Mechanisms of action of antidepressants and their combination for major depressive disorder treatment: a theoretical and clinical approach

John Michael Tabaka

Department of Psychiatry

McGill University, Montreal, Quebec, Canada

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Abstract

Background: Annually, an estimated 8.2% of Canadians aged 18 or older are affected by major depressive disorder (MDD). Nearly half of those suffering from MDD will fail to achieve remission while also having an inadequate response to an initial and continuous 6-week single antidepressant treatment. This failure to remit or respond (fully or partially) to monotherapy, referred to as treatment-resistant depression (TRD), affects more than 30% of those suffering from MDD. The addition of a second antidepressant to improve upon the effects (drug synergism) or alleviate the side-effects of the initial antidepressant has repeatedly shown encouraging therapeutic benefits. Unfortunately, the use of combination therapy in clinical settings has remained relatively low, in part because of increased pharmacological monitoring and potential life-threatening drug-drug interactions involving psychiatric and/or non-psychiatric medications.

Objective: With knowledge and understanding of the mechanisms of action of each of the seven different classes of antidepressants attained from preclinical studies (in vitro and in vivo electrophysiology) conducted at the Neurobiological Psychiatry Unit (NPU) at McGill University, the therapeutic efficacy of combination therapy can be maximized and adverse interactions and events minimized. The main goal of this thesis was to review the extensive literature concerning antidepressant studies conducted at the NPU as well as the clinical literature from PubMed and OvidSP in order to discern the most efficacious antidepressants and antidepressant combination treatments. The data collected from the literature was critically compared with the clinical database of the Mood Disorders Clinic (MDC) at the McGill University Health Centre (MUHC), which is a tertiary care psychiatric facility, in order to establish the clinical pertinence of using antidepressant combinations.

Methods: A literature review (PubMed and OvidSP) was conducted to discern the most frequently prescribed antidepressants and efficacious antidepressant combination treatments. Subsequently, we analyzed the database of the MUHC; 133 outpatients with a current DSM-IV diagnosis of major depressive disorder

aged 18 years or older were included in this study. Sociodemographic and clinical information, including current psychiatric medication prescriptions, of each patient was obtained during his or her initial diagnostic evaluation by a multidisciplinary team and chart review. Patients were also asked to complete a self-reported BDI-II questionnaire in order to assess the severity of depressive symptoms. Statistical analyses between prescribed antidepressant combinations and symptom severity were performed to determine effectiveness. A critical comparison of the findings from the literature with the clinical information obtained from patients referred to the MDC was conducted.

Results: Significantly more women than men were diagnosed with MDD ($p = 0.000$) and the mean age (in years) of all patients was 48.8 ± 14.2. Nearly three-quarters (72.4%) of the patients had at least one first-degree relative diagnosed with a psychiatric disorder. Within the six months of their initial diagnostic evaluation, 87.2% of the patients had been prescribed an antidepressant. The most frequently prescribed antidepressant was a selective serotonin reuptake inhibitor (SSRI) (42.1%), followed by a serotonin-norepinephrine reuptake inhibitor (SNRI) (30.8%) and bupropion (25.6%). Consistent with the literature, the most frequent antidepressant combination treatments were i) SSRI + bupropion, ii) SNRI + bupropion, iii) SNRI + mirtazapine, and iv) mirtazapine + bupropion. No significant difference was found between antidepressant combination treatments and mean total BDI-II scores [$F(3,19) = 1.015$, $p > 0.05$].

Conclusions: Clinical findings were generally consistent with the literature. The literature supported the use of antidepressant combinations for effective and time-efficient treatment of MDD, particularly at the beginning of treatment, yet psychiatrists still appeared hesitant on using this approach. The combinations of bupropion with an SSRI or SNRI were found to be the most efficacious combinations, receiving frequent support in the literature and in this study.

Limitations: Low completion rate of the BDI-II questionnaire resulted in the powers of performed tests to be lower than the desired powers, thus reducing the likelihood of detecting a difference when one may have actually existed. A larger

cohort of patients could allow for clinically meaningful differences to be observed.

Résumé (French Abstract)

Contexte: Chaque année, on estime que 8.2% des Canadiens âgés de 18 ans ou plus sont touchés par un trouble dépressif majeur (TDM). Près de la moitié des personnes souffrant de TDM ne parviendra pas à atteindre la rémission tout en ayant une réponse inadéquate au premier traitement antidépresseur pris seul durant 6 semaines continues. Ce défaut de rémission ou de répondre (entièrement ou partiellement) à la monothérapie, appelée dépression résistante au traitement (DRT), affecte plus de 30% des personnes souffrant de TDM. L'ajout d'un second antidépresseur pour améliorer les effets du médicament (synergie) ou atténuer les effets secondaires de l'antidépresseur a montré à maintes reprises d'encourageants avantages thérapeutiques. Malheureusement, l'utilisation de la thérapie de combinaison dans les milieux cliniques reste relativement faible, en partie à cause de l'augmentation de la surveillance pharmacologique et des interactions médicamenteuses potentiellement mortelles associées aux médicaments psychiatriques et/ou non-psychiatriques.

Objectif: Avec la connaissance et la compréhension des mécanismes d'action de chacune des sept classes différentes d'antidépresseurs obtenus à partir des études précliniques (électrophysiologie in vitro et in vivo) menées dans l'unité de Psychiatrie Neurobiologique à l'université McGill, l'efficacité thérapeutique de la thérapie de combinaison peut être maximisées et les interactions et les effets indésirables réduits au minimum. L'objectif principal de ce travail de recherche était de revisiter la littérature scientifique, à partir des études précliniques menées sur les antidépresseurs chez l'unité de Psychiatrie Neurobiologique et des études cliniques à partir de PubMed et OvidSP, afin de comprendre le mécanisme d'action des antidépresseurs et les combinaisons possibles les plus efficaces. Ces données de la littérature ont été ensuite comparées avec une banque de données cliniques du programme de troubles de l'humeur du centre universitaire de santé McGill (CUSM), qui est un centre de soins tertiaires psychiatriques, avec le but d'établir la pertinence clinique de l'utilisation des combinaisons d'antidépresseurs.

Méthodes: Une revue des études publiés dans la littérature (PubMed et OvidSP) a été effectuée pour discerner les antidépresseurs les plus prescrits et les combinaisons d'antidépresseurs les plus efficaces. Par la suite, nous avons analysé la banque de données du CUSM; 133 patients en consultation externe ayant un diagnostic du DSM-IV de trouble dépressif majeur âgés de 18 ans ou plus ont été inclus dans cette étude. Les renseignements sociodémographiques et cliniques, y compris les prescriptions actuelles de médicaments psychiatriques, de chaque patient ont été obtenus au cours de sa première évaluation diagnostique par une équipe pluridisciplinaire et examen des dossiers. Les patients ont également été invités à remplir un questionnaire d'auto-évaluation de BDI-II afin d'évaluer la sévérité des symptômes dépressifs. Des analyses statistiques entre les combinaisons d'antidépresseurs prescrits et la sévérité des symptômes ont été effectuées. Une comparaison critique de ces résultats de la littérature avec les données cliniques de la banque du CUSM a été réalisée.

Résultats: Beaucoup plus de femmes que d'hommes ont été diagnostiqués avec TDM ($p = 0.000$) et l'âge moyen (en années) de tous les patients était de 48.8 ± 14.2. Près des trois quarts (72.4%) des patients avaient au moins un parent au premier degré diagnostiqué avec un trouble psychiatrique. Dans les six mois suivant leur diagnostic initial, 87.2% des patients avaient été prescrit un antidépresseur. L'antidépresseur le plus prescrit est un inhibiteur sélectif de la recapture de la sérotonine (ISRS) (42.1%), suivi par un inhibiteur de la recapture de la sérotonine et de la noradrénaline (IRSNa) (30.8%) et le bupropion (25.6%). Conformément à la documentation, les traitements combinés d'antidépresseurs les plus fréquents étaient i) ISRS + bupropion, ii) IRSNa + bupropion, iii) IRSNa + mirtazapine, et iv) la mirtazapine + bupropion. Aucune différence significative n'a été observée entre les traitements de combinaisons d'antidépresseurs et la moyenne totale des résultats du BDI-II [$F(3,19) = 1.015$, $p>0.05$].

Conclusions: Les résultats cliniques étaient généralement conformes aux études passées. Les études sont favorables à l'utilisation de combinaisons d'antidépresseurs pour le traitement efficace et plus rapide de TDM, en particulier

au début du traitement, mais les psychiatres semblaient encore hésitants sur l'utilisation de cette approche. Les combinaisons de bupropion avec un ISRS ou IRSNa se sont révélées être les combinaisons les plus efficaces, bénéficiant d'un soutien fréquent dans les études passées et dans cette étude.

Limitations: Le faible taux d'achèvement du questionnaire BDI-II ont abouti à des pouvoirs de tests effectués à être plus faible que les pouvoirs voulus, réduisant ainsi la probabilité de détecter une différence qui peut avoir réellement existé. Une cohorte plus importante de patients pourrait permettre d’observer des différences cliniquement significatives.

List of Abbreviations and Definitions

Agonists/Antagonists

[^{3}H]cyanoimipramine: *radioligand that binds to a site associated with the 5-HT transporter*

[^{3}H]nisoxetine: *radioligand that binds to a site associated with the norepinephrine transporter*

Buspirone: *a non-SSRI anxiolytic agent*

Citalopram: *a selective 5-HT uptake inhibitor (see Treatments)*

Clonidine: *an α_2-adrenergic agonist*

Clorgyline: *a monoamine oxidase inhibitor selective for MAO-A, the A form of MAO*

Cyanopindolol: *potent 5-HT_{1B} receptor antagonist*

Desipramine: *a clinically used selective noradrenaline reuptake inhibitor (NRI) known to alter the function of α–adrenoceptors and inhibit the reuptake of noradrenaline (see Treatments)*

DSP-4: *(N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride); a neurotoxin with a significantly higher affinity for noradrenergic neurons in the rat hippocampus and cortex*

Duloxetine: *a dual 5-HT/norepinephrine reuptake inhibitor (see Treatments)*

Fluoxetine: *a 5-HT reuptake blocker with insignificant affinity for monoaminergic (neurotransmitter) and pharmacological receptors such as serotoninergic (5-HT), dopaminergic, α-adrenergic, muscarinic cholinergic, and histaminergic H_1 receptors; also a current and clinically used SSRI more commonly known as Prozac (see Treatments)*

Gepirone: *a high affinity 5-HT_{1A} agonist with no affinity for dopamine receptors; an analogue of the non-SSRI anxiolytic agent buspirone*

Hydroxybupropion: *bupropion metabolite; weak inhibitor of norepinephrine (NE) reuptake*

Idazoxan: *α_2-adrenoceptor antagonist with preferred affinity for imidazoline (ex. clonidine) recognition sites*

Ipsapirone: *a highly selective 5-HT_{1A} receptor compound*

LSD: *lysergic acid diethylamide; a somatodendritic 5-*HT_{1A} *autoreceptor agonist*

LY 165163: *a highly selective 5-HT_{1A} receptor compound*

mCPP: *m-chlorophenylpiperazine; a selective 5-HT_{1B} receptor binding compound*

Mesulergine: *a 5-HT_1 receptor ligand with a strong affinity for 5-HT_{1C} receptor sites*

Methiothepin: *a terminal 5-HT autoreceptor antagonist*

(-)Mianserin: *a selective α_2-adrenergic heteroreceptor antagonist*

Pindolol: *a 5-HT_1 partial agonist/antagonist with a higher affinity for 5-HT_{1A} receptor sites over 5-HT_{1D} receptors (See Treatments)*

RU 24969: *5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole; a mixed-type 5-HT_{1A}/5-HT_{1B} receptor agonist with a higher selectivity for 5-HT_{1B}*

Spiperone: *a neuroleptic/psychoactive drug with different affinities for 5-HT_{1A} and 5-HT_{1B} receptors*

TFMPP: *trifluoromethylphenylpiperazine; a selective 5-HT_{1B} receptor binding compound*

UK 14.304: *a selective α_2-adrenoceptor agonist*

WAY 100635: *a potent and selective 5-HT_{1A} receptor antagonist*

Yohimbine: *an α_2-adrenoceptor antagonist with an affinity for 5-HT_{1D} receptors*

5-CT: *5-carboxamidotryptamine; a non-selective 5-$HT_{1A/1B/1D}$ receptor agonist*

5-MeOT: *5-methoxytryptamine; a 5-$HT_{1A/B}$ receptor agonist*

6-OHDA: *neurotoxin affecting serotonin (5-hydroxytryptamine; 5-HT) fibres as well as catecholaminergic (such as noradrenergic) fibres*

8-OH-DPAT: *8-hydroxy-2-(di-n-propylamino)tetralin; a specific 5-HT_{1A} receptor agonist*

Enzymes

MAO: *monoamine oxidase; metabolizes 5-hydroxytryptamine (5-HT; serotonin) to 5-hydroxyindolacetic acid (5-HIAA)*

TPH: *tryptophan hydroxylase; an enzyme that hydroxylates tryptophan (introduces a hydroxyl (-OH) group) to synthesize 5-hydroxytryptophan (5-HTP). 5-HTP is the rate-limiting step in synthesizing 5-hydroxytryptamine (5-HT; serotonin)*

Neurotransmitters/Chemical Elements

DA: *dopamine; a monoamine*

GABA: *γ-aminobutyric acid; an inhibitory neurotransmitter*

K^+: *potassium cation*

NE: *norepinephrine; a catecholamine neurotransmitter (and/or hormone)*

PCPA: *para-chlorophenylalanine; a tryptophan hydroxylase (TPH) inhibitor*

5-HT: *serotonin; a monoamine neurotransmitter*

Physiological/Electrophysiological Methods and Terminology

Autoreceptor: *using feedback regulation (positive or negative), these receptors which are located at neuronal nerve terminals regulate the production (synthesis) and/or release of its indigenous ligand*

DRN: *dorsal raphe nucleus*

ED_{50}: *effective dose for ½ (50%) of the sampled population; OR the dose required for 50% suppression of a neuron's firing activity*

Heteroreceptor: *using feedback regulation (positive or negative), these receptors which are located at neuronal nerve terminals regulate the production (synthesis) and/or release of ligands other than its indigenous ligand*

HPLC: *high-performance liquid chromatography*

IC_{50}: *the half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting a biological or biochemical function (Soverini, Rosti et al. 2011)*

i.p.: *intraperitoneal (injection)*

I•T_{50} method: *used to determine the responsiveness of neurons or charge [C] (C: coulombs = current [nA] x time [s]) required to obtain a 50% reduction from baseline firing rate (Blier and de Montigny 1987)*

I•T_{50} value: *index of postsynaptic 5-HT_{1A} and/or α_2-adrenergic receptor sensitivity (for example, in dorsal hippocampus pyramidal neurons) (Beique, de Montigny et al. 2000)*

i.v.: *intravenous (injection)*

K_i: *inhibition constant (used for competitive inhibition); dissociation constant of an enzyme-inhibitor (EI) complex*

LC: *locus coeruleus*

Microiontophoreses (Microiontophoretically): *experimental procedure using seven- or five- barrelled glass micropipettes loaded with fiber glass filaments of an ionized substance (such as 5-HT or NE) implanted into the brain of an anaesthetized animal (such as a rat) in order to record the responsiveness of a particular neuron (such as hippocampal CA_3 pyramidal neurons) to the ionized substance(s) (De Montigny, Wang et al. 1980)*

RT_{50}: *recovery time 50; "reliable index of the in vivo activity of the 5-HT reuptake process in the rat hippocampus and is obtained by calculating the time in seconds required for the neuron to recover 50% of its initial firing rate at the end of the microiontophoretic application of 5-HT onto a CA3 pyramidal neuron" (Haddjeri, Blier et al. 1998)*

s.c.: *subcutaneous (injection)*

VTA: *ventral tegmental area*

Statistics

ANOVA: *analysis of variance*

SD: *standard deviation*

χ^2: *Pearson chi-square test*

Treatments

Citalopram: *a clinically used SSRI more commonly known as Celexa® (see Agonists/Antagonists)*

Desipramine: *a clinically used selective noradrenaline reuptake inhibitor (NRI) known to alter the function of α–adrenoceptors and inhibit the reuptake of noradrenaline (see Agonists/Antagonists)*

Duloxetine: *a dual 5-HT/norepinephrine reuptake inhibitor (see Agonists/Antagonists)*

Escitalopram: *a clinically used SSRI more commonly known as Lexapro®*

Fluoxetine: *a clinically used SSRI more commonly known as Prozac® (see Agonists/Antagonists)*

Fluvoxamine: *a clinically used SSRI more commonly known as Luvox®*

MAOI: *monoamine oxidase inhibitor*

NaSSA: *noradrenergic and specific serotonergic antidepressant*

NDRI: *norepinephrine-dopamine reuptake inhibitor*

Paroxetine: *a clinically used SSRI more commonly known as Paxil®*

Pindolol: *a 5-HT_1 partial agonist/antagonist with a higher affinity for 5-HT_{1A} receptor sites over 5-HT_{1D} receptors (See Agonists/Antagonists)*

SARI: *serotonin-2 (5-HT_2) antagonist and reuptake inhibitors*

Sertraline: *a clinically used SSRI more commonly known as Zoloft®*

SNRI: *serotonin-norepinephrine reuptake inhibitor*

SSRI: *selective-serotonin reuptake inhibitor*

TCA: *tricyclic antidepressant*

Trazodone: *a clinically used SARI approved by the FDA as an antidepressant (at high doses ranging between 150 – 600mg/day) but not as a low dose hypnotic*

Units of Measurement

C: *coulombs (measurement of [a neuronal] charge)*

Hz: *hertz (measurement of [a neuronal current] frequency)*

kg: *kilogram*

M: *mole*

mg: *milligram*

ml: *millilitre*

mM: *millimole*

ms: *millisecond*

mV: *millivolt*

nA: *nanoampere (measurement of [a neuronal current] intensity)*

µA: *microampere (measurement of [a neuronal current] intensity)*

µm: *micrometre*

Dedicated to my parents:

Words cannot begin to express my gratitude for all the love and support they have provided.

Merci et je vous aime!

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Section 1

An Introduction to Antidepressant Combinations, the Neurobiological Psychiatry Unit at McGill University and the McGill University Health Centre Mood Disorders Clinic

1.1.1 *Introduction to Antidepressant Combinations*

Mental illness is one of the leading causes of disability worldwide with an annual prevalence of up to 26.4% (Demyttenaere, Bruffaerts et al. 2004). Every year in Canada, an estimated 8.2% of Canadians aged 18 or over are affected by major depressive disorder (MDD) while in the United States, up to 8.7% of adults are affected (Kessler, Berglund et al. 2003; Vasiliadis, Lesage et al. 2007; Sung, Haley et al. 2012). It is important to note that the rates of MDD along with the rates of mental health service utilization do not significantly differ between Canada and the United States (Vasiliadis, Lesage et al. 2007). By the year 2020, the World Health Organization (WHO) estimates that this debilitating disorder will be the second greatest burden and cause of disability in developed countries, led only by ischemic heart disease (Demyttenaere, Bruffaerts et al. 2004). Burdens associated with major depression include, but are not limited to, low levels of education, loss of productivity in the workplace, reduced quality of life for the individual and family, and in Canada, account for some of the highest expenses in medical care costs (Merikangas and Low 2004; Vasiliadis, Lesage et al. 2007; Fleury, Grenier et al. 2010). In developing countries, MDD is almost twice as common in women than in men (Culbertson 1997; Merikangas and Swendsen 1997; Nolen-Hoeksema 2001; Merikangas and Low 2004) and the co-morbidity most strongly associated with major depression is anxiety, which in turn is associated with an increased risk of suicide (Merikangas and Low 2004). As a result, mental health in this country has evolved from an isolated and neglected health practice to an essential and fundamental aspect of primary healthcare.

The main goal of antidepressant medication is for the patient to achieve remission, as opposed to response, as the former implies a nearly complete absence of depressive symptoms while the latter only provides a clinically acceptable reduction of at least 50% of the baseline symptoms in which co-morbid and depressive debilitations may continue to persist (Trivedi, Rush et al. 2006). Studies have shown that up to 46% of those suffering from MDD will not only fail to achieve remission, but have an inadequate response to their initial antidepressant treatment consisting of an adequate drug dose and continual acute treatment of at least 6-weeks (Nierenberg and Amsterdam 1990; Fava and Davidson 1996; Lam, Hossie et al. 2004; Parikh and Lebowitz 2004; Trivedi, Rush et al. 2006). As a result of these observed failures for patients to respond (fully or partially) or remit to adequate initial antidepressant treatment, treatment-resistant depression (TRD) has unfortunately become more prevalent, affecting more than one-third of those suffering from MDD (Fava and Davidson 1996).

Those who are affected by MDD often receive initial antidepressant monotherapy medication that is frequently under-dosed and the duration of use before any significant health benefits are observed is unacceptably long, commonly leading to high medication dropout rates (Trivedi, Rush et al. 2006). In fact, due to the frustrating delays in observing any substantial mood improvement with their initial antidepressant medication, an estimated 50% of patients do not comply with their doctor's orders and stop taking their medication within the first 12-weeks of treatment (Lin, Von Korff et al. 1995; Melfi, Chawla et al. 1998; Blier, Gobbi et al. 2009). Furthermore, when asked to assess their own clinical skills and performance, mental health professionals appear to overestimate the rates of improvement of their patients while concurrently underestimating their patients' rates of decline (Walfish, McAlister et al. 2012). Based on these unsatisfactory results, the addition of a second antidepressant (or drug agent) to improve upon the efficacy and/or partial response of the initial antidepressant was warranted and explored in the early 2000s (Frye, Ketter et al. 2000). The era of mainstream antidepressant combination therapy was now in its infancy.

Although psychiatrists began to observe substantial therapeutic benefits of poly-pharmacotherapy compared to mono-pharmacotherapy, the use of antidepressant combination treatments increased rather unhurriedly (Frye, Ketter et al. 2000). Nevertheless, one of the main advantages of combination therapy was the potential synergism between the two (or more) agents. Combining antidepressants could allow the mechanisms of action of each agent to complement each other, allowing for the added medication to help improve upon the partial response of the first drug while not interfering with its mechanism of action. Thus, the therapeutic benefits of the first antidepressant would not be tainted by the second antidepressant and could even boost the therapeutic effects of the first agent, potentially leading to a faster therapeutic onset (Lam, Hossie et al. 2004). A faster onset of action could help reduce the problem of medication compliance and adherence as those unwilling to take one antidepressant due to a discouraging delayed onset of action may be more motivated to take a combination if the time course of its effects are significantly faster. An obvious caveat however, is that some may find it more difficult and confusing to adhere to a multiple medication schedule, especially if they are to be taken at different times during the day.

The financial situation of a patient may also further compound compliance to a multiple medication regimen, as the costs associated with buying numerous medications may not conform to the budget of the individual, especially if there is no therapeutically convincing evidence to support it.

Further, the overall side effect burden of the medication(s) could be alleviated (Lam, Hossie et al. 2004) as each antidepressant could off-set, or minimize, the side-effect(s) of the other (such as adding bupropion to a serotonergically-mediated antidepressant in order to combat the sexual dysfunction side-effects commonly associated with serotonergic antidepressant agents (Zisook, Rush et al. 2006)).

However, for combination treatment to be effective, knowledge and understanding of the mechanism of action of each individual antidepressant

remains imperative in order to maximize not only the therapeutic potential of any given combination, but also to avoid any and all potentially dangerous, and possibly fatal, drug-drug interactions involving psychiatric and/or non-psychiatric medications.

Although the use of combination antidepressant therapy has increased over the years, the clinical significance and overall impact of this approach remains to be elucidated as more evidence is needed to support its observable efficacy.

1.1.2 *Introduction to the Neurobiological Psychiatry Unit at McGill University and the McGill University Health Centre Mood Disorders Clinic*

The Neurobiological Psychiatry Unit at McGill University, established in 1980 by Drs. Claude de Montigny, Pierre Blier and Guy Debonel, is a translational psychiatry laboratory that applies the "bench-to-bedside" research approach by utilizing, among others, in vivo electrophysiological techniques. The McGill University Health Centre (MUHC) Mood Disorders Clinic (MDC) is a teaching-based tertiary care centre specializing in the treatment of major depression and bipolar disorder in outpatients, and is located at the Allan Memorial Institute of the Royal Victoria Hospital in Montreal, Quebec, Canada.

Together, these two institutions provide professional expertise in the field of psychopharmacology by translating, or converting, pre-clinical research into relevant and clinically meaningful applications for psychiatric patients who have failed to respond to conventional treatments. However, due to diverse pharmacological approaches in the treatment of mood disorders, namely major depressive disorder and bipolar disorder, patients undergo an initial diagnostic assessment by a multidisciplinary team composed of one or more psychiatrists, social workers, occupational therapists, MDC staff members and students in order to diagnose and evaluate the severity of the patient's illness along with his or her clinical needs. This process allows the psychiatrist, along with the multidisciplinary team, to maintain a small and highly manageable patient cohort

in order to establish personalized-drug combinations and easily accessible communication benefits for the individual. Treatment at tertiary care centres is preferred over primary and secondary care clinics by many patients, and their families, due to the quality of communication and decreased medical errors (Dy, Rubin et al. 2005; Radwin 2006).

During the initial diagnostic assessment, the medication history of the patient is learned. A study in 2007 (Cascade, Kalali et al. 2007) found that 85.0% of patients treated for major depressive disorder were prescribed only one antidepressant. If a second medication was added, the most prevalent combination was with another antidepressant (6.5%) (Cascade, Kalali et al. 2007), although the addition of an antipsychotic has been found to be similarly effective (Blier and Blondeau 2011). While the effectiveness of combination therapy has been repeatedly demonstrated (Dam, Ryde et al. 1998; DeBattista, Solvason et al. 2003; Lam, Hossie et al. 2004; Nelson, Mazure et al. 2004; Raisi, Habibi et al. 2006; Blier, Gobbi et al. 2009; Blier, Ward et al. 2010; Rocha, Fuzikawa et al. 2012), its use in clinical settings remains limited among primary care physicians and psychiatrists (Cascade, Kalali et al. 2007; Lenderts and Kalali 2009), in part because of possible undesirable reactions.

The use of combination therapy for the treatment of MDD has been shown to increase with heightened symptom severity (Cascade, Kalali et al. 2007), but in order for combination treatment to be effective, knowledge and understanding of the mechanisms of action of each of the seven classes of antidepressants remains imperative. This knowledge will allow physicians and psychiatrists to custom-tailor the patient's treatment and maximize the therapeutic potential of any given combination while avoiding potentially dangerous, and possibly fatal, drug-drug interactions. This thesis will provide the knowledge of these mechanisms of action attained from preclinical in vitro and in vivo electrophysiological studies conducted in the Neurobiological Psychiatry Unit and expand upon the therapeutic profile of antidepressant mono- and poly-pharmacy. This will be done by reviewing the literature from the Neurobiological Psychiatry Unit, in addition

to an extensive PubMed and OvidSP literature review, in order to discern the most efficacious antidepressants and antidepressant combination treatments, and critically compare these discoveries with clinical information presenting the frequency of their use and effectiveness in psychiatric outpatients referred to the MDC.

Section 2

Brief History on the Discovery of Antidepressants, Antidepressant Mechanism of Action Categories & A Short Neuroanatomical Review Important for the Mechanism of Action of Antidepressants: Human Brain Anatomy and Physiology

1.2.1 *Brief History on the Discovery of Antidepressants*

Treatment for tuberculosis in the early 1950s led researchers to a serendipitous discovery that showed patients given an antimycobacterial agent, iproniazid, had an enhanced sense of well-being as they were more cheerful, had more optimistic outlooks and even had increased physical energy (Lieberman 2003). Beginning in 1952, Zeller et al. (Zeller and Barsky 1952; Zeller, Barsky et al. 1952; Griesemer, Barsky et al. 1953; Zeller, Barsky et al. 1955; Zeller 1960; Zeller and Sarkar 1962) showed that iproniazid reduced the metabolic breakdown of serotonin (5-HT), norepinephrine (NE) and/or dopamine (DA) (subsequently leading to increased concentrations of these monoamines) by inhibiting the enzyme, monoamine oxidase (MAO), responsible for their degradation. The first class of antidepressants, the monoamine oxidase inhibitors (MAOIs), had been proclaimed. However, while clinical improvements of depressive disorders were observed with patients taking these agents, an explanation of the mechanisms of action underlying their therapeutic benefits remained to be elucidated. As a result, these discoveries led to the monoamine theory of depression (Schildkraut 1965), which proposed that a deficiency in one or more of the three important biogenic monoamines (5-HT, NE and/or DA) could be the underlying cause of depression. As more studies were conducted, a new theory, the monoamine receptor sensitivity hypothesis (Charney, Menkes et al. 1981), emerged. The proposal that antidepressants increase 5-HT_{1A} receptor desensitization and/or cause neuroadaptive plastic changes in the NE and/or DA systems not only helped

explain how antidepressant agents increase the synaptic concentration of these monoamines, but also proposed an explanation for why the therapeutic effect of all antidepressants was delayed; the time it took for these neuroplastic changes to occur or for the 5-HT_{1A} receptor to desensitize correlated with the delayed therapeutic effect of the antidepressants. This delay was also believed to allow the body to become tolerant of the drug, thus reducing the likelihood and/or severity of adverse events (Stahl 1998).

1.2.2 *Antidepressant Mechanism of Action Categories*

The mechanisms of action of the more than 24 available antidepressants (Stahl and Grady 2003), while unique, can be categorized into seven distinct groups: i) monoamine oxidase inhibitors (MAOIs), ii) tricyclic antidepressants (TCAs), iii) selective-serotonin reuptake inhibitors (SSRIs), iv) serotonin-norepinephrine reuptake inhibitors (SNRIs), v) norepinephrine-dopamine reuptake inhibitors (NDRIs), vi) noradrenergic and specific serotonergic antidepressants (NaSSAs), and vii) serotonin-2 antagonist and reuptake inhibitors (SARIs). MAOIs and TCAs are known as "classical" agents as they were the first generation of antidepressants, while the latter five groups are commonly referred to as "second-generation" antidepressants. However, while antidepressants may differ in their mechanisms of action, they all share a common end effect, that of enhancing (to varying degrees) monoaminergic functioning (Stahl, Pradko et al. 2004).

The first of the second-generation antidepressants was fluoxetine, an SSRI, which was introduced into the United States market in January 1988 (Pamer, Hammad et al. 2010). In 1989, the Canadian market also approved the SSRI fluoxetine as its first second-generation antidepressant (Hemels, Koren et al. 2002). This was a significant game-changer in antidepressant treatment as the total prescriptions for SSRIs from 1989 – 2002 [*fluoxetine* (introduced in 1989), *fluvoxamine* (introduced in 1991), *sertraline* (introduced in 1993), *paroxetine* (introduced in 1994) and *citalopram* (introduced in 1999/2000)] increased by 6.7

million and obtained the largest Canadian market share by volume (46.3%) of all prescribed antidepressants, which also included MAOIs and TCAs (Hemels, Koren et al. 2002).

1.2.3 *A Short Neuroanatomical Review Important for the Mechanism of Action of Antidepressants: Human Brain Anatomy and Physiology*

The human (mammalian) brain is a very intricate and complex system. During development, the brain folds into four (4) distinct regions known as the cerebrum, diencephalon, brainstem, and cerebellum. The cerebrum and diencephalon are collectively known as the forebrain. The cerebrum component of the forebrain is divided into left and right cerebral hemispheres, which includes the cerebral cortex, of which the neocortex engrosses the majority. The neocortex is composed of an outer grey area called grey matter and an inner layer consisting of six distinct layers of white matter. Both the left and right cerebral hemispheres are further divided into four (4) lobes known as the frontal lobe, parietal lobe, occipital lobe, and temporal lobe. (Widmaier, Raff et al. 2005).

The cerebral cortex "is the most complex integrating area of the (central) nervous system" (Widmaier, Raff et al. 2005). The frontal lobe (which encompasses the prefrontal cortex and neocortex) is involved with higher functioning such as emotion and motivation, and is therefore implicated in mood disorders (Widmaier, Raff et al. 2005).

The prefrontal cortex, neocortex and hippocampus are believed to be involved in processing cognitive feelings of guilt, worthlessness, and hopelessness, which can therefore strongly influence suicidal ideations (Nestler, Barrot et al. 2002; Widmaier, Raff et al. 2005). Through computer imaging, it can be seen that the prefrontal cortex has a high degree of neuronal activity during depressing thoughts (Drevets 2001).

Emotion, along with memory storage and retention, are believed to be mediated by the amygdala (located in the medial temporal lobe) and nucleus

accumbens (a region of the ventral striatum that receives neuronal input from the cerebral cortex) (Nestler, Barrot et al. 2002; Widmaier, Raff et al. 2005). Therefore, these areas influence emotional memory and if impaired, could lead to anxiety, anhedonia and decreased motivation (Nestler, Barrot et al. 2002).

Further, the thalamus and hypothalamus (components of the diencephalon situated below the cerebral cortex and above the midbrain) (Widmaier, Raff et al. 2005) are involved in controlling appetite, mediating sleep and providing motivation to seek pleasurable and stimulating activities, such as exercise and sex (Nestler, Barrot et al. 2002; Widmaier, Raff et al. 2005). Neurological impairments in these areas could result in a loss of interest in many normal and healthy activities for prolonged periods of time, potentially leading to irreversibly debilitating consequences.

Also, there is increased cerebral blood flow in patients with major depression compared with healthy individuals in the medial orbital cortex (region of the prefrontal cortex), amygdala and medial thalamus (Price, Carmichael et al. 1996; Drevets 2001).

1.2.3.1 *Monoaminergic Neuronal Activity in Depression*

Understanding the complex interconnections among the various areas of the brain implicated in major depression is imperative in mitigating the effects of this illness. The intricate network of monoaminergic neurons and their innervations can be (cautiously) simplified.

1.2.3.2 *Serotonin (5-HT) Innervation*

From the dorsal raphe nuclei (DRN), 5-HT neurons innervate the amygdala, nucleus accumbens (NAc), hippocampus, and hypothalamus, while

also providing serotonergic input to the ventral tegmental area (VTA) and prefrontal cortex (PFC) (Nestler, Barrot et al. 2002).

1.2.3.3 *Norepinephrine (NE) Innervation*

From the locus coeruleus (LC), NE neurons provide noradrenergic input to the VTA and PFC while innervating the amygdala, NAc, hippocampus, and hypothalamus (Nestler, Barrot et al. 2002).

1.2.3.4 *Dopamine (DA) Innervation*

From the ventral tegmental area (VTA), DA neurons innervate the amygdala and NAc, while also providing dopaminergic input to the PFC (Nestler, Barrot et al. 2002).

Section 3

Mechanism of Action of First-Generation Antidepressants:

Monoamine Oxidase Inhibitors (MAOIs)
&
Tricyclic Antidepressants (TCAs)

1.3.1 Monoamine Oxidase Inhibitors (MAOIs)

1.3.1.1 *Non-Selective & Irreversible MAOIs*

The serendipitous discovery in the early 1950s (Zeller and Barsky 1952; Zeller, Barsky et al. 1952; Griesemer, Barsky et al. 1953; Zeller, Barsky et al. 1955; Zeller 1960; Zeller and Sarkar 1962) that iproniazid reduced the metabolic breakdown of serotonin (5-HT), norepinephrine (NE) and/or dopamine (DA) by inhibiting monoamine oxidase (MAO), the enzyme responsible for their degradation, led to the monoamine theory of depression (Schildkraut 1965), an hypothesis proposing that this mood disorder was caused by a deficiency in one or more of these monoamine neurotransmitters. Consequently, one of the first clinical agents used to combat depression targeted the activity of the MAO enzyme (Coppen 1967). In the body, this enzyme is responsible for the metabolic breakdown of endogenous monoamines (5-HT, NE and/or dopamine) in nerve terminals in the brain and exogenous amines (such as dietary tyramine) in the liver, kidneys and intestinal wall via oxidative deamination (Wells and Bjorksten 1989; Wimbiscus, Kostenko et al. 2010). There are two subtypes (isoenzymes) of this enzyme, MAO-A, which is found in the intestinal tract, liver and peripheral adrenergic neurons (Wimbiscus, Kostenko et al. 2010), and MAO-B, which is found in the liver and brain (Wimbiscus, Kostenko et al. 2010). By blocking the activity of MAOs, and consequently reducing the degradation of these monoamines, the synaptic levels of these monoamines would increase, thus

alleviating the symptoms of depression (Stahl 1998). Therefore, these first-generation antidepressants were known as monoamine oxidase inhibitors (MAOIs).

The original MAOIs were non-selective and irreversible, meaning that the drug substrate had no isoenzyme preference, and thus reacted with both MAO subtypes (Wimbiscus, Kostenko et al. 2010). Further, as the substrates were (irreversibly) bound to the isoenzyme for its entire 14-28 day lifespan, the MAO could not regenerate and therefore had to be re-synthesized by the body in order to restore the homeostatic enzymatic function (Wimbiscus, Kostenko et al. 2010). Since this synthesis requires a few weeks to complete, when MAOI administration has stopped and the drug has been removed from the body, the effects of the MAOI continue until regeneration of the monoamine oxidase enzyme is complete (Fiedorowicz and Swartz 2004). Therefore, in order to avoid any potentially devastating pharmacologic drug interactions and to avoid life-threatening serotonin toxicity (serotonin syndrome), a minimum 2-week washout period is recommended when switching from an MAOI to another agent that increases the synaptic concentration of 5-HT, such as an SSRI or SNRI (Fiedorowicz and Swartz 2004). Further, when switching from a serotonergic antidepressant to an MAOI, the general rule is to allow for a washout period of at least 5 half-lives of the initial antidepressant (and its active metabolites) before taking an MAOI (Fiedorowicz and Swartz 2004). This is because most serotonergic antidepressants, while increasing synaptic 5-HT levels, do not concomitantly antagonize postsynaptic 5-HT_{2A} receptors, which when activated by 5-HT have been shown to mediate the serotonin syndrome (Spina, Santoro et al. 2008).

1.3.1.2 *MAOI Dietary Restrictions and the "Cheese Reaction"*

Due to the distinct enzymatic mechanism of action of MAOIs, along with careful drug-to-drug combination consideration, strict dietary compliance is also critical, especially when taking non-selective MAOIs. In the intestine, 80% of

MAO is of the MAO-A subtype, which degrades the naturally occurring monoamine tyramine (Yamada and Yasuhara 2004; Wimbiscus, Kostenko et al. 2010). Therefore, proper first-pass metabolism of exogenous tyramine in the liver by MAO-A could be hindered by a non-selective MAOI resulting in an excess amount of tyramine in the body (Yamada and Yasuhara 2004). The ingestion of foods rich in tyramine, most notably cheese, would further increase the concentration of tyramine in the body, which could lead to liver toxicity and a potentially fatal hypertensive crisis, commonly referred to as the "cheese effect" (Yamada and Yasuhara 2004). Although the majority of individuals taking traditional MAOIs may safely ingest up to 6mg of tyramine per serving, foods that are "absolutely restricted" include aged cheeses and meats, banana peels, broad bean pods, marmite, sauerkraut, soy bean products including soy sauce, and the tyramine content of wine and beer must be consulted before consumption (Fiedorowicz and Swartz 2004).

1.3.1.3 *Selective & Reversible MAOIs and Reversible Inhibitors of MAO-A (RIMAs)*

Due to the vigilant and strict dietary restrictions, along with the careful monitoring of drug combinations/switches, medication compliance and adherence with traditional MAOIs has made this treatment option quite inconvenient for both clinicians and patients. In fact, a study in 2009 (Shulman, Fischer et al. 2009) reported that 1.4 out of every 100,000 prescriptions in Ontario were for an irreversible MAOI and that for every 500 adults over the age of 65 who were prescribed an antidepressant, one (1) was given an irreversible MAOI. The careful monitoring of medication combination with irreversible MAOIs was exposed and the results were quite troubling as 18.1% of the patients in this study were found to be on a combination of an irreversible MAOI with at least one serotonergic agent (Shulman, Fischer et al. 2009).

Fortunately, to avoid these potential disasters, researchers have developed newer MAOIs that can selectively inhibit MAO-A or MAO-B (Stahl and Grady 2003), although no clinically beneficial antidepressant effects have been observed by the selective inhibition of MAO-B (Blier and de Montigny 1994). The inhibition of MAO-A however, has been suggested to have antidepressant qualities as the predominant effect of this isoenzyme is to increase the availability of 5-HT and NE in the nerve terminal while also catabolising tyramine (Fiedorowicz and Swartz 2004; Yamada and Yasuhara 2004; Youdim, Edmondson et al. 2006). Therefore, reversible inhibitors of MAO-A (RIMAs) have been introduced as newer-generation MAOIs. RIMAs not only selectively block the MAO-A isoenzyme, but unlike irreversible MAOIs, do not bind to MAO-A for the entire duration of the enzyme's life, enabling the MAO-A to continue its metabolic functioning (Stahl and Grady 2003). Since RIMAs can detach themselves from the tyramine-catabolising MAO-A, and thus allow for the immediate restoration of the isoenzyme's metabolic functioning, the synthesis of new MAO-A to restore homeostatic enzymatic levels in the body is unnecessary (Fiedorowicz and Swartz 2004). As a result, patients are not required to follow strict dietary restrictions, which could increase the likelihood of medication compliance (Stahl and Grady 2003). RIMAs have also been safely and effectively combined with serotonergic-agents such as SSRIs, suggesting they may have a lower potential for adverse drug interactions than the classic MAOIs (Fiedorowicz and Swartz 2004).

1.3.1.4 *Concluding Remarks*

While many experts and current guidelines continue to strongly recommend the usage of irreversible and non-selective MAOIs for the treatment of major depression (Gillman 2011), many psychiatrists either never prescribe these agents (Fiedorowicz and Swartz 2004) or choose to use them as a second or third option following treatment failure with another antidepressant (Shulman, Fischer et al. 2009). Although a meta-analysis (Lotufo-Neto, Trivedi et al. 1999)

also suggested that traditional MAOIs were slightly more effective than RIMAs in the treatment of depression, the effective and safe medication combinations of RIMAs with serotonergic-agents make these reversible and selective MAOIs a viable treatment option (Fiedorowicz and Swartz 2004).

1.3.2 Tricyclic Antidepressants (TCAs)

1.3.2.1 *Introduction*

In 1958, researchers at the Allan Memorial Institute in Montreal, Quebec, Canada presented a novel and clinically effective potent antidepressant agent called imipramine (Azima and Vispo 1958). The second of the first-generation antidepressants, the tricyclic antidepressants (TCAs), had been introduced. However, while their current usage has declined in favour of the newer antidepressants with lower risks for adverse events (Stahl and Grady 2003), the historical efficacy profile of many TCAs has encouraged psychiatrists to continue prescribing them, usually in combination with a serotonergic-agent such as an SSRI (Nelson, Mazure et al. 2004) due to their ability to inhibit the reuptake of NE into the synaptic nerve terminals in the brain (Glowinski and Axelrod 1964). Nonetheless, the combination of a TCA with an SSRI has not been proven clinically effective in treatment-resistant major depression (Fava, Rosenbaum et al. 1994).

1.3.2.2 *TCA Mechanism of Action*

Although some of the first experiments analyzing the mechanism of action of tricyclic antidepressants revealed them to be potent NE reuptake inhibitors (Glowinski and Axelrod 1964), their varying ability to inhibit the reuptake of serotonin in the central nervous system (CNS) must not be overlooked (Bradshaw,

Roberts et al. 1971). Long-term (14-days), but not short-term (2-days), administration of TCAs has been shown to increase the responsiveness of postsynaptic hippocampus and ventral lateral geniculate (VLG) neurons to 5-HT, which also correspond with their delayed onset of therapeutic antidepressant effects (de Montigny and Aghajanian 1978). However, the response of presynaptic dorsal raphe 5-HT neurons to long-term administration of TCAs was unmodified, as was the mean firing rate of these neurons (Blier and de Montigny 1980). These observations led the authors to propose that long-term TCA administration does not affect the sensitivity of the presynaptic 5-HT autoreceptor and therefore, tricyclics do not directly modify the function of 5-HT containing neurons (Blier and de Montigny 1980; Blier and de Montigny 1994). This mechanism of action is of stark contrast to selective serotonin reuptake inhibitors (SSRIs) (please see Section 4), which increase 5-HT neurotransmission by enhancing the effectiveness of 5-HT-containing neurons as opposed to increasing the responsiveness of postsynaptic neurons to 5-HT, as demonstrated by the tricyclics.

It must be noted however, that not all TCAs have the same therapeutic and efficacy profile on monoamines as they each have varying degrees of presynaptic 5-HT and NE reuptake inhibition (Stahl 1998). Although the first TCA introduced, imipramine, has been shown to have an effect on the presynaptic reuptake of both 5-HT and NE, another TCA, desipramine, is a potent reuptake inhibitor of only NE, while another tricyclic antidepressant, chlorimipramine, has been found to preferentially inhibit the reuptake of only 5-HT (de Montigny and Aghajanian 1978).

Despite TCAs being potent 5-HT and NE reuptake inhibitors in the CNS, and thus demonstrating beneficial therapeutic benefits in alleviating symptoms of depression, their antagonism of α_1-adrenergic, cholinergic and histaminergic (H_1) receptors create many undesired side effects (Stahl 1998), thus limiting their clinical usage. In addition to drowsiness caused by blockade of all these receptors, α_1-adrenoceptor inhibition also induces dizziness and decreases blood pressure;

cholinergic blockade can lead to blurred vision, constipation and dry mouth; and histamine H_1 receptor antagonism can further lead to weight gain (Stahl 1998).

1.3.2.3 *Concluding Remarks*

While TCAs are not significantly less efficacious than SSRIs in treating major depression (von Wolff, Holzel et al. 2013), their unwanted side-effects and patient tolerability result in low medication compliance by the patients (Stahl 1998), making it more uncommon for psychiatrists to prescribe these agents (Olfson and Marcus 2009).

Section 4

General Mechanism of Action of

Selective Serotonin Reuptake Inhibitors (SSRIs)

1.4.1 *Introduction to SSRI Mechanism of Action*

Selective serotonin reuptake inhibitors or serotonin specific reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressant medication (Lam, Hossie et al. 2004; Widmaier, Raff et al. 2005) and account for more than half of all antidepressant prescriptions in the United States (Stahl 1998). Of all prescribed antidepressants in Canada, SSRIs constitute the largest market share by volume at 46.3% (Hemels, Koren et al. 2002). Since the introduction of the first SSRI, fluoxetine, in Canada in 1989 along with the introduction of subsequent SSRIs [fluvoxamine (1991), sertraline (1993), paroxetine (1994) and citalopram (2000)], the number of prescribed antidepressants rose from 3.2 million in 1980 to 14.5 million in 2000, an increase of 353% with an annual increase of 16.8% (Hemels, Koren et al. 2002).

Since the proposal that a deficiency of monoamines contribute to the aetiology of depression (Schildkraut 1965), countless studies have analyzed the hypothesis that the monoamine serotonin (5-hydroxytryptamine; 5-HT), along with the function of the 5-HT neuronal system, play a fundamental role in the neurobiological modifications observed in depression. While chemically unrelated, several potent and selective SSRIs, including citalopram and paroxetine (Blier, Chaput et al. 1988), have been shown to accomplish their therapeutic effect via presynaptic sites on 5-HT neurons (Blier, de Montigny et al. 1987). More specifically, both somatodendritic and terminal 5-HT autoreceptors as well as terminal α_2-adrenoceptors on 5-HT-containing neurons, which provide negative feedback control, are believed to play different, yet equally important functions in the antidepressant response (Blier, Chaput et al. 1988). It is of importance to note

however, that the overall concentration of 5-HT in the brain does not increase as a result of SSRI treatment and that adaptive changes of 5-HT-containing neurons following long-term SSRI administration may better explain the enhanced 5-HT-mediated neurotransmission believed to underlie the delayed (2 – 3 weeks) therapeutic effect (Blier and de Montigny 1994).

1.4.2 Presynaptic Neurons

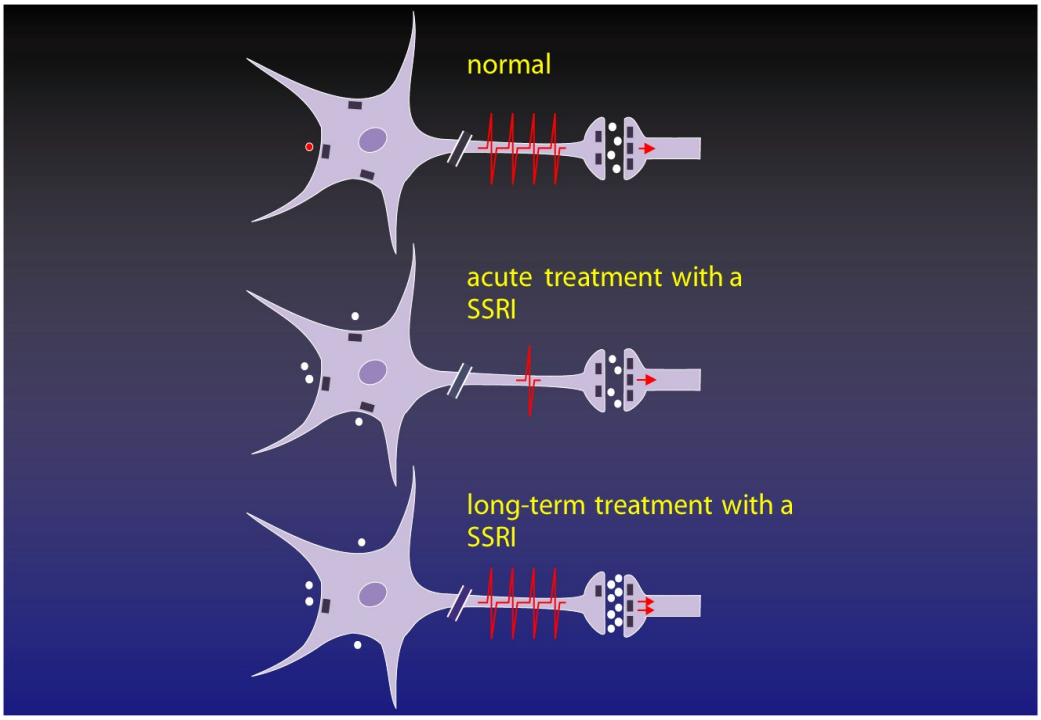
1.4.2.1 *Introduction*

Studies conducted in the late 1980s provided evidence to support the hypothesis that increased 5-HT neurotransmission is critical for the neurobiological modifications that underlie the delayed antidepressant response (Blier and de Montigny 1987). Most SSRIs are chemically unrelated and 5-HT reuptake inhibition is their only common trait. In its simplest of terms, SSRIs increase the amount of 5-HT available in the synaptic cleft and therefore, enhance 5-HT neurotransmission. However, this does not explain the delayed therapeutic response of antidepressants since these neuronal changes occur almost instantaneously. Numerous electrophysiological studies have provided convincing evidence to explain this paradox.

Mounting evidence supports the theory that adaptive changes of 5-HT neurons underlie the delayed therapeutic effect of SSRIs and that these changes are consistent with the observed delay (Blier and de Montigny 1994). Indeed, numerous electrophysiological studies have proven that the 5-HT system is exceptionally adaptable to different environments (Blier and de Montigny 1987) and one must extrapolate data from both acute and long-term effects since repeated administration of an antidepressant is required for a therapeutically significant improvement in mood.

The hippocampus receives 5-HT input from 5-HT-containing dorsal raphe neurons which contain somatodendritic and terminal 5-HT autoreceptors (Blier and de Montigny 1994). There are however, numerous subtypes of 5-HT receptors that are located in various regions of these neurons with each subtype eliciting a different electrophysiological response. The identification and location of these 5-HT autoreceptors is crucial for the development of effective SSRIs.

1.4.2.2 *Figure 1-1: Firing rate adaptation of presynaptic dorsal raphe 5-HT-containing neurons following acute and long-term treatment with an SSRI*



1.4.2.2 Figure 1-1: Acute SSRI administration results in firing activity suppression of presynaptic dorsal raphe 5-HT-containing neurons due to the desensitization of somatodendritic 5-HT_{1A} autoreceptors located on the cell body of these neurons. 5-HT_{1A} autoreceptors have a negative feedback mechanism (inhibitory characteristic) and regulate the firing activity of 5-HT-containing neurons. Terminal 5-$HT_{1B/D}$ autoreceptors, like their somatodendritic 5-HT autoreceptor counterpart, are inhibitory receptors that have a negative feedback control system and regulate the release of 5-HT into the synaptic cleft. An increased release of 5-HT into the synaptic cleft will cause 5-HT to bind to the somatodendritic 5-HT_{1A} autoreceptors and terminal 5-$HT_{1B/D}$ autoreceptors, activating the inhibitory mechanism of these receptors and initiating their negative feedback control. Consequently, the immediate (or acute) response of the 5-HT-containing neuron is to reduce neuronal firing activity in an attempt to restore a homeostatic amount of 5-HT released into the synaptic cleft. However, with sustained administration of an SSRI (minimum 14-days), extracellular 5-HT increases and with the constant bombardment of 5-HT on 5-HT_{1A} autoreceptors and terminal 5-$HT_{1B/D}$ autoreceptors, these receptors undergo modification and become desensitized to 5-HT. As a result of this desensitization, their negative feedback control is halted, allowing the firing activity of presynaptic dorsal raphe 5-HT-containing neurons to gradually recover and return to normal. Consequently, this neuronal firing activity restoration releases more serotonin into the synaptic cleft where it binds to 5-HT_{1A} receptors located on postsynaptic CA_3 dorsal hippocampus pyramidal neurons, which is a hallmark aspect of the antidepressant effect (Blier, Bergeron et al. 1997). Furthermore, the gradual recovery of the firing activity of dorsal raphe 5-HT-containing neurons is consistent with the delayed onset of action of antidepressants (Blier and de Montigny 1994). The white circles represent 5-HT while the black rectangles represent somatodendritic 5-HT_{1A} autoreceptors and terminal 5-$HT_{1B/D}$ autoreceptors located on presynaptic dorsal raphe 5-HT-containing neurons and 5-HT_{1A} receptors located on postsynaptic CA_3 dorsal hippocampus pyramidal neurons. Figure provided with courtesy from Dr. Gabriella Gobbi.

1.4.2.3 *5-HT Neurotransmission (Brief Overview)*

SSRIs heighten 5-HT neurotransmission by enhancing the effectiveness of 5-HT neurons as opposed to amplifying postsynaptic neuron responsiveness to 5-HT, which the tricyclic antidepressants (TCAs) accomplish (please see Section 3). These modifications of 5-HT neurons have been shown by assessing the effects of acute and long-term administration of gepirone, an analogue of the non-SSRI anxiolytic agent buspirone (Blier and Blondeau 2011) as well as a high affinity 5-HT_{1A} agonist with no affinity for dopamine receptors, through electrophysiological studies (Blier and de Montigny 1987).

To study the effect that gepirone has on 5-HT neurotransmission, Blier and de Montigny (Blier and de Montigny 1987; Blier, de Montigny et al. 1987) implanted subcutaneous minipumps into male Sprague-Dawley rats that released 15mg/kg/day of gepirone or saline (control pumps) for 2- (acute administration), 7-, or 14- (long-term administration) days and took recordings from either 5-HT-containing neurons in the dorsal raphe or dorsal hippocampus pyramidal neurons.

1.4.2.4 *(Presynaptic) Dorsal Raphe 5-HT-containing Neurons*

In order to record the number of spontaneously active dorsal raphe 5-HT-containing neurons along with their firing rate (Hz), single-glass micropipettes or five-barrelled microiontophoretic pipettes with solutions containing 5-HT, lysergic acid diethylamide (LSD; a somatodendritic 5-HT_{1A} autoreceptor agonist), gepirone, 8-OH-DPAT, or γ-aminobutyric acid (GABA; an inhibitory neurotransmitter) were descended into the dorsal raphe immediately below the ventral border of the Sylvius aqueduct of rats (Blier and de Montigny 1987) and identified using criteria established by Aghajanian (Aghajanian 1978; Blier and de Montigny 1987).

The mean number of spontaneously active 5-HT-containing neurons per 1mm electrode tract in control rats was 6.1 $\pm$ 0.9 neurons (Blier and de Montigny 1987). This value was significantly decreased to a mean value of 2.2 $\pm$ 0.4 active 5-HT-containing neurons per tract following an acute administration of gepirone (2-days, 15mg/kg/day), the first indication that there may possibly be changes occurring to these serotonergic neurons (Blier and de Montigny 1987). Midway through gepirone treatment (7-days, 15mg/kg/day), the mean number of active 5-HT neurons progressively increased towards normal values (5.7 $\pm$ 0.7 active 5-HT neurons/tract), which were not significantly different compared to baseline (Blier and de Montigny 1987). Following long-term administration of gepirone, the number of these 5-HT neurons per tract, surprisingly, lowered slightly to 5.5 $\pm$ 0.6 neurons/tract (Blier and de Montigny 1987). However, these results were not significantly lower than control rats, indicating that these serotonergic neurons had undergone modification (Blier and de Montigny 1987).

To begin the process of specifically identifying the(se) modification(s), the mean firing rates of dorsal raphe 5-HT-containing neurons in rats during different time periods (2-, 7-, and 14-days) and with differing treatment solutions (5-HT, LSD, 8-OH-DPAT, saline) were recorded (Blier and de Montigny 1987). In control rats, the mean firing activity of spontaneously active dorsal raphe 5-HT neurons was 1.1 $\pm$ 0.1 Hz (Blier and de Montigny 1987). Following acute gepirone treatment, the mean firing rate of these neurons dramatically decreased to approximately 0.2 $\pm$ 0.1 Hz (Blier and de Montigny 1987). Although noticeable recovery of neuronal firing rate was observed after 7-days of gepirone administration, it was still significantly lower (0.8 $\pm$ 0.1 Hz) than normal (Blier and de Montigny 1987). Full recovery of normal firing rate of these neurons was however, observed following long-term (14-days) gepirone treatment, indicating adaptive qualities of these 5-HT neurons (Blier and de Montigny 1987). The authors also found no significant "overshoot" in the firing rate of 5-HT neurons 48-hours post-minipump removal when compared to control rates (Blier and de Montigny 1987).

1.4.2.5 *Presynaptic 5-HT$_{1A}$ Somatodendritic Autoreceptor*

The activation of somatodendritic 5-HT autoreceptors decreases the firing activity of 5-HT-containing neurons (Aghajanian 1978) and thus plays a crucial role in regulating the firing activity of 5-HT-containing dorsal raphe neurons. Intravenous administration of highly selective 5-HT$_{1A}$ receptor compounds ipsapirone and LY 165163 in chloral-hydrate-anesthetised rats hyperpolarized raphe cell membranes while also dose-dependently suppressing the spontaneous firing rate of dorsal raphe neurons (Sprouse and Aghajanian 1987). Selective 5-HT$_{1B}$ receptor binding compounds m-chlorophenylpiperazine (mCPP) and trifluoromethylphenylpiperazine (TFMPP) however, demonstrated weak or irregular firing activity of these dorsal raphe neurons (Sprouse and Aghajanian 1987). As a result, these studies provide evidence that somatodendritic autoreceptors of dorsal raphe neurons are specifically 5-HT$_{1A}$ autoreceptors.

The somatodendritic 5-HT$_{1A}$ autoreceptor agonist LSD was used to determine if the adaptive recovery of 5-HT$_{1A}$ autoreceptor firing activity was caused by a desensitization of these 5-HT$_{1A}$ autoreceptors (Blier and de Montigny 1987). If the observed recovery of 5-HT neuronal firing activity seen in long-term treatment with gepirone was due to a desensitization of these 5-HT$_{1A}$ autoreceptors, then one would expect a decreased responsiveness of dorsal raphe 5-HT neuronal firing activity to LSD (Blier and de Montigny 1987).

Upon intravenous administration of LSD, the authors found a significant decrease in the response (firing activity) of dorsal raphe 5-HT neurons in rats treated with long-term gepirone (Blier and de Montigny 1987). In control rats, the effective dose (ED_{50}) of LSD in decreasing dorsal raphe 5-HT neuronal firing activity was 5 ± 1 μg/kg (Blier and de Montigny 1987). However, following long-term administration of gepirone, the ED_{50} of LSD was significantly increased to 15 ± 2 μg/kg (Blier and de Montigny 1987). By determining ED_{50} values of LSD 48-hours after removal of the minipumps in long-term gepirone treated rats, the authors were able to verify that the decreased neuronal responsiveness was due to autoreceptor desensitization as opposed to competition of LSD and gepirone at the

5-HT_{1A} autoreceptor site (Blier and de Montigny 1987). This was verified as the ED_{50} value for LSD at this 48-hour time point was 13 $\pm$ 1 μg/kg, which was not significantly different than the ED_{50} value calculated following long-term administration of gepirone via the osmotic minipump (15 $\pm$ 2 μg/kg) (Blier and de Montigny 1987).

To further validate that 5-HT_{1A} autoreceptor desensitization was indeed responsible for the decreased neuronal response to intravenous LSD, microiontophoretic applications of 5-HT, LSD, 8-OH-DPAT, and gepirone (all 5-HT_{1A} agonists) were administered once daily for 14-days in control rats and in rats treated with gepirone (15mg/kg/day, s.c.) for 14-days (Blier and de Montigny 1987). When compared to control rats, 5-HT neuron responsiveness to all 5-HT_{1A} agonists in rats treated with gepirone was significantly lower (Blier and de Montigny 1987).

Ample confirmation that desensitization of the 5-HT_{1A} autoreceptor was responsible for decreased 5-HT neuron responsiveness came when GABA, an inhibitory neurotransmitter (with no 5-HT agonistic properties), was microiontophoretically administered once daily for 14-days in rats treated with long-term gepirone (15mg/kg/day, s.c.) and was not significantly different than control (Blier and de Montigny 1990). This verified that the decreased sensitivity of these 5-HT neurons was not in response to nonspecific inhibitory agents but rather, it was the 5-HT agonists that were responsible for the desensitization of these 5-HT neurons (Blier and de Montigny 1990).

1.4.2.6 *Concluding Remarks: Presynaptic 5-HT_{1A} Somatodendritic Autoreceptor*

These experiments provided fundamental insight into how 5-HT_{1A} somatodendritic autoreceptors adapt to their environment in an attempt to modify the serotonergic neuronal system in response to depression. However, these are not the sole receptors responsible for the antidepressant response of SSRIs. In fact, one could argue that presynaptic 5-HT_{1A} somatodendritic autoreceptors

function in unison with presynaptic 5-$HT_{1B/D}$ nerve terminal autoreceptors to elicit the complete antidepressant response.

1.4.2.7 *Presynaptic 5-$HT_{1B/D}$ Nerve Terminal Autoreceptor*

Terminal 5-HT autoreceptor activation is responsible for the regulation of 5-HT release into the synaptic cleft (Blier, Chaput et al. 1988). Studies have demonstrated that the 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) ligand is an agonist specific for 5-HT_{1A} receptors (Middlemiss and Fozard 1983) while RU 24969 is a mixed-type 5-HT_{1A}/5-HT_{1B} receptor agonist with a higher selectivity for 5-HT_{1B} (Doods, Kalkman et al. 1985; Maura, Roccatagliata et al. 1986). Examining the radioactivity released by K^+ in rat hippocampal nerve endings, which was used as a determinant of un-metabolized [^{3}H]5-HT (Maura, Roccatagliata et al. 1986), showed that a concentration of 0.1μM of RU 24969 inhibited the release of [^{3}H]5-HT by close to 60% while a 0.001μM concentration of RU 24969 inhibited the release of [^{3}H]5-HT by just over 25% (Maura, Roccatagliata et al. 1986). Further, a 1μM concentration of 8-OH-DPAT inhibited the release of [^{3}H]5-HT by less than 10%, rendering it almost ineffective (Maura, Roccatagliata et al. 1986). Since released neurotransmitter binds to its autoreceptor in order to inhibit neurotransmitter release, these results indicate that RU 24969 (which has a higher preference for 5-HT_{1B} receptor) dose-dependently activates the serotonergic autoreceptor in rat hippocampal nerve endings, inhibiting the release of 5-HT. In contrast, 8-OH-DPAT (a selective 5-HT_{1A} receptor agonist) does not activate these autoreceptors, even at the commodious dose of 1μM (Maura, Roccatagliata et al. 1986). Therefore, it is shown that 5-HT_{1B} autoreceptors are located at rat hippocampal nerve terminals (Maura, Roccatagliata et al. 1986). Similar studies have also found nerve terminal 5-HT_{1B} autoreceptors in the cerebral cortex (Engel, Gothert et al. 1986) and cerebellum (Bonanno, Maura et al. 1986) of rats. In humans, the equivalent nerve terminal autoreceptor is 5-HT_{1D} (Blier and de Montigny 1994).

Previous studies (Blier and de Montigny 1987) have shown that long-term treatment with a 5-HT_{1A} agonist causes a modification of somatodendritic 5-HT_{1A} autoreceptors in 5-HT-containing neurons by decreasing their firing activity through receptor desensitization. However, these 5-HT autoreceptors are not the sole regulator of 5-HT neuronal transmission. 5-HT-containing neurons are also endowed with terminal 5-HT autoreceptors that play a critical role in modulating the release of 5-HT into the synapse. Like their somatodendritic counterpart, these 5-HT terminal autoreceptors achieve this regulation through receptor modification. To study this effect, Blier and colleagues continued to expand their knowledge of 5-HT-containing neurons by attempting to verify the effects of long-term treatment (14-days) of 5-HT reuptake inhibitors on terminal 5-HT autoreceptors (Blier, Chaput et al. 1988).

In order to analyze the function of the terminal 5-HT autoreceptor, it was deemed more appropriate to focus the majority of experimental efforts to evaluating the firing activity of postsynaptic CA_3 dorsal hippocampus pyramidal neurons as opposed to directly testing the terminal 5-HT autoreceptor (Blier, Chaput et al. 1988). However, the authors were also interested in determining whether fluoxetine, a 5-HT reuptake blocker devoid of affinity for monoaminergic receptors as well as a currently used SSRI, had the ability to decrease the function of terminal 5-HT autoreceptors, thereupon increasing the amount of 5-HT released into the synaptic cleft (Blier, Chaput et al. 1988).

It was postulated that frequency stimulation (one at 0.8 Hz and another at 5 Hz) would be a viable method to indirectly evaluate terminal 5-HT autoreceptor function based on the assumption that a low frequency stimulation (0.8 Hz) of terminal 5-HT autoreceptors would result in a lack of receptor negative feedback producing a high availability of synaptic 5-HT (Chaput, Blier et al. 1986). Conversely, a high frequency stimulation (5 Hz) of terminal 5-HT autoreceptors would result in greater activation of terminal 5-HT autoreceptor negative feedback, consequently resulting in a decreased amount of available 5-HT in the synaptic cleft (Chaput, Blier et al. 1986). With each subsequent high frequency

pulse, less 5-HT would be released into the synapse, thus reducing the effect on postsynaptic neurons (Chaput, Blier et al. 1986). Therefore, a stimulator delivering 0.5ms square pulses at a frequency of either 0.8 Hz or 5 Hz applied a minimum of 150 pulses in each trial at intensities of either 80 or 160 μA (Blier, Chaput et al. 1988).

Fluoxetine, a 5-HT reuptake blocker with insignificant affinity for monoaminergic (neurotransmitter) and pharmacological receptors such as serotoninergic (5-HT), dopaminergic, α-adrenergic, muscarinic cholinergic, and histaminergic H_1 receptors (Wong, Bymaster et al. 1983; Blier, Chaput et al. 1988), and methiothepin, a terminal 5-HT autoreceptor antagonist, were used to help study the function of terminal 5-HT autoreceptors following both acute (2-days) and long-term (14-days) fluoxetine treatment in rats (Blier, Chaput et al. 1988). A single intravenous injection of methiothepin (1mg/kg/day) was given to both control rats and rats treated with fluoxetine for 14-days (10mg/kg/day, i.p.) (Blier, Chaput et al. 1988). Since methiothepin is an antagonist of the terminal 5-HT autoreceptor, it was expected to block the inhibitory function of the receptor (terminal 5-HT autoreceptor blockade), thus hindering the receptors' negative feedback mechanism resulting in an increased release of 5-HT per stimulation pulse (Blier, Chaput et al. 1988). Furthermore, for each stimulation pulse, methiothepin was expected to enhance the effectiveness of 5-HT neurotransmission in 5-HT-containing neurons, resulting in considerable 5-HT release as a consequence of its terminal 5-HT autoreceptor blockade properties (Blier, Chaput et al. 1988).

Prior to the administration of fluoxetine, it was hypothesized that long-term treatment with this selective serotonin reuptake inhibitor would block the function of the inhibitory terminal 5-HT autoreceptor, thereby enhancing 5-HT neurotransmission and consequently, increasing the release of 5-HT into the synapse (Blier, Chaput et al. 1988).

Based on these theories, stimulation pulses at a higher frequency (ie. 5 Hz vs. 0.8 Hz) would greatly increase the function of the inhibitory terminal 5-HT

autoreceptor with each subsequent pulse. This augmentation of terminal 5-HT autoreceptor function would in turn induce a smaller quantity of 5-HT to be released into the synapse, consequently producing a smaller effect on postsynaptic dorsal hippocampus pyramidal neurons (in the rat) (Chaput, Blier et al. 1986; Blier, Chaput et al. 1988).

At the lowest studied stimulation (80 μA) administered at the lowest frequency (0.8 Hz), methiothepin's 5-HT neurotransmission enhancement was significantly reduced in long-term (14 days) fluoxetine-treated rats (39 ms $\pm$ 8) compared to controls (18 ms $\pm$ 2) (Blier, Chaput et al. 1988). Recordings from control rat dorsal hippocampus pyramidal neurons also showed that when the frequency of stimulation was increased from 0.8 Hz to 5 Hz, there was a significant reduction (61%) in the duration of suppression of firing, which was used as a gauge for 5-HT neurotransmission (Blier, Chaput et al. 1988). However, when compared to fluoxetine-treated rats (10 mg/kg/day, i.p., 14 days), there was only a 28% reduction in the duration of suppression of firing (Blier, Chaput et al. 1988). Taken together, it was ascertained that the negative feedback control of the terminal 5-HT autoreceptors was less efficient in rats administered with fluoxetine, thereby producing a high availability of 5-HT in the synaptic cleft (Blier, Chaput et al. 1988).

1.4.3 Postsynaptic Neurons

1.4.3.1 *5-HT_{1A} Autoreceptors on (Postsynaptic) Dorsal Hippocampus Pyramidal Neurons*

Postsynaptic dorsal hippocampus pyramidal neurons in rats also possess a significant amount of 5-HT_{1A} receptors (Deshmukh, Yamamura et al. 1983), however, these receptors exhibit different traits compared to presynaptic somatodendritic 5-HT_{1A} autoreceptors located on dorsal raphe 5-HT neurons

(Blier and de Montigny 1987) and when activated by 5-HT, are believed to mediate the antidepressant effect (Blier, Bergeron et al. 1997). By microiontophoretically applying 5-HT, gepirone and 8-OH-DPAT to these pyramidal neurons in rats treated for 14-days with gepirone (15mg/kg/day, s.c.), it was found that long-term gepirone treatment does not significantly modify (ie. desensitize) the responsiveness of postsynaptic dorsal hippocampus pyramidal 5-HT-containing neurons (Blier and de Montigny 1990). I•T_{50} values (control [C: coulombs] vs. post 14-days gepirone [C: coulombs]) for microiontophoretically administered 5-HT (~ 150 C vs. 155 C), gepirone (~ 170 C vs. 200 C) and 8-OH-DPAT (~ 225 C vs. 175 C) validate that the responsiveness of dorsal hippocampus pyramidal neurons in rats remain largely unchanged following long-term gepirone treatment in rats (Blier and de Montigny 1990).

1.4.3.2 *Postsynaptic 5-HT_{2A} Receptors*

SSRIs activate postsynaptic 5-HT_{2A} receptors which, when activated, are believed to be the main contributing factor leading to antidepressant-associated sexual dysfunction such as ejaculatory or orgasmic delay (Nutt 1997; Coleman, Cunningham et al. 1999; Zisook, Rush et al. 2006). To counteract this side effect, mirtazapine or bupropion have been concomitantly prescribed in order to act as an "antidote for sexual dysfunction occurring with SSRI or venlafaxine (SNRI) therapy" (Zisook, Rush et al. 2006). These combinations will be further investigated in this thesis (Sections 10 and 11).

1.4.4 ***Currently Used SSRIs for the Treatment of Depression***

Commonly prescribed antidepressants are listed below using their generic name followed by their commonly used brand name (in parentheses) (Fava 2003):

- **Citalopram (Celexa®)**
- **Escitalopram (Lexapro®)**
- **Fluoxetine (Prozac®)**
- **Fluvoxamine (Luvox®)**
- **Paroxetine (Paxil®)**
- **Sertraline (Zoloft®)**

1.4.5 *Citalopram*

1.4.5.1 *Introduction*

Celexa® (citalopram) was introduced into the Canadian market in late 1999/early 2000 (Hemels, Koren et al. 2002), but in 2003 the pharmaceutical company Lundbeck lost its patent protection for this SSRI (Huskamp, Donohue et al. 2008), opening the door for other companies to produce the (cheaper) generic form. This consequently encouraged healthcare professionals to promote SSRIs, especially citalopram, as a first-step agent in the treatment of major depressive disorder (MDD) (Rush, Fava et al. 2004). Due to the popularity of SSRIs along with citalopram's minimal drug interactions with other agents and relatively short half-life (allowing a low-risk switch from citalopram to another agent without a washout or tapering off period), the STAR*D study designers chose this SSRI as its Level 1, first-line treatment agent (Rush, Fava et al. 2004). (For information on STAR*D, please see Section 9). As a result of its popular prescription profile and

usage as a first-step treatment option in a study involving numerous subsequent treatment steps, the mechanism of action of citalopram is warranted.

1.4.5.2 *Mechanism of Action of Citalopram*

In 1977, John Hyttel explored the biochemical properties of Lu 10-171, a bicyclic phthalane derivative [1-(3-(di-methylamino)propyl)-1-(*p*-fluorophenyl)-5-phthalan-carbonitrile], more commonly known as citalopram, on the 5-HT neuronal system (Hyttel 1977). Citalopram is a chiral compound that is a racemic mixture composed of an S(+)- and R(-)-enantiomer (escitalopram and R-citalopram, respectively) in a 1:1 ratio (Hyttel 1977). Upon providing evidence that it "is a very potent and completely selective inhibitor of the 5-HT reuptake mechanism" (Hyttel 1977), countless studies have been conducted to determine the acute and long-term effects of citalopram administration on the 5-HT neuronal system, while concurrently analyzing its efficacy in the treatment of depression.

To assess acute and long-term effects, 20mg/kg intraperitoneal (i.p.) injections of citalopram were administered to male Sprague-Dawley rats for 2, 7 or 14 consecutive days (ie. acute to long-term administration) (Chaput, de Montigny et al. 1986). This dose is substantially higher than that used in clinical trials as the plasma single-dose half-life of citalopram in rats is close to 3 hours, whereas in humans, it is approximately 30 hours (Kragh-Sorensen, Overo et al. 1981; Fredricson Overo 1982; Melzacka, Rurak et al. 1984; Chaput, de Montigny et al. 1986). Approximately 10-12 hours after the final citalopram injection (after 2, 7 or 14 days), rats were anesthetised with 400mg/kg, i.p., chloral hydrate and a tail vein injection of 2.5 – 25μg/kg, i.p., of lysergic acid diethylamide (LSD), a somatodendritic 5-HT autoreceptor agonist with inhibitory effects (de Montigny, Chaput et al. 1990), or citalopram (0.05 – 0.5mg/kg) was administered once a stable dorsal raphe 5-HT neuron recording was established (Chaput, de Montigny et al. 1986). Another group of rats received 20mg/kg, i.p., of citalopram daily for 14 days, and 24 hours after the final injection, rats were anesthetised with

400mg/kg, i.p., chloral hydrate and injected with methiothepin (1mg/kg, i.v.), a non-selective 5-$HT_{1B/D}$ receptor antagonist that enhances 5-HT neurotransmission (Slassi 2002), while CA_3 hippocampal pyramidal neurons were recorded (Chaput, de Montigny et al. 1986). The 24-hour post final-injection juncture was chosen since, at this time point, only trace amounts of citalopram are present in the rat brain following consistent administration (Arnt, Overo et al. 1984; Hyttel, Overo et al. 1984; Chaput, de Montigny et al. 1986).

1.4.5.3 *Effects of Citalopram on the Electrically-Evoked Release of 5-HT*

The effects of citalopram on the electrically-evoked release of [^{3}H]-5-HT from rat hypothalamic slices were demonstrated in 1989 (Blier, Ramdine et al. 1989). It had been reported (Langer and Moret 1982) that doses of citalopram (0.001 to 10 μM) when added 20 minutes before the second period of stimulation did not significantly modify the electrically-evoked release of [^{3}H]-5-HT between the second period of stimulation and the first control period in slices of the rat hypothalamus. In this experiment (Blier, Ramdine et al. 1989), it was also found that the solo addition of citalopram (0.01 to 1 μmol/L) 20 minutes before the second period of stimulation did not significantly alter the fractional electrically-evoked release of [^{3}H]-5-HT in slices of the rat hypothalamus when these slices were stimulated at 3 Hz. The same result was also found with the addition of another SSRI (paroxetine) (0.1 μmol/L) 20 minutes before the second period of stimulation at a frequency of 3 Hz (Blier, Ramdine et al. 1989). As inferred, it was found that slices of the rat hypothalamus stimulated at 1 Hz did produce significant changes in the fractional release of [^{3}H]-5-HT (Blier, Ramdine et al. 1989). The addition of citalopram (0.01 to 1 μmol/L) (and paroxetine; 0.1 μmol/L) 20 minutes before the second period of stimulation significantly decreased the fractional electrically-evoked release of [^{3}H]-5-HT in slices of the rat hypothalamus when these slices were stimulated at a frequency of 1 Hz (Blier, Ramdine et al. 1989).

Furthermore, in an attempt to gauge any potential 5-HT autoreceptor agonist properties of citalopram, the authors tested the effect of citalopram (1 μmol/L) on the fractional electrically-evoked release of [^{3}H]-5-HT in slices of the rat hypothalamus in the presence of the terminal 5-HT autoreceptor antagonist methiothepin (1 μmol/L) at frequencies of 1 Hz and 3 Hz (Blier, Ramdine et al. 1989). A significant increase in the fractional release of [^{3}H]-5-HT overflow propagated by citalopram in the presence of the 5-HT autoreceptor blocker methiothepin was nearly identical at both frequencies (1 Hz and 3 Hz) (Blier, Ramdine et al. 1989).

Based on these results, the authors were able to conclude (at least in the rat hypothalamus) that serotonin reuptake inhibitors, such as citalopram, have the ability to augment the negative feedback mechanism exerted by 5-HT released into the synapse on inhibitory terminal 5-HT autoreceptors (Blier, Ramdine et al. 1989).

1.4.5.4 *Acute Administration*

By injecting 11 naïve rats via the tail vein, a median effective dose (ED_{50}) (mg/kg $\pm$ S.E.M.) of 0.23 $\pm$0.03mg/kg citalopram was found to suppress the spontaneous firing activity of dorsal raphe 5-HT neurons by 50% (Chaput, de Montigny et al. 1986).

10-12 hours after the final acute citalopram administration (20mg/kg/day, 2-days), both the firing activity and number of spontaneously active dorsal raphe 5-HT neurons were markedly decreased (Chaput, de Montigny et al. 1986). However, when observed 15-17 hours after the final acute administration, both variables returned to near control values (Chaput, de Montigny et al. 1986). The authors speculate that following 2-day administration of citalopram in rats, 5-HT reuptake is no longer inhibited after this time point (Chaput, de Montigny et al. 1986).

1.4.5.5 *Long-Term Administration*

Ten to twelve hours following 7-day administration of citalopram (20mg/kg/day, i.p.), dorsal raphe 5-HT neurons had moderately regained their firing activity back towards control values (Chaput, de Montigny et al. 1986). The number of spontaneously active dorsal raphe 5-HT neurons following 7-day administration had greatly increased from what was found following 2-day citalopram treatment, to the point where these values had almost completely returned to control levels (Chaput, de Montigny et al. 1986).

Ten to twelve hours following 14-day administration of citalopram (20mg/kg/day, i.p.), both the firing activity and number of spontaneously active dorsal raphe 5-HT neurons had recovered and returned to control values (Chaput, de Montigny et al. 1986).

To determine the sensitivity of somatodendritic dorsal raphe 5-HT autoreceptors to long-term administration of citalopram (20mg/kg/day, i.p., 14-days), LSD was administered in various doses 10-12 hours following the final citalopram treatment (Chaput, de Montigny et al. 1986). Compared to controls, a significantly higher dose of LSD ($ED_{50's}$: 10.0 $\pm$1.4µg/kg + citalopram vs. 5.3 $\pm$0.5µg/kg, control) was needed to suppress the spontaneous firing activity of dorsal raphe 5-HT neurons following long-term citalopram treatment (Chaput, de Montigny et al. 1986). As a result of the reduced ability of LSD to inhibit the firing activity of these 5-HT neurons in citalopram-administered rats, it appeared that long-term treatment with citalopram caused somatodendritic 5-HT autoreceptor desensitization (Chaput, de Montigny et al. 1986).

Long-term administration of citalopram (20mg/kg/day, i.p., 14-days) was shown to substantially enhance 5-HT synaptic neurotransmission (Chaput, de Montigny et al. 1986). However, this was not found in acute citalopram treatment (Chaput, de Montigny et al. 1986). The injection of 1mg/kg/day, i.v., citalopram in naïve rats for 2 days found that the mean firing activity duration of postsynaptic CA_3 hippocampal pyramidal neurons in response to the electrical stimulation of

the ascending 5-HT pathway was not significantly modified by acute administration (Chaput, de Montigny et al. 1986). This shows that the enhanced effect of 5-HT synaptic neurotransmission seen in long-term treatment is not the result of 5-HT reuptake blockade by citalopram (Chaput, de Montigny et al. 1986). Using methiothepin (1mg/kg, i.v.), the firing activity of terminal 5-HT autoreceptors on CA_3 hippocampal pyramidal neurons in response to long-term citalopram treatment was measured (Chaput, de Montigny et al. 1986). 14-day administration of citalopram (20mg/kg/day, i.p.) significantly hindered the ability of methiothepin to enhance the duration of firing suppression of CA_3 hippocampal pyramidal neurons in response to 320μA stimulations of the ascending 5-HT pathway when compared to controls, indicating terminal 5-HT autoreceptor desensitization (Chaput, de Montigny et al. 1986).

1.4.5.6 *Concluding Remarks*

The desensitization of the somatodendritic, and especially the terminal, 5-HT autoreceptors is of crucial importance to the efficacy of citalopram as an effective antidepressant. Desensitization of these 5-HT autoreceptors is expected to halt the negative feedback control that decreases the amount of 5-HT neurotransmitter available for release into the synapse and increase the firing activity of 5-HT-containing neurons. As a result, one would expect an increased amount of 5-HT available within the presynaptic neuron to be released by an electrical impulse and be available to bind to 5-HT postsynaptic receptors. Further, it has been shown that the increased effect of 5-HT neurotransmission in long-term citalopram treatment is not the result of 5-HT reuptake blockade. Therefore, it appears that the efficacy of long-term citalopram treatment as an effective antidepressant lies more on the desensitization of 5-HT autoreceptors, especially terminal 5-HT autoreceptors, than on 5-HT reuptake blockade. (For information pertaining to citalopram's involvement in the STAR*D study, please see Section 9).

Section 5

Serotonin Norepinephrine Reuptake Inhibitors (SNRIs)

Venlafaxine

1.5.1 *Introduction*

During the late 1990s, it became evident that a more efficacious medication was needed in order to more effectively relieve symptoms related to treatment-resistant depression (TRD). Near the turn of the century, a phenylethylamine derivative known as venlafaxine (1-[2-(dimethylamino)-1-(4-methyoxyphenyl)-ethyl]cyclohexanol) appeared to display convincing evidence in the effective treatment of major depression while also exhibiting a faster onset of therapeutic action (Schweizer, Weise et al. 1991). Later studies concluded that, at high doses, venlafaxine was significantly efficacious in TRD (Nierenberg, Feighner et al. 1994; de Montigny, Silverstone et al. 1999) and therefore, much attention was given to understanding its mechanism of action.

1.5.2 *Mechanism of Action*

The main distinguishing characteristic of the mechanism of action of venlafaxine is that it is a dual reuptake inhibitor as it blocks the reuptake of both serotonin (5-HT) and norepinephrine (NE) (or noradrenaline (NA)). The antidepressant effect of most medications lies in their ability to increase 5-HT neurotransmission (Blier and de Montigny 1994) either by acting directly on 5-HT neurons (Chaput, de Montigny et al. 1986; Blier, de Montigny et al. 1987; Blier, Galzin et al. 1990) or indirectly via noradrenergic neurons (Blier, Galzin et al. 1990; Mongeau, Blier et al. 1993; Haddjeri, Blier et al. 1995). Therefore, an agent capable of acting simultaneously on both 5-HT and NE systems should, theoretically, be a clinically and more rapidly effective medication option. It has

been suggested that the dual inhibition of 5-HT and NE reuptake does indeed provide greater therapeutic benefit (Beique, de Montigny et al. 1998) and studies have shown superior therapeutic benefits of SNRIs compared to SSRIs (Clerc, Ruimy et al. 1994; Poirier and Boyer 1999; Blier 2006; Nemeroff, Entsuah et al. 2008). Therefore, understanding the exact nature of venlfaxine's reuptake inhibition is imperative to learning how it exerts its effective and well tolerated antidepressant effect (de Montigny, Silverstone et al. 1999).

Initial in vivo electrophysiological studies in rats (Beique, De Montigny et al. 1996) indicated that venlafaxine potently inhibited the reuptake of both 5-HT and NE, however, venlafaxine appeared to have a greater potency in inhibiting 5-HT reuptake than NE reuptake, both in vitro (Beique, Lavoie et al. 1998) and in vivo (Beique, de Montigny et al. 1998; Beique, de Montigny et al. 2000).

1.5.3 *In Vitro Radioligand Binding Studies*

Radioligand binding studies in rat brains were conducted in order to determine the affinity of venlafaxine, in vitro, for both the 5-HT transporter and NE transporter using the selective radioligands [^{3}H]cyanoimipramine and [^{3}H]nisoxetine, respectively (Beique, Lavoie et al. 1998). [^{3}H]cyanoimipramine has been shown to bind to a site associated with the 5-HT transporter (Burkard 1980) while [^{3}H]nisoxetine has been shown to bind to a site associated with the norepinephrine transporter (Tejani-Butt, Brunswick et al. 1990). Competition studies were used to measure these affinities by determining the ability of venlafaxine to displace [^{3}H]cyanoimipramine from the 5-HT transporter and [^{3}H]nisoxetine from the NE transporter (Beique, Lavoie et al. 1998).

1.5.3.1 *5-HT Transporter Binding Sites*

Based on K_i values (inhibition constant/dissociation constant of an enzyme-inhibitor (EI) complex; for definition, please see List of Abbreviations

and Definitions), it was found that the affinity of venlafaxine for the [^{3}H]cyanoimipramine binding site was 74 $\pm$ 1.9 nM while the affinity of duloxetine (another dual 5-HT and norepinephrine reuptake inhibitor) for the [^{3}H]cyanoimipramine binding site was 1.8 $\pm$ 0.1 nM, indicating that, in vitro, venlafaxine has only a moderate affinity for the 5-HT transporter compared to duloxetine, which has a high affinity for the 5-HT transporter (Beique, Lavoie et al. 1998).

1.5.3.2 *NE Transporter Binding Sites*

The affinity of venlafaxine for the [^{3}H]nisoxetine binding site was 1260 $\pm$ 144 nM while that of duloxetine was 3 $\pm$ 0.3 nM, indicating that, in vitro, the affinity of venlafaxine for the norepinephrine transporter is less than 420 times that of duloxetine (de Montigny, Silverstone et al. 1999). Interestingly, for what has been shown to be an effective dual serotonin and norepinephrine reuptake inhibitor (de Montigny, Silverstone et al. 1999), these in vitro K_i values reveal venlafaxine to have only a moderate to low 5-HT and norepinephrine reuptake inhibition profile when compared to another dual 5-HT and norepinephrine reuptake inhibitor.

These in vitro findings are surprising as venlafaxine has not only been shown to be quite effective and well tolerated in patients with treatment-resistant depression (de Montigny, Silverstone et al. 1999) but also has a much higher probability of being among the most efficacious antidepressants (22.3%) than duloxetine (0.9%) (Cipriani, Furukawa et al. 2009) (please see Section 10). In order to better grasp the neurobiology of this drug and to further understand the mechanism of action of venlafaxine, in vivo electrophysiological experiments were performed on CA_3 pyramidal neurons in the rat dorsal hippocampus (Beique, de Montigny et al. 1998; Beique, de Montigny et al. 2000).

1.5.4 ***Acute Administration of Venlafaxine***

1.5.4.1 *In Vivo Electrophysiological Studies*

In vivo electrophysiological studies (Beique, de Montigny et al. 1998) were carried out in male Sprague-Dawley rats (250 – 300g) and secured in a stereotaxic device while anaesthetized with chloral hydrate (400 mg/kg, i.p.). Before being secured in the stereotaxic apparatus, the rats were implanted subcutaneously with osmotic mini-pumps that microiontophoretically delivered either venlafaxine (10, 20 or 40 mg/kg/day) or vehicle for 48 hours (for definition of microiontophoretic applications, please see List of Abbreviations and Definitions) (Beique, de Montigny et al. 1998). The recovery time 50 (RT_{50}) (for definition, please see List of Abbreviations and Definitions) was used to determine both of the monoamines reuptake activity (Beique, de Montigny et al. 1998) as the RT_{50} has been shown to be a reliable in vivo index of the activity of 5-HT reuptake in the rat hippocampus (Pineyro, Blier et al. 1994; Haddjeri, Blier et al. 1998), amygdala and lateral geniculate body (Wang, de Montigny et al. 1979) as well as in vivo reuptake activity of NA in the rat hippocampus (De Montigny, Wang et al. 1980).

Using the same neurons to determine how venlafaxine affects the recovery time from intravenous microiontophoretic applications of 5-HT and NA, the authors found that a dose of only 1 mg/kg of venlafaxine at a current of 5 nA was sufficient enough to significantly increase the RT_{50} value for 5-HT but not for NA (Beique, de Montigny et al. 1998). In order to significantly increase the RT_{50} value for NA, a venlafaxine dose of 10 mg/kg at a current of 3 nA was required, providing evidence to support the in vitro affinity studies and show that in vivo, venlafaxine displays a greater influence in blocking the 5-HT transporter than it does the NA transporter (Beique, de Montigny et al. 1998) .

Following an acute (2-day) administration of 10 mg/kg/day of venlafaxine after microiontophoretic applications of 5-HT at currents of both 1 and 5 nA, it

was found that the RT_{50} values were significantly increased compared to controls (Beique, de Montigny et al. 1998). However, this was not found following microiontophoretic applications of NA at currents of both 3 and 10 nA (Beique, de Montigny et al. 1998). Further, when the dose of venlafaxine was increased to 20 mg/kg/day or 40 mg/kg/day, the RT_{50} values following microiontophoretic applications of 5-HT at currents of 1 or 5 nA and NA at currents of 3 and 10 nA were all significantly increased when compared to controls (Beique, de Montigny et al. 1998). It was important to note that these findings were not dose-dependent as there were no significant increases in RT_{50} values for either 5-HT or NA applications when the high dose was compared to the minimal effective dose (Beique, de Montigny et al. 1998).

The authors concluded that, in vivo, venlafaxine has an affinity for both 5-HT and NA due to its reuptake inhibition of these two monoamines and that this affinity is much greater for 5-HT reuptake inhibition than NA reuptake inhibition (Beique, de Montigny et al. 1998).

1.5.4.2 *In Vitro versus In Vivo Affinities for 5-HT and NA*

In order to begin to confidently understand the mechanism of action of venlafaxine, it is important to compare the affinity values of venlafaxine for 5-HT and norepinephrine found in vitro with those found in vivo. When these values are compared, the discrepancy between in vitro and in vivo reuptake binding affinities for venlafaxine become clearly evident and blurs our understanding of its effectual mechanism of action in treatment-resistant depression.

In 1999, an in vivo electrophysiological experiment in rats (Beique, de Montigny et al. 1999) used ED_{50} values (effective dose-50; for definition, please see List of Abbreviations and Definitions) to compare the ability of venlafaxine (SNRI) and paroxetine (an SSRI) to suppress the firing activity of dorsal raphe 5-HT neurons as well as venlafaxine and desipramine (a selective noradrenaline reuptake inhibitor (SNRI) known to alter the function of α–adrenoceptors

(Checkley, Slade et al. 1981) and inhibit the reuptake of noradrenaline (Shur and Checkley 1982)) to suppress the firing activity of locus coeruleus NE neurons (Beique, de Montigny et al. 1999).

Extracellular unitary recordings of at least one (1) minute revealed that a total of 33 dorsal raphe 5-HT neurons fired at a mean basal rate of 1.32 $\pm$ 0.11 Hz while a total of 56 locus coeruleus NE neurons had a mean basal firing rate of 2.6 $\pm$ 0.18 Hz (Beique, de Montigny et al. 1999).

1.5.4.3 *Venlafaxine and Paroxetine (Effects on Dorsal Raphe 5-HT Neuron Firing Activity)*

An acute (2-day), single i.v. dose of venlafaxine injected into the lateral tail vein of 14 naïve rats showed a dose-dependent suppression of the firing activity of dorsal raphe 5-HT neurons (Beique, de Montigny et al. 1999). These dose-dependent results were duplicated by paroxetine (Beique, de Montigny et al. 1999).

In addition, the doses of venlafaxine and paroxetine required for 50% firing activity suppression of the dorsal raphe 5-HT neurons (ED_{50}) was 233 µg/kg, i.v. and 211 µg/kg, i.v., respectively, revealing that these two drugs were equipotent at suppressing the firing activity of dorsal raphe 5-HT neurons (Beique, de Montigny et al. 1999).

However, when these results were compared with prior in vitro studies (Beique, de Montigny et al. 1998), the affinity (K_i) of paraoxetine for the 5-HT transporter ([^{3}H]cyanoimipramine) was 0.04 $\pm$ 0.004 nM, which was 1,850 times greater than the affinity of venlafaxine for the 5-HT transporter (74 $\pm$ 1.9 nM) (Beique, de Montigny et al. 1999). Also, as previously mentioned, the affinity of duloxetine (a dual 5-HT/norepinephrine reuptake inhibitor) for this same transporter was 1.8 $\pm$ 0.1 nM, which was 41 times greater than venlafaxine (Beique, Lavoie et al. 1998). It was further concluded that the affinity of

venlafaxine for the 5-HT transporter was only mediocre (Beique, Lavoie et al. 1998). These in vitro and in vivo results strongly suggest that the ability of venlafaxine to inhibit the reuptake of 5-HT is not as influential as its ability to suppress the firing activity of 5-HT neurons and that the antidepressant effect of this drug may be more complex than simply binding to its reuptake transporter site (Beique, de Montigny et al. 1999). This will be discussed in more depth later in this section.

1.5.4.4 *Venlafaxine and Desipramine (Effects on Locus Coeruleus NE Neuron Firing Activity)*

An acute (2-day), single i.v. dose of venlafaxine revealed a dose-dependent suppression of the firing activity of locus coeruleus NE neurons (Beique, de Montigny et al. 1999). However, low doses (<400 µg/kg, i.v.) of venlafaxine appeared to not only have very minimal abilities to suppress the firing activity of these NE neurons, but was unable to completely suppress the firing of these neurons (Beique, de Montigny et al. 1999). Desipramine, on the other hand, was not only able to suppress the firing activity of locus coeruleus NE neurons in a dose-dependent manner, but was able to completely suppress the neurons firing activities (Beique, de Montigny et al. 1999).

The ED_{50} for venlafaxine suppressing 50% of locus coeruleus NE neuron firing activity was 737 $\pm$ 68 µg/kg, i.v. while the ED_{50} for desipramine was 240 $\pm$ 54 µg/kg, i.v., indicating that venlafaxine was only 3 times less potent at suppressing these NE neurons (Beique, de Montigny et al. 1999).

When these results were compared to previous in vitro affinity studies (Beique, Lavoie et al. 1998), the affinity (K_i) of venlafaxine for the NE transporter ([^{3}H]nisoxetine) was 1260 $\pm$ 144 nM, which was almost 3000 times less than the affinity of desipramine for the NE transporter (0.55 $\pm$ 0.04 nM) (Beique, de Montigny et al. 1999). The study also concluded that venlafaxine had a low affinity for the NE transporter (Beique, Lavoie et al. 1998). Taken together, these

in vitro and in vivo studies provide more potent suggestions that venlafaxine's ability to inhibit the reuptake of NE is not as strong as its ability to suppress the firing activity of NE neurons (much like with 5-HT) and that the mechanism of antidepressant action of this drug appears to be more complex than simply binding to its NE (and 5-HT) reuptake transporter site (Beique, de Montigny et al. 1999). This will also be explored in more depth later in this section.

1.5.5 ***Long-Term Administration of Venlafaxine***

Although studies based on the acute administration of venlafaxine in rats provided fundamental insight into the mechanism of action of this SNRI, the conclusions led to confusion as its in vivo 5-HT and NE reuptake blocking properties were not a reliable predictive marker for venlafaxine's affinity for the 5-HT and NE transporters (Beique, de Montigny et al. 2000). Therefore, observation of long-term venlafaxine administration was imperative to deducing its antidepressant mechanism of action.

1.5.5.1 *Venlafaxine RT_{50} Values*

Using microiontophoretic applications of 5-HT and NE in CA_3 pyramidal neurons in the rat dorsal hippocampus, the uptake activity of these two monoamines following long-term (21-day) administration was observed by analyzing their respective neuronal firing activity recovery time-50 (RT_{50}) values (see List of Abbreviations and Definitions) (Beique, de Montigny et al. 2000). At a 10 mg/kg/day subcutaneous (s.c.) dose of venlafaxine for 21 days, an ejection current of 1 nA did not significantly effect dorsal hippocampal neuronal firing rates after microiontophoretic 5-HT applications (Beique, de Montigny et al. 2000). In contrast, when the current was increased to 5 nA, the RT_{50} value significantly increased by 111% when compared to baseline values (Beique, de Montigny et al. 2000). At the same venlafaxine dose of 10 mg/kg/day,

microiontophoretic applications of NE at a current of either 1 nA or 5 nA did not significantly increase the dorsal hippocampal neuronal firing activity compared to baseline (Beique, de Montigny et al. 2000)

However, a 40 mg/kg daily subcutaneous (s.c.) dose of venlafaxine for 21 days displayed significantly different results at both currents and for both monoamines (Beique, de Montigny et al. 2000). Following microiontophoretic applications of 5-HT at a current of 1 nA or 5nA, the RT_{50} values significantly increased by 126% and 163%, respectively (Beique, de Montigny et al. 2000). Moreover, after microiontophoretic applications of NE at a current of 1 nA or 5nA, the RT_{50} values were also significantly increased by 186% and 90%, respectively (Beique, de Montigny et al. 2000)

1.5.5.2 *Long-Term Administration of Venlafaxine on Postsynaptic 5-HT_{1A} Receptors, Postsynaptic α_2-adrenergic Heteroreceptors and Terminal 5-HT_{1B} Autoreceptors*

Based on $I \bullet T_{50}$ values, both postsynaptic 5-HT_{1A} and α_2-adrenergic receptor sensitivity of CA_3 dorsal hippocampus pyramidal neurons were unchanged following 10 and 40 mg/kg/day, s.c. treatments of venlafaxine (Beique, de Montigny et al. 2000). However, 40 mg/kg/day, s.c. of venlafaxine did increase the tonic activation of these postsynaptic 5-HT_{1A} receptors while also desensitizing terminal 5-HT_{1B} autoreceptors (Beique, de Montigny et al. 2000). These results were not observed with the 10 mg/kg/day, s.c. venlafaxine treatment (Beique, de Montigny et al. 2000). Therefore, the high dose, but not the low dose, of this drug decreased the responsiveness of the terminal 5-HT_{1B} receptor, which led to 5-HT reuptake blockade (Beique, de Montigny et al. 2000). This consequently enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors, whose activation are believed to play an extremely important role in the therapeutic antidepressant effect (Haddjeri, Blier et al. 1998; Beique, de Montigny et al. 2000).

1.5.5.3 *Dorsal Raphe 5-HT Neurons and Somatodendritic 5-HT$_{1A}$ Autoreceptors*

Dorsal raphe 5-HT neurons exhibited a mean habitual spontaneous firing rate of approximately 1 Hz, which was lowered by 47% following an acute (2-day) venlafaxine administration of 10 mg/kg/day, indicating 5-HT reuptake blockade (Beique, de Montigny et al. 2000). However, after 21-days the firing activity of these 5-HT neurons had completely recovered (Beique, de Montigny et al. 2000). Not only was the mean firing rate of these neurons suppressed following a 2-day treatment of venlafaxine at a low dose of 10mg/kg/day, but the somatodendritic 5-HT$_{1A}$ autoreceptors underwent a desensitization and the authors believed that this desensitization allowed for the full recovery of the firing activity of these dorsal raphe 5-HT neurons seen following long-term (21-day) administration of venlafaxine (Beique, de Montigny et al. 2000).

1.5.5.4 *Locus Coeruleus NE Neurons*

Spontaneously active locus coeruleus NE neurons displayed a mean habitual firing rate close to 2.5 Hz (Beique, de Montigny et al. 2000). An acute (2-day) treatment of venlafaxine (10 mg/kg/day) produced a significantly small firing reduction of 21%, while the long-term (21-day) regimen suppressed the firing activity of these neurons by 50%, and this suppression was significantly greater than that seen following the acute treatment of venlafaxine at this dose (Beique, de Montigny et al. 2000).

When the dose of venlafaxine was increased to 40 mg/kg/day, the firing activity of locus coeruleus NE neurons was significantly decreased by 61% following acute (2-day) administration (Beique, de Montigny et al. 2000). Results of neuronal firing suppression obtained following long-term (21-day) administration of venlafaxine at the high dose of 40 mg/kg/day revealed a 54% reduction in firing activity and were not significantly different than what was

found following acute venlafaxine administration at this dose (Beique, de Montigny et al. 2000). However, unlike α_2-adrenergic receptors on 5-HT neurons, α_2-adrenergic receptors on NE neurons do not undergo desensitization under these circumstances and therefore, when the NE transporter is blocked and the firing activity of these NE neurons is suppressed, there is no neuronal recovery (Blier 2006).

As neuronal firing suppression is indicative of monoamine reuptake blockade (De Montigny, Wang et al. 1980; Pineyro, Blier et al. 1994; Blier 2006), these results (21% vs. 61% neuronal firing activity suppression following acute, 2-day treatment of 10 and 40 mg/kg/day doses, respectively) demonstrated that NE reuptake was not very pronounced following a low dose (10 mg/kg/day) treatment of venlafaxine, however, inhibition of NE reuptake was distinctly evident at the high dose (40 mg/kg/day) treatment (Beique, de Montigny et al. 2000).

1.5.6 ***Concluding Remarks***

The dual reuptake inhibition of 5-HT and NE makes venlafaxine an intriguing and efficacious antidepressant. Indeed, it ranks among one of the highest efficacious antidepressants with a 22.3% probability of being therapeutically effective (Cipriani, Furukawa et al. 2009) (see Section 10) and studies have shown superior therapeutic benefits of SNRIs compared to SSRIs (Clerc, Ruimy et al. 1994; Poirier and Boyer 1999; Blier 2006; Nemeroff, Entsuah et al. 2008). However, venlafaxine does not block the reuptake of both monoamines with equal potencies and therefore, may be used as an SSRI at low doses and as an SNRI at high doses.

The low dose of venlafaxine (10 mg/kg/day in rats and 75mg/day in clinical therapy) only affects the 5-HT neuornal pathway, and therefore, acts as an SSRI when administered at these doses due to its sole function of inhibiting the reuptake of 5-HT. As the dose of venlafaxine increases (40 mg/kg/day in rats and

250 mg/day in clinical therapy), its mechanism of action diversifies and begins to significantly affect the NE system in conjuction with the 5-HT system by inhibiting the reuptake of both 5-HT and NE, thus “converting” this drug from an SSRI into an SNRI.

Section 6

Bupropion

1.6.1 *Introduction*

In 1981, a new and clinically effective compound with an "atypical" mechanism of antidepressant activity was discovered (Ferris, White et al. 1981; Ferris and Beaman 1983; Ferris, Cooper et al. 1983) and introduced in the United States for clinical trial in 1989 (Stahl, Pradko et al. 2004). This novel agent was called bupropion (2-tert-butylamino-3'-chloropropiophenone hydrochloride) and unlike currently available antidepressants at the time, did not significantly inhibit monoamine oxidase (MAO) in the brain (as seen in MAOIs) (Ferris, Cooper et al. 1983), nor did it appear to possess clinically significant pre- or post-synaptic serotonergic neurotransmission (as seen in SSRIs) (Ascher, Cole et al. 1995; Stahl, Pradko et al. 2004). The compound, unlike tricyclic antidepressants (TCAs), also did not have significant affinity for cholinergic, histaminergic, α- or β-adrenergic, serotonergic (5-HT_2), imipramine or nicotinic receptor binding sites in the rat brain (Raisman, Briley et al. 1979; Ferris and Beaman 1983; El Mansari, Ghanbari et al. 2008), thus categorizing bupropion as an "atypical" antidepressant. However, while many classify this agent as an "atypical" antidepressant with no clinically significant dopaminergic profile (El Mansari, Ghanbari et al. 2008), some (Stahl, Pradko et al. 2004) consider bupropion to be a norepinephrine-dopamine reuptake inhibitor (NDRI).

1.6.2 *Mechanism of Action*

As previously mentioned, the mechanism of action of bupropion is unlike that of other clinically available antidepressants whose activity contains, at the very least, a minor enhancement of monoaminergic functioning (Stahl, Pradko et al. 2004). Although bupropion does enhance monoaminergic function, it nor its

metabolites (hydroxybupropion, threohydrobupropion, and erythrohydrobupropion) do not significantly affect postsynaptic serotonergic neurotransmission (Stahl, Pradko et al. 2004), while also demonstrating a low affinity profile for the 5-HT transport system (El Mansari, Ghanbari et al. 2008). In other words, unlike other clinically available antidepressants, the antidepressant effects observed in bupropion are not directly mediated by serotonin. The primary mechanism of action of bupropion is believed to be noradrenergically-mediated as it acts via NE neurons in the LC, which in turn indirectly increase 5-HT neuronal firing activity (Cooper, Wang et al. 1994; Ascher, Cole et al. 1995; Dong and Blier 2001; El Mansari, Ghanbari et al. 2008). Further, and highly debatable, bupropion was shown to have a very low affinity for DA transporters and did not significantly inhibit the reuptake of DA in rats (El Mansari, Ghanbari et al. 2008).

It is important to note however, that a clinical review by Stahl in 2004 (Stahl, Pradko et al. 2004) suggested that bupropion acted as a dual norepinephrine-dopamine reuptake inhibitor (NDRI) in humans and increased DA neurotransmission in the nucleus accumbens and prefrontal cortex while displaying no clinically significant effects on the serotonergic pathway. These discrepancies will be discussed.

1.6.3 *Short Term (2-day) Administration*

Although no dose of bupropion was shown to have a direct effect on the firing activity of 5-HT in the dorsal raphe nucleus (Cooper, Wang et al. 1994), it has been shown to indirectly increase 5-HT firing activity via it's primary mode of action on norepinephrine (NE) neurons in the locus coeruleus (LC) (Dong and Blier 2001).

Male Sprague-Dawley rats (250 – 300g) were anaesthetised with chloral hydrate (400mg/kg, i.p.) in order to measure the spontaneous firing activity of NE, 5-HT and dopamine (DA) neurons in the brain following subcutaneous (SC) implantation of a mini-pump delivering bupropion at a maximal dose of 30 mg/kg

for 2 days (Dong and Blier 2001). Following a 2-day administration of 30mg/kg of bupropion, the mean spontaneous firing activity of NE neurons in the LC had significantly decreased by 80% when compared to controls (Dong and Blier 2001). Concomitantly, the mean firing activity of 5-HT in the dorsal raphe was twice as high in this group (30mg/kg/day bupropion, 2-days) than controls (Dong and Blier 2001). When DSP-4 (a neurotoxin with a highly significant affinity for noradrenergic neurons in the rat hippocampus and cortex, see List of Abbreviations and Definitions) was administered, there was no significant difference in the mean firing activity of 5-HT neurons in these NE-lesioned rats when compared to controls, indicating indirect 5-HT neuronal activity in the dorsal raphe via NE neurons in the LC (Dong and Blier 2001). It was also found that somatodendritic 5-HT_{1A} autoreceptors underwent desensitization while α_2-adrenergic autoreceptors were over-activated following 2-day administration (El Mansari, Ghanbari et al. 2008). When the dose of bupropion was decreased to 15 mg/kg/day, the mean spontaneous firing activity of NE neurons was 0.77 ± 0.08 Hz and when the dose of bupropion was further decreased to 7.5mg/kg/day, the mean spontaneous firing activity of NE neurons was increased to 1.70 ± 0.16 Hz (Dong and Blier 2001). These results expanded upon, and further validated, previous findings (Ascher, Cole et al. 1995) that acute doses of bupropion not only dose-dependently reduced the firing activity of NE neurons in the LC, but that this atypical antidepressant did not directly modify the firing activity of dorsal raphe 5-HT neurons (Dong and Blier 2001). It was also found that this atypical antidepressant had no significant effect on the firing activity of dopaminergic neurons in the ventral tegmental area (VTA) (Dong and Blier 2001), leading to discrepancies in the literature regarding dopamine's role in the mechanism of action of bupropion.

1.6.4 *Dopamine Discrepancies*

In 1994, Cooper et al. (Cooper, Wang et al. 1994) provided evidence that the mechanism of action of bupropion was not only mediated by NE, but by DA

as well. However, the IC_{50} dose (the half maximal inhibitory concentration; see List of Abbreviations and Definitions) of bupropion needed to significantly inhibit the firing activity of DA neurons was 42mg/kg, which was more than three times higher than the IC_{50} dose of bupropion (13mg/kg) needed to significantly inhibit the firing activity of NE neurons in the LC, leading the authors to suggest that dopamine was not clinically relevant to bupropion's antidepressant mechanism of action (Cooper, Wang et al. 1994). This conclusion was further validated one year later when Ascher et al. (Ascher, Cole et al. 1995) also found bupropion to reduce the firing activity of dopaminergic neurons in brain areas A9 and A10 but at doses higher than the dose required to reduce the firing activity of NE neurons in the LC. The authors suggested that bupropion may have activated the central nervous system via dopaminergic mechanisms and therefore, DA may have played a role in the overall antidepressant effects of this drug (Ascher, Cole et al. 1995). Microdialysis studies conducted in the hippocampus, hypothalamus, nucleus accumbens and frontal cortex of rats revealed that at clinically relevant doses, bupropion increased extracellular concentrations of NE and DA in these areas of the brain (Nomikos, Damsma et al. 1992; Li, Perry et al. 2002; Piacentini, Clinckers et al. 2003; El Mansari, Ghanbari et al. 2008). Human in vitro studies later concluded bupropion inhibited the reuptake of both NE and DA at clinically relevant doses "without affecting release or transport of other neurotransmitters and without binding to other neurotransmitter receptors" (Stahl, Pradko et al. 2004). Later studies however, disputed this and concluded that "bupropion is not an effective dopamine reuptake inhibitor" (El Mansari, Ghanbari et al. 2008). This study by El Mansari et al. in 2008 was the first in vivo electrophysiological study to observe long-term effects of bupropion on the firing activity of 5-HT, NE and DA neurons (El Mansari, Ghanbari et al. 2008), and will be reviewed in the next section.

1.6.5 *Long Term (14-day) Administration*

Many of the previous studies were based on short-term bupropion administration, rendering the long-term effects of this drug on these three monoamines (5-HT, NE and DA) unknown. The following study (El Mansari, Ghanbari et al. 2008) was undertaken to observe the long-term effects of this atypical antidepressant, since it's administration and onset of action are longer than 2 days.

1.6.5.1 *5-HT Firing Activity*

Using the previously reviewed 2001 acute bupropion study (Dong and Blier 2001) as a template, El Mansari et al. (El Mansari, Ghanbari et al. 2008) were interested in observing the effects of long-term administration of bupropion on the spontaneous firing activity of dorsal raphe nucleus (DRN) 5-HT, LC NE and VTA DA neurons. Similar to the 2001 acute bupropion study (Dong and Blier 2001), 2-day subcutaneous (s.c.) administration of bupropion at 30mg/kg/day resulted in a mean firing rate of DRN 5-HT neurons (2 Hz) that was significantly doubled when compared to controls (1 Hz), resulting in a 100% increase in firing activity (El Mansari, Ghanbari et al. 2008). Following 7- and 14- days administration of bupropion (30mg/kg/day, s.c.), the mean firing activity of these DRN 5-HT neurons remained significantly increased (by 80%) when compared to controls (El Mansari, Ghanbari et al. 2008).

1.6.5.2 *NE Firing Activity*

It was found that following 2-days of bupropion administration (30mg/kg/day, s.c.), α_2-adrenergic autoreceptors became over-activated and significantly decreased the mean firing rate of NE neurons by 46% when compared to controls (El Mansari, Ghanbari et al. 2008). After 7-days, there was a partial recovery of these neurons, but the mean firing activity of these NE neurons

was still significantly decreased (by 78%) when compared to controls (El Mansari, Ghanbari et al. 2008). However, following 14-days of bupropion administration (30mg/kg/day, s.c.), there was a complete recovery in the mean firing activity of these NE neurons compared to controls (El Mansari, Ghanbari et al. 2008). It was also found that following a 14-day treatment with bupropion (30mg/kg/day, s.c.), α_2-adrenergic autoreceptors on NE cell bodies in the LC underwent desensitization (El Mansari, Ghanbari et al. 2008).

1.6.5.3 *DA Firing Activity*

Contrary to previous findings, bupropion (30mg/kg/day, s.c.) had no significant effect on the mean spontaneous firing activity of DA neurons in the VTA following 2-, 7- or 14- day administration (El Mansari, Ghanbari et al. 2008).

1.6.6 *Combination of Bupropion with SSRIs and SNRIs*

Serotonergically-mediated antidepressants such as SSRIs and SNRIs (venlafaxine) activate postsynaptic 5-HT_{2A} receptors which, when activated, are believed to greatly contribute to antidepressant-associated sexual dysfunction (Nutt 1997; Coleman, Cunningham et al. 1999; Zisook, Rush et al. 2006). Therefore, bupropion may be prescribed in combination with an SSRI or SNRI in order to alleviate sexual dysfunction associated with SSRI or SNRI (venlafaxine) therapy (Zisook, Rush et al. 2006).

It has been reported that bupropion given in combination with an SSRI or SNRI has significant clinical efficacy in not only counteracting sexual dysfunction side-effects associated with serotonergic antidepressant agents (Zisook, Rush et al. 2006), but when combined with citalopram (an SSRI) also improves the antidepressant response compared to switching to SSRI or SNRI monotherapy

(Lam, Hossie et al. 2004). (For a review of bupropion combination with an SSRI or SNRI, please see Section 11).

1.6.7 ***Concluding Remarks***

Although there is still much to be elucidated concerning the mechanism of action of this "atypical" antidepressant, numerous studies have begun to shed light on how bupropion exerts its antidepressant effect. There is evidence demonstrating that 5-HT neuronal activity in the DRN is significantly increased following 2-, 7- and 14-day treatment with bupropion (30mg/kg/day, s.c.) while also desensitizing somatodendritic 5-HT_{1A} autoreceptors (El Mansari, Ghanbari et al. 2008). 5-HT_{1A} autoreceptors possess negative feedback characteristics and as a result of their desensitization, the inhibitory qualities of these autoreceptors are removed and the firing activity of these DRN 5-HT neurons is restored (El Mansari, Ghanbari et al. 2008). 5-HT_{1A} autoreceptor desensitization after only 2-days of bupropion is important since these autoreceptors undergo desensitization after 14-days of treatment with an SSRI, leading one to postulate that the onset of antidepressant action of bupropion may be much faster compared to SSRIs (El Mansari, Ghanbari et al. 2008). However, caution is warranted not to believe that this implies 5-HT neurotransmission to be increased in postsynaptic neurons during this time period as 5-HT transporters and terminal 5-$HT_{1B/D}$ autoreceptors also influence serotonin transmission (El Mansari, Ghanbari et al. 2008).

At the same time, it was also shown that following 14-days of bupropion administration (30mg/kg/day, s.c.), α_2-adrenergic autoreceptors on NE cell bodies in the LC underwent desensitization (El Mansari, Ghanbari et al. 2008). This desensitization after 14-days came after α_2-adrenergic autoreceptors were over-activated following 2-days of bupropion administration (30mg/kg/day, s.c.) (El Mansari, Ghanbari et al. 2008). It was observed that over the course of 14-days, the over-activated α_2-adrenoceptors became desensitized synchronously with the gradual and complete recovery of NE neuron firing activity from 2-days (short-

term administration) to 14-days (long-term administration) (El Mansari, Ghanbari et al. 2008). These observations led the authors to hypothesize that long-term administration of bupropion increases the synaptic concentration of NE via a "sustained increase in NE neurotransmission" (El Mansari, Ghanbari et al. 2008) and this, along with the rapid increase in 5-HT neuronal firing activity, may be the pivotal mechanism by which this atypical antidepressant "exerts its delayed therapeutic effect in depression" (El Mansari, Ghanbari et al. 2008).

The in vivo electrophysiological study (El Mansari, Ghanbari et al. 2008) also found that 2-, 7- or 14-day bupropion administration (30mg/kg/day, s.c.) had no significant effect on the firing activity of VTA DA neurons (El Mansari, Ghanbari et al. 2008). As previously mentioned, the dose of bupropion needed to inhibit the firing activity of DA neurons was more than three times the dose of bupropion needed to inhibit the firing activity of NE neurons (Cooper, Wang et al. 1994), indicating that at clinically relevant doses, bupropion does not have a significant dopaminergic effect (El Mansari, Ghanbari et al. 2008).

Section 7

Noradrenergic and Specific Serotonergic Antidepressant (NaSSA)

Mirtazapine

1.7.1 *Introduction to Mirtazapine Mechanism of Action*

Traditional antidepressants are generally enzyme or reuptake inhibitors, such as monoamine oxidase inhibitors (MAOIs) or selective serotonin reuptake inhibitors (SSRIs). Mirtazapine, however, is a dual-acting receptor-blocking antidepressant that is a noradrenergic and specific serotonergic antidepressant (NaSSA). This agent has α_2-receptor antagonistic properties, affects both norepinephrine (NE) and serotonin (5-HT) systems in the central nervous system (CNS), and does not possess monoamine (eg. serotonin) or catecholamine (eg. norepinephrine) reuptake properties (Haddjeri, Blier et al. 1997). Unlike the tricyclic antidepressants (TCAs), mirtazapine does not have anticholinergic or cardiovascular effects, decreasing the potential for unwanted side effects such as blurred vision, dry mouth, constipation, vertigo, hypotension, palpitations and/or tachycardia (Nutt 1997).

Mirtazapine is a racemic mixture of S(+)- and R(-)-1,2,3,4,10,14b-hexahydro-2-methylpyrazino-[2,1-a]-pyrido[2,3-c][2]benzazepine (Fawcett and Barkin 1998) and both enantiomers are pharmacologically active; the former is responsible for the majority of mirtazapine's pharmacological profile (Holm and Markham 1999).

1.7.2 α_2-adrenoceptors

1.7.2.1 α_2-adrenergic Autoreceptors

Evidence suggests that mirtazapine acts primarily as an antagonist on inhibitory presynaptic α_2-adrenergic autoreceptors at two main sites; at the axon terminal, which causes an increase in the amount of norepinephrine released into the synaptic cleft, while in the cell body, cell firing and synthesis of neurotransmitter are greatly augmented (Nutt 1997). As a result, although there is an increased availability of postsynaptic norepinephrine, reuptake inhibition of norepinephrine by mirtazapine does not occur (Nutt 1997).

Mirtazapine also indirectly increases serotonergic transmission via presynaptic α_2-adrenergic autoreceptors on noradrenergic nerve terminals (Nutt 1997). Studies have shown that in the dorsal raphe nucleus (DRN), which innervates limbic areas of the brain implicated in depression such as the dorsolateral prefrontal cortex (DLPFC) and hippocampus (HC), and where the 5-HT system originates (Lopez-Figueroa, Norton et al. 2004), noradrenergic nerve terminals innervate serotonergic cell bodies (Haddjeri, Blier et al. 1995). The blockade of inhibitory α_2-adrenergic autoreceptors at these noradrenergic nerve terminals causes an increase of norepinephrine to be released (Nutt 1997). Norepinephrine then binds to postsynaptic α_1-adrenoceptors located on DRN serotonergic cell bodies and dendrites, stimulating an increase in 5-HT cell firing (Haddjeri, Blier et al. 1998). The firing activity of 5-HT neurons in the dorsal raphe is inhibited by noradrenergic-lesioned rats, indicating that this effect is indeed mediated through noradrenergic neurons (Haddjeri, Blier et al. 1998). Mirtazapine has a low affinity for these postsynaptic α_1-adrenoceptors, and therefore, does not compete with the actions of norepinephrine (Haddjeri, Blier et al. 1998). The increased serotonergic cell firing enhances the release of serotonin at the axon terminal and into the limbic areas of the brain (Nutt 1997).

1.7.2.2 *α_2-adrenergic Heteroreceptors*

Additionally, these previously mentioned presynaptic 5-HT neurons also contain inhibitory adrenergic α_2-heteroreceptors at their nerve terminals and must not be overlooked as they also impact the release of 5-HT into the synaptic cleft. Since α_2-adrenoceptors are heterotrimeric G-protein-coupled membrane receptors (Gilman 1987), they have the ability to promptly stimulate an effector system (Maze and Tranquilli 1991). When these inhibitory α_2-heteroreceptors are activated in 5-HT-containing neurons (by endogenous noradrenaline or NE, for example), they attenuate the electrically evoked release of 5-HT from the nerve terminals (Gothert and Huth 1980; Blier, Galzin et al. 1990; Mongeau, Blier et al. 1993), resulting in a decreased release of 5-HT. However, when these α_2-adrenergic heteroreceptors are hindered by an α_2-adrenergic heteroreceptor antagonist, the amount of 5-HT released into the synapse increases. Furthermore, it must be noted that the inhibitory activation of α_2-adrenergic heteroreceptors on 5-HT nerve terminals is *independent* of the synaptic concentration of 5-HT, whereas the activation of 5-HT autoreceptors on 5-HT nerve terminals is *dependent* on the synaptic concentration of 5-HT, which is determined by the frequency of 5-HT neuronal depolarization (Blier, Ramdine et al. 1989).

Mirtazapine, having similarly high affinity for both α_2-autoreceptors and α_2-heteroreceptors, pK_i 7.7 and 8.0, respectively, antagonizes presynaptic α_2-heteroreceptors at the 5-HT nerve terminal resulting in increased release of serotonin (Anttila and Leinonen 2001). It has been shown that the R(-)-enantiomer of mirtazapine is selective for these α_2-heteroreceptors (Haddjeri, Blier et al. 1995).

1.7.3 *Serotonergic (5-HT) Receptor Subtypes*

1.7.3.1 *5-HT_2 and 5-HT_3 Receptors*

The affinity of mirtazapine for 5-HT receptors is highest for 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptor subtypes, pK_i 8.2, 7.9, 8.1, respectively, where it acts as an antagonist (Anttila and Leinonen 2001). The affinity of serotonin for the 5-HT_{1A} receptor is much higher than that for mirtazapine, pK_i 8.4 and 5.3, respectively (van Wijngaarden, Tulp et al. 1990; Anttila and Leinonen 2001), and therefore, mirtazapine does not compete with the binding of serotonin to this receptor. The S(+)-enantiomer of mirtazapine is responsible for its antagonistic effects on 5-HT_{2A} and 5-HT_{2C} receptors while the R(-)-enantiomer is responsible for its antagonistic effects on 5-HT_3 receptors (de Boer 1996; Fawcett and Barkin 1998). 5-HT_{2A} receptors are found predominately in deep layers of the neocortex in humans (Burnet, Eastwood et al. 1995), while 5-HT_{2C} receptors are mainly concentrated in the choiroid plexus (Nutt 1997).

In 1987, Idzikowsi et al. (Idzikowski, Cowen et al. 1987) showed that 5-HT_2 receptor antagonists, such as ritanserin, are capable of reducing anxiety and increasing the duration of slow-wave sleep. When taken at bedtime, fixed incremental treatments of mirtazapine beginning at 30mg/day doses, and not 15mg/day, were found to not only improve sleep initiation, but also prolonged sleep duration while receiving equivalent tolerability when compared to those who began the fixed incremental treatments at 15mg/day (Radhakishun, van den Bos et al. 2000).

Most SSRI antidepressants activate 5-HT_2 receptors, which may lead to sexual dysfunction such as ejaculatory or orgasmic delay (Nutt 1997; Coleman, Cunningham et al. 1999). To combat this side effect, many psychiatrists will also prescribe a 5-HT_2 receptor blocker. However, as mirtazapine already possesses 5-HT_2 antagonistic properties in the brain, sexual side effects are rarely reported (Fawcett and Barkin 1998).

5-HT_3 receptors have been shown to play a role in gastrointestinal functioning and 5-HT_{3A} subunits are expressed in the myenteric plexus ganglia of the human intestine (Bottner, Bar et al. 2010), while specific antibodies for both 5-HT_{3A} and 5-HT_{3B} receptor subtypes have confirmed their existence in the human hippocampus (Brady, Dover et al. 2007). 5-HT_3 receptors are also found in the vagus nerve, are the only 5-HT receptor belonging to the Cys-loop superfamily of ligand-gated pentameric ion channels (Walstab, Rappold et al. 2010) (5-HT_{1A} and 5-HT_2 are transmembrane, helical receptors), and when activated, cause a rapid influx of sodium into the cell which depolarizes the neuron (Nutt 1997). This, in turn, stimulates the vagus nerve and may mediate the nauseous side-effects seen during 5-HT_3 receptor stimulation (Nutt 1997). Further, activation of 5-HT_{3A} receptors in the human intestine may increase gastrointestinal motility, producing an "upset stomach" and diarrhea (Nutt 1997). As a result, 5-HT_3 antagonists, such as mirtazapine, have less adverse gastrointestinal and nauseating side effects than other antidepressants affecting serotonin.

A study examining human gene variations found that a single-nucleotide polymorphism in the HTR3B gene was responsible for a high frequency variation of the 5-HT_{3B} subunit, 5-HT_{3B}(Y129S), of the 5-HT_{3AB} receptor within the general population (Krzywkowski, Davies et al. 2008). Individuals who carry the Y129S allele show differences in neurotransmitter release, while females with major depression appear to have a haplotype block of the Y129S polymorphism in the HTR3B gene (Krzywkowski, Davies et al. 2008). It has been suggested that the 5-HT_{3B}(Y129S) polymorphism may make individuals less susceptible to depression (Yamada, Hattori et al. 2006). Thus, the HTR3B gene provides encouraging therapeutic potential for depression at the genomic level.

1.7.3.2 *5-HT$_{1A}$ Receptor*

Serotonin released by presynaptic 5-HT nerve terminals causes the exclusive enhancement of this monoamine (de Boer 1996) and activation of postsynaptic 5-HT$_{1A}$ receptors is believed to be of paramount importance in alleviating depressive symptoms by 5-HT-acting antidepressant treatments (Blier, Bergeron et al. 1997; Richer, Hen et al. 2002). As previously mentioned, the affinity of mirtazapine for the 5-HT$_{1A}$ receptor is much lower than that for serotonin, pK_i 5.3 and 8.4, respectively, and therefore, does not interfere with the activation of postsynaptic 5-HT$_{1A}$ receptors (van Wijngaarden, Tulp et al. 1990; Anttila and Leinonen 2001). 5-HT$_{1A}$ mRNA expression in the DLPFC and HC of depressed patients was found to be significantly lower than normal controls (Lopez-Figueroa, Norton et al. 2004) and in humans, 5HT$_{1A}$ mRNA are abundantly high in raphe neurons, the CA1 region of the hippocampus and in superficial layers of the neocortex (Burnet, Eastwood et al. 1995). As previously mentioned, both the DLPFC and HC have been found to play important roles in affecting mood (Lopez-Figueroa, Norton et al. 2004).

1.7.3.3 *Summary: Mirtazapine and 5-HT receptor subtypes*

The antagonist effects of mirtazapine on 5-HT$_{2A}$, 5-HT$_{2C}$ and 5-HT$_3$ receptor subtypes improve its side effect profile over other 5-HT-acting drugs, while its ability to increase postsynaptic 5-HT$_{1A}$ transmission greatly contributes to its antidepressant profile.

1.7.4 *Histamine H_1 Receptor*

Histamine is a monoamine neurotransmitter responsible for physiological activity of the immune, gastrointestinal, and peripheral and central nervous systems (Widmaier, Raff et al. 2005). Histaminergic neurons are located within the tuberomamillary nucleus of the posterior hypothalamus and are responsible for

transmitting histamine to numerous brain regions (Tashiro, Mochizuki et al. 2002). Through histamine H_1 receptors in the central nervous system, histamine plays a role in a variety of functions including the regulation of the sleep-wake cycle and the H_1 receptor is thought to be responsible for the drowsy and sedative side effects of several antidepressants, including some TCAs (Stahl 2000). Histamine binding to postsynaptic H_1 receptors activates phosphatidyl inositol via a G-protein-linked second messenger system which then leads to activation of the cFOS transcription factor, causing wakefulness and cognitive alertness (Stahl 2000). H_1 receptor antagonists in the brain, sometimes referred to as antihistamines, block the activation of phosphatidyl inositol and consequently, the activation of the cFOS transcription factor, leading to sedation and drowsiness. The affinity of mirtazapine for H_1 receptors is very high (pK_i 9.3) and acts as an antagonist, thus explaining its somnolent side effects (Stahl 2000).

Although the mechanism of action is not currently well understood, antagonism of H_1 receptors is also believed to be responsible for weight gain, a common side effect of numerous antidepressants, including mirtazapine (Stahl 2000). In fact, significant increases in weight have been reported after just one week of treatment with mirtazapine (Kraus, Haack et al. 2002).

1.7.5 *Acute vs. Long-Term Administration*

The physiological effects of mirtazapine on neurotransmitter release occur far ahead of any antidepressant activity. In fact, understanding the delayed onset of action for all antidepressant drugs remains a major limitation for improving antidepressant efficacy. While one must be cautious when extrapolating animal results for human benefit, studies conducted on male Sprague-Dawley rats have provided invaluable knowledge into possible explanations for mirtazapine's antidepressant activity (Haddjeri, Blier et al. 1998)(a).

1.7.5.1 *Acute (2-day) Administration*

Clonidine, an α_2-adrenergic (α_2-adrenoceptor) agonist, inhibits the firing of norepinephrine neurons in the locus coeruleus (LC) while concomitantly, and indirectly, inhibiting the firing of 5-HT neurons in the DRN (Svensson, Bunney et al. 1975). As previously mentioned, mirtazapine enhances the endogenous release of 5-HT by blocking adrenergic α_2-heteroreceptors at 5-HT nerve terminals. In rat studies, it has also been shown to prevent both low (10 μg/kg, i.v.) and high dose (100 μg/kg, i.v.) effects of clonidine, revealing the antagonistic properties of mirtazapine on presynaptic adrenergic α_2-auto- and heteroreceptors during acute administration (Haddjeri, Blier et al. 1998)(a). Much of the previous discussion has alluded to acute, presynaptic mirtazapine mechanism of action. However, one must not neglect its effect on postsynaptic neurons. Studies in rats have demonstrated that an acute administration of mirtazapine results in inhibition of CA_3 dorsal hippocampus pyramidal neurons by NE, providing evidence for mirtazapine's antagonistic effect on postsynaptic α_2-adrenoceptors (Haddjeri, Blier et al. 1998)(a,b).

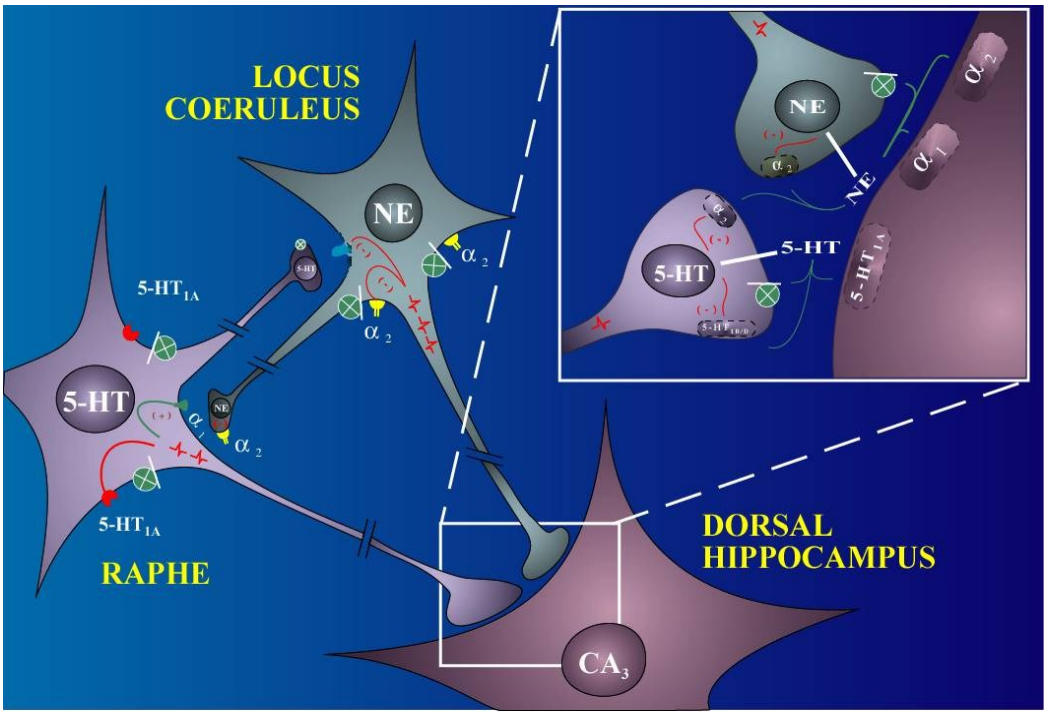
1.7.5.2 *Long-Term (21-day) Administration*

Antidepressant treatment is unfortunately not a quick solution and patients taking antidepressant medications do so for extended periods of time. Therefore, it is imperative to understand long-term effects of any, and all, antidepressant drugs in order to maintain efficacy and safety. Unfortunately, literature on the long-term mechanism of action of mirtazapine has been restricted to studies in rats, but still provides invaluable insight into its long-term effects.

To mimic long-term administration in humans, 21-day mirtazapine treatment via an osmotic minipump in rats was conducted in 1998 (Haddjeri, Blier et al. 1998)(a). These tests revealed an increase in the firing activity of NE neurons in the LC as well as increased firing activity of DRN 5-HT neurons, effects that were both abolished 48-hours after removal of the osmotic minipump

(Haddjeri, Blier et al. 1998)(a). This indicates the ability of mirtazapine to maintain blockage of α_2-adrenergic autoreceptors on NE neurons that project to 5-HT neurons in the DRN (Haddjeri, Blier et al. 1998)(a). The increase in NE also caused the desensitization of α_2-adrenergic heteroreceptors on 5-HT terminals, increasing the synaptic release of 5-HT (Haddjeri, Blier et al. 1998)(a). This inactivation of α_2-adrenergic heteroreceptors caused a sustained increase in the firing activity of 5-HT neurons in the dorsal hippocampus, resulting in tonic activation of postsynaptic $5HT_{1A}$ receptors (Haddjeri, Blier et al. 1998)(a). These alterations may be of paramount importance in contributing to the antidepressant efficacy of mirtazapine (Haddjeri, Blier et al. 1998)(a).

1.7.5.3 *Figure 1-2: Summary of the Mechanism of Action of Mirtazapine*



1.7.5.3 Figure 1-2: Mirtazapine acts mainly as an antagonist for α_2-adrenergic auto- and hetero-receptors, as well as possessing antagonistic properties for serotonergic 5-HT_2, 5-HT_3 and histamine H_1 receptors. At presynaptic α_2-adrenergic autoreceptors on locus coeruleus NE neurons, the antagonistic properties of mirtazapine not only allows for release of norepinephrine into the synapse, but also indirectly aids in the release of 5-HT by NE binding to α_1-adrenoceptors located at 5-HT neuron cell bodies in the dorsal raphe. This, in turn, stimulates the dorsal raphe 5-HT-containing neuron, releasing serotonin into the synaptic cleft where it binds to 5-HT_{1A} receptors located on postsynaptic CA_3 dorsal hippocampus pyramidal neurons, a hallmark aspect for antidepressant effects (Blier, Bergeron et al. 1997). The release of serotonin is further enhanced by mirtazapine binding to α_2-heteroreceptors at the 5-HT nerve terminal on dorsal raphe 5-HT-containing neurons, which also releases serotonin into the synaptic cleft, allowing for 5-HT to bind to 5-HT_{1A} receptors on postsynaptic CA_3 dorsal hippocampus pyramidal neurons. The affinity of mirtazapine for 5-HT_2 and 5-HT_3 receptors is higher than its affinity for 5-HT_{1A} receptors, allowing for mirtazapine to antagonize the former receptors, improving upon the side effects usually associated with 5-HT_2 and 5-HT_3 activation. Terminal 5-$HT_{1B/D}$ autoreceptors on dorsal raphe 5-HT-containing neurons also influence serotonin transmission, however, mirtazapine has a low affinity for these receptors, pK_i 4.9 and 5.3, respectively (Anttila and Leinonen 2001), and therefore, does not bind to them. The circles with a white "X" represent serotonin transporters (SERTs). Figure provided with courtesy from Dr. Gabriella Gobbi.

Section 8

Serotonin-2 (5-HT_2) Antagonist and Reuptake Inhibitors (SARIs)

Trazodone

1.8.1 *Introduction*

In its most simplified explanation, the mechanisms of action of serotonin-2 (5-HT_2) antagonist and reuptake inhibitors (SARIs), such as trazodone, are quite similar to those of the selective serotonin reuptake inhibitors (SSRIs) (please see Section 4) (Stahl 1998). However, the main therapeutic difference between these antidepressants rests in their interaction with postsynaptic 5-HT_{2A} and 5-HT_{2C} receptors, where SARIs function as antagonists and SSRIs do not (Stahl 1998; Stahl 2009). 5-HT reuptake inhibition by SSRIs increases the synaptic concentration of serotonin, allowing for 5-HT to bind to not only postsynaptic 5-HT_{1A} receptors required to mediate the antidepressant response (Haddjeri, Blier et al. 1998), but also bind to postsynaptic 5-HT_{2A} and 5-HT_{2C} receptors, which cause adverse side effects such as anxiety and sexual dysfunction (Stahl 2009). 5-HT reuptake inhibition by SARIs also increases the synaptic concentration of serotonin, but because of the 5-HT_{2A} and 5-HT_{2C} antagonistic properties of this agent, 5-HT is only able to bind to 5-HT_{1A} receptors, thus mediating the antidepressant response and inhibiting the adverse events seen with SSRIs (Stahl 2009). While trazodone has the ability to block 5-HT reuptake and is a potent antagonist of 5-HT_{2A} receptors, it also has moderate antagonistic affinities for α_1-adrenergic receptors, making it a dose-dependent multifunctional pharmacologic agent (Stahl 2009).

1.8.2 *Mechanism of Action*

1.8.2.1 *Low Dose Hypnotic*

At low doses of trazodone (25 – 150mg/day), this SARI functions as a hypnotic. This is because its antagonistic affinities for 5-HT_{2A} and α_1-adrenergic receptors are much higher than its affinity for the serotonin transporter (SERT) responsible for 5-HT reuptake and contributing to the antidepressant effect, allowing this drug to bind to these receptors at doses too small to have any effect on SERTs (Stahl 2009). In fact, the ability of trazodone to bind to and antagonize 5-HT_{2A} receptors is 100 times more potent than its ability to bind to SERTs and inhibit 5-HT reuptake (Stahl 2009). Therefore, these low doses of trazodone (50mg/day) will fully saturate 5-HT_{2A} receptors and almost completely saturate α_1-adrenergic receptors before saturating SERTs (Stahl 2009). SERTs must almost be completely saturated in order for there to be any therapeutic antidepressant effects (Stahl 2009). Because trazodone acts as an antagonist of 5-HT_{2A} and α_1-adrenergic, the adverse events associated with their activation, such as anxiety and sexual dysfunction, are non-existent (Stahl 2009). Also, due to its 5-HT_{2A} antagonism, trazodone may be combined with serotonergic agents, such as SSRIs and SNRIs, to combat the sexual dysfunction side effects associated with 5-HT_{2A} receptor activation by SSRIs and SNRIs (Stahl 2009). Although trazodone has not been approved as a sleeping agent at low doses by the Food and Drug Administration (FDA) in the United States, it remains one of, if not, the most frequently prescribed off-label hypnotics in that country (Stahl 2009).

1.8.2.2 *High Dose Antidepressant*

While trazodone has not been approved by the FDA as a low dose hypnotic, it has been approved as an antidepressant at higher doses (150 – 600mg/day) (Stahl 2009). As previously mentioned, its antidepressant mechanism

of action is similar to that of SSRIs or SNRIs (Stahl 1998), but with the added benefit of acting as an antagonist at 5-HT_{2A} and 5-HT_{2C} postsynaptic receptors, therefore alleviating the adverse effects such as anxiety and sexual dysfunction associated with the activation of these 5-HT_2 receptors (Stahl 2009).

The mechanism of action of these agents to increase the synaptic concentration of 5-HT is cyclic, and therefore, this overview will begin the cycle at the somatodendritic 5-HT_{1A} autoreceptor located on the cell body of a presynaptic dorsal raphe nuclei (DRN) 5-HT neuron in the midbrain raphe. 5-HT_{1A} autoreceptors have a negative feedback mechanism and regulate the firing activity of 5-HT-containing neurons. An increased release of 5-HT into the synaptic cleft will cause 5-HT to bind to the somatodendritic 5-HT_{1A} autoreceptors, activating the inhibitory mechanism of the receptor and initiating negative feedback control. Consequently, the immediate (or acute) response of the 5-HT-containing neuron is to reduce neuronal firing activity in an attempt to restore a homeostatic amount of 5-HT released into the synaptic cleft. However, with sustained administration of an SSRI (minimum 14-days) on terminal 5-HT autoreceptors (explanation to follow), extracellular 5-HT increases and with the constant bombardment of 5-HT on 5-HT_{1A} autoreceptors, these receptors undergo modification and become desensitized to 5-HT, failing to habitually respond to this monoamine. As a result of this desensitization, the 5-HT-containing neuron is "tricked" into believing there is now an insufficient amount of 5-HT in the synaptic cleft, thus restoring the firing activity of the neuron to levels required for 5-HT release from the terminus of 5-HT-containing neurons.

Terminal 5-HT autoreceptors, like their somatodendritic 5-HT autoreceptor counterpart, are inhibitory receptors that have a negative feedback control system and regulate the release of 5-HT into the synaptic cleft. If 5-HT from the synapse binds to these (inhibitory) terminal 5-HT autoreceptors, their negative feedback control will "believe" there to be a sufficient amount of 5-HT in the synaptic cleft and consequently inhibit 5-HT from being released.

Acute administration (2-days) of 5-HT reuptake inhibitors will bind to these terminal 5-HT autoreceptors and produce an initial decrease in the release of 5-HT, similar to the way SSRIs achieve this result. However, repeated administration of these serotonergically-mediated agents (at least 14-days) will cause adaptive changes and reduce the function (become desensitized) of these inhibitory terminal 5-HT autoreceptors, halting the negative feedback control mechanism and allowing 5-HT to once again be released into the synaptic cleft.

This 5-HT can then bind to postsynaptic 5-HT receptors, such as the 5-HT_{1A} receptors which are responsible for antidepressant effects (Cipriani, Furukawa et al. 2009), and also to 5-HT_2 receptors, which are responsible for anxiety and sexual side effects. While the antagonistic properties of SARIs block 5-HT from binding to 5-HT_2 receptors, SSRIs and SNRIs do not block these postsynaptic 5-HT_2 receptors, allowing the serotonin in the synapse to bind and activate these receptors.

As SERTs must be at least 70% - 80% saturated in order for there to be any antidepressant effects (Stahl 2009), these high doses are required for trazodone to adequately saturate the SERTs and bind to 5-HT_{2C} receptors since their affinities for trazodone are much lower than the affinities for trazodone of 5-HT_{2A} and α_1-adrenergic receptors (Stahl 2009). The ability of trazodone to block postsynaptic 5-HT_{2C} receptors, which when activated by 5-HT cause anxiety, insomnia and sexual dysfunction, are another reason why this SARI at high doses may be prescribed in combination with an SSRI or SNRI (Stahl 2009). Therefore, the synergistic antidepressant effects of these combinations must further be explored.

**Please note that although many psychiatrists prescribe the SARI trazodone as an off-label hypnotic, because it has been approved by the FDA as an antidepressant (at high doses ranging between 150 – 600mg/day) and not as a low dose hypnotic (Stahl 2009), this thesis will classify trazodone only as an antidepressant.*

Section 9

Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Study

1.9.1 *Introduction*

Prior to 2001, there had been no large-scale clinical trial(s) conducted to effectively measure subsequent treatment options for persons suffering from major depressive disorder (MDD) who did not remit, or respond, after the primary treatment had failed. From July 2001 until April 2004, an American consortium of 23 psychiatric and 18 primary care clinics overseen by 14 university-based centres (Gaynes, Rush et al. 2008) collectively conducted and participated in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, coordinated by the Principal Investigator, A. John Rush, MD, and funded by the National Institute of Mental Health (NIMH) (Fava, Rush et al. 2003; Rush, Fava et al. 2004). The STAR*D design employed "an equipoise, stratified, randomized design to evaluate the relative efficacy and tolerability of various antidepressant treatments for outpatients with non-psychotic major depressive disorder who had a lack of remission or could not tolerate the selective serotonin-reuptake inhibitor (SSRI) citalopram (Celexa®) or subsequent treatments" (Rush, Trivedi et al. 2006).

Celexa® (or citalopram) was introduced into the Canadian market in late 1999/early 2000 (Hemels, Koren et al. 2002), but between 2001 and 2006, pharmaceutical companies for four commonly-prescribed SSRIs (Celexa®, Paxil®, Prozac® and Zoloft®) lost their patent protection (Huskamp, Donohue et al. 2008). This opened the door for other companies to produce the (cheaper) generic form (Huskamp, Donohue et al. 2008), encouraging health systems to promote SSRIs, especially citalopram, as a first-step agent in the treatment of major depressive disorder (Rush, Fava et al. 2004). For this reason, along with its minimal drug interactions with other agents and relatively short half-life between 32-35 hours (allowing a low-risk switch from citalopram to another agent without a two-week

washout or tapering off period), the STAR*D study designers chose citalopram as its Level 1, first-step treatment option (Rush, Fava et al. 2004). (For a review on the mechanism of action of citalopram, please see Section 4).

The study protocol was comprised of four (4) treatment levels with a sub-level at the second stage; Level 1, Level 2, (Level 2a), Level 3, and Level 4 with the duration of each lasting up to 14 weeks (acute-phase treatment was defined as treatment lasting 6 – 8 weeks) (Rush, Fava et al. 2004).

The 17-item Hamilton Rating Scale for Depression (HAM-D_{17}) (Hamilton 1960) and the 16-item Quick Inventory of Depressive Symptomatology – Self-Report (QIDS-SR_{16}) (Rush, Trivedi et al. 2003) were primarily used by clinicians in their assessment of non-psychotic MDD severity in outpatients during the STAR*D study. It must be noted however, that three (3) different versions of the Quick Inventory of Depressive Symptomatology (QIDS), a scale derived from the 30-item Inventory of Depressive Symptomatology (IDS) clinician rating scale (Rush, Giles et al. 1986; Rush, Gullion et al. 1996), exist; 1) a clinician rating (QIDS-C_{16}), 2) a self-report (QIDS-SR_{16}), and 3) an automated, interactive voice response telephone system (QIDS-IVR_{16}), (Rush, Bernstein et al. 2006). While usual clinician assessment of non-psychotic MDD severity in outpatients was traditionally evaluated using the HAM-D_{17} and/or QIDS-C_{16}, it was found that either of these scales could be "successfully replaced" by the QIDS-SR_{16} and/or QIDS-IVR_{16} (Rush, Bernstein et al. 2006). Therefore, some flexibility was warranted in how different clinicians in different clinics assessed their patients.

Previous guidelines and reports on depression (Rush 1993; Schulberg, Katon et al. 1998; Schulberg, Katon et al. 1999) recommended that *remission* be the primary outcome and preferred goal of antidepressant treatment since remission has been shown to have a favourable prognosis and provide better daily functioning than *response* (Miller, Keitner et al. 1998; Judd, Paulus et al. 1999; Rush, Trivedi et al. 2006).

*Note: Definitions of Remission, Response and Relapse According to STAR*D:*

Remission was defined in the STAR*D as a total score ≤7 on the 17-item Hamilton Rating Scale for Depression ($HAM\text{-}D_{17}$), and also a total score ≤5 on the 16-item Quick Inventory of Depressive Symptomatology – Self Report ($QIDS\text{-}SR_{16}$) at the conclusion of the study (McGrath, Stewart et al. 2006; Trivedi, Fava et al. 2006).

Response to treatment was defined in the STAR*D as a ≥50% reduction from the baseline 16-item Quick Inventory of Depressive Symptomatology – Self Report ($QIDS\text{-}SR_{16}$) score at the conclusion of the study (Trivedi, Fava et al. 2006).

Relapse was defined in the STAR*D as a score ≥11 on the $QIDS\text{-}SR_{16}$ (equivalent to a score ≥14 on the $HAM\text{-}D_{17}$) at the conclusion of an interactive voice response system during the follow-up after exiting the STAR*D (Rush, Trivedi et al. 2006).

Time to remission (in weeks) was determined in the STAR*D from the time of treatment initiation until the first obtainment of a score ≤5 on the $QIDS\text{-}SR_{16}$ (Rush, Trivedi et al. 2006).

*STAR*D Participant Requirements*

In order to have been considered for participation in the study, the following criteria were required (Rush, Trivedi et al. 2006; Gaynes, Rush et al. 2008):

- Age between 18 and 75 years old
- Patients diagnosed by a clinician to have non-psychotic major depressive disorder (MDD) based on the definition from the revised DSM-IV-TR (A.P.A. 2000)
- Clinician recommended treatment for the patient was antidepressant(s)

- Obtain a score ≥14 on the Hamilton Rating Scale for Depression (HAM-D_{17}) (Hamilton 1960)

- Patients *could not* be undergoing the following concomitant treatments at entry into the study:

    - Anticonvulsants, antipsychotic agents, mood stabilizers, stimulants, non-protocol antidepressants, and/or possible augmenting antidepressant agents (Rush, Trivedi et al. 2006)

- Patients *could* be undergoing the following concomitant treatments at entry into the study:

    - Medication(s) for the treatment of concurrent general medical conditions, side effects of protocol antidepressants, anxiolytic agents and/or sedatives (Rush, Trivedi et al. 2006)

1.9.2 STAR*D Treatment Levels

1.9.2.1 *Level 1*

Of the 4,484 patients screened for participation, 4,041 entered Level 1 of the STAR*D study (Rush, Trivedi et al. 2006). All participants in this level received the SSRI citalopram with a mean dose of 40.6 ± 16.6mg/day and 42.5 ± 16.8mg/day in primary care clinics and psychiatric clinics, respectively (Gaynes, Rush et al. 2008). The dosing range for citalopram is 20 – 60 mg/day (Cipriani, Furukawa et al. 2009), so the mean dose used was very near the middle. It should be noted that the required score for entry into the first level was ≥14 on the 30-item HAM-D_{17} (Rush, Trivedi et al. 2006). Remission rates in this level were 27% and 33% based on scores from the HAM-D_{17} and QIDS-SR_{16}, respectively, and the response rate, based on the QIDS-SR_{16}, was 47% (Gaynes, Rush et al. 2008). Further, of those who achieved remission, it took, on average, 49 days in a

psychiatric clinic and 44 days in a primary care clinic (Gaynes, Rush et al. 2008). It should be noted that there were no significant differences in remission rates and duration, and response rates and duration of patients treated in either a psychiatric clinic or a primary care clinic (Gaynes, Rush et al. 2008) .

Entry into Level 2 of STAR*D was highly recommended for those who did not remit following a 14-week treatment of only citalopram and who did not pre-maturely exit the study due to intolerable side effects to treatment (Rush, Trivedi et al. 2006). Those who did achieve symptom remission however, were asked to enter a 12-month naturalistic follow-up phase that occurred every 2 months (Rush, Trivedi et al. 2006).

1.9.2.2 *Level 2*

Following failure to achieve remission after an initial treatment with an SSRI for depression (Level 1 with citalopram), or were unable to tolerate this SSRI, 1,439 patients participated in the second step of the STAR*D treatment (Level 2) (Rush, Trivedi et al. 2006). This step however, included a variety of different treatment options available, and randomly assigned, to the patients in an effort to effectively mimic clinical settings through the use of an equipoise-stratified randomized design (Lavori, Rush et al. 2001; Fava, Rush et al. 2003; Rush, Fava et al. 2004; Rush, Trivedi et al. 2006; Rush, Trivedi et al. 2006). This particular study design was implemented to allow the patient the choice of accepting and/or rejecting medication augmentations and/or switches, similar to real-life clinical decisions (Lavori, Rush et al. 2001).

Level 2 allowed the patients to accept or decline random assignment into one of three medication augmentation treatments and/or one of four medication switch treatments. There were four medication switch treatment options, including cognitive therapy, and three citalopram augmentation treatment options. However, only those patients who's randomly assigned treatment included cognitive therapy (either as an augmentation to citalopram treatment or as a sole switch treatment

option) were eligible to enter into a "special" third treatment step (Level 2A) (Rush, Trivedi et al. 2006). To be eligible for Level 2A, these participants must not have achieved remission or were intolerant to either cognitive therapy alone or cognitive therapy with citalopram treatment (Rush, Trivedi et al. 2006).

Medication Switch (excluding switch option of cognitive therapy alone):

Three (3) second-step medication switches (excluding the fourth switch option; cognitive therapy alone) were classified as either an in-class switch (SSRI to another SSRI: sertraline), out-of-class switch (SSRI to a non-SSRI: bupropion sustained-release) or a "dual-action" switch (Nierenberg, Feighner et al. 1994; de Montigny, Silverstone et al. 1999) in that the medication inhibited both serotonin and norepinephrine reuptake (SSRI to SNRI: venlafaxine extended-release) (Rush, Trivedi et al. 2006).

Bupropion-Sustained Release (Bupropion-SR):

Remission rates were 21.3% and 25.5% based on scores from the HAM-D_{17} and QIDS-SR_{16}, respectively, with a response rate, based on the QIDS-SR_{16}, of 26.1% (Rush, Trivedi et al. 2006). The mean time to remission was 5.4 $\pm$ 4.5 weeks and the mean time to response was 5.5 $\pm$ 3.5 weeks (Rush, Trivedi et al. 2006).

Sertraline:

Remission rates were 17.6% and 26.6% based on scores from the HAM-D_{17} and QIDS-SR_{16}, respectively, with a response rate, based on the QIDS-SR_{16}, of 26.7% (Rush, Trivedi et al. 2006). The mean time to remission was 6.2 $\pm$ 5.0 weeks while the mean time to a response was 6.6 $\pm$ 4.3 weeks (Rush, Trivedi et al. 2006).

Venlafaxine-Extended Release (Venlafaxine-XR):

Remission rates were 24.8% and 25.0% based on scores from the HAM-D_{17} and QIDS-SR_{16}, respectively, with a response rate, based on the QIDS-SR_{16}, of 28.2% (Rush, Trivedi et al. 2006). The mean time to remission was 5.5 $\pm$ 4.7 weeks and the mean time to a response was 7.0 $\pm$ 4.3 weeks (Rush, Trivedi et al. 2006).

Concluding Remarks: Medication Switch (excluding switch option of cognitive therapy alone)

Statistical analyses revealed that among the three switch treatments, they did not differ significantly in terms of patient intolerability and/or side effects of each medication, suggesting that patient intolerance towards one SSRI does not predict intolerance and/or efficacy of another SSRI (Rush, Trivedi et al. 2006). Further, there were no significant differences in remission rates and duration, and response rates and duration, among the three switch treatments (Rush, Trivedi et al. 2006). Therefore, it was concluded that after failure of an initial treatment with an SSRI (citalopram) for non-psychotic MDD, there was no significant benefit in antidepressant treatment if a patient switched to another SSRI (sertraline), bupropion or an SNRI (venlafaxine) (Rush, Trivedi et al. 2006).

Medication Augmentation (excluding augmentation with cognitive therapy):

Two (2) second-step medication citalopram augmentations (excluding augmentation with cognitive therapy) were citalopram augmentation with bupropion sustained-release or citalopram augmentation with buspirone (a non-SSRI anxiolytic agent) (Trivedi, Fava et al. 2006). Buspirone may have been chosen as a second-step medication option since approximately 46% of the

participants had MDD with concomitant anxious features (Rush, Kilner et al. 2008).

Citalopram Augmentation with Bupropion-SR:

Remission rates were 29.7% and 39.0% based on scores from the HAM-D_{17} and QIDS-SR_{16}, respectively, with a response rate, based on the QIDS-SR_{16}, of 31.8% (Trivedi, Fava et al. 2006). The mean time to remission was 6.3 $\pm$ 4.8 weeks and the mean time to a response was 6.3 $\pm$ 4.6 weeks (Trivedi, Fava et al. 2006).

Citalopram Augmentation with Buspirone:

Remission rates were 30.1% and 32.9% based on scores from the HAM-D_{17} and QIDS-SR_{16}, respectively, with a response rate, based on the QIDS-SR_{16}, of 26.9% (Trivedi, Fava et al. 2006). The mean time to remission was 5.4 $\pm$ 4.4 weeks and the mean time to a response was 6.8 $\pm$ 3.9 weeks (Trivedi, Fava et al. 2006).

Concluding Remarks: Medication Augmentation (excluding augmentation w/ cognitive therapy):

Statistical analyses showed that there were significant differences in medication cessation due to patient intolerability, with a 20.6% intolerable rate with buspirone augmentation compared to only 12.5% intolerability with bupropion-SR augmentation (Trivedi, Fava et al. 2006). Although there were no significant differences in MDD symptom remission rates, response rates or treatment duration between the two augmentation treatments, it was found that augmentation with bupropion-SR had significantly lower mean total QIDS-SR_{16} scores at the study's end compared to augmentation with buspirone, scores of 8.0

and 9.1, respectively (Trivedi, Fava et al. 2006). Therefore, although it was concluded that augmentation of the SSRI citalopram with either bupropion-SR or buspirone were not significant in overall treatment, there were advantages, such as medication tolerance, to augmentation with bupropion-SR over buspirone (Trivedi, Fava et al. 2006). The literature also showed convincing evidence supporting the superior efficacy of citalopram (SSRI) and bupropion-SR combination therapy in the treatment of patients with treatment-resistant depression compared to switching to monotherapy (Lam, Hossie et al. 2004). (For more information on bupropion combination with an SSRI, please see Section 11).

Cognitive Therapy

Cognitive therapy has become a more prevalent option among treatment alternatives for resistant-depression. Consequently, the effectiveness of cognitive therapy versus pharmacotherapy was a studied variable in the STAR*D (Rush, Trivedi et al. 2006; Thase, Friedman et al. 2007). Of all 1,439 participants in Level 2, only 369 (26%) were willing to accept one or both of the cognitive therapy options while the remaining 1,070 participants refused (Thase, Friedman et al. 2007; Wisniewski, Fava et al. 2007). Those who consented to cognitive therapy were more likely to have had a family history of depression or bipolar disorder, had obtained a college degree and/or spent a significant time in Level 1 (Wisniewski, Fava et al. 2007). Of the 369 patients willing to accept cognitive therapy as a treatment option, 85 were randomly assigned into *i)* a group consisting of medication augmentation of citalopram with cognitive therapy while 62 were randomly assigned into *ii)* a medication switch to cognitive therapy alone (Rush, Trivedi et al. 2006). (Only patients assigned into either treatment option containing cognitive therapy had the possibility of entering into the sub-level, Level 2A; depending on treatment outcome.)

Medication Switch to Cognitive Therapy Alone:

For the 62 patients who discontinued medication and received only cognitive therapy as a second-step treatment option, based on scores from the QIDS-SR_{16} (HAM-D_{17} scores were unavailable), the remission rate was 41.9% and the response rate was 30.6% (Rush, Trivedi et al. 2006). For those who did remit, the mean time to remission was 5.2 weeks while the mean time to a response was 7.8 weeks (Rush, Trivedi et al. 2006).

Medication Augmentation of Citalopram with Cognitive Therapy:

Of the 85 patients who received cognitive therapy augmentation to citalopram, the remission rate was 29.4% with a response rate of 34.1%, both based on QIDS-SR_{16} scores (Rush, Trivedi et al. 2006). Unfortunately, HAM-D_{17} scores were not published. The mean time to remission was 7.2 weeks (for those who remitted) and of those who responded, the mean time to response was 7.9 weeks (Rush, Trivedi et al. 2006).

Concluding Remarks: Level 2:

Researchers of the STAR*D trial concluded that the effectiveness of either of the two cognitive psychotherapy strategies in remission or response rates were comparable with the five pharmacotherapy treatments (Thase, Friedman et al. 2007). Augmenting citalopram with cognitive therapy was shown to not have a significantly greater tolerance quota when compared to augmentation with medication, but was also found to have similar symptomatic improvements compared with bupropion-SR or buspirone (Thase, Friedman et al. 2007) . Further, no significant difference was found in the rate of remission between both treatment methods (Thase, Friedman et al. 2007). However, a significant difference was observed in the time it took for cognitive therapy to exhibit any opportune remission effects when compared to pharmacotherapy treatments,

taking an average of 20 days longer (Thase, Friedman et al. 2007). Interestingly, this variable was not found to be significant when comparing results of patients who switched treatments to either pharmacotherapy or psychotherapy (Thase, Friedman et al. 2007).

Another rather surprising result showed no significant difference in the welfare of those who switched to cognitive therapy compared with those who switched to the pharmacotherapy treatment options, which included a switch to either bupropion-SR, venlafaxine-XR or sertraline, but cognitive therapy was slightly better tolerated (Thase, Friedman et al. 2007).

When taken together, these results appear to indicate that after initial treatment failure with citalopram, an augmentation or switch to pharmacotherapy compared to a cognitive therapy augmentation or switch was not advantageous to the overall effectiveness of treatment. However, if time is a concern, then pharmacotherapy appears to be a more viable option, but if not, cognitive therapy has the advantage of alleviating the risk of treatment intolerance.

1.9.2.3 *Level 2A (Treatment with Either Bupropion-SR or Venlafaxine-XR)*

Due to the lack of evidence for the "mechanism of action" of cognitive therapy, those who did receive this treatment (either as an augmentation to citalopram or as a complete "medication" switch) may have been eligible for a "special, third step" known as Level 2A (Rush, Trivedi et al. 2006). Those who entered Level 2A (N=31) were randomly assigned treatment with either bupropion (N=15) or venlafaxine (N=16) based on remission failure or an intolerance to either of the two aforementioned treatments, and justification for this option was to ensure that all patients who entered Level 3 of the STAR*D had not achieved remission after two similar, yet different pharmacotherapy treatment strategies (Rush, Trivedi et al. 2006).

Treatment with Bupropion-SR:

Of the 15 patients who were randomly treated with bupropion after an ineffective cognitive therapy treatment step, QIDS-SR_{16} scores revealed that 6.7% achieved remission with an equivalent percentage responding to treatment (Rush, Trivedi et al. 2006). However, it took an average of 1 week of those who remitted to achieve remission scores on the QIDS-SR_{16} while it took a mean time of 10.2 weeks for those who responded to respond. (Rush, Trivedi et al. 2006).

Treatment with Venlafaxine-XR:

For the 16 patients given venlafaxine following an ineffective treatment step with cognitive therapy, based on QIDS-SR_{16} scores, 6.3% remitted and the response rate was 12.5% (Rush, Trivedi et al. 2006). It took an average of 8.0 and 7.8 weeks for remission and response rates, respectively. (Rush, Trivedi et al. 2006).

1.9.2.4 *Level 3*

Following the failure to achieve remission of non-psychotic MDD symptoms after two pharmacotherapy treatments, 377 patients were encouraged to enter Level 3 of the STAR*D study (Rush, Trivedi et al. 2006). After consecutive failures, a medication switch or augmentations to the current antidepressant have become the two most preferred treatment alternatives (Gaynes, Dusetzina et al. 2012). Patients had the option of choosing to be randomly assigned into either i) medication augmentation (current medication with the addition of lithium or T_3 (thyroid hormone)), ii) medication switch (nortriptyline or mirtazapine) or iii) consent to random assignment into either medication augmentation or switch. The two STAR*D studies reported here (Fava, Rush et al. 2006; Nierenberg, Fava et

al. 2006) compared outcomes of patients who were randomly assigned in a 1:1 ratio into a medication switch or augmentation group, respectively, based on the patient's willingness to accept placement into a treatment group consisting of at least one (ie. any switch OR any augmentation treatment), or both (switch AND augmentation treatments) of the treatment options.

Why Use Lithium or T_3 Augmentation?

Serotonin has been established as an integral part of antidepressant efficacy and lithium has been shown to increase 5-HT presynaptic synthesis, storage and release (De Montigny, Grunberg et al. 1981; Nierenberg, Fava et al. 2006). It should be no surprise then that lithium augmentation has gained popularity as an acute treatment option for major depression in augmenting antidepressant medication(s) after initial treatment failure(s) (Rouillon and Gorwood 1998; Bschor, Lewitzka et al. 2003; Blier, Gobbi et al. 2009). The addition of lithium has further been shown to potentiate the antidepressant effects of TCAs in depressed patients who failed to respond to a minimum 3-week treatment with tricyclic antidepressants (De Montigny, Grunberg et al. 1981). Meta-analysis studies have also shown lithium augmentation to be effective in treatment-resistant depression (Bschor, Lewitzka et al. 2003) and its augmentation has been shown to have a clinical response rate of up to 40% before 6-weeks of augmentation treatment (Crossley and Bauer 2007; Blier, Gobbi et al. 2009). From this body of evidence, it appeared rational to use lithium as an augmentation treatment option after two failed medication treatments in the STAR*D.

Since the late 1880s, and although relative to only a select few, a consistent correlation has been found between patients suffering from a mood disorder and who also have abnormal thyroid function (Iosifescu, Howarth et al. 2001). However, beneficial antidepressant treatment in depressed patients with abnormal thyroid function has been an inconsistent variable with some studies reporting a correlation between thyroid abnormalities, namely hypothyroidism, in

treatment-resistant depression (Joffe and Levitt 1992; Sullivan, Wilson et al. 1997) while others do not share this view (Vandoolaeghe, Maes et al. 1997; Joffe 1999). Thyroid hormone augmentation has the potential to be clinically beneficial in treatment-resistant depression and its inclusion in the STAR*D as an almost "last-ditch effort" is warranted.

Medication Augmentation (Nierenberg, Fava et al. 2006):

Of the 377 patients who entered Level 3, 142 began lithium or T_3 augmentation treatments. (N=127 for augmentation only) + [(N=29 for either augmentation or switch) – (N=14 who were randomly assigned into switch group) =15 who were randomly assigned for lithium or T_3 augmentation] = 127 + 15 = 142. (Nierenberg, Fava et al. 2006).

Of the 142 patients treated with medication augmentation, 69 patients had their current medication augmented with lithium while 73 had their medication augmented with T_3. (Nierenberg, Fava et al. 2006).

Based on HAM-D_{17} scores, 15.9% and 24.7% achieved remission in the lithium and T_3 groups, respectively. The authors calculated that the difference in these scores were not significant. QIDS-SR_{16} scores revealed remission rates to be 13.2% for lithium and 24.7% for T_3. Again, these differences were found to not be statistically significant. There was also no significant difference in patients who responded to lithium and thyroid hormone augmentation, 16.2% and 23.3% respectively. Further, in those who did achieve remission, it was concluded that the antidepressant being augmented with either lithium or T_3 (bupropion-SR, citalopram, sertraline or venlafaxine-XR) had no significant impact on the mean rates of remission. (Nierenberg, Fava et al. 2006).

The mean time to achieve remission with lithium was 7.4 weeks while those taking T_3 achieved remission in 6.6 weeks. For responsive patients, the mean time to response was 5.7 weeks for those taking lithium and 6.0 weeks for

thyroid hormone augmentation. The mean time differences between both augmenting medications in patients who achieved remission or responded were found to not be significantly different. (Nierenberg, Fava et al. 2006).

However, patients who were receiving T_3 augmentation appeared to better tolerate the medication compared with those taking lithium. Although not significant, lithium users did experience maximal intensity and burden of medication side effects, whereas T_3 users did not reach these levels. The frequency of lithium side effects was significantly greater than T_3 and as a result, significantly more patients receiving lithium augmentation prematurely exited the study. (Nierenberg, Fava et al. 2006).

Although there was no significant difference between medication augmentation with lithium or thyroid hormone with respect to the proportion of those achieving remission, of those who responded and also to the time it took to achieve either remission or response, thyroid hormone augmentation continually had higher proportions of those achieving remission and responses, along with consistently improved scores (lower scores) on both the HAM-D_{17} and QIDS-SR_{16}. Further, T_3 was shown to have a slightly superior tolerance threshold when compared to lithium augmentation, making T_3 augmentation a more desirable treatment option than lithium augmentation following two previously unsuccessful pharmacotherapy treatments. (Nierenberg, Fava et al. 2006).

Medication Switch (Fava, Rush et al. 2006):

Of the 377 patients who were treated in Level 3, 235 had a medication switch to either mirtazapine (an atypical antidepressant) or nortriptyline (a TCA). (N=221 for switch only) + [(N=29 for either switch or augmentation) – (N=15 who were randomly assigned into augmentation group) = 14 who were randomly assigned into switch group] = 221 + 14 = 235. (Fava, Rush et al. 2006).

Of the 235 patients treated by a medication switch, 114 patients randomly had their medication switched to mirtazapine while the other 121 patients had their medication switched to nortriptyline. (Fava, Rush et al. 2006).

HAM-D_{17} scores at exit revealed that 12.3% and 19.8% of patients achieved remission in the mirtazapine and nortriptyline groups, respectively, and were not significantly different. Based on QIDS-SR_{16} scores at exit, remission rates for mirtazapine and nortriptyline were 8.0% and 12.4%, respectively. These differences were also not significantly different. (Fava, Rush et al. 2006).

Based on QIDS-SR_{16}, with mirtazapine, it took an average of 5.7 weeks for those who remitted to achieve remission while those in the nortriptyline group took an average of 6.3 weeks. Response rates for those who responded were 6.9 weeks for mirtazapine and 6.3 weeks for nortriptyline. The mean time to remission and response were not significant between the two groups. Further, both switch medications were tolerated to a similar extent, as were the probabilities of any major adverse events occurring, which were all below or equal to 3.5% of the patients. (Fava, Rush et al. 2006).

Based on these results, there appears to be no conclusively significant advantage when choosing mirtazapine or nortriptyline as a switch option for antidepressant treatment (Fava, Rush et al. 2006). However, the treating physician may want to review the patient's medication history and take into account what types of antidepressants the patient was on previously since the mechanism of action for mirtazapine and nortriptyline vary greatly, and may result in disastrous pharmacokinetic interactions. For a more in-depth review on the mechanism of action of mirtazapine, please see Section 7.

Concluding Remarks for Level 3:

Analysis of patient outcomes between medication switch and augmentation do not provide clinically significant evidence for either as a

preferred next-step treatment (Gaynes, Dusetzina et al. 2012). Remission and response rates, as well as time to remission and response, did not greatly differ between switch and augmentation. Although not significant, T_3 augmentation was seen as a more desirable treatment option over lithium (Nierenberg, Fava et al. 2006). As antidepressants and thyroid treatment are still not agreeably correlated, along with the already overall low percentage rate of remission in Level 3 (14%) (Rush, Trivedi et al. 2006; Gaynes, Rush et al. 2008; Rush, Kilner et al. 2008), the treating physician may consider a medication switch (based on the medication's mechanism of action and patient's previous pharmacological profile) over augmenting the current antidepressant.

1.9.2.5 *Level 4*

A total of 109 out of the initial 4,041 patients enrolled in the STAR*D study either failed to achieve remission or were intolerant to medication (citalopram and two subsequent medication treatments) following three antidepressant medication treatments and entered into the study's final level, Level 4 (Rush, Trivedi et al. 2006). The purpose of this final level was to compare tolerance and effectiveness of an MAOI treatment compared to combination treatment for patients with highly resistant MDD (McGrath, Stewart et al. 2006).

Therefore, 58 patients were randomly assigned treatment with the MAOI tranylcypromine and 51 were randomly assigned to a combination treatment of venlafaxine-XR + mirtazapine (McGrath, Stewart et al. 2006).

<u>Tranylcypromine:</u>

Based on scores from the HAM-D_{17} and QIDS-SR_{16}, remission rates were 6.9% and 13.8%, respectively (McGrath, Stewart et al. 2006). The response rate, based on the QIDS-SR_{16}, was 12.1% (McGrath, Stewart et al. 2006). The mean

time to remission was 8.6 weeks and the mean time to a response was 11.4 weeks (McGrath, Stewart et al. 2006).

Venlafaxine-XR + Mirtazapine:

Remission rates, based on scores from the HAM-D_{17} and QIDS-SR_{16}, were 13.7% and 15.7%, respectively (McGrath, Stewart et al. 2006). The response rate, based on the QIDS-SR_{16}, was 12.1% (McGrath, Stewart et al. 2006). The mean time to remission was 8.1 weeks and the mean time to a response was 8.6 weeks (McGrath, Stewart et al. 2006).

Concluding Remarks for Level 4:

Total (N=109) remission rates based on HAM-D_{17} and QIDS-SR_{16} scores were 10.1% and 14.7%, respectively, and the response rate was 17.4% based on a $\geq$50% reduction from level 4 baseline scores on the QIDS-SR_{16} (McGrath, Stewart et al. 2006). Although the remission and response rates in both treatments were extremely low, they were not found to be significantly different (McGrath, Stewart et al. 2006). The mean time to remission and the mean time to response for both treatments were also not found to be significantly different (McGrath, Stewart et al. 2006). However, when comparing the reduction in QIDS-SR_{16} scores from level 4 baseline to exit, there was a substantial reduction in the percentage of QIDS-SR_{16} scores for those treated with venlafaxine-XR + mirtazapine (-25.0%) compared to tranylcypromine (-6.2%), indicating a significantly greater improvement of depressive symptoms for those who received the combination treatment (McGrath, Stewart et al. 2006).

It was of interest to note that because of the dietary restrictions associated with MAOI medications (see Section 3), a mandatory 2-week washout period for those treated with tranylcypromine was required after exiting level 3 and before entering level 4 (McGrath, Stewart et al. 2006). This was the only situation in the

STAR*D study in which a washout period was warranted between any medication switch/augmentation. However, even with this 2-week washout period included in tranylcypromine treatment duration calculations, significantly more of these patients were treated for less than 4 weeks compared to the venlafaxine-XR + mirtazapine group, 29.3% and 7.8% respectively (McGrath, Stewart et al. 2006). Therefore, excluding the washout period and taking into account the amount of time patients treated with tranylcypromine were actually taking the medication, close to 30% had less than 2 weeks of treatment (McGrath, Stewart et al. 2006). Further, 46.6% of those treated with tranylcypromine compared to 21.6% of those treated with venlafaxine-XR + mirtazapine were in treatment for less than 8 weeks (and these 8 weeks included the 2-week washout period) (McGrath, Stewart et al. 2006). Therefore, almost 50% of patients taking tranylcypromine were in treatment for less than 6 weeks. While no significant differences were found in frequency or intensity of treatment side effects between the two groups, it was found that significantly more patients who received tranylcypromine than venlafaxine-XR + mirtazapine exited level 4 because of medication intolerance, 41.4% compared to 21.6%, respectively (McGrath, Stewart et al. 2006).

Although there were no significant differences in remission rates or symptom improvement between the two treatment groups, which is in the end what the study was attempting to find, based on the numerous inconveniences of MAOIs (constant dietary monitoring and restrictions, a possible restless 2-week washout period before MAOI treatment can safely begin, along with other significant differences found between these two treatment groups), combination treatment with venlafaxine-XR + mirtazapine was found to be a more favourable option when deciding on a new course of action in the treatment of highly-resistant depression (McGrath, Stewart et al. 2006).

1.9.3 STAR*D Concluding Remarks

While the STAR*D study was unable to establish a paramount alternative regimen following the failure of the first antidepressant treatment, it was able to shed some light on viable and justifiable next-step treatment options following the failure of one or more antidepressant treatments. After four treatment levels, the cumulative remission rate (theoretical) was found to be 67% (Gaynes, Rush et al. 2008). However, with subsequent antidepressant medication treatment failures, the rate of achieving remission diminished, with a significant decline in achieving remission following two failed antidepressant treatments (Gaynes, Rush et al. 2008).

Remission Rates Following Exit from Each Level (Rush, Trivedi et al. 2006; Gaynes, Rush et al. 2008; Rush, Kilner et al. 2008) :

Level 1: 37%

Level 2: 31%

Level 3: 14%

Level 4: 13%

The STAR*D was unable to provide a definitive explanation to account for such a sharp decline in rates of remission following two unsuccessful antidepressant treatment trials (~30% to ~13%), however, the results were able to provide physicians, clinicians and patients with encouraging information about treatment alternatives after initial, or subsequent, failures. It further showed innate human compromises towards frustrations in both professionals and patients towards multiple failures by revealing that the more resistant a patient's depression, and hence the more treatment levels and time required, both physician and patient would seemingly be content with simply a response rather than remission (Gaynes, Rush et al. 2008). This could also help explain such drastic

drops in remission rates seen in the last two levels (13.7% and 13.0% at Levels 3 and 4, respectively) and the slightly higher response rates (16.8% and 16.3% at Levels 3 and 4, respectively) seen in these final two treatment steps. Further, following the first and second treatment steps, 20.9% and 29.7 % of participants exited the study, respectively, while following the third treatment step (Level 2A and Level 3), 42.3% dropped out (Rush, Trivedi et al. 2006).

Longer-term results from these last two levels not only revealed significant unlikeliness that those patients would enter remission, but their scores on the QIDS-SR_{16} were significantly higher as were their rates of relapse during the follow-up phase after achieving remission (Rush, Trivedi et al. 2006).

The rather effective combination treatment of venlafaxine-SR + mirtazapine seen in Level 4 has raised the question as to whether or not combination treatment should be used as an initial treatment option or at the very least, chosen as a second-step treatment following failure of the first. This combination at treatment initiation was consequently studied (Blier, Ward et al. 2010) and will be reviewed in Section 11.

Section 10

Meta-Analysis: Antidepressant Combination versus Monotherapy Treatment

1.10.1 *Introduction*

Recently, the combination of antidepressant medication to treat major depressive disorder (MDD) has gained substantial and increasing interest. It has been suggested (Blier, Gobbi et al. 2009; Blier, Ward et al. 2010) that the use of antidepressant combination therapy from the start of treatment has a greater efficacy in achieving remission rates and displays no significant differences in medication adverse effects and tolerability when compared to treatment with a single antidepressant. However, since not all antidepressants are equally effective, much thought must be directed towards understanding the mechanisms of action of the various types of antidepressants and finding medications that may complement one another (ie. antidepressant synergy).

1.10.2 *Review of Efficacy and Tolerability of 12 Current Antidepressants*

While many psychiatrists and researchers have had biased opinions towards a particular antidepressant, or antidepressants, it was not until 2009 when a meta-analysis (Cipriani, Furukawa et al. 2009) systematically reviewed and analyzed 117 randomized-controlled experiments (with a combined participation of 25, 928 individuals) from 1991 until November 30th, 2007, by comparing the efficacy and tolerability of 12 antidepressants in their treatment of acute-phase (8 weeks) unipolar major depression. The following lists the 12 antidepressants along with their respective antidepressant class and dosing ranges (mg/day) in parentheses, as assigned by Cipriani, Furukawa et al. 2009; bupropion (atypical, 150 – 450mg/day); citalopram (SSRI, 20 – 60mg/day); duloxetine (SNRI, 60 – 100mg/day); escitalopram (SSRI, 10 – 30mg/day); fluoxetine (SSRI, 20 – 60mg/day), fluvoxamine (SSRI, 50 – 300mg/day); milnacipran (SNRI, 50 –

300mg/day); mirtazapine (NaSSA, 15 – 45mg/day); paroxetine (SSRI, 20 – 60mg/day); reboxetine (NRI, 4 – 12mg/day); sertraline (SSRI, 50 – 200mg/day); venlafaxine (SNRI, 75 – 250mg/day).

For all the studies analyzed, the mean duration of treatment was found to be 8.1 weeks (the upper duration for acute depression treatment) and the mean HAM-D_{17} score at entry into the study was 23.47 (SD 4.27) (Cipriani, Furukawa et al. 2009). The required score for entry into Level 1 of the STAR*D was $\geq$14 on the 30-item HAM-D_{17} (See Section 9) so this score is closer to the upper limit, indicating greater severity of symptoms.

The results of this meta-analysis revealed that the SSRI sertraline (Zoloft®) was the most versatile antidepressant as it had the best overall balance between efficacy and tolerance, as well as, according to the authors, having a reasonably affordable acquisition cost (Cipriani, Furukawa et al. 2009). At the other end of this spectrum, it was found that the NRI reboxetine was consistently and significantly less efficacious than all the other studied antidepressants, and although it was not found to be significant, was consistently the least tolerated of all 12 antidepressants (Cipriani, Furukawa et al. 2009).

In terms of treatment efficacy, mirtazapine (NaSSA), escitalopram (SSRI), venlafaxine (SNRI) and sertraline (SSRI) were found to be significantly more efficacious than duloxetine (SNRI), fluoxetine (SSRI), fluvoxamine (SSRI), paroxetine (SSRI) and reboxetine (NRI) (Cipriani, Furukawa et al. 2009). Further, escitalopram (SSRI) and sertraline (SSRI) were significantly better tolerated [while bupropion (atypical) and citalopram (SSRI) were not significantly but also better tolerated] than duloxetine (SNRI), fluvoxamine (SSRI), paroxetine (SSRI), reboxetine (NRI) and venlafaxine (SNRI) (Cipriani, Furukawa et al. 2009) . Fluoxetine (SSRI) and mirtazapine (NaSSA) were also among the least tolerated, but this was found to not be significant (Cipriani, Furukawa et al. 2009).

From these results, although mirtazapine and venlafaxine were among two of the most efficacious medications, their significantly (venlafaxine) and non-

significantly (mirtazapine) acceptability profile compared to tolerance levels of the others makes them viable options, but not the best overall. That distinction, as previously mentioned, went to sertraline (Cipriani, Furukawa et al. 2009). However, as previously reported (Section 9), the STAR*D revealed that the combination of mirtazapine with venlafaxine-SR was rather effective in the treatment of depression (McGrath, Stewart et al. 2006); results obtained during Level 4 of the study and once again reviving the "what-if" adage had this combination been used at the beginning of treatment. Consequently, this combination at treatment initiation was studied in 2010 (Blier, Ward et al. 2010) and will be reviewed in Section 11.

Because of its noradrenergic and specific serotonergic qualities, whose mechanisms of action appear to complement one another, mirtazapine has been used quite frequently as a first-step medication option. A meta-analysis on the antidepressant effect of mirtazapine (Bech 2001), which included 7 trials with a mean of 45 patients (varied between 38 – 64) using the HAM-D_{17} to evaluate depressive symptoms, found that it does indeed possess a pure antidepressant effect in terms of improving depressed mood following 6-weeks of treatment. Its combination with other antidepressants has also been studied (Blier, Gobbi et al. 2009; Blier, Ward et al. 2010) and as already mentioned, will be reviewed in Section 11.

Of note were the results of citalopram. While it was among the top tolerable medications (18.7%), which was found to not be significant, citalopram had a very low efficacy profile (3.4%) (Cipriani, Furukawa et al. 2009). In the previous section of this thesis (Section 9), the STAR*D chose citalopram as its initial, first-step Level 1 antidepressant. Justification for the use of citalopram as the Level 1 entry medication was based on its SSRI profile to which it was argued, "health care systems are likely to encourage use of an SSRI as a first agent" (Rush, Fava et al. 2004), its low drug-drug interactions and a half-life between 32 – 35 hours, meaning no wash-out period would be required when switching medications (Rush, Fava et al. 2004). The "what-if" scenario in this

case becomes very intriguing as one might ponder the results of the STAR*D had, for example, sertraline been chosen as the initial Level 1 entry antidepressant.

While the results of this meta-analysis (Cipriani, Furukawa et al. 2009) provide invaluable in-sight into the efficacy and tolerability of a variety of antidepressants, studies (Dam, Ryde et al. 1998; Nelson, Mazure et al. 2004; Raisi, Habibi et al. 2006; Blier, Gobbi et al. 2009; Blier, Ward et al. 2010; Rocha, Fuzikawa et al. 2012) have repeatedly shown that the combination of two antidepressants at the beginning of treatment is much more robust, especially in terms of remission, than the use of a single antidepressant at the initiation of treatment.

Section 11

Antidepressant Combination Treatment

1.11.1 *Meta-Analysis Review*

Published in April 2012, a systematic review and meta-analysis (Rocha, Fuzikawa et al. 2012) assessed the efficacy of antidepressant combination therapy versus single antidepressant therapy from treatment initiation for major depression. Based on the review's inclusion criteria for all randomized controlled trials that compared combination versus single antidepressant treatment from Day 1 dating back to 1966 and ending in August 2010, only 5 studies were found to completely satisfy the review's required criteria for inclusion. A more in-depth analysis of these studies will be reviewed later in this section, along with a review on the use of bupropion in combination with SSRIs and SNRIs.

The meta-analysis indeed found the combination of antidepressants at the beginning of treatment to be more effective than a single antidepressant. It was further suggested however, that the majority of combination treatments may cause more medication side effects than those of a single antidepressant, but also mentioned that the 2 antidepressants used in these combination treatments might off-set each other's side effects, such as sexual dysfunction from the long-term use of an SSRI being countered by adding bupropion to the SSRI treatment regimen (Demyttenaere and Jaspers 2008; Rocha, Fuzikawa et al. 2012).

1.11.2 *Review of the 5 Antidepressant Combination Studies*

To be consistent with efficacy and tolerability results previously mentioned, HAM-D_{17} scores will be used when discussing rates of remission and severity of depressive symptoms. The medication doses used in each study will be

compared to the suggested dose ranges given in Section 10 (Cipriani, Furukawa et al. 2009).

*Recall MDC = Mood Disorders Clinic of the McGill University Health Centre

1.11.2.1 *Antidepressant Combination Study 1*

The earliest study (Dam, Ryde et al. 1998) dated back to 1998 and compared the combination of fluoxetine (an SSRI) and mianserin (a NaSSA that was gradually replaced by its successor and structural analogue, mirtazapine) efficacy with the efficacy of fluoxetine alone in the acute (6-weeks) treatment of major depression. Since mianserin enhances noradrenergic neurotransmission by antagonizing pre-synaptic α_2-adrenoceptors (Ferreri, Lavergne et al. 2001), it was reasonable to use this medication to complement the serotonergic neurotransmission via 5-HT reuptake inhibition of fluoxetine (please see Section 4 for a review on the general mechanism of action of SSRIs), and possibly increase the overall antidepressant efficacy while counteracting one another's side effects (Ferreri, Lavergne et al. 2001). Inclusion criteria required a score >16 on the HAM-D_{17}, which is higher than that used for the STAR*D which required a score $\geq$14. The dosage of fluoxetine given was 20mg/day and falls on the lower end of the suggested dose range (20 – 60 mg/day). Mianserin was given at 30mg/day, which appeared to be at the low end of the dose range since other studies (Ferreri, Lavergne et al. 2001) used a mianserin dose of 60mg/day along with 20mg/day of fluoxetine. Moreover, the combination of an SSRI with mirtazapine at treatment initiation was explored (Blier, Gobbi et al. 2009) and will be discussed later in this section (see *Antidepressant Combination Study 4*). The results of this study however, concluded that the difference in HAM-D_{17} scores from baseline to the end of the study in patients taking a combination of mianserin and fluoxetine were significantly different ($p < 0.05$) than the HAM-D_{17} score differences obtained from the fluoxetine alone group (Dam, Ryde et al. 1998). Another study also found these differences to be significant when comparing the combination group

with a fluoxetine group ($P \leq 0.03$) (Ferreri, Lavergne et al. 2001). Augmentation of fluoxetine with mianserin also demonstrated acceptable tolerability and significantly better efficacy ratings ($HAM\text{-}D_{17}$ scores from baseline to end as well as quality of life ratings) when compared to those simply taking fluoxetine.

1.11.2.2 *Antidepressant Combination Study 2*

A second study (Nelson, Mazure et al. 2004) analyzed the efficacy and duration to onset of action in the treatment combination of desipramine (a TCA regarded as "the most potent norepinephrine reuptake inhibitor available" (Nelson, Mazure et al. 2004) with fluoxetine (a commonly available SSRI) compared to the efficacy of treatment with desipramine-only or fluoxetine-only from treatment initiation. The authors believed that the down-regulation of β-adrenergic receptors resulted in an antidepressant response and cited a study (Baron, Ogden et al. 1988) which suggested that combining desipramine with fluoxetine produced a more rapid down-regulation of β-adrenergic receptors than desipramine or fluoxetine taken individually. Further justification for this particular combination was given by the report (Weilburg, Rosenbaum et al. 1989) that suggested that patients who did not respond to TCA treatment showed that 86.7% of patients' symptoms improved when the TCA was augmented with fluoxetine.

Pharmacokinetic Interaction Between Fluoxetine and TCAs

A major concern of this combination was its pharmacokinetic interaction in which fluoxetine has been shown to potently inhibit the CYP2D6 isozyme-mediated hydroxylation of any TCA (including desipramine). When fluoxetine is administered at its dosing range between 20 – 60mg/day, it has been shown that TCA plasma concentrations can increase 2- to 4-fold while displaying toxic adverse effects such as sedation and urinary retention (Aranow, Hudson et al.

1989; Westermeyer 1991; Bergstrom, Peyton et al. 1992; Preskorn, Alderman et al. 1994; Spina, Santoro et al. 2008). Therefore, much care must be taken in order to avoid a potentially devastating situation, a requirement that a psychiatrist with many patients may be unable to safely and properly manage.

Inclusion criteria required psychiatric in-patients 21 years of age or older with a unipolar nonpsychotic major depression diagnosis using the Yale Depression Inventory (Mazure, Nelson et al. 1986) who obtained a score $\geq$18 on the HAM-D_{17}. Treatment lasted for 6-weeks (acute treatment). The final and full therapeutic dose of desipramine administered during the study was calculated to be 160ng/mL while fluoxetine was given at a dose of 20mg/day, which fell on the lower end of its dosing range (20 – 60mg/day). Although this combination was previously shown to be successful, as previously mentioned, a major deterrent seemed to come in the very meticulous dose adjustments based on desipramine plasma concentrations throughout treatment. Blood was drawn from these patients weekly and HPLC was used to determine plasma concentrations of desipramine. Appropriate and proportional reductions of desipramine were made based on the extent to which each participant metabolized the drug; in high metabolizers, fluoxetine increased desipramine levels 3.5 times while in low metabolizers, desipramine levels rose 1.5 times due to fluoxetine. Further, a third group was found to have desipramine levels increase 2.5 times as a result of fluoxetine combined with the patient's rate of metabolism. Already, this antidepressant combination has shown that considerable caution and constant observation by the treating psychiatrist is imperative to the safety and health of the patient. This does not seem to be clinically reasonable as psychiatrists not only have many patients to treat, but also have other duties that greatly increase their workload. The addition of weekly blood collection and monitoring of desipramine plasma levels when augmented with fluoxetine would definitely be an unnecessary task.

Nevertheless, based on Montgomery-Asberg Depression Rating Scale (MADRS) scores which were used to determine rates of remission and response,

it was shown that those in the combination treatment were significantly more likely to achieve remission than those taking only desipramine (X^2=6.50, p=0.01) and those in the combination group were also more likely, although not significant, to achieve remission when compared to those taking only fluoxetine (X^2=4.99, p=0.025). Rates of response were found to be significantly different among all three treatment groups with response rates in the combined group being significantly higher than those in the fluoxetine-only group [$X^2(3)$=8.47, p=0.04] as well as when compared to those in the desipramine-only group [$X^2(3)$=13.4, p=0.004]. Rates of remission and response based on HAM-D_{17} scores also revealed greater rates of remission and response in the combination group when compared to the other two groups; however, these differences were found not to be significant. This is an interesting result because the HAM-D_{17} is regarded as the "gold standard" (Riedel, Moller et al. 2010) as it is the most universally used scale in assessing depressive symptoms. Therefore, these non-significant HAM-D_{17} results may potentially negate any argument about the superiority of this combination when compared to other antidepressant combinations showing significance in both the HAM-D_{17} and MADRS scales, indicating very robust and superior combinations. The authors concluded that for all patients who had an improvement of symptoms $\geq$25% (based on analysis of MADRS and HAM-D_{17} as continuous variables), patients in the combination group had significantly higher rates of improvement. Finally, differences in tolerance levels in all three groups were not significant.

As previously mentioned, combining fluoxetine with a TCA does not appear to be a clinically practical antidepressant combination. Nonetheless, although this particular serotonin and norepinephrine reuptake inhibitor combination is not an optimal choice, this study did demonstrate that indeed, the combination of an SSRI with a potent norepinephrine reuptake inhibitor (NRI) is significantly more efficacious than a single SSRI or NRI in improving depressive symptoms in an acute, 6-week treatment regimen. Therefore, finding other NRIs to augment single SSRI treatment based on more practical mechanism of action

interactions between the two appears to be of important clinical significance and must be further explored.

1.11.2.3 *Antidepressant Combination Study 3*

Another study (Raisi, Habibi et al. 2006) assessed the effectiveness and tolerability of combining citalopram with nortriptyline compared to citalopram alone in the treatment of moderate to severe depression. This combination was selected based on the theory of depression implicating irregular serotonin and noradrenaline neurotransmission (Correa, Duval et al. 2001; Raisi, Habibi et al. 2006). The authors believed that the serotonergic qualities exhibited by the SSRI citalopram (for a review of the mechanism of action of citalopram, please see Section 4) combined with the noradrenergic qualities displayed by nortriptyline (a TCA with noradrenergic enhancement qualities) would demonstrate a more robust efficacy and tolerability profile when compared to an agent that simply affects serotonergic neurotransmission.

The HAM-D_{17} scale was used to measure the severity of depressive symptoms and inclusion criteria required a score $\geq$20. This was much higher than scores previously reported, however, the study design was to treat moderate to severe depression and therefore, higher HAM-D_{17} scores (which indicate increased severity of depressive symptoms) was warranted. Citalopram (in both the combination and single groups) was given at a dose of 40mg/day (which falls directly between its dose range of 20 – 60mg/day) while nortriptyline was administered at 50mg/day. Of note was that in both groups, both citalopram and nortriptyline were titrated to their respective doses over the course of three days.

While both the combination and single treatment groups displayed significant decreases in HAM-D_{17} scores during the 8-weeks of treatment ($p=0.001$), treatment efficacy at week 8 for the combination group was significantly better than the citalopram-only group ($t=3.34$, $df=36$, $P=0.001$). It should be further noted that the results also suggested that this combination could

show significant decreases in HAM-D_{17} scores after only 2 weeks. This finding was not encountered in the citalopram-only group. No difference was reported in treatment tolerability as the frequency of side effects between the two groups was not significant.

Therefore, it can be concluded from this study that although treatment with a single SSRI, such as citalopram, does significantly improve depressive symptoms over an 8-week treatment period, its combination with a noradrenergic enhancing agent, such as the TCA nortriptyline, demonstrates a superior efficacy profile and can significantly decrease the time it takes for depressive symptoms to diminish if prescribed at treatment initiation. A lack of significant adverse reactions between the combination and citalopram-only groups further aids in the suggestion that initial treatment with an SSRI augmented with a TCA is much better than an initial treatment with only an SSRI. Other studies have also shown this superiority in terms of remission and response rates (Nelson, Mazure et al. 2004), which was discussed earlier in this section (see *Antidepressant Combination Study 2*).

1.11.2.4 *Antidepressant Combination Study 4*

A fourth study (Blier, Gobbi et al. 2009) assessed the combined efficacy of mirtazapine (a NaSSA) with paroxetine (an SSRI) at the beginning of treatment and compared these results with efficacy results found in treatment initiation with mirtazapine-only and paroxetine-only. Rationale for this combination was based on the synergistic and complementary mechanisms of action of paroxetine and mirtazapine on serotonin and norepinephrine neurotransmission. (For a review of SSRI and mirtazapine mechanisms of action, please see Sections 4 and 7, respectively.)

Inclusion criteria required a score $\geq$18 on the HAM-D_{17}, which was again higher than that used for inclusion into the STAR*D. The mean HAM-D_{17} score of all participants was approximately 24. The study conducted an acute treatment

of major depression as treatment was provided for 8-weeks (56 days). There was also the option for patients to continue their treatment regimen long-term (a 4 month treatment extension) if by day 42 they showed a substantial rate of response (an improvement of 50% or more on the Montgomery-Asberg Depression Rating Scale (MADRS)).

The MADRS was used to determine rates of remission and response in this study. Remission was defined as a score $\leq$10 on the MADRS on any day of treatment. Response, however, was defined as an improvement of 30% or more on the MADRS at day 28.

Mirtazapine was given in the morning and was administered via two 15mg tablets, making the dose of mirtazapine to be 30mg/day. This falls within the middle of mirtazapine's dosing range (15 – 45mg/day). To keep the study design consistent, paroxetine was also given in two tablets, each with a dose of 10mg. Therefore, the daily dose of paroxetine was 20mg/day, which falls at the lower end of its dosing range (20 – 60mg/day).

Based on HAM-D_{17} scores, there was a significant decrease from baseline HAM-D_{17} scores at day 35 ($F=4.38$, $P=0.02$) in the combination group when compared to mirtazapine-only ($P=0.005$) but was not found to be significant when the combination group was compared to the paroxetine-only group at day 35. However, at day 42, a significant decrease from baseline HAM-D_{17} scores in the combination group was found ($F=5.42$, $P=0.007$) when compared to both mirtazapine-only ($P=0.002$) and paroxetine-only ($P=0.04$) groups. An apparently common and, for some, worrisome side effect of mirtazapine (as well as paroxetine) is weight gain. There appears to be conflicting evidence with some studies stating that mirtazapine does not cause significant weight gain (Carpenter, Yasmin et al. 2002; Blier, Gobbi et al. 2009) while others argue that significant weight gain is indeed a side effect of mirtazapine (Wheatley, van Moffaert et al. 1998; Kraus, Haack et al. 2002), not only in acute treatment, but in long-term treatment as well (Fava 2000; Anttila and Leinonen 2001). In this acute treatment study of 56 days, no significant difference in weight gain was found in any of the

three treatment groups (at days 28, 42 and 56) when compared to each group's respective baseline weight (weight at day 0). However, of note was the significant weight gain observed in patients who were being treated in the 4 month prolongation.

Based on MADRS scores, by day-42, 43% of patients in the combination group, 26% of patients in the paroxetine-only group and 19% of those in the mirtazapine-only group achieved remission. The difference in rates of remission between these groups was not significantly different (X^2=0.22, P >0.05). The rate of response by day 28 was not significantly different between the three groups, however, at day 7, there was significant improvement on MADRS scores in the combination group (F=3.61, P=0.033) when compared to the paroxetine-only group (P=0.013), but not when compared to the mirtazapine-only group. Moreover, at day 28, there was a significant improvement on the MADRS in the combination group (F=3.28, P=0.045) when compared to both the paroxetine-only (P=0.02) and mirtazapine-only (P=0.046) groups. Among all three groups, no significant differences were found in the mean time to remission for those who achieved remission, or in the mean time to response. Further, no significant differences were found in adverse events between the three groups and tolerability was comparable. Combination therapy did however, fail to produce a quicker onset of action within the first 42 days of treatment.

Although the data was not shown in this review, the authors found that the addition of paroxetine to the mirtazapine-only group in patients not responding to monotherapy, and vice versa, remission was seen in about 50% of patients after 14 days. This, along with the significant efficacy and comparable tolerability results, helps strengthen the argument that the combination of mirtazapine with paroxetine provides a superior treatment efficacy when compared to either mirtazapine or paroxetine monotherapy.

1.11.2.5 *Antidepressant Combination Study 5*

In the meta-analysis (Cipriani, Furukawa et al. 2009) of 12 current antidepressants previously reviewed (Section 10), mirtazapine was shown to have the highest probability of efficacy at 24.4%. In addition, and as previously reported (Section 9), the STAR*D showed that the combination of mirtazapine with venlafaxine-SR was efficacious in the treatment of depression with HAM-D_{17} and QIDS-SR_{16} remission rates of 13.7% and 15.7%, respectively (McGrath, Stewart et al. 2006). However, these results were obtained when this combination was prescribed following several previous treatment failures, leaving one to ponder how efficacious this combination may be if prescribed at treatment initiation. The literature suggests that the combination of serotonergic and noradrenergic agents has a superior efficacy profile (achieving remission and a response) compared to when these agents are administered alone. Along with the hypothesis that combination treatment with two antidepressants at the beginning of therapy is superior to a single antidepressant at treatment initiation, this acute (6-weeks) double-blind randomized antidepressant combination study (Blier, Ward et al. 2010) attempted to answer the mirtazapine/venlafaxine question. In addition, the study evaluated the superiority of antidepressant combination treatment at the beginning of therapy and also provided further evidence to the more robust efficacy profile of an antidepressant combination which implicates both serotonin and norepinephrine neurotransmission when compared to a single antidepressant which only affects serotonergic neurotransmission. Therefore, this study randomly assigned participants into four different treatment groups; i) mirtazapine [NaSSA] + bupropion [atypical], ii) mirtazapine + fluoxetine [SSRI], iii) mirtazapine + venlafaxine [SNRI], and iv) fluoxetine-only.

Inclusion criteria required a score $\geq$18 on the HAM-D_{17} as well as a diagnosis of major depressive disorder based on requirements established in the DSM-IV (A.P.A. 2000). For 6-weeks, the daily dosage of mirtazapine was 30mg/day, which falls at the median of its dosing range (15 – 45mg/day). Also for 6-weeks, bupropion was administered in capsules containing 150mg of bupropion

powder mixed with methylcellulose which gave a slow-release of bupropion noted to be as effective as a 300mg dose of bupropion (Reimherr, Cunningham et al. 1998; Blier, Ward et al. 2010). 300mg/day is the median dosage of bupropion (dosing range of 150 – 450mg/day). Fluoxetine was also administered consistently for 6-weeks at a dose of 20mg/day, which falls on the low end of its therapeutic dosing range (20 – 60mg/day). Based on previous studies that assessed the effects of varying doses of venlafaxine on both serotonin and norepinephrine reuptake inhibition (Redrobe, Bourin et al. 1998; Debonnel, Saint-Andre et al. 2007), the dose of venlafaxine was gradually titrated to ensure proper norepinephrine reuptake inhibition (Blier, Ward et al. 2010). (A review of the mechanism of action of venlafaxine can be found in Section 5). This regimen required patients taking venlafaxine to be given 75mg/day for the first week, 150mg/day during the second week and 225mg/day for the remaining four weeks. These three doses cover the entire therapeutic dosing range of venlafaxine (75 – 250mg/day) and were administered in this manner because it has been found (Redrobe, Bourin et al. 1998; Debonnel, Saint-Andre et al. 2007) that at a lower dose (such as 75mg/day), venlafaxine acts simply as a serotonin reuptake inhibitor, while at higher doses (such as 250mg/day) it acts as both a serotonin and norepinephrine reuptake inhibitor.

The patients who participated in this study (Blier, Ward et al. 2010) suffered from moderate to severe depression as the mean HAM-D_{17} score was found to be 23. The mean MADRS score was approximately 32, further reinforcing the diagnosis of moderate to severe depression. At the conclusion of the acute (6-weeks) treatment, HAM-D_{17} scores revealed that from day 7 onward, the fluoxetine-only group displayed significant improvement in symptoms compared to their HAM-D_{17} scores at treatment initiation. However, the three groups involving combination treatment with mirtazapine exhibited a significant improvement beginning from day 4 onward, when comparing HAM-D_{17} scores from baseline. Arguably, the most important result was that, based on HAM-D_{17} scores (the "gold standard" in assessing depressive symptoms (Riedel, Moller et al. 2010)), there was a significant difference in the improvement of depressive

symptoms when the fluoxetine-only group's scores were compared to all three combination groups, indicating significantly greater improvement in all combination groups compared to the monotherapy group ($F=3.87$, $df=3, 101$, $p=0.011$).

When the symptom improvements in the three groups were compared to symptom improvements in the fluoxetine-only group, the mirtazapine + bupropion group was the first to have a significant difference in improvement, which began on day 21. The mirtazapine + venlafaxine group was next to show a significant difference beginning on day 28. Finally, the mirtazapine + fluoxetine group began to show a significant difference in improved symptoms compared to fluoxetine-only on day 35. The percentage of patients who achieved sustainable remission was significantly greater in the fluoxetine + mirtazapine (52%) and venlafaxine + mirtazapine (58%) groups when compared to the rates of sustained remission in the fluoxetine-only group (25%). Although the proportion of patients who achieved sustained remission in the bupropion + mirtazapine group (46%) was greater than the fluoxetine-only group (25%), it was not statistically significant. There were no significant differences among the four groups in terms of mean time to remission as they all produced this result in either 23 or 24 days. The proportion of patients who responded in each group was not significantly different, nor was the mean time to sustained response. In terms of tolerability, there were also no significant differences in the combination groups compared with the fluoxetine-only group; all were similarly tolerated.

The results suggested that combination treatment at the beginning of therapy, namely mirtazapine combined with either bupropion, fluoxetine or venlafaxine, not only improved depressive symptoms more robustly than fluoxetine-alone during a 6-week period, but these combinations also increased the probability of achieving remission when compared to fluoxetine monotherapy and although not significant, seem to achieve remission in a more time-efficient manner. A notable limitation of these results however, was that the long-half life of fluoxetine combined with the low dose used (a dose which may not have been

beneficial to some) in a short term treatment (6-weeks) may have contributed to the delayed onset of action and therefore, a longer term treatment may have produced higher remission rates in the fluoxetine-only groups (Nelson, Mazure et al. 2004; Rush 2010).

Weight increase has been a constant shortcoming for the use of mirtazapine in the treatment of depression, and the following results only further these concerns. While no significant weight gain was found in the fluoxetine-only group after 42 days of treatment (mean=+0.1kg, SD=1.5, N=25), patients in all three mirtazapine combination groups did experience significant weight gain after 42 days, with those in the fluoxetine combination group experiencing the highest increase (mean=3.1kg, SD=2.5, N=25), followed by the bupropion combination group (mean=2.7kg, SD=2.4, N=22) and finally, the venlafaxine combination group (mean=2.2kg, SD=2.5, N=24). The weight increases in the three mirtazapine combination groups were all significantly greater when compared to the fluoxetine-only group ($F=9.1$, $df=3, 92$, $p<0.001$). The six-month prolongation treatment revealed no significant weight change in the fluoxetine-only group (mean=-0.7, SD=1.9, N=10) and during this long-term treatment, all combination groups were effectively switched to mirtazapine-only treatment regimens and showed no significant weight changes (mean=$\pm$0.2, SD=3.5, N=12).

It is of note that in a previous study (Blier, Gobbi et al. 2009), the combination of mirtazapine with paroxetine did not report significant weight differences after 28, 42 and 56 days of (acute) treatment but did so during the four month prolongation.

When comparing mirtazapine combination results at day 42 from both studies (Blier, Gobbi et al. 2009; Blier, Ward et al. 2010), it appears that in short-term treatment, the mechanism of action of paroxetine either counters that of mirtazapine, or is dominant over mirtazapine since this NaSSA is associated with weight gain during acute treatment while paroxetine is associated with weight gain over the long-term (Blier, Gobbi et al. 2009). This suggests that the mechanism of action of mirtazapine in the short-term is just as influential as that

of paroxetine. Further, weight gain is not a common side effect associated with bupropion, fluoxetine or venlafaxine treatment, suggesting that in acute antidepressant combination treatment, the mechanism of action of mirtazapine is more influential than the three respective agents.

On the other hand, as previously mentioned, paroxetine has been implicated in weight gain during long-term treatment (Demyttenaere and Jaspers 2008; Blier, Gobbi et al. 2009) and the study which combined mirtazapine with paroxetine reported that, "there was a significant increase in patients taking the combination between the beginning and end of the four month prolongation (prior to: 68.1+4.0kg, after: 71.4+4.0kg, n=16, t=4.91, $p < 0.001$)" (Blier, Gobbi et al. 2009) . It appears then, that mirtazapine becomes less influential and yields to the mechanism of action of paroxetine. Further evidence suggesting that the mechanism of action of mirtazapine (at least the mechanism that influences weight) appears to functionally diminish over time can be seen by observing those patients who were assigned to the mirtazapine combination (with bupropion, fluoxetine or venlafaxine) acute treatment groups who took part in the long-term, six month prolongation (Blier, Ward et al. 2010). These individuals had their combination treatment changed during this time to mirtazapine-only and did not experience a significant change in weight from the beginning to the end of the six month prolongation, suggesting a weakened mechanism of action over the long-term. These values however, did not take into consideration the weight gains experienced during the initial, acute treatment study. In other words, the individual's weight at the conclusion of the first study (which may have been higher) became the new baseline weight when observing weight fluctuations during the six month prolongation.

1.11.3 *Review: Bupropion Used in Combination with SSRIs and SNRIs*

A review on the use of bupropion in combination with SSRIs and SNRIs was conducted in 2006 and concluded that the combination of bupropion with an SSRI or SNRI was found to not only augment the antidepressant response of an SSRI or SNRI alone, but was well tolerated and helped to alleviate sexual dysfunction side-effects commonly associated with serotonergic-mediated antidepressants such as SSRIs and SNRIs (Zisook, Rush et al. 2006).

Numerous studies (Bodkin, Lasser et al. 1997; Spier 1998; Coleman, Cunningham et al. 1999; Mischoulon, Nierenberg et al. 2000; Perlis, Fava et al. 2002; Ramasubbu 2002; DeBattista, Solvason et al. 2003; Lam, Hossie et al. 2004) have provided evidence supporting bupropion combination therapy with either of these two serotonergically-mediated antidepressants to be superior than monotherapy.

At the turn of the millennium, 801 clinicians (630 of whom were psychiatrists) were surveyed at the annual psychopharmacology review course at the Massachusetts General Hospital and asked how they would continue treatment for an MDD patient who had partially responded to SSRI monotherapy (Mischoulon, Nierenberg et al. 2000). 445 clinicians responded to the survey for 56% survey participation. When asked about what medication they would use as an augmenting agent, bupropion was found to be the most popular choice (n = 134, 30%) followed by lithium (n = 98, 22%). It was also interesting to note that the mean practice years of these clinicians was 16.6 years (SD = 10.7) and that the more experienced clinicians chose bupropion as their first-choice augmenting agent while the less experienced clinicians chose lithium as their first-choice.

In 2003, an acute (6-weeks) open-label study (DeBattista, Solvason et al. 2003) found evidence to support the combination of bupropion with serotonergic antidepressants. Inclusion criteria required patients to have a DSM-IV (A.P.A. 2000) diagnosis of MDD along with a failure to respond to serotonergically-mediated antidepressant monotherapy (fluoxetine, paroxetine, sertraline or

venlafaxine) after at least 4 weeks of treatment based on a score $\geq$15 on a 24-item HAM-D scale. The mean HAM-D score of all 28 participants (12 males, 16 females) who entered the study was 23.46 (SD = 5.99). The dose of bupropion given to each patient ranged between 150 – 300mg/day, which falls within its accepted dosing range (150 – 450mg/day). Of the 28 patients, 54% showed a response after 6-weeks based on a decrease of more than 50% on their HAM-D scores compared to baseline scores.

A study in 2004 (Lam, Hossie et al. 2004) further demonstrated the superiority of combining bupropion-SR with an SSRI (citalopram) compared to a monotherapy switch in patients whose treatment with at least 1 antidepressant for at least 6 weeks had failed. 32 patients were included in the acute (6-week) combination treatment and 29 patients were treated in the acute monotherapy switch (citalopram to bupropion-SR or vice versa). The mean doses of medication in the combination group were 33.1 $\pm$ 9.7 mg/day of citalopram and 248.4 $\pm$ 72.4mg/day of bupropion-SR. The mean doses of medication in the monotherapy group were 38.8 $\pm$ 13.2 mg/day of citalopram and 283.3 $\pm$ 68.5 mg/day of bupropion-SR. These mean doses fall between the lower to mid-range of the suggested daily dose range for each medication (20 – 60mg/day for citalopram and 150 – 450mg/day for bupropion). An adapted HAM-D scale called the 29-item Structured Interview Guide for the Hamilton Depression Rating Scale, Seasonal Affective Disorders Version (SIGH-SAD), which included the 21-item depression scale along with an 8-item supplement for atypical symptoms (Lam, Hossie et al. 2004) was administered to the patients before and 6 weeks after treatment. Compared to the monotherapy switch group, the combination group showed a significant improvement in the SIGH-SAD scores (-14.8 $\pm$ 10.1 in combination group compared to -10.1 $\pm$ 6.8 in the monotherapy group; t=2.1, df=59, p <0.04). No significant differences in SIGH-SAD scores were found in the monotherapy groups who switched from citalopram to bupropion-SR or vice versa. Rates of response based on SIGH-SAD scores, although higher in the combination group than monotherapy group (56% and 38%, respectively), were found to not be significantly different [X^2=2.0, df=1, p >0.15]. Rates of remission,

defined as an improvement $\geq$50% in SIGH-SAD score and a post-treatment SIGH-SAD score $\leq$10 (Lam, Hossie et al. 2004), were however, significantly greater in the combination group than monotherapy group (28% and 7%, respectively; X^2=4.6, df=1, p <0.05). Finally, no significant differences were found in the severity of adverse events between the different groups (Mann-Whitney U=404, p >0.35). These results led the authors to conclude the superior efficacy of citalopram (SSRI) and bupropion-SR combination therapy over an antidepressant monotherapy switch for the acute (6-week) treatment of patients with treatment-resistant depression (Lam, Hossie et al. 2004).

It must also be noted that the combination of bupropion-SR and citalopram was given to patients in Level 2 of the STAR*D after failing to achieve remission following an initial treatment with citalopram. Although not significant, this combination was found to be more advantageous in the overall treatment of these patients (please see Section 9) (Trivedi, Fava et al. 2006). As the literature shows, there is convincing evidence for bupropion combination with an SSRI or SNRI compared to SSRI or SNRI monotherapy.

CHAPTER 2

ANTIDEPRESSANT COMBINATION AND MONOTHERAPY TREATMENT IN MAJOR DEPRESSIVE DISORDER

Antidepressant Combination and Monotherapy Treatment in Major Depressive Disorder

2.1 *Introduction*

The McGill University Health Centre (MUHC) Mood Disorders Clinic (MDC) is an outpatient tertiary care centre specializing in the treatment of major depression and bipolar disorder. All patients undergo an initial diagnostic assessment by a multidisciplinary team composed of psychiatrists, social workers, MDC staff members and students in order to diagnose and evaluate the severity of the patient's illness and his or her clinical needs.

Patients with major depressive disorder (MDD) referred to the MDC usually present increased symptom severity as a result of a failure to remit or respond (fully or partially) to previous antidepressant treatment. As a consequence of the delay in observing any substantial mood improvement with their initial antidepressant medication, an estimated 50% of patients stop taking their medication within the first 12-weeks of treatment (Lin, Von Korff et al. 1995; Melfi, Chawla et al. 1998; Blier, Gobbi et al. 2009). Evidence-based guidelines suggest medication compliance for at least 6 months in order to allow time for the antidepressant to be effective and reduce the possibility of relapse (Anderson, Ferrier et al. 2008). These failures have led some clinicians to study the efficacy of antidepressant combination therapy and although shown to be effective (Dam, Ryde et al. 1998; DeBattista, Solvason et al. 2003; Lam, Hossie et al. 2004; Nelson, Mazure et al. 2004; Raisi, Habibi et al. 2006; Blier, Gobbi et al. 2009; Blier, Ward et al. 2010; Rocha, Fuzikawa et al. 2012), the use of combination treatment strategies remains limited (Cascade, Kalali et al. 2007; Lenderts and Kalali 2009).

Having previously reviewed the literature in Chapter 1 for the most efficacious and well-tolerated antidepressants and antidepressant combination treatments, we will now critically compare these findings with clinical

information obtained from MDD outpatients referred to the MDC. A critical comparison of the most efficacious and well-tolerated antidepressant combination treatments from the literature were found to be i) SSRI with bupropion, ii) SSRI with mirtazapine, iii) SNRI with bupropion, iv) SNRI with mirtazapine, and v) mirtazapine with bupropion. The combination of certain SSRIs with a TCA was not found to be clinically practical due to the concern for adverse pharmacokinetic interactions between these drugs (Nelson, Mazure et al. 2004) along with studies showing this combination to not be clinically effective in treatment-resistant depression (Fava, Rosenbaum et al. 1994).

2.2 *Objectives*

Objective 1: To present sociodemographic information of patients with major depressive disorder including i) age, ii) gender, iii) marital status, iv) level of education, v) employment, and vi) living arrangement.

Objective 2: To present the clinical psychiatric history of patients with major depressive disorder including i) currently prescribed psychiatric medications, ii) age of 1st psychiatric consultation, iii) age of 1st psychiatric hospitalization, iv) age of most recent psychiatric hospitalization, v) number of psychiatric hospitalizations, vi) number of suicide attempts, and vii) number of 1st degree relatives with a psychiatric illness. Psychiatric medications included i) antidepressants, ii) antipsychotics, iii) mood stabilizers and iv) other psychiatric medications.

Objective 3: To report the prescription frequency of each class of antidepressant to patients with MDD. Antidepressants were categorized as i) MAOIs, ii) TCAs, iii) SSRIs, iv) SNRIs, v) NDRIs, vi) NaSSAs, and vii) SARIs.

Objective 4: To report the frequency of prescribed antidepressant combinations in patients referred to a tertiary care centre with major depressive disorder.

Objective 5: Based on a critical review of the literature, the most efficacious and recommended antidepressant combination treatments were discerned. The objective was to compare and report the clinical psychiatric history of patients prescribed these antidepressant combinations.

Moreover, we also reported scores of the Beck Depression Inventory-II (BDI-II) correlated with antidepressant combinations. However, as only 60 patients completed the BDI-II, it was not possible to correlate with statistical analysis.

2.3 *Sample*

Patients with a current (defined as within 6-months) DSM-IV (A.P.A. 2000) diagnosis of major depressive disorder aged 18 years or older were included in this study. Patients with a diagnosis of MDD prior to the 6 months of their initial diagnostic evaluation or with a diagnosis of bipolar disorder (BD) were excluded. A total of 133 patients met the inclusion criteria and were analyzed in this study.

2.4 *Procedures and Measures*

A medical clinical record (MCR) of the patient was completed during the initial diagnostic evaluation and any missing information was obtained by chart review. The Psychiatry/Psychology (PSY) Research Ethics Board (REB) of the MUHC provided approval for chart review. The MCR contained sociodemographic information, psychiatric diagnoses, psychiatric medications, and clinical psychiatric history concerning psychiatric hospitalizations, consultations, suicidal behaviour and familial psychiatric history.

The sociodemographic information of each patient analyzed included i) age, ii) gender, iii) marital status, iv) level of education, v) employment, and vi) living arrangement.

Only patients with a current (within the past 6 months) diagnosis of MDD were included in the analysis.

Psychiatric medication categories were divided into i) antidepressants, ii) antipsychotics, iii) mood stabilizers and iv) other psychiatric medications. Only currently prescribed (within the past 6 months) psychiatric medications were included in the analysis. *Antidepressants included* i) MAOIs, ii) TCAs, iii) SSRIs, iv) SNRIs, v) NDRIs, vi) NaSSAs, and vii) SARIs. *Antipsychotics included* i) typical antipsychotics, ii) olanzapine, iii) quetiapine, iv) risperidone, v) paloperidone, vi) ziprasidone, and vii) long-acting/depot injections. *Mood stabilizers included* i) lithium, ii) valproic acid, iii) lamotrigine, iv) carbamazepine/oxicarbamazepine (CBZ/OXCBZ), v) topiramate and vi) gabapentin. *Other psychiatric medications included* i) benzodiazepines, ii) hypnotics, iii) busparone, iv) pindolol, v) tryptanophan, vi) T3 and vii) T4. Please note that while many psychiatrists prescribe the SARI trazodone as an off-label hypnotic, because it has been approved by the FDA as a high dose antidepressant and not as a low dose hypnotic (Stahl 2009), *this study classified trazodone only as an antidepressant*.

Only currently prescribed (within the past 6 months) antidepressants were included in the drug combination treatment analysis. Evidence-based guidelines suggest that patients continue taking an antidepressant for at least 6 months in order to allow time for the medication to be effective and to reduce the possibility of relapsing back into depression (Anderson, Ferrier et al. 2008). Antidepressants included i) MAOIs, ii) TCAs, iii) SSRIs, iv) SNRIs, v) NDRIs, vi) NaSSAs, and vii) SARIs.

The clinical psychiatric history of patients with MDD included i) age of 1st psychiatric consultation, ii) age of 1st psychiatric hospitalization, iii) age of most

recent psychiatric hospitalization, iv) number of psychiatric hospitalizations, v) number of suicide attempts, and iv) number of 1st degree relatives with a diagnosed psychiatric illness.

In addition to the MCR, at the initial diagnostic assessment, each patient was asked to complete a modified 20-item Beck Depression Inventory-II (BDI-II) questionnaire (Beck, Ward et al. 1961; Beck, Steer et al. 1993; Beck, Steer et al. 1996). The BDI-II has been shown to be positively correlated with the Hamilton Rating Scale for Depression (HAM-D) (Beck, Steer et al. 1996), which is the most universally used scale in assessing depressive symptoms and generally regarded as the "gold standard" (Riedel, Moller et al. 2010). Each item (or question asked) on the 20-item BDI-II was rated according to a 4-point scale with the score of each item ranging from 0 – 3 and the total BDI-II score ranging from 0 – 60. Total BDI-II scores reflected the severity of depressive symptoms and were evaluated as follows: 0 – 13, minimal depression; 14 – 19, mild depression; 20 – 28, moderate depression; 29 – 60, severe depression (Beck, Ward et al. 1961; Beck, Steer et al. 1993; Beck, Steer et al. 1996). Those who did not complete the BDI-II during the initial diagnostic evaluation were asked to return using a clinic-addressed stamped envelope provided by the MDC.

Furthermore, there were four selected antidepressant combination treatments that were analyzed from the MDC database, i) SSRI + bupropion, ii) SNRI + bupropion, iii) SNRI + mirtazapine, and iv) mirtazapine + bupropion. The combination of an SSRI + mirtazapine was omitted due to very low clinical usage (<3.0%).

2.5 *Statistical Analysis*

Sociodemographic, clinical psychiatric history and BDI-II data were analyzed using IBM® SPSS® statistical program version 20 and Systat Software Inc.® Sigma Plot® version 12.0 for Windows®. Pearson chi-square (χ^2) and Pearson correlation tests were conducted on categorical variables and Yates'

correction for continuity was used when appropriate. Age, total BDI-II scores and clinical psychiatric history variables are expressed as means ± SD (standard deviation). Means were compared using analysis of variance (ANOVA) testing and Student-Newman-Keuls post hoc comparisons were performed when necessary. A significance level of $p < 0.05$ was used.

2.6 *Results*

Data was analysed for one hundred thirty-three patients (87 female, 46 male) with MDD and medication history was limited to the six months prior to the patients' initial diagnostic assessment at the MDC. Complete sociodemographic information and clinical psychiatric history are presented in Table 1. A sizable gender difference was observed as significantly more women than men suffered from MDD, 65.4% and 34.6%, respectively, $p = 0.000$. However, there was no significant difference between the mean age of women and men (in years), 48.5 ± 14.2 and 49.4 ± 14.5, respectively, while the mean age (in years) of all patients was 48.8 ± 14.2. Just over one-third of the patients were either married (36.6%) or single (35.1%) while close to one-fifth of the patients were divorced/separated (19.1%). The most common level of education was at the university level where 40.5% had obtained a bachelor's degree. High school or college/CEGEP completion had similar completion rates, 19.8% and 19.0%, respectively. Furthermore, about one-half of the patients were unemployed (51.1%). The majority of patients did not live alone (63.6%) and most lived with a partner/spouse with or without children (60.7%) while 17.9% lived with their parents.

The clinical psychiatric history of these patients showed that 87.2% had been prescribed an antidepressant within the past 6 months and 38.3% of the patients had been prescribed an antipsychotic. The mean age (in years) of the first psychiatric consultation of all the patients was 34.5 ± 13.2. Half of the patients had been admitted into a psychiatric hospital (49.6%) and the mean age (in years)

of these patients' first psychiatric hospitalization and most recent psychiatric hospitalization was 39.6 $\pm$ 11.6 and 44.3 $\pm$ 11.6, respectively. About one-fifth of the patients (21.4%) had visited a psychiatric ER once within the 12 months of their initial assessment at the MDC. Just over one-third of the patients diagnosed with MDD (34.6%) had attempted suicide and of those, over one-half (55.8%) had tried once while one-quarter (25.6%) had attempted suicide twice. The vast majority of all patients diagnosed with MDD (72.4%) had at least one first-degree relative diagnosed with a psychiatric illness.

The mean numbers of prescribed psychiatric medications are presented in Table 2 and the mean total number of prescribed psychiatric medications was 2.5 $\pm$ 1.4. The mean number of prescribed antidepressants was 1.3 $\pm$ 0.7, antipsychotics (0.4 $\pm$ 0.6), mood stabilizers (0.2 $\pm$ 0.5) and other psychiatric medications (0.5 $\pm$ 0.6). A one-way between groups ANOVA conducted on the mean number of prescribed psychiatric medications yielded a significant difference between the medication treatment categories, $F(3,528) = 75.9$, $p < 0.001$. Student-Newman-Keuls post hoc tests showed that antidepressants were prescribed significantly more often to patients diagnosed with MDD than antipsychotics, mood stabilizers and other psychiatric medications, $p < 0.05$. Furthermore, antipsychotics and other psychiatric medications were prescribed at a significantly higher frequency rate than mood stabilizers, $p < 0.05$. The difference in mean numbers of prescriptions between antipsychotics and other psychiatric medications were not significant, $p > 0.05$.

Table 3 reports the percentages of patients prescribed each class of antidepressant. The percentage of patients prescribed an SSRI was 42.1%, SNRI (30.8%), bupropion (25.6%), mirtazapine (12.0%), TCA (8.3%), MAOI (3.0%) and SARI (9.8%). A one-way between groups ANOVA conducted on the data yielded a statistically significant difference among the seven different antidepressant classes, $F(6,924) = 19.9$, $p < 0.001$. Student-Newman-Keuls post hoc analyses revealed that significantly more patients were prescribed an SSRI than all other antidepressant classes, $p < 0.001$. Significantly more patients were

prescribed an SNRI than mirtazapine, TCA, MAOI or SARI, $p < 0.05$, but not an SSRI or bupropion, $p > 0.05$. Furthermore, significantly more patients were prescribed bupropion than mirtazapine, TCA, MAOI or SARI, $p < 0.05$, but not an SSRI or SNRI, $p > 0.05$.

The percentages of patients prescribed an antidepressant in combination with another antidepressant are reported in Table 4. Pearson chi-square tests indicated that the most frequent antidepressant combinations were bupropion with an SNRI or SSRI, as 9.8% and 8.3% of patients, respectively, were prescribed these combinations. Mirtazapine prescribed with an SNRI or bupropion were the next most common antidepressant combinations, 4.5% and 3.0% of patients, respectively. Pearson correlation tests conducted on the data revealed a significant correlation between an SSRI and mirtazapine at the 0.05 level and a significant correlation between an SSRI and SNRI at the 0.01 level.

Table 5 presents the correlation between the four selected antidepressant combination treatments and select clinical psychiatric history variables [i) mean age (years) of first psychiatric consultation, ii) mean age (years) of first psychiatric hospitalization, and iii) mean age (years) of most recent psychiatric hospitalization]. No statistical significance was found between any of the four antidepressant combination treatments and the mean age of first psychiatric consultation ($p = 0.620$), first psychiatric hospitalization ($p = 0.260$) or most recent psychiatric hospitalization ($p = 0.109$).

The mean total BDI-II scores, and in turn the severity of depressive symptoms, for patients prescribed the four selected antidepressant combination treatments are reported in Table 6. The mean total BDI-II scores, and symptom severity, for patients prescribed each antidepressant combination treatment were as follows, SSRI + bupropion (21.4 ± 16.0, moderate depression), SNRI + bupropion (31.6 ± 13.1, severe depression), SNRI + mirtazapine (28.4 ± 8.8, moderate/severe depression), and mirtazapine + bupropion (33.8 ± 11.4, severe depression). A one-way between groups ANOVA revealed no statistically

significant difference between antidepressant combination treatments and mean total BDI-II scores, $F(3,19) = 1.015$, $p > 0.05$.

2.7 *Tables*

Table 1: Sociodemographic and Clinical Characteristics of 133 Patients with Major Depressive Disorder (1/2)

Characteristic	MDD (N)	n (%)	Mean ± SD	*P*-value
Age, y	N = 132		48.8 ± 14.2	
18 - 29		14 (10.6)		
30 - 39		19 (14.4)		
40 - 49		31 (23.5)		
50 - 59		36 (27.3)		
60 - 69		25 (18.9)		
≥ 70		7 (5.3)		
Gender	N = 133			**0.000***
Female		87 (65.4)		
Male		46 (34.6)		
Mean Gender Age	N = 132			0.707
Female (N = 86)			48.5 ± 14.2	
Male (N = 46)			49.4 ± 14.5	
Marital Status	N = 131			
Married		48 (36.6)		
Common Law		7 (5.3)		
Single		46 (35.1)		
Widow(er)		5 (3.8)		
Divorced/Separated		25 (19.1)		
Education	N = 116			
Elementary		2 (1.7)		
High School		23 (19.8)		
College/CEGEP		22 (19.0)		
Bachelor's Degree		47 (40.5)		
Master's Degree		14 (12.1)		
Doctorate		4 (3.4)		
Technical/Vocational Program/DEP		4 (3.4)		
Employed	N = 131			
Yes		64 (48.9)		
No		67 (51.1)		
Lives Alone	N = 132			
Yes		48 (36.4)		
No		84 (63.6)		
Lives With	N = 84			
Partner/Spouse (+/- Children)		51 (60.7)		
Parents		15 (17.9)		
Other Relatives		5 (5.9)		
Friends		4 (4.8)		
Group Home/Foster Home/Supervised Housing		1 (1.2)		
Children/Offspring		8 (9.5)		
Currently Prescribed Psychiatric Medication(s)	N = 133			
Antidepressants		116 (87.2)		
Antipsychotics		51 (38.3)		
Mood Stabilizers		29 (21.8)		
Other Medications		56 (42.1)		
Age of 1st Psychiatric Consultation	N = 117		34.5 ± 13.2	
< 18		11 (9.4)		
18 - 29		36 (30.8)		
30 - 39		28 (23.9)		
40 - 49		24 (20.5)		
50 - 59		13 (11.1)		
60 - 69		5 (4.3)		
≥ 70		0 (0.0)		

*p <0.05

Table 1: Sociodemographic and Clinical Characteristics of 133 Patients with Major Depressive Disorder (2/2)

Characteristic	MDD (N)	n (%)	Mean ± SD	*P*-value
Psychiatric Hospitalizations	N = 125			
Yes		62 (49.6)		
No		63 (50.4)		
Number of Psychiatric Hospitalizations	N = 59			
1 - 3		49 (83.1)		
4 - 6		7 (11.9)		
7 - 10		1 (1.7)		
> 10		2 (3.4)		
Age of 1st Psychiatric Hospitalization	N = 61		39.6 ± 11.6	
< 18		1 (1.6)		
18 - 29		11 (18.0)		
30 - 39		21 (34.4)		
40 - 49		14 (23.0)		
50 - 59		12 (19.7)		
60 - 69		2 (3.3)		
≥ 70		0 (0.0)		
Age of Most Recent Psychiatric Hospitalization	N = 61		44.3 ± 11.6	
< 18		1 (1.6)		
18 - 29		3 (4.9)		
30 - 39		22 (36.1)		
40 - 49		15 (24.6)		
50 - 59		12 (19.7)		
60 - 69		7 (11.5)		
≥ 70		1 (1.6)		
Number of Psychiatric ER Visitis in the Past Year	N = 103			
0		77 (74.7)		
1		22 (21.4)		
2		2 (1.9)		
3		1 (1.0)		
≥ 4		1 (1.0)		
Ever Attempted Suicide	N = 130			
Yes		45 (34.6)		
No		85 (65.4)		
Number of Suicide Attempts	N = 43			
1		24 (55.8)		
2		11 (25.6)		
3 - 5		5 (11.6)		
> 5		3 (7.0)		
Any 1st Degree Relatives with a Psychiatric Illness	N = 123			
Yes		89 (72.4)		
No		34 (27.6)		
Number of 1st Degree Relatives with a Psychiatric Illness	N = 89			
1		37 (41.6)		
2		29 (32.6)		
3		15 (16.9)		
4		4 (4.5)		
5		2 (2.2)		
6		2 (2.2)		

*p <0.05

Table 2: Number of Current Psychiatric Medications Prescribed to 133 Patients with Major Depressive Disorder

	Mean ± SD
Total Number of Prescribed Psychiatric Medications	2.5 ± 1.4
*Prescribed Psychiatric Medication Category**	
Number of Prescribed Antidepressants	1.3 ± 0.7
Number of Prescribed Antipsychotics	0.4 ± 0.6
Number of Prescribed Mood Stabilizers	0.2 ± 0.5
Number of Other Prescribed Psychiatric Medications	0.5 ± 0.6

*$p < 0.001$

Table 3: Percentage of Patients with Major Depressive Disorder (N = 133) Prescribed Each Class of Antidepressant

Antidepressant	Percentage of Patients (%)*
SSRI	42.1
SNRI	30.8
Bupropion	25.6
Mirtazapine	12.0
TCA	8.3
MAOI	3.0
SARI	9.8

* $p < 0.001$

Table 4: Percentage of Patients with Major Depressive Disorder (N = 133) Precribed an Antidepressant in Combination with a Second Antidepressant

	Antidepressant						
	SSRI	SNRI	Bupropion	Mirtazapine	TCA	MAOI	SARI
Antidepressant							
SSRI		3.8%**	8.3%	2.3%*	3.0%	0.0%	3.8%
SNRI	3.8%**		9.8%	4.5%	1.5%	0.8%	3.0%
Bupropion	8.3%	9.8%		3.0%	0.8%	0.0%	2.3%
Mirtazapine	2.3%*	4.5%	3.0%		0.8%	0.8%	0.8%
TCA	3.0%	1.5%	0.8%	0.8%		0.0%	1.5%
MAOI	0.0%	0.8%	0.0%	0.8%	0.0%		0.8%
SARI	3.8%	3.0%	2.3%	0.8%	1.5%	0.8%	

**Pearson correlation is significant at the 0.01 level

*Pearson correlation is significant at the 0.05 level

Table 5: Correlation Between Select Antidepressant Combination Treatments and Clinical Psychiatric History for Patients with Major Depressive Disorder

Combination	Age (years) of 1st Psychiatric Consultation (N = 117) N	(Mean ± SD)	Age (years) of 1st Psychiatric Hospitalization (N = 61) N	(Mean ± SD)	Age (years) of Most Recent Psychiatric Hospitalization (N = 61) N	(Mean ± SD)
SSRI + Bupropion (N = 11)	11	(30.7 ± 11.6)	5	(39.4 ± 9.5)	5	(40.0 ± 8.9)
SNRI + Bupropion (N = 13)	12	(31.9 ± 11.6)	5	(42.6 ± 8.8)	5	(49.0 ± 10.2)
SNRI + Mirtazapine (N = 6)	6	(39.5 ± 19.6)	4	(53.3 ± 11.8)	4	(54.5 ± 12.4)
Mirtazapine + Bupropion (N = 4)	4	(31.5 ± 14.2)	2	(44.5 ± 10.6)	1	(37.0)
P-value		0.620		0.260		0.109

Table 6: Mean Total BDI-II Scores of Select Antidepressant Combination Treatments for Patients with Major Depressive Disorder

	BDI (N = 60)		
Combination	Total Score (Mean ± SD)	Severity of Depression*	*P*-value
			0.408
SSRI + Bupropion (N = 7)	21.4 ± 16.0	Moderate	
SNRI + Bupropion (N = 7)	31.6 ± 13.1	Severe	
SNRI + Mirtazapine (N = 5)	28.4 ± 8.8	Moderate/Severe	
Mirtazapine + Bupropion (N = 4)	33.8 ± 11.4	Severe	

*BDI-II Total Scores (Beck, Ward et al. 1961; Beck, Steer et al. 1993; Beck, Steer et al. 1996):
0 - 13: Minimal Depression
14 - 19: Mild Depression
20 - 28: Moderate Depression
≥ 29: Severe Depression

2.8 *Discussion*

These results not only present the MDC with in-depth sociodemographic and clinical psychiatric histories of their outpatients, but also provide insight into the clinical prescription patterns of psychiatrists, and clinicians, in treating patients with MDD.

Before communicating medication prescription patterns, it must be noted that a significant gender difference among patients referred to the MDC was found as nearly two-thirds were female, which is consistent with the literature indicating that women are twice as likely to suffer from major depression than men (Culbertson 1997; Merikangas and Swendsen 1997; Nolen-Hoeksema 2001; Merikangas and Low 2004). Childhood adversity, socio-cultural roles and a predisposition to stress (Merikangas and Low 2004) are some examples used to explain the underlying factors associated with this gender difference and more research is needed in order to obtain a better understanding of this occurrence.

It was also interesting to observe the rather high level of education and employment reported by these outpatients since major depressive disorder has been associated with low education and employment levels (Merikangas and Low 2004; Vasiliadis, Lesage et al. 2007; Fleury, Grenier et al. 2010). This finding may, in part, be a consequence of the MDC being a teaching-based clinic associated with a distinguished university located in the downtown core of a major Canadian city. It would be interesting to compare the educational and employment levels of outpatients at non-teaching based psychiatric tertiary care centres not affiliated with educational institutions and located in lower socio-economic status communities.

The high percentage of patients with a first degree relative diagnosed with a psychiatric illness is consistent with the literature suggesting that a positive family history is one of the strongest predictors for MDD (Weissman, Gammon et al. 1987; Birmaher, Ryan et al. 1996; Merikangas and Low 2004). In fact, it has been reported that there is a 60% probability that an individual who has a parent

diagnosed with a psychiatric illness will suffer from major depressive disorder by 25 years of age (Beardslee, Keller et al. 1993; Merikangas and Low 2004). This is almost 10 years younger than the mean age of the first psychiatric consultation reported by patients referred to the MDC, however, there has been evidence suggesting that the delay in seeking psychiatric treatment following the initial onset of a mood disorder, including MDD, ranges from 1.0 to 14.0 years (Wang, Angermeyer et al. 2007). Further, the mean ages of the patients referred to the MDC, as well as the mean ages of their first psychiatric consultation, first psychiatric hospitalization and most recent psychiatric hospitalization, revealed the gradual rate of relapse and progression of symptom severity in majorly depressed patients whose initial and subsequent antidepressant treatments had failed, leading to treatment-resistant depression (TRD).

The first chapter of this thesis reviewed the literature of antidepressant studies conducted at the Neurobiological Psychiatry Unit, and from PubMed and OvidSP, to determine the most efficacious antidepressants and antidepressant combination treatments in order to critically compare these findings with the clinical information obtained from outpatients referred to the MDC. With respect to prescribed psychiatric medications, antidepressants remained the preferred medication option for the treatment of major depression with SSRIs being the most prescribed antidepressant followed by an SNRI and bupropion. These are consistent with findings that SSRIs and SNRIs are an effective option not only for persons suffering from moderate to severe depression, but also for those suffering from TRD (Andrews, Ninan et al. 1996). Despite the fact that treatment with SNRIs, including venlafaxine, has been found to be therapeutically superior to SSRIs, including citalopram, fluvoxamine, paroxetine and sertraline (Clerc, Ruimy et al. 1994; Poirier and Boyer 1999; Blier 2006; Nemeroff, Entsuah et al. 2008), the efficacy profile and tolerance of SSRIs has remained high and their frequent use in the treatment of major depressive disorder is still justified.

Moreover, the addition of an antipsychotic to an antidepressant has been shown to be similarly effective as the addition of a second antidepressant (Blier

and Blondeau 2011) and therefore, it was not surprising to observe that these agents were prescribed at a significantly higher rate than mood stabilizers but at a significantly lower rate than antidepressants. However, the mean total number of prescribed psychiatric medications was lower than expected since the number of medications prescribed has been shown to increase with heightened symptom severity (Cascade, Kalali et al. 2007), and it is these patients who are usually referred to tertiary care clinics.

Evidence from the literature (Dam, Ryde et al. 1998; Nelson, Mazure et al. 2004; Raisi, Habibi et al. 2006; Blier, Gobbi et al. 2009; Blier, Ward et al. 2010; Rocha, Fuzikawa et al. 2012) has reported that the use of antidepressant combination therapy at treatment initiation has increased rates of remission in a more time-efficient manner and displays no significant differences in medication adverse effects and tolerability when compared to a single antidepressant treatment. A critical review of the literature found the most efficacious and well-tolerated antidepressant combination treatments to be the combination of an SSRI with bupropion, an SSRI with mirtazapne, an SNRI with bupropion, an SNRI with mirtazapine, and mirtazapine in combination with bupropion. It was interesting however, that although the combination of an SSRI with mirtazapine has been shown to be clinically therapeutic and well tolerated (Blier, Gobbi et al. 2009), only 2.3% of the patients had been prescribed this combination. This finding may, in part, be explained by the possible sexual dysfunction side-effects associated with an SSRI as well as weight gain commonly observed with mirtazapine treatment.

Despite an ample amount of evidence supporting the effectiveness of the combination of bupropion with either an SSRI or SNRI (Bodkin, Lasser et al. 1997; Spier 1998; Coleman, Cunningham et al. 1999; Mischoulon, Nierenberg et al. 2000; Perlis, Fava et al. 2002; Ramasubbu 2002; DeBattista, Solvason et al. 2003; Lam, Hossie et al. 2004; Zisook, Rush et al. 2006), these combinations were prescribed rather infrequently to patients who had been referred to the MDC. However, in spite of their low usage, bupropion with an SNRI or SSRI remained

the two most prescribed medication combinations, respectively. Moreover, the large difference in mean baseline total BDI-II scores between these two combinations, although not significant, was rather unexpected. Based on these mean BDI-II scores, patients who were taking bupropion in combination with an SSRI were moderately depressed while those taking bupropion with an SNRI were severely depressed. The low sample size of patients with a complete BDI-II may have been due to the fact that if the patient did not complete the BDI-II during his or her initial diagnostic assessment at the MDC, the patient was asked to return it to the clinic once completed at home. As there was no deadline, many patients may have delayed its completion and subsequent return to the clinic. The discrepancy in BDI-II means between the four antidepressant combination treatments may have been due to the methodology involved in MCR collection and BDI-II reporting as the MCR and BDI-II were not always completed at the same time. The mood of a person constantly changes, which could have altered the score on their BDI-II depending on how they were feeling when they completed it. Therefore, an increased population along with completion of the BDI-II before leaving the MDC may alter, drastically or moderately, the scores of the BDI-II, and consequently, the reported severity of depressive symptoms. This would allow the MDC to obtain a clearer picture of the severity of depression together with the medication combinations of their patients.

Patients prescribed an SSRI with bupropion were the only ones to be on the lower-end of the moderately depressed scale while patients taking the other three combinations were either on the higher-end of the moderately depressed scale or lower-end of the severely depressed scale. Again, these scores were based on low population sizes and the BDI-II may have been completed at home several days or weeks after the initial diagnostic assessment. Therefore, the tendency for people to "self-enhance" on self-report questionnaires (Pronin 2007; Fiske 2009) combined with the possibility that the individuals completed the BDI-II when they were feeling "good" or "up to it" may have resulted in the lower BDI-II score for bupropion with an SSRI. As previously mentioned, there was no reliable way of knowing when the patients completed the BDI-II after they had left the clinic.

However, it is interesting to note that the mean BDI-II score of the SSRI and bupropion combination found in this study was very similar to the mean baseline BDI score obtained in a previous study, 21.31 $\pm$ 9.47 (DeBattista, Solvason et al. 2003), providing further evidence to the efficacy of this combination.

Further, SNRIs in combination with either bupropion or mirtazapine had mean BDI-II scores that were similar, but still higher than the mean score of an SSRI with bupropion. Since bupropion and mirtazapine both block 5-HT_2 receptors, the sexual dysfunction side effects associated with serotonergically-mediated drugs may indeed have been avoided. However, the increased BDI-II scores of an SNRI with either bupropion or mirtazapine were surprising as several studies, including the STAR*D, have shown these combinations to be quite efficacious (McGrath, Stewart et al. 2006; Zisook, Rush et al. 2006; Blier, Ward et al. 2010). As the dosages of the SNRIs were not ascertained, it was not possible to verify if the SNRI only blocked the reuptake of 5-HT or was administered at the higher doses required to significantly inhibit the reuptake of both 5-HT and NE.

While the combination of mirtazapine and bupropion has a faster onset of symptom improvement compared with the other combinations in acute treatment of MDD, over a longer duration, it has been shown to be slightly less efficacious than the other previously reported combinations (Blier, Ward et al. 2010). In this study, it was associated with the highest mean BDI-II score amongst the analyzed combinations. However, because mirtazapine with bupropion has been shown to improve depressive symptoms faster than other combinations (Blier, Ward et al. 2010), based on the clinical psychiatric history and past treatment failures, some patients diagnosed with MDD may be more compliant to adhere to this combination regimen if symptom improvement is prompt.

2.9 *Limitations and Future Considerations*

As previously mentioned, the methodology involved in the timing of

MCR collection and BDI-II reporting was not synchronized, possibly skewing the association between antidepressant combination treatments and scores of the BDI-II along with the reported severity of depressive symptoms. Further, due to the low completion rate of the BDI-II, the powers of performed tests were lower than the desired powers and therefore, a difference may not have been detected despite one actually existing. In addition, due to the tendency of people to "self-enhance" on self-report questionnaires (Pronin 2007; Fiske 2009) and the fact that descriptive approaches remain the only tool for psychiatric diagnoses (Merikangas and Low 2004), continuation of this study would be to observe Clinical Global Impression (CGI) Severity of Illness scores from the date of initial diagnostic assessment and 6 months thereafter, along with a 6-month CGI Global Improvement score, in order to observe the long-term efficacy of these combinations on symptom improvements. Further, a larger cohort of patients in each combination treatment will allow for more definitive and clinically meaningful differences between these treatment strategies.

CHAPTER 3

GENERAL DISCUSSION

3.1 General Discussion

Antidepressants remain the leading medication choice for the treatment of major depressive disorder and although numerous studies (Dam, Ryde et al. 1998; Nelson, Mazure et al. 2004; McGrath, Stewart et al. 2006; Raisi, Habibi et al. 2006; Rocha, Fuzikawa et al. 2012) have repeatedly supported the use of antidepressant combination strategies for effective and time-efficient treatment, especially at the beginning of therapy (Blier, Gobbi et al. 2009; Blier, Ward et al. 2010), many psychiatrists and primary care clinicians still appear hesitant on using this approach. This may be due to potentially dangerous, and sometimes lethal, drug-drug interactions involving psychiatric and/or non-psychiatric medications.

The combination of bupropion with serotonergically-mediated antidepressants, namely SSRIs and SNRIs, received abundant support in the literature (Bodkin, Lasser et al. 1997; Spier 1998; Coleman, Cunningham et al. 1999; Mischoulon, Nierenberg et al. 2000; Perlis, Fava et al. 2002; Ramasubbu 2002; DeBattista, Solvason et al. 2003; Lam, Hossie et al. 2004; Zisook, Rush et al. 2006). As a result, their use was warranted and accordingly, these combinations, although still infrequently used, remained the most prescribed medication combinations for patients referred to the MDC. Other studies, including the STAR*D (McGrath, Stewart et al. 2006; Zisook, Rush et al. 2006; Blier, Ward et al. 2010), have shown the efficacy of mirtazapine with an SNRI (such as venlafaxine), yet only few patients referred to the MDC had been prescribed this combination. Furthermore, while the first-generation antidepressants (MAOIs and TCAs) have been shown to be just as efficacious as the second-generation antidepressants (but with more adverse events) (Gillman 2011), their combination with serotonergically-mediated drugs such as SSRIs and SNRIs warrants caution and inconveniently strict clinical observation. This is due to the potentially life-threatening pharmacological interactions between these first- and second-generation antidepressants.

Patients in antidepressant combination treatments appear to achieve remission in a more time-efficient manner than those taking a single antidepressant. Further, the use of combination treatment at the beginning of therapy was found to improve depressive symptoms more robustly and also increased the probability of achieving remission compared to antidepressant monotherapy (Blier, Gobbi et al. 2009; Blier, Ward et al. 2010). These factors could help improve rates of medication compliance and adherence as it has been found that up to 50% of patients do not comply with their treatment regimen and stop taking their medication within the first 12-weeks of therapy (Lin, Von Korff et al. 1995; Melfi, Chawla et al. 1998; Blier, Gobbi et al. 2009).

While the financial situation and healthcare coverage of patients, along with the cost of antidepressants, were not reviewed in this thesis, the likelihood of an individual not adhering to a medication regimen may increase if no noticeable symptom improvements are noticed within a reasonable amount of time. Some combinations have been shown to expedite symptom improvement compared to antidepressant monotherapy and therefore, patients may be more willing to include these medications in their budget and/or purchase healthcare insurance if it is perceived to be beneficial.

Among the different classes of antidepressants, it was found that SSRIs and SNRIs were the two most commonly prescribed antidepressants, followed by bupropion and mirtazapine. The combinations of these two former serotonergically-mediated antidepressants with bupropion were found to be the most commonly prescribed antidepressant combinations at the MDC. These findings not only agree with the literature supporting their efficacy but are also consistent with studies that demonstrate that the use of bupropion impedes the sexual dysfunction side-effects commonly associated with serotonergic drugs (Zisook, Rush et al. 2006). Effective combinations have also been shown to be as tolerant as single antidepressants with no significantly greater adverse events (Blier, Gobbi et al. 2009; Blier, Ward et al. 2010).

However, in order for antidepressant combinations to be effective, those

treating the patient must be aware of the mechanisms of action of each individual drug. This will maximize the combination's therapeutic potential without hindering the benefits of each individual medication and diminishing the possibility for any dangerous interactions. While all antidepressants share the same end result of enhancing monoaminergic functioning (Stahl, Pradko et al. 2004), the mechanisms of action of the more than two-dozen available antidepressants in North America (Stahl and Grady 2003) can be categorized into one of seven distinct groups (MAOIs, TCAs, SSRIs, SNRIs, NDRIs, NaSSAs and SARIs). Knowledge and understanding of these unique mechanisms of action will allow the psychiatrist, or clinician, to custom-tailor the treatment according to the patient's symptoms.

The STAR*D provided viable, but not conclusive, alternative treatment options following the failure of one or more antidepressant treatments. It also suggested that the frustration of multiple treatment failures may result in health care professionals and their patients being content with a response as the rate of achieving remission was found to significantly diminish following two failed antidepressant treatments (Gaynes, Rush et al. 2008). Moreover, insight into better and more effective treatment options can avoid the self-assessment bias in mental health professionals in which there is a tendency to overestimate the improvement rates while simultaneously underestimating the rates of decline of their patients (Walfish, McAlister et al. 2012).

Combination therapy has been shown to be effective, yet few patients referred to the MDC had been prescribed antidepressant combinations within the six months prior to their initial diagnostic assessment. To increase and/or improve rates of remission in a time efficient manner, psychiatrists should consider combination therapy at the beginning of their patients' treatment.

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