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Platelet [^{3}H]Imipramine and [^{3}H]Paroxetine Binding Sites in Depression, Anxiety and Stress, and their Association with Symptoms and Risk Factors for Depression

Linda Joy Iny

A Thesis

in

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ABSTRACT

Platelet [^{3}H]Imipramine and [^{3}H]Paroxetine Binding Sites in Depression, Anxiety and Stress, and their Association with Symptoms and Risk Factors for Depression

Linda Joy Iny, Ph.D.
Concordia University, 1992

The literature has strongly suggested a role for serotonin (5-HT) in the link between depression, anxiety and stress. Alterations in 5-HT neurotransmission have also been associated with specific symptoms and psychosocial risk factors for depression. The present series of studies was conducted in order to further investigate these findings, using [^{3}H]imipramine and [^{3}H]paroxetine binding on blood platelets as indirect markers of 5-HT function. Previous studies have indicated a relationship between these binding sites and the 5-HT transporter complex.

A pilot study provided preliminary evidence of a significant, positive association between the maximal binding capacity (B_{max}) of [^{3}H]paroxetine, a recently developed ligand, and the maximum velocity of 5-HT uptake on blood platelets of healthy volunteers. Platelet [^{3}H]imipramine and [^{3}H]paroxetine binding were then measured in patients with major depression, dysthymia, generalized anxiety and panic disorder, and in healthy volunteers. Compared to controls, the B_{max} for [^{3}H]imipramine was lower in all patient groups. The density of [^{3}H]paroxetine binding was significantly reduced in platelets from patients with anxiety disorders, but not in platelets of patients with depression or dysthymia. No differences in the affinity constant (K_d) were observed. There was a

significant positive correlation between hopelessness and the K_d for [^{3}H]imipramine, however, no other associations between hopelessness and biological measures were found. In the final study, a significant decrease in platelet B_{max} values for [^{3}H]imipramine and [^{3}H]paroxetine was observed in students during examination stress compared to a period after vacation, but no change in K_d. In addition, perception of social support correlated with the [^{3}H]imipramine B_{max} in the expected direction during examinations, and contributed significantly to the prediction of B_{max} values by depressive symptomatology.

The results are discussed in relation to the notion that there is a neurochemical link between depression, anxiety and stress, and that disturbances in neurochemical functioning may be associated with depressive symptomatology, independent of psychiatric diagnosis. In addition, it is suggested that psychosocial factors may be associated with alterations in serotonergic neurotransmission, thus rendering an individual more physiologically vulnerable to psychological disturbance.

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DEDICATION

To my parents, for their all around, whole-hearted, never-ending support, throughout the course of my, at times, seemingly never-ending, academic training.

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INTRODUCTION

During the past several years there has been growing interest in the phenomenological and neurobiological relationship between depression and anxiety. This interest follows from clinical observations that patients with an anxiety disorder commonly receive a secondary diagnosis of depression (Dealy, Ishiki, Avery, Wilson and Dunner, 1981), and that patients with an initial diagnosis of anxiety often develop a depressive syndrome (Clancy, Noyes, Hoenk and Slymen, 1979). Similarly, patients with major depression often have concommitant symptoms of anxiety or history of an anxiety disorder (Clayton, Grove, Coryell, Keller, Hirschfeld and Fawcett, 1991; Leckman et al., 1983). Further evidence of a link between depression and anxiety is available from reports of the effects of antidepressant drugs in the treatment of patients with anxiety disorders (Fontaine, Chouinard and Iny, 1985; Kahn et al., 1986; Klein, 1964; Thoren et al., 1980) as well as in animal studies of anxiety (e.g. Bodnoff, Suranyi-Cadotte, Quirion and Meaney, 1989).

Another body of literature which links depression with heightened states of emotional arousal is that which describes the relationship between depression and stress. Several investigators have reported an association between stressful life events and the onset of depression (Brown, Harris and Peto, 1973; Paykel, 1978; Paykel et al., 1969), especially with events that are uncontrollable (Leff, Roatch and Bunney, 1970; Lloyd, 1980), or that involve the loss of a significant person from one's social environment (Fava, Maas and Dekirmenjian, 1981; Paykel et al.,

1969). A link between depression and stress is also suggested from observations that changes occurring in response to stress, such as dysphoric mood, irritability, diminished ability to concentrate, decreased appetite and libido, and increased hypothalamic-pituitary-adrenal and sympathetic activity are features commonly seen in depression (Breier et al., 1987; Gold, Goodwin and Chrousos, 1988).

Indeed, acute inescapable stress in animals has been shown consistently to produce behavioral responses as well as neurochemical deficits that are also observed in depression (see Table 1). When exposed to stress, animals are reported to exhibit early-morning awakening, decreased feeding and libido, decreased grooming, and helplessness (Anisman, de Catanzaro and Remington, 1978; Stone, 1978; Weiss, Goodman, Losito, Corrigan, Charry and Bailey, 1981). These behavioral changes are accompanied initially by an increase in the utilization and synthesis of brain norepinephrine (NE), dopamine (DA) and serotonin (5-HT; Thierry, 1973; Johnston, Demarest and Moore, 1984), and can lead to amine depletion (Anisman and Zacharko, 1982; Weiss, Glazer and Pohorecky, 1976). Under continued, short-term stress, a series of adaptive neurochemical changes takes place, including a decrease in utilization rates while increased synthesis persists, resulting in increased concentrations of monoamines to levels observed in nonstressed animals (Irwin and Anisman, 1984). In addition, down-regulation of ß-noradrenergic (ß-NE) receptors (Nomura, Watanabe, Ukei and Nakazawa, 1981; Stone, 1983) and reduced NE-stimulated cyclic AMP

Table 1

Similarities Between Stress, Depression and Antidepressant Treatments

Acute Stress	Depression
- Arousal, alertness	- Dysphoric hyperarousal and anxiety
- Increased vigilance, focussed attention	- Hypervigilance, constricted focus, obsessionalism
- Decreased eating	- Decreased eating
- Decreased libido, sexual behavior	- Decreased libido, sexual behavior
- Appropriate caution, restraint	- Excessive caution

Short-term Stress	Antidepressant Treatments
- Decreased density of ß-NE receptors	- Decreased density of ß-NE receptors
- Reduced responsiveness of the NE-sensitive adenylate cyclase system	- Reduced responsiveness of the NE-sensitive adenylate cyclase system
- Reduction in anorexia, body weight loss, passivity	- Reduction in depressive symptomatology

formation have been reported to occur (Stone, 1983). These changes have been proposed to compensate for sustained monoaminergic turnover (Anisman and Zacharko, 1982). Long-term, chronic or repeated stress results from failure of the animal to cope behaviorally with the stressor following neurochemical adaptation, leading to monoaminergic depletion and exhaustion of the animal. In long-term, chronic stress, the animal continues to respond to repeated exposure to the stressor as though it is seeing it for the first time.[1]

Stone (1983) suggested that the increased release of brain NE, at levels which occur during acute exposure to stress, could lead to depression under conditions of chronic stress, and that receptor subsensitivity might act to mitigate such an effect He further suggested that short-term stressors would precipitate NE down-regulation as an adaptive response, much in the same way as tricyclic antidepressants (Sulser, 1984; Golden et al., 1988). Thus, when an antidepressant drug induces subsensitivity to NE in the brain, it is thought to be mimicking an adaptive neurochemical change that normally occurs during successful adaptation to stress. This is supported by observations that a gradual reduction in the behavioral signs of distress occurring in response to short-term stress parallel the reduction in depressive symptomatology seen in patients over the course of

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[1]The term "acute" stress in this thesis refers to single exposure to a stressor of reasonably short duration, and is measured in minutes or hours rather than days. "Short-term" stress refers to exposure to stressors of longer duration, but which terminate prior to evidence of behavioral or neurochemical deficits. The terms "chronic" and "repeated" stress are used interchangably to refer to long-term exposure to a stressor.

chronic treatment with antidepressants (Stone, 1979; see Table 1). Interestingly, both the behavioral and neurochemical effects of stress are prevented by treatment with antidepressant drugs (see Anisman and Zacharko, 1982).

Taken together, these observations provide considerable evidence for an association between depression, anxiety and stress. Further support for a link between affective and anxiety disorders and stress is available from neurochemical studies.

Neurochemical Alterations in Depression, Anxiety and Stress

Early investigations into the neurochemistry of depression were prompted by observations of the effects of various drugs on mood or behavior. The antihypertensive drug, reserpine, which produces a marked depletion of monoamines, was reported to cause severe depression in some patients (Harris, 1957). Conversely, elevation of mood was observed in pulmonary tuberculosis patients treated with iproniazid (Selikoff, Robityk and Ornstein, 1952; Bloch, Dooneief, Buchberg and Spellman, 1954), an inhibitor of monoamine oxidase, an enzyme involved in the catabolism of monoamines (Zeller, Barsky and Berman, 1955). Subsequent investigations (Crane, 1957; Kline, 1958) indicated that monoamine oxidase inhibitors (MAOIs) may exert their antidepressant effect by increasing the availability of monoamines. These observations led to the formulation of the catecholamine hypothesis of depression (Schildkraut, 1965), which suggested that a functional deficit of NE in brain regions involved in the control of emotion may be responsible for depression in man. Shortly thereafter,

Coppen (1967) presented the indoleamine hypothesis of depression, which proposed a functional deficit of 5-HT in endogenous depressive illness. More recent studies have supported the roles of altered NE, and especially 5-HT function in depression, and suggest that such alterations may form a common link between depression, anxiety and stress. The focus of the present investigation is on the 5-HT system.

Depression and 5-HT. The first direct observation linking depression and 5-HT was the finding that tryptophan, an amino acid necessary for the synthesis of 5-HT, potentiated the antidepressant activity of MAOIs (Coppen, Shaw and Farrell, 1963). The results of recent studies indicate that dietary tryptophan depletion specifically reduces brain 5-HT function (Young, Ervin, Pihl and Finn, 1989) and leads to mild impairment in attention and increased subjective reports of negative mood, without clinical depression, in healthy male subjects (Smith, Pihl, Young and Ervin, 1987; Young, Smith, Pihl and Ervin, 1985). Recently, the depletion of tryptophan through dietary methods was found to lead to a clinically significant return of depressive symptoms in 14 of 21 remitted depressed patients receiving antidepressants, with return to the remitted state once regular food intake was resumed (Delgado et al., 1990). Similarly, depletion of 5-HT levels with the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) has been shown to reverse antidepressant drug effects within 24 hours in patients with major depression (Shopsin, Gershon, Goldstein, Friedman and Walk, 1975; Shopsin, Friedman and Gershon, 1976).

Reduced levels of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) have also been associated with depression in several studies (Asberg, Thoren, Traskman, Bertilsson and Rineberger, 1975; Banki, 1977; Coppen, Prange, Whybrow and Noguerra, 1972), suggesting a deficiency in the synthesis, release, or metabolism of 5-HT in depressed patients. Evidence for altered 5-HT function in depression is also available from studies based on post-mortem examinations in which low levels of 5-HT and 5-HIAA have been found in the brains of depressed suicide victims (Shaw, Camps and Eccleston, 1967; Lloyd, Farley, Dick and Hornyiewicz, 1974). A compensatory increase in 5-HT_2 receptor binding, which is considered to be secondary to reduced serotonergic activity, has also been observed in victims of suicide (Arango et al., 1990).

Studies using blood platelets, an accessible peripheral model for studying the presynaptic uptake, storage and release of 5-HT, have generally found that 5-HT uptake by platelets is significantly reduced among depressed patients (Coppen, Swade and Wood, 1978; Tuomisto, Tukianen and Ahlfors, 1979; Pecknold, Suranyi-Cadotte, Chang and Nair, 1988), resulting in increased synaptic levels of 5-HT. Both platelets and serotonergic neurons possess a high-affinity active transport mechanism for 5-HT located in the cellular membrane (Keyes and Rudnick, 1982). Furthermore, the biochemical, kinetic and pharmacological properties of the 5-HT uptake site are similar in both platelets and serotonergic synaptosomes (Sneddon, 1973; Stahl and Meltzer, 1978). Blood platelets of

depressed patients have also been determined by several investigators to be deficient in high-affinity binding sites for the tricyclic antidepressant, [^{3}H]imipramine (Briley, Langer, Raisman, Sechter and Zarifian, 1980; Paul, Rehavi, Skolnick, Ballenger and Goodwin, 1981; Suranyi-Cadotte, Wood, Schwartz and Nair, 1983). [^{3}H]Imipramine binding sites have been functionally and structurally associated with the 5-HT transporter complex, and it has been suggested that these sites regulate the uptake of 5-HT (Langer, Moret, Raisman, Dubocovich and Briley, 1980; Paul et al., 1981). A decrease in the density of [^{3}H]imipramine binding sites has also been reported in post-mortem analyses of the brains of depressed patients (Perry, Marshall, Blessed, Tomlinson and Perry, 1983; Stanley, Virgilio and Gershon, 1983). In addition, in recent studies, 5-HT$_2$ receptor binding has been reported to be increased in platelets of depressed patients compared to normal controls (Arora and Meltzer, 1989; Bigeon, Weizman, Karp, Ram and Wolf, 1987; Mikuni et al., 1991).

Taken together, the results of these studies have formed the basis for the 5-HT hypothesis of depression. However, probably the strongest line of evidence in support of the role for 5-HT has emerged from studies on the mechanism of action of antidepressant treatments. The results of these studies suggest that the therapeutic effects of chronic antidepressant treatments, including electroconvulsive shock therapy (ECT) and a broad range of antidepressant drugs occur through the enhancement of 5-HT neurotransmission, primarily within the raphe-hippocampal 5-HT system. These findings are derived mainly from single-

cell recording studies in rat brain by de Montigny and colleagues using in vivo electrophysiology with microiontophoretic application of 5-HT or 5-HT agonists, a technique which permits direct assessment of the effect of antidepressant treatments on identified pre- and postsynaptic neurons (Blier and de Montigny, 1985; see Figure 1).

Studies on the mechanism of action of antidepressants have found that animals treated with various types of tricyclic antidepressant drugs for 14 days showed an increase in the firing rate of postsynaptic hippocampal neurons in response to the microiontophoretic application of 5-HT (de Montigny, Chaput and Blier, 1989; Wang and Aghajanian, 1980). Similar results have been reported for animals receiving a series of ECT treatments (de Montigny et al., 1989). The clinical relevance of this effect is indicated by observations that the increased responsiveness of postsynaptic 5-HT hippocampal neurons to microiontophoretically-applied 5-HT does not occur following acute treatment, but rather follows a time course consistent with the delayed onset of therapeutic effect in humans (Oswald, Brezinova and Dunleavy, 1973).

Enhancement of 5-HT neurotransmission has also been obtained by MAO type "A" inhibitors, drugs which deaminate NE and 5-HT in addition to DA (Yang and Neff, 1974), thus increasing the availability of these amines in the synaptic cleft (Aghajanian, Graham and Sheard, 1969). Acute treatment with MAO-A inhibitors has been reported to initially decrease the firing rate of 5-HT neurons in the dorsal raphe, followed by the normalization of firing activity after treatment

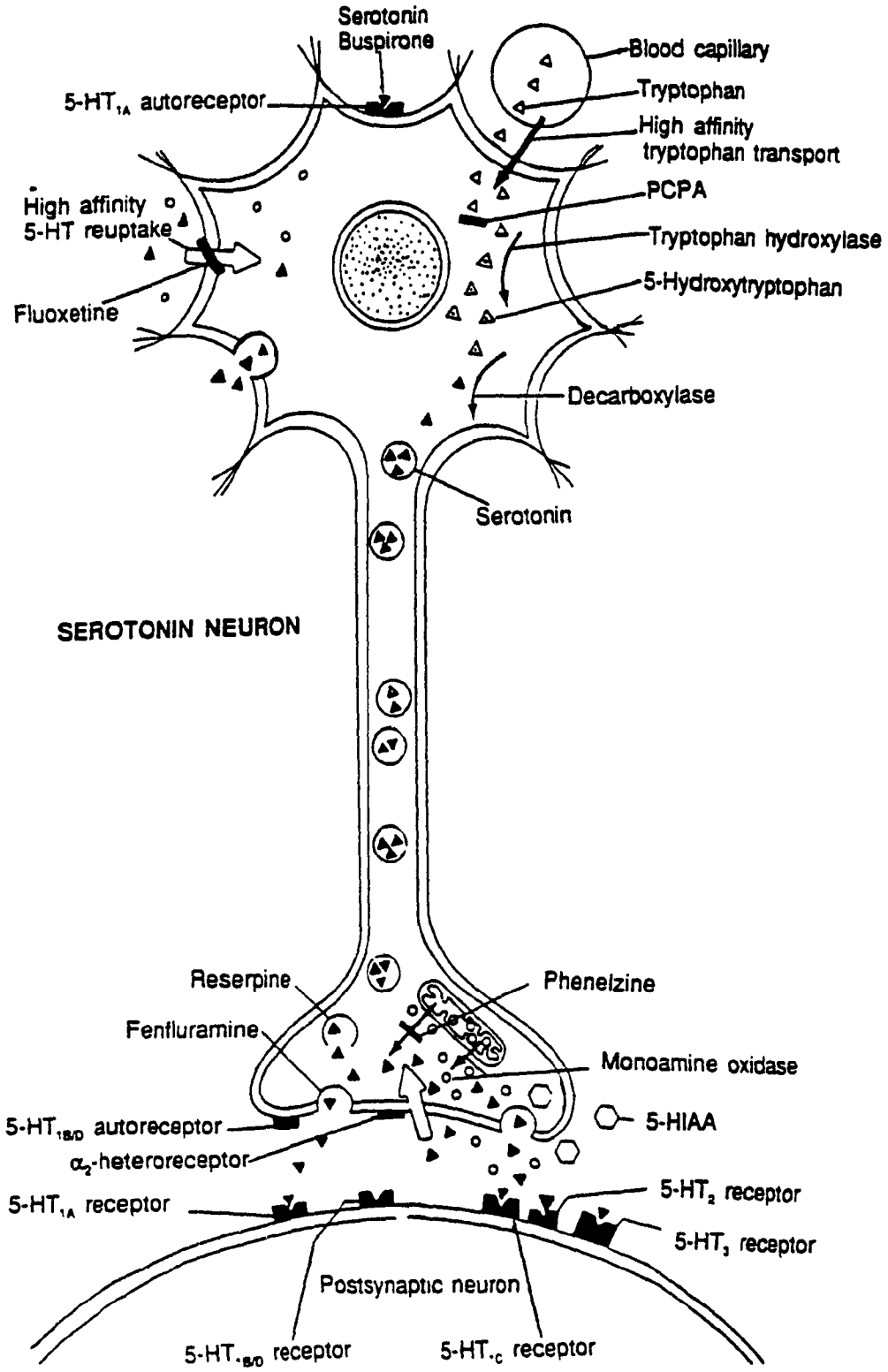


Figure 1. Serotonergic neuron (from P. Blier, 1991, p. 90).

for 21 days. Recovery of the firing rate is accompanied by the reduced responsiveness of the somatodendritic 5-HT autoreceptor, which is responsible for decreasing 5-HT release (Blier, de Montigny and Azarro, 1986).

Acute treatment with selective 5-HT reuptake blockers such as fluoxetine initially decrease the firing rate of 5-HT neurons, while gradual recovery of the firing activity is observed in animals treated for 14 days. In addition, chronic treatment reportedly decreases the responsiveness of 5-HT neurons to intravenous administration of the somatodendritic 5-HT autoreceptor agonist lysergic acid diethylamide (LSD), thus leading to increased 5-HT release at the terminal site. The attenuated function of the terminal 5-HT autoreceptor is suggested to be due to a sustained occupation of the [^{3}H]imipramine binding site (Blier, Chaput and de Montigny, 1988).

Gepirone and buspirone, both 5-HT_{1A} receptor agonists, are also effective in the treatment of depression (Amsterdam, Berwish, Potter and Rickels, 1987; Schweizer, Amsterdam, Rickels, Kaplan and Droba, 1986). Similar to the effects of MAOIs and 5-HT reuptake blockers, treatment with gepirone for 2 days markedly decreased the firing rate of dorsal raphe 5-HT neurons, while after 14 days of treatment recovery was complete. Chronic treatment was accompanied by a decrease in the response of the somatodendritic 5-HT autoreceptor to the microiontophoretic application of 5-HT (Blier and de Montigny, 1989).

Electrophysiological studies on the effects of long-term administration of antidepressant treatments are consistent with investigations of the quantification

of 5-HT_{1A} receptors following chronic treatment with antidepressant drugs (Welner, de Montigny, Desroches, Desjardins and Suranyi-Cadotte, 1989). The highest densities of 5-HT_{1A} receptors in the brain are in the raphe and hippocampus; in raphe nuclei these binding sites are localized on the soma of 5-HT neurons, while in the hippocampus these sites are found on the postsynaptic membrane (Verge et al., 1985; Pazos and Palacios, 1985; Hoyer, Pazos, Probst and Palacios, 1986). Welner and collegues (1989) reported that treatment for 21 days with the tricyclic antidepressant amitriptyline resulted in a significant increase in the density of 5-HT_{1A} receptors in the dorsal hippocampus. This finding is consistent with reports that tricyclic antidepressants enhance hippocampal postsynaptic 5-HT neurotransmission (de Montigny and Aghajanian, 1978), and that this effect is mediated by postsynaptic 5-HT_{1A} receptors (Andrade and Nicoll, 1987). Treatment for 21 days with fluoxetine and gepirone was observed to decrease the number of 5-HT_{1A} receptors in the dorsal raphe; this is consistent with evidence that long-term administration of these drugs decreases the responsiveness of terminal and somatodendritic 5-HT autoreceptors (Blier et al., 1988; Blier and de Montigny, 1989).

Thus, antidepressant treatments may exert their therapeutic effect through the serotonergic system via different mechanisms: 1) sensitization of postsynaptic neurons to 5-HT; 2) desensitization of somatodendritic 5-HT autoreceptors, and; 3) desensitization of 5-HT autoreceptors located on 5-HT nerve terminals. These changes result in an overall increase in 5-HT neurotransmission within the raphe-

hippocampal system in response to chronic antidepressant treatment. While the focus of the present investigation is on the 5-HT system, it should be noted that other monoaminergic systems are also implicated in the response to antidepressants, owing to the functional links between these systems. For example, the presence of an intact NE system appears to be essential for the development of postsynaptic sensitization to 5-HT by ECT or tricyclic antidepressants, as this enhanced responsiveness cannot be obtained in NE-denervated animals (Green and Deakin, 1979).

Anxiety and 5-HT. Interestingly, drugs which affect 5-HT_{1A} activity have also been reported to be effective in the treatment of generalized anxiety. This follows observations of the anxiolytic properties of 5-HT_{1A} partial agonists such as buspirone, gepirone and ipsapirone (Feighner, Meredith and Hendrickson, 1982; Traber and Glaser, 1987), and animal studies demonstrating that serotonergic lesions block the anxiolytic effects of these drugs (Eison, Eison, Stanley and Riblet, 1986). Serotonergic lesions also inhibit the anxiolytic properties of benzodiazepines (Stein, Wise and Belluz, 1975; Tye, Everitt and Iverson, 1977), drugs commonly used in the treatment of anxiety disorders. Further support for a role for 5-HT in anxiety is provided by increasing evidence that serotonergic reuptake inhibitors are effective in the treatment of patients with panic disorder (Westenberg and denBoer, 1989). These findings support the hypothesis that decreased 5-HT function may be anxiogenic, since chronic administration of 5-HT reuptake inhibitors appears to enhance serotonergic neurotransmission. Support

for a role for 5-HT in anxiety is also provided by Gray (1982), who proposed the importance of the ascending 5-HT system in anxiety based upon its innervation of the septo-hippocampal system, and the results of a series of lesion studies.

Nevertheless, a number of studies involving the pharmacological manipulation of serotonergic neurotransmission in animal models of anxiety have generally found that drugs which diminish 5-HT function have anxiolytic effects, whereas compounds which stimulate the 5-HT system are anxiogenic (Colpaert, Meert, Niemegeers and Janssen, 1985; Critchley and Handley, 1986; Costall, Domeney, Gerrard, Kelly and Naylor, 1988; Meert and Colpaert, 1986; Stein et al., 1975; File and Hyde, 1977; Tye et al., 1977; Chopin and Briley, 1987). Evidence linking decreased 5-HT neurotransmission to reduced anxiety is inconsistent, however, with clinical observations of an association between reduced serotonergic function and an increase in anxiety symptoms. Banki (1977) observed a negative correlation between CSF levels of 5-HIAA in depressed women and scores on the anxiety items of the Hamilton Rating Scale for Depression. Shopsin and collegues (1976) found that when PCPA was added to treatment with an MAOI in depressed patients, it resulted in increased anxiety. In addition, clinical studies with the 5-HT precursors l-tryptophan (L-trp) and 5-hydroxytryptophan (5-HTP) have failed to induce anxiety (Trimble, Chadwick, Reynolds and Marsden, 1975; Puhringer, Wirz-Justice and Laneranjian, 1976). Moreover, L-trp has been reported to be useful in the treatment of patients with obsessive compulsive disorder (Yaryura-Tobias and Bhagavan, 1977). Taken

together, these findings suggest that 5-HT does play a role in the control of anxiety, although the exact nature of the relationship is not yet clear. A resolution of this apparent conflict emerges from studies examining the effects of stress on monoamine activity.

Stress and 5-HT. As previously mentioned, there is evidence that the relationship between stress and psychiatric disorder may be due, in part, to the effects of stressors on central nervous system neurotransmitters, including 5-HT. Animal studies have demonstrated that exposure to continued stressors, such as inescapable shock, results in behavioral and neurochemical alterations (Weiss, Bailey, Pohorecky, Korzeniowski and Grillone, 1980). Initially, animals respond to shock with agitation, struggling, vocalization and unsuccessful attempts at escape; long-term, chronic exposure to shock produces apathy and withdrawal.

Alterations in neurochemical activity also vary, depending upon the acute or chronic nature of stress exposure. Initially, the utilization of NE, DA and 5-HT in various brain regions increases, as does the rate of monoamine synthesis (Thierry, 1973; Anisman and Zacharko, 1982). Over the short-term, continued stress results in a further series of adaptive changes, including decreased utilization rates of monoamines, increased rates of monoamine synthesis (Weiss, Glazer and Pohorecky, 1976), down-regulation of ß-NE receptors, and reduced NE-stimulated cyclic AMP formation (Stone, 1983), which enables the animal to deal effectively with the stressor.

However, continued failure to cope behaviorally with the stressor results in long-term, chronic exposure to stress. Such continued exposure leads to a reduction in levels of monoamines as continued demands on the neurochemical compensatory systems result in the inability of monoamine synthesis to keep pace with utilization (Weiss et al., 1976), resulting in exhaustion of the animal. Neurochemical reductions occur more readily when the organism does not have methods available to cope behaviorally with the stressor (Anisman, Pizzino and Sklar, 1980; Weiss et al., 1976); consequently, greater demands are placed on physiological processes. It is thought that reduced concentrations of monoamines may render the organism less well prepared to biologically respond to the further demands that may be placed upon it, thus increasing its vulnerability to psychological disturbances (Anisman, 1984; Weiss et al., 1979).

Weiss and collegues (1981) measured levels of NE, DA and 5-HT in various brain regions of rats after the animals had been exposed to controllable and uncontrollable shocks. Compared to rats that had control over the shocks, those that did not have control exhibited reduced levels of NE in the locus coeruleus, a reduction of NE and DA in the anterieur cortex, and significantly less 5-HT in the brain stem. Similarly, Kitayama and collegues (1989) reported that severe immobilization stress resulted in a marked decrease of 5-HT in various serotonergic cell groups and at nerve terminals, indicating a reduction of 5-HT synthesis, and possibly reduced 5-HT release. Indeed, Sherman and Petty (1980)

found that chronic, uncontrollable shock decreased the calcium-dependent release of 5-HT in slice preparations from rat neocortex and septum.

Evidence that the behavioral deficits are mediated by these neurochemical changes (Anisman and Lapierre, 1982; Anisman and Zacharko, 1982; Weiss et al., 1981) is suggested by observations that protection of neurochemical depletion during stress by drug treatment can prevent the behavioral depression from occurring (Anisman, Remington and Sklar, 1979; Anisman, Suissa and Sklar, 1980). Furthermore, treatment with antidepressant drugs has been shown to reverse the behavioral deficit once it has occurred. Sherman, Sacquitne and Petty (1982) tested responsiveness to several types of antidepressants, and to a number of psychoactive drugs which are not effective in treating depression in humans. They exposed rats to learned helplessness training, and then injected them intraperitoneally with one of the drugs daily for five days; the animals were then tested for the escape deficit characteristic of helplessness. The results indicated that daily administration of tricyclic antidepressants, atypical antidepressants, and MAOIs were effective in reversing learned helplessness. Animals receiving ECT twice daily for four consecutive days also responded to treatment. On the other hand, chronic treatment with anxiolytics, neuroleptics, or stimulants was not effective in the treatment of helplessness.

The difference in monoamine levels following exposure to acute versus long-term, chronic stress leads one to suggest that perhaps the discrepancy regarding the role of 5-HT in animal and human studies of anxiety is due to the

fact that animal anxiety studies often use acute, single situations, in which an initial, high level of 5-HT may in fact be adaptive. For example, File and Hyde (1977) found that the decrease in social interaction observed in pairs of rats placed in an unfamiliar condition was blocked by the acute administration of PCPA, a 5-HT synthesis inhibitor, suggesting an association between anxiety and increased concentrations of 5-HT. In actuality, reduced interaction with an unknown animal in an unfamiliar environment is an adaptive response that can serve to protect the animal from a potentially dangerous situation. One would think that only once the animal has familiarized itself with the situation and has determined that there is no imminent danger, would it cautiously proceed to interact with its surroundings. This leads one to suggest that the effect of PCPA on social interaction during or after this process of acclimatization would probably not be the same as when the animals are first placed in the test box. The animal's anxiety would be maladaptive when such a response continues to occur in relation to inappropriate stimuli, when it's survival is not in danger.

Thus, one may speculate that with repeated, chronic exposure of the animals in the anxiety paradigms, 5-HT levels would decrease as synthesis of the amine would be unable to keep pace with utilization. In this instance, high levels of anxiety may be associated with lower levels of 5-HT neurotransmission, as has been reported in anxious strains of mice (File, Curle, Baldwin and Neal, 1987) and in human anxiety (Briley, Chopin and Moret, 1991; Yaryura-Tobias and Bhagavan, 1977).

Despite the implication of serotonergic mechanisms in the pathophysiology of psychiatric disorders, little human data is available concerning the effects of stressful events on 5-HT neurotransmission. Palermo and collegues (1986) reported a decrease in platelet 5-HT content, possibly due to massive release of the neurotransmitter, following administration of a laboratory stressor, the cold pressor test, in healthy subjects. Stress immediately prior to gynecological surgery has been observed to significantly increase levels of plasma 5-HT, compared to 24 hours before or four days after the operation (Evron, Pfeifer, Sadovsky and Sulman, 1982). Furthermore, an increase in whole blood 5-HT has been demonstrated as a result of stress immediately prior to a mid-term academic examination, compared to three days pre-examination (Davis, Dunlop, Shea, Brittain and Hendrie, 1985). These studies suggest an increase in the peripheral synthesis and/or release of 5-HT in response to acute or short-term stress.

Swann and collegues (1990) investigated the relationship between stressful events and biological variables in depressed and manic patients. They found that unipolar depressed patients reporting a high level of perceived stress had lower CSF 5-HIAA levels than patients who reported that stress played a reduced role in their illness. These results suggest an association between stress and lowered synthesis or metabolism of 5-HT. Again, these apparent inconsistencies concerning the role of 5-HT in human stress may be due to the lack of differentiation in the literature between the effects of stressors of varying duration and intensity on monoaminergic function.

The Present Studies

In light of these findings, the purpose of this investigation was to further examine the idea that alterations in 5-HT neurotransmission form the basis for the relationship between depression, anxiety and stress, and that such alterations may account for the symptomatology common to these conditions. Specifically, the hypothesis is that depression, anxiety and chronic stress are all associated with decreases in serotonergic function, and that these decreases may account for common symptoms such as changes in mood, appetite and sleep.

Similarly, evidence that other symptoms characteristic of depression, such as hopelessness, are present in psychiatric patients with a range of diagnoses, suggests a neurochemical substrate for the experience and expression of these symptoms that is independant of psychiatric diagnosis. Thus, another aim of this investigation was to examine the relationship between these variables and neurochemical markers, through the study of heterogeneous populations of psychiatric patients. This approach emphasizes the importance of looking beyond diagnostic classification in investigations of psychopathology, and further suggests a dimensional 5-HT hypothesis, linking 5-HT disturbances to symptoms across diagnoses, rather than a categorical view of 5-HT dysfunction in behavior.

It was also of interest to attempt to determine the specific mechanisms through which psychosocial risk factors for depression, such as stress and low social support, are associated with the depressed state, and whether this effect involves regulation of the 5-HT system. Specifically, are stress and low social

support associated with neurochemical changes, such as alterations in 5-HT function, that are observed in depression? This question follows directly from the high risk literature. If such relationships are found, they would clarify the nature of the association between psychosocial risk factors and the onset of a depressive episode, and suggest a mechanism through which such factors may regulate depression. The hypothesis is that risk factors for depression are associated with neurochemical changes which underlie symptoms of affective disorders, thereby rendering an individual more physiologically vulnerable to a depressive episode.

Measures of 5-HT function. The measures of 5-HT function used in these studies are [^{3}H]imipramine and [^{3}H]paroxetine binding on blood platelets. Previous studies have indicated a relationship between these binding sites and the 5-HT transporter complex (Briley, Langer and Sette, 1981; Habert, Graham, Tahraoui, Claustre and Langer, 1985). These sites on blood platelets have analagous properties to sites found in the brain (Paul et al., 1980; Habert, Graham and Langer, 1986), and may thus serve as peripheral models for central 5-HT function. Studies have already emphasized the significance of the [^{3}H]imipramine binding site in the pathophysiology of depression (Briley et al., 1980). [^{3}H]Paroxetine is a recent, selective inhibitor of 5-HT uptake that is considered to be a more potent and specific marker of 5-HT function than [^{3}H]imipramine, and is thought to label directly the substrate recognition site for 5-HT uptake in brain and platelets (Buus-Lassen, 1978). To date, little is known about either the function of the [^{3}H]paroxetine binding site in psychopathology, or

concerning the relationship between the [^{3}H]imipramine and [^{3}H]paroxetine binding sites.

Importantly, it must be emphasized that although the sites labeled by [^{3}H]imipramine and [^{3}H]paroxetine have been associated with serotonergic function and may be useful in looking at the pathophysiology of depression, anxiety and stress, neither [^{3}H]imipramine nor [^{3}H]paroxetine binding on blood platelets are direct markers of the central 5-HT system. A fuller discussion of the use of platelet markers, and the sites labeled by [^{3}H]imipramine and [^{3}H]paroxetine, is provided in the introduction to the first study.

Suicidal behavior, hopelessness and 5-HT. A number of studies have implicated suicidal behavior with a decrease in 5-HT neurotransmission. The most direct support comes from findings of a bimodal distribution of 5-HIAA in the CSF of depressed patients, in which the low 5-HIAA subgroup was found to be at higher risk for suicide (Asberg, Traskman and Thoren, 1976; Traskman, Asberg, Bertillson and Sjostrand, 1981). In addition, post-mortem examinations of the brains of suicide victims have generally reported low levels of 5-HT and 5-HIAA, localized to regions rich in serotonergic neurons, including the brainstem and the median and dorsal raphe nuclei of the midbrain (Lloyd et al., 1974; Shaw et al., 1967). Recent studies have also found a significant reduction in the number of [^{3}H]imipramine binding sites in the brains of suicide victims (Stanley et al., 1982).

Some studies suggest that a decrease in 5-HT function is characteristic of suicide not only in primary depression, but in suicide in general. Low 5-HIAA was found to correlate significantly with a history of suicidal behavior in a sample of Navy enlisted men with personality disorders (Brown, Goodwin, Ballenger, Goyer and Major, 1979). A similar pattern of low 5-HIAA and history of a suicide attempt has emerged in a study of patients with borderline personality disorder (Brown et al., 1982) and in suicidal schizophrenic patients (Ninan et al., 1985). Recently, Coccaro and collegues (1989) reported reduced central 5-HT function, as determined by the prolactin response to fenfluramine hydrochloride, associated with a history of suicide attempt in a subgroup of patients with major affective and personality disorders. In a review of studies on 5-HT and depression, Goodwin and Post (1983) concluded that CSF 5-HIAA is not low in all depressed patients, but that those in the low subgroup may have a different profile of depressive symptoms and behavioral vulnerabilities, including suicide. These studies suggest that altered 5-HT metabolism may be a highly significant factor contributing to suicidal behavior, in whatever diagnostic group it is observed.

Hopelessness, one of the major psychological correlates of suicide, has similarly been observed in patients with various diagnoses. Defined as a system of negative expectations concerning oneself and one's future (Stotland, 1969), hopelessness is an important concept in the understanding and prediction of suicidal behavior. It has been identified as a core characteristic of depression

(Beck, 1967; Melges and Bowlby, 1969), and has been suggested to serve as the link between depression and suicide (Beck, 1967). For example, Beck, Kovacs and Weissman (1975) found that hopelessness accounted for 76% of the variance between depression and suicidal intent in hospitalized suicide attempters. Other studies have found that the seriousness of suicidal intent is more highly correlated with negative expectations than with depression (Minkoff et al., 1973; Wetzel, 1976). Indeed, several authors have suggested that hopelessness is not specific to depression, but rather is represented in a number of psychiatric conditions (Akiskal, Hirschfeld and Yerevanian, 1983; Stotland, 1969).

Beck, Steer, Kovacs and Garrison (1985) conducted a prospective study in which they examined depression, hopelessness and suicidal ideation in 207 hospitalized patients with various diagnoses, including affective, anxiety, and personality disorders, and schizophrenia. They found that only hopelessness significantly differentiated those patients who eventually committed suicide; a score of 9 or more on the Hopelessness Scale (Beck, Brown, Barchick, Stewart and Steer, 1974) correctly identified over 90% of the eventual suicides. Similar results were obtained in a prospective study of almost 2,000 psychiatric outpatients (Beck et al., 1990), in which the high-risk group, identified by a score of 9 or above on the Hopelessness Scale, was 11 times more likely to commit suicide than the rest of the patients. This was double the rate predicted by a depression inventory.

In summary, these findings indicate that suicidal behavior, a symptom of depression which is observed in various diagnostic categories, is associated with alterations in 5-HT neurotransmission irrespective of psychiatric diagnosis. Hopelessness, a psychological correlate of suicide, is also a symptom of depression which cuts across diagnostic boundaries. Since a strong association has been reported to exist between suicidal behavior and hopelessness, it is hypothesized that hopelessness, like suicidal behavior, may similarly be associated with decreases in 5-HT neurotransmission, regardless of primary psychiatric diagnosis.

Stress, social support and 5-HT. As previously mentioned, several investigators have suggested that stressful life events may precipitate the onset of depression (Brown et al., 1973). These findings have led to extensive investigation of the effects of stress on the rat, including examination of stressor effects on 5-HT function (Anisman and Zacharko, 1990), due to evidence suggesting that alterations in 5-HT may have a causal or predisposing role in depression (Asberg et al., 1975; Van Praag, 1982).

Previous findings indicate that acute stressors lead to an increase in 5-HT synthesis, release and utilization (eg. Thierry, 1973). Few investigators, however, have examined the effects of continued, long-term stress on brain 5-HT function, in spite of the fact that such studies may be of more relevance to the postulated link between stress and depression in humans. Results from studies that have been conducted suggest reduced levels of 5-HT function in animals exposed to chronic stressors. Minchin and collegues (Minchin, Williams, Bowdler and Green,

1983) measured 5-HT uptake in brain slices from rats that had been exposed to a series of five electroshocks over ten days. They found a significant decrease in 5-HT uptake in the treated animals. These investigators suggested that the observed decrease in 5-HT uptake may reflect an adaptation to a fall in 5-HT levels in the synaptic cleft, reflecting an increase in the duration of action of synaptically-released 5-HT. Kennett, Dickinson and Curzon (1985) investigated the effects of repeated immobilization on 5-HT dependent behaviors, following administration of the 5-HT releaser p-chloroamphetamine (PCA) and the 5-HT agonist 5-methoxy-N,N-dimethyltryptamine (5-MEODMT). These investigators observed an enhancement of behavioral abnormalities after seven days immobilization, suggesting a sensitization of 5-HT postsynaptic functions, which may be secondary to a decrease in the release of 5-HT after repeated immobilization. These results are similar to studies in depressed patients in which decreases in the uptake of 5-HT (Coppen et al., 1978), and increases in the binding of postsynaptic 5-HT receptors (Arango et al., 1990) have been observed. Together, these findings suggest that stressful life events may precipitate the onset of a depressive episode by causing alterations in 5-HT neurotransmission similar to those observed in depression.

Another factor which has been identified as rendering an individual more vulnerable to depression is the lack of social support, especially lack of the support of an intimate who can be confided in (Brown and Harris, 1978). Two models of social support have been proposed which may account for the

association between support and depression. The buffering model suggests that support helps mitigate against the impact of life stress upon the individual's psychological state (Cohen and Wills, 1985). Beneficial buffering effects of social support are thought to arise from an enhanced sense of mastery in coping with life stress and from avoidance of helpless feelings (Cohen and Wills, 1985; Heitzman and Kaplan, 1988). The main effects model suggests that the lack of support can predispose an individual to depression in its own right, even under conditions of low stress (Aneshensel and Stone, 1982), thus increasing risk of illness (Thoits, 1982). The implication is that social reinforcement contributes to psychological well-being by providing a sense of self-worth, and by fulfilling an individual's needs for affiliation, respect and affection (Berkman, 1985; Cohen and Wills, 1985). The main effect and buffering hypotheses are not mutually exclusive.

Evidence that social factors may influence serotonergic function is available from a study of changes in social status among adult male vervet monkeys. Raleigh, McGuire, Brammer and Yuwiler (1984) observed that both the spontaneous and induced transition of male monkeys from the subordinate to the dominant position in a group was accompanied by a corresponding increase in the concentration of whole-blood 5-HT, while 5-HT concentrations declined as the initially dominant males became subordinate. The investigators also found that a 30-day isolation period reduced whole-blood 5-HT concentrations in dominant male monkeys to levels exhibited by subordinate males; after returning to their

groups, these animals regained their dominant positions, and their concentrations of whole blood 5-HT returned to pre-isolation levels.

Monoamine oxidase, an enzyme involved in the metabolism of 5-HT and measured in blood platelets and plasma, and which may result in a decrease in the availability of 5-HT for synaptic transmission, has also been reported to correlate negatively with social interaction in both monkeys (Redmond and Murphy, 1975; Redmond, Murphy and Baulu, 1979) and humans (Coursey, Buchsbaum and Murphy, 1979). Moreover, studies of the effects of isolation in rodents have consistently found that a low level of social contact is associated with reduced 5-HT function. Compared to grouped animals, socially-isolated mice show reductions in the turnover rate of brain 5-HT (Garattini, Giacolone and Valzelli, 1967; Giacalone, Tansella, Valzelli and Garattini, 1968; Essman, 1969; Welch and Welch, 1971; Kempf, Puglisi-Allegra, Cabib, Schleef and Mandel, 1984) and concentrations of 5-HIAA (Welch and Welch, 1968; Garattini et al., 1969), decreased sensitivity of neurons to 5-HT in various brain regions (Oehler, Jahkel and Schmidt, 1987), as well as reductions in brain tryptophan (Miller, Pachter and Valzelli, 1979) and tryptophan hydroxylase activity (Yanai and Sze, 1983; Segal, Knapp, Kuczenski and Mandell, 1973), the rate-limiting enzyme in 5-HT synthesis.

Taken together, these findings provide considerable evidence for the influence of stress and social factors on 5-HT neurotransmission. These studies further suggest that alterations in serotonergic function associated with

psychosocial risk factors for depression may underlie the onset of a depressive episode.

Design of the Investigation

The present investigation consisted of three experiments. Study 1 was a preliminary investigation aimed at establishing the association between the binding density of [^{3}H]paroxetine, a newly developed radioligand that is reported to be a specific marker of the 5-HT uptake site, and [^{14}C]5-HT uptake in blood platelets of healthy volunteers. Confirmation of a significant association between these markers in human platelets was necessary in order to provide evidence that [^{3}H]paroxetine binding may serve as a useful indicator of 5-HT function in the present studies.

Study 2 compared [^{3}H]imipramine and [^{3}H]paroxetine binding measured in parallel, in patients with depressive and anxiety disorders, as well as in healthy, age-matched volunteers. The study included determinations of both [^{3}H]imipramine and [^{3}H]paroxetine binding, since: 1) [^{3}H]imipramine binding sites have been reported to modulate the uptake of 5-HT, and the number of sites has been suggested as a biochemical marker of depression, and; 2) little is known as yet about the relationship between [^{3}H]imipramine and [^{3}H]paroxetine binding sites on human platelets, especially in patients with psychiatric disorders. Objective and subjective measures of depression and anxiety were also obtained for all subjects, and correlated with binding values.

Another aim of Study 2 was to examine the biochemical basis of hopelessness, a symptom of depression, independent of psychiatric diagnosis. This approach emphasizes a dimensional rather than a categorical view of the interaction between neurochemical function and behavior. Given that several studies have implicated suicidal behavior with a decrease in 5-HT neurotransmission, and since hopelessness is significantly associated with suicide, it was predicted that [^{3}H]imipramine and [^{3}H]paroxetine binding would be significantly decreased in subjects with a high degree of hopelessness. In this analysis, ratings from depressed and anxious patients and healthy controls were pooled, in order to examine the association between hopelessness and binding values in a heterogeneous sample of subjects. Altered 5-HT function in individuals with a high degree of hopelessness may indicate a neurochemical substrate for the experience of hopelessness, and provide further support for hopelessness as an indicator of suicidal risk.

Study 3 examined the effects of a naturalistic stressor, year-end academic examinations, on [^{3}H]imipramine and [^{3}H]paroxetine binding. In light of the findings that: a) stressful events may precipitate depression, and; b) affective illness may be associated with variations in serotonergic activity, it was expected that a lower density of [^{3}H]imipramine and [^{3}H]paroxetine binding sites would be observed in individuals during the stress of examinations than in the period following vacation, that is, in the absence of stressful conditions. This finding would support the hypothesis that a high level of stress may lead to a decrease in

serotonergic function, which would render an individual more physiologically vulnerable to a depressive episode. Ratings of the students' perception of their overall level of stress, and daily positive and negative events were obtained in addition to objective and subjective measures of depression and anxiety. Concentrations of plasma cortisol were also obtained to confirm that subjects were exposed to a physiologically relevant amount of stress.

The relationship between social support and [^{3}H]imipramine and [^{3}H]paroxetine binding was also examined in Study 3 by administering a questionnaire on perception of social support to students during their examinations and after vacation. Social support may act as a buffer between stressful events and the onset of depression, by decreasing the influence of stress on serotonergic function. In light of evidence that social factors can alter serotonergic function, it was expected that perception of social support would correlate significantly with markers of the 5-HT system used in the present study.

Summary

The bulk of the literature strongly suggests a role for 5-HT in the link between depression, anxiety and stress. Alterations in 5-HT neurotransmission have also been associated with specific symptoms and psychosocial risk factors for depression. The present series of studies was conducted in order to further examine these findings, using [^{3}H]imipramine and [^{3}H]paroxetine binding on blood platelets as indirect markers of 5-HT function. The results are discussed in relation to the notions that a neurochemical link exists between depression,

anxiety and stress, and that a disturbance in neurochemical functioning may be associated with depressive symptomatology, independent of psychiatric diagnosis. In addition, it is suggested that psychosocial factors may be associated with alterations in serotonergic neurotransmission, thus rendering an individual more physiologically vulnerable to psychological disturbance.

STUDY 1

The Association between [^{3}H]Paroxetine Binding and [^{14}C]5-HT Uptake in Blood Platelets of Healthy Volunteers

Paroxetine is a new antidepressant which is a potent and selective inhibitor of 5-HT uptake (Buus Lassen, 1978). In its labeled form, paroxetine is considered to be a more specific marker of the 5-HT transporter complex than [^{3}H]imipramine (Habert et al., 1985; Sette et al., 1983). While several investigators have characterized the site labeled by [^{3}H]paroxetine on rat neuronal membranes and platelets (eg. Habert et al., 1985), few studies have examined the [^{3}H]paroxetine binding site on blood platelets in humans. Thus, the aim of the present study was to establish the relationship between [^{3}H]paroxetine binding and [^{14}C]5-HT uptake on blood platelets of healthy volunteers. An association between [^{3}H]paroxetine binding and [^{14}C]5-HT uptake would suggest that [^{3}H]paroxetine might serve as a reliable marker of the 5-HT uptake site in the subsequent studies.

Platelets as Peripheral Models of CNS 5-HT Terminals

Peripheral models of 5-HT functioning are of great interest due to the difficulty involved in directly studying 5-HT neurotransmission in the CNS. Many investigators have proposed that the blood platelet can serve as a model for the 5-HT presynaptic nerve terminal (Lingjaerde, 1977; Pletscher, 1968; Sneddon, 1973),

owing to its morphological and biochemical similarities with the serotonergic neuron. The platelet, like the neuron, possesses a cytoplasmic membrane with an active transport system for 5-HT, with binding sites for several drugs and neurotransmitters. The platelet also contains mitochondria with monoamine oxidase, and dense-cored vesicles which store 5-HT and other monoamines (Sneddon, 1973; Da Prada et al., 1988). In addition, platelets appear to have storage and release properties similar to those of other secretory cells, and the process of 5-HT release from platelets is reported to be analagous to neuronal release (Douglas, 1968; see Figure 2). Thus, platelets may also serve as a model for the firing of central serotonergic neurons. The process of platelet 5-HT uptake has been shown to be similar, if not identical, to the 5-HT uptake mechanism in brain 5-HT nerve endings. It is a saturable, high-affinity process that is energy-dependant, temperature sensitive and requires the presence of Na^+ and Cl^- ions (Lingjaerde, 1977). Under analagous conditions, the kinetic constants for 5-HT transport into the brain and platelet are very similar (Stahl, 1985). In addition, many typical and atypical 5-HT blockers inhibit, with the same relative potency, the uptake of 5-HT into platelets and synaptosomes (Todrick and Tate, 1969). A passive diffusion mechanism for 5-HT accumulation contributes significantly to total 5-HT accumulation when concentrations of 5-HT outside the platelet or neuron are increased (Da Prada, Pletscher and Bartholini, 1965).

Specific, high affinity binding sites for [^{3}H]imipramine have also been reported on both platelet and neuronal membranes, and the kinetic and

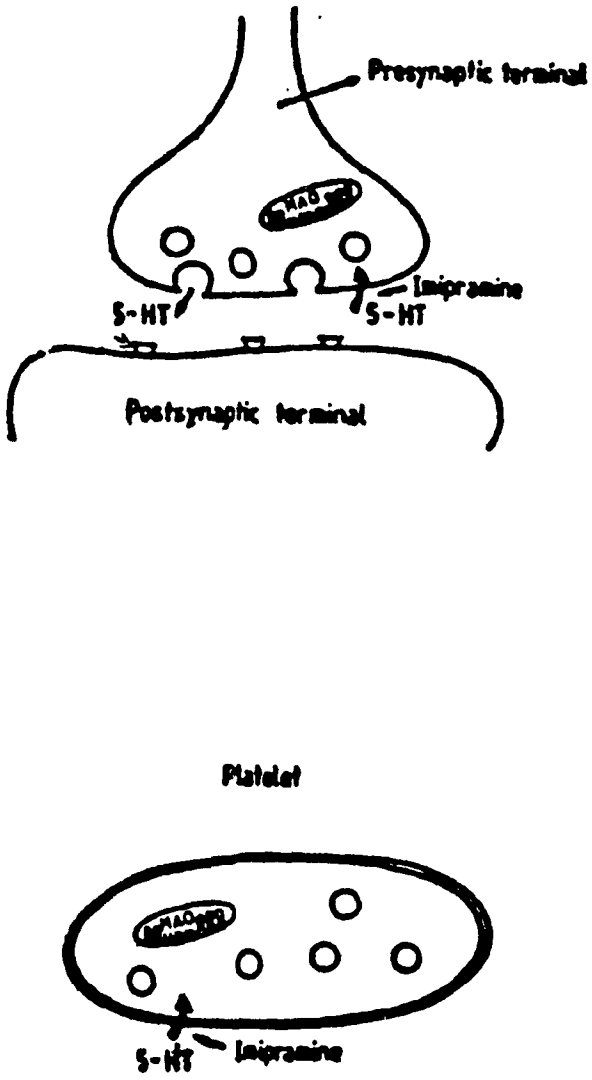


Figure 2. Uptake of 5-HT by brain synaptosomes and blood platelets (from A. Rotman, 1983, p.136).

pharmacological properties of binding are nearly identical in both (Briley, Raisman and Langer, 1979; Paul et al., 1980). Evidence that the [^{3}H]imipramine binding site can be modified in parallel comes from a report that cats treated chronically with imipramine for three weeks showed a similar down-regulation of binding sites in both brain and platelet membranes (Briley, Raisman, Arbilla, Casadamont and Langer, 1982). Similarly in Fawn-Hooded rats, a strain of rats with defective platelet 5-HT storage, [^{3}H]imipramine binding sites were found to be absent or severely reduced in both brain and platelets (Dumorille-Ross and Tang, 1981; Arora, Tong, Jackman, Stoff and Meltzer, 1983). An association between the [^{3}H]imipramine binding site and the uptake of 5-HT in platelets and neurons has also been found (Paul et al., 1980). Recently, platelets have also been shown to contain the 5-HT_2 membrane receptor (Peters and Grahame-Smith, 1980; McBride, Mann, McEwen and Bigeon, 1983), which is reported to have many binding properties similar to 5-HT_2 receptors in central 5-HT synaptosomes (De Clerck, David and Janssen, 1983).

Unlike neurons, however, platelets are enucleated, and do not contain the enzymes necessary for the biosynthesis of monoamines (Sorimachi, Kataoka, Hori and Fugisawa, 1973). Hence, the platelet is not a good model for the synthesis of 5-HT by the brain, nor for 5-HT concentrations or turnover by CNS serotonergic neurons. Rather, evidence suggests that platelet 5-HT originates from the enterochromaffin cells of the intestinal mucosa, which is then taken into the platelet by transport mechanisms (Sneddon, 1973). In addition, while the platelet

may be considered as a model for the presynaptic serotonergic nerve terminal, it is not a model synaptosome since the postsynaptic element of the synapse is lacking (Stahl, 1985).

Taken together, these findings suggest that blood platelets accumulate, store and release 5-HT in a manner analagous to the CNS serotonergic synaptosome, and in addition possess receptors with binding characteristics similar to some of the sites on neuronal 5-HT membrane. This suggests that blood platelets are a valid and relatively simple model for selected pharmacological studies on central serotonergic neurons.

[^{3}H]Imipramine Binding Sites in Brain and Platelets

A specific, high-affinity binding site for [^{3}H]imipramine was first demonstrated on 5-HT neuronal membrane by Raisman and collegues (1979a,b), who reported a single population of binding sites in rat cerebral cortex. The [^{3}H]imipramine binding site fulfills many of the criteria for a receptor. The site labeled by [^{3}H]imipramine is saturable, reversible and ion-sensitive, and is selectively inhibited by tricyclic antidepressants. In addition, the site correlates with the inhibition of 5-HT uptake by clinical doses of tricyclic antidepressants, and is altered by chronic antidepressant treatments (Davis, 1984; Langer and Briley, 1981).

Rainbow, Bigeon and McEwen (1982) conducted an autoradiographic study of [^{3}H]imipramine binding in rat brain, and found that the highest densities of sites were located in the dorsal and median raphe nuclei, the dorsomedial nucleus

of the hypothalamus, the superficial layer of the superior colliculus, the interpeduncular nucleus, the locus coeruleus, and the central gray. Moderate densities of specific binding sites were observed in the caudate-putamen, all layers of the cerebral cortex, and all subregions and laminae of the hippocampus, while low concentrations were found in the cerebellum, substantia nigra and limbic areas. The regional distribution of [^{3}H]imipramine binding sites has been reported to correlate significantly with endogenous levels of 5-HT (Palkovits, Raisman, Briley and Langer, 1981) and 5-HT nerve terminals in the rat brain (Dawson and Warnsley, 1983), and the binding sites are decreased in parallel with 5-HT content and uptake following stereotaxic lesion (Sette, Raisman, Briley and Langer, 1981) and administration of the specific 5-HT neurotoxin, 5,7-DHT (Gross, Gothert, Ender and Schuman, 1981; Luine, Frankfurt, Rainbow, Bigeon and Azmitia, 1983). These findings indicate an association between the [^{3}H]imipramine binding site and central serotonergic neurotransmission.

Specific, high-affinity binding sites for [^{3}H]imipramine have also been found on human platelet membrane (Briley et al., 1979). These sites are reported to have binding characteristics similar to those described for rat and human brain (Paul et al., 1980), and a comparison of the potency of various drugs for the inhibition of [^{3}H]imipramine binding in rat brain, and human brain and platelets (Langer, Briley, Raisman, Henry and Morselli, 1980; Langer, Agid, Raisman, Briley and Agid, 1981; Rehavi et al., 1980) suggests th. e [^{3}H]imipramine binding sites from these sources are identical.

Evidence for an association between the [^{3}H]imipramine binding site and the substrate recognition site for 5-HT on the 5-HT transporter complex has also led some investigators to suggest that the [^{3}H]imipramine binding site and the recognition site for 5-HT on the transporter complex are identical (Paul et al., 1981). Others suggest that the site labelled by [^{3}H]imipramine is separate, situated close to the 5-HT recognition site, and exerts an allosteric regulatory effect on 5-HT uptake (Briley et al., 1981; Wennogle and Meyerson, 1983; see Figure 3). Support for an allosteric coupling between the [^{3}H]imipramine-labeled and 5-HT sites is suggested by inhibition studies in which tricyclic antidepressants were observed to inhibit the binding of [^{3}H]imipramine in rat brain in a competitive manner, while the inhibition of [^{3}H]imipramine binding by 5-HT and non-tricyclic 5-HT uptake inhibitors was complex (Sette, Briley and Langer, 1983; Meyerson, Ieni and Wennogle, 1987). This suggests that tricyclics bind directly to the [^{3}H]imipramine binding site, whereas the site through which 5-HT inhibits [^{3}H]imipramine binding is the 5-HT recognition site of the 5-HT uptake transporter. Further evidence that the site labeled by [^{3}H]imipramine represents a separate site is available from clinical studies which show a lack of association between the [^{3}H]imipramine binding site and 5-HT uptake in platelets from untreated depressed patients and healthy volunteers (Raisman et al., 1982), and in patients suffering from alcoholic cirrhosis (Ahtee et al., 1981). Reports that the site labelled by [^{3}H]imipramine is distinct has led to an attempt to identify endogenous ligands for this putative receptor. Recent investigations indicate that

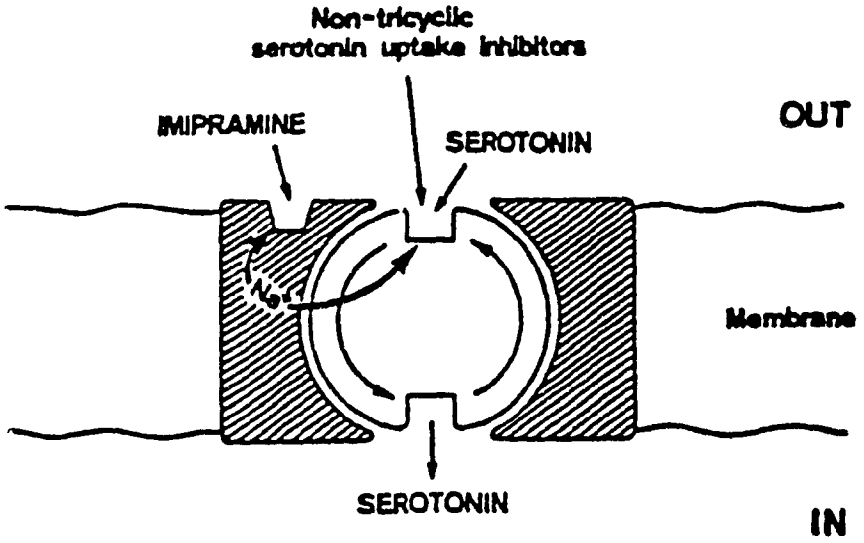


Figure 3. Proposed interaction between the [^{3}H]imipramine binding site and the 5-HT uptake complex (from M. Briley, 1985, p. 64).

this ligand may be α1-acidic glycoprotein (Abraham, Ieni and Meyerson, 1987; Nemeroff et al., 1990).

Shortly after the discovery of the specific high-affinity binding sites for [^{3}H]imipramine, it was found that these sites were decreased in platelets from depressed patients, with no change in the affinity constant (Briley et al., 1980). A decrease in the concentration of [^{3}H]imipramine-labeled sites has also been observed in post-mortem studies of the brains of patients with depressive illness (Perry et al., 1983). Numerous investigators have reported similar findings (Suranyi-Cadotte et al., 1983; Suranyi-Cadotte, Quirion, Nair, Lafaille and Schwartz, 1981; Raisman, Sechter, Briley, Zarifian and Langer, 1981; Poirier et al., 1986; Langer and Raisman, 1983; Arora, Wunnicke and Meltzer, 1985; Paul et al., 1981). Decreased [^{3}H]imipramine binding has been reported in patients with reactive, and unipolar and bipolar endogenous depression (Raisman et al., 1982), and appears to be independant of the severity of depressive symptoms (Briley et al., 1980; Paul et al., 1981).

Although initial studies suggested that the reduced density of [^{3}H]imipramine binding sites was a stable biochemical trait (Raisman et al., 1982; Gay et al., 1983), perhaps linked genetically to a susceptibility to depression, recent studies indicate that [^{3}H]imipramine binding is a state-dependent phenomenon which returns to normal following remission of the clinical signs of depression. Suranyi-Cadotte and collegues (Suranyi-Cadotte, Wood, Nair and Schwartz, 1982) reported that the decrease observed in platelet [^{3}H]imipramine

binding in depressed patients returned to normal levels after patients were in remission for at least two months. Similarly, Langer, Sechter, Loo, Raisman and Zarifian (1986) found that the [^{3}H]imipramine B_{max} in depressed patients reached control values 12 to 18 months following treatment with ECT; B_{max} values immediately following treatment were slightly increased, but still significantly below those of controls. Euthymic bipolar patients reportedly show no differences in the B_{max} or K_d of [^{3}H]imipramine binding when compared with controls (Berrettini, Nurnberger, Post and Gershon, 1982). These findings suggest that normalization of [^{3}H]imipramine binding values lags behind the apparent clinical improvement and becomes evident only during and following the period of full remission.

Taken together, these findings strongly suggest that the [^{3}H]imipramine binding site is functionally associated with central serotonergic neurotransmission, and may play a role in the pathophysiology of affective disorders.

[^{3}H]Paroxetine Binding Sites

Specific, saturable, high-affinity binding sites for [^{3}H]paroxetine have been described on rat (Habert et al., 1986; De Souza and Kuyatt, 1987) and human neuronal membranes (Laruelle, Vanisberg and Maloteaux, 1988; Cortes, Soriano, Pazos, Probst and Palacios, 1988), and the relative potencies of various drugs in displacing [^{3}H]paroxetine is reported to be similar in both tissues (Habert et al., 1986). High-affinity [^{3}H]paroxetine binding sites have also been identified on human platelets (Mellerup, Plenge and Engelstoft, 1983), and are reported to

have a pharmacological profile comparable to [^{3}H]paroxetine sites on neuronal tissue (Habert et al., 1985; Mellerup and Plenge, 1986a).

The distribution of [^{3}H]paroxetine binding sites in rat and human brain is similar to that of [^{3}H]imipramine, and is highly consistent with the organization of 5-HT terminals, axons and cell bodies. de Souza and Kuyatt (1987) examined the distribution of [^{3}H]paroxetine-labeled sites in rat brain by autoradiography. They found the highest concentrations of [^{3}H]paroxetine binding sites in dorsal and median raphe nuclei, the central gray, the superficial layer of the superior colliculus, lateral septal nuclei, the paraventricular nucleus of the hypothalamus, as well as in the substantia nigra and locus coeruleus. Moderate densities of [^{3}H]paroxetine binding sites were observed in various regions including the hippocampus, hypothalamus, thalamus, amygdala, septum and some areas of the cortex. The lowest levels of binding were observed in the cerebellum and in white matter.

An association between the [^{3}H]paroxetine binding site and the 5-HT uptake transporter complex is suggested by findings that [^{3}H]paroxetine selectively labels only sites associated with the 5-HT transporter in the brain (Habert et al., 1985; Mellerup and Plenge, 1986a). Furthermore, several investigators have reported an excellent correlation between the specificity of various drugs for [^{3}H]paroxetine binding and synaptosomal 5-HT uptake (de Souza and Kuyatt, 1987; Habert et al., 1985), whereas there was no correlation between the potencies of a variety of drugs in inhibiting [^{3}H]paroxetine binding and their

relative potencies in inhibiting either NE or DA uptake into synaptosomes (Laruelle et al., 1988).

The site labeled by [^{3}H]paroxetine is considered to be distinct from the [^{3}H]imipramine binding site. This follows from reports that the inhibition of [^{3}H]imipramine binding by paroxetine or vice versa is complex, indicating an allosteric, rather than a competetive interaction (Sette et al., 1983; Mellerup et al., 1983). Plenge and collegues (Plenge, Mellerup, Honore and Honore, 1987) found no correlation between the inhibition of [^{3}H]paroxetine binding and the affinity of the compounds for the [^{3}H]imipramine binding site, suggesting that these binding sites are situated on different parts of the 5-HT transport system. In addition, the molecular weight for the polypeptide chain containing the binding site for [^{3}H]imipramine is reported to be higher than that for the polypeptide chain which binds [^{3}H]paroxetine (Mellerup, Plenge and Nielsen, 1984). Furthermore, selective destruction of 5-HT nerve terminals with chemical lesions was reported to lead to almost total destruction of [^{3}H]paroxetine binding sites (De Souza and Kuyatt, 1987; Habert et al., 1985; Hrdina, Foy, Hepner and Summers, 1990), but only a 42% reduction in [^{3}H]imipramine binding (Sette et al., 1983). As well, Mellerup and Plenge (1986a) reported that while both ligands label the same number of sites on platelets, in neuronal membranes the B_{max} for [^{3}H]imipramine is somewhat higher than that for [^{3}H]paroxetine. These findings indicate that although a major part of the high-affinity [^{3}H]imipramine binding is located on the 5-HT transport mechanism, [^{3}H]imipramine binding occurs to other sites in

brain tissue as well. Indeed, there is accumulating evidence that [^{3}H]imipramine binds to more than one population of binding sites: 1) a high-affinity site which is located at the presynaptic serotonergic nerve terminals and is associated with the 5-HT uptake complex, and; 2) a low-affinity site which may be related to various postsynaptic neurotransmitter receptors, and which may also be located extraneuronally (Hrdina, 1984).

Taken together, these findings suggest that [^{3}H]paroxetine may be a useful biochemical marker of the 5-HT transporter complex. The present study, which measured [^{3}H]paroxetine binding and [^{14}C]5-HT uptake in parallel, on blood platelets from healthy volunteers, provides further support for the use of [^{3}H]paroxetine as a label for the 5-HT uptake site, since a significant correlation between [^{3}H]paroxetine binding and [^{14}C]5-HT uptake was found.

Method

Subjects

Eleven healthy volunteers were recruited from the Douglas Hospital-McGill University Research Centre for participation in the study. The subjects included six men and five women, ranging in age from 24 to 38 years (mean age $\pm$ SEM = 29 $\pm$ 2 years). Report of physical or psychiatric illness, including affective disorder, schizophrenia, organic mental disorder, or substance abuse served as exclusion criteria. Volunteers with evidence of recent use of alcohol or medication were also excluded.

Procedures

Platelet preparation. All blood samples were collected in a single month, in ethylenediamine tetra-acetic acid (EDTA) vacutainers following an overnight fast. Platelet-rich plasma (PRP) was obtained by centrifugation at 272 g for 15 minutes. An aliquot (6 ml) of PRP from each subject was further processed for 5-HT uptake, while the remainder of the PRP was centrifuged at 18,000 g for ten minutes to isolate the platelets (Wood, Suranyi-Cadotte, Nair, Lafaille and Schwartz, 1983). In brief, platelets were washed twice in 50 mM Tris-HCl, 20 mM EDTA, 150 mM NaCl (pH 7.5), lysed in 5 mM Tris-HCl, 5 mM EDTA (pH 7.5), and then the membranes were isolated by centrifugation at 39,000 g for ten minutes. The membranes were washed again with 70 mM Tris-HCl (pH 7.5), finally suspended in 50 mM Tris-HCl, 5 mM KCl and 120 mM NaCl (pH 7.4), and frozen at -80°C until the [^{3}H]paroxetine binding assay was performed.

[^{14}C]5-HT uptake. The uptake of [^{14}C]5-HT was conducted according to the method of Malmgren (1981). Undiluted PRP (500 ul) was preincubated for 5 minutes at 37°C with and without 100 uM desipramine, and then incubated with 20 ul of [^{14}C]5-HT (52.8 mCi/mmol; New England Nuclear, Boston, MA) at concentrations of 0.10, 0.25, 0.45, 0.9 and 1.8 uM, respectively. The incubation was interupted after 2.5 minutes by filtration on filters (0.65 u), followed by 3 x 3 ml washes with cold 50 mM Tris-HCl, 20 mM EDTA (pH 7.5), and counted in a liquid scintillation cocktail by scintillation spectrometry. Specific uptake was defined as the difference in uptake in the presence and absence of desipramine

and represented 90% of the total counts. In order to establish the degree of linearity between concentrations of [^{14}C]5-HT and initial uptake velocities of 5-HT, Scatchard analysis was performed. The K_m and V_{max} of [^{14}C]5-HT uptake were then obtained by linear regression using the Lineweaver-Burke plot.

[^{3}H]Paroxetine binding. The [^{3}H]paroxetine binding assay was performed according to the method described by Langer, Schoemaker and Segonzac (1985). The lysed membranes (0.5 - 1.5 mg protein/ml) were incubated in a final volume of 300 ul in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM KCl and 120 mM NaCl, with 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0 and 6.0 nM [^{3}H]paroxetine (19 Ci/mmol; New England Nuclear) for 90 minutes at 20°C. Samples were filtered on GF/B Whatman filters (Fisher Scientific; Montreal, Que.) and washed with 3 x 3 ml of cold incubation buffer. Specific binding was calculated as the difference in [^{3}H]paroxetine binding observed in the presence and absence of 3 uM citalopram (Lundbeck Pharmaceuticals; Copenhagen, Denmark), and represented 80 to 90% of the total binding. The inter-assay variability of the [^{3}H]paroxetine binding assay was 6%. The B_{max} and affinity constant (K_d) of [^{3}H]paroxetine binding were calculated by linear regression of Scatchard plots (see Appendix A for a more detailed description of receptor binding and analysis).

Results

Scatchard analysis of [^{3}H]paroxetine binding was linear, indicating the presence of a single class of binding sites, with a mean ($\pm$ S.E.M.) B_{max} of 1375 $\pm$ 108 fmol/mg protein, and an apparent K_d of 0.43 $\pm$ 0.03 nM. A representative

Scatchard plot is shown in Figure 4. The mean maximum velocity (V_{max}) of 5-HT uptake was 139 $\pm$ 20 fmol/10^5 platelets/2 minutes, with a K_m of 2.58 $\pm$ 0.37 nM (see Figure 5 for a representative Lineweaver-Burke plot). Figure 6 shows that a significant positive relationship exists between [^{3}H]paroxetine binding and [^{14}C]5-HT uptake on blood platelets of normal volunteers (r = .60, p<.05). The correlation between the affinity constants for [^{3}H]paroxetine binding and [^{14}C]5-HT uptake was not significant (r = .12).

Discussion

Although [^{3}H]paroxetine binding has been well-correlated with the 5-HT system in animal studies, no literature exists on the nature of the relationship between the site labeled by [^{3}H]paroxetine and 5-HT function in human platelets. Indeed, the present study is the first known investigation of the association between platelet [^{3}H]paroxetine binding and 5-HT function in humans. The results of this study indicate a significant correlation between [^{3}H]paroxetine binding and the uptake of [^{14}C]5-HT carried out in the same blood samples of healthy volunteers. These findings extend results from previous studies by indicating a significant relationship between these markers in human platelets.

The significance of the association between platelet [^{3}H]paroxetine binding and [^{14}C]5-HT uptake in the present study is limited, however, by the small number of subjects and the modest range of variability in the data. For example, if the two subjects with a high maximal velocity of [^{14}C]5-HT uptake were removed from the analysis, the association between [^{3}H]paroxetine binding and

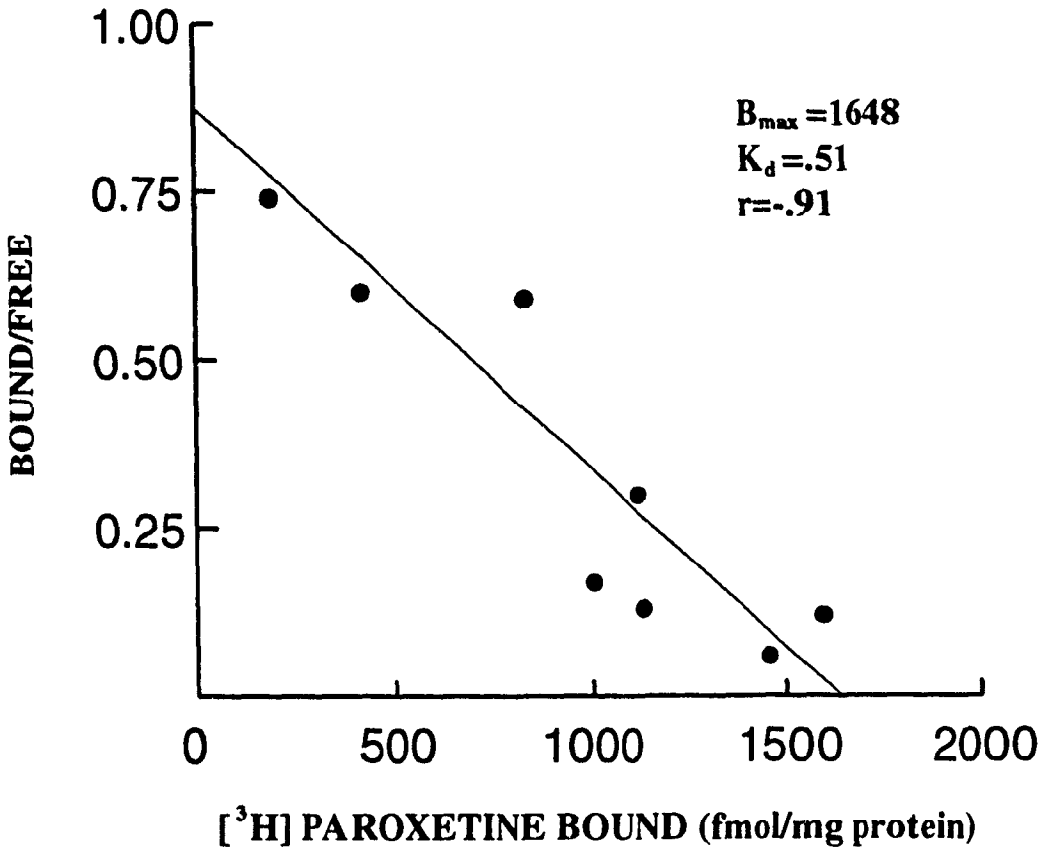


Figure 4. Representative scatchard plot for [^{3}H]paroxetine binding.

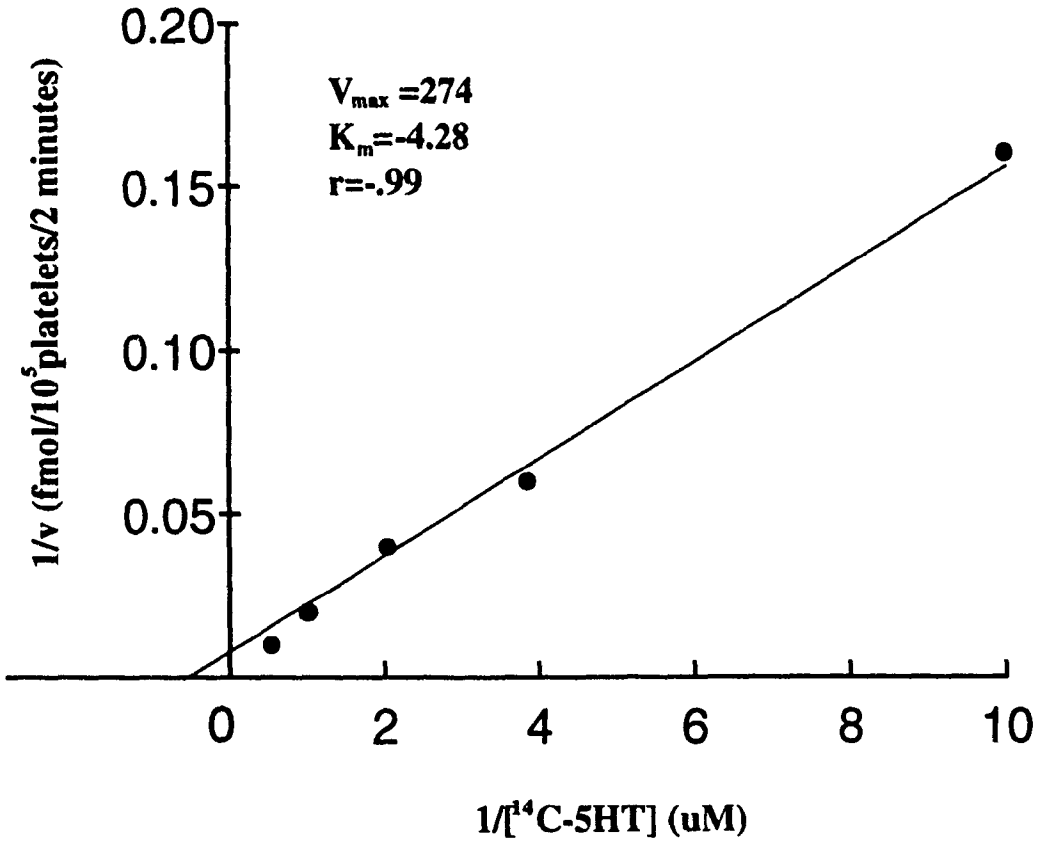
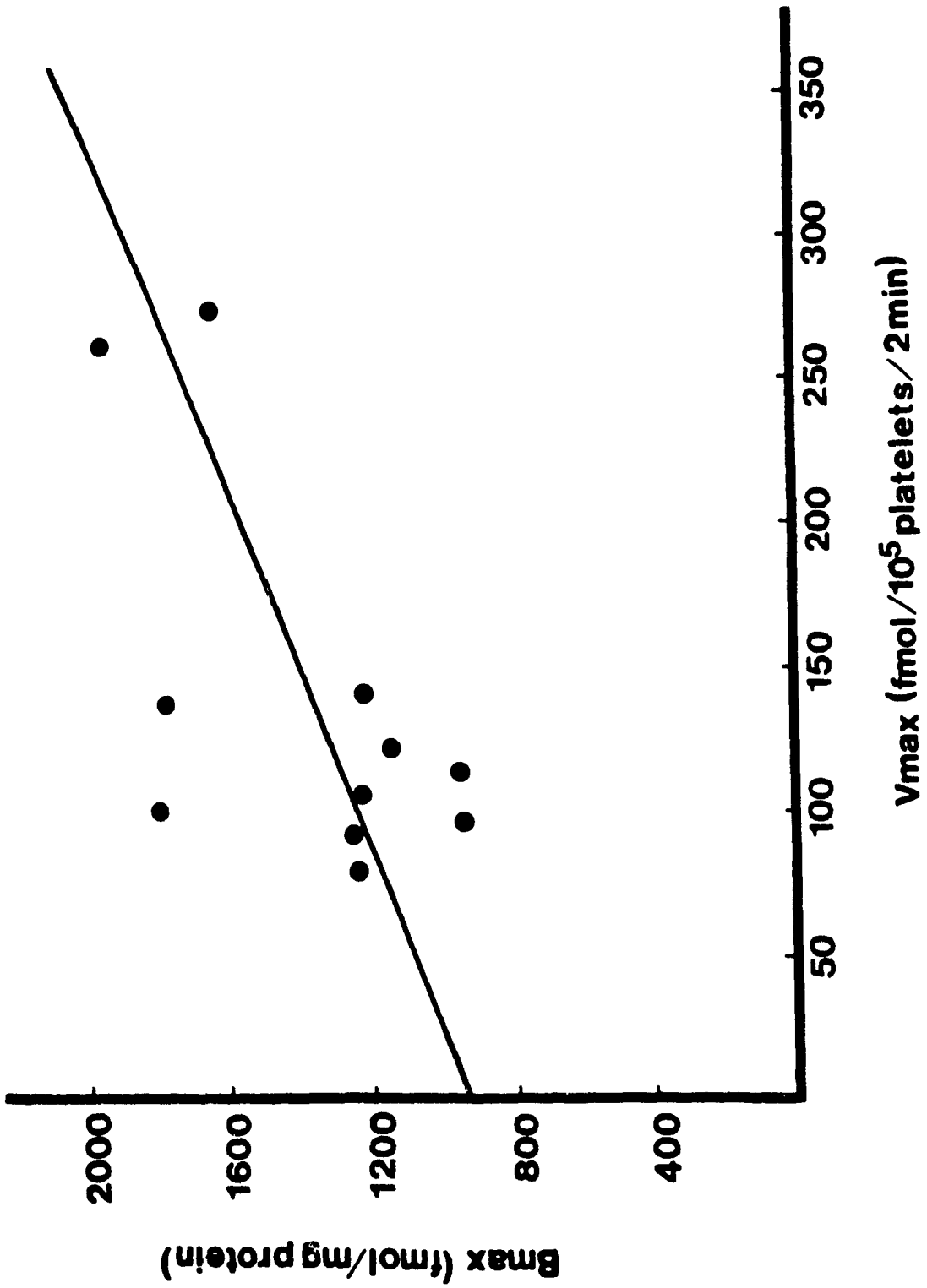


Figure 5. Representative Lineweaver-Burke plot for the analysis of 5-HT uptake.

Figure 6. Significant correlation between the uptake of [^{14}C]5-HT and [^{3}H]paroxetine binding on blood platelets of healthy volunteers (Pearson's r = .60, p<.05).


Bmax (fmol/mg protein)
2000
1600
1200
800
400
Vmax (fmol/10^5 platelets/2min)
50
100
150
200
250
300
350

[^{14}C]5-HT uptake would no longer be significant. Additional subjects would need to be added in order to determine whether the significance of the association would be strengthened or reduced. Thus, the results of the present study may only be considered to provide preliminary evidence of an association between [^{3}H]paroxetine binding and [^{14}C]5-HT uptake on human platelets.

Both 5-HT uptake (Tuomisto and Tukianen, 1976) and [^{3}H]imipramine binding sites (Suranyi-Cadotte et al., 1983) on blood platelets are reportedly decreased in clinically depressed patients as compared to healthy volunteers, and high-affinity [^{3}H]imipramine binding has been proposed as a useful biochemical marker of depression (Langer et al., 1981). This is of interest since disturbances in the 5-HT system have been proposed to underlie depression (eg. Van Praag, 1982). Recent investigations indicate that [^{3}H]paroxetine binding may serve as a more reliable marker for the 5-HT transporter system than [^{3}H]imipramine (Buus-Lassen, 1978; Habert et al., 1985; Mellerup et al., 1983). As previously mentioned, [^{3}H]paroxetine selectively labels only sites associated with the 5-HT transporter in the brain (Habert et al., 1985). Furthermore, the selective destruction of 5-HT nerve terminals reportedly leads to almost total destruction of [^{3}H]paroxetine binding sites (Habert et al., 1985), but to only a 42% reduction in [^{3}H]imipramine binding sites (Sette et al., 1983). There is also evidence that [^{3}H]imipramine labels a site which is not associated with the 5-HT transporter complex (Hrdina, 1984), and [^{3}H]imipramine binding has not been consistently associated with 5-HT uptake on blood platelets (Ahtee et al., 1981; Wood et al.,

1983). In addition, unlike [^{3}H]imipramine binding, [^{3}H]paroxetine binding assays can be performed at physiological temperatures, such as those used to measure [^{14}C]5-HT uptake.

In summary, the results of the present study provide evidence that [^{3}H]paroxetine binding is associated with [^{14}C]5-HT uptake in human blood platelets. These findings provide further support for the notion that [^{3}H]paroxetine may serve as a reliable marker for the 5-HT transporter system. In addition, the results suggest that [^{3}H]paroxetine binding may be useful in further characterizing the alterations in 5-HT uptake presumed to underlie various psychiatric and neurological disorders.

STUDY 2

[^{3}H]Imipramine and [^{3}H]Paroxetine Binding on Blood Platelets of Patients with Depressive and Anxiety Disorders: Association with Hopelessness

The literature reviewed in the introduction to this investigation suggests that alterations in serotonergic function may serve as a common neurochemical link in the relationship between depression and anxiety. Serotonin has long been thought to be involved in the pathophysiology of affective disorders (Coppen et al., 1972). Evidence implicating 5-HT as an important monoamine in the control of anxiety is more recent, following the demonstration of the anxiolytic properties of drugs which affect 5-HT_{1A} activity (Feighner et al., 1982).

Alterations in 5-HT function have also been associated with suicidal behavior (Asberg et al., 1976), not only in primary depression, but in suicide in general (eg. Brown et al., 1979). Hopelessness, a core characteristic of depression, has recently emerged as an important concept in the understanding and prediction of suicide (Beck et al., 1985). Like suicidal behavior, hopelessness is not specific to depression, but rather is represented in a number of psychiatric conditions (Akiskal et al., 1983).

In light of these findings, the aims of this study were two-fold: 1) to further examine the nature of 5-HT function in depressive and anxiety disorders, and; 2) to examine the association between hopelessness and 5-HT function,

independant of psychiatric diagnosis. The hypothesis that hopelessness may be associated with 5-HT function follows from observations of a strong association between hopelessness and suicidal behavior, which suggests that hopelessness, like suicide, may similarly be associated with decreases in 5-HT neurotransmission. Importantly, while the first aim of this study examines 5-HT function within specific psychiatric diagnoses, the second looks beyond diagnostic classification in investigations of psychopathology; this latter approach supports a dimensional rather than a categorical view of 5-HT dysfunction in behavior.

The measures of 5-HT function used in this study were [^{3}H]imipramine and [^{3}H]paroxetine binding on blood platelets. As mentioned above, previous studies have indicated a relationship between these binding sites and the 5-HT transporter complex (Briley et al., 1981; Habert et al., 1985). Again, it must be emphasized that although the [^{3}H]imipramine, and particularly the [^{3}H]paroxetine binding sites have been closely associated with serotonergic function, these sites do not represent direct markers of the central 5-HT system.

Method

Subjects

All patients were recruited through outpatient research facilities at the Douglas Hospital and met DSM-III-R (American Psychiatric Association, 1987) criteria for depressive or anxiety disorders. In addition, to be included in the study, patients with Major Depression (n = 11) had to meet Research Diagnostic Criteria for endogenous depression (Spitzer, Endicott and Robins, 1978) and had

to obtain a score of at least 18 on the 17-item Hamilton Rating Scale for Depression (HAM-D; Hamilton, 1960); those meeting diagnostic criteria for Dysthymic Disorder (n = 9) had to achieve a score of at least 13 on the HAM-D. Patients meeting the criteria for Generalized Anxiety Disorder (GAD; n = 18) had to obtain a score of at least 18 on the Hamilton Rating Scale for Anxiety (HAM-A; Hamilton, 1959). Patients who met the DSM-III-R criteria for Panic Disorder (n = 10) must have experienced a minimum of one unexpected (spontaneous) panic attack and/or one situational panic attack per week for the past three weeks.

Physical illness and signs or symptoms of substance abuse disorder, schizophrenia, or organic mental disorder served as exclusion criteria. All patients were medication-free for at least two weeks before participation in the study. Healthy, age-matched volunteers (n = 13) were recruited from Concordia University and were screened to exclude those with previous or current psychiatric disorder or drug intake. A total of 61 subjects between the ages of 18 and 71 years old were entered into the study between October 1988 and October 1989 (see Table 2).

Procedures

Following written informed consent (see Appendix B), subjects were interviewed by a psychiatrist to determine their personal and family history of psychiatric disorder, and history of suicidal behavior. They were then rated on the Hamilton scales for depression and anxiety. The HAM-D is a 17-item scale which

Table 2

Clinical Data for Subgroups of Patients and Healthy Volunteers

	Depression	Dysthymia	GAD	Panic	Controls
Total no.	11	9	18	10	13
Sex					
Female	6	4	8	8	7
Male	5	5	10	2	6
Age	46.5±4.2	45.0±4.8	47.0±2.7	34.7±3.3[a]	39.0±2.2
[^{3}H]Imipramine[b]					
B_{max}(n)	1285±137(9)	1248±130(8)	1240±95(11)	1190±123(9)	1784±119(13)
K_d	2.7±1.5	1.4±0.3	2.4±1.3	2.2±1.3	1.5±0.1
[^{3}H]Paroxetine[c]					
B_{max}(n)	1225±102(10)	1295±124(8)	936±74(18)	963±119(10)	1513±84(13)
Kd	0.7±0.2	0.6±0.1	0.8±.09	0.5±.09	0.7±.08

Note. Values represent mean ± SEM.

[a]Patients with Panic Disorder were significantly ($p < .05$) younger than patients with Depression and GAD, however, no significant difference with the control group was found.

[b]B_{max} indicates maximal platelet binding (fmol/mg protein); K_d indicates the dissociation constant (nmol/l). The [^{3}H]imipramine B_{max} for all patient groups was significantly lower than for controls ($p < .05$).

[c]Only patients with GAD and Panic Disorder were significantly lower than controls ($p < .05$). No significant differences for K_d were observed.

includes factors for psychomotor retardation, anxiety and sleep. The HAM-A includes 14 items which assess the psychic and somatic components of anxiety. All psychiatrists participated in inter-rater reliability sessions, to ensure that an adequate level of reliability on the Hamilton scales was achieved.

In addition, subjects completed the Hopkins Symptom Checklist (SCL-58; Derogatis, Lipman, Rickels, Uhlenhuth and Covi, 1974), a self-rating scale scored on five symptom dimensions: depression, anxiety, somatization, obsessive-compulsive and interpersonal sensitivity. Subjects were instructed to rate themselves on each symptom using a four-point scale of distress, with "not at all" being scored 1 and a score of 4 representing "extreme" distress. Subjects also completed the Hopelessness Scale (HS; Beck, Weissman, Lester and Trexler, 1974), a 20 item true-false self-report inventory used to assess the extent of pessimism. Each of the 20 items is scored 1 or 0; nine of the items are keyed false and 11 true. The total score is the sum of the individual item scores, and the possible range of scores is from 0 to 20. Samples of the rating scales are shown in Appendix C.

Platelet Binding

Seventy to 80 ml of blood were collected between 8:30 a.m. and 11:30 a.m. in ethylenediamine tetra-acetic acid (EDTA) vacutainers following an overnight fast. Platelet-rich plasma (PRP) was obtained by centrifugation at 272 x g for 15 minutes. The PRP was then processed for the isolation of platelet membranes as described in the previous experiment, according to the method of Wood and

colleagues (1983). The membranes were divided into two equal aliquots and stored at -80°C until analysis.

[^{3}H]Imipramine binding. The [^{3}H]imipramine binding assay was carried out as described by Briley and collegues (1980). The lysed membranes (0.5 to 1.5 mg protein/ml) were incubated in a final volume of 250 ul with 0.3, 1.0, 2.0, 3.0, 4.0 and 5.0 nM [^{3}H]imipramine (77 ci/mmol; New England Nuclear, Boston, MA) for 60 minutes at 4°C. Samples were filtered on GF/B Whatman filters and washed with 3 x 5 ml of 50 mM Tris-HCl (pH 7.4) cold incubation buffer containing 5 mM KCl and 120 mM NaCl, and counted in 5 ml of a liquid scintillation cocktail by scintillation spectometry. Specific binding was defined using 100 uM of desipramine and represented 70 to 85% of the total binding. Inter-assay variability was 7%.

[^{3}H]Paroxetine binding. The [^{3}H]paroxetine binding assay was performed according to the method of Langer et al. (1985), as described in the previous study. Briefly, the lysed membranes were incubated in a final volume of 300 ul in the 50 mM Tris-HCl buffer described above, with 0.1 to 6.0 nM [^{3}H]paroxetine (19 Ci/mmol) for 90 minutes at 20°C. Samples were filtered on GF/B Whatman filters, washed with 3 x 3 ml of the 50 mM Tris-HCl buffer at 4°C, and counted as described above. Specific binding was calculated as the difference in [^{3}H]paroxetine binding observed in the presence and absence of 3 uM citalopram and represented 80 to 90% of the total binding. As previously mentioned, the inter-assay variability for [^{3}H]paroxetine binding was 6%.

The B_{max} and K_d of [^{3}H]imipramine and [^{3}H]paroxetine binding were calculated by linear regression of Scatchard plots. Protein assays were performed using the Lowry (Lowry, Rosebrough, Farr and Randall, 1951) method.

Due to difficulties with blood sampling and technical problems with laboratory equipment and supplies, determinations of [^{3}H]imipramine and [^{3}H]paroxetine binding are not available for all subjects (see Table 2 for final sample sizes for each binding assay).

Statistical Methods

Analyses of variance with Duncan's multiple comparison procedure, t-tests and Pearson correlations were performed using the SAS statistical package. Probability values were not adjusted for multiple comparisons, as the study was intended as a descriptive analysis.

Results

[^{3}H]Imipramine Binding

Scatchard analyses revealed a single population of high affinity binding sites. Typical Scatchard plots for a healthy volunteer and a depressed patient are shown in Figure 7. The B_{max} for [^{3}H]imipramine was significantly ($F[4,45] = 4.92$, $p<.002$) lower on platelets from patients with Major Depressive, Dysthymic, GAD and Panic disorders than in healthy volunteers (Duncan Multiple Range Test, $p<.05$ for all groups; see Figure 8). No significant differences were found between patients and controls for the apparent affinity constant ($F[4,45] = 2.34$, n.s.). K_d values for the various groups are shown in Table 2.

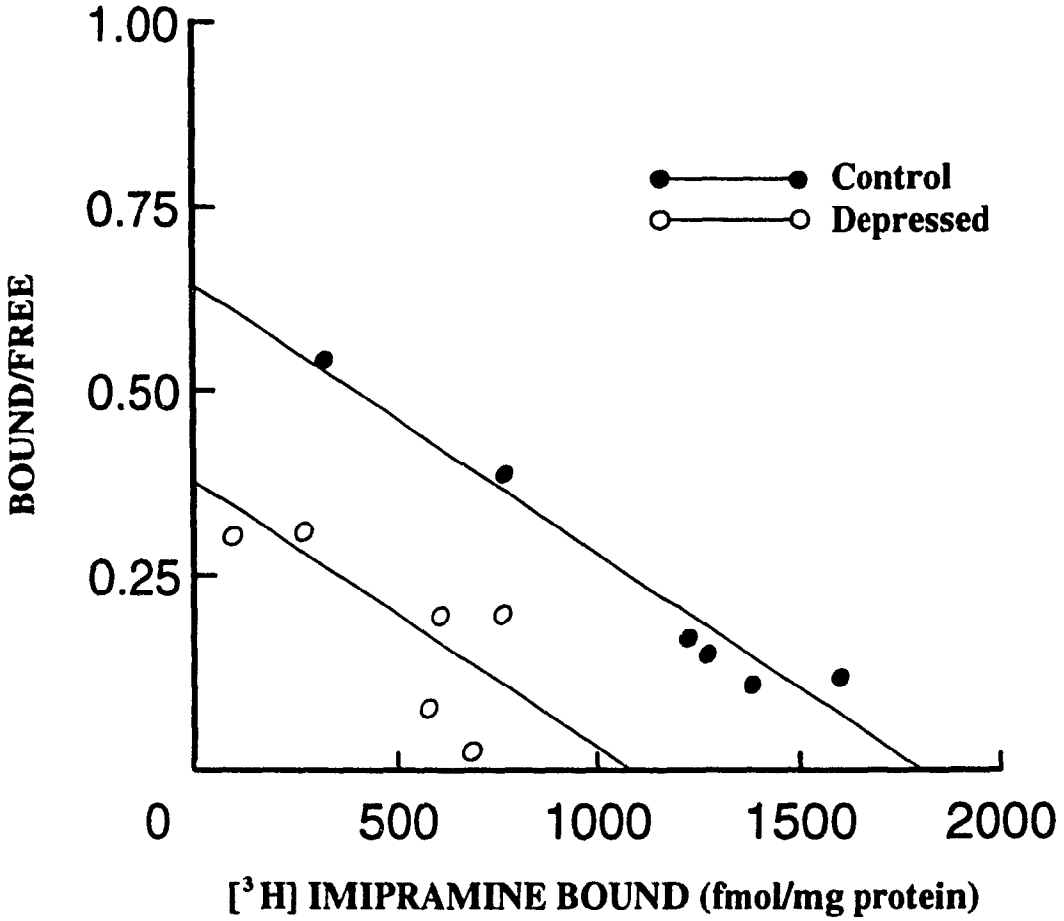
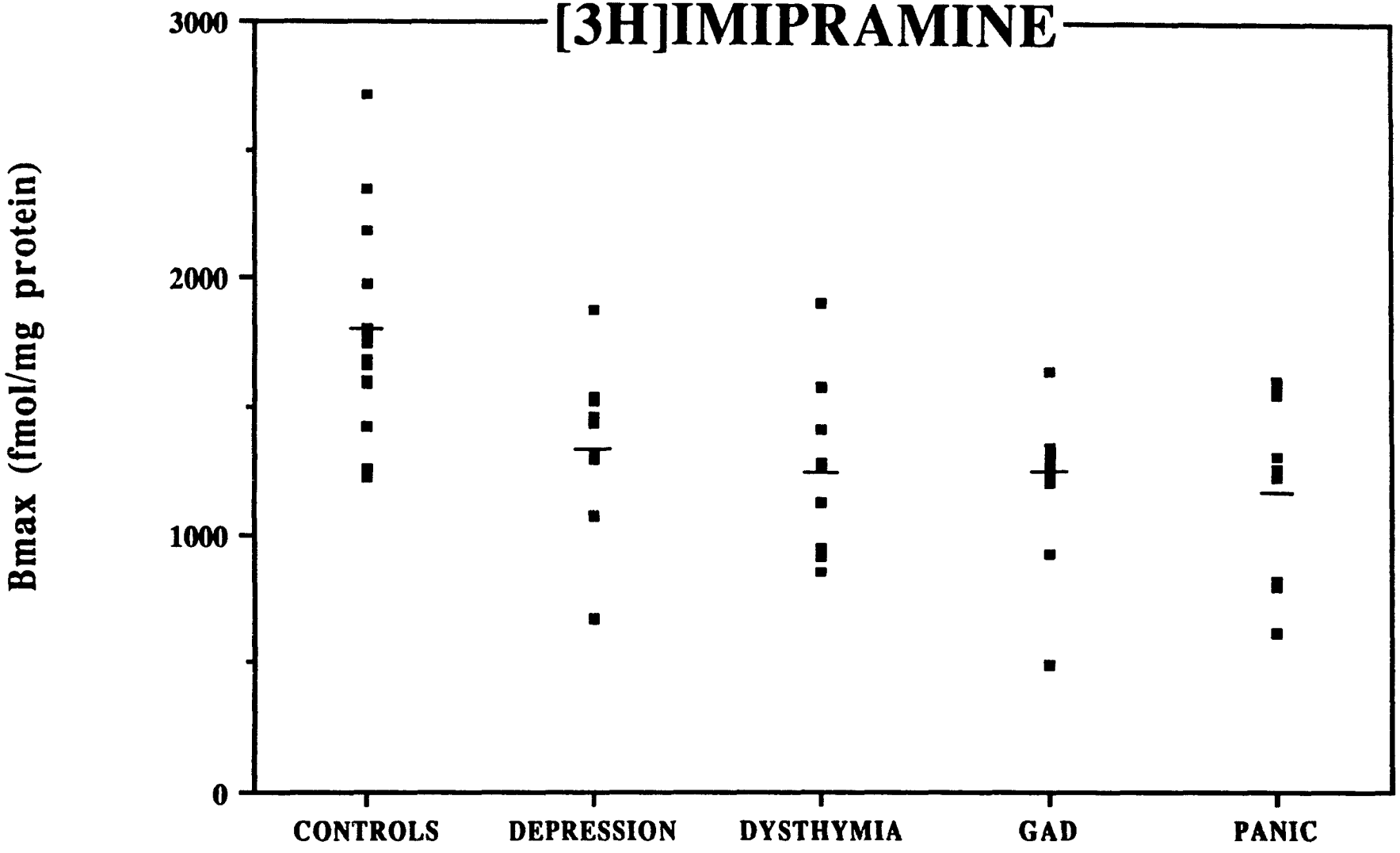


Figure 7. Representative scatchard plots for [^{3}H]imipramine binding in a healthy volunteer (B_{max} = 1786, K_d = 1.69, r = -.99) and a depressed patient (B_{max} = 1089, K_d = 2.57, r = -.82).

Figure 8. [^{3}H]Imipramine binding capacity (B_{max}) on platelets from controls (mean $\pm$ SEM = 1784 $\pm$ 119 fmol/mg protein, n = 13) and patients with depression (1285 $\pm$ 137, n = 9), dysthymia (1248 $\pm$ 130, n = 8), GAD (1240 $\pm$ 95, n = 11) and panic disorder (1190 $\pm$ 123, n = 9); the Bmax for all patient groups was significantly lower than for controls (Duncan, $p < .05$).


[3H]IMIPRAMINE
Bmax (fmol/mg protein)
3000
2000
1000
0
CONTROLS
DEPRESSION
DYSTHYMIA
GAD
PANIC

[^{3}H]Paroxetine binding

A single population of high affinity binding sites was also revealed by Scatchard analyses of [^{3}H]paroxetine binding (see Figure 9 for representative Scatchard plots from a healthy control and a depressed patient). The density of [^{3}H]paroxetine binding differed significantly across groups ($F[4,54] = 7.15$, $p<.001$; see Figure 10). Post-hoc analysis revealed that binding was significantly reduced only in patients with Generalized Anxiety and Panic disorders (Duncan, $p<.05$). Although B_{max} values of depressed and dysthymic patients showed a trend toward a decrease compared to healthy volunteers, no significant differences were found. No differences between the groups for the apparent affinity constant were observed ($F[4,54] = 0.97$, n.s.; see Table 2 for K_d values).

Relationship of [^{3}H]Imipramine and [^{3}H]Paroxetine Binding

No significant correlations were observed between the B_{max} or the K_d for [^{3}H]imipramine and [^{3}H]paroxetine binding measured in parallel in individual subjects, in any of the groups. Similarly, when the groups were pooled together, no significant correlation between the B_{max} ($r = .19$, $n = 48$) or the K_d ($r = .24$, $n = 48$) for [^{3}H]imipramine and [^{3}H]paroxetine binding were found.

Age, Sex, Personal and Family History

Consistent with previous literature, no significant correlations were observed between age and the parameters of [^{3}H]imipramine (B_{max}: $r = -.01$; K_d: $r = -.24$, $n = 50$) or [^{3}H]paroxetine binding (B_{max}: $r = .19$; K_d: $r = .05$, $n = 59$) when the data was examined overall. In addition, no significant differences in

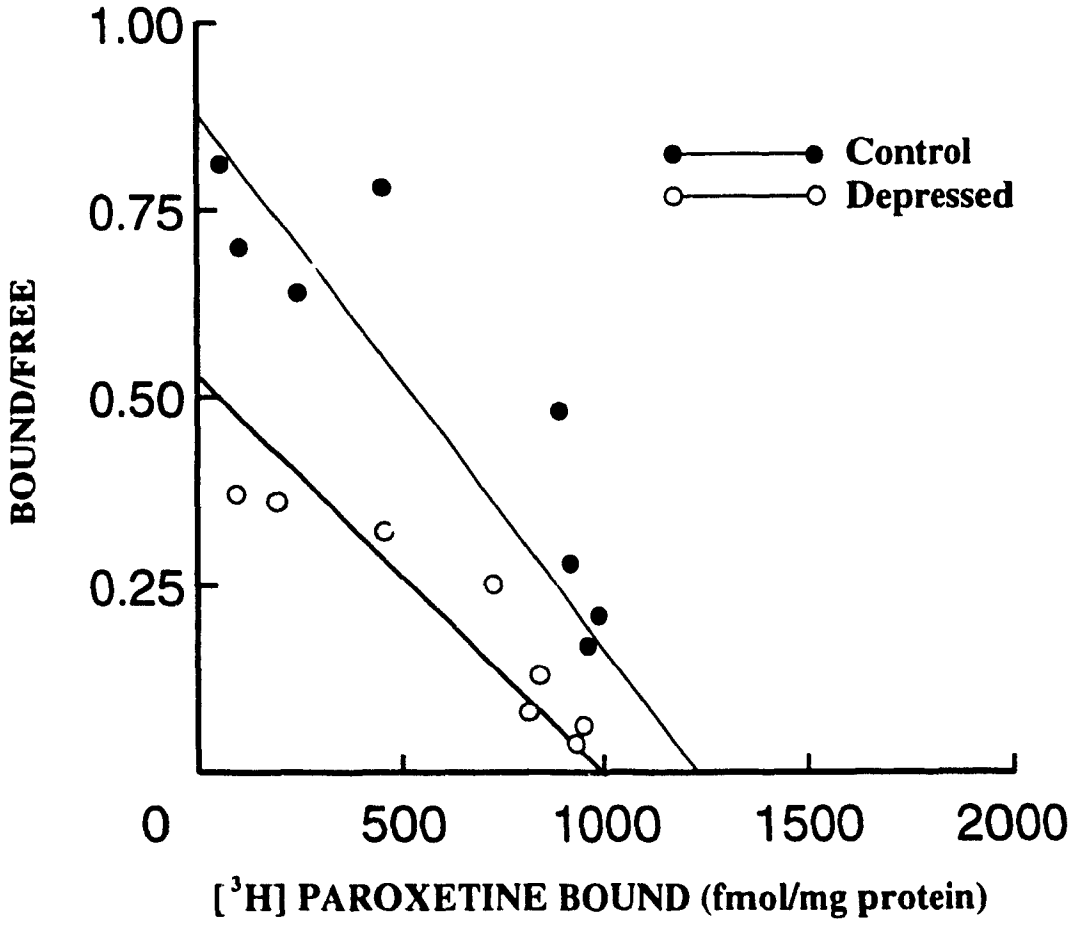
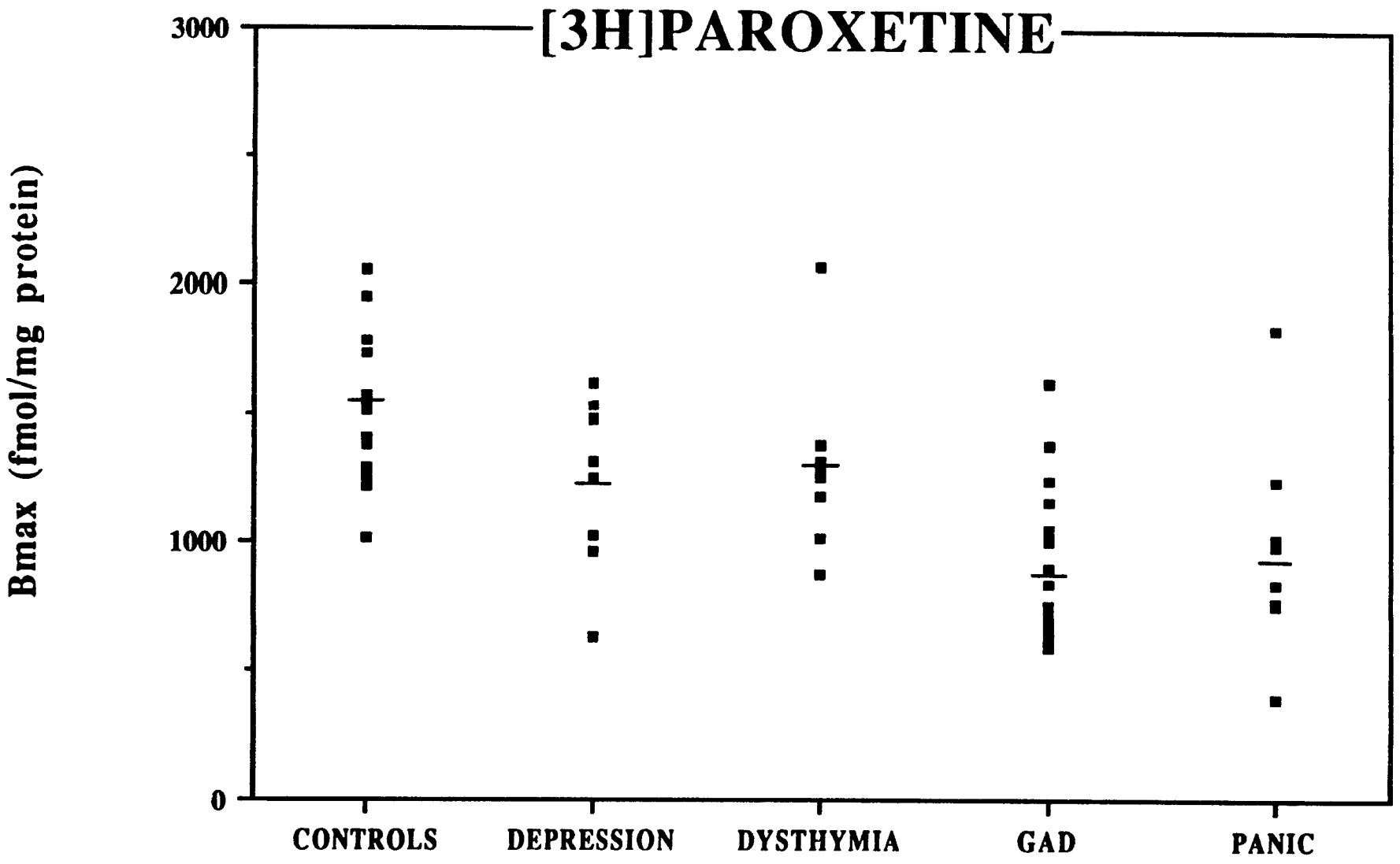


Figure 9. Representative scatchard plots for [^{3}H]paroxetine binding in a healthy volunteer (B_{max} = 1215, K_d = .86, r = -.90) and a depressed patient (B_{max} = 956, K_d = .59, r = -.94).

Figure 10. [^{3}H]Paroxetine B_{max} on platelets from controls (mean $\pm$ SEM = 1513 $\pm$ 84 fmol/mg protein, n = 13), and patients with depression (1226 $\pm$ 102, n = 10), dysthymia (1296 $\pm$ 130, n = 8), GAD (936 $\pm$ 95, n = 18) and panic disorder (963 $\pm$ 123, n = 10). Patients with GAD and panic disorder had significantly less binding than controls (Duncan, $p<.05$); reduced binding in depression and dysthymia were not significant.


[3H]PAROXETINE
Bmax (fmol/mg protein)
3000
2000
1000
0
CONTROLS
DEPRESSION
DYSTHYMIA
GAD
PANIC

binding between male and female subjects were observed across the groups (all t values $\leq$ 1.15).

The observed frequencies for personal and family history variables for each group are presented in Table 3. Examination of the data from all subjects together revealed no significant differences in binding values related to personal or family history of depression, or other psychiatric illness (all t values $\leq$ 1.78). Individuals who had taken psychotropic medication in the three months prior to the study had lower B_{max} values for [^{3}H]paroxetine binding than subjects without recent psychopharmacologic treatment (964 $\pm$ 102, n = 10, vs. 1207 $\pm$ 56, n = 49, fmol/mg protein respectively, $p < .05$); however, no significant difference in [^{3}H]imipramine B_{max} values were observed (1237 $\pm$ 138, n = 10, vs. 1418 $\pm$ 69, n = 40, n.s.). The distribution of subjects with a history of medication usage in the three months prior to the study was spread throughout the patient groups (see Table 3).

Rating scales

HAM-D. As expected, total scores on the HAM-D for patients with Major Depression were significantly ($p < .05$) higher than for the other patient groups; scores for all patient groups were significantly greater than for controls ($p < .05$; see Table 4 for total and factor scores on the HAM-D).

When correlations between the HAM-D and binding values were examined by group, only patients with Panic Disorder showed a significant correlation between HAM-D total and the B_{max} for [^{3}H]imipramine (r = -.70, $p < .05$, n = 8).

Table 3

Observed Frequencies of Personal and Family History Variables by Group: Frequency (% of occurrence)

	Depression	Dysthymia	GAD	Panic	Controls
Personal History					
Depression	9(82)	3(33)	12(67)	1(10)	0(0)
Suicidal behavior	3(27)	1(14)	3(17)	0(0)	0(0)
Prior medications	2(18)	3(33)	3(17)	2(20)	0(0)
Family History					
Depression	4(36)	4(44)	10(56)	3(30)	3(23)
Other psych. illness	1(9)	2(22)	8(20)	2(8)	1(8)
n	11	9	18	10	13

Table 4

Mean (±SEM) Total and Factor Scores on the HAM-D by Group

	Depression	Dysthymia	GAD	Panic	Controls
HAM-D Total	23±1.2	18±1.3	11±0.9	15±2.6	1.3±0.4[a]
HAM-D Factors					
Psychomotor	7.5±0.5	5.9±0.7	3.6±0.3	3.2±0.7	0.3±0.2[b]
Anxiety	6.6±0.3	6±0.8	4.3±0.5	7.0±1.0	0.6±0.2[c]
Sleep	3.5±0.5	2.5±0.4	2.3±0.3	2.9±0.6	0.3±0.1[c]
n	11	9	18	9	13

Note. The sum of the factors does not equal the total score, as all items are not included in these factors.

[a]Scores for depressed patients are significantly higher than for other patient groups ($p < 0.5$); scores for all patient groups are significantly greater than for controls ($p < .05$).

[b]Scores for depressed and dysthymic patients are significantly higher than for other patient groups ($p < .05$); all patient groups are significantly greater than controls ($p < .05$).

[c]All groups are significantly different from controls ($p < .05$).

No significant associations between [^{3}H]imipramine or [^{3}H]paroxetine binding values and factor scores on the HAM-D were observed for individual groups. When all groups were combined, however, total scores on the HAM-D correlated significantly with the B_{max} for [^{3}H]imipramine (r = -.52, p<.001) and [^{3}H]paroxetine (r = -.29, p<.05), as well as with the affinity constant for [^{3}H]imipramine binding (r = .38, p<.01). The K_d for [^{3}H]paroxetine binding, however, was not significantly associated with scores on the HAM-D (r = -.10). See Appendix D for correlations between ratings and platelet binding, as well as intercorrelations between the various scales in combined groups.

HAM-A. Patients with Generalized Anxiety and Panic disorders had significantly (p<.05) higher total scores on the HAM-A than patients with depression and dysthymia. Total scores on the HAM-A for all patient groups were significantly greater than for healthy volunteers (p<.05; see Table 5). When examined by group, HAM-A scores were not found to be significantly associated with binding values. When subjects from various groups were pooled, however, HAM-A scores were observed to correlate significantly with the B_{max} for [^{3}H]imipramine (r = -.54, p<.01) and [^{3}H]paroxetine (r = -.55, p<.01), and with the K_d for [^{3}H]imipramine binding (r = .37, p<.01). The affinity constant for [^{3}H]paroxetine binding was not significantly associated with HAM-A total scores (r = .05).

SCL-58. Factor and total scores on the SCL-58 for each group are presented in Table 6. All patient groups had a significantly higher total score on

Table 5

Mean (±SEM) Total and Factor Scores on the HAM-A by Group

	Depression	Dysthymia	GAD	Panic	Controls
HAM-A Total	18±2.9	11±1.5	26±0.9[a]	24±2.3[a]	2.1±0.7[b]
HAM-A Factors					
Psychic anxiety	11±1.8	8±1.0	13±0.7[a]	13±1.4[a]	1.5±0.5[b]
Somatic anxiety	7±1.0	3±0.7	13±0.4[a]	11±1.0[a]	0.6±0.3[b]
n	4	9	18	9	13

[a]Scores for GAD and Panic Disorder patients are significiantly higher than for patients with depression and dysthymia ($p < 0.5$).

[b]Scores for all patient groups are significantly higher than for healthy controls ($p < 0.5$).

Table 6

Mean (±SEM) Total Raw Scores and Subscale Item Scores of the SCL-58 by Group

	Depression	Dysthymia	GAD	Panic	Controls
Total Raw Score	130±7	116±5	135±8	135±11	72±3[a]
Factors					
Depression	2.5±0.2	2.5±0.2	2.4±0.2	2.3±0.4	1.4±0.1[a]
Anxiety	2.8±0.2	2.0±0.2	3.0±0.2	3.7±0.3	1.3±0.2[b]
Somatization	2.1±0.3	1.6±0.1	2.2±0.2	2.3±0.2	1.2±0.1[b]
Obsessive	2.5±0.1	2.3±0.1	2.6±0.2	2.1±0.2	1.3±0.1[a]
Interpersonal	2.3±0.1	2.3±0.3	2.3±0.1	2.3±0.3	1.4±0.1[a]
n	10	9	18	6	13

[a]Scores for all patient groups are significiantly higher than for controls ($p < .05$).

[b]Scores for all patient groups except Dysthymia are significantly higher than for controls ($p < 0.5$).

the SCL-58 than healthy controls (Duncan, $p < .05$), and, in addition, scored significantly higher on the Depression and Anxiety factors of the scale than healthy volunteers. No significant difference between the patient groups was observed on the Depression subscale. On the Anxiety factor of the scale, patients with Major Depression and GAD did not differ significantly.

Analysis of correlational relationships by group revealed a significant negative relationship between the B_{max} for [^{3}H]imipramine and the total score on the Anxiety factor of the scale for patients with Generalized Anxiety ($r = -.79$, $p < .01$, $n = 11$). Overall, however, significant correlations were observed in the expected directions between binding values and scores on the SCL-58, with the exception of the [^{3}H]paroxetine K_d.

Hopelessness Scale. Total scores on the HS ranged from 3 to 20 (mean $\pm$ SEM = 9.35 $\pm$ 0.8; $n = 49$); totals by group are shown in Table 7. Patients with Major Depression, Dysthymia and GAD had significantly higher scores on the HS than controls (Duncan, $p < .05$); patients with Panic Disorder had scores similar to those of controls.

When all the subjects were pooled, the HS correlated significantly with total scores on the HAM-D ($r = .34$, $n = 49$, $p < .01$) and the SCL-58 ($r = .36$, $n = 49$, $p < .01$), as well as with the depression factor of the SCL-58 ($r = .53$, $n = 47$, $p < .0001$). No significant associations between HS scores and total score on the HAM-A ($r = .24$, $n = 44$), or the anxiety factor of the SCL-58 were found ($r = .14$, $n = 48$).

Table 7

Mean (±SEM) Total Raw Score on the Hopelessness Scale by Group

	Depression	Dysthymia	GAD	Panic	Controls
Hopelessness	11±2*	14.5±2*	10.6±1.5*	6.5±1.0	5.7±0.6
n	8	6	16	6	13

*Significantly higher than controls ($p < .05$).

Unexpectedly, scores on the HS were not associated with the B_{max} for [^{3}H]imipramine (r = .06, n = 40) or [^{3}H]paroxetine (r = -.11, n = 47), or with the K_d for [^{3}H]paroxetine binding (r = .19, n = 47). A significant correlation was found however, between the HS and the [^{3}H]imipramine K_d (r = .40, n = 40, p<.01).

Suicidal behavior. Of the subjects included in the study, six had a history of attempted suicide. These subjects scored significantly higher on the HS than individuals with no history of a suicide attempt (t[47] = 2.9, n = 49, p<.006). No significant differences in [^{3}H]imipramine or [^{3}H]paroxetine binding were found, however, according to history of suicidal behavior (t values $\leq$ 1.4).

Discussion

Consistent with previous literature, the density of [^{3}H]imipramine binding was observed to be lower in patients with Major Depression as compared to healthy controls. The B_{max} for [^{3}H]imipramine binding was also significantly decreased in Dysthymic patients, and in patients suffering from Generalized Anxiety and Panic disorders. In addition, patients with anxiety disorders demonstrated a significant reduction in the density of [^{3}H]paroxetine binding compared to normal subjects. Depressed and dysthymic patients also had lower B_{max} values for [^{3}H]paroxetine than healthy controls, but the differences did not attain statistical significance. No differences in the apparent affinity constants were found. The decreased density of [^{3}H]imipramine and [^{3}H]paroxetine binding, and thus presumably a decrease in the modulatory capacity or inhibition

of 5-HT uptake, are likely to be associated with a decrease in synaptic levels of 5-HT, and may thus explain the appearance of symptomatology.

These findings suggest that alterations in serotonergic function are associated with anxiety, and that these alterations are similar to changes observed in depressed patients. The results support the long recognized overlap between symptoms of depression and anxiety, and further suggest a neurochemical link between depressive and anxiety disorders, via the serotonergic system. Importantly, alterations in measures of 5-HT function observed in patients with Generalized Anxiety and Panic disorders in the present study were found to be independant of personal or family history of depression; due to the preliminary nature of this study, however, corroborating historical evidence was not obtained from informants or medical records. In addition, among patients with generalized anxiety, B_{max} values for [^{3}H]imipramine and [^{3}H]paroxetine were not associated with ratings of depressive symptomatology; these findings suggest that the observed decrease in the density of binding sites cannot be attributed to the presence of depressive symptoms. In patients with Panic disorder, however, a significant relationship was observed between the [^{3}H]imipramine B_{max} and total score on the HAM-D, suggesting that concurrent depressive symptoms may play a role in the decreased density of binding sites observed in these patients.

Previous studies on [^{3}H]imipramine binding in anxiety disorders have produced inconsistent results, with investigators reporting no difference (Uhde, Berrettini, Roy-Byrne, Boulenger and Post, 1987; Schneider, Munjack, Severson

and Palmer, 1987; Nutt and Fraser, 1987; Innis, Charney and Heninger, 1987; Pecknold et al., 1988) or decreased (Lewis, Noyes, Coryell and Clancey, 1985; Weizman et al., 1986) density of [^{3}H]imipramine binding sites in anxious patients relative to normal controls. The role of 5-HT in the pathophysiology of anxiety disorders has remained unclear. In the present study, findings of a reduction in the density of platelet [^{3}H]imipramine and [^{3}H]paroxetine binding sites in anxious patients compared to healthy controls suggest that anxiety is associated with a decrease in 5-HT function. Clearly, further studies are needed to address the discrepancies between animal and human data, and to elucidate the exact nature of 5-HT function in patients with anxiety disorders. The validity of animal models of anxiety, the acute versus chronic effects of serotonergic drugs, the distinction between various types of anxiety disorders and their differential responsiveness to 5-HT related drugs, and the presence of depressive symptomatology must be considered.

Lack of a significant reduction in [^{3}H]paroxetine binding in depressed patients is consistent with previous results (Suranyi-Cadotte, Iny, Desjardins, Yassa and Welner, 1989) showing that compared to controls, the reduction in platelet [^{3}H]paroxetine binding in depression is not statistically significant. D'Haenen, De Waele and Leyson (1988) also observed no differences in the B_{max} or K_d of [^{3}H]paroxetine in depressed patients in comparison to controls. In addition, Lawrence and collegues (Lawrence et al., 1990) failed to observe a reduction in [^{3}H]paroxetine binding values during post-mortem analyses in various brain

regions of depressed suicide victims compared to control subjects. These findings suggest that although [^{3}H]paroxetine is reported to be a more potent and specific marker of the 5-HT uptake site than [^{3}H]imipramine (Buus-Lassen, 1978), and is associated with 5-HT uptake in blood platelets, the site labeled by [^{3}H]paroxetine may be dissociated from the 5-HT uptake site in depressive illness. In addition, the lack of correlation observed between the binding parameters for [^{3}H]imipramine and [^{3}H]paroxetine in individual subjects supports previous findings that the site labeled by [^{3}H]paroxetine is distinct from the [^{3}H]imipramine binding site.

In the present study, no association was observed between hopelessness and the density of platelet [^{3}H]imipramine or [^{3}H]paroxetine binding sites, nor with the apparent affinity constant for [^{3}H]paroxetine. The observation that B_{max} values for [^{3}H]imipramine or [^{3}H]paroxetine were not associated with self-ratings of hopelessness was unexpected, since hopelessness has previously been associated with suicide and depression (Beck et al., 1985), and both suicidal behavior and depression have been associated with a reduced density of [^{3}H]imipramine binding sites (Stanley et al., 1982; Briley et al., 1980). Furthermore, in the present study hopelessness was significantly positively correlated with measures of depression, and was greater in subjects with a history of suicidal behavior.

A significant positive correlation was observed, however, between hopelessness and the affinity constant for [^{3}H]imipramine binding. This finding suggests that individuals who are more hopeless have a higher K_d. A high K_d

represents lower affinity of the platelet receptor for [^{3}H]imipramine and for any putative endogenous imipramine-like inhibitor of 5-HT uptake. Reduced inhibition of 5-HT uptake, because of low affinity for an endogenous uptake inhibitor, could produce more 5-HT uptake and less 5-HT at the synapse. This is consistent with the hypothesis that hopelessness is related to decreased 5-HT function. Meltzer and Arora (1986) reported a positive association between the K_d, but not the B_{max}, for [^{3}H]imipramine binding and Hamilton Depression suicide ratings in nonpsychotic and psychotic depressed patients. Further studies may consider examination of the relationship between hopelessness and a broader range of parameters thought to reflect central 5-HT activity, such as CSF 5-HIAA and behavioral responses to 5-HT agonists and antagonists. Similarly, the relationship of [^{3}H]imipramine and [^{3}H]paroxetine binding with other cognitive and behavioral dimensions may also be examined.

De Jong and Roy (1990) found that two cognitive items from the Beck Depression Inventory, self-accusation and expectation of punishment, were strongly predictive of CSF levels of corticotropin-releasing hormone (CRH) in depressed patients. Hypersecretion of CRH from the paraventricular nucleus of the hypothalamus has been suggested to play a role in the dysregulation of the hypothalamic-pituitary-adrenal (H-P-A) axis found in depression (Gold et al., 1986; Nemeroff et al., 1984). Further investigations may focus on the relationship between [^{3}H]imipramine and [^{3}H]paroxetine binding and the variables identified

by De Jong and Roy (1990) as being associated with H-P-A function, both during the depressive episode and following remission.

No difference in [^{3}H]imipramine or [^{3}H]paroxetine binding was found in subjects with a history of suicidal behavior. These results are consistent with the findings of Meltzer and Arora (1986), who reported no significant difference in platelet [^{3}H]imipramine binding in subjects with a lifetime history of a suicide attempt. Reports that platelet [^{3}H]imipramine binding may be state-dependant (Suranyi-Cadotte et al., 1982) suggest that the lack of a significant association between platelet antidepressant binding and history of suicidal attempt in the present study may be due to the fact that blood sampling did not directly follow the suicide attempt. Indeed, the density of [^{3}H]imipramine binding sites appears to be more consistently altered in post-mortem studies of suicide completers (Stanley et al., 1982).

Taken together, these data suggest that alterations in serotonergic function are similar in depression and anxiety, suggesting a neurochemical link between depressive and anxiety disorders by way of the serotonergic system. When the data from the various groups were combined, ratings of hopelessness were observed to correlate only with the affinity constant for [^{3}H]imipramine binding; hopelessness was not significantly associated with the density of [^{3}H]imipramine or [^{3}H]paroxetine binding sites. Additional studies need to be conducted using other markers in order to further investigate the hypothesis that psychiatric symptoms may be associated with a specific biological state, regardless of diagnosis.

STUDY 3

The Effects of Examination Stress on [^{3}H]Imipramine and [^{3}H]Paroxetine Binding: Association with Social Support

The present study was conducted in order to examine whether a naturalistic stressor, such as year-end academic examinations, may be associated with alterations in serotonergic activity in healthy individuals which are similar to the changes observed in depression. This hypothesis follows evidence reviewed earlier that stressful events may precipitate depression (eg. Brown et al., 1973), and that the depressed state is associated with variations in serotonergic activity (Coppen et al., 1972).

The relationship between social support and 5-HT function was also examined in this study, as supportive relationships have been suggested to act as a buffer between stressful events and the onset of depression by decreasing the impact of stress on the individual (Cohen and Wills, 1985). It is hypothesized here that the buffering effect of social support associated with stress is related to an association between perceived support and serotonergic function, as previous studies have reported a correlation between social factors and 5-HT activity (Raleigh et al., 1984). As in the previous study, platelet [^{3}H]imipramine and [^{3}H]paroxetine binding were used as indicators of serotonergic function.

Method

Subjects

Nineteen first-year medical students, aged 20 to 25 years (mean $\pm$ SEM = 23 $\pm$ 1) were tested in the middle of their final examination period in June on a day when there were no scheduled examinations (stress condition), and re-tested three weeks following their return to class in September (control condition). Evidence of physical illness, substance abuse and major affective disorder served as exclusion criteria. An honorarium was awarded for participation in the study.

Procedures

All participants were interviewed briefly to explain the nature of the study and obtain written informed consent. As in the previous experiment, subjects were rated on the Hamilton scales for depression and anxiety, and completed the SCL-58. They also completed a validated, shortened version of the Social Support Questionnaire (SSQ; Sarason, Levine, Basham and Sarason, 1983). The SSQ yields scores for perceived number of social supports (quantity) and satisfaction with the social support that is available (quality). In addition, subjects completed the Daily Hassles and Uplifts scales (DeLongis, 1985) to assess the degree of their daily stress, and an analog scale on which they rated their current overall level of stress (Perceived Stress) on a scale from 0 to 100.

Biochemical Assays

Blood samples were obtained in the fasting state between 8 and 10 a.m. as previously described, for the measurement of platelet [^{3}H]imipramine and

[^{3}H]paroxetine binding; the assays were conducted as described above. Samples from five subjects, to be analyzed for the determination of [^{3}H]paroxetine binding, could not be found following isolation of the platelet membranes, resulting in a total of 14 samples for this binding assay.

Blood was also obtained during the same sampling for the measurement of plasma cortisol, and was measured using the radioimmunoassay of Krey and colleagues (Krey, Butler, Hotchkiss, Piva and Knobil, 1975), with a cortisol antiserum (F3314) purchased from Endocrine Sciences, California and [^{3}H]cortisol (New England Nuclear; Boston, MA) as tracer. The minimum level of detection with the assay is 10 pg/ml. The intra-assay coefficient of variation is 8.9%.

Statistical Methods

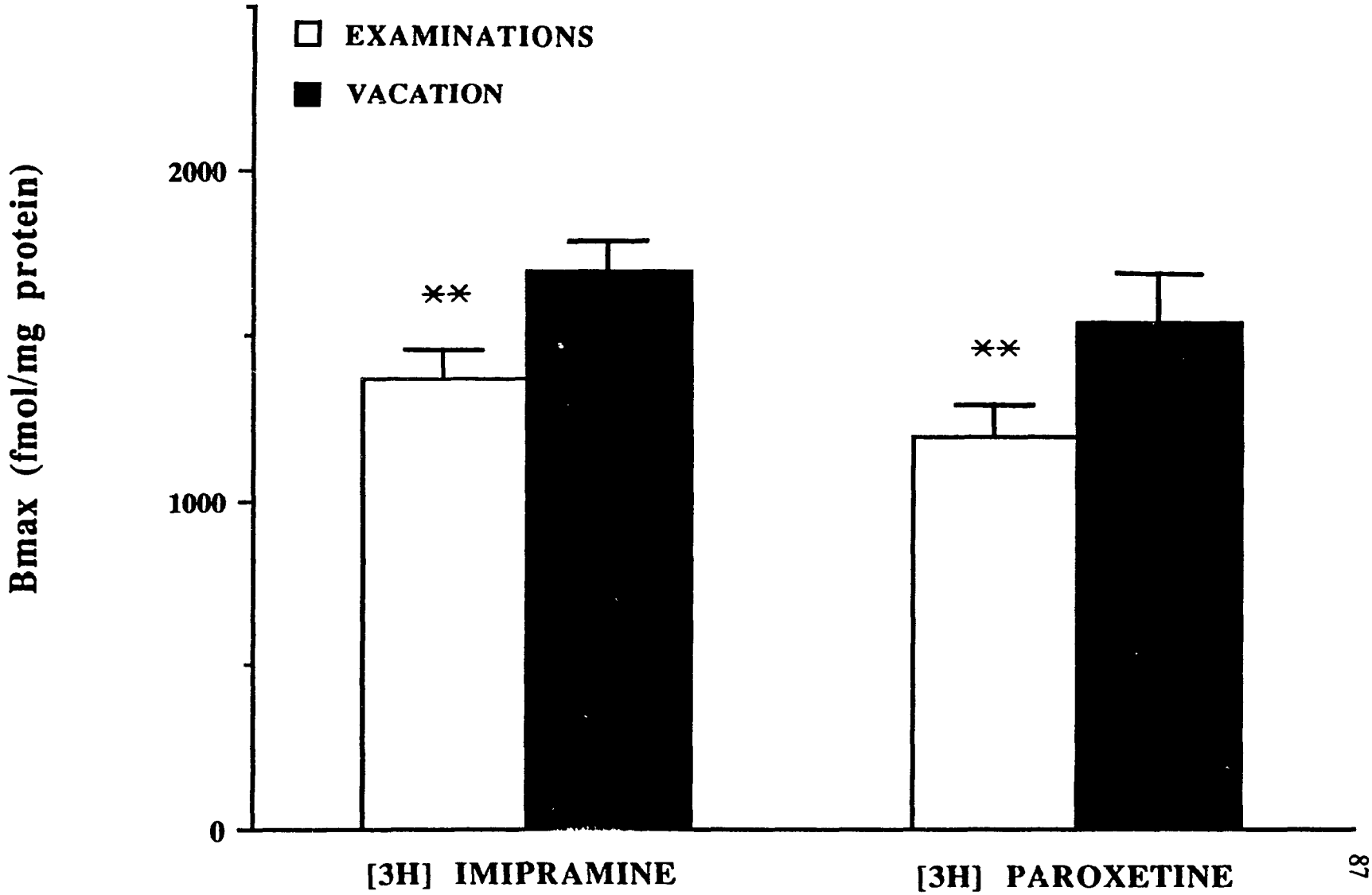
Analyses of variance with Duncan's multiple comparison procedure, t-tests, Pearson correlations and hierarchical regression were performed using the SAS statistical package. Probability values were not statistically adjusted for multiple comparisons, as the study was intended as a descriptive analysis.

Results

[^{3}H]Imipramine Binding

The B_{max} for [^{3}H]imipramine was significantly lower during examinations (1365 $\pm$ 72 fmol/mg protein) than after vacation (1694 $\pm$ 88 fmol/mg protein, $t[17] = 3.82$, $n = 19$, $p<.005$; see Figure 11). Following vacation, 16 of the 19 students showed an increase in [^{3}H]imipramine B_{max} values. No significant change in K_d was observed (1.2 $\pm$ 0.07 nM during examinations vs. 1.3 $\pm$ 0.07 nM

Figure 11. B_{max} for [^{3}H]imipramine (n = 19, p<.001) and [^{3}H]paroxetine binding (n = 14, p<.005) in medical students during and after a period of stress.


EXAMINATIONS
VACATION
Bmax (fmol/mg protein)
2000
1000
0
**
**
[3H] IMIPRAMINE
[3H] PAROXETINE

following vacation, $t[17] = -0.9$, n.s.). Representative scatchard plots for [^{3}H]imipramine binding in the same individual during examinations and after vacation are shown in Figure 12.

[^{3}H]Paroxetine Binding

The B_{max} for [^{3}H]paroxetine was also significantly decreased during examinations (1196 $\pm$ 66 fmol/mg protein) compared to after vacation (1543 $\pm$ 117 fmol/mg protein, $t[12] = 3.3$, $n = 14$, $p<.005$; see Figure 11), with no change in affinity (1.1 $\pm$ 0.1 nM during examinations vs. 1.1 $\pm$ 0.1 nm after vacation; $t[14] = -0.3$, n.s.). Following vacation, 12 of the 14 students showed an increase in the density of [^{3}H]paroxetine binding. Typical scatchard plots for [^{3}H]paroxetine binding in the same individual during examinations and after vacation are shown in Figure 13.

As in the previous study, no significant correlation was observed between the B_{max} ($r = .13$) or the K_d ($r = .03$) of [^{3}H]imipramine and [^{3}H]paroxetine binding for individual subjects in the control condition.

Cortisol

Plasma cortisol levels were significantly increased during examinations (39.6 $\pm$ 7 ug%) compared to after vacation (7.6 $\pm$ 3 ug%, $t[17] = 4.3$, $n = 19$, $p<.001$; see Figure 14), thereby confirming that students were indeed stressed during the examination period. Plasma cortisol levels were not significantly correlated with the B_{max} or the K_d of [^{3}H]imipramine or [^{3}H]paroxetine at either time period (see Table 8). In addition, changes in cortisol values between the

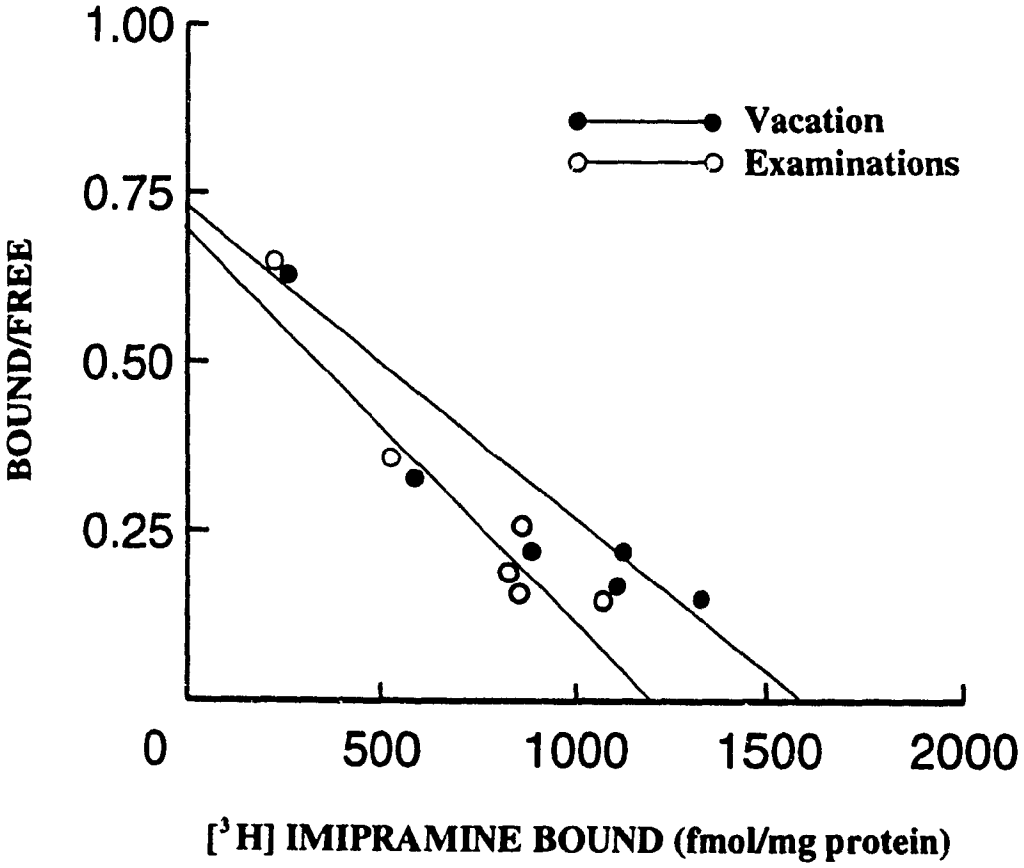


Figure 12. Representative scatchard plots for [^{3}H]imipramine binding in the same individual during examinations (B_{max} = 1213, K_d = .95, r = -.95) and after vacation (B_{max} = 1572, K_d = 1.16, r = -.94).

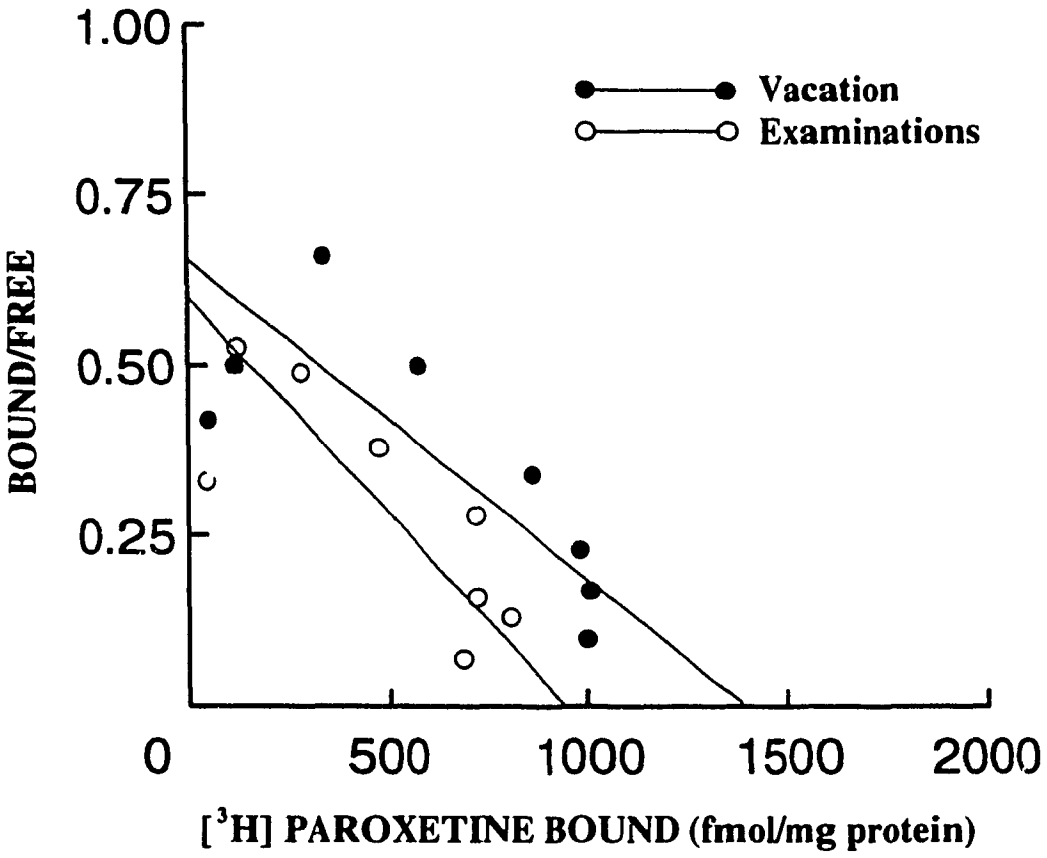


Figure 13. Representative scatchard plots for [^{3}H]paroxetine binding in the same individual during examinations (B_{max} = 962, K_d = .88, $\underline{r}$ = -.79) and after vacation (B_{max} = 1349, K_d = .88, $\underline{r}$ = -.78).

Figure 14. Plasma cortisol values in 19 medical students during examinations and after vacation ($p < .0004$).

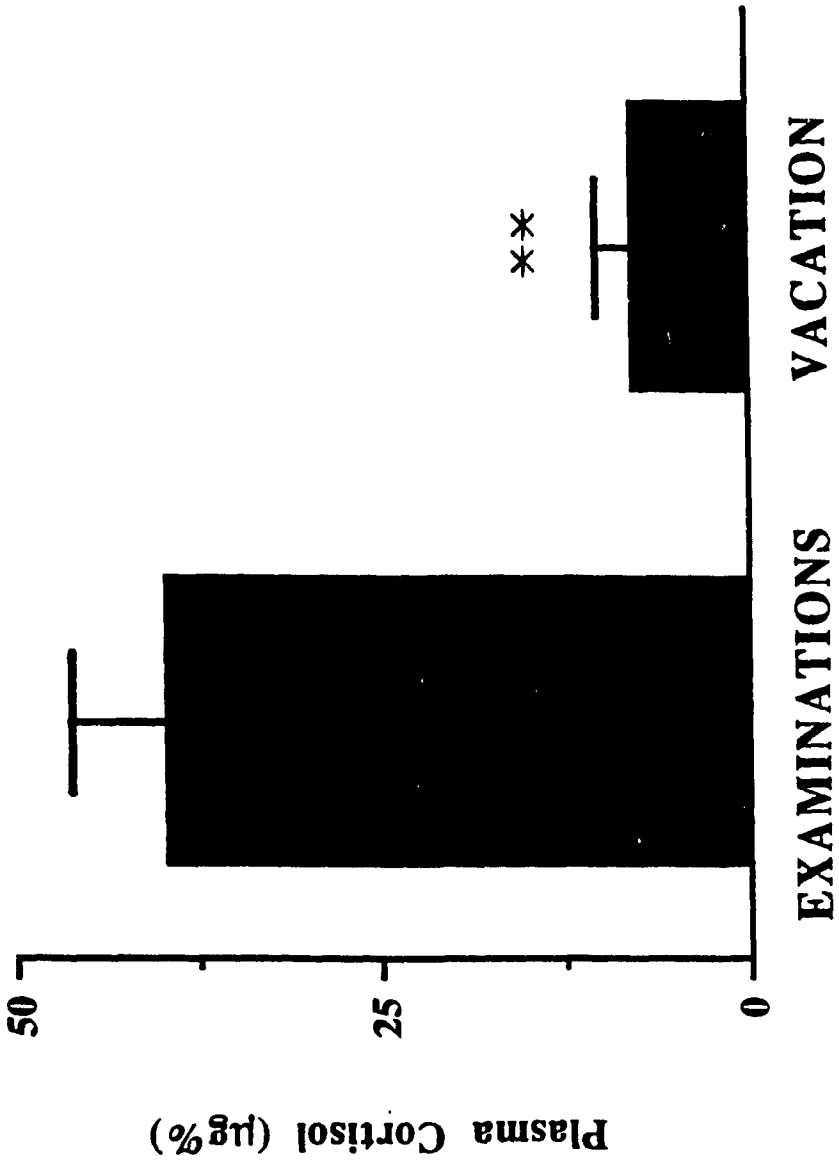

Plasma Cortisol (µg%)
50
25
0
EXAMINATIONS
VACATION
**

Table 8

Correlations between [^{3}H]Imipramine and [^{3}H]Paroxetine Binding and Plasma Cortisol Levels during Examinations and after Vacation

	Plasma Cortisol	
Binding	Examinations	Vacation
[^{3}H]Imipramine[a]		
B_{max}	-.02	-.14
K_d	-.09	-.08
[^{3}H]Paroxetine[b]		
B_{max}	.14	.42
K_d	-.02	.30

[a] n = 19. [b] n = 14.

examination and vacation periods were not associated with changes in the B_{max} of [^{3}H]imipramine (r = -.06) or [^{3}H]paroxetine (r = -.42) binding sites.

Rating Scales

Symptomatology was significantly greater during final examinations, as indicated by total scores on the HAM-D (t[17] = 4.01, p<.0008), HAM-A (t[17] = 4.79, p<.0001) and SCL-58 (t[17] = 5.44, p<.0001), as well as on most factors of these scales (see Table 9). Scores on the Hassles (t[17] = 3.53, p<.002) and Perceived Stress (t[17] = 6.60, p<.0001) scales also decreased significantly after vacation compared to the stress condition. The increase in the number of uplifts following the vacation period, however, was not significant (t[17] = 0.63, n.s.). T-tests of difference scores for social support during the examination period and following vacation revealed that total perceived social support (t[17] = -3.96, p<.0009) was significantly reduced during examinations as compared to the post-vacation period. Intercorrelations between all rating scales during examinations and following vacation are shown in Appendix E.

During the examination period, the B_{max} for [^{3}H]imipramine was significantly correlated with total perceived social support (r = .46, p<.05; see Table 10). Correlations between [^{3}H]imipramine B_{max} and satisfaction with support (r = .29), and between total scores on the HAM-D (r = -.34) and HAM-A (r = -.31) were in the predicted directions, but did not attain significance. After vacation, no significant associations between [^{3}H]imipramine binding values and scores on the rating scales were observed (see Table 11). No correlations

Table 9

Mean (±SEM) Scores on the Rating Scales for Students[a] during Examinations and after Vacation

	Examinations	Vacation
HAM-D Total[b]	4.0±0.5	1.0±0.4***
Psychomotor	0.7±0.2	0.2±0.1*
Anxiety	2.3±0.4	0.5±0.2***
Sleep	0.5±0.1	0.3±0.2
HAM-A Total	5.2±0.8	1.3±0.4***
Psychic	3.0±0.4	0.9±0.3***
Somatic	2.2±0.4	0.4±0.2***
SCL-58 Total[c]	94±4	73±2.7***
Depression	1.7±0.1	0.3±0.1***
Anxiety	0.4±0.1	0.3±0.1***
Somatization	0.3±0.1	0.3±0.1***
Obsessive-compulsive	0.4±0.1	0.3±0.1***
Interpersonal sensitivity	0.4±0.1	0.3±0.1***

[a]n = 19.

[b]The sum of the HAM-D factors does not equal the total score, as all items are not included in these factors.

[c]Factor scores on the SCL-58 represent item means, and not the raw score.

*p < .05. **p < .01. ***p < .001.

table continues

Table 9 (continued)

Mean (±SEM) Scores on the Rating Scales for Students[a] during Examinations and after Vacation

	Examinations	Vacation
Social Support Total	10.4±0.5	11.1±0.5***
Quantity	5.3±0.4	5.9±0.4**
Quality	5.1±0.1	5.3±0.1*
Hassles	49±6	26±5.3**
Uplifts	96±16	106±13
Perceived Stress	61±5	22±4***

[a]n = 19.

*p < .05. **p < .01. ***p < 001.

Table 10

Correlations between Rating Scales and Biochemical Data during Examinations

	[^{3}H]Imipramine[a]		[^{3}H]Paroxetine[b]		
Scale	B_{max}	K_d	B_{max}	K_d	Cortisol[a]
HAM-D Total	-.34	-.09	-.52*	-.23	-.33
Psychomotor	-.17	-.09	-.22	-.03	-.29
Anxiety	-.27	-.08	-.57*	-.16	-.13
Sleep	-.11	.09	.07	-.28	-.39
HAM-A Total	-.31	-.08	-.51	-.09	-.31
Psychic	-.24	-.07	-.38	-.12	-.59**
Somatic	-.31	-.07	-.54*	-.05	-.01
SCL-58 Total	.08	.26	-.41	-.13	-.12
Depression	.03	.12	-.33	-.06	-.21
Anxiety	.12	.35	-.48	-.01	-.05
Somatization	.01	.02	-.35	-.03	-.24
Obsessive	.07	.24	-.43	-.18	.12
Interpersonal	-.03	.17	-.31	-.18	-.09

[a]n = 19. [b]n = 14.

*p < .05. ** p < .01.

table continues

Table 10 (continued)

Correlations between Rating Scales and Biochemical Data during Examinations

	[^{3}H]Imipramine[a]		[^{3}H]Paroxetine[b]		
Scale	B_{max}	K_d	B_{max}	K_d	Cortisol[a]
Social Support Total	.46*	.13	.31	.46	-.09
Quantity	.46*	.16	.21	.34	-.12
Quality	.29	.01	.48	.26	.02
Hassles	-.12	.27	-.72**	-.15	-.27
Uplifts	-.13	.07	-.26	.34	-.07
Perceived stress	-.29	-.01	-.41	.06	-.19

[a]n = 19. [b]n = 14.

*p < .05. **p < .01.

Table 11

Correlations between Rating Scales and Biochemical Data after Vacation

	[^{3}H]Imipramine[a]		[^{3}H]Paroxetine[b]		
Scale	B_{max}	K_d	B_{max}	K_d	Cortisol[a]
HAM-D Total	.01	-.13	-.11	.03	-.12
Psychomotor	-.09	-.15	-.16	.01	-.18
Anxiety	-.10	-.24	-.22	-.03	-.19
Sleep	.01	.02	.07	-.10	-.06
HAM-A Total	-.21	-.24	-.31	-.04	-.31
Psychic	-.18	-.22	-.31	-.13	-.24
Somatic	-.21	-.21	-.24	.09	-.34
SCL-58 Total	-.19	-.28	.08	.07	-.08
Depression	-.21	-.29	-.08	-.11	-.22
Anxiety	-.24	-.34	.28	.13	-.06
Somatization	-.22	.16	-.02	.30	.07
Obsessive	-.07	-.25	.11	.14	-.04
Interpersonal	-.24	-.31	-.27	-.14	-.15

[a]n = 19. [b]n = 14.

table continues

Table 11 (continued)

Correlations between Rating Scales and Biochemical Data after Vacation

	[^{3}H]Imipramine[a]		[^{3}H]Paroxetine[b]		
Scale	B_{max}	K_d	B_{max}	K_d	Cortisol[a]
Social Support Total	.20	.15	.11	.26	.37
Quantity	.16	.19	.09	.29	.32
Quality	.31	-.02	.16	.13	.12
Hassles	.08	-.35	-.17	-.12	-.42
Uplifts	.13	-.24	-.08	.13	-.15
Perceived stress	-.35	.09	.07	.05	.12

[a]n = 19. [b]n = 14.

between the affinity of [^{3}H]imipramine for its binding site and any other variables were found.

During examinations, the B_{max} for [^{3}H]paroxetine binding was significantly associated with total score on the HAM-D (r = -.52, n = 14, p<.05), the Anxiety factor of the scale (r = -.57, n = 14, p <.05), the Somatic Anxiety factor of the HAM-A (r = -.54, n = 14, p<.05), and with the Hassles scale (r = -.72, n = 14, p<.01) in the expected directions (see Table 10). Correlations between the density of [^{3}H]paroxetine binding and total perceived social support (r = .31), total score on the HAM-A (r = -.51) and SCL-58 (r = -.41), and Perceived Stress (r = -.41) were in the predicted directions, but were not statistically significant. No significant relationships were observed for [^{3}H]paroxetine binding and other measures during the period following vacation (see Table 11). A significant negative correlation between plasma cortisol levels and scores on the rating scales were observed during the examination period, in relation to the Psychic Anxiety factor of the HAM-A (r = -.59, n = 19, p<.01).

Hierarchical regression was employed to determine if the addition of total perceived social support improved the prediction of binding density beyond that accounted for by depressive symptoms (scores on the HAM-D). Each step in the hierarchy was added in a seperate run. Assumptions of normality, linearity and homoscedasticity were verified for each regression equation.

During examination stress, HAM-D scores were entered first into the equation and accounted for 12% of the variance in [^{3}H]imipramine binding

values, R^2 = .12, $F(1,17)$ = 2.27, n.s. Addition of perceived social support to the prediction of B_{max} by HAM-D scores resulted in a significant increment in R^2 (R^2 = .33, $F[2,16]$ = 3.94, $p<.04$). After vacation, when examined together, HAM-D and social support did not significantly predict the B_{max} for [^{3}H]imipramine (R^2 = .05, $F[2,16]$ = .65, n.s.). Table 12 displays the standardized regression coefficients (B), t values, semi-partial correlations (sr^2), and the R^2 amd adjusted R^2 after entry of both variables for the periods during examinations and following vacation.

During examinations, Hamilton depression scores accounted for a significant portion of the variance in [^{3}H]paroxetine binding density (R^2 = .27, $F[2,11]$ = 4.48, $p<.056$). However, perceived social support did not contribute significantly to the prediction of binding values following HAM-D (R^2 = .38, $F[2,11]$ = 3.38, n.s). Together, Hamilton depression ratings and social support did not account for a significant portion of the variance in [^{3}H]paroxetine binding density after vacation (R^2 = .02, $F[2,11]$ = .10, n.s.; see Table 13).

Discussion

A significant (20%) reduction in the density of [^{3}H]imipramine and [^{3}H]paroxetine binding sites was observed in the medical students during their final examination period compared to after vacation. In comparison with the findings of the previous study, the binding densities observed for students during examinations were in the same range as values obtained for the depressed and anxious patients, while after vacation the B_{max} values for students were no different from those of healthy volunteers. These findings suggest that exposure

Table 12

Hierarchical Regression of Student[a] HAM-D Scores and Perception of Social Support on [^{3}H]Imipramine Binding Density during Examinations and after Vacation

Predictor	Beta	t	Prob	sr^2 (incremental)
Examinations				
HAM-D Total	-.34	-1.65	n.s.	.12
Total Perceived Support	.46	2.24	.04	.21*
			R^2 = .33	Adj. R^2 = .25
			$F(2,16) = 3.94, p < .04$	
Vacation				
HAM-D Total	.13	.46	n.s.	.00
Total Perceived Support	.26	.95	n.s.	.05
			R^2 = .05	Adj. R^2 = -.06
			$F(2,16) = 0.65$, n.s.	

[a] $n = 19$.

* $p < .05$.

Table 13

Hierarchical Regression of Student[a] HAM-D Scores and Perceived Quantity of Social Support on [^{3}H]Paroxetine Binding Density during Examinations and after Vacation

Predictor	Beta	t	Prob	sr^2 (incremental)
Examinations				
HAM-D Total	-.53	-2.24	.05	.27*
Total Perceived Support	.31	1.25	n.s.	.09
			R^2 = .38	Adj. R^2 = .27
			$F(2,11) = 3.38, p < .07$	
Vacation				
HAM-D Total	-.08	-.24	n.s.	-.08
Total Perceived Support	.08	.25	n.s.	.005
			R^2 = .02	Adj. R^2 = -.16
			$F(2,11) = .10$, n.s.	

[a]n = 19.

* $p < .05$.

to stress can lead to neurochemical changes observed in psychopathological states, including depression, and may thus help to explain the mechanism through which stressful events may place an individual at risk for the development of an affective disorder. Specifically, the reduced densities of [^{3}H]imipramine and [^{3}H]paroxetine binding sites observed during exposure to stress in the present study are likely associated with a decrease in the inhibition of 5-HT uptake, resulting in decreased synaptic levels of 5-HT. This reduction in serotonergic neurotransmission may explain the appearance of symptoms and the development of psychiatric disorders associated with stressful events.

The present findings are consistent with the observations of Sherman and Petty (1984) of a decrease in the maximal density of [^{3}H]imipramine binding in rat cortex with learned helplessness, and with the findings of Minchin and collegues (Minchin, Williams, Bowdler and Green, 1985) of a decrease in [^{3}H]imipramine binding to rat platelets after repeated electroconvulsive shock. Recently, a significant reduction in the maximal density of [^{3}H]paroxetine binding sites was reported in the hypothalamus of learned helpless rats as compared to naive controls, following chronic, uncontrollable shock training (Edwards, Harkins, Wright and Henn, 1991), providing further evidence that stress can lead to alterations in serotonergic function associated with behavioral changes.

Some investigators have questioned the validity of decreased density of [^{3}H]imipramine binding sites as a biological marker for endogenous depression (Bech et al., 1988; Mellerup and Plenge, 1988). This concern is based on

observations that some studies have found no difference in platelet [^{3}H]imipramine binding between depressed patients and controls (Whitaker, Warsh, Stancer, Persad and Vint, 1984; Tang and Morris, 1985; Kanof, Coccaro, Johns, Siever and Davis, 1987), and that even in studies which found significant differences in the density of binding between the two groups, there was considerable overlap in B_{max} values between patients and controls. In the present study, comparison of platelet [^{3}H]imipramine and [^{3}H]paroxetine binding values in medical students during examinations and after vacation suggests that stress influences the density of antidepressant binding sites and may contribute to the overlap in B_{max} values that is observed between patients and controls.

While stress has been identified as playing a role in the development of psychiatric disorders (Brown et al., 1973; Paykel et al., 1969), having a psychiatric illness such as anxiety or depression can be a stress in itself and can lead to a higher number of life stressors such as work and relationship difficulties. Thus, the reduction in B_{max} values observed in depressed and anxious patients could be due to the stress involved in having a psychiatric disorder. These findings suggest that it is important to consider stress as an influence on platelet antidepressant binding in future studies, and further emphasize the importance of identifying factors other than psychiatric diagnosis which may distinguish between subgroups of individuals on parameters of platelet antidepressant binding.

The decreased densities of [^{3}H]imipramine and [^{3}H]paroxetine binding sites during examination stress in normal subjects also suggest that these sites reflect

the biochemical state of the individual, and are not a reflection of trait markers of genetic vulnerability. These data are consistent with the observations of several investigators (Suranyi-Cadotte et al. 1982; Langer et al., 1986; Maj et al., 1988) of normalization of B_{max} in clinical remission, and of Berrettini and collegues (1982) who found no significant difference in platelet [^{3}H]imipramine binding between bipolar patients in remission and healthy controls.

The differences observed in platelet binding in the medical students during examinations and after vacation do not appear to be due to the effects of seasonal changes. This can be seen from the comparison of values for [^{3}H]imipramine and [^{3}H]paroxetine binding for the control group in Study 2 with results from the medical students during their final examinations; both sets of samples were obtained in the month of June. Maximal binding for both [^{3}H]imipramine and [^{3}H]paroxetine were significantly lower in medical students during examinations than in healthy controls measured in the same month. In September when the students were no longer under the stress of examinations, their binding values were indistinguishable from those of controls obtained in June. If the decreased density of platelet binding observed during examinations was due to seasonal variation, one would expect the values of the control group, from whom samples were obtained during the same month, to be low also.

Plasma cortisol levels were significantly increased in the medical students during examinations compared to after vacation. Increased adrenocortical secretion is also observed in many patients with depression (Carroll and Mendels,

1976) and anxiety (Rosenbaum et al., 1983). There is evidence that increased plasma cortisol levels are relevant to the decreased density observed in platelet antidepressant binding sites. Arora and Meltzer (1986) adrenalectomized rats and found an increase in the B_{max} and K_d of [^{3}H]imipramine binding, while administration of corticosterone decreased [^{3}H]imipramine binding. In a sample of depressed patients, Roy and collegues (1987) found significant negative correlations between B_{max} values for [^{3}H]imipramine binding and plasma cortisol levels following dexamethasone administration. These findings indicate an inhibitory effect of glucocorticoids on the synthesis or availability of [^{3}H]imipramine binding sites.

The lack of association between cortisol levels and platelet binding observed in the present study indicates that although a single plasma cortisol level is sensitive to the effects of stress, it is not precise enough to permit investigation of the relationship between glucocorticoids and antidepressant binding. Further studies using more exact measures of adrenocortical activity, such as corticosterone binding globulin, a plasma protein which is inversely correlated with levels of free, physiologically active corticosterone, over an entire diurnal cycle are needed to examine more closely the relationship between glucocorticoids and the [^{3}H]imipramine and [^{3}H]paroxetine binding sites.

In the present study, total perceived social support was positively associated with the density of [^{3}H]imipramine binding sites during examination stress; together with ratings of depressive symptomatology, these variables accounted for

a significant portion of the variance in [^{3}H]imipramine binding values during the examination period. No significant correlations between [^{3}H]imipramine binding and ratings of social support or depressive symptoms were observed following vacation. The positive association between perceived social support and [^{3}H]imipramine binding values during the examination period suggests that social support may protect individuals from the potentially adverse effects of stressful events. For the students in the present study, the results suggest that perception of social support served as a buffer for the effects of examination stress; a greater amount of perceived support was associated with higher B_{max} values, and contributed significantly to the prediction of B_{max} values beyond that afforded by depressive symptoms.

Reports of increases in adrenocortical activity in isolated animals compared to those that are group-housed (Greco et al., 1989) are consistent with the idea that the association between social support and the density of [^{3}H]imipramine binding sites observed in the present study may result from enhanced adrenocortical function. As previously discussed, increased plasma glucocorticoid levels have been related to the decreased density observed in [^{3}H]imipramine binding sites (Arora and Meltzer, 1986).

The density of [^{3}H]paroxetine binding was not observed to correlate significantly with measures of social support in the present study. This finding may be partially explained by the smaller sample size for [^{3}H]paroxetine binding than for [^{3}H]imipramine. In addition, however, the lack of association between

[^{3}H]paroxetine B_{max} values and social support follows observations from Study 2, that the site labeled by [^{3}H]paroxetine is distinct from the [^{3}H]imipramine binding site, and is not as closely associated with depression as the site labeled by [^{3}H]imipramine. Further studies need to be conducted in order to more closely examine the relationship between [^{3}H]paroxetine binding, psychosocial risk factors for depression, and the depressed state.

There is some indication in the literature that the relationship between life events or stress and social support is confounded (Thoits, 1982), since many important events, such as death of a spouse, divorce, or marriage are interpretable as losses or gains of supportive relationships. Thus, life events or stress may be conceptually and operationally identical to change in social support, in addition to producing other alterations in the individual's social support system as well. Thoits (1982) has suggested that an interaction between stress and social support can serve to bias results in support of the buffering hypothesis. In the present study, the finding that social support was selectively correlated with [^{3}H]imipramine binding values during examinations, but not following vacation, may reflect an interaction between year-end examination stress and perceived social support. During examinations, students spend more time alone in order to prepare for their examinations, and therefore are more socially isolated as a result. In addition, the stress during the examination period may affect the way the students perceive or interpret the social support that is available. Further studies need to be conducted which assess actual social contact, rather than the

amount of support perceived, in order to confirm the neurobiological link between low social support and depression, and to clarify whether indeed an interaction between stress and social support exists under these conditions.

In conclusion, the findings from the present study indicate that stress influences the density of antidepressant binding sites on blood platelets, and may contribute to the reductions in binding density observed in depression and anxiety. These findings suggest a common neurochemical pathway in depression, anxiety, and stress. In addition, the findings of an association between the B_{max} for [^{3}H]imipramine and social support during academic examinations suggests that low social support is associated with neurochemical changes that are observed in the depressed state. Taken together, these findings suggest that neurochemical alterations associated with stress and a decrease in social support may render an individual more vulnerable to depression.

GENERAL DISCUSSION

The major results of the present investigation were: 1) similarities in [^{3}H]imipramine and [^{3}H]paroxetine binding in depression, anxiety and stress, and; 2) an association between perception of social support with the density of [^{3}H]imipramine binding during examination stress, and the significant contribution of social support to the prediction of binding density by depressive symptomatology.

These results suggest that stress is associated with a reduction in antidepressant binding sites, and may thus serve as a common factor which predicts the reduced number of sites observed in depressive and anxiety disorders. Furthermore, the finding that social support was associated with [^{3}H]imipramine binding during a period of examination stress suggests that the [^{3}H]imipramine binding site may be a marker for low social support and depression under conditions of stress.

The findings that stress and social support were associated with platelet antidepressant binding in the present studies provide support for the hypothesis that psychosocial factors may be associated with neurochemical changes in healthy individuals which are associated with psychopathology. These data are consistent with previous literature suggesting that the density of [^{3}H]imipramine binding is a state-dependant marker, and is not a characteristic of the individual. Thus, the results suggest that psychosocial factors remain very important in determining the

development and course of a depressive episode, as they may confer susceptibility for depression in individuals with no genetic predisposition to the disorder.

Importantly, the associations between stress, social support and biochemical variables in this investigation were observed in healthy volunteers during a natural stressor, year-end academic examinations, and were thus unrelated to psychiatric diagnosis. These findings point to a need to understand biochemical alterations not only at the extreme ends of psychopathology, but also within the realm of normal human experience, and further indicate that such information may increase our understanding of the neurochemical pathways leading to psychiatric disorder. Moreover, the implication that factors such as stress may contribute to alterations in antidepressant binding in both depressive and anxiety disorders suggests the importance of looking beyond diagnostic classification in investigations of the relationship between behavioral and biochemical variables.

Indeed, Goodwin and Post (1983) suggest that particular dimensions of behavior may be related to specific biochemical states, regardless of psychiatric diagnosis. This idea corresponds with mounting evidence that altered 5-HT function is associated with aggression in nonpsychiatric volunteers (Asberg, Schalling, Traksman-Bendz and Wagner, 1987; Roy, Adinoff and Linnoila, 1988), criminal offenders (Linnoila et al., 1983; Lidberg, Tuck, Asberg, Scalia-Tomba and Bertilsson, 1985; Virkkunen, Nuutila, Goodwin and Linnoila, 1987) and psychiatric patients with various diagnoses (Brown et al., 1982; Coccaro et al., 1989; Asberg et al., 1987; Van Praag, 1986).

Moreover, there is an extensive body of literature which supports the role of central 5-HT in the regulation of aggression in rodents. Muricidal behavior (Katz, 1980), shock-induced fighting (Sewell, Gallus, Gault and Cleary, 1982) and pup killing (Copenhaver, Schalock and Carver, 1978) are reported to be increased by the chemical or electrolytic lesioning of central 5-HT neurons, and are reversed, or blocked, by agents that increase 5-HT activity (Copenhaver et al., 1978, Broderick and Lynch, 1982). Interestingly, prolonged social isolation has also been reported to produce aggressive behavior in mice, but only in animals where 5-HT activity is reduced (Valzelli and Bernasconi, 1979). Taken together, these studies suggest that behaviors such as aggression may be associated with alterations in central 5-HT activity irrespective of psychiatric diagnosis, thus providing further support for a dimensional rather than a categorical view of central 5-HT dysfunction in human behavior.

Reports that the decreased density of [^{3}H]imipramine binding sites is not specific to depression, as has previously been suggested (Suranyi-Cadotte et al., 1985), support the idea that studies relating psychosocial and biochemical variables are best examined across psychiatric diagnoses. In addition to reduced concentrations of [^{3}H]imipramine binding sites in depression (Briley et al., 1980), and, more recently, anxiety disorders (Lewis et al., 1985; Weizman et al., 1986), a decreased density of [^{3}H]imipramine binding sites has also been reported on platelets of patients with migraine headaches (Geaney et al., 1984), and in chronic pain patients with anxiety and depressive symptoms (Mellerup, Poulsen, Beck and

Plenge, 1984). Impulsive and aggressive children (Birmaher et al., 1990), conduct-disordered children (Stoff, Pollock, Vitiello, Behar and Bridger, 1987), enuretic children and adolescents (Weizman, Carel, Tyano and Rehavi, 1984) and adolescent females suffering from anorexia nervosa (Weizman, Carmi, Tyano, Apter and Rehavi, 1986) have also been reported to have lower B_{max} values for platelet [^{3}H]imipramine binding than controls of the same age. Recently, reduced concentrations of [^{3}H]imipramine binding sites have been reported on the platelets of alcoholics (Javors, Blaisdell, Lee and Bowden, 1987; Suranyi-Cadotte, Dongier, Lafaille and Luthe, 1989) and subjects at risk for alcoholism (Suranyi-Cadotte et al., 1989), and in various brain regions of patients with Alzheimer's disease (Marcusson et al., 1987) and parkinsonism (Raisman, Cash and Agid, 1986). Serotonergic neurotransmission is reported to be decreased in several of these populations (Arai, Kosaka and Iizuka, 1984; Bhagavan, Coleman and Coursin, 1975; Scatton, Javoy-Agid, Montfort & Agid, 1984). The results of the present investigation suggest that it is not inconceivable that stress is a common neurochemical link between these various disorders, which leads to difficulties in the differential diagnosis of patients according to biochemical criteria.

In the present studies, both [^{3}H]imipramine and [^{3}H]paroxetine were used as markers for the 5-HT transporter system, since previous investigations demonstrated an association between these ligands and the 5-HT transporter complex. The first experiment provided preliminary evidence for a positive correlation between the density of [^{3}H]paroxetine binding and [^{14}C]5-HT uptake

on blood platelets of healthy controls. This finding is in contrast to previous studies which reported a lack of association between the B_{max} for [^{3}H]imipramine and the uptake of [^{14}C]5-HT (Ahtee et al., 1981; Wood et al., 1983). These results provide further evidence that [^{3}H]paroxetine is a more specific label of the 5-HT uptake site than [^{3}H]imipramine. Moreover, since the [^{3}H]paroxetine binding assay takes place at physiological temperatures, it is suggested that the site labeled by [^{3}H]paroxetine may be more representative for 5-HT uptake inhibitory activity than [^{3}H]imipramine, for which binding is determined at 0°C (Segonzac, Schoemaker and Langer, 1987). Further investigation of the association between the [^{3}H]paroxetine binding site and the serotonergic system in blood platelets are needed to confirm these findings.

The present studies also provide further evidence that [^{3}H]imipramine and [^{3}H]paroxetine do not label identical sites, since no correlation was observed between B_{max} or K_d values for [^{3}H]imipramine and [^{3}H]paroxetine binding among psychiatric patients, healthy volunteers, or medical students. Furthermore, the profile of [^{3}H]imipramine and [^{3}H]paroxetine binding density differed in psychiatric patients with various diagnoses. While [^{3}H]imipramine B_{max} values were significantly lower for all patient groups than for controls, the binding density for [^{3}H]paroxetine was only significantly reduced in patients with generalized anxiety and panic disorders; decreases in B_{max} values observed for depressed and dysthymic patients were not significantly lower than those seen in controls. A further difference in [^{3}H]imipramine and [^{3}H]paroxetine binding was

that only the site labeled by [^{3}H]imipramine was observed to significantly correlate with quantity of perceived support during examination stress in medical students. Moreover, unlike [^{3}H]imipramine, K_d values for [^{3}H]paroxetine were not associated with self-ratings of hopelessness in psychiatric patients, and also failed to show a similar pattern of association as the affinity constant for [^{3}H]imipramine on other rating scales administered.

Initial studies on [^{3}H]imipramine binding pointed to a homogeneous population of binding sites (Rehavi, Paul, Skolnick and Goodwin, 1980; Langer et al., 1981) that was intimately associated with the serotonergic system. However, more recent studies have demonstrated two distinct classes of [^{3}H]imipramine binding in the brain: a high affinity site of protein nature which correlates with the regional distribution of 5-HT uptake, and a low affinity site with a K_d approximately 50-100 times greater than that observed for the high affinity component, and a ten-fold greater density of binding sites, which is not of protein nature and is not associated with the 5-HT uptake site (Hrdina, 1984; Marcusson, Fowler, Hall, Ross and Winblad, 1985). Only the high affinity [^{3}H]imipramine site is sodium-dependant and is located on serotonergic nerve terminals (Marcusson, Backstrom and Ross, 1986, Hrdina, 1987a). Low affinity sites have been suggested to be located extraneuronally on glial cells (Whitaker, Vint and Morin, 1983). The parameters of the high affinity binding site are suggested to be influenced by the proportion of low affinity sites present even at relatively low

[^{3}H]imipramine concentrations when 100 uM desipramine, rather than 5-HT, is used as a displacer in the binding assay (Hrdina, 1984).

High and low affinity components of [^{3}H]imipramine binding have also been demonstrated on human platelets, using a wide range of ligand concentrations, and excess desipramine to define specific binding (Ieni, Zukin and Van Praag, 1984; Phillips, Wood and Williams, 1984). In a recent study by Hrdina (1989), the specific [^{3}H]imipramine binding defined by 100 uM desipramine, using a low concentration of [^{3}H]imipramine, was found to consist of 30% sodium-independant binding; the density of sodium-dependant [^{3}H]imipramine sites in platelets was significantly lower than, and not correlated with, the B_{max} of desipramine-defined binding sites (Hrdina, 1989). Taken together, these findings suggest that the lack of association between [^{3}H]imipramine and [^{3}H]paroxetine binding observed in the present investigation may be accounted for by the heterogeneity of the [^{3}H]imipramine site, particularly since desipramine was used to define specific [^{3}H]imipramine binding. In a recent study, however, Marcusson and Tiger (1988) observed no differences between specific [^{3}H]imipramine binding in human platelets defined as that sensitive to 100 uM 5-HT and that sensitive to 100 uM desipramine, when a low concentration of [^{3}H]imipramine was used; furthermore, binding was of protein nature, suggesting that the ligand labeled the high affinity, sodium-dependant component of [^{3}H]imipramine binding. Clearly, further studies on the nature of the [^{3}H]imipramine binding sites and their relation to serotonergic neurotransmission

are needed in order to establish the usefulness of platelet [^{3}H]imipramine as a reliable marker of 5-HT function.

As already mentioned, the lack of a significant reduction in [^{3}H]paroxetine binding in patients with depressive disorders is consistent with previous results from our laboratory (Suranyi-Cadotte et al., 1989) showing that compared to controls, the reduction in platelet [^{3}H]paroxetine binding in depression is not statistically significant. These findings are surprising, considering the well-documented role of 5-HT in affective disorders, and suggest that the [^{3}H]imipramine binding site is more closely associated with the pathophysiology of depression than the site labeled by [^{3}H]paroxetine.

Nevertheless, observations that the density of [^{3}H]paroxetine binding was significantly decreased in anxious patients, as well as in students during a period of examination stress, indicates that [^{3}H]paroxetine may be a useful biochemical marker of the states associated with anxiety and stress. Further studies are needed to characterize [^{3}H]paroxetine binding along various behavioral dimensions in order to clarify its role in psychopathology. Other measures of 5-HT function, such as platelet 5-HT$_2$ receptors, CSF 5-HIAA, and response to 5-HT agonists and antagonists can also serve as useful indicators of 5-HT neurotransmission, and provide a more complete picture of serotonergic function, given the dynamic nature of neurotransmitter systems.

Importantly, given the state-dependent nature of platelet antidepresent binding, factors which may influence binding parameters, such as diet and plasma

levels of 5-HT also need to be more fully addressed. For instance, affective patients often have poor appetites which could result in lower tryptophan uptake, lower 5-HT levels and, perhaps, lower platelet binding. This issue could be addressed using a tryptophan-depleting paradigm (eg. Delgado et al., 1990) with plasma 5-HT levels as a correlate. As well, more extensive correlative studies of brain and platelet uptake binding during different physiological conditions would also serve to address this issue.

In addition, findings of high concentrations of [^{3}H]paroxetine-labeled sites in brainstem areas rich in catecholaminergic neurons, including the substantia nigra, the ventral tegmental area, and the locus coeruleus (De Souza and Kuyatt, 1987), suggest the importance of interactions between neurotransmitter systems. Monoaminergic neurons, including NE, DA and 5-HT, project diffusely throughout the brain. All three systems have been linked to depression or to antidepressant action, and are affected by stress. Noradrenergic neurons in the locus coeruleus project to 5-HT neurons in the dorsal raphe (Baraban and Aghajanian, 1981), and NE and 5-HT have reciprocal effects on each other's turnover (Agren, Koulu, Saavedra, Potter and Linnoila, 1987). Furthermore, the characteristic down-regulation of NE postsynaptic ß-adrenoceptors caused by long-term antidepressant treatment does not occur if 5-HT projections to the NE system are cut (Stockmeier, Martino and Kellar, 1985). In addition, 5-HT neurons project to DA neurons in the substantia nigra and striatum and modulate DA turnover (Fuenmayer and Bermudez, 1985), while dopaminergic receptors

found on 5-HT nerve terminals in the substantia nigra affect 5-HT release (Benkirane, Arbilla and Langer, 1987). Taken together, these findings have led some investigators to suggest that neither the pathophysiology of affective disorders nor the mechanism of action of antidepressants can be explained entirely by changes in a single neurotransmitter system (Hsaio et al., 1987).

In conclusion, the results of the present investigation suggest that alterations in the serotonergic system do play a significant role in the link between depression, anxiety and stress, and in addition, may serve as an important component of the neurochemical mechanisms which link psychosocial risk factors for depression with the onset of a depressive episode.

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APPENDIX A

Measurement and Analysis of Receptor Binding

To measure the density of [^{3}H]imipramine and [^{3}H]paroxetine binding sites, saturation experiments were performed. These experiments are based on the principle that biological tissue contains only a limited number of receptor sites for any given substance. Thus, specific receptor binding should plateau with increasing concentrations of ligand, indicating that all of the available receptors are occupied. The fundamental assumption made when performing a binding assay is that the specific binding detected represents attachment of the labeled (radioactive) ligand to the receptor of interest.

In the present experiments, platelet membranes and buffer were incubated in a series of tubes containing various concentrations of labeled ligand ([^{3}H]imipramine or [^{3}H]paroxetine). These tubes were used together with a parallel set which, in addition, contained a fixed, high concentration of unlabeled ligand (desipramine or citalopram). Concentrations of the labeled ligand were the same for each pair of tubes. The tubes were incubated for a period of time until equilibrium was reached, that is, the time that is determined to be necessary for specific binding to reach maximum. The receptor binding reaction was then terminated by pouring the incubation mixture over a glass fiber filter which was placed over a vacuum tank and was, therefore, continuously exposed to a negative pressure. The vacuum pulls the incubation solution through the filter but leaves the tissue membrane plus attached radioligand on the surface, thus separating the

ligand bound to receptors from the unbound ligand present in the incubation medium. The platelets were then washed free of nonreceptor-bound radioactivity by rinsing three times with cold buffer. Each filter was subsequently placed in a counting vial with liquid scintillation cocktail, and counted by scintillation spectometry.

In saturation experiments, the excess concentration of nonlabeled ligand competes for the same receptor site as the labeled ligand, which it displaces, and also binds to sites that are of low affinity. Binding of the nonlabeled ligand is not saturable, and so probably represents nonspecific attachment to the tissue, as well as counting background; thus, it is referred to as nonspecific binding. Radioligand binding that takes place in the absence of nonlabeled ligand is also nonsaturable, and is referred to as total binding, that is, the sum of radioligand binding to the (specific) receptor of interest in addition to binding to nonspecific sites. Thus, specific binding is calculated as the difference between the amount bound in the presence and absence of unlabeled ligand, that is, the difference between nonspecific and total binding; it represents attachment of the radioligand to the receptor being studied, and is saturable, indicating that there are a limited number of sites available for attachment. Free ligand is defined as the concentration of total radioligand added to the tubes, minus the concentration of total radioligand bound.

Values for nonspecific binding and free radioligand, as determined by the amount of radioactivity in tubes containing labeled plus unlabeled ligand, are used

to calculate a regression line that defines nonspecific binding as a function of the concentration of free radioligand. The concentration of free labeled ligand in total-binding tubes can then be used to calculate the nonspecific binding in those tubes via the line determined by regression. This value can then be subtracted from total binding, and the result defined as nonspecific binding. This method assumes that nonspecific binding is linear, and that a regression analysis of nonspecific binding data will yield a good estimate of nonspecific binding as a function of free ligand.

Scatchard (1949) analysis of nonspecific binding, which plots concentrations of nonspecific bound / free ligand versus the concentration of nonspecific bound ligand (that is, B/F versus B), results in a horizontal line when binding is linear. It yields information relating to the maximum density of binding sites in the tissue (B_{max}) and the affinity of the receptor for the ligand (K_d). The intercept of the abscissa (ie., when B/F = 0) is B_{max}. The K_d is the negative reciprocal of the slope of the line, and refers to the concentration of radioligand which occupies one half of the total receptors. Thus, it is relatively easy to calculate K_d and B_{max} either graphically or by linear regression of B/F vs B.

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APPENDIX B

Consent Forms

I, _____, hereby consent to participate in a study directed by Drs. Michael Meaney and Barbara Suranyi-Cadotte on the biological state associated with symptoms of depression and anxiety. I understand that this will involve, on two separate occasions, being assessed by an interview with a clinician, and by filling in questionnaires. At the same time, I understand that an authorized registered nurse or technician will withdraw seventy (70) cc of blood (eight tubes) from me.

I understand that while this may eventually provide useful information regarding biological markers in depression and anxiety, as well as information regarding the causes of emotional disorders, I may not benefit directly from this procedure.

I further understand that the risks associated with this procedure are the same as a routine blood test.

I understand that my participation in this study is voluntary, and that I may withdraw this consent at any time.

_____ _____

Signature Date

_____ _____

Witness Date

Consent Form

I, _____, hereby consent to participate in a study directed by Drs. Michael Meaney and Barbara Suranyi-Cadotte on the biological state associated with stress. I understand that this will involve, on two separate occasions, being assessed by an interview with a clinician, and by filling in questionnaires. At the same time, I understand that a doctor, an authorized registered nurse, or a technician will withdraw eight (80) cc of blood (nine tubes) from me. I understand that the risks associated with this procedure are the same as a routine blood test.

I further understand that while this may eventually provide useful information regarding the biochemical effects of stress, as well as information concerning biological markers in depression and anxiety, I may not benefit directly from this procedure.

I also understand that my participation in this study is voluntary, and that I may withdraw this consent at any time.

_____ _____

Signature Date

_____ _____

Witness Date

APPENDIX C

Scales and Inventories

RDC Criteria for Endogenous Depression

			Present
A.	(1)	Distinct quality to depressed mood, i.e., depressed mood is perceived as distinctly different from the kind of feeling he/she would have or has had following the death of a loved one.	____
	(2)	Lack of reactivity to environmental changes (once depressed doesn't feel better, even temporarily, when something good happens).	____
	(3)	Mood is regularly worse in the morning.	____
	(4)	Pervasive loss of interest or pleasure (some loss in all areas).	____
B.	(1)	Feelings of self-reproach or excessive or inappropriate guilt.	____
	(2)	Early morning awakening or middle insomnia.	____
	(3)	Psychomotor retardation or agitation (more than mere subjective feeling of being slowed down or restless).	____
	(4)	Poor appetite.	____
	(5)	Weight loss (2 lbs. a week over several weeks or 20 lbs. in a year when not dieting).	____
	(6)	Loss of interest or pleasure (may or may not be pervasive) in usual activities or decreased sexual drive.	____

Probable symptom	(a total of at least 4 symptoms, including at least one from group A).	____
Definite symptom	(a total of at least 6 symptoms, including at least one from group A).	____
Absent		____

DOUGLAS HOSPITAL

HAMILTON PSYCHIATRIC RATING SCALE FOR DEPRESSION

DATE		Patient Number	
Patient Initials		M = 001-499	
First	Last	F = 500-998	
		Form Number	049
Time Units		Period Number	
1 = hour		Time Unit	
2 = day		Rater Number	
3 = week			
4 = month			
0 = pretreatment			

INSTRUCTIONS.
For each item select the "cue" which best characterizes the patient

1. DEPRESSED MOOD *(Sadness, hopeless, helpless, worthless)*

0 = Absent
1 = These feeling states indicated only on questioning
2 = These feeling states spontaneously reported verbally
3 = Communicates feeling states non-verbally — i.e., through facial expression, posture, voice, and tendency to weep
4 = Patient reports VIRTUALLY ONLY these feeling states in his spontaneous verbal and non-verbal communication

2. FEELINGS OF GUILT

0 = Absent
1 = Self-reproach, feels he has let people down
2 = Ideas of guilt or rumination over past errors or sinful deeds
3 = Present illness is a punishment. Delusions of guilt
4 = Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations

3. SUICIDE

0 = Absent
1 = Feels life is not worth living
2 = Wishes he were dead or any thoughts of possible death to self
3 = Suicidal ideas or gesture
4 = Attempts at suicide *(any serious attempt rates 4)*

4. INSOMNIA EARLY

0 = No difficulty falling asleep
1 = Complains of occasional difficulty falling asleep — i.e., more than 1/2 hour
2 = Complains of nightly difficulty falling asleep

5. INSOMNIA MIDDLE

0 = No difficulty
1 = Patient complains of being restless and disturbed during the night
2 = Waking during the night — any getting out of bed rates 2 *(except for purposes of voiding)*

6. INSOMNIA LATE

0 = No difficulty
1 = Waking in early hours of the morning but goes back to sleep
2 = Unable to fall asleep again if he gets out of bed

7 WORK AND ACTIVITIES

0 = No difficulty
1 = Thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies
2 = Loss of interest in activity, hobbies or work — either directly reported by patient or indirectly in listlessness, indecision and vacillation *(feels he has to push self to work or activities)*
3 = Decrease in actual time spent in activities or decrease in productivity. In hospital, rate 3 if patient does not spend at least three hours a day in activities *(hospital job or hobbies)* exclusive of ward chores
4 = Stopped working because of present illness. In hospital rate 4 if patient engages in no activities except ward chores, or if patient fails to perform ward chores unassisted

8. RETARDATION *(Slowness of thought and speech impaired ability to concentrate, decreased activity)*

0 = Normal speech and thought
1 = Slight retardation at interview
2 = Obvious retardation at interview
3 = Interview difficult
4 = Complete stupor

9. AGITATION

0 = NONE
1 = FIDGETINESS
2 = PLAYING WITH HANDS, HAIR, ETC.
3 = MOVING ABOUT, CAN'T SIT STILL
4 = HAND WRINGING, NAIL BITING, HAIR-PULLING, BITING OF LIPS

10. ANXIETY PSYCHIC

0 = No difficulty
1 = Subjective tension and irritability
2 = Worrying about minor matters
3 = Apprehensive attitude apparent in face or speech
4 = Fears expressed without questioning

11. ANXIETY SOMATIC

0 = Absent
1 = Mild
2 = Moderate
3 = Severe
4 = Incapacitating

Physiological concomitants of anxiety such as

Gastro-intestinal — dry mouth, wind, indigestion, diarrhea, cramps, belching
Cardio-vascular — palpitations, headaches
Respiratory — hyperventilation, sighing
Urinary frequency
Sweating

12 SOMATIC SYMPTOMS GASTROINTESTINAL

0 = None
1 = Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen
2 = Difficulty eating without staff urging. Requests or requires laxatives or medication for bowels or medication for G.I. symptoms

PAGE 2

DOUGLAS HOSPITAL

HAMILTON PSYCHIATRIC RATING SCALE FOR DEPRESSION

DATE:	Patient Number
Patient Initials: First / Last	M = 001-499 F = 500-998
Time Units: 1 = hour 2 = day 3 = week 4 = month 0 = pretreatment	Form Number: 049 Period Number Time Unit Rater Number

13. SOMATIC SYMPTOMS GENERAL

0 = None
1 = Heaviness in limbs, back or head. Backaches, headaches, muscle aches. Loss of energy and fatigability
2 = Any clear-cut symptom rates 2

14. GENITAL SYMPTOMS

0 = Absent
1 = Mild
2 = Severe

Symptoms such as: Loss of libido, Menstrual disturbances

15. HYPOCHONDRIASIS

0 = Not present
1 = Self-absorption *(bodily)*
2 = Preoccupation with health
3 = Frequent complaints, requests for help, etc.
4 = Hypochondriacal delusions

16. LOSS OF WEIGHT *Rate either a or b*

a. When rating by history:

0 = No weight loss
1 = Probable weight loss associated with present illness
2 = Definite *(according to patient)* weight loss
3 = Not assessed

b. On weekly ratings by ward psychiatrist, when actual weight changes are measured:

0 = Less than 1 lb. weight loss in week
1 = Greater than 1 lb. weight loss in week
2 = Greater than 2 lb. weight loss in week
3 = Not assessed

17. INSIGHT

0 = Acknowledges being depressed and ill
1 = Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
2 = Denies being ill at all

18. DIURNAL VARIATION

a. Note whether symptoms are worse in morning or evening. If NO diurnal variation, mark none.

0 = No variation
1 = Worse in [illegible]
2 = Worse in P.M.

b. [illegible] severity of the variation. Mark none if NO variation.

0 = None
1 = Mild
2 = Severe

19. DEPERSONALIZATION AND DEREALIZATION

0 = Absent
1 = Mild
2 = Moderate
3 = Severe
4 = Incapacitating

Such as: Feelings of unreality, Nihilistic ideas

20. PARANOID SYMPTOMS

0 = None
1 = Suspicious
2 = Ideas of reference
3 = Delusions of reference and persecution

21. OBSESSIONAL AND COMPULSIVE SYMPTOMS

0 = Absent
1 = Mild
2 = Severe

DOUGLAS HOSPITAL

HAMILTON ANXIETY SCALE

DATE		Patient Number		
Patient Initials		M = 001-499		
First	Last	F = 500-998		7-9
Time Units		Form Number	048	10-12
1 = hour		Period Number		13-14
2 = day		Time Unit		15
3 = week		Rater Number		16-17
4 = month				
5 = pretreatment				

INSTRUCTIONS:

Rate each symptom construct by the term which best describes the patient's present condition.

0 = Not Present 1 = Mild 2 = Moderate 3 = Severe 4 = Very Severe

1. ANXIOUS MOOD Worries, anticipation of the worst, fearful anticipation, irritability		18
2. TENSION Feelings of tension, fatigability, startle response, moved to tears easily, trembling, feelings of restlessness, inability to relax		19
3. FEARS Of dark, of strangers, of being left alone, of animals, of traffic, of crowds		20
4. INSOMNIA Difficulty in falling asleep, broken sleep, unsatisfying sleep and fatigue on waking, dreams, nightmares, night terrors		21
5. INTELLECTUAL Difficulty in concentration, poor memory		22
6. DEPRESSED MOOD Loss of interest, lack of pleasure in hobbies, depression, early waking, diurnal swing		23
7. SOMATIC (Muscular) Pains and aches, twitchings, stiffness, myoclonic jerks, grinding of teeth, unsteady voice, increased muscular tone		24
8. SOMATIC (Sensory) Tinnitus, blurring of vision, hot and cold flushes, feelings of weakness, pricking sensation		25
9. CARDIOVASCULAR SYMPTOMS Tachycardia, palpitations, pain in chest, throbbing of vessels, fainting feelings, sighing, dyspnea		26
10. RESPIRATORY SYMPTOMS Pressure or constriction in chest, choking feelings, sighing, dyspnea		27
11. GASTROINTESTINAL SYMPTOMS Difficulty in swallowing, wind, abdominal pain, burning sensations, abdominal fullness, nausea, vomiting, borborygmi, looseness of bowels, loss of weight, constipation		28
12. GENITOURINARY SYMPTOMS Frequency of micturition, urgency of micturition, amenorrhea, menorrhagia, development of frigidity, premature ejaculation, loss of libido, impotence		29
13. AUTONOMIC SYMPTOMS Dry mouth, flushing, pallor, tendency to sweat, giddiness, tension headache, raising of hair		30
14. BEHAVIOR AT INTERVIEW Fidgeting, restlessness or pacing, tremor of hands, furrowed brow, strained face, sighing or rapid respiration, facial pallor, swallowing, etc.		31

SELF RATING SYMPTOM SCALE

INSTRUCTIONS

USE THESE CODES TO RATE SYMPTOMS LISTED:

NOT AT ALL = 1 A LITTLE = 2 QUITE A BIT = 3 EXTREMELY = 4

DURING THE PAST WEEK: HOW MUCH WERE YOU BOTHERED BY	SEVERITY
1. Headaches	___
2. Nervousness or shakiness inside	___
3. [illegible] able to get rid of bad thoughts or ideas	___
4. Faintness or dizziness	___
5. Loss of sexual interest or pleasure	___
6. Feeling critical of others	___
7. Bad dreams	___
8. Difficulty in speaking when excited	___
9. Trouble remembering things	___
10. Worried about sloppiness or carelessness	___
11. Feeling easily annoyed or irritated	___
12. Pains in heart or chest	___
13. Itching	___
14. Feeling low in energy or slowed down	___
15. Thoughts of ending your life	___
16. Sweating	___
17. Trembling	___
18. [illegible] eeling confused	___
19. Poor appetite	___
20. Crying easily	___
21. Feeling shy or uneasy with the opposite sex	___
22. A feeling of being trapped or caught	___
23. Suddenly scared for no reason	___
24. Temper outburst you could not control	___
25. Constipation	___
26. Blaming yourself for things	___
27. [illegible] ins in the lower part of your back	___
28. Feeling blocked in getting things done	___
29. Feeling lonely	___

HOW MUCH WERE YOU BOTHERED BY	SEVERITY
30. Feeling blue	___
31. Worrying too much about things	___
32. Feeling no interest in things	___
33. Feeling fearful	___
34. Your feelings being easily hurt	___
35. Having to ask others what you should do	___
36. Feeling others do not understand you or are unsympathetic	___
37. Feeling that people are unfriendly or dislike you	___
38. Having to do things very slowly in order to be sure you were doing them right	___
39. Heart pounding or racing	___
40. Nausea or upset stomach	___
41. Feeling inferior to others	___
42. Soreness of your muscles	___
43. Loose bowel movements	___
44. Trouble falling asleep	___
45. Having to check and double-check what you do	___
46. Difficulty making decisions	___
47. Wanting to be alone	___
48. Trouble getting your breath	___
49. Hot or cold spells	___
50. Having to avoid certain things, places or activities because they frighten you	___
51. Your mind going blank	___
52. Numbness or tingling in parts of your body	___
53. A lump in your throat	___
54. Feeling hopeless about the future	___
55. Trouble concentrating	___
56. Feeling weak in parts of your body	___
57. Feeling tense or keyed up	___
58. Heavy feelings in your arms or legs	___

Initials __/__/__ Date _____

Please mark each statement as TRUE or FALSE as it applies to you.

	TRUE	FALSE
1. I look forward to the future with hope and enthusiasm	( )	( )
2. I might as well give up because I can't make things better for myself	( )	( )
3. When things are going badly, I am helped by knowing they can't stay that way forever	( )	( )
4. I can't imagine what my life would be like in ten years ..	( )	( )
5. I have enough time to accomplish the things I most want to do	( )	( )
6. In the future, I expect to succeed in what concerns me most	( )	( )
7. My future seems dark to me	( )	( )
8. I expect to get more of the good things in life than the average person	( )	( )
9. I just don't get the breaks, and there's no reason to believe I will in the future	( )	( )
10. My past experiences have prepared me well for my future ..	( )	( )
11. All I see ahead of me is unpleasantness rather than pleasantness	( )	( )
12. I don't expect to get what I really want	( )	( )
13. When I look ahead to the future, I expect I will be happier than I am now	( )	( )
14. Things just won't work out the way I want them to	( )	( )
15. I have great faith in the future	( )	( )
16. I never get what I want so it's foolish to want anything	( )	( )
17. It is very unlikely that I will get any real satisfaction in the future	( )	( )
18. The future seems vague and uncertain to me	( )	( )
19. I look forward to more good times than bad times	( )	( )
20. There's no use in really trying to get something I want because I probably won't get it	( )	( )

Initials __/__/__ Date _____

Instructions

The following questions ask about people in your environment who provide you with help or support. Each question has two parts. For the first part, list all the people you know, excluding yourself, whom you can count on for help or support in the manner described. Give the person's initials and their relationship to you (see example). Do not list more than one person next to each of the numbers beneath the question.

For the second part, circle how satisfied you are with the overall support you have. If you have no support for a question, check the words "no one", but still rate your level of satisfaction. Do not list more than nine persons per question.

Please answer all questions as best you can. All your responses will be kept confidential.

Example:

a. Who do you know whom you can trust with information that could get you into trouble?

No one	1) T.N. (brother)	4) L.N. (father)	7)
	2) L.M. (friend)	5) P.T. (employer)	8)
	3) R.S. (friend)	6)	9)

b. How satisfied are you with the overall support?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

1a. Who can you really count on to listen to you when you need to talk?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with the overall support?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

2a. Whose lives do you feel that you are an important part of?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with this overall?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

3a. Whom could you really count on to help you out in a crisis situation, even though they would have to go out of their way to do so?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with the overall support?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

4a. Whom can you talk with frankly, without having to watch what you say?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with this overall?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

5a. Whom can you really count on to be dependable when you need help?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with the overall support?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

6a. Whom can you really count on to give you useful suggestions that help you to avoid making mistakes?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with this overall?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

7a. Who will comfort you when you need it by holding you in their arms?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with this overall?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

8a. Whom do you feel would help if a family member very close to you died?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with this overall?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

9a. Who are the people that you could expect to let you know when they like your ideas or the things that you do?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with this overall?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

10a. Who are the people that you get together with to have fun or to relax?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

b. How satisfied are you with this overall?

very satisfied	fairly satisfied	a little satisfied	a little dissatisfied	fairly dissatisfied	very dissatisfied
6	5	4	3	2	1

11. Who are the people that you can expect to have some unpleasant disagreements with or people that you can expect to make you angry and upset?

No one	1)	4)	7)
	2)	5)	8)
	3)	6)	9)

Name: _____

Date: _____

THE HASSLES SCALE

Directions: Hassles are irritants that can range from minor annoyances to fairly major pressures, problems, or difficulties. They can occur few or many times. Listed in the center of the following pages are a number of ways in which a person an feel hassled. First, circle the hassles that have happened to you in the past month. Then look at the numbers on the right of the items you circled. Indicate by circling a 1,2, or 3 how SEVERE each of the circled hassles has been for you in the past month. If a hassle did not occur in the last month do NOT circle it.

	HASSLES	SEVERITY 1. Somewhat Severe 2. Moderately Severe 3. Extremely Severe		
(1)	Misplacing or losing things	1	2	3
(2)	Troublesome neighbors	1	2	3
(3)	Social obligations	1	2	3
(4)	Inconsiderate smokers	1	2	3
(5)	Troubling thoughts about your future	1	2	3
(6)	Thoughts about death	1	2	3
(7)	Health of a family member	1	2	3
(8)	Not enough money for clothing	1	2	3
(9)	Not enough money for housing	1	2	3
(10)	Concerns about owing money	1	2	3
(11)	Concerns about getting credit	1	2	3
(12)	Concerns about money for emergencies	1	2	3
(13)	Someone owes you money	1	2	3
(14)	Financial responsibility for someone who doesn't live with you	1	2	3
(15)	Cutting down on electricity, water, etc	1	2	3
(16)	Smoking too much	1	2	3
(17)	Use of alcohol	1	2	3
(18)	Personal use of drugs	1	2	3
(19)	Too many responsibilities	1	2	3
(20)	Decisions about having children	1	2	3
(21)	Non-family members living in your house	1	2	3
(22)	Care for pet	1	2	3
(23)	Planning meals	1	2	3
(24)	Concerned about the meaning of life	1	2	3
(25)	Trouble relaxing	1	2	3

	HASSLES	SEVERITY 1. Somewhat Severe 2. Moderately Severe 3. Extremely Severe		
(26)	Trouble making decisions	1	2	3
(27)	Problems getting along with fellow workers	1	2	3
(28)	Customers or clients give you a hard time	1	2	3
(29)	Home maintenance (inside)	1	2	3
(30)	Concerns about job security	1	2	3
(31)	Concerns about retirement	1	2	3
(32)	Laid-off or out of work	1	2	3
(33)	Don't like current work duties	1	2	3
(34)	Don't like fellow workers	1	2	3
(35)	Not enough money for basic necessities	1	2	3
(36)	Not enough money for food	1	2	3
(37)	Too many interruptions	1	2	3
(38)	Unexpected company	1	2	3
(39)	Too much time on hands	1	2	3
(40)	Having to wait	1	2	3
(41)	Concerns about accidents	1	2	3
(42)	Being lonely	1	2	3
(43)	Not enough money for health care	1	2	3
(44)	Fear of confrontation	1	2	3
(45)	Financial security	1	2	3
(46)	Silly practical mistakes	1	2	3
(47)	Inability to express yourself	1	2	3
(48)	Physical illness	1	2	3
(49)	Side effects of medication	1	2	3
(50)	Concerns about medical treatment	1	2	3
(51)	Physical appearance	1	2	3
(52)	Fear of rejection	1	2	3
(53)	Difficulties with getting pregnant	1	2	3
(54)	Sexual problems that result from physical problems	1	2	3
(55)	Sexual problems other than those resulting from physical problems	1	2	3
(56)	Concerns about health in general	1	2	3
(57)	Not seeing enough people	1	2	3
(58)	Friends or relatives too far away	1	2	3

	HASSLES	SEVERITY 1. Somewhat Severe 2. Moderately Severe 3. Extremely Severe		
(59)	Preparing meals	1	2	3
(60)	Wasting time	1	2	3
(61)	Auto maintenance	1	2	3
(62)	Filling out forms	1	2	3
(63)	Neighborhood deterioration	1	2	3
(64)	Financing children's education	1	2	3
(65)	Problems with employees	1	2	3
(66)	Problems on job due to being a woman or man	1	2	3
(67)	Declining physical abilities	1	2	3
(68)	Being exploited	1	2	3
(69)	Concerns about bodily functions	1	2	3
(70)	Rising prices of common goods	1	2	3
(71)	Not getting enough rest	1	2	3
(72)	Not getting enough sleep	1	2	3
(73)	Problems with aging parents	1	2	3
(74)	Problems with your children	1	2	3
(75)	Problems with persons younger than yourself	1	2	3
(76)	Problems with your lover	1	2	3
(77)	Difficulties seeing or hearing	1	2	3
(78)	Overloaded with family responsibilities	1	2	3
(79)	Too many things to do	1	2	3
(80)	Unchallenging work	1	2	3
(81)	Concerns about meeting high standards	1	2	3
(82)	Financial dealings with friends or acquaintances	1	2	3
(83)	Job dissatisfactions	1	2	3
(84)	Worries about decisions to change jobs	1	2	3
(85)	Trouble with reading, writing, or spelling abilities	1	2	3
(86)	Too many meetings	1	2	3
(87)	Problems with divorce or separation	1	2	3
(88)	Trouble with arithmetic skills	1	2	3
(89)	Gossip	1	2	3
(90)	Legal problems	1	2	3
(91)	Concerns about weight	1	2	3
(92)	Not enough time to do the things you need to do	1	2	3

	HASSLES	SEVERITY 1. Somewhat Severe 2. Moderately Severe 3. Extremely Severe		
(93)	Television	1	2	3
(94)	Not enough personal energy	1	2	3
(95)	Concerns about inner conflicts	1	2	3
(96)	Feel conflicted over what to do	1	2	3
(97)	Regrets over past decisions	1	2	3
(98)	Menstrual (period) problems	1	2	3
(99)	The weather	1	2	3
(100)	Nightmares	1	2	3
(101)	Concerns about getting ahead	1	2	3
(102)	Hassles from boss or supervisor	1	2	3
(103)	Difficulties with friends	1	2	3
(104)	Not enough time for family	1	2	3
(105)	Transportation problems	1	2	3
(106)	Not enough money for transportation	1	2	3
(107)	Not enough money for entertainment and recreation	1	2	3
(108)	Shopping	1	2	3
(109)	Prejudice and discrimination from others	1	2	3
(110)	Property, investments or taxes	1	2	3
(111)	Not enough time for entertainment and recreation	1	2	3
(112)	Yardwork or outside home maintenance	1	2	3
(113)	Concerns about news events	1	2	3
(114)	Noise	1	2	3
(115)	Crime	1	2	3
(116)	Traffic	1	2	3
(117)	Pollution	1	2	3

HAVE WE MISSED ANY OF YOUR HASSLES? IF SO, WRITE THEM IN BELOW:

(118) _____ _____ 1 2 3

ONE MORE THING: HAS THERE BEEN A CHANGE IN YOUR LIFE THAT AFFECTED HOW YOU ANSWERED THIS SCALE? IF SO, TELL US WHAT IT WAS:

_____

Do not write here:

FRQ _____
CHS _____
INT _____

Name: _____

Date: _____

THE UPLIFTS SCALE

Directions: Uplifts are events that make you feel good. They can be sources of peace, satisfaction, or joy. Some occur often, others are relatively rare.

On the following pages, circle the events that have made you feel good in the past week. Then look at the numbers on the right of the items you circled. Indicate by circling a 1, 2, or 3 how OFTEN each of the circled uplifts has occurred in the last week. If an uplift did not occur in the last week, do NOT circle it.

	UPLIFTS	HOW OFTEN 1. Somewhat often 2. Moderately often 3. Extremely often		
(1)	Getting enough sleep	1	2	3
(2)	Practicing your hobby	1	2	3
(3)	Being lucky	1	2	3
(4)	Saving money	1	2	3
(5)	Nature	1	2	3
(6)	Liking fellow workers	1	2	3
(7)	Not working (on vacation, laid-off, etc.)	1	2	3
(8)	Gossiping: "shooting the bull"	1	2	3
(9)	Successful financial dealings	1	2	3
(10)	Being rested	1	2	3
(11)	Feeling healthy	1	2	3
(12)	Finding something presumed lost	1	2	3
(13)	Recovering from illness	1	2	3
(14)	Staying or getting in good physical shape	1	2	3
(15)	Being with children	1	2	3
(16)	"Puling something off"; getting away with something	1	2	3
(17)	Visiting, phoning, or writing someone	1	2	3
(18)	Relating well with your spouse or lover	1	2	3
(19)	Completing a task	1	2	3
(20)	Giving a compliment	1	2	3
(21)	Meeting family responsibilities	1	2	3
(22)	Relating well with friends	1	2	3
(23)	Being efficient	1	2	3
(24)	Meeting your responsibilities	1	2	3
(25)	Quitting or cutting down on alcohol	1	2	3

	UPLIFTS	HOW OFTEN 1. Somewhat often 2. Moderately often 3. Extremely often		
(26)	Quitting or cutting down on smoking.....	1	2	3
(27)	Solving an ongoing practical problem.....	1	2	3
(28)	Daydreaming.....	1	2	3
(29)	Weight.....	1	2	3
(30)	Financially supporting someone who doesn't live with you.	1	2	3
(31)	Sex.....	1	2	3
(32)	Friendly neighbors.....	1	2	3
(33)	Having enough time to do what you want.....	1	2	3
(34)	Divorce or separation.....	1	2	3
(35)	Eating out.....	1	2	3
(36)	Having enough (personal) energy.....	1	2	3
(37)	Resolving inner conflicts.....	1	2	3
(38)	Being with other people.....	1	2	3
(39)	Finding no prejudice or discrimination when you expect it	1	2	3
(40)	Cooking.....	1	2	3
(41)	Capitalizing on an unexpected opportunity.....	1	2	3
(42)	Using drugs or alcohol.....	1	2	3
(43)	Life being meaningful.....	1	2	3
(44)	Being well-prepared.....	1	2	3
(45)	Eating.....	1	2	3
(46)	Relaxing.....	1	2	3
(47)	Having the "right" amount of things to do.....	1	2	3
(48)	Being visited, phoned, or sent a letter.....	1	2	3
(49)	The weather.....	1	2	3
(50)	Thinking about the future.....	1	2	3
(51)	Spending time with family.....	1	2	3
(52)	Home (inside) pleasing to you..	1	2	3
(53)	Being with younger people.....	1	2	3
(54)	Buying things for the house.....	1	2	3
(55)	Reading.....	1	2	3
(56)	Shopping.....	1	2	3
(57)	Smoking.....	1	2	3
(58)	Buying clothes.....	1	2	3

	UPLIFTS	HOW OFTEN 1. Somewhat often 2. Moderately often 3. Extremely often		
(59)	Giving a present	1	2	3
(60)	Getting a present	1	2	3
(61)	Becoming pregnant or contributing thereto	1	2	3
(62)	Having enough money for health care	1	2	3
(63)	Traveling or commuting	1	2	3
(64)	Doing yardwork or outside housework	1	2	3
(65)	Having enough money for transportation	1	2	3
(66)	Health of a family member improving	1	2	3
(67)	Resolving conflicts over what to do	1	2	3
(68)	Thinking about health	1	2	3
(69)	Being a "good" listener	1	2	3
(70)	Socializing (parties, being with friends, etc.)	1	2	3
(71)	Making a friend	1	2	3
(72)	Sharing something	1	2	3
(73)	Having someone listen to you	1	2	3
(74)	Your yard or outside of house is pleasing	1	2	3
(75)	Looking forward to retirement	1	2	3
(76)	Having enough money for entertainment and recreation	1	2	3
(77)	Entertainment (movies, concerts, TV, etc.)	1	2	3
(78)	Good news on local or world level	1	2	3
(79)	Getting good advice	1	2	3
(80)	Recreation (sports, games, hiking, etc.)	1	2	3
(81)	Paying off debts	1	2	3
(82)	Using skills well at work	1	2	3
(83)	Past decisions "panning out"	1	2	3
(84)	Growing as a person	1	2	3
(85)	Being complemented	1	2	3
(86)	Having good ideas at work	1	2	3

	UPLIFTS	HOW OFTEN 1. Somewhat often 2. Moderately often 3. Extremely often		
(87)	Improving or gaining new skills	1	2	3
(88)	Job satisfying despite discrimination due to your sex	1	2	3
(89)	Free time	1	2	3
(90)	Expressing yourself well	1	2	3
(91)	Laughing	1	2	3
(92)	Vacationing without spouse or children	1	2	3
(93)	Liking work duties	1	2	3
(94)	Having good credit	1	2	3
(95)	Music	1	2	3
(96)	Getting unexpected money	1	2	3
(97)	Changing jobs	1	2	3
(98)	Dreaming	1	2	3
(99)	Having fun	1	2	3
(100)	Going someplace that's different	1	2	3
(101)	Deciding to have children	1	2	3
(102)	Enjoying non-family members living in your house	1	2	3
(103)	Pets	1	2	3
(104)	Car working/running well	1	2	3
(105)	Neighborhood improving	1	2	3
(106)	Children's accomplishments	1	2	3
(107)	Things going well with employee(s)	1	2	3
(108)	Pleasant smells	1	2	3
(109)	Getting love	1	2	3
(110)	Successfully avoiding or dealing with bureaucracy or institutions	1	2	3
(111)	Making decisions	1	2	3
(112)	Thinking about the past	1	2	3
(113)	Giving good advice	1	2	3

	UPLIFTS	HOW OFTEN 1. Somewhat often 2. Moderately often 3. Extremely often		
(114)	Praying	1	2	3
(115)	Meditating	1	2	3
(116)	Fresh air	1	2	3
(117)	Confronting someone or something	1	2	3
(118)	Being accepted	1	2	3
(119)	Giving love	1	2	3
(120)	Boss pleased with your work	1	2	3
(121)	Being alone	1	2	3
(122)	Feeling safe	1	2	3
(123)	Working well with fellow workers	1	2	3
(124)	Knowing your job is secure	1	2	3
(125)	Feeling safe in your neighborhood	1	2	3
(126)	Doing volunteer work	1	2	3
(127)	Contributing to a charity	1	2	3
(128)	Learning something	1	2	3
(129)	Being "one" with the world	1	2	3
(130)	Fixing/repairing something (besides at your job)	1	2	3
(131)	Making something (besides at your job)	1	2	3
(132)	Exercising	1	2	3
(133)	Meeting a challenge	1	2	3
(134)	Hugging and/or kissing	1	2	3
(135)	Flirting	1	2	3

HAVE WE MISSED ANY OF YOUR UPLIFTS? IF SO, WRITE THEM IN BELOW:

(136) _____ 1 2 3

ONE MORE THING: HAS THERE BEEN A CHANGE IN YOUR LIFE THAT AFFECTED HOW YOU ANSWERED THIS SCALE? IF SO, TELL US WHAT IT WAS:

_____

Do not write here:

FRQ _____
INT _____

Name: _____ Date _____

Please place a mark on the line below to represent, on this continuum, the degree of stress you are currently experiencing:

Not at all stressed Extremely stressed

APPENDIX D

Correlations between Rating Scales and Platelet Binding, and Intercorrelations between Rating Scales in Combined Groups for Subjects in Study 2

Table D-1

Correlations between HAM-D Scores and Platelet Binding in Combined Groups

	[^{3}H]Imipramine[a]		[^{3}H]Paroxetine[b]	
Scale	B_{max}	K_d	B_{max}	K_d
HAM-D Total	-.52***	.38**	-.29*	-.10
Psychomotor	-.45***	.39**	-.29*	-.06
Anxiety	-.50***	.37**	-.30*	-.19
Sleep	-.48***	.12	-.46***	-.04

[a]n = 49. [b]n = 58.

*p < .05. **p < .01. ***p < .001.

Table D-2

Correlations between HAM-A Scores and Platelet Binding in Combined Groups

	[^{3}H]Imipramine[a]		[^{3}H]Paroxetine[b]	
Scale	B_{max}	K_d	B_{max}	K_d
HAM-A Total	-.53***	.37**	-.55***	.05
Psychic	-.57***	.42**	-.52***	.02
Somatic	-.47***	.31*	-.54***	.07

[a]n = 42. [b]n = 52.

*p < .05. **p < .01. ***p < .001.

Table D-3

Correlations between SCL-58 Scores and Platelet Binding in Combined Groups

	[^{3}H]Imipramine[a]		[^{3}H]Paroxetine[b]	
Scale	B_{max}	K_d	B_{max}	K_d
SCL-58 Total	-.54***	.33*	-.36**	.01
Depression	-.38**	.30*	-.27*	.01
Anxiety	-.60***	.30*	-.39**	-.05
Somatization	-.53***	.38**	-.33**	-.10
Obsessive	-.54***	.38**	-.38**	.05
Interpersonal	-.39**	.16	-.31*	.07

[a]n = 46. [b]n = 54.

*p < .05. **p < .01. ***p < .001.

Table D-4

Intercorrelations between Rating Scales for Patients and Healthy Volunteers

	HAM-D			
Scale	Total	Psychomotor	Anxiety	Sleep
HAM-D Total	-	-	-	-
Psychomotor	.93***	-	-	-
Anxiety	.89***	.76***	-	-
Sleep	.77***	.59***	.66***	-
HAM-A Total	.53***	.45***	.58***	.53***
Psychic	.65***	.57***	.66***	.59***
Somatic	.37**	.30*	.46***	.44***
SCL-58 Total	.51***	.49***	.51***	.38***
Depression	.52***	.54***	.45***	.30*
Anxiety	.45***	.42***	.50***	.34**
Somatization	.48***	.47***	.50***	.41***
Obsessive	.54***	.53***	.48***	.41**
Interpersonal	.35**	.35**	.33**	.19
Hopelessness	.31*	.33*	.22	.17

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table D-4 (continued)

Intercorrelations between Rating Scales for Patients and Healthy Volunteers

	HAM-A			SCL-58
Scale	Total	Psychic	Somatic	Total
HAM-A Total	-	-	-	-
Psychic	.97***	-	-	-
Somatic	.97***	.88***	-	-
SCL-88 Total	.72***	.75***	.66***	-
Depression	.53***	.56***	.46***	.90***
Anxiety	.76***	.75***	.73***	.88***
Somatization	.73***	.73***	.68***	.50***
Obsessive	.66***	.69***	.59***	.48***
Interpersonal	.47***	.53***	.39***	.33
Hopelessness	.24	.26	.20	.22

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table D-4 (continued)

Intercorrelations between Rating Scales for Patients and Healthy Volunteers

	SCL-58				
Scale	Depression	Anxiety	Somatization	Obsessive	Interpersonal
SCL-58					
Depression	-	-	-	-	-
Anxiety	.70***	-	-	-	-
Somatization	.60***	.82***	-	-	-
Obsession	.80***	.73***	.69***	-	-
Interpersonal	.80***	.54***	.47***	.76***	-
Hopelessness	.53***	.14	.10	.36**	.45***

* $p < .05$. ** $p < .01$. *** $p < .001$.

APPENDIX E

Intercorrelations between Rating Scales for Subjects in Study 3

Table E-1

Intercorrelations between Students'[a] Ratings during Examinations

	HAM-D			
Scale	Total	Psychomotor	Anxiety	Sleep
HAM-D Total	-	-	-	-
Psychomotor	.83***	-	-	-
Anxiety	.91***	.62**	-	-
Sleep	.09	-.05	-.15	-
HAM-A Total	.82***	.65**	.27***	.07
Psychic	.85***	.73***	.71***	.18
Somatic	.63**	.45*	.67***	-.03
SCL-58 Total	.29	.08	.38	-.09
Depression	.37	.28	.35	-.15
Anxiety	.13	-.11	.26	-.18
Somatization	.36	.15	.42	.04
Obsessive	.27	.04	.47*	-.37
Interpersonal	.10	-.04	.16	-.05

[a] $n = 19$.

*$p < .05$. **$p < .01$. ***$p < .001$.

table continues

Table E-1 (continued)

Intercorrelations between Students'[a] Ratings during Examinations

	HAM-D			
Scale	Total	Psychomotor	Anxiety	Sleep
Social Support Total	-.01	.26	-.06	-.27
Quantity	.12	.37	.07	-.24
Quality	-.36	-.12	-.38	-.23
Hassles	.62**	.37	.60**	.07
Uplifts	.04	-.06	.03	.02
Perceived Stress	.41	.18	.54	-.19

[a] $n = 19$.

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table E-1 (continued)

Intercorrelations between Students'[a] Ratings during Examinations

	HAM-A			SCL-58
Scale	Total	Psychic	Somatic	Total
HAM-A Total				
Psychic	.86***	-	-	-
Somatic	.91***	.57**	-	-
SCL-58 Total	.48*	.31	.52*	-
Depression	.44	.28	.49*	.82***
Anxiety	.23	.08	.31	.81***
Somatization	.53	.49*	.46*	.79***
Obsessive	.46*	.24	.56*	.83***
Interpersonal	.43	.19	.54*	.86***
Social Support Total	.07	.23	-.08	-.13
Quantity	.12	.31	-.06	-.10
Quality	-.10	-.08	-.09	-.33
Hassles	.75***	.59**	.73***	.71***
Uplifts	.13	.05	.17	.05
Perceived Stress	.56**	.52*	.48*	.39

[a] $n = 19$.

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table E-1 (continued)

Intercorrelations between Students'[a] Ratings during Examinations

	SCL-58				
Scale	Depression	Anxiety	Somatization	Obsessive	Interpersonal
Social Support Total	-.17	-.16	-.03	-.17	-.29
Quantity	-.11	-.11	.11	-.12	-.28
Quality	-.25	-.23	-.21	-.24	-.18
Hassles	.60**	.49*	.61**	.61**	.58**
Uplifts	.02	.09	.07	-.01	-.08
Perceived Stress	.18	.40	.46	.43	.32

[a] $n = 19$.

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table E-1 (continued)

Intercorrelations between Students'[a] Ratings during Examinations

	Social Support				
Scale	Total	Quantity	Quality	Hassles	Uplifts
Social Support Total					
Quantity	.96***	-	-	-	-
Quality	.71***	.51*	-	-	-
Hassles	-.23	-.13	-.40	-	-
Uplifts	-.19	-.28	.13	.35	-
Perceived Stress	.10	.14	-.04	.35	-.18

[a] $n = 19$.

* $p < .05$. ** $p < .01$. *** $p < .001$.

Table E-2

Intercorrelations between Students[a] Ratings following Vacation

	HAM-D			
Scale	Total	Psychomotor	Anxiety	Sleep
HAM-D Total	-	-	-	-
Psychomotor	.94***	-	-	-
Anxiety	.71***	.51*	-	-
Sleep	.82***	.89***	.22	-
HAM-A Total	.86***	.81***	.82***	.53*
Psychic	.86***	.76***	.82***	.56**
Somatic	.64**	.69***	.61**	.35
SCL-58 Total	.84***	.83***	.58**	.65**
Depression	.73***	.72***	.60**	.51*
Anxiety	.69***	.71***	.43	.56**
Somatization	.30	.25	.29	.14
Obsessive	.78***	.74***	.55*	.59**
Interpersonal	.88***	.87***	.70***	.66**

[a] $n = 19$.

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table E-2 (continued)

Intercorrelations between Students[a] Ratings following Vacation

	HAM-D			
Scale	Total	Psychomotor	Anxiety	Sleep
Social Support Total	-.47*	-.47*	-.43	-.32
Quantity	-.48*	-.49*	-.40	-.37
Quality	-.36	-.33	-.45*	-.13
Hassles	.78***	.72***	.74***	.48*
Uplifts	.05	.10	.13	-.07
Perceived Stress	.46*	.44*	.26	.52*

[a]n = 19.

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table E-2 (continued)

Intercorrelations between Students[a] Ratings following Vacation

	HAM-A			SCL-58
Scale	Total	Psychic	Somatic	Total
HAM-A Total				
Psychic	.94***	-	-	-
Somatic	.85***	.61**	-	-
SCL-58 Total	.80***	.75***	.67***	-
Depression	.79***	.78***	.60**	.89***
Anxiety	.67***	.63**	.56**	.90***
Somatization	.37	.30	.39	.50*
Obsessive	.69***	.61**	.63**	.82***
Interpersonal	.88***	.85***	.72***	.86***
Social Support Total	-.50*	-.47*	-.42*	-.66***
Quantity	-.48*	-.45*	-.40	-.64**
Quality	-.49*	-.45*	-.42	-.64**
Hassles	.86***	.86***	.64**	.75***
Uplifts	.18	.04	.34	-.04
Perceived Stress	.40	.58**	.04	.53**

[a] $n = 19$.

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table E-2 (continued)

Intercorrelations between Students[a] Ratings following Vacation

	SCL-58				
Scale	Depression	Anxiety	Somatization	Obsessive	Interpersonal
Social Support Total	-.67***	-.77***	-.27	-.46	-.61**
Quantity	-.63**	-.76***	-.19	-.47	-.60**
Quality	-.68***	-.69***	-.50*	-.37	-.56**
Hassles	.80***	.67***	.19	.62**	.81***
Uplifts	.10	-.22	-.28	-.05	.07
Perceived Stress	.55**	.49*	.39	.30	.45*

[a]n = 19.

* $p < .05$. ** $p < .01$. *** $p < .001$.

table continues

Table E-2 (continued)

Intercorrelations between Students'[a] Ratings after Vacation

	Social Support				
Scale	Total	Quantity	Quality	Hassles	Uplifts
Social Support Total					
Quantity	.99***	-	-	-	-
Quality	.88***	.79***	-	-	-
Hassles	-.56**	-.57**	-.47*	-	-
Uplifts	.34	.32	.34	.25	-
Perceived Stress	-.48*	-.46*	-.47*	.38	-.34

[a] $n = 19$.

* $p < .05$. ** p .01. *** $p < 001$.