Blocks of Two Trials: Adrenalectomy, Tianeptine and One-Day Learning Task.

The repeated measures ANOVA showed that there was a significant BLOCKS effect ($F [6, 378] = 39.314$, $p < 0.0001$). There was also a significant BLOCKS x STRESS interaction effect ($F [6, 378] = 5.869$, $p < 0.0001$). The repeated measures ANOVA also revealed a BLOCKS x DRUG interaction effect ($F [6, 378] = 3.060$, $p < 0.006$). Also, a significant BLOCKS x STRESS x DRUG interaction effect was shown ($F [6, 378] = 3.833$, $p < 0.001$. A test of linearity among all groups by block revealed a significant linear trend for BLOCKS ($F [1, 63] = 127.877$, $p < 0.0001$). A significant linear trend was also evident within the BLOCKS x DRUG effect ($F [1, 63] = 6.276$, $p < 0.015$). The between subjects analysis showed significant STRESS x DRUG interaction effect ($F [1, 63] = 9.098$, $p < 0.004$), and a significant SURGERY x STRESS x DRUG three-way interaction ($F [1, 63] = 4.760$, $p < 0.033$). All groups showed a significant learning curve across the six two-block acquisition trials.

Results: Retention Trial Analysis

The means and Standard Error of the Mean (SEM) for Retention Trial (RT) performance for all groups are as follows: Sham/Saline = 0.333 ± 0.188; ADX/Saline = 0.25 ± 0.179; Sham/Stress = 2.444 ± 0.669; ADX/Stress = 3.5 ± 0.619; Sham/TIA = 0.667 ± 0.333; ADX/TIA = 0.667 ± 0.422; Sham/Stress/TIA = 0.875 ± 0.295; ADX/Stress/TIA = 0.625 ± 0.375.

The analysis of variance (ANOVA) performed on the RT error rates between groups indicated that there was a significant STRESS main effect, ($F [1, 63] = 20.637$, $p < 0.0001$). The ANOVA also showed that there was a significant DRUG main effect by the tianeptine, ($F [1, 63] = 9.218$, $p < 0.003$). Conversely, the ANOVA found that there was not a significant main effect of ADX ($F [1, 63) = 0.352$, $p < 0.555$). In addition, the

ANOVA indicated that there was a significant STRESS x DRUG two-way effect, (F [1, 63] = 18.223, $p < 0.0001$). The remaining two-way effects, ADX x STRESS (F [1, 63] = 0.534, $p < 0.468$) and ADX x DRUG (F [1,63} = 1.009, $p < 0.319$) were not significant. The three way interaction ADX x STRESS x DRUG was not significant (F [1.63) = 1.303, $p < 0.258$ (see Figure Fourteen). The cat exposure significantly increased errors in the ADX-STR and Sha-STR groups. These data indicate that ADX did not block the stress effect on memory. Also, the data indicate that in the presence of ADX and sham surgery tianeptine significantly lowered memory errors in the ADX-STR-TIA and Sha-STR-TIA groups.

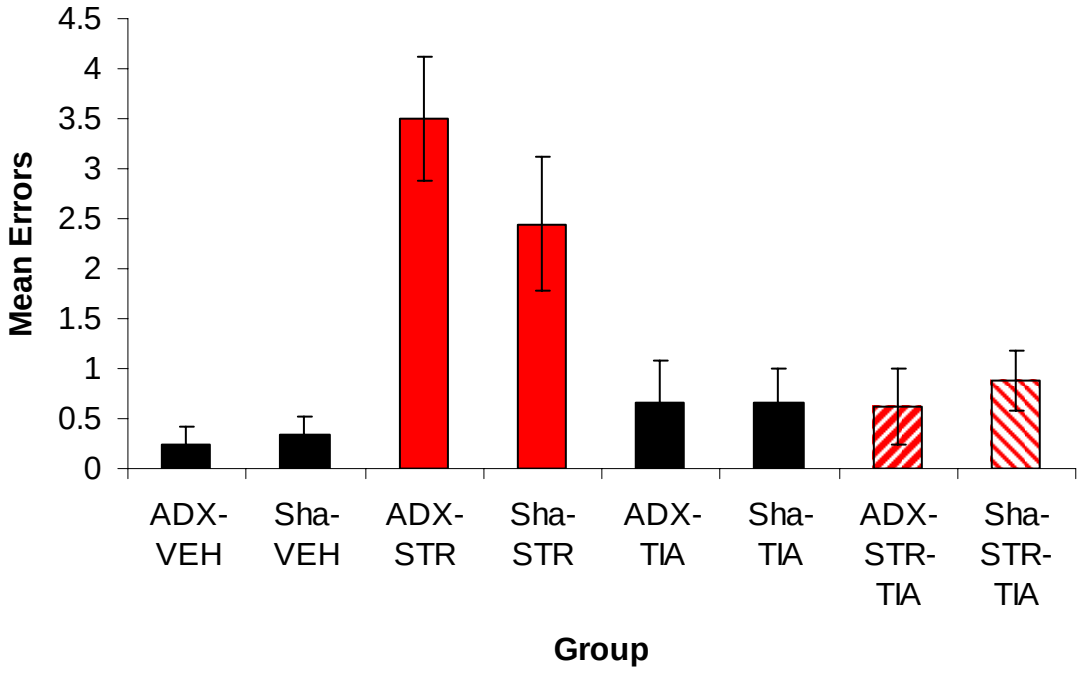

Figure Fourteen: Errors on Retention Trial for all Groups: Adrenalectomy, Tianeptine and One-Day Learning Task.

Results: Blood/CORT Analysis for ADX Experiments

Means were calculated and a one-way ANOVA was performed for all groups. The following are the means and standard errors of the mean for all groups: Sham = 13.45 ± 0.76; ADX = 3.092 ± 0.45; ADX/Stress = 3.792 ± 0.442; Sham/Stress = 43.55 ± 5.323; ADX/TIA = 4.273 = 0.385; Sham/TIA = 14.159 ± 1.588; ADX/Stress/TIA = 4.738 ± 0.826; Sham/Stress/TIA = 44.97 ± 6.164 (see Figure Fifteen).

The ANOVA revealed that there was a significant ADX main effect ($F [1, 41] = 129.714$, $p < 0.0001$). There was also a significant STRESS main effect ($F [1, 41] = 49.749$, $p < 0.0001$). There was also a significant ADX x STRESS interaction effect ($F [1, 41] = 46.083$, $p < 0.0001$). All other effects and interactions were not significant. The data show that stress significantly increased CORT levels in Sham-STR and the Sham-STR-TIA groups.

In summary, Experiment Five showed that ADX alone did not impair spatial memory performance in rats. The ADX/Vehicle and Sham/Vehicle groups were not significantly different and both exhibited mean error rates significantly low than both ADX/Stress and Sham/Stress groups. Experiment Five showed that stress-induced memory errors were produced in the presence of ADX. ADX/Stress and Sham/Stress groups showed a significant increase in mean error rate and both groups' error rates were significantly higher than all other groups. Tianeptine was shown to maintain efficacy in reducing stress-induced memory errors even in the presence of ADX. ADX/Stress/TIA and Sham/Stress/TIA groups were both significantly lower than ADX/Stress and Sham/Stress groups, and were significantly similar to all Home Cage groups. Experiment Five also showed that the ADX/STR group exhibited significantly higher error rates and

the group's CORT levels were at low ADX levels. These data suggest that high stress levels of CORT are not necessary for stress-induced errors to occur.

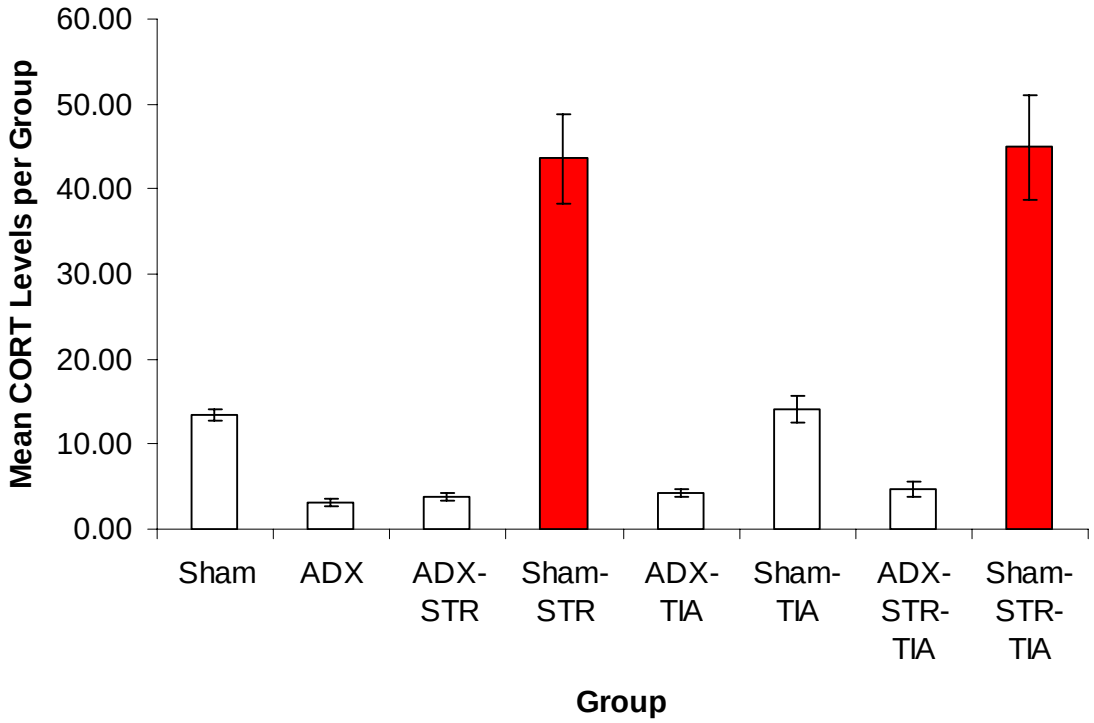

Figure Fifteen: Mean Plasma CORT Levels for All Groups: Adrenalectomy, Tianeptine and One-Day Learning Task.

Chapter Seven

Discussion

Tianeptine Blocks Stress-Induced Memory Errors

The first four experiments in the current series examined the effects of the antidepressant tianeptine on stress-induced memory errors in rats. Tianeptine has been shown to reverse the stress-induced blockade of synaptic-plasticity (Kole et al, 2002; Shakesby et al, 2002) and has reversed spatial memory deficits in rats (Conrad et al, 1996; Luine et al, 1994). The current research found that acute administration of tianeptine was effective in blocking stress-induced memory errors in two different training paradigms within the radial-arm water maze (RAWM). In both the criterion-based training regimen (Experiment One) and the one-day learning regimen (Experiment Three), tianeptine blocked the stress-induced memory deficit on the post-stress retention trial. In both experiments tianeptine was given thirty minutes before the first acquisition or learning trial. Tianeptine administration blocked stress-induced memory errors on the retention trial, but did not affect acquisition. Both vehicle and tianeptine groups showed a significant learning curve over the acquisition trials prior to any stress during the thirty minute manipulation period.

Serotonergic Mechanism of Tianeptine Action

Research suggests that tianeptine exerts its effects through a serotonergic mechanism (Mennini et al, 1987, Mocaer et al, 1988, de Simoni et al, 1992), namely increasing the reuptake of serotonin from the synapse, in turn reducing the amount of synaptic serotonin. The serotonergic theory is attractive due to the idea that stress increases serotonin levels in the synapse (Matsuo et al, 1996; Yoshioka et al, 1995).

Tianeptine would then be hypothesized to alleviate the stress effects by reducing the serotonin due to the stress.

NMDA Receptor Mediated Mechanism of Tianeptine Action

While the serotonin hypothesis is still a viable possibility, recent research has shown that tianeptine may also act through an NMDA receptor mechanism, buffering the NMDA receptor from the effects of stress (Kole et al, 2002; Shakesby et al, 2002). The glutamatergic NMDA receptor is a logical mechanism for stress-induced effects on memory and the blockade of these effects by tianeptine based on the idea that the NMDA receptor has been shown to mediate cellular and functional effects of stress. Following a stressful event, receptor binding and receptor subunit expression for hippocampal NMDA receptors are enhanced (Bartanusz et al, 1995; Krugers et al, 1993). Also, stress lowers the threshold for long-term depression (LTD), a form of hippocampal synaptic plasticity, via NMDA receptor activation (Kim et al, 1996). The administration of an NMDA receptor antagonist prevents stress-induced dendritic remodeling of CA3 pyramidal neurons (Magarinos and McEwen, 1995). Dendritic remodeling is known to be a consequence of chronic stress.

Other research indicates that the diminishing of NMDA receptor function may be linked to the activity of antidepressant drugs (Skolnick et al, 1999; Petrie et al, 2000; Krystal et al, 2002). For instance, the expression of hippocampal NMDA receptor subunits is reduced after chronic administration of antidepressants (Skolnick et al, 1999). Recent research reviewed by Petrie et al (1999), indicates that in animal models of depression NMDA antagonists exhibit similar potencies as antidepressant medications. Also, recent work by Berman et al (2000) showed that ketamine, an NMDA-antagonist,

produced a transient improvement in the mood state of patients with major depression. As seen here, both preclinical and clinical work has shown that the NMDA receptor may be heavily linked to stress effects and disorders closely associated to stress such as major depressive disorder (MDD). A further discussion of MDD and stress will follow below.

Another line of research involving hippocampal synaptic plasticity has indicated that stress blocks long-term potentiation (LTP) in the hippocampus and this blockage is reversed by tianeptine (Shakesby et al, 1999). The work with tianeptine and LTP suggests that tianeptine may work via NMDA receptors by increasing the amount of glutamate available to the receptor, thus buffering it from the effects of stress. Tianeptine would then be seen as a drug that would protect the receptor from the effects of stress while also setting a set of chemical preconditions, through increased release of glutamate that would make the receptor less susceptible to the effects of stress.

Testing the Anti-Anxiety Properties of Tianeptine

In Experiment Two, tianeptine was given after the stressful experience within the criterion-based regimen. Tianeptine was not effective in reducing stress-induced memory errors on the retention trial when administered after the stressful event. Thus tianeptine was effective in reducing the effects of stress on memory only when administered in a proactive manner. Tianeptine seems to set a series of chemical and receptor-based conditions that make the cell less susceptible to stress. This is the case mentioned above in Experiments One and Three when tianeptine was given thirty minutes before the first training trial. Tianeptine is also thought to exhibit anti-anxiety properties as well as antidepressant properties (Wilde et al, 1995; Rodgers et al, 1997; Drobizhev et al, 2000; Lepine et al, 2001; Rumiantseva et al, 2003). The effects of tianeptine given after the

stressful experience are useful in gauging the anti-anxiety properties of the drug. It would be possible that the errors exhibited by the stress/vehicle groups could be caused by increases in anxiety levels. An increase in anxiety levels could affect performance of the rat in the maze by increasing the overall speed of motor function (i.e. swimming). If this was the case, giving the tianeptine after the stressful event should have reduced anxiety levels in the rat, thus reducing the error rate on the retention trial. The fact that errors were not reduced in stressed rats given tianeptine after the stressful event suggests that the reduction in stress-induced errors was due to stress-related factors other than increased levels of anxiety and related changes in motor function. However, we did not measure anxiety behavior, per se, in the rats. Therefore, whether tianeptine blocked post-cat exposure anxiety can not yet be determined.

Experiment Four also examined the role of anxiety and performance by administering the beta-blocker drug propranolol. Two doses of propranolol (5 mg/kg and 10 mg/kg) were ineffective in reducing stress-induced errors on the retention trial on the one-day learning task. The ineffectiveness of propranolol on blocking stress-induced memory errors on the one-day learning task suggests that the beta-adrenergic system is not a viable mechanism for stress-induced memory change under the current RAWM-cat conditions. The idea that propranolol did not block stress effects on memory is not consistent with previous research which showed that propranolol blocked the memory enhancing effects of glucocorticoids and epinephrine. Previous research showed that glucocorticoid injection enhanced memory for an inhibitory avoidance task and that this enhancement was blocked by propranolol (Roozendaal et al, 1999). The blockade of enhancement of the stressful memory would be analogous to the enhancement of the

stressful memory of the cat in the current studies. The enhancement of the cat stress memory would cause the stress –induced impairment of the peripheral memory, the memory of the platform location. However, in the current studies propranolol was ineffective in blocking stress-induced memory impairment of the peripheral memory. This suggests that the blockade of beta-adrenergic activity at the time of stress is not effective in the predator exposure stress situation.

Stress and Major Depressive Disorder (MDD): Pre-Clinical Applications of Tianeptine

The effects of tianeptine detailed here are important to the investigation of the pathophysiology and treatment of major depressive disorder (MDD). There are similar neurological effects seen in stressed subjects and those with MDD. Namely in both instances, stress and MDD, hippocampal and prefrontal cortical functioning is impaired whereas amygdaloid functioning is enhanced (Burghardt et al, 2003; Vouimba et al, 2003). Also certain biomarkers including neurotransmitter abnormalities and hormone levels, including the stress hormone corticosterone, and brain structure morphology (Hindmarch et al, 2001; Phillips et al, 2003; Sapolsky, 2000) have commonalities among stress and MDD. It is known that during a stress response and with MDD corticosterone levels in rats or cortisol levels in humans are elevated (Boyer et al, 2000; Moghaddam, 2002, Parker et al, 2003). This commonality between stress and MDD makes stress a prime area of study to better understand the etiology and treatment of MDD. It is also intriguing to note that severe life stressors can sometimes lead to the occurrence of clinical depression. The study of stress and its neurological and chemical substrates will lead to better understanding of how stressful life experiences can lead to depressive

disorders. The study of stress can also lead to a better understanding of effective treatments for depressive disorders.

Also it must be taken into account the different mechanisms by which tianeptine and other antidepressants such as selective serotonin reuptake inhibitors (SSRIs) work. SSRIs are thought to alleviate symptoms of depression over time by increasing the amount of serotonin available in the synapse. Increases in serotonin are achieved by the inhibition of reuptake of serotonin back into the pre-synaptic cell. Conversely, tianeptine has been shown to be a serotonin reuptake enhancer in rats and in humans (deSimoni et al, 1992; Wilde et al, 1995). After treatments ranging in duration from 4 weeks to 3 months at the time of testing, tianeptine was seen to have the same efficacy as amitriptyline, imipramine, and fluoxetine (Wilde et al, 1995). As stated above, tianeptine enhances the reuptake of serotonin into the pre-synaptic cell. The pre-synaptic cell would then have an increase in the amount of serotonin that could possibly be released. Over time, it is possible that SSRIs and tianeptine may indeed have the same mechanism of action, increased amounts of serotonin in the synapse. SSRIs accomplish this by blocking reuptake, whereas tianeptine may accomplish this by having more serotonin to release from the pre-synaptic cell due to the enhancement of reuptake. It is important to keep in mind the possible long-term pharmacological effects of drugs with seemingly different mechanisms of action in the treatment of depression.

Involvement of Corticosterone In the Formation and Blockade of Stress Effects

One area of interest in the current series of experiments was the actions of corticosterone and its effects on stress and stress-induced memory change. In Experiment One it was found that rats that were stressed and received a vehicle treatment exhibited

elevated levels of CORT. The finding of elevated CORT levels is consistent with other data indicating raised levels in response to cat exposure (Diamond et al, 1999). In addition, rats that were stressed and received tianeptine thirty minutes prior to training also exhibited statistically significant elevated levels of CORT, similar to the levels found in stressed rats that were administered vehicle treatment. The fact that CORT was elevated in the STRESS/TIA rats suggests that tianeptine exerts its effects independent of modifying circulating blood CORT levels. That is, the blocking of stress-induced memory errors in Experiment One within the criterion-based multi-day training regimen was not due to a reduction in circulating blood levels of CORT. In Experiment Three, similar elevated CORT levels were found in stressed rats trained on the one-day learning regimen. Thus, tianeptine did not lower CORT levels in either training regimen, the multi-day training nor the one-day learning task.

The current series of experiments showed that stress-induced memory deficits can be alleviated by tianeptine in the presence of high CORT or in the absence of CORT via ADX. The current data lend to the discussion of the interaction between CORT levels and memory performance. Extensive research has shown that stimuli that are considered arousing can enhance memory (Cahill, 2000; LeDoux, 2000; McGaugh, 2000). Conversely, stimuli that increase the emotionality of the subject, such as stress also impair memory (Diamond and Park, 2000; Kim and Diamond, 2002) and cause amnesia (Loftus and Kaufman, 1992; Joseph, 1999). An apparent paradox exists therefore between the type of arousing stimulus and its effects on memory and behavior. While arousing stimuli such as predator exposure cause amnesic effects on a spatial memory task in rats (Diamond et al, 1996, 1999b) other arousing stimuli that do not contain a fear

element , such as giving a male rat access to an estrous female rat, do not disrupt spatial memory (Woodson et al, 2003). Woodson et al (2003) found that rats that were stressed with a cat and those that were given access to an estrous female both exhibited high levels of CORT. But whereas the cat exposed rats showed an increase in errors the rats given access to the female did not show an increase in error rate. The findings of Woodson et al (2003) are consistent with the current findings because certain groups of rats in both studies exhibited high levels of CORT while not showing spatial memory impairments. Other research (Park et al, 2001) found that rats that were not stressed with the cat but were injected with stress levels of exogenous CORT showed no spatial memory impairment when tested in the RAWM, suggesting that CORT alone does not cause spatial memory impairment. This hypothesis suggests that the nature of the arousing or stressful stimulus must be taken into account much like that in the Woodson et al (2003) findings. Past studies have shown that certain stressors including shock, restraint and exposure to a novel environment have resulted in spatial (hippocampal-dependent) memory impairment (Diamond et al, 1994; Kuroda et al, 1998; Magarinos et al, 1995). And impairment of spatial memory (Kim and Diamond, 2002) and the impairment of synaptic plasticity (Foy et al, 1987; Shors et al, 1989; Kim et al, 2002) in the hippocampus have been associated with high stress levels of CORT. The current experiments and the work of Woodson et al (2003) extend these findings by showing memory impairment is not always tied to the presence of high CORT. The current data elucidates the nature of the interaction between elevated CORT levels and memory impairment by showing that high CORT alone does not lead to spatial memory impairment. Experiment Five in the current series also finds that stress effects and the

alleviation of stress-induced memory deficits by tianeptine occur in the absence of CORT via ADX. As discussed below, the findings of Experiment Five are consistent with the idea that elevated CORT is not the sole cause of spatial memory impairment.

The Role of Adrenal Hormones on Stress and Memory

Experiment Five further examined the effects of CORT and other adrenal hormones on stress and its effects on memory. Adrenalectomized (ADX) rats that were not stressed showed acquisition of the one-day learning task in a manner statistically equal to that of sham operated controls. Previous research has shown the ADX rats exhibited impaired learning (Vaher et al, 1994). The difference between previous research and the current experiment is that ADX rats in the current experiment received CORT replacement in their drinking water. The amount of CORT given in the replacement therapy was designed to maintain the integrity of hippocampal cells that would normally be at risk under ADX conditions, namely granule dentate gyrus cells within the hippocampus. Previous research showing memory impairment did not wish to maintain hippocampal cell integrity thus impairments were seen. It was imperative that the hippocampal cells of Experiment Five rats remain intact, allowing the effects of stress and tianeptine to be studied independent of the effects of hippocampal cell atrophy processes. Data from Experiment Five also showed that ADX rats that were exposed to the cat displayed stress-induced memory errors on the retention trial. The stress effect in ADX rats implies that adrenal hormones, most notably CORT, are not necessary for a stress-induced memory impairment to occur. Also in Experiment Five, tianeptine exerted its effects on stressed rats in both sham and ADX conditions. As in the results from Experiments One and Three, in which tianeptine was effective in reducing stress-induced

memory impairments in the *presence* of elevated levels of CORT, Experiment Five showed that tianeptine was effective in reducing errors in the *absence* of adrenal hormones, including CORT. The evidence given here puts into debate the necessity of CORT to the production of a stress effect. In the current experiments stress effects were found in the absence of circulating CORT. The current research showing the induction of a stress effect in the absence of CORT leads to the analysis of the reactions of the hypothalamic-pituitary-adrenal (HPA) axis during a stressful event. The HPA axis and hippocampus act as a negative feedback mechanism for circulating CORT levels. If CORT levels are elevated, as in a stressful situation, the CORT-receptor rich hippocampus will signal the HPA-axis to reduce the amount of stress hormones that are being released. If the feedback mechanism is disrupted as in the case of ADX the actions of the hypothalamus and the pituitary gland remain unchecked. In this case, the release of corticothophin-releasing hormone (CRH) by the hypothalamus and andrenocorticotrophic hormone (ACTH) by the pituitary gland continue to be released in stress-induced quantities. Research has shown that administration of CRH and ACTH can lead to the production of stress effects similar to those of CORT (Wang et al, 1998).

Summary and Conclusions

The current set of experiments investigated tianeptine's ability to block stress-induced memory errors. Experiment One found that tianeptine blocked stress-induced errors on a criterion-based memory task. The rats were very well trained in the RAWM at the point of tianeptine administration. The criterion-based task dictates that the rat be trained for a period of approximately two weeks. In Experiment Three tianeptine blocked stress-induced memory deficits on a one-day learning task. On the one-day learning task,

the rats were not as well trained to the task as in Experiment One. Taken together, the results of Experiments One and Three suggest that the level of training on the task, extended criterion-based vs. one-day training, did not alter the efficacy of tianeptine administration. Experiment Two indicated that when tianeptine was given after the stressful event, stress-induced memory errors were not blocked. The idea that tianeptine given after the stressful event did not block errors suggests that tianeptine acted in a proactive manner, in essence setting a set of chemical and/or electrical conditions before the stress occurs. The preconditions set by the administration of tianeptine may serve to strengthen the memory of the hidden platform location making the hidden platform memory less susceptible to stress. If, as in Experiment Two, the tianeptine was given after the stressor, there was no opportunity for the setting of preconditions and the strengthening of the platform memory via NMDA receptor activation. Experiment Two also elucidated the idea that tianeptine was not merely reducing the level of anxiety in response to the cat. If this were so giving tianeptine after the stressful event should lower the anxiety level and, in turn, promote the retrieval of the hidden platform memory.

Also, propranolol, at two doses, was not effective in reducing errors on the retention trial. It is also noted that the amygdala has a large population of beta-adrenergic receptors, and even though the propranolol was administered globally, in theory the blockage of beta-adrenergic receptors by propranolol should compromise the functioning of the amygdala. In fact, Experiment Four showed that rats given propranolol exhibited good retention memory. Thus, the blockade of beta-adrenergic receptors did not disrupt spatial memory function, suggesting the amygdala may not play a role in tianeptine's blockade of stress-induced memory effects. Also, Roozendaal et al (1999) found that

propranolol blocked the glucocorticoid-mediated enhancement of memory in the inhibitory avoidance task. The glucocorticoid enhanced the memory of the stressful event, the context in which the shock was administered. In Experiment Four, it was hypothesized that the beta-adrenergic antagonist would have similar effects in the cat exposed rats. That is, the propranolol would block the fear-intensified memory of the cat experience, and, in turn, not cause an impairment of the memory peripheral to the stress memory, the memory of the platform location. In Experiment Four, neither the 5 mg/kg nor the 10 mg/kg dose of propranolol blocked stress-induced errors. Since the peripheral memory was impaired, it can be stated that propranolol was not effective in reducing the effect of the memory of the cat on the memory of the location of the platform. Propranolol also exhibits anxiolytic effects by acting on serotonin receptors. More specifically, propranolol acts on the 5-HT_1 receptor. Graeff et al (1990) showed that propranolol exhibited anxiolytic effects on elevated plus-maze performance when administered into the midbrain central gray region. Nevertheless, rats in Experiment Four when administered propranolol at both 5 mg/kg and 10 mg/kg doses did not show a blockage of stress-induced memory errors.

It is interesting to note that in Experiment Five, tianeptine blocked stress effects on memory. Tianeptine blocked the effects of stress in the absence of adrenal hormones including corticosterone and peripheral epinephrine. The idea that stress effects occurred in the absence of epinephrine suggests that stress effects in the current series may not depend on the actions of either corticosterone or adrenergic (or noradrenergic) substrates. The CORT data submitted in the current experiments illuminates the idea that CORT is not necessary for a stress effect to occur with respect to memory. In Experiments One and

Three CORT levels were elevated in both stress groups, Stress/Saline and Stress/TIA. But it was shown that tianeptine reduced errors in the Stress/TIA group. Thus, tianeptine blocked stress-induced working memory errors *in the presence* of stress levels of CORT. CORT data in Experiment Three shows a similar effect in the one-day learning task. That is, tianeptine blocked stress-induced memory errors *in the presence* of stress levels of CORT. The idea that errors were reduced in the presence of stress-induced elevations in CORT suggests that CORT alone is not responsible for the induction of working memory errors. Interestingly, in Experiment Four, Home Cage rats that received propranolol showed elevated levels of CORT. Nevertheless, the Home Cage/PROP rats (at both 5 and 10 mg/kg doses) exhibited control levels of memory errors, again showing that high levels of CORT alone do not directly induce working memory errors. To compound the idea that CORT may not be the causal influence for memory errors, Experiment Five tested rats in the presence of adrenalectomy, essentially eliminating circulating levels of CORT. In Experiment Five, stress effects occurred *in the absence* of CORT and other adrenal hormones, and tianeptine blocked stress-induced memory errors *in the absence* of adrenal hormones including CORT. Taken together, the CORT data indicate that CORT is not essential to the production and alleviation of a stress effect.

In summary, the current set of experiments identified that tianeptine was effective in blocking stress-induced memory errors in two different working memory training tasks. Also, the current experiments found that the reduction in errors was not due to the lowering of anxiety levels, but rather a possible strengthening of NMDA receptors within the hippocampus. The experiments also found that the formation of a stress effect is not dependent on CORT or adrenal hormones. These experimental results will lead to further

investigation to examine the possible mechanisms of tianeptine's actions as well as lead to examination of the non-CORT/non-adrenal theory on the formation of a stress effect on working memory. The current set of experiments may also lead to a better understanding and future research on the mechanism of action and the efficacy of tianeptine as a treatment for Major Depressive Disorder (MDD).

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Adam M. Campbell received his Bachelor's degree in psychology from the University of Cincinnati in 1994. In 1997 he received a Master's degree in Experimental Psychology from East Tennessee State University. At ETSU Adam served as vice-president of Psi Chi, and was accepted into the Gamma Beta Phi and Phi Kappa Phi Honor Societies. Adam began teaching psychology courses while at ETSU and continued teaching for four years after enrolling in the Cognitive and Neural Sciences program in the Department of Psychology at the University of South Florida in 1997. While at USF, Adam continued his doctoral research at the James A. Haley VA Hospital in Tampa, and was awarded the Outstanding Research Poster at the 1st Annual College of Arts and Sciences Graduate Research Symposium in 2002. Adam has also made several presentations at the annual Society for Neuroscience meetings.