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The Role of Anti-Depressant Treatment in an Animal Model of Anxiety

Shari Ruth Bodnoff

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montréal, Québec, Canada

January 1987

ISBN 0-315-35567-0

ABSTRACT

The Role of Anti-Depressant Treatment in an Animal Model of Anxiety

Shari Ruth Bodnoff

Clinical and anecdotal evidence indicates that anti-depressants are effective in alleviating certain sub-types of anxiety, such as panic disorders and agoraphobia, and suggesting that these drugs possess intrinsic anxiolytic properties. The purpose of the present experiments was to compare the anxiolytic effects of anti-depressants with those of drugs known to have anti-anxiety effects. The acute and chronic effects of these drugs were examined in an animal model of anxiety. In this paradigm, rats were food-deprived for 48 hours prior to being placed in a novel environment containing food. The rationale behind the paradigm was that the fear associated with the novel environment would suppress consummatory behavior, and any drug that was effective in alleviating the fear elicited by novelty would also reduce the latency to begin eating in the novel environment relative to vehicle controls.

Acute injections of diazepam or adinazolam, given one hour prior to behavioral testing, significantly reduced the latency to begin eating. Desmethylimipramine and amitriptyline, when administered chronically, but not acutely, were effective in reducing eating latencies,

although the anxiolytic effect was moderate compared to diazepam and adinazolam. A time course study indicated that desmethylimipramine or amitriptyline significantly reduced the latencies to begin eating only after treatment for 14 or 21 days.

In a non-pharmacological manipulation, pre-exposure to the novel environment significantly reduced eating latencies relative to controls, and the anxiogenic ligand, FG-7142, reversed the effects of pre-exposure. These data suggest that benzodiazepines and chronic anti-depressants were effective in the paradigm because of their ability to reverse the inhibitory effects of novelty.

The findings are discussed in terms of possible common mechanisms of action for the benzodiazepines and anti-depressants.

Acknowledgements

I would like to thank my supervisor, Michael Meaney, for offering me the chance to take advantage of the excellent scientific opportunities, both at Concordia University and the Douglas Hospital. He has provided me with the technical, financial, and often motivational resources necessary to achieve a well-rounded background in the sciences.

My collaborations with Barbara Suranyi-Cadotte have been both informative and enjoyable. Her enthusiasm in my work and confidence in my skills have helped me to get through many of those valleys that occur all too frequently during a master's thesis.

One significant advantage of working at the Douglas Hospital was having access to one of the best pharmacology libraries in Montreal - Remi Quirion's filing cabinet. His technical advice during the writing of this thesis, especially on the serotonin story, was much appreciated.

David Aitken has been both friend and foe during the writing of this thesis, and both roles were much appreciated. It is rare to find such a knowledgeable and helpful technician in any laboratory, and I consider myself very fortunate to have had him around.

I would sincerely like to thank my external committee members, Roy Wise and Jackie Crawley, for taking time to offer objective and insightful commentary on this research.

Their suggestions have provided me with ideas for several experiments to keep me out of trouble for quite some time.

While she was soaking up the sun and "culture" of Sarasota, my mother's bi-weekly phone calls have often made the difference between sanity and suicide. The full extent of her love and support, especially during last year's difficulties, is immeasurable. I just hope she lives long enough to see me get a real job.

This research has been generously funded by Fonds de la Recherche en Sante du Quebec.

Dedication

This thesis is dedicated to the loving memory of my father and grandmother.

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The Role of Anti-Depressant Treatment in an Animal Model of Anxiety

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-III, American Psychiatric Association, APA; 1980), there exists several diagnostic categories of anxiety. These categories include generalized anxiety disorder, panic disorder, and phobic disorder (which includes agoraphobia). Although the precipitating stimuli for these disorders differ, there are several common behavioral and physiological symptoms, such as motor tension, autonomic hyperactivity, apprehension, and vigilance. Another feature associated with each of these syndromes is the occurrance of mild depressive symptoms (APA, 1980). Interestingly, feelings of anxiety, along with panic attacks and phobias, are also common symptoms in major depressive disorder (APA, 1980).

There is a considerable amount of clinical evidence for the idea that the anxious and depressive states may be related (Stavrakaki and Vargo, 1986). Dealy, Ishiki, Avery, Wilson, and Dunner (1981) found that 64% of patients with anxiety neuroses were also diagnosed with secondary depression compared with only 29% of patients diagnosed as having anxiety neuroses without the secondary depression. Similarly, Clancy, Noyes, Hoenk, and Slymen (1979) found that after a six-year follow-up, 44% of

patients with an initial diagnosis of primary anxiety were eventually diagnosed as having secondary depression. These clinical data suggest a link between the anxiety and depression.

The close association between anxiety and depression, along with the anecdotal evidence that anti-depressants are effective in reducing both anxiety and depression in these depressed patients, suggests that anti-depressants may have anxiolytic properties. In fact, the results of some clinical studies indicate that the tricyclic anti-depressants and monoamine oxidase inhibitors are actually more effective than the benzodiazepines in alleviating panic attacks and agoraphobia (Kelly, 1973). Moreover, recent research suggests that imipramine may also be effective in the treatment of generalized anxiety disorders (Kahn, McNair, Lipman, Covi, Rickels, Downing, Fisher, and Frankenthaler (1986). Although the onset of action of the anti-anxiety effects of anti-depressants is approximately two weeks, there have been fewer reports of tolerance and withdrawal that have been associated with chronic use of the benzodiazepines. This research suggests that the anti-depressants may serve as a viable alternative to the benzodiazepines in the chronic treatment of anxiety.

This thesis was designed to assess the role of anti-depressant treatment in anxiety. In general, the

benzodiazepines are the drug of choice in the treatment of anxiety disorders. Unfortunately, there are several sub-types of anxiety, including panic disorders and agoraphobia, that are non-responsive to such drugs. Moreover, recent research indicates both the development of tolerance to the anxiolytic properties of the benzodiazepines and the incidence of seizures following sudden withdrawal from protracted use of the drugs. This has led to interest in developing a single class of drugs that is capable of alleviating all forms of anxiety without producing tolerance or severe withdrawal consequences. The literature offers evidence to suggest that the anti-depressants may well serve this function.

Treatment of Anxiety

Many compounds, including alcohol, barbiturates, meprobamate, and the benzodiazepines alleviate the symptoms of anxiety. The common feature among these anti-anxiety agents is their ability to depress central nervous system activity (Haefely, 1978; Skolnick and Paul, 1981a). The class of drugs that has received the most attention is the 1,4-benzodiazepines, discovered by Leo H. Sternbach in the 1950's (Sternbach, 1983). These drugs have four distinct properties. They are anxiolytic, muscle-relaxant, sedative-hypnotic, and anti-convulsant (Haefely, 1978). The lowest, pharmacologically-active

dose of the benzodiazepines alleviates symptoms of anxiety, while increasing doses produce muscle-relaxation and sedation (Haefely, 1978). The sedative effects usually occur during the early phase of treatment and tolerance generally develops to these effects, although this is not always true for the anxiolytic effect (Greenblatt and Shader, 1978; Rickels, 1978).

The prevalence of anxiety in today's society is best exemplified by the number of prescriptions written each year for anxiolytic drugs. The benzodiazepines, chlordiazepoxide (Librium) and diazepam (Valium) are the two most widely prescribed anxiolytic drugs, accounting for greater than 40% of all the prescriptions written each year (Hollister, 1978). In Canada, more than 10% of the population receives a prescription for anti-anxiety drugs each year (Martin, 1982). The distribution of these drugs in hospitals is even greater. Approximately 30% of all hospitalized patients receive anxiolytics (Ban, Brown, Da Silva, Gagnon, Lamont, Leeman, Lowy, Ruedy, and Sellers, 1981).

Yet, despite their widespread use, there is considerable disagreement regarding the usefulness of the benzodiazepines in the long-term treatment of chronic anxiety. Originally, the early research suggested that the benzodiazepines had a low potential for abuse, tolerance, toxicity, and withdrawal symptoms (Bellantuono,

Reggi, Tognoni, and Garattini, 1980; Marks, Ayd, Bowden, Fisher, Laughren, Rickels, and Smith, 1981; Rickels, Case, Downing, and Winokur, 1985), but this is now under question. Recently, seizures and psychotic reactions have been observed following abrupt discontinuation of these drugs (Owens and Tyrer, 1983). Other reports describe milder manifestations of withdrawal, including anxiety and insomnia (Ashton, 1984; Busto, Sellers, Naranjo, Cappell, Sanchez-Craig, and Sykora, 1986). Thus, while the benzodiazepines remain the treatment of choice in anxiety, clinicians and researchers are being made increasingly aware of the problems associated with these drugs and are presently searching for alternative methods of treatment for chronic anxiety.

The Mechanism of Action of the Benzodiazepines

Since the discovery of the benzodiazepines, research has focussed upon their effects on most of the major neurotransmitter systems. Although the benzodiazepines have been found to decrease the synthesis or turnover of norepinephrine, serotonin, and dopamine (Hoehn-Saric, 1982), their effects upon gamma aminobutyric acid (GABA), the major inhibitory neurotransmitter in the mammalian central nervous system (Iversen and Bloom, 1972), have received the most attention.

There is considerable electrophysiological data to suggest a relationship between the benzodiazepines and GABA. In general, this research suggests that benzodiazepines modulate the efficacy of GABA'ergic transmission. Schmidt, Mohler, and Haefely, (1967) demonstrated that pre-synaptic inhibition in the cat spinal cord was mediated by GABA. The presence of GABA at the cell membrane increased the permeability of the membrane to negatively-charged chloride ions. The influx of chloride ions produced a greater negative charge inside the cell and subsequent hyperpolarization (Guidotti, Baraldi, Leon, and Costa, 1980). Therefore, GABA reduced the probability of an action potential. In the spinal cord preparation, the addition of diazepam to the preparation increased this inhibition. Moreover, this diazepam-induced potentiation of pre-synaptic inhibition was blocked by the GABA-receptor antagonist, bicucculine (Polc, Mohler, and Haefely, 1974) suggesting that a functional GABA'ergic system was necessary for the benzodiazepine potentiation of GABA's inhibitory action.

Another electrophysiological technique used to study the interactions between benzodiazepines and GABA involves the stimulation of the dorsal root ganglion. Stimulation of the dorsal root ganglion evokes an anti-dromic discharge consisting of a short latency dorsal root reflex (DRR) followed by a long duration dorsal root potential

(DRP), in adjacent dorsal roots. Both the DRR and DRP discharge are mediated by GABA (O'Brien, Schlosser, Spirt, Franco, Horst, Polc, and Bonetti, 1981). O'Brien et al. (1981) demonstrated that the benzodiazepine, midazolam, increased the dorsal root reflex. Using the same electrophysiological technique, Polc et al. (1974) showed that diazepam increased the dorsal root potentials that were elicited by stimulation of peripheral afferents. This effect was blocked by the GABA-receptor antagonist, biccuculine. Similarly, O'Brien et al. (1981) demonstrated that the benzodiazepine, midazolam, increased the dorsal root reflex, although there was no effect on the dorsal root potential. These electrophysiological data offered clear support of the ability of the benzodiazepines to potentiate the inhibitory action of GABA, and thereby enhanced the action of this neurotransmitter.

In summary, the electrophysiological data offer two important features regarding the nature of the relationship between GABA and the benzodiazepines. First, the benzodiazepines significantly enhance the inhibitory action of GABA transmission. Second, a functional GABA'ergic system must exist for the benzodiazepines to augment the inhibitory actions of GABA.

The enhancement of GABA'ergic activity by the benzodiazepines could result from one of several

mechanisms. The benzodiazepines might interact directly with the GABA-receptor, or might act through some specific benzodiazepine site associated with the GABA-receptor. Olsen, Ticku, Van Ness, and Greenlee (1978) demonstrated that the benzodiazepines were only weak inhibitors of [^{3}H]GABA binding in mouse brain and therefore were not acting as GABA agonists. Moreover, Olsen et al. (1978) demonstrated that the benzodiazepines did not influence the synthesis of GABA, nor as was shown by Iversen and Johnston (1971), do the benzodiazepines enhance GABA'ergic activity by inhibiting its re-uptake. Rather, the mechanism by which the benzodiazepines do enhance GABA functioning is by increasing the affinity of GABA, and GABA-mimetics, for the GABA receptor. In the presence of the benzodiazepines, the affinity of GABA for the GABA receptor increases from 120 nM to 20 nM (Toffano, Guidotti and Costa, 1978). Therefore, the addition of benzodiazepines potentiates the action of GABA at its receptor. These data suggest a close, pharmacological relationship between the benzodiazepines and GABA.

In 1977, two independent research groups discovered benzodiazepine binding to specific sites in membrane preparations (Mohler and Okada, 1977; Squires and Braestrup, 1977). Braestrup and Squires (1977) reported saturable, specific high-affinity binding of [^{3}H]diazepam in brain tissue, with an apparent binding affinity, or Kd,

of approximately 3 nM. The highest receptor densities (approximately 1.0 pmol/mg protein) were found in cerebral cortex, hippocampus, and cerebellum and the lowest densities (approximately 0.2 pmol/mg protein) were found in the spinal cord and white matter. The hypothalamus, pons-medulla, corpus striatum, and midbrain contained intermediate densities (approximately 0.4 pmol/mg protein) of binding sites (Muller, 1982; Richards and Mohler, 1984).

Mohler and Okada (1978) reported a positive rank order correlation ($r = 0.83$) between the affinity of 12 benzodiazepines for the in vitro [^{3}H]diazepam site in cortical tissue and their average therapeutic dose in an animal model of anxiety. This suggested a strong relationship between the binding affinity of the benzodiazepines for the [^{3}H]diazepam binding site and a behavioral measure of anxiolytic potency. This finding clearly implies the importance of [^{3}H]diazepam binding site in the clinical effects of the benzodiazepines.

Additionally, Mohler and Okada (1978) reported that at low concentrations of the [^{3}H]diazepam (100 nM), there was no uptake of the drug into rat cortical slices, suggesting that the benzodiazepine's site of action was localized on the cell surface rather than within the cell. These findings were supported by fractionation studies, performed by differential centrifugation. The highest

[^{3}H]diazepam binding was found in the P_2 pellet, associated with the synaptosomal fraction containing "pinched-off" nerve terminals (Braestrup and Squires, 1977; Mohler and Okada, 1978). This finding suggested, that since the greatest [^{3}H]diazepam binding occurred in synaptosomal fractions, these specific binding sites might play an important role in synaptic transmission.

In summary, the original data from both research groups suggests that the [^{3}H]diazepam binding represents a pharmacologically-relevant receptor since it demonstrates high affinity, proper ligand selection, saturability, regional distribution, and correlates well with functional response (Levine, 1983).

In addition to CNS receptor sites, Braestrup and Squires (1977) reported [^{3}H]diazepam binding in peripheral tissue such as, lung, liver, and kidney that differed from that found in the CNS. Surprisingly, Schoemaker, Boles, Horst, and Yamamura (1983) later reported the existence of these "peripheral-type" receptors in brain tissue. There were four differences in the binding properties between the central and peripheral sites. First, [^{3}H]diazepam had a lower affinity for the peripheral sites (Kd = 40 nM). Second, the two sites had different pharmacological specificities. Clonazepam, a potent benzodiazepine anxiolytic displaced [^{3}H]diazepam binding in brain with an IC_{50} = 5 nM, yet was ineffective in displacing peripheral

[^{3}H]diazepam binding (IC_{50} = 2900 nM). In contrast, the therapeutically-inactive benzodiazepine, Ro5-4864, was found to be a potent displacer of [^{3}H]diazepam binding in peripheral tissue (IC_{50} = 5 nM), whereas it was rather weak in brain (IC_{50} > 0.1 mM). Third, fractionation studies revealed that the greatest concentration of [^{3}H]diazepam binding in peripheral tissue was associated with the P_1 or nuclear fraction, rather than the P_2 fraction, suggesting different cellular localization for the two binding sites. The fourth difference between the two binding sites was their distribution in the CNS. The highest densities of [^{3}H]Ro5-4864 binding were found in the olfactory bulb and pituitary with the lowest densities in the hippocampus, striatum, cortex, and cerebellum (Schoemaker et al., 1983). Thus, the distribution of [^{3}H]Ro5-4864 binding in the CNS was considerably different from that seen with [^{3}H]diazepam binding. Although the physiological relevance of the peripheral benzodiazepines sites is still unknown, these sites are not considered to be directly involved in the anxiolytic properties of the benzodiazepines (Schoemaker et al., 1983).

A Model of the Benzodiazepine-GABA Interaction

Although it was established that the benzodiazepines enhanced GABA'ergic transmission by increasing the affinity of GABA for its receptor, the exact relationship

between benzodiazepines, GABA, and their respective receptors remained unclear. Various research groups proposed models to explain the nature of this relationship (Costa and Guidotti, 1979; Paul, Marangos, and Skolnick, 1981). In general, the basic unit, the GABA-benzodiazepine-chloride ionophore complex, located on post-synaptic macromolecules, had three major recognition sites. They included a GABA-receptor (GABA-R) that could alternate between a high (Kd = 20 nM) and low (Kd = 120 nM) affinity state (Toffano et al., 1978), a benzodiazepine receptor, and a picrotoxin site (which was also the recognition site for the barbiturates). These sites mutually interacted with each other and with a two-state (open-closed) chloride ion channel (Paul et al., 1981).

Based on the GABA-benzodiazepine-chloride ionophore complex, Braestrup, Schmiechen, Neff, Nielsen, and Petersen (1982) proposed a possible mode of action of the benzodiazepines. In the 'ground state' of the complex, the GABA-R was in the low-affinity state (i.e unactivated) and the chloride ion channel was closed. This low-affinity state for the GABA-R was defined by the presence of GABA-modulin, a thermostable, acidic protein with a molecular weight of 15,000 daltons (Tallman, Paul, Skolnick, and Gallager, 1980; Toffano et al., 1978). This endogenous inhibitor bound competitively to the

benzodiazepine receptor (Costa, Guidotti and Toffano, 1978), suggesting that the presence of GABA-modulin prevented the occupation of the benzodiazepine receptor by the benzodiazepines. It has been shown that if access to the benzodiazepine receptor was blocked, the benzodiazepines could not enhance the affinity of the GABA receptor for GABA and the receptor remained in the low-affinity state (Mohler and Richards, 1981). These data suggest that if access to the benzodiazepine receptor is blocked, functioning of the GABA'ergic systems is also diminished. When GABA-modulin is displaced from the benzodiazepine receptor by benzodiazepines, the GABA-R converts to the high affinity state, and GABA and GABA agonists such as muscimol can then bind to the receptor with a greater affinity. GABA-R binding then activates the opening of the chloride ionophore, enhances the flow of the negatively charged chloride ions into the cell, and subsequently hyperpolarizes the cell.

The mechanism by which the benzodiazepines potentiated GABA's inhibitory action involved the conductance of chloride ions across the membrane. Study and Barker (1982) demonstrated that the benzodiazepines potentiated GABA's inhibitory action primarily by increasing the frequency of the chloride channel openings with only a modest increase in the amount of time the channel remained open. Thus, the increased ionic

conductance for chloride ions is due to an increase in the opening of the chloride ion channels.

Although a functional GABA system must exist for the benzodiazepines to exert a full anxiolytic effect, GABA and GABA-mimetics are not, in themselves, considered effective anxiolytics (Sanger, 1985). This finding is easily explained within the proposed GABA-benzodiazepine-chloride ionophore complex. The binding of the benzodiazepines to their receptors displaces GABA-modulin and produces a conformational change in the GABA-receptor. If the benzodiazepines are not present, or if access to their receptors is blocked, GABA-modulin is not displaced from the benzodiazepine receptor and the GABA-receptor remains in the low-affinity state. As long as the GABA-receptor remains in this state, GABA will not produce the increase in frequency of opening of the chloride ion channels.

In conclusion, the GABA-benzodiazepine-chloride ionophore complex represents a functionally-interacting unit that can explain the potentiation of GABA'ergic functioning by the benzodiazepines. Moreover, each system must be fully functional in order for the benzodiazepines to exert their anxiolytic properties.

Alternative Forms of Treatment in Anxiety

The efficacy of benzodiazepines in the treatment of anxiety is well-established. Unfortunately, there are several sub-types of anxiety that do not respond successfully to benzodiazepine treatment. These include panic disorders and agoraphobia. Considerable evidence exists to suggest that that these sub-types of anxiety respond favorably to tricyclic anti-depressants.

Klein (1964) described a group of patients that were referred for tranquilizers because there were complaints of spontaneous panic attacks. These subjects were not responding to psychotherapy or sedatives. Interestingly, although these patients were not agoraophobic, they were afraid of leaving the home environment in case they were unable to care for themselves. Reports of helplessness, a subjective state often associated with depression (i.e. Maier and Seligman, 1976), along with apathy and depression were frequent amongst these patients. The use of anti-depressant therapy for the treatment of these panic attacks was initiated in response to these reports of depression-like symptoms. Imipramine, but not the monoamine oxidase inhibitors, tranylcypromine and phenelzine produced a cessation of the panic attacks within 3-14 days. In contrast, electroconvulsive therapy (ECT), another mode of treatment for depression, was ineffective in alleviating their panic attacks. The

subjects were taken off the medication only after the attacks had been absent for three months. In almost half of the patients, there was a recurrence of the symptoms within one month. Klein concluded that chronic anti-depressant treatment served a 'prophylactic' role in the treatment of panic attacks and that these drugs were not effective in 'curing' the patients of these panic attacks.

Early behavioral techniques used in the treatment of agoraphobia included systematic densensitization and flooding (Rohs and Noyes, 1978). Recently, pharmacotherapy has been incorporated into these behavioral interventions. In contrast to the finding of Klein (1964), monoamine oxidase inhibitors were demonstrated to be extremely effective in reducing the panic attacks that generally accompany the agoraphobic syndrome (Kelly, 1973). Moreover, the benzodiazepines were considerably less effective in controlling these attacks (Kelly, 1973). Ballenger, Sheehan, and Jacobsen (cited in Rohs and Noyes, 1978) compared the efficacy of imipramine, phenelzine, and placebo in the treatment of agoraphobia. Ninety-four percent of phenelzine-treated patients and 89 percent of imipramine-treated patients were rated as significantly improved. The drugs also produced improvements that were significantly greater than group therapy alone.

In conclusion, these data clearly indicate that several sub-types of anxiety that are non-responsive to benzodiazepine treatment, are extremely responsive to anti-depressant and monoamine oxidase inhibitor therapy. These findings suggest that the symptoms of anxiety occurring in panic attacks and agoraphobia are very different from those anxiety states that are responsive to benzodiazepine treatment.

While both tricyclics and MAO inhibitor had been shown to be clinically effective in panic disorders and agoraphobia, until recently, there had been no documented evidence for the efficacy of these drugs in treating other forms of anxiety. Recent evidence has suggested that the tricyclics may also be effective in treating generalized anxiety disorders. In a double-blind, placebo-controlled study, Kahn et al. (1986) compared the efficacy of chlordiazepoxide, imipramine, and placebo over an eight-week period, in 242 patients diagnosed with anxiety disorders. The findings suggested that by the second week of treatment, the anxiolytic effects of imipramine were actually superior to those of both chlordiazepoxide and placebo. Moreover, patients taking the anti-depressant continued to improve over the course of study, while the efficacy of benzodiazepine treatment was limited to the first few weeks of treatment. These findings are also consistent with those of other research groups which

suggest that tolerance did develop to the anxiolytic activity of benzodiazepines with long-term use (Shapiro, Struening, Shapiro, and Milcarek, 1983).

In conclusion, the clinical research strongly suggests that anti-depressants are extremely effective in alleviating anxiety of various diagnostic categories. Moreover, the discovery that tolerance develops to the anxiolytic effects of benzodiazepines with long-term usage, and that seizures might occur following sudden discontinuation of these drugs, indicate that the benzodiazepines should not necessarily be the automatic drug of choice in the treatment of anxiety.

Common Mechanism of Action Between Anti-Depressants and Benzodiazepines

The efficacy of the anti-depressants in the alleviation of anxiety suggests that these drugs may have inherent anxiolytic properties. Therefore, the next question is whether the anti-depressants and the anxiolytics share a common mechanism of action.

The mechanism of action of anti-depressants has focussed upon post-synaptic events (Charney, Menkes, and Heninger, 1981; Sulser, Vetulani, and Mobley, 1978). In general, chronic, but not acute, anti-depressant treatment produces a down-regulation of post-synaptic B-adrenergic receptors (Banarjee, Kung, Riggi, and Chandra, 1977;

Sellinger-Barnette, Mendels, and Frazer, 1980) as well as serotonin receptors (Kellar, Cascio, Butler, and Kurtzke, 1981; Peroutka and Snyder, 1980). Down-regulation of these receptors is considered a marker for increased noradrenergic and serotonergic functioning respectively (Charney et al., 1981). The importance of the down-regulation of these receptors has come under scrutiny as this effect is not selective only to those clinically-effective anti-depressants (Banarjee, Sharma, Kung-Cheung, Chandra, and Riggi. 1979). Moreover, some anti-depressants fail to down-regulate these receptors (Peroutka and Snyder, 1980; Suranyi-Cadotte, Dam, Bodnoff, and Quirion, 1985). These data clearly suggest that since changes in either the B-receptor, or the serotonin receptor are insufficient to explain the efficacy of all anti-depressants, these systems are unlikely to be the common mechanism of action of the anxiolytics and the anti-depressants.

One system that had received virtually no attention was the benzodiazepine receptor system. This was quite surprising based on the clinical evidence, presented earlier, that anti-depressants were extremely effective in the treatment of anxiety disorders. There have been relatively few attempts to examine if a common mechanism of action existed between those drugs generally used for the treatment of anxiety (i.e. benzodiazepines) and those

for depression (i.e anti-depressants). In a recent attempt to examine this question, Suranyi-Cadotte, Dam, and Quirion (1984) treated animals for 21 days with a range of anti-depressant drugs, including DMI (a selective norepinephrine-uptake inhibitor), zimelidine (a selective serotonin-uptake inhibitor), buproprion (a selective inhibitor of dopamine uptake), and adinazolam (a triazolobenzodiazepine with both anxiolytic with anti-depressant activity), or vehicle control. They found a significant decrease in [^{3}H]flunitrazepam binding (decrease in Bmax without any change in the Kd) in all groups relative to controls following chronic treatment. In contrast, acute treatment with anti-depressants had no effect on [^{3}H]flunitrazepam binding (Suranyi-Cadotte, unpublished data). These researchers postulated a role for the benzodiazepine/GABA system in the therapeutic action of anti-depressants. Moreover, the down-regulation of the receptors with chronic treatment corresponded to the delayed onset of action of the anxiolytic properties of the anti-depressants (Kahn et al., 1986).

Animal Models of Anxiety

The data presented above suggest that the benzodiazepine receptor system may be the common mechanism of action through which the anti-depressants exert their anxiolytic activity. Unfortunately, a pharmacological

model such as the down-regulation of the benzodiazepine receptors provides little information regarding function, and the clinical importance of a change in receptor density, is as yet, unknown. Moreover, it represents the state of the animal in a home-cage condition rather than under conditions of stress or anxiety. Therefore, it is imperative to address the issue of the role of anti-depressants in anxiety in a behavioral model of anxiety. Will these drugs have anxiolytic activity in such a paradigm?

Geller and Seifter (1960) developed a paradigm, the Geller conflict test, that was found to reflect the anxiolytic effects of the benzodiazepines, barbiturates, alcohol, and meprobamate. Briefly, food-deprived rats were trained to bar-press for the delivery of sweetened condensed milk under a continuous reinforcement schedule (CRF) until a steady rate of responding was achieved. Once this baseline level was attained, a new condition was introduced. In the presence of a discriminitive stimulus, (tone) every bar-press produced both a reward and a mild footshock. This was described as a conflict situation and under such conditions, (tone or shock) rats showed little or no responding. Geller and Seifter found that clinically effective anxiolytics, including benzodiazepines, meprobamate, and barbiturates increased the number of responses made in the presence of the

discriminitive cue without producing any changes in the rate of unpunished or baseline responding. Therefore, the anxiolytics had very selective effects. In the conflict situation, every reward was paired with punishment and the tendency to avoid punishment was much stronger than the tendency to receive both reward and punishment. Anxiolytics were assumed to decrease the anxiety or fear associated with this punishment without affecting responding in the absence of the fear (unpunished responding).

Following these experiments, the first obvious question raised was whether the anti-punishment effects of the anxiolytics were due to the possible analgesic properties of these drugs. This explanation was easily ruled out. First, morphine, a conventional analgesic, even if given chronically, was ineffective in the Geller conflict test. Second, in a tail-flick test, which is extremely sensitive to conventional analgesics (i.e. morphine), benzodiazepines, meprobamate, and barbiturates did not increase the latency of the tail-flick in response to a heat stimulus (Treit, 1985). Finally, the anti-conflict effect of anxiolytics had been demonstrated using stimuli other than non-painful stimuli, such as entry into a novel, brightly-lit open-field (Crawley and Goodwin, 1980). These data suggested that it was not necessary to

use analgesia as an explanatory concept for the mechanism of action of anxiolytics in the conflict paradigm.

A second problem that was raised concerned the observation that drugs such as the benzodiazepines produced effects on food-motivated behavior that were directionally similar to their effects in the conflict situation. That is, diazepam increased feeding, the same behavior being measured in the conflict test, in both sated (Cooper, 1985), and food-deprived (Cole, 1983; Cooper, 1980) animals. There are data that suggest that the anxiolytics influenced behavior in the conflict paradigm rather than feeding. First, with the Geller paradigm, if diazepam increased punished responding because of its effects upon feeding, there should also be a similar increase in feeding in the unpunished condition. The increase in responding for food, induced by diazepam, should not have had a selective effect on punished responding. Second, since the anti-conflict effect of diazepam has been demonstrated in non-food-motivated behaviors, such as exploration (Crawley and Goodwin, 1980), it is not necessary to propose that benzodiazepine-induced hyperphagia explained the anti-conflict effect of these drugs. The evidence suggests that the anti-punishment effects of the benzodiazepines are not associated with their effects upon feeding or analgesia.

One complaint of the Geller paradigm is its inability to achieve construct validity. That is, the paradigm does not reflect the clinical anxiety state. How relevant is it to require a rat to endure shock to receive a food reward? A good (or poor) example of this problem is exemplified in a study by Meert and Colpaert (1986). In this paradigm, a probe was inserted into the testing cage and a baseline measure of physical contact with the probe was quantified. When the probe was electrified, there was a significant decrease in the amount of contact with the probe. Meert and Colpaert (1986) reported that the benzodiazepines significantly increased the amount of contact these animal made with the electrified probe. Clearly, the administration of benzodiazepines in this model is allowing the animal to behave maladaptively. This same example can be applied to the clinical state of anxiety. Benzodiazepines would never be prescribed to a patient who complains that he is afraid to place his hand on a hot stove. This fear is highly adaptive for the survival of this individual. On the other hand, if this patient was agoraphobic, and afraid to leave the house, this behavior would be classified as maladaptive. Clearly, the use of anxiolytics in this instance would be appropriate. In an attempt to represent the clinical anxiety state, it is imperative to select paradigms that represent a maladaptive fear. Since the 1960's, many

other paradigms were developed in an attempt to address the issue of conflict or fear and the actions of anxiolytics using clinically-relevant models.

Crawley and Goodwin (1980) examined the exploration of mice between a brightly-lit open field and darkened enclosure. Under baseline conditions, mice generally avoided exploration of the novel environment and remained in the darkened area. Crawley and Goodwin (1980) found that both clonazepam and chlordiazepoxide increased the amount of active exploration of the open-field. Ro5-4864 (peripheral benzodiazepine receptor agonist), clorgyline (an MAO inhibitor) and chlorpromazine (an anti-psychotic) were ineffective in increasing exploration (Crawley, 1981). Interestingly, there was no drug effect in a standard open-field without the dark compartment, suggesting that the benzodiazepines increased exploration rather than simply producing an increase in overall behavioral activation. These data suggested that the benzodiazepines function by decreasing the fear response (i.e avoidance) elicited by the brightly-lit open-field.

In a potentiated startle response (PSR) paradigm, Davis (1979) presented rats with 45 light-shock pairing to determine the baseline startle response. Twenty-four hours later, either an acoustic stimulus was presented alone or the original light stimulus preceeded the tone, and the startle response to the tone was measured. Davis

found that the startle response to the tone was enhanced or potentiated in those animals for which the light preceeded the tone, compared to those animals for which the tone was presented alone. Davis also found that diazepam decreased the potentiated startle response in a dose-dependent manner without having an effect upon those animal that had not received the light-tone pairings.

A final paradigm, designed by Britton and Thatcher-Britton (1981) incorporated the concept of conflict between behaviors without using shock. In this experiment, food-deprived rats were presented with a single food pellet placed on a pedestal in the middle of a novel open field. The rationale behind this design was that in a situation where hunger conflicted with the rat's tendency to avoid the centre of an open field, anxiolytics should reduce the fear of the novel environment and subsequently eliminate the conflict (increase feeding). The experimenters measured the amount of food eaten per 15-minute session and found that diazepam increased this response in a dose-dependent manner. There were similar findings for ethanol and pentobarbital, but morphine was ineffective in this test. The experimenters also found that diazepam did not increase food consumption in the home cage (in the absence of novelty or conflict) suggesting that the anxiolytic effect of diazepam could not be explained by hyperphagia. The authors concluded

that in this paradigm, anxiolytics reduced the fear associated with a novel environment and thereby allowed the resolution of the conflict between novelty-related behaviors and feeding. Moreover, the anxiolytic effect was specific to a fear-inducing situation in that the anxiolytics did not increase feeding in a familiar environment (the home cage).

In summary, there are many animal models available that are sensitive to the anxiolytic effects of the benzodiazepines (Treit, 1984). The differences between these paradigms exist mainly at the procedural level. For example, the Geller conflict paradigm requires and extensive training to achieve stabilized baseline responding, while exploration of a brightly-lit open-field requires no training or major equipment. More important is the question of the validity of the paradigms. In general, most models meet the criteria necessary for face validity. The tests are sensitive only to those drugs known to be effective in alleviating anxiety clinically, while false-positives are minimized. Construct validity is much more difficult to achieve. That is, do the paradigms measure what that are intended to measure, anxiety? Regardless of whether a behavioral test is sensitive to the effects of anxiolytic drugs, this pharmacological profile is less meaningful if the behavior is not reflective of the anxious state. The paradigm

developed by Britton and Thatcher-Britton (1981) represents a clinically-relevant animal model of anxiety. It is important to bear in mind that these models attempt to mimic conditions of clinical anxiety; a situation where a fear response that has no adaptive value is interferring with other, desirable behaviors. In the Britton and Thatcher-Britton paradigm, the benzodiazepines are found to reduce the fear associated with the center of the novel, but innocuous environment. In this situation, the animal is not faced with any threat and therefore it is adaptive for the animal to suppress the fear response and begin eating. In the other paradigms, the fear is not maladaptive; the animal is faced with a very real threat. The findings that the benzodiazepines are able to reduce this adaptive behavior, is at best, interesting, but less-obviously related to the clinical situation. The advantage of the paradigm by Britton and Thatcher-Britton (1981) is that it achieves both face and construct validity.

Objectives of this Study

There has been little systematic animal research into the anxiolytic actions of anti-depressant treatment at the behavioral level. This is somewhat surprising considering the evidence presented earlier suggesting that anxiety is a prominant feature of the depressive state and that

chronic anti-depressant treatment can eliminate both conditions.

The goal of the present research is to directly examine the efficacy of chronic anti-depressant treatment in an animal model of anxiety. Moreover, this work is an attempt to provide a behavioral model for the anxiolytic effects of chronic anti-depressant treatment as the basis for subsequent work examining the neuropharmacological basis for this effect.

Under conditions of fear or novelty, consummatory behaviors such as eating or drinking are suppressed (Gray, 1982). The strongest experimental evidence for this statement was derived for the conditioned emotional response (CER) paradigm. Estes and Skinner (1941) trained rats to lever-press for food. Once stable baseline responding was achieved, a buzzer was introduced that was paired with shock. Initial shocks produced marked suppression of lever-pressing. Interestingly, following a few buzzer-shock pairings, the animal's responding was suppressed in response to the buzzer alone, prior to the delivery of shock. That is, the animal anticipated the delivery of shock in the presence of the buzzer and did not respond. These data suggested that under fearful conditions, such as the anticipation of shock, responding for food was suppressed.

While fear inhibits consummatory behaviors such as feeding, it facilitates others. For example, when placed into a novel environment, the predominant behaviors will be ambulation (Whimbey and Denenberg, 1967) or freezing (Bolles, 1970). Based upon this evidence, a very hungry rat, placed in a novel environment containing food, is an ideal conflict paradigm. When a rat is exposed to a fearful situation, ambulation or freezing will predominate. Either of these behaviors is incompatible with feeding. Once the fear of novelty has decreased, the probability that a hungry animal will begin eating will increase. It was this premise upon which the present behavioral paradigm focussed. Animals were food-deprived for 48 hours prior to behavioral testing, thereby creating a strong physiological requirement for food, and appetitive motivation towards food. Then, when placed into a novel environment, two opposing incentive motivational states should be apparent: One related to novelty and the other to hunger. As mentioned earlier, novelty-related behaviors would predominate until the fear-related motivational state has diminished. Only then would the animal begin to eat.

This paradigm was quite similar to that of Britton and Thatcher-Britton (1981). The animals were similarly food-deprived and then placed into a novel environment containing food. There were two differences between the

paradigms. As described earlier, Britton and Thatcher-Britton used a single food pellet placed on a pedestal in the middle of an open-field. In the present experiment, food pellets were placed throughout a much smaller cage. Second, the measures of interest were different. Britton and Thatcher-Britton were interested in both the number of approaches made towards the food pellet (in the center of the open field) and the amount of food consumed in a 15-minute period. The variable of interest here was the latency for the animals to begin eating. The rationale behind the two paradigms might offer a further explanation of the differences. It was demonstrated that when a rat was placed in the open-field, it initially explores the outer perimeter of the environment. Only after the animal's fear had been reduced would it enter the centre of the field (Roth and Katz, 1979). Britton and Thatcher-Britton hypothesized that anxiolytics would reduce the fear-inducing cues of the novel environment, decrease the amount of exploration of the outer squares and increase the number of approaches to the centre of the field and the food. In the present experiment, it was hypothesized that an unfamiliar environment produced novelty-related behaviors (exploration or freezing) that were incompatible with feeding. According to Gray (1982), anxiolytics decreased these novelty-related behaviors and should

thereby decrease the latency for the animals to begin eating in an unfamiliar environment.

To determine that the behavioral paradigm was selective to anxiolytics, only those drugs found to alleviate anxiety in humans or animal models when given acutely should be effective in reducing the latency to begin feeding. Once it has been established that this paradigm is a valid model of anxiety, it is then possible to examine the anxiolytic action of chronic anti-depressant treatment. Based upon the clinical and anecdotal evidence presented earlier, it was postulated that chronic, but not acute anti-depressant treatment had anti-anxiety properties. Therefore, like the benzodiazepines, chronic anti-depressants should reduce the behavioral responses towards fearful stimuli and effectively reduce the latency to begin eating in a novel environment.

Experiment 1

The exact conflict procedure used in the present experiment had not been previously established in the literature. Therefore, in original testing, it was important to determine that the paradigm was sensitive to diazepam, a prototypical benzodiazpine. Did diazepam decrease the latency to begin eating in a novel

environment relative to vehicle controls? An anxiolytic effect was defined as a significant decrease in mean latency to begin eating compared with vehicle controls. Diazepam consistently has been found to attenuate anxiety in both human and animals and thus was the drug of choice in establishing the validity of a conflict paradigm. The dosage chosen, 2 mg/kg, was shown to produce significant anti-conflict effects in rats (File, 1986).

Methods

Subjects. Ten adult male Long Evans New Colony rats (Charles Rivers Breeding Laboratories, St. Constant, Quebec) were used in the experiment. Their body weight upon arrival at the laboratory was between 275 and 300 grams. The animals were group-housed, six per cage. The cages, 43 x 45 x 25 cm, were in a colony room maintained at 22°C, with a 12:12 hour light:dark cycle (lights on at 0800 hours). With the exception of the food deprivation period, standard Purina Lab Chow pellets and water were available ad libitum. All animals weighed between 300 and 325 grams by the time of testing. Animals were handled daily for approximately one week prior to testing to allow for adaptation to the new housing conditions.

Apparatus. The testing apparatus consisted of individual plexiglas cages, 54 x 28 x 21 cm, with stainless steel grid lids. The floor of each cage was

covered with approximately 1.5 cm of beta chips and 12 evenly-arranged lab chow pellets.

Drugs. Diazepam Injection U.S.P. (Sabex International, Montreal, Canada), was obtained in ampoule form in a concentration of 10 mg/2ml. The drug was dissolved in 40% propylene glycol and 10% alcohol. The dosage used was 2 mg/kg. The vehicle control was a solution of 50% saline and 50% propylene glycol (Fisher Scientific, Montreal), with a pH of approximately 6.5.

Procedure. Forty-eight hours prior to behavioral testing, all food was removed from the home cage, however, water was still available to the animals. Sixty minutes prior to testing, animals were given a single intraperitoneal injection of one of the above-mentioned drugs. All injections were administered between 0930 and 1030 hours and testing began between 1080 and 1130 hours. The animals were placed in a plastic bucket and transported to a small testing room, with overhead fluorescent lighting. Each subject was placed into a separate plexiglas cage. The stainless-steel grid lids were closed and a stopwatch was immediately started. The measure of interest was the latency to begin eating and this was defined as chewing of the food, not simply sniffing or playing with a pellet. If animals had not eaten within 360 seconds, the test was terminated.

Results and Discussion.

Figure 1 represents the mean latency, in seconds, for each group to begin eating. A single dose of diazepam produced an 81% decrease in eating latency relative to controls. A t-test of independent means revealed that this difference was highly significant ($t = 5.27$, $df = 7$, $p < .001$).

The conflict test appeared to be sensitive to the effects of an acute dose of a benzodiazepine. Diazepam produced a significant decrease in latency to begin eating in a novel environment and suggested that this paradigm may be a useful conflict model.

Experiment 2

The purpose of this experiment was to assess the role of the central-type versus the peripheral-type benzodiazepine receptor in mediating the anxiolytic effect of diazepam in the conflict paradigm. The effect of diazepam, an effective anxiolytic in humans and animals, which binds to the central benzodiazepine receptor, was compared to Ro5-4864, the peripheral-type benzodiazepine receptor agonist, in the conflict test. As discussed earlier, the peripheral receptor had not been implicated in the mediation of the anxiolytic effects of the benzodiazepines. It was hypothesized that only those

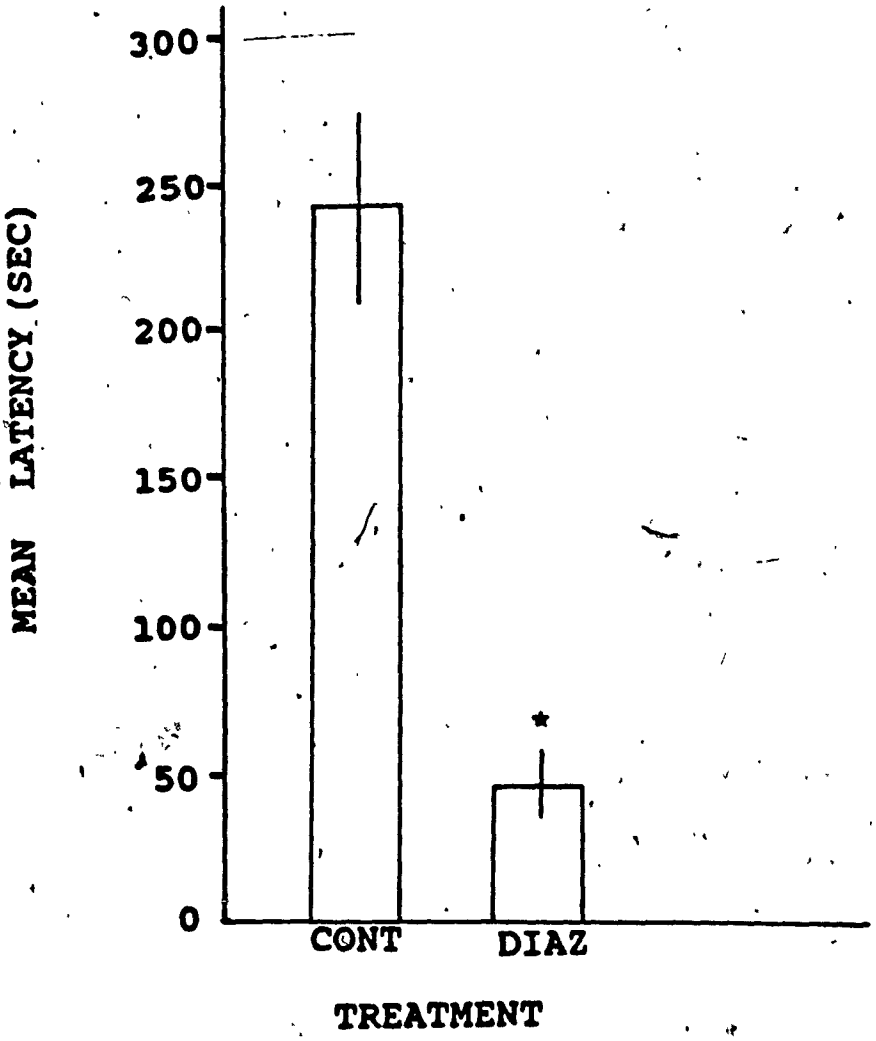

Figure Caption

Figure 1. Mean latency (sec) to begin eating in a novel environment following a single dose of either vehicle control (CONT) or diazepam (DIAZ). An asterisk indicates a mean that is significantly different from the control group ($p < .05$).

drugs known to bind to the central-type benzodiazepine receptor should be effective in reducing the latency for animals to begin eating in the novel environment. The particular dose of Ro5-4864 selected for the experiment was determined by its affinity to the peripheral receptor relative to the affinity of diazepam for the central-type receptor. Thus, since diazepam and Ro5-4864 have similar affinities for their respective receptors, it was appropriate to use the same dosage (2 mg/kg) of each drug in the paradigm.

Methods

Subjects. Twelve male Long Evan rats were used in this experiment. All housing, handling, and feeding conditions were identical to those outlined in Experiment 1.

Apparatus. The testing apparatus was the same as described in Experiment 1.

Drugs. The dosage of diazepam was 2 mg/kg, identical to Experiment 1. The Ro5-4864, 2 mg/kg, generously supplied by Hoffmann-La Roche, Basel, Switzerland, was dissolved in distilled water and one drop of Tween 80 (Fisher Scientific, Montreal, Canada) per 5 ml of water). The vehicle was the same saline/propylene glycol solution described in Experiment 1.

Procedure. The procedure was identical to that described in Experiment 1. Forty-eight hour food-deprived animals were injected with either diazepam, Ro5-4864, or vehicle. One hour later, all animals were tested in the conflict paradigm and the latency to begin eating was recorded.

Results and Discussion

Figure 2 represents the mean latency, in seconds, for the groups to begin eating relative to vehicle controls. An acute dose of diazepam produced a 72% decrease, while Ro5-4864 produced a 30% increase in mean latency to begin eating. A one-way analysis of variance revealed a significant difference between the means ($F = 8.16$, $df = 2,9$, $p < .01$; see Table 1). Post-hoc Newman-Keuls comparisons, tested at the 5% level of significance, revealed that the diazepam group ate significantly faster than both the Ro5-4864 and control animals. The latter two groups did not differ from each other.

The results of this experiment suggested that the central-type benzodiazepine receptor was important in mediating the effects of the benzodiazepines in the paradigm used here. Only diazepam, which has a high affinity for the central-type receptor, was effective in decreasing the latency to begin eating. Ro5-4864, with a very low affinity for the central-type receptor, did not

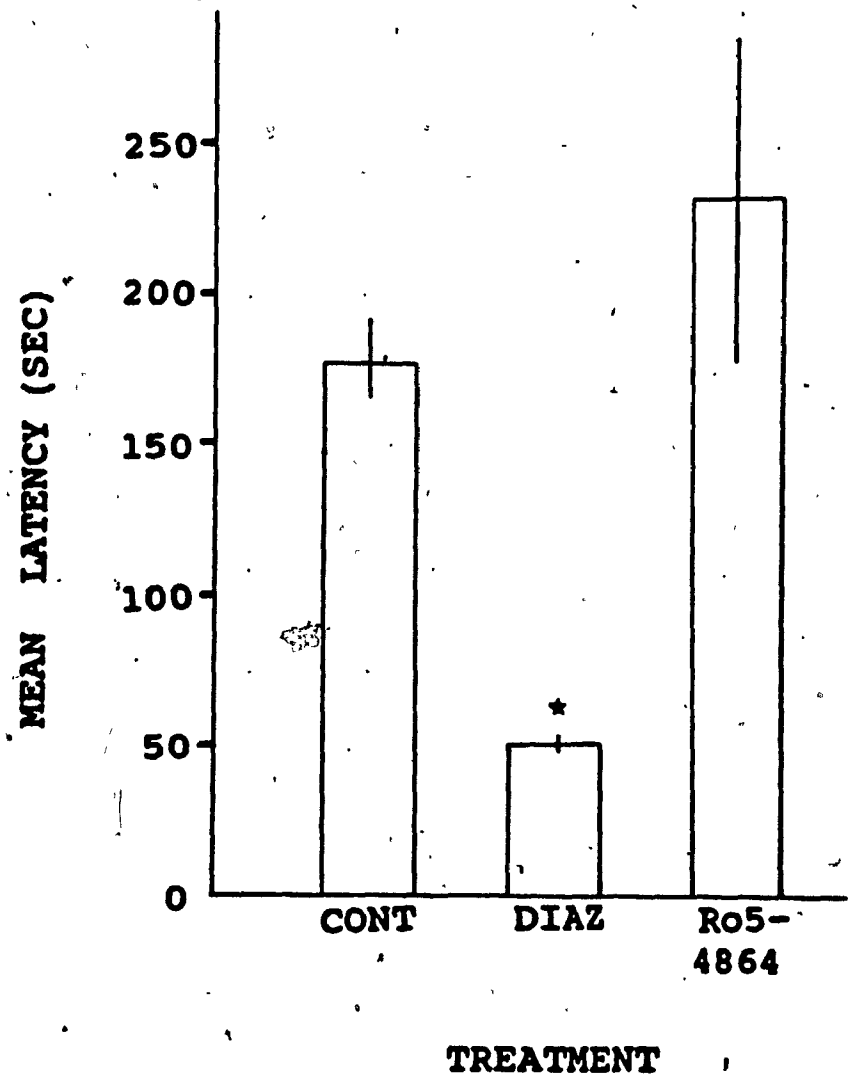

Figure Caption

Figure 2. Mean latency (sec) to begin eating in a novel environment following a single dose of vehicle control (CONT), diazepam (DIAZ), or Ro5-4864. An asterisk indicates a mean that is significantly different from control ($p < .05$).

Table 1

Source Table for the ANOVA of Acute Control, Diazepam, and Ro5-4864

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	2	71780.66	35890.33	8.16	.010
Within	11	39564.25	4396.03		
Adjusted	13	111244.90			

produce an anxiolytic effect. This finding was not surprising, considering that the peripheral-type receptor, to which Ro5-4864, binds with high affinity, had not been implicated in the mediation of the anxiolytic effects of the benzodiazepines.

Experiment 3

Drugs such as the benzodiazepines have strong effects on food-motivated behavior that were directionally the same as their anxiolytic effect. Extensive research indicated that diazepam increased feeding in sated rats (Cooper and Yerbury, 1986). It was possible to separate the hyperphagic and anxiolytic effects of the benzodiazepines. As mentioned earlier, it was postulated that the anxiolytics were effective in the conflict paradigm because they were able to attenuate the fear associated with the novel environment. One technique frequently used to reduce the fear of a new apparatus is pre-exposure. By continually exposing an animal to an innocuous environment, animals no longer manifest behaviors associated with fear (Britton and Thatcher-Britton, 1981). If diazepam was effective in the conflict paradigm because of its fear-reducing properties, rather than simply increasing feeding behavior, then pre-exposure should produce responses similar to those of diazepam.

Another issue of interest was the role of an anxiogenic substance in the conflict paradigm. The beta-carboline, FG-7142, a partial "inverse agonist" of the benzodiazepine receptor produced behavioral and physiological responses associated with fear (Beck and Cooper, 1986; Crawley, Ninan, Pickar, Chrousos, Linnoila, Skolnick, and Paul). In cats, a 10 mg/kg dose of FG-7142 produced an increased startle response, increased attention to the environment, and piloerection (Ognini, Barzaghi, and Marzanatti (1983). In man, a large dose of this anxiogenic drug produced sudden attacks of anxiety approximately 50 minutes following administration, feelings of intense inner strain and excitation, coupled with an increase in heart rate and blood pressure (Dorow, Horowski, Paschelke, and Amin (1983). Could pre-exposure reduce fear-related behaviors and latency to begin eating, and would the anxiogenic drug reinstate the anxiety or fear that pre-exposure had reduced?

Methods

Subjects. Fifteen male Long Evans rats were used in this experiment. The housing, handling, and feeding conditions were the same as described in Experiment 1.

Apparatus. The apparatus was the same as described in Experiment 1.

Drugs. The dosage of FG-7142, 10 mg/kg, (Hoffmann-La Roche, Basel, Switzerland) was selected because it had been demonstrated to reduce punished drinking in rats (Petersen, Jensen, Honore, and Braestrup, 1983). The drug was dissolved in the saline/propylene glycol solution and sonicated until the drug went into suspension. The saline/propylene glycol solution was also used for the vehicle control group.

Procedure. Three groups were tested in the conflict paradigm. The vehicle control were handled on 4 consecutive days. On the third day, food was removed from the home cage and one hour prior to testing, each animal was given an i.p. injection of the vehicle. The pre-exposure + vehicle animals were placed into the individual plexiglas cages that were subsequently used for behavioral testing, for one hour on 4 consecutive days. During these sessions, all food was removed from the testing apparatus. Again, food deprivation began on the third day and one hour prior to behavioral testing, each animal received a single injection of the vehicle. The pre-exposure + FG-7142 group was also exposed to the testing apparatus for 4 consecutive days and food-deprivation began on Day 3. One hour prior to testing, each animal received a single i.p. injection of FG-7142 (10 mg/kg).

Results and Discussion

Figure 3 represents the mean latency for each group to begin eating. Four days of pre-exposure to the test environment produced a 60% decrease in the mean latency for animals to begin eating relative to vehicle controls. Moreover, an acute dose of FG-7142 was able to completely reverse the effects of four days of habituation. A one way analysis of variance revealed a significant main effect ($F = 7.81$, $df = 2,11$, $p < .008$; see Table 2). Newman-Keuls tests revealed that the pre-exposure + vehicle animals ate significantly faster than both the vehicle control and pre-exposure + FG-7142 groups. The latter two groups did not differ significantly from each other.

These data clearly suggested that pre-exposure was capable of decreasing the latency for animals to begin eating in the testing apparatus. Therefore, exposing an animal to a novel environment over a period of four days, apparently reduced the fear associated with the test cage and on test day, these animals ate significantly faster than controls. Although they were not compared in the same experiment, a single dose of diazepam (Experiments 1 and 2) produced a slightly greater mean decrease in eating latency than 4 days of pre-exposure to the testing environment. An earlier study by Britton and Thatcher-Britton (1981) found similar results, but a methodological

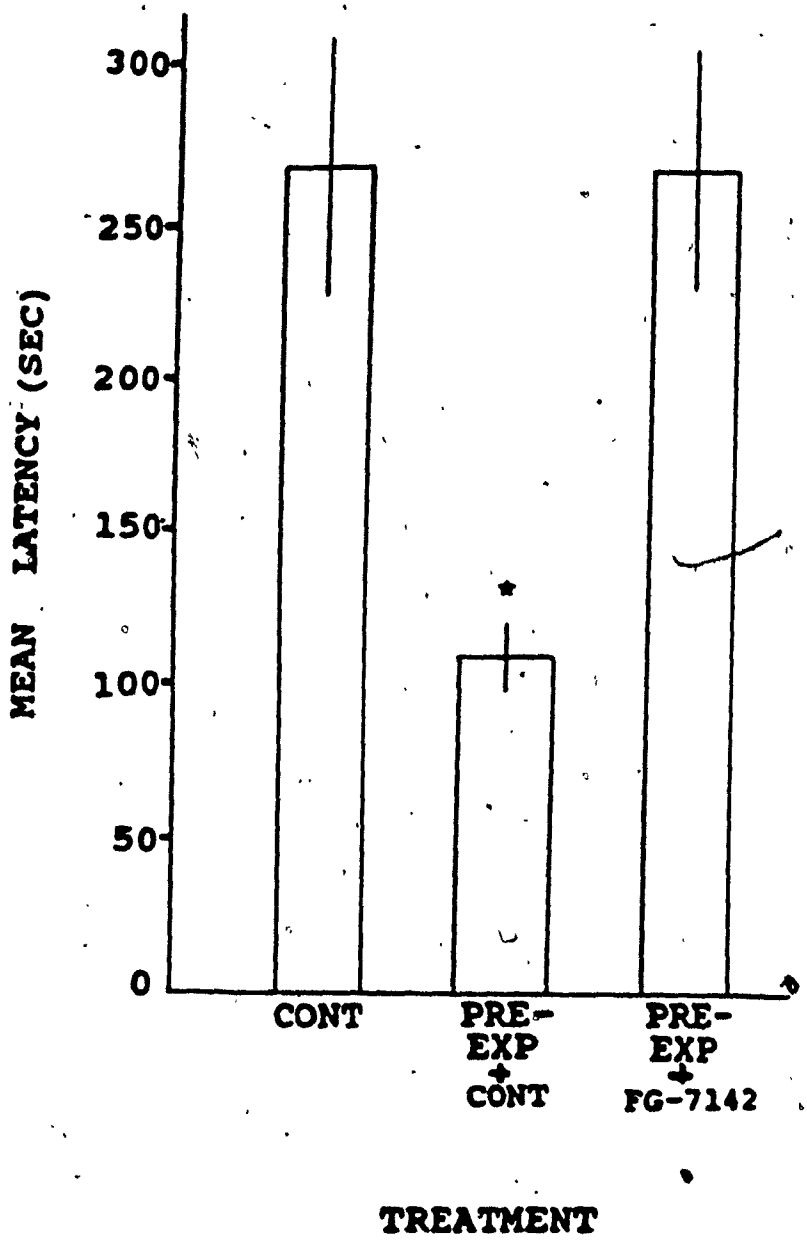

Figure Caption

Figure 1. Mean latency (sec) to begin eating in a novel environment following a single dose of vehicle control (CONT), four days of pre-exposure to the testing apparatus + a single dose of vehicle control (PRE-EXP + CONT), or four days of pre-exposure to the testing apparatus + a single dose of FG-7142 (PRE-EXP + FG-7142). An asterisk indicates a mean that is significantly different from control ($p < .05$).

Table 2

Source Table for the ANOVA of Control, Pre-Exposure + Vehicle, and Pre-Exposure + FG-7142

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	2	80954.61	40472.81	7.81	.000
Within	11	57030.75	5184.61		
Adjusted Total	13	137976.40			

change between the two studies could offer a possible explanation for the difference in effectiveness between the two conditions. These experimenters looked at the amount of food eaten in a testing environment over 7 consecutive days. Obviously with each subsequent exposure to the cage, the animals habituated to all the stimuli of the testing condition including the food. In the present experiment, animals were pre-exposed to the cages without food being present. Therefore, when the actual conflict behavior was measured, the presence of the food produced some fear-arousing cues thereby increasing latencies to begin eating. Also, the experiments of Britton and Thatcher-Britton, (1981) indicated that at least seven days of pre-exposure was necessary to produce an anxiolytic effect that was comparable to diazepam. Despite these procedural differences, pre-exposure to the testing apparatus for four days did produce a strong anxiolytic effect.

Interestingly, a single dose of the anxiogenic drug, FG-7142 is able to reverse the effects of pre-exposure in that these animals were not significantly different from controls. Since FG-7142 is an "inverse agonist" at the benzodiazepine receptor (Braestrup, Nielsen, Honore, Jensen, and Petersen, 1983), it is possible that the effects of pre-exposure to the testing apparatus were related to the benzodiazepine receptor. This could be

determined by administering the benzodiazepine receptor antagonist, Ro15-1788 prior to the injection of FG-7142. If the benzodiazepine receptor is important in mediating the effects of pre-exposure, then Ro15-1788 should reverse the effects of FG-7142.

Experiment 4

The purpose of the present experiment was to examine the effects of acute treatment with anti-depressants in the conflict test. As mentioned at the outset, chronic, but not acute anti-depressant treatment down-regulated the benzodiazepine receptor (Suranyi-Cadotte et al., 1984). Moreover, in many animal models of anxiety, acute anti-depressant treatment was found to be ineffective (Kilts, Commissaris, and Rech, 1981; Marriott and Smith, 1972; Poschel, 1971). The drugs of interest were: diazepam, adinazolam, desmethylimipramine (DMI), amitriptyline, chlorpromazine (CPZ), and vehicle control.

Adinazolam, a triazolobenzodiazepine in the development research phase, has been found to be a highly effective anxiolytic with anti-depressant activity (Amsterdam, Kaplan, Potter, Bloom, and Rickels, 1986). The tricyclic antidepressants, DMI and AMI were chosen to assess the specificity of the conflict paradigm. Only those drugs used in the alleviation of clinical anxiety should reduce the latency to begin eating in the test. In

general, acute anti-depressant treatment is without anxiolytic activity (Kilts, Commissaris, and Rech, 1981; Poschel, 1971). Chlorpromazine, an anti-psychotic with strong sedative effects, was chosen to assess the nature of the specificity of the anxiolytics in the conflict test. That is, were the anti-conflict effects of the benzodiazepines reflecting the anxiolytic or the sedative properties of these drugs? If the test was a sensitive measure of anxiety, and the effectiveness of anti-anxiety drugs, then CPZ should be ineffective.

Methods

Subjects. Thirty-six male Long Evans rats, were used in this experiment. The housing and feeding schedules were identical to those described in Experiment 1.

Apparatus. The testing apparatus was the same as described in Experiment 1.

Drugs. The drugs used in the experiment were diazepam, 2 mg/kg; CPZ (Largactil 50, Rhone-Poulenc Pharma Inc., Montreal, Canada, available in a concentration of 25 mg/ml), 5 mg/kg); adinazolam (Upjohn Pharmaceuticals, Kalamazoo, Michigan), 20 mg/kg; DMI (Merrell Dow Pharmaceuticals, Richmond Hill, Canada), 10 mg/kg; and amitriptyline, 10mg/kg (Merck, Sharp, and Dome Pharmaceuticals, Kirkland, Canada). Adinazolam, DMI, and

amitriptyline were dissolved in the saline/propylene glycol solution.

Procedure. The entire procedure for this experiment was the same as described in Experiment 1. Briefly, animals were food-deprived for 48 hours. One hour prior to behavioral testing, animals in each group received a single i.p. injection of the appropriate drug or vehicle. The mean latency to begin eating in the novel environment was determined one hour later.

Results and Discussion

Figure 4 represents the mean latency to begin eating, in seconds, for each treatment group. Both diazepam and adinazolam produced shorter eating latencies relative to controls (81% and 72.5% decrease respectively. Second, neither of the anti-depressants was effective in producing an anxiolytic effect. DMI produced a 14% increase while amitriptyline produced a 9% decrease in eating latencies relative to the controls. CPZ produced an 89% increase in eating latency relative to controls. In fact, only one of the six animals began eating within the 360-second time limit. A one-way analysis of variance indicated a significant treatment effect ($F = 15.35$, $df = 5,28$, $p < .001$; see Table 3). Post hoc Newman-Keuls tests revealed both diazepam and adinazolam groups ate significantly faster than the controls, DMI, and amitriptyline groups.

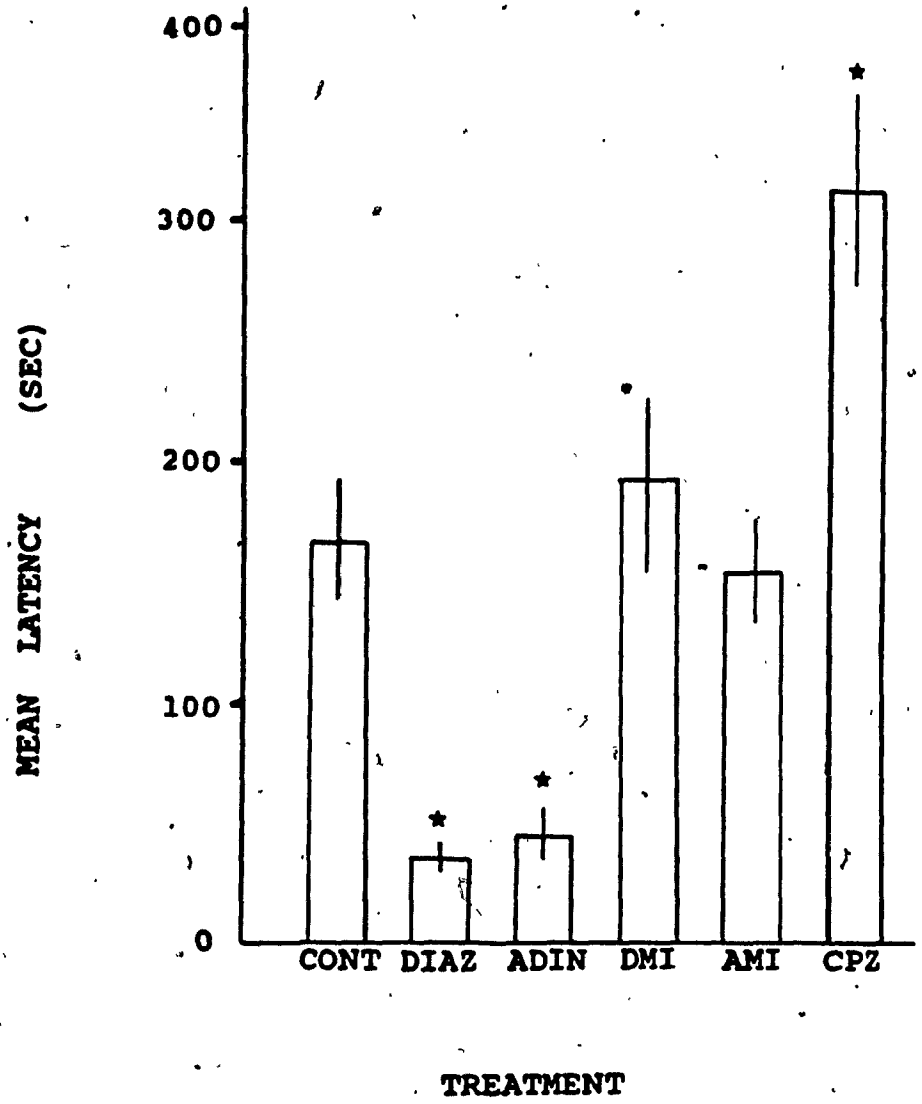

Figure Caption

Figure 4. Mean latency (sec) to begin eating in a novel environment following a single dose of vehicle control (CONT), diazepam (DIAZ), adinazolam (ADIN), desmethylimipramine (DMI), amitriptyline (AMI), or chlorpromazine (CPZ). An asterisk indicates a mean that is significantly different from control ($p < .05$).

Table 3

Source Table for the ANOVA of Acute Control, Diazepam, Adinazolam, Desmethylimipramine, Amitriptyline, and Chlorpromazine

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	5	333071.9	66614.38	15.35	.000
Within	28	121500.6	4339.31		
Adjusted Total	33	454572.5			

Neither of the anti-depressants differed significantly from the controls. A single dose of CPZ produced eating latencies that were significantly greater than control values.

The data clearly suggested that both diazepam and adinazolam produced significant anti-conflict effects inthis paradigm. Neither of the anti-depressants, nor CPZ (given at a dose producing sedation) were capable of attenuating the fearful cues associated with the novel environment and thereby minimizing the conflict. These data confirmed the findings from several other experiments (Kilts et al., 1981; Poschel, 1981).

Experiment 5

The issue being addressed here was the possible anti-anxiety effects of chronic anti-depressant treatment. Experiment 4 demonstrated that a single dose of a tricyclic anti-depressant had no anxiolytic activity in the novel environment. Could chronic anti-depressant treatment attenuate the novelty cues of an unfamiliar test environment and decrease the latency to begin eating? A second problem that was addressed here was the possible development of tolerance to the anxiolytic effects of chronic benzodiazepine treatment. There was some evidence at the behavioral (Treit, 1985) and neurochemical

(Vellucci and File, 1979) level, suggesting that the anxiolytic effects of the benzodiazepines diminished over time. Thus, in this experiment, we compared the latency to begin eating in animal treated for 21 days with diazepam, adinazolam, DMI, amitriptyline, CPZ, or vehicle control.

Methods.

Subjects. Forty-two male Long Evans rats were used in this experiment. The the beginning of the injections the rats weighed between 250-275 grams. The housing, feeding, and handling regimes were identical to those described in Experiment 1.

Apparatus. The testing apparatus was the same as described in Experiment 1.

Drugs. The drugs and dosages were identical to those described in Experiment 4.

Procedure. In pilot testing, the dosage of DMI used was 20 mg/kg. Unfortunately, two the the five animals died before ten days of injections were completed and the remaining animals weighed between 150-180 grams at the time of testing. It was therefore decided that the dosage should be reduced to 10 mg/kg. At this dosage, the mortality rate was 0%. Body weights were monitored during the course of study and careful attention was paid to the health of the animals.

Rats were injected daily for 21 consecutive days. On the 19th injection day, all food was removed from the home cage and on the 21st day, the final injection was given one hour prior to testing. The conflict paradigm was carried out in the manner described in Experiment 1.

Results and Discussion.

The behavioral effects of chronic anxiolytics or anti-psychotic, and chronic anti-depressant administration were determined on two different days, with a control group run on each day to control for daily variations in uncontrollable variables such as background noise. Although the anxiolytics and anti-depressants cannot be directly compared, a control group was run on both days and a common anxiolytic effect (expressed as a percentage of control values) could therefore be determined for each group. Figure 5 represents the mean latency to begin eating following chronic treatment of diazepam, adinazolam, CPZ, or vehicle control. These drugs produced an 83%, 85%, and 30%, decrease respectively, relative to the controls. A one-way ANOVA revealed a significant main effect ($F = 20.48$, $df = 3,19$, $p < .001$; see Table 4). Newman-Keuls tests revealed that both diazepam and adinazolam produced significant anti-conflict effects. The decrease in eating latency seen by CPZ was not significant.

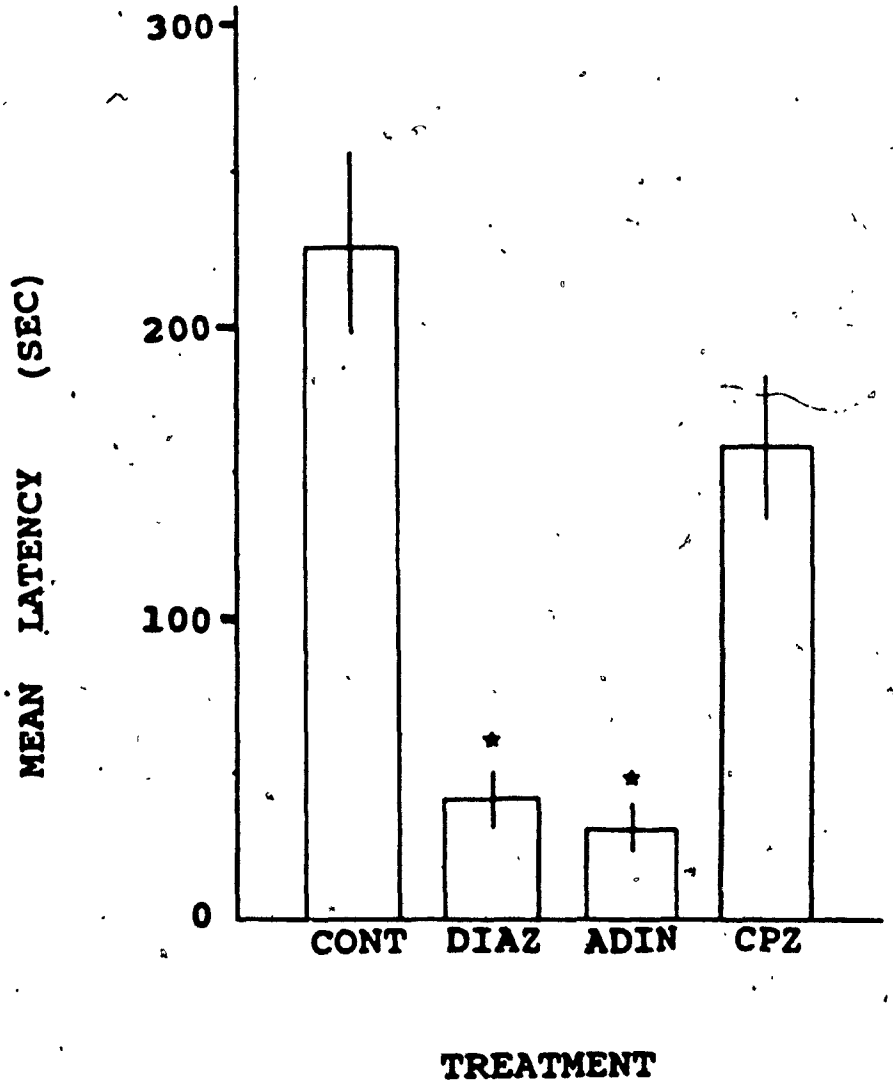

Figure Caption

Figure 5. Mean latency (sec) to begin eating in a novel environment following 21 days of treatment with either vehicle control (CONT), diazepam (DIAZ), adinazolam, (ADIN), or chlorpromazine (CPZ). An asterisk indicates a mean that is significantly different from the control group ($p < 05$).

Table 4

Source Table for the ANOVA for Day 21 Control, Diazepam, Adinazolam, and Chlorpromazine

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	3	161740.40	53913.48	20.48	.000
Within	19	50014.87	2632.36		
Adjusted Total	22	211755.30			

Figure 6 represents the mean eating latencies of the anti-depressants and vehicle control. Chronic DMI and amitriptyline treatment produced a 47% and 48% decrease respectively in latency to begin eating in the novel environment. A one-way analysis of variance revealed a significant treatment effect ($F = 16.2$, $df = 2,17$, $p < .001$; see Table 5). Post hoc Newman-Keuls tests indicated that both the DMI and the amitriptyline groups ate significantly faster than the controls.

Although the percentage decrease in eating latency following chronic anti-depressant treatment was not as great as that of chronic benzodiazepines, these animals did eat significantly faster than controls. By definition, this significant decrease represents an anxiolytic effect. Also, there did not appear to be any tolerance to the anxiolytic effect of chronic benzodiazepine administration, at least in the paradigm used here. In fact, it appears that adinazolam becomes more effective with 21 days of treatment.

Of interest was the observation that the CPZ animals did eat during the test compared with the acute condition in which only one animal ate within 360 seconds. Some motor coordination problems were noticeable, but the animals did explore the cage and rearing was also evident. Another difference between CPZ and the anxiolytics and anti-depressants was the pattern of the eating bouts.

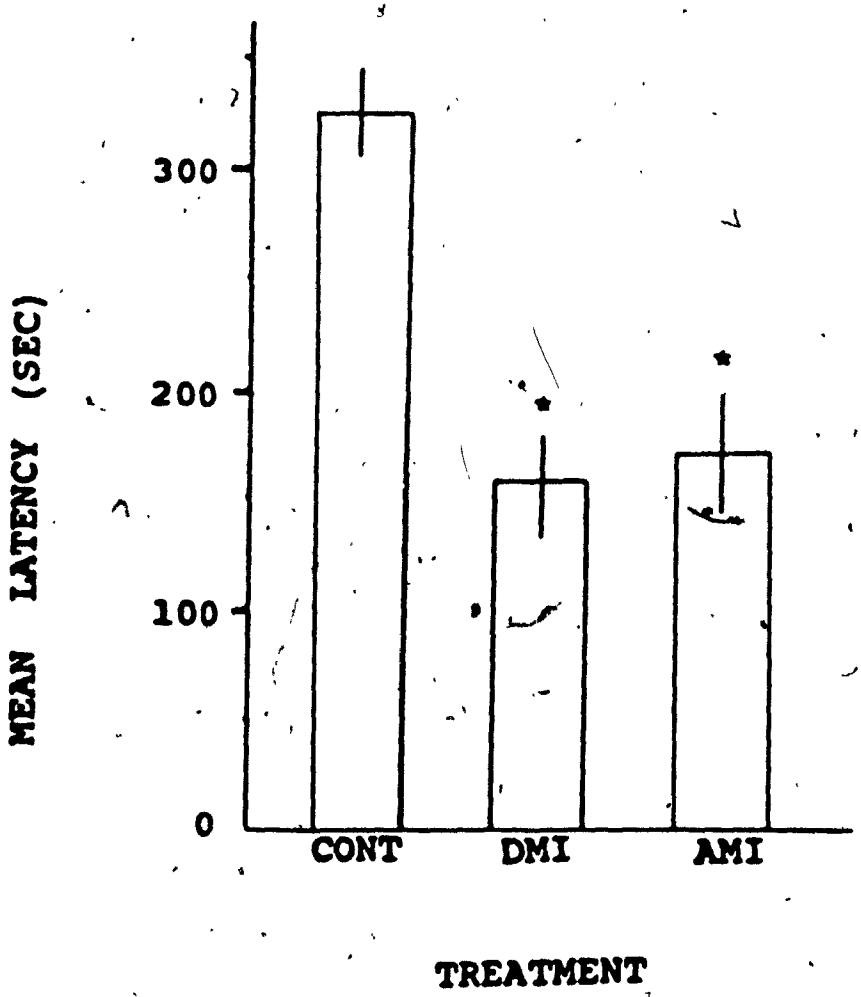

Figure Caption

Figure 6. Mean latency (sec) to begin eating in a novel environment following 21 days of treatment with either vehicle control (CONT), desmethylimipramine (DMI), or amitriptyline (AMI). An asterisk indicates a mean that is significantly different from control ($p < .05$).

Table 5

Source Table for the ANOVA of Day 21 Control, Desmethylimipramine, and Amitriptyline

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	2	93009.23	46504.61	16.2	.000
Within	17	48789.98	2869.99		
Adjusted Total	19	141799.20			

Experiment 6

The data from Experiments 4 and 5 suggested that the anxiolytic action of the anti-depressants occurred sometime between 1 and 21 consecutive days of treatment. As mentioned earlier, studies indicated that panic attacks, treated with imipramine, ceased within 3-14 days of treatment (Klein, 1964). Moreover, Kahn et al. (1986) demonstrated that the anxiolytic properties of imipramine occurred only after at least 14 days of treatment. Therefore, the purpose of Experiment 6 was to determine whether the time course of the development of the anxiolytic effect of anti-depressant administration followed the clinical literature. Another issue being addressed here was the possible role of the benzodiazepine receptor in mediating the anxiolytic effect of chronic anti-depressant treatment. Suranyi-Cadotte et al. (1984) demonstrated a down-regulation of the benzodiazepine receptor following chronic anti-depressant therapy and suggested that the benzodiazepine receptor might be the locus of action of the anxiolytic effects of these drugs. At low doses (less than 2 mg/kg), Ro15-1788, a BZR antagonist, had no pharmacological effect on its own, but could block the anxiolytic activity of the benzodiazepines if the two drugs are administered concurrently (Corda, Blaker, Mendelson, Guidotti, and Costa 1983). At doses greater than 8 mg/kg, the drug became anxiogenic in nature

and if administered alone, would decrease punished drinking (Corda et al., 1983). If chronic anti-depressant treatment attenuated anxiety in a manner similar to that of diazepam (i.e. via the benzodiazepine receptor), then it was expected that a single dose of Ro15-1788 would similarly block the anxiolytic effect of chronic DMI treatment. If the benzodiazepine receptor was not the mechanism of action for the anxiolytic action of the anti-depressants, then the receptor antagonist should not block the anti-conflict effects of these drugs.

Methods.

Subjects. Sixty male Long Evans rats were used in this experiments. The housing, feeding, and handling procedures were identical to those described in Experiment 1.

Apparatus. The testing apparatus was the same as described in Experiment 1.

Drugs. The drugs used were diazepam, DMI, and vehicle control. The dosages were identical to those in Experiment 4. A dosage of 1 mg/kg for Ro15-1788, was chosen to ensure that the drug did not produce an anxiogenic effect (greater than 8 mg/kg). Ro15-1788 was generously donated by Hoffmann-La Roche, Basel, Switzerland. It was dissolved in distilled water and a drop of Tween 80 per 5ml of water.

Procedure. Animals were injected with diazepam, DMI, or vehicle for either 1, 7, 14, or 21 consecutive days. Food deprivation began 48 hours prior to behavioral testing. Again, all animals were given a final injection 60 minutes prior to behavioral testing. For the final injection in the 21-day treatment group, the procedure was changed slightly. One hour prior to testing, half of the animals in each group received a preliminary injection of Ro15-1788, while the remaining animals received an initial injection of the vehicle. Two minutes later, all animals received a second injection of diazepam, DMI, or vehicle. All animals were observed in the conflict paradigm one hour later.

Results and Discussion.

Figures 7, 8, 9, and 10 represent the four individual test days with each drug group being compared to its control. Following 1, 7, 14 and 21 days of diazepam treatment, there was a decrease of 85%, 84%, 79.6% and 64% decrease in eating latency respectively. The time course of DMI treatment produced an initial 31% increase in eating latency following an acute dose, while 7, 14, and 21 days treatment produced an 8%, 44% and 37% decrease respectively. A one-way analysis of variance was conducted for each treatment duration. There was a significant main treatment effect following acute

treatment ($F = 7.43$, $df = 2,11$, $p < .009$; see Table 6). The Newman-Keuls tests revealed diazepam animals ate significantly faster than DMI and control groups. Moreover, DMI and control animals did not differ from each other. The data replicated the findings of Experiment 4. Following 7 days of treatment, there was a significant effect of treatment ($F = 6.21$, $df = 2,11$, $p < .016$; see Table 7). Post hoc Newman-Keuls tests revealed that only the diazepam group ate significantly faster than the controls. Seven days of DMI did not produce an anxiolytic effect.

Fourteen and twenty-one days of treatment produced significant main effects ($F_{14} = 14.82$, $df = 2,12$, $p < .001$; see Table 8; $F_{21} = 6.51$, $df = 4,23$, $p < .001$; see Table 9). Newman-Keuls tests showed that following either two or three weeks of both diazepam and DMI treatment, significant anxiolytic effects were observed. These data replicate the findings of Experiment 5.

The benzodiazepine receptor antagonist, Ro15-1788, reduced the latency to begin eating by 40% when givenprior to the 21st injection of diazepam, and by 30% when given prior to DMI. The ANOVA determined above for 21 days treatment included these 2 groups. The Newman-Keuls tests revealed that Ro15-1788 was not successful in blocking the anxiolytic effects of chronic diazepam or DMI treatment.

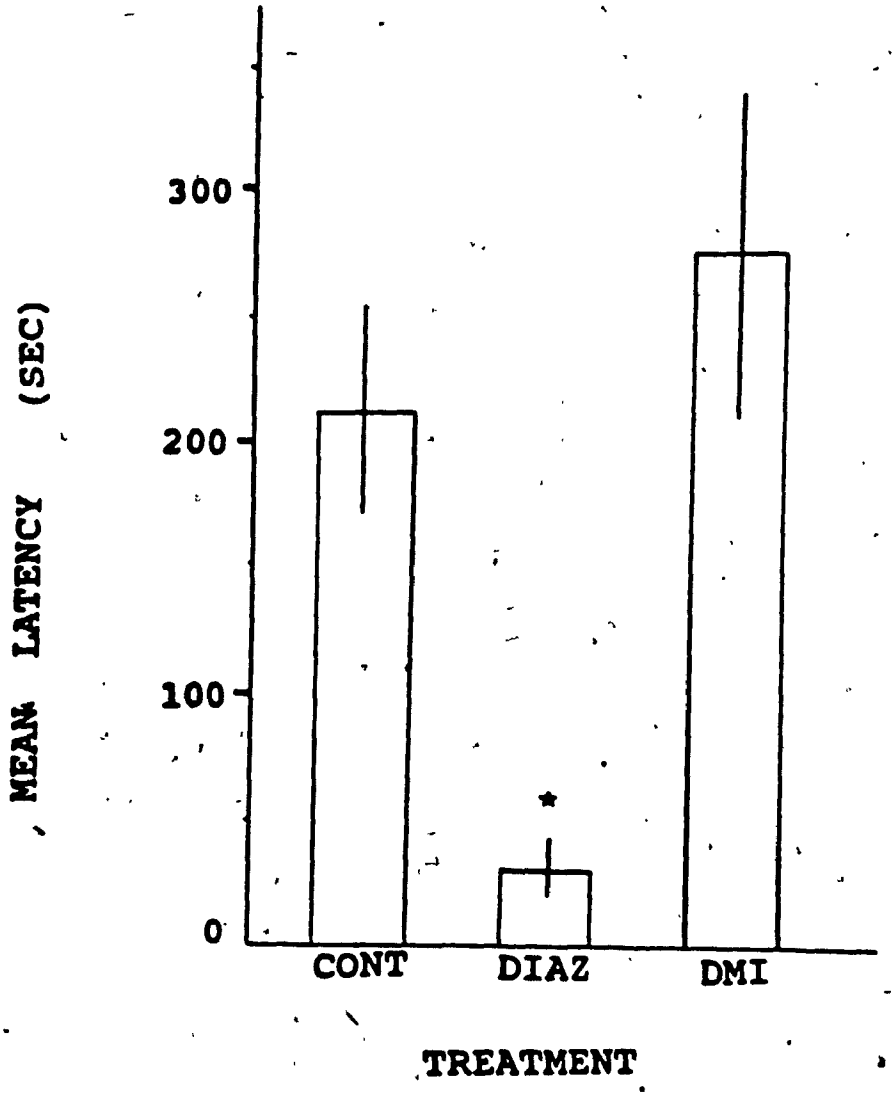

Figure Caption

Figure 7. Mean latency (sec) to begin eating in a novel environment following a single treatment with either vehicle control (CONT), diazepam (DIAZ), or desmethylimipramine (DMI). An asterisk indicates a mean that is significantly different from control ($p < .05$).

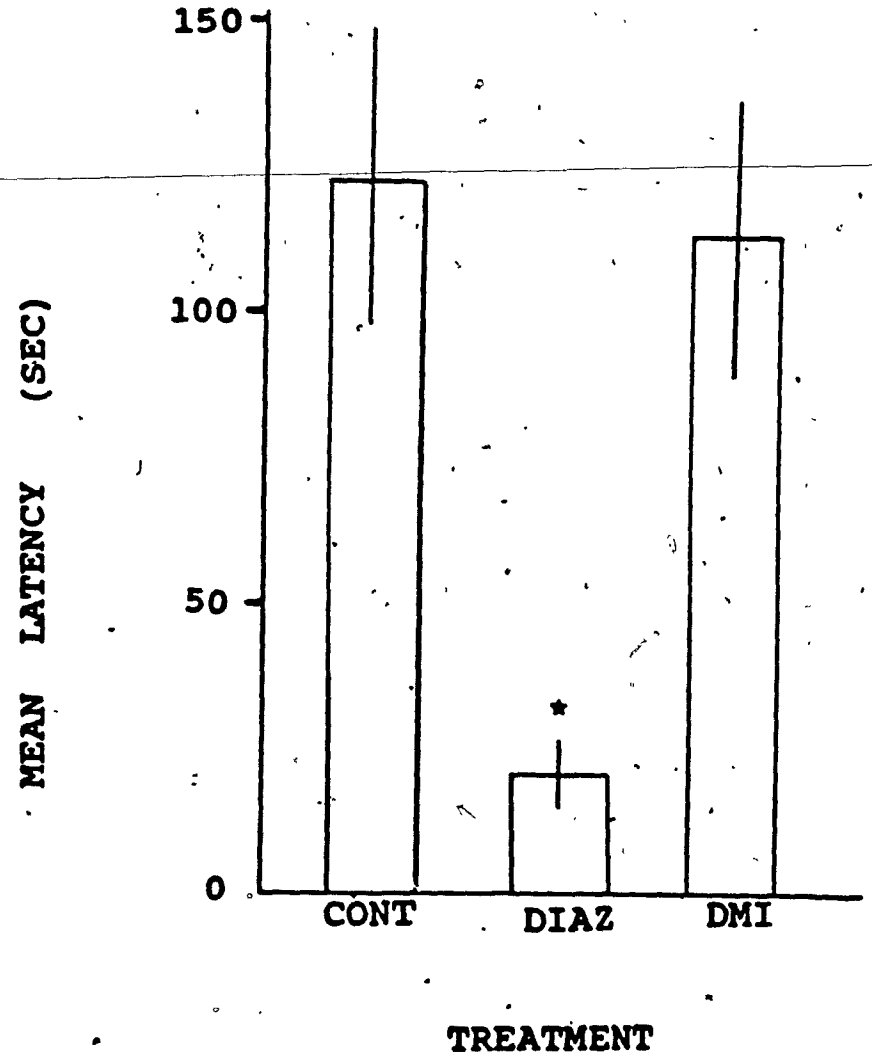

Figure Caption

Figure 8. Mean latency (sec) to begin eating in a novel environment following 7 days of treatment with either vehicle control (CONT), diazepam (DIAZ), or desmethylimipramine (DMI). An asterisk indicates a mean that is significantly different from control ($p < .05$).

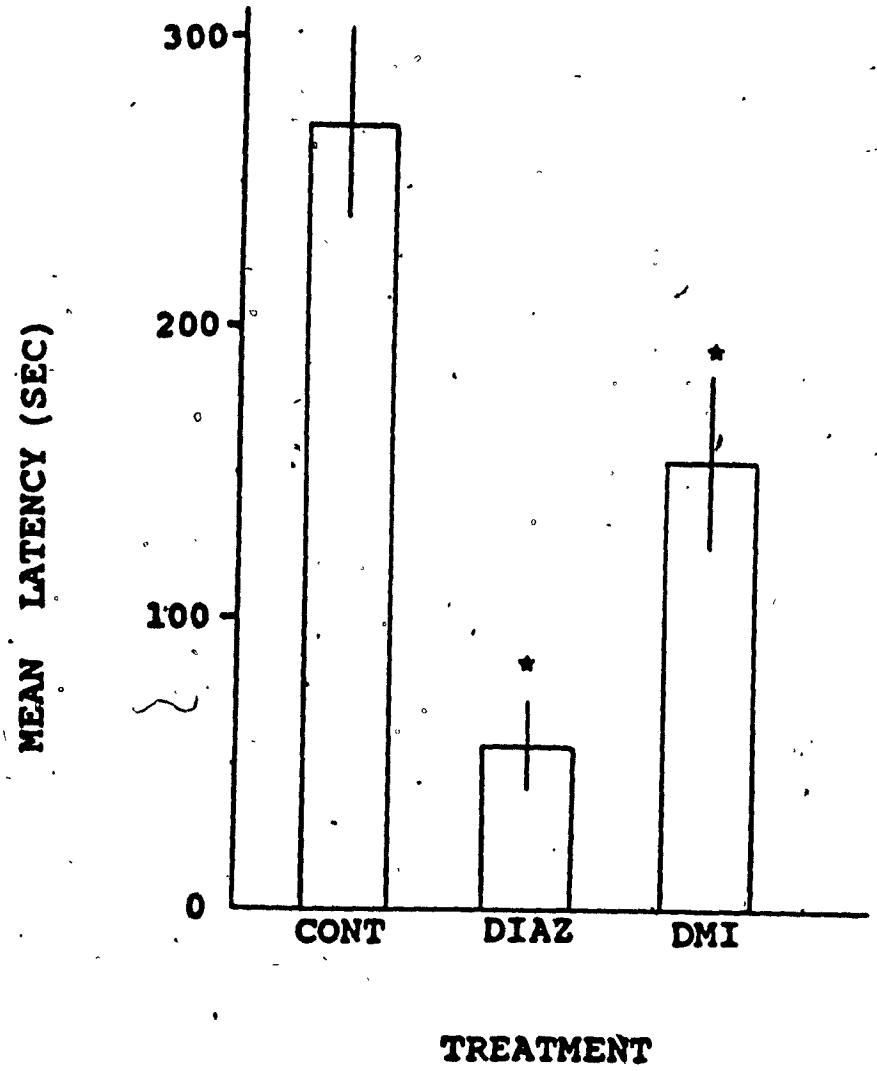

Figure Caption

Figure 9. Mean latency (sec) to begin eating in a novel environment following 14 days of treatment with either vehicle control (CONT), diazepam (DIAZ), or desmethylimipramine (DMI). An asterisk indicates a mean that is significantly different from control ($p < .05$).

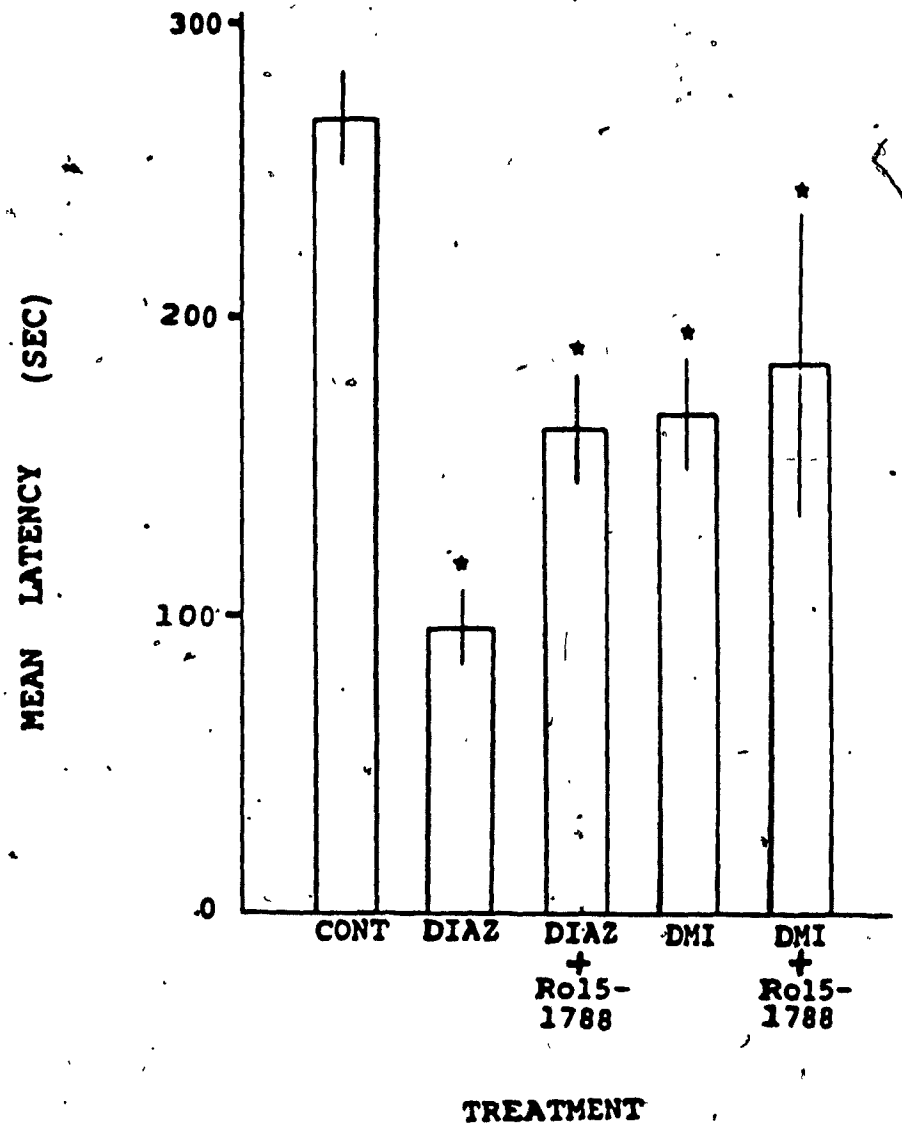

Figure Caption

Figure 10. Mean latency (sec) to begin eating in a novel environment following 21 days of treatment with either vehicle control (CONT), diazepam (DIAZ), diazepam + Ro15-1788 (DIAZ + Ro15-1788), desmethylimipramine (DMI), or desmethylimipramine + Ro15-1788 (DMI + Ro15-1788). An asterisk indicates a mean that is significantly different from control ($p < .05$).

Table 6

Source Table for the ANOVA of Day 1 Control, Diazepam, and Desmethylimipramine

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	2	102280.7	51140.33	7.43	.009
Within	11	75672.2	6879.29		
Adjusted Total	13	177952.9			

Table 7

Source Table for the ANOVA of Day 7 Control, Diazepam, and Desmethylimipramine

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	2	27057.86	13528.93	6.21	.016
Within	11	23977.00	2179.73		
Adjusted Total	13	51034.86			

Table 8

Source Table for the ANOVA of Day 14 Control, Diazepam, and Desmethylimipramine

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	2	116805.7	58402.87	14.82	.001
Within	12	47285.2	3940.43		
Adjusted Total	14	164090.9			

Table 9

Source Table for the ANOVA of Day21 Control, Diazepam, and Desmethylimipramine

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Between	4	100147.6	58402.87	6.51	.001
Within	23	88394.5	3843.43		
Adjusted Total	27	188542.1			

Clearly, the antagonist was able to partially block the anxiolytic effect of diazepam. The difference in mean latency to begin eating between diazepam + Ro15-1788 and diazepam alone was 24%. Interestingly, Ro15-1788, at a dose of 1mg/kg, was not able to block the anxiolytic effect of chronic DMI treatment.

The data suggested that the development of the anxiolytic activity of the anti-depressant, DMI, closely followed the time course established in the clinical literature. The literature consistently showed that a minimum of two weeks of treatment with anti-depressants was necessary for the alleviation of anxiety (Kahn et al., 1986). Acute and 7 days of treatment produced similar results. No anti-conflict effect of the anti-depressant was observed. In contrast, 14 and 21 days of DMI treatment produced a significant anxiolytic effect. Interestingly, in contrast to the findings of Experiment 5, the effectiveness of diazepam treatment decreased progressively over time. Acute treatment resulted in an 85% decrease in eating latency to a 64% decrease following chronic treatment. These data appeared to suggest that over time, diazepam may indeed begin to lose some of its effectiveness in the conflict paradigm. It was important to note that diazepam was still an extremely potent anxiolytic in this test.

Finally, although the particular dosage of Ro15-1788 did not produce a full blockade of the anti-conflict effect of diazepam, it did partially attenuate the anxiolytic effect of the benzodiazepine. In contrast, the benzodiazepine receptor antagonist was without effect when given prior to the 21st injection of DMI. That is, both DMI and DMI + Ro15-1788 produced similar decreases in eating latencies. In order to accurately assess the role of the benzodiazepine receptor in mediating the anxiolytic effect of chronic antidepressant treatment, it is imperative to select a dose that fully blocks the effect of diazepam in the conflict paradigm.

General Discussion

In a conflict test that is sensitive to the anxiolytic effects of diazepam and adinazolam, two weeks of treatment with DMI and three weeks of treatment with either DMI or amitriptyline significantly reduced the latency for rats to begin eating in a novel environment. Acute or seven days of treatment with these anti-depressants was without effect. These data offer behavioral verification of the anxiolytic effects of chronic, anti-depressant treatment.

A second issue concerns the magnitude of the anxiolytic effect of the anti-depressant treatment. In comparison to diazepam and adinazolam, the anxiolytic effect of chronic DMI or amitriptyline treatment could be considered as being moderate. Although diazepam, adinazolam, DMI, and amitriptyline all produced significant anxiolytic effects, the percentage decrease in latency to begin eating relative to controls, was greater in the diazepam group. Chronic benzodiazepine treatment produced a 64-85% decrease, while chronic anti-depressant treatment produced a 37-48% decrease in the latency to begin eating. The question here is whether anti-depressants could be as effective as diazepam in the conflict test. This could be resolved by a dose-response curve, measuring latencies to begin eating following

chronic administration with various doses of anti-depressants. Relevant here, are the results of an initial pilot study (see Experiment 5) using a 20 mg/kg dose of DMI. Although this dosage resulted in severe decreases in body weight over the 21 day injection period, the mean percentage decrease in eating latencies relative to controls in the conflict paradigm was 75+10.6%, well within the range of the benzodiazepines. This finding suggests that higher doses of DMI might produce latencies to begin eating that are comparable to those seen in benzodiazepine-treated animals and would underscore the importance of a dose-response study.

The impetus for this thesis was to assess the role of anti-depressants in anxiety and to begin to address the question of whether the anti-depressants and benzodiazepines reduced anxiety via the same mechanism. As mentioned earlier, this question was not answered adequately, since the results of the benzodiazepine receptor antagonist, Ro15-1788, were inconclusive. Although the role of the benzodiazepine receptor as a common mechanism of action between the benzodiazepines and anti-depressants remains to be clarified, the importance of this receptor in the anxiolytic effects of anti-depressants can still be addressed. Suranyi-Cadotte et al. (1984) demonstrated a significant decrease in benzodiazepine binding sites following chronic anti-

depressant treatment and suggested that this reduced receptor density might be correlated with the anxiolytic effect of these drugs. Such a relationship could be perfectly reasonable, if one assumes that the endogenous ligand for the benzodiazepine receptor is anxiogenic. Thus, a decrease in the number of binding sites available for an anxiogenic endogenous ligand, released under conditions of fear, might well result in a decrease in the degree of anxiety.

Speculation regarding the existence of an endogenous ligand is based upon the finding that the phylogenetic appearance of benzodiazepine receptor occurs with the boney fishes (Skolnick and Paul, 1981b). One is hard-pressed to believe that these receptors existed in anticipation of the development of the benzodiazepines millions of years later. The ligand might be viewed as being released during a situation associated with fear-inducing stimuli, and may bind to the benzodiazepine receptor. The search for the endogenous ligand has been extensive, and the benzodiazepine ligand, B-carboline-3-carboxylic acid ethyl ester (B-CCE), first discovered in human urine (Braestrup, Nielsen, and Olsen, 1980), was thought to be a naturally-occurring anxiogenic. Insel, Ninan, Aloi, Jimerson, Skolnick, and Paul (1984) demonstrated that the administration of low doses of B-CCE to rhesus monkeys produced a marked increase in behavioral

agitation, heart rate, blood pressure, and plasma cortisol levels. These findings were confirmed by Crawley, Ninan, Pickar, Chrousos, Linnoila, Skolnick, and Paul (1985). Moreover, these effects were similar to those experienced by healthy males who had received a closely-related analogue, FG-7142 (Dorow et al., 1983). The affinity of B-CCE for the benzodiazepine receptor (labelled by [^{3}H]clonazepam) was approximately equal to that of clonazepam (IC_{50} = 1.6 vs 1.2 nM) (Insel et al., 1984). Unfortunately, B-CCE has been found to be an artifactual by-product of the extraction technique, formed non-enzymatically from tryptophan-containing proteins that were subjected to the specific extraction and purification procedures used in these studies. The B-carbolines, therefore, do not appear to be the endogenous anxiogenic ligand (Braestrup and Nielsen, 1980). To date, the nature of the endogenous ligand(s) remains an open question.

Although the idea of anti-depressants reducing the availability of binding sites for an endogenous anxiogenic ligand remains attractive, it is difficult to reconcile with existing data on two animal models. The first of these models concerns two strains of rats selectively bred for fearfulness. The Maudsley reactive (MR) and the Maudsley non-reactive (MNR) rats have been selectively bred for high and low fearfulness respectively in the open field (Broadhurst, 1975). Thus, the MR rats show high

levels of ambulation and defecation compared to the MNR animals. Robertson, Martin, and Candy (1978) demonstrated significantly lower benzodiazepine binding (without a change in binding affinity) in the MR compared with the MNR rats. These differences were most pronounced in the hippocampus, hypothalamus, midbrain, and medulla-pons. These data suggest that low receptor levels correspond to high fearfulness in a novel environment.

A similar result has been observed in a paradigm involving the environmental regulation of benzodiazepine receptors during development. Animals were either handled (H) or left undisturbed (non-handled; NH) during the first 21 days of life. At approximately 90 days of age, males from both groups were sacrificed and [^{3}H]flunitrazepam binding was measured in whole brain. It was found that NH animals had significantly lower concentrations of benzodiazepine receptors relative to H animals (Bodnoff, Suranyi-Cadotte, Quirion, and Meaney, 1987). When tested in the conflict paradigm described here, H animals ate significantly faster than did NH animals (Bodnoff et al., 1987). Taken together, the data from the handling manipulations again suggest that a decrease in benzodiazepine receptor concentrations is associated with increased fear or anxiety in the conflict paradigm.

Unfortunately, the hypothesis proposed earlier implied that decreased benzodiazepine receptors render an

animal less susceptible to the endogenous anxiogenic ligand. This is, of course, opposite to the data derived from the Maudsley strain of rats and the handling manipulations. Non-handled animals, with lower benzodiazepine receptors relative to handled animal, showed increased fear (and increased eating latencies) in the novel environment. Moreover, anti-depressant treated animals, also with reduced benzodiazepine receptors, showed decreased fear (and decreased eating latencies) in the novel environment. Although both groups of animals have significantly reduced benzodiazepine receptor concentrations relative to their respective control groups, their behavior in the conflict paradigm is exactly opposite.

This ambiguity will remain until the exact nature of the endogenous ligand(s) is known. At that point it will also, of course, be important to examine its release under aversive conditions. For example, it may be that "non-anxious" animals, such as the MNR and H's, do not release the endogenous anxiogenic ligand. This would explain both the higher receptor densities (i.e. up-regulation in the absence of endogenous ligand) and the absence of fear responses either in the open-field or the conflict test. Moreover, the non-handled and Maudsley reactive rats are highly anxious because the endogenous anxiogenic ligand is hyperactive. Again, this could explain the decreased

benzodiazepine receptor levels and their fearfulness in the open-field and conflict test. Animals treated chronically with anti-depressant have decreased levels of benzodiazepine receptors (a pharmacological manipulation, that would, in this case, mimic a normal, adaptive response), but would otherwise be like the handled or non-reactive animals (low levels of endogenous anxiogenic ligand). Therefore, in the conflict paradigm, these animals behave similarly to the non-anxious animals. The receptor levels could then be considered as being less relevant in animals with low levels of the endogenous anxiogenic.

Since variations in benzodiazepine receptor densities may be unrelated to the anxiolytic effects of chronic anti-depressant treatment in the conflict paradigm, it becomes necessary to examine other systems through which the benzodiazepines and anti-depressants may exert their anxiolytic effects. The question, then, is whether the benzodiazepines and anti-depressants share a common mechanism of action in addition to any actions these drugs may have on the the GABA-benzodiazepine-Cl ionophore complex.

Of interest to the present discussion is the role of serotonin in anxiety, and there is a significant body of literature to implicate this neurotransmitter in anxiety (Iversen, 1984). Gray (1982) proposed the importance of

the ascending 5-HT system in anxiety based upon its innervation of the septo-hippocampal system (see Figure 11). Serotonergic innervation of limbic regions originates in median raphe nuclei, with a smaller contribution from the dorsal raphe. Efferents from the dorsal raphe innervate the ventral hippocampus and lateral septum. Ascending fibres from the median raphe enter the septum from the medial forebrain bundle and then reach the dorsal hippocampus via either the fimbria-fornix or the cingulum bundle. Finally, the median raphe innervates the medial septum via the medial forebrain bundle (Gray, 1982).

The role of serotonin in animal models of anxiety has been approached from several perspectives. For example, Soubrie, Thiebot, Jobert, and Hamon (1981) examined the effects of microiontophoretic application of chlordiazepoxide (CDP) and 5-HT, in the conditioned emotional response (CER) paradigm. In the CER paradigm, animals are trained to bar-press for food. Once stable baseline responding is achieved, an auditory stimulus is introduced, and this tone is paired with the delivery of shock. On subsequent trials, the tone is presented in the absence of the shock and the degree of behavioral suppression (i.e. reduced bar-pressing for food) is measured in response to the tone. Soubrie et al. (1981) found that injections of 5-HT into the dorsal raphe

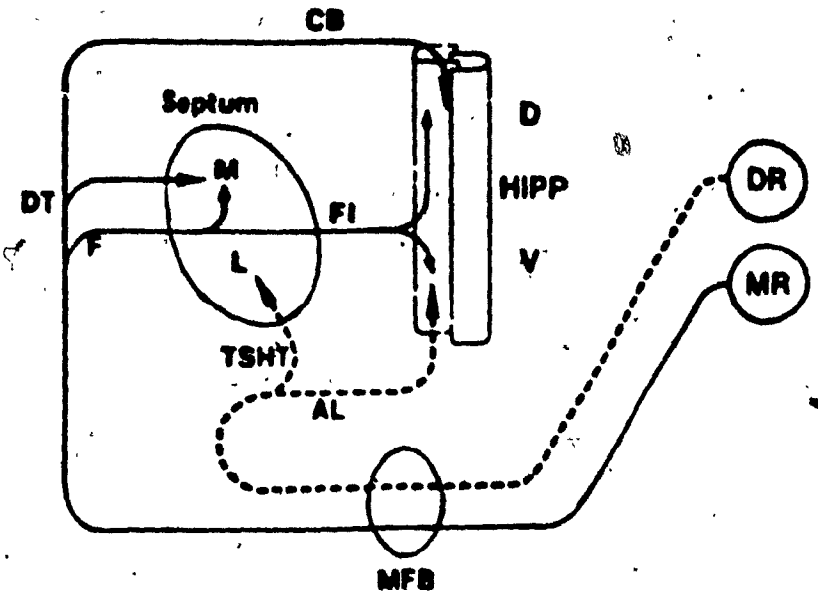

Figure Caption

Figure 11. Diagrammatic representation of the main projections of the serotonin axons to the septo-hippocampal complex. AL, ansa lenticularis; CB, cingulum bundle; D, dorsal and V, ventral hippocampus; DR, dorsal raphe; DT, diagonal tract; F, fornix column; FI, fimbria; L, lateral and M, medial septal nuclei; MFB, medial forebrain bundle; MR, median raphe; TSHT, septohypothalamic tract. (From Gray, 1982.)

produced an increase in responding that had previously been suppressed by the tone. The application of 5-HT into the raphe produces a decrease in serotonergic activity at limbic projection sites, perhaps due to its inhibitory effects upon serotonin receptors. The stimulation of these receptors by 5-HT or an agonist produces a decrease in release and turnover of 5-HT and decreased serotonergic activity in the septum and hippocampus (Haigler and Aghajanian, 1977). Therefore, if serotonin is applied at the level of the cell body, 5-HT turnover is decreased and there is a concomittant increase in responding that was originally suppressed by a tone. In a similar CER paradigm, Hartmann and Geller (1971) demonstrated that systemic administration of p-chlorophenylalanine (PCPA) reversed the behavioral suppression produced by the tone. PCPA decreases 5-HT levels by blocking the activity of the serotonin precursor, 5-hydroxytryptophan (5-HTP) (Feldman and Quenzer, 1984).

Another method of decreasing central 5-HT activity is by injecting 5,7-dihydroxytryptamine (5,7-DHT) into the ascending 5-HT fibres. This neurotoxin selectively destroys the 5-HT fibres (Feldman and Quenzer, 1984). Tye, Everitt and Iversen (1977) reported that 5,7-DHT significantly increased bar-pressing that had previously been suppressed by shock.

Conversely, increasing central serotonergic activity produces behavioral suppression. Aprison and Ferster (1961) trained pigeons to key-peck for food and subsequently paired delivery of food with shock. Iproniazid (a monoamine oxidase inhibitor) and 5-HTP produced significant decreases in responding when food delivery was paired with shock. Moreover, Stein and Berger (1973) using a Geller conflict paradigm, found that the increase in punished responding produced by the benzodiazepine, oxazepam, was blocked by an i.c.v. injection of 5-HT. Collectively, these data clearly indicate that antagonism of serotonin produces an increase in responding that was previously suppressed by punishment, while the enhancement of the serotonergic system appears to produce an anxiogenic effect.

The mechanism by which the benzodiazepines might produce their anxiolytic effects through the serotonergic system was examined by Wise, Berger, and Stein (1972). Rats received either one (acute) or six consecutive treatments with oxazepam, and the effects upon serotonin and norepinephrine (NE) turnover were subsequently determined. Following an acute dose, both 5-HT and NE turnover were reduced. Interestingly, following 6-days of treatment with the benzodiazepine, only the serotonergic turnover was still depressed. Tolerance had developed towards the effect upon norepinephrine. These findings

were also demonstrated at the behavioral level. Using the Geller conflict test, Margules and Stein (1968) found that the initial response to a high dose of oxazepam was sedation. That is, the benzodiazepines decreased both punished and unpunished responding. Drug-sophisticated rats (22-daily injections) demonstrated a different behavioral pattern. The decrease in unpunished responding, a measure reflective of sedation, was absent, whereas the increase in punished responding was maximal. These data suggest that the sedative effects of the benzodiazepines persist in drug-naive animals, while drug-sophisticated animals have become tolerant to these effects. These data, in conjunction with Wise et al. (1972) appear to attribute the anxiolytic action of the benzodiazepines to their continual suppressive effects upon serotonin. Moreover, the tolerance measured in norepinephrine turnover parallels the tolerance to the observed sedative effects of the benzodiazepines.

All of these data appear to suggest that the limbic system, especially the septum and hippocampus, plays an important role in the expression of anxiety and may suggest a neurological site of action for the anxiolytic drugs. Moreover, serotonergic projections from the raphe to these brain regions also appear to be implicated in anxiety, which suggests that this neurotransmitter may

represent a second mechanism (in addition to GABA) upon which the anxiolytics act.

The role of serotonin in anxiety has received considerable support. Interestingly, this neurotransmitter has also been implicated in the depressive state. New anti-depressants such as zimelidine, indalpine, and fluvaxamine, are relatively-selective serotonin uptake inhibitors (de Montigny and Blier, 1984). Their effectiveness suggests that low levels of central serotonin may exacerbate the depressive state. Understanding the mechanism of action of these drugs has received an increasing amount of attention. Presumably, administration of such drugs would produce an immediate increase in the availability of serotonin, yet the clinical research demonstrates that treatment must persist for at least two to three weeks before any alleviation of depressive symptoms is observable. Thus, it is unlikely that the re-uptake effect is central to the anti-depressant action of these drugs. This is a phenomenon common to all anti-depressants and the explanation can be found at the level of the receptor.

Several research groups have demonstrated that the acute administration of tricyclic anti-depressants and MAO inhibitors depress the firing rate of neurons in raphe nuclei (Sheard, Zolovick, and Aghajanian, 1972). De Montigny and Blier (1984) examined the responses of dorsal

raphe neurons in anesthetized rats following acute intraveneous injections of zimelidine or indalpine. With both drugs, there was a significant reduction of firing in these serotonin neurons compared to baseline firing rates. As discussed earlier, the decrease in firing is associated with the activation of serotonergic autoinhibition, which subsequently produces a decrease in the amount of the transmitter available post-synaptically (i.e. limbic structures). Moreover, animals were treated for either 2, 7, or 14 days with either zimelidine, indalpine or vehicle, and the spontaneous firing rate of the dorsal raphe nuclei was determined. Following acute treatment, the spontaneous firing rate was dramatically decreased. This finding replicates those of Sheard et al. (1972). By seven days of treatment, the activity of the dorsal raphe nuclei had increased significantly, but was still below control values. Two weeks of treatment returned the spontaneous firing of these neurons to control values, indicating a progressive recovery of spontaneous firing rate in the raphe cells. This recovery process was explained as a decreased sensitivity of the neurons to the presence of serotonin. This was demonstrated by measuring the firing rate of these raphe neurons to an i.v. injection of LSD in rats that were treated for 14 days with either indalpine or zimelidine. De Montigny and Blier found that the ED_{50} for LSD was increased 2.5 fold

in those animals that had been pre-treated with these serotonergic uptake inhibitors. That is, significantly more LSD was required in the anti-depressant treated animals to produce a 50% decrease in firing rate relative to controls. These data suggested that the raphe neuron was less sensitive to the inhibitory influences of serotonin. The densensitization of the raphe is also observed following chronic treatment with tricyclic anti-depressants (DMI and AMI) and MAO inhibitors (tranylcypromine). Three weeks treatment with these drugs produces a significant decrease in [^{3}H]spiroperidol binding, which, under the appropriate conditions, is a selective ligand for the 5-HT_2 receptor (Kellar, Cascio, Butler, and Kurtzke, 1980; Peroutka and Snyder, 1980). This receptor down-regulation is not found following acute treatment. Together, these data suggest that both the receptor down-regulation and desensitization of these raphe neurons correlates with the increase in central 5-HT levels following chronic anti-depressant treatment.

The development of the clinical anti-depressant effect of these uptake inhibitors closely follows the desensitization of the serotonergic raphe neurons. Acute treatment with the drugs results in an increase in the availability of serotonin in the raphe nuclei due to blockade of uptake of the transmitter. This produces a significant decrease in the firing of the raphe neurons.

Continued administration of the uptake blockers results in the desensitization of the raphe neurons and a subsequent return to normal firing rates of these neurons. Once the autoregulatory function of the serotonin receptor has become desensitized, these drugs will increase the availability of serotonin and alleviate the symptoms of depression.

The data from the serotonergic manipulations offer another paradox in the present thesis. That is, chronic anti-depressant treatment, which <u>increases</u> central serotonin levels, also produces an anxiolytic effect in the conflict paradigm. The evidence presented earlier suggests that anti-anxiety effects are produced by <u>blocking</u> serotonin, and that low levels of serotonin would be anticipated for an anti-conflict effect. The only way to address this issue is to suggest that the effects of anti-depressants upon serotonin levels in a home-cage experiment differs markedly to the effects these drugs have under conditions of stress or anxiety. That is, the work by de Montigny and Blier demonstrates that chronic anti-depressants decrease raphe firing rates and thereby increase serotonin levels. These experiments are performed on anesthetized animals. Moreover, the observed down-regulation of 5-HT_2 receptors following chronic anti-depressant treatment is found in a home-cage situation. Perhaps in an anxiety-provoking situation, serotonergic

activity following chronic anti-depressant treatment is different from that seen in home-cage or anesthetized conditions.

Clearly, both the benzodiazepine receptor and serotonin data leave unresolved the question of how anti-depressants exert their anxiolytic activity. Perhaps one approach is to reformulate the question. In addition to examining a common mechanism of action between the benzodiazepines and anti-depressants, it would also be fruitful to examine whether there is a common underlying basis for anxiety and depression. If anti-depressants are effective in treating depression because of their ability to reduce anxiety, then one might question whether the benzodiazepines might also be effective in the treatment of depression. Schatzberg and Cole (1978) reviewed 20 double-blind controlled studies in which a benzodiazepine and placebo, was compared with either a tricyclic anti-depressant or an MAO inhibitor in the treatment of depression. In none of the studies was the benzodiazepine significantly superior to the anti-depressant or placebo. The authors suggested that although the benzodiazepines could lessen the anxiety associated with the depressive state, these drugs were unable to alleviate the core symptom of depression.

There is some exciting research that may provide insight into the role of anxiety in depression. It has

been demonstrated in clinical studies that the triazolobenzodiazepine, alprazolam, is as effective as diazepam (Maletzky, 1980; Rickels, Csanalosi, Greisman, Cohen, Werblowsky, Ross, and Harris, 1983) or lorazepam (Ruiz, 1983) in the treatment of severe anxiety. The advantages of alprazolam is that there have been no indications of tolerance (Fawcett and Kravitz, 1982), a lower incidence of side effects, including sedation and drowsiness (Maletzky, 1980; Rickels et al., 1983) and absence of seizures following withdrawal from the drug (Fawcett and Kravitz, 1982).

Even more impressive than its efficacy in the treatment of anxiety is alprazolam's potential as an anti-depressant. Aden (1983) reported upon the efficacy of alprazolam in treating clinically anxious patients with associated depressed mood. More interesting are the controlled studies in which alprazolam was compared with imipramine in the treating of major depressive disorders (Fabre and McLendon, 1980; Rickels, Cohen, Csanalosi, Harris, Koepke, and Werblowsky, 1982). Both studies indicated that alprazolam was as effective as imipramine in alleviating the depressive symptoms. Moreover, there were several important differences in favor of alprazolam over imipramine. First, in terms of mg/kg daily dosages, the mean alprazolam dosage was 2.6 mg/kg versus 128.4 mg/kg for imipramine patients (Fabre and McLendon, 1980).

Second, the incidence of anti-cholinergic side-effects associated with tricyclics, such as dizziness and dry-mouth, were noticeably absent with alprazolam (Fabre and McLendon, 1980; Rickels et al., 1982). Finally, alleviation of the depressive symptoms by alprazolam occurred much earlier in the treatment than with imipramine. Significant improvements on the Hamilton Psychiatric Rating Scale for Depression were observed by the end of the first week of treatment with alprazolam (Fabre, 1976; Fabre and McLendon, 1980; Rickels et al., 1982). Consistent with earlier literature, at least 14 to 21 days of treatment was necessary to observe any improvement with imipramine.

The efficacy of alprazolam in the treatment of depression, combined with the data suggesting that the 1,4-benzodiazepines are considered ineffective in these disorders, strongly suggest that alprazolam has anti-depressant activity that is independent from its anxiolytic effects. Unfortunately, relatively little is known about the mechanism of action of alprazolam. The drug does not block reuptake of NE at the synaptic cleft, inhibit monoamine oxidase, or affect 5-HT metabolism (Fawcett and Kravitz, 1982), suggesting that the mechanism of action is different from other anti-depressants. Once the mechanism of action of this anxiolytic-anti-depressant

is determined, it may provide further insight into a common link between anxiety and depression.

There have been relatively few animal studies addressing this relationship between anxiety and depression. An study by Drugan, Maier, Skolnick, Paul, and Crawley (1985) offers some interesting insight into this issue. Rats received 80 inescapable tail-shocks and 24-hours later were tested for escape learning in a two-way shuttlebox. In general, previous exposure to inescapable shock produces a deficit in the ability to learn the appropriate behavioral response when escape becomes possible (Maier and Seligman, 1976). Moreover, this model has been considered analagous to the depressive state (Maier and Seligman, 1976). Another group of rats received a single i.p. injection of the anxiogenic B-carboline, FG-7142 and were similarly tested in the shuttle-box 24-hours later. Both inescapable shock and FG-7142 produced marked deficits in escape learning relative to controls. Interestingly, the behavioral deficit was blocked by the benzodiazepine receptor antagonist, Ro15-1788 suggesting the deficit was mediated through this receptor. These data offer support that anxiety may predispose an animal to display behaviors associated with depression when exposed to an inescapable, stressful situation.

In another study, Little, Stanford, and Taylor demonstrated that a single dose of FG-7142 produced a significant up-regulation of B-adrenergic receptors one week later. Increased B-receptors densities are a consistent marker of depression (Biegon, 1983) Again, these data suggest a correlation between anxiety and depression.

For future studies, it would be very important to address the issue of the predisposing influence of anxiety on the development of depression (Anisman and Zacharko, 1982). Moreover, what are the behavioral effects of chronic anxiety. Does chronic anxiety produce behavioral depression? If so, is that depression responsive to benzodiazepines or anti-depressant treatment?

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