

Impact of Medications Used in the Treatment of Mood Disorders on Monoaminergic Systems

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DEDICATION

Dedicated to anyone who is battling the dark clouds of depression; in memory of those for whom it all became too much; in celebration of those who have overcome.

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LIST OF ABBREVIATIONS

3-MT	3-methoxytyramine
5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytryptamine (serotonin)
5-HTT	serotonin transporter
5-HTP	5-hydroxytryptophan
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino) tetralin
AMPT	α -methyl-para-tyrosine
ANOVA	analysis of variance
ANCOVA	analysis of covariance
cAMP	cyclic adenosine monophosphate
CSF	cerebrospinal fluid
CNS	central nervous system
COMT	catechol-O-methyl-transferase
DA	dopamine
DAT	dopamine transporter
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride
DOPAC	dihydroxyphenylalanine
DRN	dorsal raphe nucleus
DSM-IV	diagnostic and statistical manual of mental disorders
GPCR	G protein-coupled receptor
HAMD	Hamilton Rating Scale for Depression
HVA	homovanillic acid
i.v.	intravenous
LC	locus coeruleus
L-dopa	L-dihydroxyphenylalanine
LSD	lysergic acid diethylamide
MADRS	Montgomery-Asberg Depression Rating Scale
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MDD	major depression disorder
MDL 100,907	R-(+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidinem ethanol
NE	norepinephrine
NRI	norepinephrine reuptake inhibitor
PCPA	<i>p</i> -chlorophenylalanine
PET	positron emission tomography
S.E.M.	standard error of mean
SSRI	selective serotonin inhibitor
TTX	tetrodotoxin
TCA	tricyclic antidepressant
TPH	tryptophan hydroxylase
VTA	ventral tegmental area
WAY 100,635	N-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride

LIST OF MANUSCRIPT PUBLICATIONS

I. El Mansari M, **Ghanbari R**, Janssen S, Blier P (2008) Sustained administration of bupropion alters the neuronal activity of serotonin, norepinephrine but not dopamine neurons in the rat brain. *Neuropharmacology*. 55(7):1191-8. Epub 2008 Jul 30.

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V. **Ghanbari R**, El Mansari M, Shahid M, Blier P (2009) Electrophysiological characterization of the effects of asenapine at 5-HT_{1A}, 5-HT_{2A}, α_2 -adrenergic and D₂ receptors in the rat brain. *Eur Neuropsychopharmacol*. 19(3):177-87. Epub 2008 Dec 29.

VI. El Mansari M, Guiard BP, Chernoloz O, **Ghanbari R**, Katz N, Blier P (2010) Relevance of norepinephrine-dopamine interactions in the treatment of major depressive disorder. *CNS Neuroscience & Therapeutics*. 16(3):e1-17. Epub 2010 Apr 8.

ABSTRACT

While selective serotonin (5-HT) reuptake inhibitors (SSRIs) are utilized as the first-line strategy in treating depression, new approaches are still desired. Using *in vivo* electrophysiological techniques, the effects of co-administration of bupropion with the SSRI escitalopram on the firing rate of dorsal raphe 5-HT and locus coeruleus norepinephrine (NE) neurons were investigated. Escitalopram significantly decreased the firing of 5-HT and NE neurons at day 2. The 5-HT firing rate, unlike that of NE, recovered after the 14-day escitalopram regimen. Bupropion did not increase 5-HT firing but decreased that of NE after 2 days. Following 14-day bupropion, 5-HT firing was markedly enhanced, and NE firing was back to baseline. Co-administration of escitalopram and bupropion doubled 5-HT firing after 2 and 14 days, whereas NE neurons were inhibited after 2, but partially recovered after 14 days.

Although sustained bupropion administration did not alter the sensitivity of 5-HT_{1A} receptors in hippocampus, the tonic activation of postsynaptic 5-HT_{1A} receptors was enhanced in 14-day bupropion-treated rats to a greater extent than in the 2-day and control rats. The function of terminal 5-HT_{1B} autoreceptors was not changed. The inhibitory action of α_2 -adrenergic receptors on 5-HT terminals was, however, diminished. The function of terminal α_2 -adrenergic autoreceptors was also attenuated in rats given bupropion for 14 days.

Administration of the antidepressant trazodone suppressed the 5-HT firing at day 2, which recovered to baseline following 14 days. Prolonged trazodone-administration enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors in hippocampus, and decreased the function of terminal 5-HT_{1B} autoreceptors.

Finally, a novel psychotropic agent asenapine showed potent antagonistic activity at 5-HT_{2A}, D₂, and α_2 -adrenoceptors. Asenapine, however, acted as a partial agonist at 5-HT_{1A} receptors in dorsal raphe and hippocampus.

Overall, the therapeutic effects of various antidepressants may be, at least in part, due to the enhancement of 5-HT and/or NE neurotransmission.

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Lastly, I offer my regards to all of those who supported me in any respect during the completion of the thesis.

CONTRIBUTIONS OF COLLABORATORS

It is important to indicate that Ramez Ghanbari, Mostafa El Mansari and Pierre Blier designed all the studies. Ramez Ghanbari carried out the experiments, and collected the data. It is, however, vital to emphasize on the contribution and technical expertise of Dr. Mostafa El Mansari on various experiments, in particular with the measurement of tissue/medium ratios of radioactivity *in vitro* for the trazodone experiments. Drs. Daniel Gustafson, Susan Hudachek and Ryan Hansen (Colorado State University, Fort Collins, CO) assessed trazodone and mCPP levels in the rat brain. All authors, on page xiii, assisted in drafting the published articles, and approved the final manuscript. Drs. Damon Smith, Corinne Benquet and Dorothee LeGarrec provided insight on the trazodone manuscript.

1 INTRODUCTION

1.1 Scope of the problem

Depression was described as the “common cold” of psychiatry by the American psychologist Martin Seligman in 1975. Today, thirty five years later, the situation remains problematic. Indeed, major depressive disorder (MDD) is one of the most debilitating disorders of all medical conditions, afflicting more than 120 million people worldwide (WHO, 2007). It is currently ranked as the third leading cause of disability globally (WHO, 2008). Tragically, the magnitude of the problem is expected to worsen in the future, as the World Health Organization projects that by 2030, MDD will become the first leading cause of disability worldwide (WHO, 2008). Currently, it is, however, the leading factor for the largest burden of all disorders in middle- to high-income countries, ahead of ischemic heart disease (WHO, 2008). Studies indicate that depression comorbid with other medical conditions adversely affects the prognosis of other illnesses and creates a substantial burden of additional impairment (Musselman *et al.*, 1998; Glassman *et al.*, 2002; Simon, 2003). Moreover, the direct medical costs and the lost productivity associated with major depression were estimated approximately at 83 billion dollars in U.S. in the year 2000 alone (Greenberg *et al.*, 2003). In addition to the lost productivity and cost, suicidal ideation is a common symptom in patients with MDD. Approximately one million people commit suicide globally each year as a result of this burdensome illness (WHO, 2007). In 1999, in the U.S. alone, the number of suicides was twice the number of homicides (IOM, 2002). It has become the third leading cause of death in individuals 15 to 24 years old in the U.S. (NCIPC, 2000). It is worth noting that a significant number of individuals who die from suicide suffer from MDD (Conwell and Brent, 1995; Schulz *et al.*, 2000).

Despite the intensive research efforts over the last 40 years, the precise neurobiological mechanism underlying MDD is not fully understood. This simply illustrates the complexity of the illness and the challenge for an accurate diagnosis. Currently, psychiatrists mainly rely on the patient’s signs, symptoms of mental status and behavioral observations in line with the diagnostic categories listed in the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV; APA, 2000). Although in

the broad sense, the term depression may refer to feeling down or blue, a clinical episode of MDD is characterized by depressed mood, feelings of worthlessness or guilt, anhedonia, social withdrawal, agitation, concentration difficulties, sleep disturbances, and in severe cases suicidal ideation. The MDD diagnosis can be made if the person exhibits such symptoms for at least two weeks. Most MDD patients experience multiple episodes. Indeed, majority of MDD patients who recover from the first episode of depression will experience at least one subsequent episode of depression (Mueller *et al.*, 1999). Furthermore, each subsequent episode increases the risk of recurrence depression (Mueller *et al.*, 1999; Solomon *et al.*, 2000). Therefore, maintenance therapy is as important as achieving remission to prevent relapse and recurrent depression. Although the genetic and environmental factors may increase the susceptibility to this disorder, both basic and clinical research over the last 4 decades have implicated the abnormalities of monoaminergic, that is serotonin (5HT), norepinephrine (NE) and dopamine (DA) systems in the pathophysiology of MDD. Thus, the monoaminergic systems and their abnormalities in depression will be discussed.

1.2 Serotonin system

1.2.1 Localization

Although the existence of an endogenous vasoconstrictor agent in blood serum had been reported in the early days of the 20th century, it was in 1948 that Rapport and colleagues discovered the unidentified serum substance as 5-HT to indicate its origin from blood serum and its effect on vascular muscle tone. Shortly after, it became evident that the amine is also present in the central nervous system (CNS; Twarog and Page, 1953; Bogdanski *et al.*, 1956). The discovery of 5-HT neurons and their projections in the brain sparked a tremendous interest on the 5-HT system (Dahlström and Fuxe, 1964; Fuxe, 1965).

Most 5-HT neurons are localized on the midline of the brain stem called *raphe* nuclei, adopted from Latin for midline. The raphe nuclei are further divided into rostral and caudal regions. The rostral raphe nuclei consist of dorsal and median raphe which contain about 50% and 5% of 5-HT neurons in mammalian CNS, respectively (Wiklund and Björklund, 1980; Descarries *et al.*, 1982). Although all brain regions receive 5-HT innervation

(Dahlström and Fuxe, 1964), the D system containing thin fibers originates from dorsal raphe nucleus (DRN) and projects primarily to frontal cortex and striatum. The median raphe, on the other hand, forms the thick afferent fibers projecting to cerebral cortex and hippocampus (Kosofsky and Molliver, 1987; Mulligan and Tork, 1988).

1.2.2 Synthesis, storage and release

L-tryptophan, the precursor of 5-HT synthesis, is an essential amino acid that is actively taken up into neurons. The first and rate-limiting step in the 5-HT synthesis involves hydroxylation of the precursor to 5-hydroxytryptophan (5-HTP), catalyzed by an enzyme mainly known as tryptophan hydroxylase (TPH). This crucial enzyme is synthesized in 5-HT cell bodies and transported to the 5-HT terminals where most of the neurotransmitter biosynthesis occurs (Meek and Neff, 1972). It is important to note that two genes encode two different TPH isoforms, commonly known as TPH1 and TPH2. TPH1 is primarily expressed in peripheral tissues such as gut and skin. Nevertheless, it is also expressed in the CNS, mainly in the pineal gland. TPH2, on the other hand, is exclusively expressed in 5-HT neurons in the raphe where it acts as the rate-limiting enzyme in 5-HT synthesis (Walther *et al.*, 2003, Zhang *et al.*, 2004). 5-HTP is then converted to 5-HT by amino acid decarboxylase. Most of the synthesized 5-HT is packaged and stored in secretory vesicles. The 5-HT pool is released into the extracellular synaptic cleft in a Ca^{+2} -dependent exocytotic manner (Sanders-Bush and Martin, 1982; Smith and Augustine, 1988). Upon release from presynaptic terminal, 5-HT can interact with both G protein-coupled receptors (GPCRs) as well as ionotropic receptors to induce cellular changes (Fig. 1). 5-HT is cleared from the extracellular space by an active reuptake process *via* 5-HT transporter (5-HTT) located on presynaptic neuronal membranes (Kuhar *et al.*, 1972). Once in the presynaptic terminal, 5-HT can be repackaged into the synaptic vesicles for recycling. 5-HT may also be metabolized to 5-hydroxyindoleacetaldehyde which is rapidly oxidized to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase (MAO). 5-HIAA, the primary metabolite of 5-HT, diffuses out of the neuron and enters the cerebrospinal fluid (CSF).

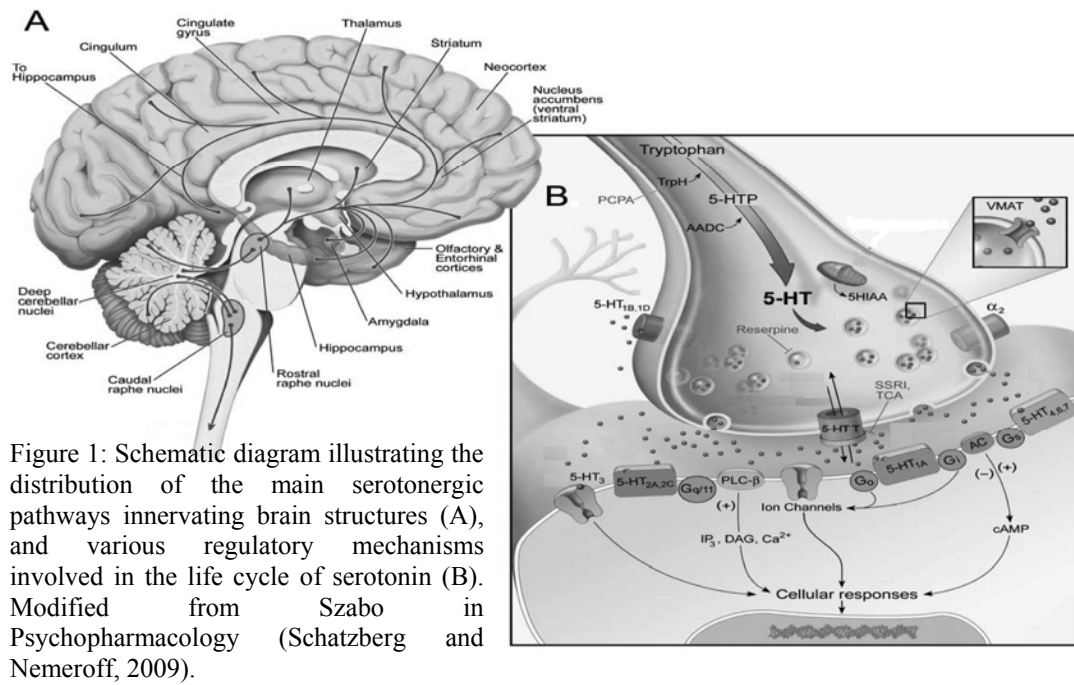


Figure 1: Schematic diagram illustrating the distribution of the main serotonergic pathways innervating brain structures (A), and various regulatory mechanisms involved in the life cycle of serotonin (B). Modified from Szabo in Psychopharmacology (Schatzberg and Nemeroff, 2009).

The DRN 5-HT neurons exhibit a slow (0.5 - 2.5 Hz), regular discharge pattern with long duration action potential (Aghajanian, 1978; Aghajanian and Vandermaelen, 1982a; Fig. 2). These neurons sometimes discharge in brief bursts of action potentials, mostly doublets and triplets, with a very short interspike time interval (Hajos and Sharp, 1996). The firing activity in bursting mode is believed to increase the amount of 5-HT release for the same number of spikes delivered at regular intervals (Gartside *et al.*, 2000). The firing activity of 5-HT neurons has physiological importance since the 5-HT release is dependent on the firing activity (Blier *et al.*, 1989; Sharp *et al.*, 1989a). Interestingly, the release of neurotransmitters in the postsynaptic region is directly proportional to firing rate of neurons (Gonon, 1988; Garris *et al.*, 1994). In line with this, local application of 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) into the dorsal or median raphe reduced extracellular 5-HT release in the corresponding projection areas (Sharp *et al.*, 1989a; Hutson *et al.*, 1989; Bonvento *et al.*, 1992). Furthermore, local application of the action potential blocker tetrodotoxin (TTX) into DRN decreases the spontaneous release of [³H]5-HT in rats (Bosker *et al.*, 1994).

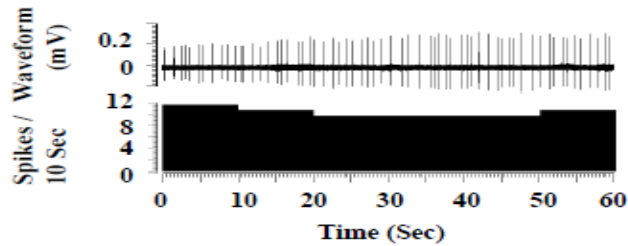


Figure 2: The lower panel represents the integrated histogram of the firing activity of a DRN 5-HT neuron (upper panel) recorded in the brain of an anesthetized rat.

Although there are 14 different 5-HT receptors known to date, the firing rate of raphe 5-HT neurons is regulated by somatodendritic 5-HT_{1A} autoreceptors (Aghajanian *et al.*, 1972; Vandermaelen *et al.*, 1986; Blier and de Montigny, 1987). These receptors are abundantly expressed on the cell body of 5-HT neurons in the dorsal raphe as well as the postsynaptic structures in hippocampus and cortex (Pazos and Palacios, 1985). All 5-HT receptor subtypes, except 5-HT₃ which is an ionotropic receptor, belong to the broad family of GPCR. These receptors mediate cellular activity by regulating various second messengers. For instance, somatodendritic 5-HT_{1A} receptors, in this case, are coupled to G_i, the generic name for the G protein containing an α_i subunit. The G_i protein inhibits adenylyl cyclase, the enzyme that catalyzes synthesis of the second messenger cyclic adenosine monophosphate (cAMP). It is, however, worth noting that the inhibitory action of 5-HT and 5-HT_{1A} agonist on the firing rate of raphe 5-HT neurons is mediated by opening the K⁺ channels, thereby increasing conductance to potassium (Aghajanian and Lakoski, 1984). Nevertheless, the response of DRN 5-HT neurons to 5-HT and 5-HT_{1A} agonists were completely abolished in rats treated with G_{i/o} protein-sensitive pertussis toxin, indicating a modulatory interaction between the G_i protein and the ion channel (Innis and Aghajanian, 1987a).

Another important 5-HT receptor subtype that plays a crucial role on the 5-HT transmission is 5-HT_{1B} autoreceptors. These autoreceptors are localized on 5-HT neuron terminals where they mediate 5-HT release *via* a negative feedback mechanism. Indeed, activation of 5-HT_{1B} autoreceptors induced a greater negative feedback on the release of 5-HT both *in vitro* and *in vivo* (Göthert, 1980; Chaput *et al.*, 1986a; Blier *et al.*, 1989). In support of this, systemic administration of 5-HT_{1A} or 5-HT_{1B} agonist inhibits 5-HT release in rat hippocampus, as measured by microdialysis (Sharp *et al.*, 1989b). It is important to note that although 5-HT_{1B} receptors are not detected in human brains, the human 5-HT_{1D}

receptor is the rodent 5-HT_{1B} equivalent as they share similar anatomical distribution, second messenger system and pharmacology (Hoyer *et al.*, 1990; Hamblin *et al.*, 1992).

1.2.3 Abnormalities of 5-HT system in MDD

Early studies over 30 years ago showed that introduction of small dose of tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA) reversed the antidepressant action of tricyclic imipramine and MAO inhibitor (MAOI) tranylcypromine (Shopsin *et al.*, 1975; Shopsin *et al.*, 1976) indicating that 5-HT system may be involved in inducing depressive symptoms. Tryptophan depletion paradigm, however, has enabled researchers to directly investigate the role of 5-HT in the pathophysiology of depression (Bell *et al.*, 2001). Indeed, acute dietary depletion of 5-HT precursor tryptophan rapidly lowered mood (Young *et al.*, 1985; Smith *et al.*, 1987) and induced cognitive deficits (Murphy *et al.*, 2002) in healthy individuals. Interestingly, tryptophan depletion reversed antidepressant-induced remission, and tryptophan level was negatively correlated with depression severity during depletion test (Delgado *et al.*, 1990; Smith *et al.*, 1997; Booij *et al.*, 2005), suggesting a vital role for 5-HT function in the etiology MDD. In support of this, a positron emission tomography (PET) imaging study examining the effects of tryptophan depletion on remitted patients, reported that tryptophan depletion reduced brain metabolism in brain regions implicated in the pathogenesis of depression, a phenomenon that correlated with the increased depressive symptoms as a result of the challenge test (Bremner *et al.*, 1997).

Furthermore, Meyer and colleagues (2006a, 2009) reported that MDD patients exhibited 34% higher levels of monoamine oxidase A, the enzyme responsible for metabolizing monoamines. Although controversial, this finding may explain the altered levels of monoamine metabolites during depressive state (Asberg *et al.*, 1976; Curzon, 1982; Reddy *et al.*, 1992). For instance, Asberg *et al.* (1976) reported that a large group of depressed patients had lower levels of the major 5-HT metabolite 5-HIAA, and that these patients were significantly more at the risk of committing suicide. In parallel, the observation that acute tryptophan depletion reduced CSF 5-HIAA levels, on average by about 32% in healthy subjects (Carpenter *et al.*, 1998; Williams *et al.*, 1999), may explain the detrimental effects of tryptophan depletion on mood (Young *et al.*, 1985; Smith *et al.*, 1987). In line

with this, introduction of monoamine transporter blocker reserpine, which interferes with uptake and storage mechanisms, induces depressive-like symptoms (Belmaker, 2008).

An interesting finding that puts into evidence 5-HT malfunction during depressive state is the observation that suicide victims with MDD had an increased density of 5-HT_{1A} autoreceptors in DRN (Stockmeier *et al.*, 1998, Boldirini *et al.*, 2008). It is, however, important to note that reduction of the raphe 5-HT_{1A} binding has been reported in depression (Drevets *et al.*, 1999). This observation, however, may be attributed to the loss of 5-HT neurons and cortisol hypersecretion (Drevets *et al.*, 2007). Other studies in the field have shown enhanced 5-HT_{1A} binding potentials in un-medicated MDD patients (Parsey *et al.*, 2006a, b). Indeed, Parsey *et al.*, (2006b) showed that non-remitter subjects had higher 5-HT_{1A} binding potential as well as an overexpression of homozygous G/G genotype, which prevents gene repression of 5-HT_{1A} autoreceptors (see Albert and François, 2010), a phenomenon that may explain the increased density/binding of 5-HT_{1A} receptors in MDD patients. Given that these receptors play an inhibitory role on the firing activity of 5-HT neurons, it may provide a direct link between 5-HT deficit and MDD. Another interesting abnormality in MDD patients involves genetic defects of 5-HT neuronal elements. One of the most widely reported genetic defect that increases the susceptibility to depression is 5-HTT polymorphism. A common polymorphism has been reported; the s representing short allele, and the l designating the long allele. The s variant of the 5-HTT polymorphism restricts transcriptional activity of the transporter promoter resulting in low efficiency of 5-HTT, when compared to the l/l genotype (Collier *et al.*, 1996; Heils *et al.*, 1996; Lesch *et al.*, 1996; Heils *et al.*, 1997; Praschak-Rieder *et al.*, 2007). In support of this, Neumeister and colleagues (2002) reported that healthy subjects with the s/s genotype for 5-HTT developed higher depressive symptoms in response to tryptophan depletion, regardless of family history of depression.

1.3 Dopamine system

1.3.1 Localization

Although DA was first synthesized in 1910, it was not until late 1950s, when the ground breaking work of Carlsson and colleagues showed that DA is not just a precursor of NE

and functions as a neurotransmitter in CNS (Carlsson *et al.*, 1957; Carlsson *et al.*, 1958). The early mapping studies showed that, unlike 5-HT and NE, DA neurons form discrete projection pathways (Descarries *et al.*, 1980; Fuxe *et al.*, 1985). The major dopaminergic pathways include: the nigrostriatal projection from the substantia nigra to the putamen and caudate nucleus, the mesolimbic and mesocortical fibers composed of DA neurons in ventral tegmental area (VTA) giving rise to axons that innervate many structures in limbic, and cortical regions, respectively, and the tuberoinfundibular system from the arcuate nucleus of the hypothalamus to the pituitary stalk. The presence of dense DA innervation in these brain areas suggests the role of DA in Parkinson's disease, schizophrenia, addiction as well as mood disorders.

1.3.2 Synthesis, storage and release

As shown in figure 3, the synthetic pathway of DA involves conversion of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-dopa) by the enzyme tyrosine hydroxylase. L-dopa is then readily converted to DA by L-aromatic amino acid decarboxylase. DA is, then, stored in presynaptic vesicles in the dopaminergic nerve terminals and released in the synaptic cleft in Ca^{+2} -dependent manner. Following the release, DA is transported back into the intracellular space, through DA transporters (DAT), where it is recycled or metabolized to dihydroxyphenylalanine (DOPAC) by intraneuronal MAO, located on the outer membrane of mitochondria. The extracellular DA is initially metabolized to 3-methoxytyramine by catechol-O-methyl-transferase (COMT), and further degraded to homovanillic acid (HVA) by extracellular MAO (Cooper *et al.*, 2003).

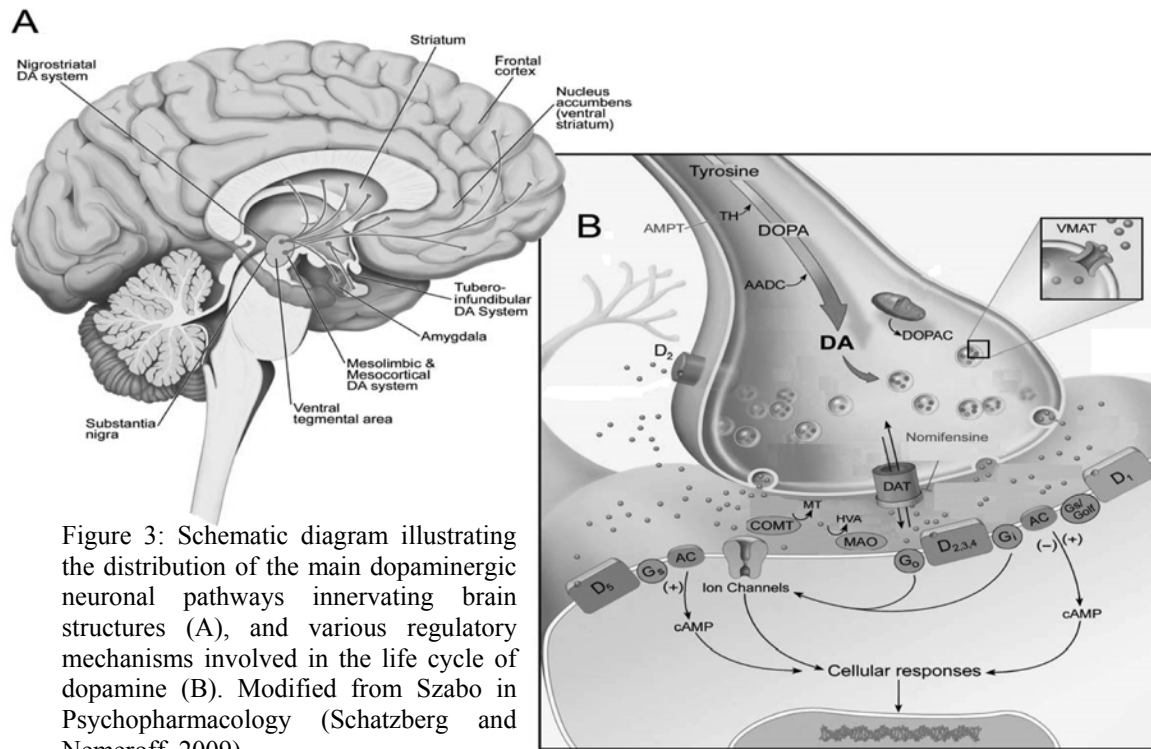


Figure 3: Schematic diagram illustrating the distribution of the main dopaminergic neuronal pathways innervating brain structures (A), and various regulatory mechanisms involved in the life cycle of dopamine (B). Modified from Szabo in Psychopharmacology (Schatzberg and Nemeroff, 2009).

The electrophysiological properties of DA neurons have been well established. These neurons, similar to 5-HT cells, discharge in a spontaneous fashion followed by a silent period due to temporary hyperpolarization. They exhibit a slow spontaneous firing rate (0.5 – 7 Hz), and a long duration (>2.5 msec) often with a notch on the rising phase. The discharge of DA neurons is mainly composed of single-spike and bursting pattern, where a burst may contain 2 to 10 action potentials. The amplitude of action potentials progressively decreases in a given burst (Freeman *et al.*, 1985; Grace and Bunney, 1984; Guiard *et al.*, 2008; Fig. 4). Burst firing mode is believed to play a critical role in the DA transmission since the amount of DA released per burst is greater than for the same number of action potentials discharged as single spikes (Gonon *et al.*, 1988; Garris *et al.*, 1994). Once in the extracellular space, DA may act on the postsynaptic dopaminergic receptors, all of which are known to belong to the GPCR superfamily. One of the most important DA receptors is D₂-autoreceptors that are expressed on the cell body and nerve terminals of DA neurons. D₂ autoreceptors regulate their cellular actions, by inhibition of adenylyl cyclase, via G_{i/o} proteins, as well as opening of K⁺ channels thereby inhibiting DA neuronal firing (Innis and Aghajanian, 1987b).

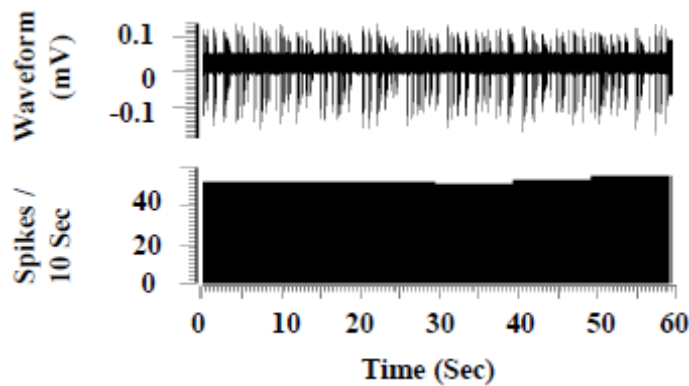


Figure 4: The lower panel represents the integrated histogram of the firing activity of a VTA DA neuron (upper panel) recorded in the brain of an anesthetized rat.

1.3.3 Abnormalities of DA system in MDD

As mentioned previously, dopaminergic neurons innervate brain regions associated with behavioral and executive functions that are altered during a depressive state. Disruption of the mesocorticolimbic DA projections may lead to core features of depression such as diminished motivation and anhedonia. A great body of literature suggests the hypoactivity of DA system in depression (Dunlop and Nemeroff, 2007). In this context, high frequency of depression has been observed in patients with Parkinson disease, which is associated with a substantial loss of DA neurons. Indeed, Tandberg and colleagues (1996) reported that, in a community-based study, about 8% of the patients with Parkinson disease exhibited MDD, with another 45.5% showing mild depressive symptoms. Most importantly, depression is the most contributing factor in elevating suicidal ideation in patients with Parkinson disorder (Kummer *et al.*, 2009). Another line of evidence suggesting the attenuated dopaminergic activity in MDD involves measurement of DA metabolite HVA. Studies comparing the HVA levels have reported marked reduction of the CSF DA metabolite in patients with major depression (Korf and Van Praag, 1971; Träskman *et al.*, 1981). In line with this, the reduced HVA was also observed in treatment-resistant depression (Lambert *et al.*, 2000). Furthermore, the CSF HVA levels were significantly lower in suicide attempters than control subjects (Engström *et al.*, 1999). Interestingly, imaging experiments have shown decreased density of DAT in MDD patients compared to control individuals (Meyer *et al.*, 2001). This observation is akin to previous reports that DAT density is reduced following prolonged DA depletion (Kilbourn *et al.*,

1992; Gordon *et al.*, 1996). Moreover, the D₂ receptor density is high in depressed patients (D'haenen and Bossuyt, 1994). This observation is consistent with the significantly high D₂ binding potential in depressed patients (Meyer *et al.*, 2006b). Intriguingly, although no significant difference between MDD patients and controls, the D₂ receptor binding markedly decreased following antidepressant therapy (Ebert *et al.*, 1996). These adaptations perhaps indicate the compensatory mechanism of DA system for the DA loss in MDD.

1.4 Norepinephrine system

1.4.1 Localization

The discovery of NE can be traced back to the early years of the 20th century even though it was identified as NE by von Euler in 1946. Indeed, initially known as *sympathin*, was shown to be released by sympathetic nerve terminals (Cannon and Uridil, 1921). It was, however, in 1954 that NE was proposed to function as a neurotransmitter in the CNS (Vogt, 1954). The NE-containing neurons are mainly located in the medulla and pons with locus coeruleus nucleus (LC) containing the highest density of NE neurons giving rise to about 90% of the ascending projections to widespread brain areas such as cerebral cortex and limbic structures (Fuxe, 1965; Fuxe *et al.*, 1970; Foote *et al.*, 1983; Levitt *et al.*, 1984).

1.4.2 Synthesis, storage and release

The NE precursor L-tyrosine is actively taken up from the circulation. Once in the brain, it is converted to L-dopa, in the cytosol of the NE neuronal cell body and its nerve terminals, through a rate-limiting step by the enzyme tyrosine hydroxylase. L-DOPA is then decarboxylated to DA by decarboxylase. DA is taken up from the cytoplasm into vesicles *via* vesicle monoamine transporters and converted to NE by the enzyme DA β -hydroxylase that is present in NE-specific neurons. Indeed, the difference between DA and NE reside only in one hydroxyl group. The synthesized NE is then stored in the synaptic vesicles and released in the synaptic cleft *via* exocytosis (Weiner and Molinoff, 1994). Upon release, the effects of NE on the postsynaptic neuron are mediated *via* two G protein superfamily of α and β adrenoreceptors (Fig. 5). Once in the extracellular space, NE can be taken up through NE transporters (NET) back into the presynaptic nerve terminal for recycling or

metabolized to normetanephrine by COMT. The NE metabolites can be further processed to form 3-methoxy-4-hydroxyphenylglycol (MHPG) by MAO (Cooper *et al.*, 2003).

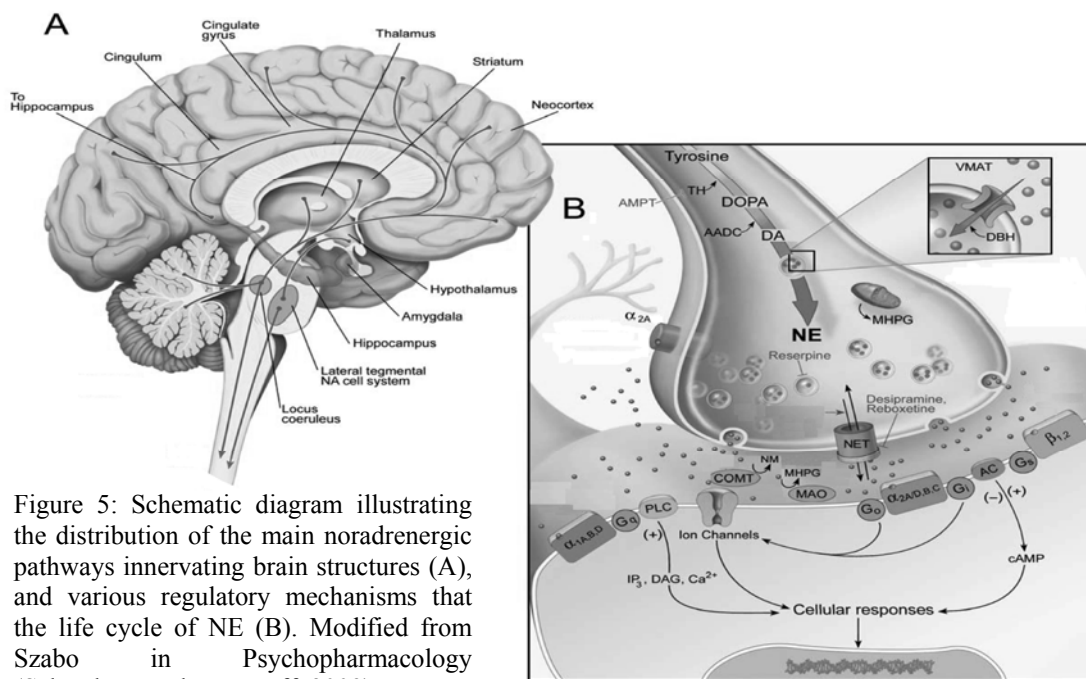


Figure 5: Schematic diagram illustrating the distribution of the main noradrenergic pathways innervating brain structures (A), and various regulatory mechanisms that the life cycle of NE (B). Modified from Szabo in Psychopharmacology (Schatzberg and Nemeroff, 2009).

The LC NE neurons discharge at a regular firing rate of 0.5 – 5 Hz. These neurons are characterized by their a biphasic action potential of long duration (0.8 - 1.2 msec) often with a notch between the initial segment and somatodendritic spike component, and a characteristic burst discharge followed by a quiescent period in response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen, 1982b; Ramirez and Wang, 1986; Fig. 6). The LC NE neurons sometimes discharge in the bursting pattern of doublets and triplets (Dawe *et al.*, 2001). The firing activity of the NE neurons is regulated by the inhibitory function of α_2 -adrenergic autoreceptors located on the NE cell body. In a way, these autoreceptors resemble the action of 5-HT_{1A} autoreceptors on the 5-HT neuronal activity. In fact, activation of the α_2 -adrenergic autoreceptors that are negatively coupled to adenylyl cyclase inhibits NE neuronal firing rate (Cedarbaum and Aghajanian, 1977; Egan *et al.*, 1983; Andrade and Aghajanian, 1985).

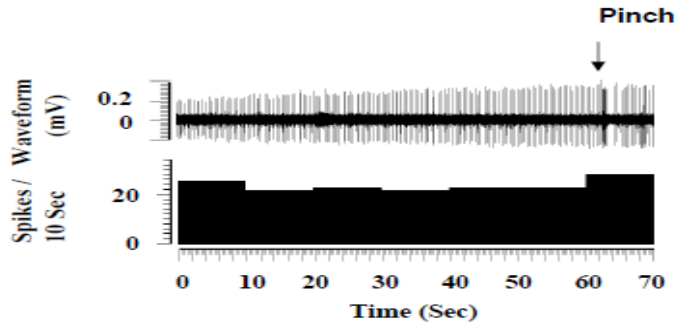


Figure 6: The lower panel represents the integrated histogram of the firing activity of a LC NE neuron (upper panel) recorded in the brain of an anesthetized rat. Note the response of the NE neuron to contralateral pinch.

1.4.3 Abnormalities of NE system in MDD

Most early evidence of NE dysfunction comes from the clonidine challenge. This neuroendocrine test that stimulates the release of growth hormone indirectly measures the sensitivity of brain α -adrenergic receptors. Indeed, earlier studies reported that depressed patients showed significantly less growth hormone responses than those of the matched healthy individuals (Checkley *et al.*, 1981; Charney *et al.*, 1982), suggesting a possible NE defect in depression. A more direct support derives from catecholamine depletion studies showing that reserpine results in lowering mood. Furthermore, a competitive tyrosine hydroxylase inhibitor α -methyl-para-tyrosine (AMPT), which inhibits NE and DA synthesis (Brodie *et al.*, 1971; Bunney *et al.*, 1971) partially, reversed antidepressant-effects of mirtazapine (Delgado *et al.*, 2002). In line with this, depressed patients who responded to NE reuptake inhibitor (NRI) desipramine had about 80% risk for relapse, as compared to about 25% of selective serotonin reuptake inhibitor (SSRI) responders, once introduced to AMPT (Delgado *et al.*, 1990; Delgado *et al.*, 1993; Miller *et al.*, 1996).

A postmortem study reported that depressed suicidal patients had higher levels of α_2 -adrenergic receptors in hypothalamus and frontal cortex, as compared to the matched healthy controls (Meana *et al.*, 1992). This observation seems akin to the hypothesis that depression may be related to supersensitive α_2 -adrenoceptor (García-Sevilla *et al.*, 1986). In line with this, prolonged administration of antidepressants decrease the density of α_2 -adrenergic receptors in the rat brain (Smith *et al.*, 1981; Giralt and García-Sevilla, 1989) as well as in depressed patients (García-Sevilla *et al.*, 1981; Charney *et al.*, 1983).

1.5 Monoaminergic-based strategies for treatment of MDD

1.5.1 Tricyclics and MAOIs

The introduction of tricyclic antidepressants (TCAs) in the late 1950s marked the beginning of an era for the treatment of depression. As suggested by the name, tricyclic agents are named after their central three-ring chemical structure. Although the antidepressant effects of this class were reported in the late 1950s (Kunh, 1958), it was years later that the mechanism of action of these agents was found to be, mostly, *via* the blockade of 5-HTT and NET (Glowinski and Axelrod, 1964; Richelson and Nelson, 1984). Another class of antidepressants that was introduced during the same period is MAOIs. These agents prevent the breakdown of monoamines thereby enhancing 5-HT, NE and DA levels. The clinical use of TCAs and MAOIs has fallen out of favor among psychiatrists primarily due to adverse side effects, dietary restrictions, and hypertensive crisis.

1.5.2 Selective Serotonin Reuptake Inhibitors

The introduction of fluoxetine as the first SSRI represents a turning-point in the treatment of depression. Indeed, SSRIs are now considered as the first-line treatment strategy in treating depression mainly due to their efficacy as well as desirable safety profiles. The action of SSRIs on 5-HT neurotransmission has been thoroughly established over the years. Short-term administration of SSRIs attenuates the firing rate of DR 5-HT neurons, due to overactivation of somatodendritic 5-HT_{1A} autoreceptors since SSRIs promptly inhibit 5-HT uptake, leading to an enhancement of 5-HT levels in the vicinity of the cell body. Prolonged administration of SSRIs, however, leads to complete recovery of 5-HT firing rate. Such phenomenon is attributable to desensitization of 5-HT autoreceptors (Blier and de Montigny, 1983), later characterized as the 5-HT_{1A} subtype (de Montigny *et al.*, 1984; Kennett *et al.*, 1987; Riad *et al.*, 2001). Indeed, desensitization of somatodendritic and terminal autoreceptors, following prolonged administration of SSRIs, enhances 5-HT levels in the synaptic cleft (Chaput *et al.*, 1986a). The gradual adaptive changes of 5-HT neuronal elements, which correspond to the delayed therapeutic action, is perhaps due to alterations in receptor gene transcription and modifications of G-protein coupled to the 5-HT_{1A} autoreceptor (Hensler, 2003; Albert and Lemonde, 2004).

1.5.3 Norepinephrine Reuptake Inhibitors

As mentioned previously, hypoactivity of NE system has been reported in major depression. A practical way to enhance the NE deficit is to enhance the NE concentrations by blocking NET. Indeed, it was shown that selective non-tricyclic NRI reboxetine induced antidepressant effects in major depression, an effect that was similar to that of TCA imipramine and the SSRI fluoxetine (Scates and Doraiswamy, 2000). Thus, it is possible to obtain an antidepressant response solely by targeting the NE transmission. The selective NRIs are very potent at inhibiting the NET *in vitro* and *in vivo* (Hyttel, 1982; Scuvée-Moreau and Dresse, 1979). Electrophysiological studies have shown that acute and short-term administration of selective NRIs inhibit LC NE neuronal firing rate due to overactivation of somatodendritic α_2 -adrenergic autoreceptors as a result of enhanced NE levels in the presence of NE reuptake inhibition (Nybäck *et al.*, 1975; Szabo *et al.*, 2000). The LC NE firing rate remains attenuated following prolonged administration of the selective NRI desipramine, due to lack of desensitization of α_2 -adrenergic autoreceptors, which may be counterproductive in clinics (Szabo *et al.*, 2000).

1.5.4 Serotonin-Norepinephrine Reuptake Inhibitors

The antidepressant efficacy of the first serotonin-norepinephrine reuptake inhibitor (SNRI) venlafaxine in the treatment of MDD (Cunningham, 1997) suggested that a greater and faster antidepressant response may be achieved once both 5-HT and NE reuptake sites are blocked. For example, Guelfi and colleagues (1995) reported that venlafaxine significantly improved the depressive symptoms measured by Montgomery-Asberg depression rating scale (MADRS) and Hamilton rating scale for depression (HAMD) scores after 4 and 7 days, respectively, with the response (50% decrease in depressive symptoms) rate of 65%. Although double-blind studies have shown the superior efficacy of the SNRI venlafaxine than SSRIs fluoxetine and paroxetine (Clerc *et al.*, 1994) there are no data indicating more rapid onset of action than SSRIs. Interestingly, the remission rate obtained with venlafaxine was twice that of the SSRI paroxetine (Poirier and Boyer, 1999). In line with this, co-administration of the SSRI fluoxetine with NRI desipramine had superior efficacy than either drug alone (Nelson *et al.*, 2004).

1.5.5 Triple reuptake inhibitors

The notion that dopaminergic abnormalities have been reported during depressive state, and that dopaminergic agents alleviate depressive symptoms merits the hypothesis that the enhancement of DA neurotransmission, in addition to 5-HT and NE, may provide benefits to some MDD patients. For instance, a double-blind, placebo-controlled study, with 174 patients, demonstrated that the D₂/D₃ receptor agonist pramipexole used as monotherapy induces antidepressant response in patients with MDD (Corrigan *et al.*, 2000). The clinical efficacy of pramipexole has also been shown as an adjunctive agent in treatment-resistant MDD patients (Sporn *et al.*, 2000; Cassano *et al.*, 2004). Preclinical experiments have also reported antidepressant-like behaviour in animal models of depression following prolonged administration of pramipexole (Breuer *et al.*, 2009; Kitagawa *et al.*, 2009). Given the reciprocal interaction among the monoaminergic neurons *in vivo* (Guiard *et al.*, 2008), the enhancement of DA transmission may have beneficial effects on the 5-HT and NE neurotransmission. With this regard, an electrophysiological study showed that prolonged administration of pramipexole increased the firing rate of DRN 5-HT neurons accompanied with desensitization of somatodendritic 5-HT_{1A} as well as LC α_2 -adrenergic autoreceptors (Chernoloz *et al.*, 2009a). Thus, introduction of the concept of triple reuptake inhibitors, also known as broad spectrum antidepressants, is aimed at achieving greater efficacy than the SSRI and SNRI class. Since triple reuptake inhibitors are a young class of antidepressants, there are limited data on their efficacy. Nevertheless, two major compounds belonging to this category are DOV 216303 and NS 2359. Preclinical experiments have shown that administration of DOV 216303 induces antidepressant-like behaviour (Breuer *et al.*, 2008), perhaps due to the enhanced 5-HT, NE and DA levels (Prins *et al.*, 2010). The clinical studies have reported that DOV 216303 yielded significant improvement in HAMD scores as early as one week, and that it is a safe and well-tolerated compound (Skolnick *et al.*, 2006). Although the clinical efficacy of this class of compounds yet to be further confirmed, it may benefit MDD patients who suffer from sexual dysfunction, as a result of the 80% 5-HTT occupancy to induce antidepressant response (Meyer *et al.*, 2004). Indeed, the threshold of 5-HTT occupancy, to have a meaningful clinical effect, may be well below the 80% in the presence of NET and DAT inhibition.

1.5.6 Augmentation/Combination Strategies

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial from the National Institute of Mental Health reported that approximately 50% of MDD patients respond to an adequate trial of a SSRI, with a remission rate of about 30% (Trivedi *et al.*, 2006). Moreover, even MDD patients who remit following a monotherapy of SSRI experience some residual symptoms (Nierenberg *et al.*, 1999). The primary goal in the treatment of MDD is remission. In the case of a failed trial, the medication is usually substituted to another antidepressant drug of the same (i.e. SSRI) or different class. This approach would require a substantial amount of time as these trials often take weeks or months. An alternative measure consists of an augmentation or combination approach. An augmentation strategy consists of adding an agent that is not recognized as an antidepressant by itself such as lithium and triiodothyronine. In line with this, addition of lithium to an ongoing standard antidepressant rapidly alleviates depressive symptoms in treatment-resistant MDD patients (de Montigny *et al.*, 1981; Bauer *et al.*, 2003). In that respect, addition of lithium to different classes of antidepressants potentiated the 5-HT transmission in hippocampus which may account for its clinical efficacy (Haddjeri *et al.*, 2000). The combination strategy is composed of adding a recognized antidepressant to an ongoing monotherapy. In terms of the clinical efficacy, there is no evidence suggesting that these two strategies have superior efficacy over each other. However, recent data suggest that combination therapy from treatment initiation may double the remission (HAMD score ≤ 7) rate compared to monotherapy (Blier *et al.*, 2009; Blier *et al.*, 2010a). Starting with the multifunctional pharmacological approach may minimize the cost and lag phase in the treatment of MDD. In this regard, two of the antidepressant drugs used as combination agents (Blier *et al.*, 2009; Blier *et al.*, 2010a) mirtazapine and bupropion will be briefly discussed below.

1.5.7 Mirtazapine

Mirtazapine is an effective antidepressant agent that functions by blocking α_2 -adrenergic autoreceptors located on the LC NE cell body and terminals that regulate the firing rate and release of NE, respectively. Electrophysiological studies showed that both acute and prolonged administration of mirtazapine enhanced the firing rate of LC NE neurons

(Haddjeri *et al.*, 1996; Haddjeri *et al.*, 1997a). Administration of mirtazapine promotes an increase in NE release in the prefrontal cortex, an effect that is attributed to the antagonistic action of mirtazapine at α_2 -adrenergic receptors since administration of α_2 -adrenoceptor agonist clonidine abolished this effect (Devoto *et al.*, 2004). In addition, mirtazapine inhibits α_2 -adrenergic heteroreceptors, present on 5-HT terminals, which have inhibitory roles on the 5-HT release. Indeed, administration of mirtazapine for 21 days enhanced the 5-HT transmission in hippocampus due to the diminished negative feedback of these heteroreceptors as well as a robust increase of DRN 5-HT firing rate (Haddjeri *et al.*, 1998a, b). Thus, the antidepressant action of mirtazapine may stem from its modulatory actions at both NE and 5-HT systems. In support of this, depressed patients who responded to mirtazapine exhibited depressive symptoms following tryptophan depletion and administration of AMPT (Delgado *et al.*, 2002).

The enhancement of both NE and 5-HT transmission by mirtazapine may suggest faster onset of action than an agent with a single action such as the SSRI class. In fact, mirtazapine, but not the SSRI paroxetine, enhanced tonic activation of postsynaptic 5-HT_{1A} receptors in hippocampus after 2 days, although both agents activated these receptors to the same extent after 21 days (Besson *et al.*, 2000). These observations are akin to the clinical reports that mirtazapine demonstrated an accelerated antidepressant response in comparison to the SSRIs fluoxetine, paroxetine and citalopram (Thompson, 1999). The beneficial action of mirtazapine at 5-HT and NE systems may promote this agent as an excellent add-on candidate. Indeed, the remission rates were considerably increased when mirtazapine was coadministered with the SSRI fluoxetine (52%), the SNRI venlafaxine (58%), or bupropion (46%) when compared to monotherapy of fluoxetine (25%, Blier *et al.*, 2010a).

1.5.8 Bupropion

Bupropion, an effective antidepressant, has been the most frequently utilized adjunctive agent for the treatment of resistant depression in North America (Mischoulon *et al.*, 2000). In addition to its great efficacy, clinical data have suggested that its antidepressant action may be endowed with low risk of sexual dysfunction, weight gain and sedation since bupropion has no affinity for 5-HT neuronal elements (DeBattista, 2006). Furthermore,

clinical trials suggest that addition of bupropion to SSRIs may enhance and accelerate the effectiveness of antidepressant response in treatment-resistant patients (Trivedi *et al.*, 2006). Although bupropion has been used in the treatment of depression since the early 1980s, the precise mechanism of its therapeutic actions is not fully understood. A number of articles suggest that bupropion exerts its antidepressant action by blocking DAT and NET. Although the mechanism of action of bupropion is not completely known, DA reuptake inhibition is unlikely because several PET scan studies have reported that clinically effective regimen of bupropion produce no to low occupancy of dopamine reuptake sites (Meyer *et al.*, 2002; Learned-Coughlin *et al.*, 2003; Kugaya *et al.*, 2003; Argyelan *et al.*, 2005). Indeed, the occupancy of the DAT is 15%, a value which is in the test-retest variability of the method. Considering that the potency of bupropion for NE reuptake sites is 100 times less than that of DA transporters (Tatsumi *et al.*, 1997), it is thus unlikely that bupropion exerts its antidepressant effects *via* the blockade of NE reuptake. Furthermore, clinical data indicate drugs that have the ability to block the NE transporter result in attenuation of the increase of systolic blood pressure since entry of intravenously infused tyramine into NE terminals is prevented *via* the NE transporter. However, subjects treated with 150-300 mg per day of bupropion did not exhibit any attenuation in the increase of systolic blood pressure produced by tyramine, whereas the NE reuptake inhibitor nortriptyline abolished this response (Gobbi *et al.*, 2003). The mechanism of action of bupropion will be discussed further in detail in chapters I and II.

2 SPECIFIC AIMS

I. To determine the effects of co-administration of escitalopram and bupropion on DRN 5-HT and LC NE neurons in the rat brain.

II. To investigate the effects of prolonged administration of bupropion on 5-HT and NE neurotransmission in the rat hippocampus.

III. To understand the effects of sustained administration of the antidepressant agent trazodone on 5-HT neurotransmission in DRN and hippocampus in the rat brain.

IV. To characterize the *in vivo* electrophysiological actions of the novel psychotropic drug asenapine at 5-HT_{1A}, 5-HT_{2A}, α_2 -adrenergic and D₂ receptors in the rat brain.

3 MATERIALS AND METHODS

3.1 Animals

The *in vivo* electrophysiological experiments were carried out in male Sprague-Dawley rats (Charles River, St. Constant, QC, Canada) weighing between 250 and 350 g at the time of recordings. The animals were kept, 2 per cage, under standard laboratory conditions (12:12 light-dark cycle with access to food and water *ad libitum*). The animals were allowed to acclimatize to their new environment for at least one week prior to start of any new treatments or experiments. All the experiments were approved by the local Animal Care Committee and conducted in accordance with the Canadian Council on Animal Care, for the care and use of laboratory animals.

3.2 Treatments

The rats were anesthetized with isoflurane to implant, subcutaneously, the osmotic Alzet minipumps (Alza, Palo Alto, Calif., USA), to ensure slow and steady release of the drugs of interest for 2 or 14 days. Such durations were chosen because these drugs require about a week to achieve steady state in humans, but only days in rats, due to their much shorter half-lives in rats (i.e. escitalopram 3 vs 33 hours in rats and humans, respectively; Kreilgaard *et al.*, 2008; Rao, 2007). Prior to the electrophysiological recordings, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Supplemental doses of the anesthetic (100 mg/kg, i.p.) were given to maintain constant anesthesia and to prevent any nociceptive reaction to pinching of the hind paws. It is noteworthy that the use of anesthetic dampens the firing activity of the spontaneously-active monoaminergic neurons since these cells fire more slowly during slow-wave sleep than the active waking (Jacobs and Fornal, 1993). Nevertheless, these neurons exhibit regular and steady firing activity that provides a stable baseline to study the impact of antidepressants on the monoaminergic firing activity. Body temperature was maintained at 37°C throughout the experiment utilizing a thermistor-controlled heating pad. Prior to the electrophysiological experiments, a catheter was inserted in a lateral tail vein for systemic intravenous (i.v.) injection of appropriate

pharmacological agents. The electrophysiological experiments were carried out with the minipumps in place.

3.3 Electrophysiological Recordings

3.3.1 Unitary extracellular recording of DRN 5-HT, LC NE and VTA DA neurons

The extracellular recordings of 5-HT, NE and DA neurons were obtained using single-barrel glass micropipettes. The tips of the electrodes were broken back to 1-3 μm and filled with 2 M NaCl solution. The impedance of the electrodes was between 4 and 7 $\text{M}\Omega$. This technique allows recording of spontaneous firing activity of a single neuron in a living rat. Since the electrode is in close proximity of a neuron, there is less concern of damage to the neuron. Furthermore, this approach provides an opportunity to record many neurons in a given rat.

3.3.2 Recording of DRN 5-HT neurons

In order to record DRN 5-HT neurons, single-barreled microelectrodes were positioned 0.9 – 1.2 mm anterior to lambda on the midline and lowered into the DRN. The presumed 5-HT neurons were encountered over a distance of 1 mm immediately below the ventral border of the Sylvius aqueduct, and identified by their slow (0.5 - 2.5 Hz), regular firing rate and long duration (0.8 - 1.2 msec) positive action potential (Aghajanian, 1978; Vandermaelen and Aghajanian, 1983).

3.3.3 Recording of LC NE neurons

LC NE neurons were recorded with single-barreled glass micropipettes positioned at 1.1 – 1.2 mm posterior to lambda and 0.9 – 1.3 to the midline suture. These neurons were encountered at the depth of 4.5 to 6.0 mm from the surface of brain. The presumed NE neurons were identified by their regular firing rate (0.5 - 5 Hz), a biphasic action potential of long duration (0.8 – 1.2 msec), and a characteristic burst discharge followed by a quiescent period in response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen, 1982b; Ramirez and Wang, 1986).

3.3.4 Recording of VTA DA neurons

The VTA DA neurons were recorded with single-barreled glass micropipettes lowered at 3.0 – 3.6 mm anterior to lambda and 0.6 – 0.8 to the midline suture. These neurons were encountered at the depth of 6.0 to 8.5 mm from the surface of brain. The presumed DA neurons were identified by established electrophysiological criteria; a regular firing rate (0.5 – 7 Hz), an irregular single spiking pattern, slow bursting activity, a long duration (>2.5 msec) often with a notch on the rising phase (Grace and Bunney, 1984; Freeman *et al.*, 1985; Guiard *et al.*, 2008).

3.3.5 Assessment of the sensitivity of 5-HT_{1A} and α_2 -adrenergic autoreceptors

In order to assess the sensitivity of 5-HT_{1A} and α_2 -adrenergic autoreceptors, dose-response curves for the alteration of 5-HT and NE neuronal firing activities were constructed using systemic administration of the 5-HT autoreceptor agonist LSD and the α_2 -adrenoreceptor agonist clonidine, respectively, in control and treated rats. LSD is a more reliable probe than the 5-HT_{1A} agonist 8-OH-DPAT for the 5-HT_{1A} autoreceptor because the latter also acts on 5-HT_{1A} receptors of cortical neurons feeding back to 5-HT neurons (Blier and de Montigny, 1987; Ceci *et al.*, 1994). Furthermore, the responsiveness of dorsal raphe 5-HT neurons to microiontophoretic application of LSD, 8-OH-DPAT and systemic injection of LSD is decreased, whereas that to systemic 8-OH-DPAT is not altered following chronic administration of the 5-HT_{1A} agonist gepirone (Blier and de Montigny, 1987). After systemic injection of LSD or clonidine the selective 5-HT_{1A} antagonist WAY 100,635 and the selective α_2 -adrenoreceptor antagonist idazoxan were injected, respectively, to reverse the decrease in firing activity produced by previously injected agonists. This also served to validate the use of the agonists which are not entirely selective for 5-HT_{1A} and α_2 -adrenergic receptors. Dose-response curves were obtained using only the initial response to the first dose of the agonists injected to a single neuron in each rat.

3.3.6 Analysis of burst firing

Studies have shown that paired or multiple impulses enhance the release of 5-HT (Gartside *et al.*, 2000), NE (Hardebo, 1992) and DA (Gonon, 1988; Garris *et al.*, 1994) neurotransmitters for the same number of single action potentials over the same period of time. Thus, the changes in the burst firing of monoamine neurons were analyzed by interspike interval burst analysis (Grace and Bunney, 1984). The onset of a burst for the DA and NE neurons was taken as the occurrence of two spikes with an interspike interval shorter than 0.08 s. The termination of the NE burst was defined as an interspike interval of 0.16 s or longer (Dawe *et al.*, 2001). Burst firing of DRN 5-HT neurons was defined by brief bursts of action potentials, mostly doublets, with a very short interspike time interval (typically < 10 ms; Hajos and Sharp, 1996).

3.3.7 Dose-response curves

The dose-response curves for the alteration of DRN 5-HT, LC NE and VTA DA neuronal firing activities were constructed using systemic administration of asenapine. First, the novel psychotropic agent asenapine was administered systemically to determine its intrinsic effect on the basal firing of 5-HT, NE and DA neurons. It is important to note that the firing rate of all the monoaminergic neurons used in these experiments was within their normal firing rate, and that a 2-minute period was allowed to obtain a stable baseline before the *i.v.* injections. The rate and pattern of firing rate were typical for 5-HT, NE and DA neurons as previously described (Aghajanian, 1978; Aghajanian and Vandermaelen, 1982a, b; Grace and Bunney, 1984). Changes are expressed as percentages of baseline firing rate. Second, in a different series of experiments, asenapine was administered, following the suppression of 5-HT, NE and DA neuronal firing, to determine whether it has *in vivo* antagonistic effects at 5-HT_{1A} autoreceptors, 5-HT_{2A} receptors, α_2 -adrenoreceptors, and D₂ autoreceptors. Consecutive *i.v.* injections of asenapine (1, 10, 100 and 1000 $\mu\text{g}/\text{kg}$) were administered at 2-minute intervals after sustained inhibition of 5-HT neuronal firing with the 5-HT_{1A} agonist 8-OH-DPAT (10 $\mu\text{g}/\text{kg}$), NE neuronal firing with the α_2 -adrenoreceptor agonist clonidine (10 $\mu\text{g}/\text{kg}$), NE firing with the 5-HT_{2A} receptor agonist DOI (50 $\mu\text{g}/\text{kg}$), and DA firing with the D₂ autoreceptor agonist apomorphine (40 $\mu\text{g}/\text{kg}$). In order to avoid the residual drug effects, the dose-response curves were generated using only one single neuron in each rat.

3.3.8 Extracellular unitary recording and microiontophoresis DRN 5-HT neurons

Five-barreled glass micropipettes were used to record spontaneous activity of DRN 5-HT neurons and to perform microiontophoretic applications of 5-HT and asenapine. The impedance of these electrodes for DRN ranged from 1.5 to 3 M Ω . The duration of the microiontophoretic applications of the pharmacological agents was kept constant throughout the experiments. The same ejection current was always used before and after each *i.v.* injection of the 5-HT_{1A} antagonist WAY 100,635. Neuronal responsiveness to the microiontophoretic ejections of 5-HT, asenapine and co-application of 5-HT and asenapine, prior to and following *i.v.* injection of WAY 100,635, was assessed by determining the number of spikes suppressed per nanoampere (nA).

3.3.9 Recording and microiontophoresis of CA₃ hippocampus pyramidal neurons

Extracellular recording and microiontophoresis of CA₃ pyramidal neurons were performed with five-barreled glass micropipettes. The central barrel, used for the unitary recording, was filled with a 2 M NaCl solution, and the impedance of these electrodes ranged from 2 to 4 M Ω . Depending on the experiments the side barrels were filled with the following solutions: 5-HT creatinine sulfate (10 mM in 200 mM NaCl, pH 4), (\pm)-NE bitartrate (10 mM in 200 mM NaCl, pH 4), asenapine (0.05 mmol/liter in 200 mmol/liter NaCl, pH 4) or trazodone hydrochloride (20 mM in 200 mM NaCl, pH 4), quisqualic acid (1.5 mM in 200 mM NaCl, pH 8), and the last barrel was filled with a 2 M NaCl solution used for automatic current balancing. The micropipettes were lowered into the dorsal hippocampus CA₃ region using the following coordinates; 4 - 4.2 mm anterior to lambda and 4 - 4.4 mm lateral (Paxinos and Watson, 1998). A small current of quisqualate +2 to -5 nA was used to activate the pyramidal neurons within their physiological firing range (10 to 15 Hz; Ranck, 1975) because these neurons do not discharge spontaneously in chloral hydrate anesthetized rats. The hippocampus CA₃ pyramidal neurons were found at a depth of approximately 4.0 \pm 0.5 mm below the surface of the brain, and identified by their large amplitude (0.5 - 1.2 mV) and long-duration (0.8 - 1.2 ms) simple action potentials, alternating with complex spike discharges (Kandel and Spencer, 1961). The duration of

local application of the agents and the ejection currents (in nA) were kept constant before and after each *i.v.* injection of 5-HT_{1A} antagonist WAY 100,635. The responsiveness of CA₃ pyramidal neurons to the microiontophoretic application of 5-HT and NE, prior to and following *i.v.* injections, was assessed by determining the number of spikes suppressed per nA for the ejection period.

3.3.10 Assessment of the tonic activation of hippocampus 5-HT_{1A} receptors

The degree of tonic activation of hippocampus CA₃ 5-HT_{1A} receptors was assessed using systemic injection of the potent and selective 5-HT_{1A} antagonist WAY 100,635 (Khawaja *et al.*, 1995; Fletcher *et al.*, 1996). Such disinhibition of the neuronal activity is best assessed when the firing rate is low. Indeed, a low stable firing baseline was obtained by lowering the ejection current of quisqualate. The baseline firing was recorded for at least 2 minutes before the administration of WAY 100,635. WAY 100,635 (100 µg/kg) was systemically administered in incremental doses of 25 µg/kg at time intervals of 2 minutes to detect the changes in the firing activity of hippocampus pyramidal neurons in control and treated rats. Such curves represent stable changes in the firing rate of CA₃ pyramidal neurons as percentages of baseline firing following each systemic drug administration. In order to avoid residual drug effects, only one neuron in each rat was studied. The electrophysiological model for the assessment of the tonic activation of 5-HT_{1A} receptors in hippocampus is presented in Figure 7. In contrast to the control condition, the systemic administration of the 5-HT_{1A} antagonist WAY 100,635 induces disinhibition of the firing rate of CA₃ pyramidal neurons, thereby indicating an enhanced tonic activation of these receptors by increased levels of endogenous 5-HT in the presence of an antidepressant (Fig. 7). Indeed, the strength of this model was put into evidence by the commonality of all classes of antidepressant treatments including tricyclics, MAOIs, SSRIs, mirtazapine, the 5-HT_{1A} agonist gepirone, agomelatine, and electroconvulsive shocks (ECS) as well as vagus nerve stimulation (Haddjeri *et al.*, 1998a; Manta *et al.*, 2009; Blier *et al.*, 2010b). In contrast, the classical antipsychotic chlorpromazine, which does not exert antidepressant activity, is devoid of the disinhibitory effect in this paradigm (Haddjeri *et al.*, 1998a). As an active control, the effects of electroconvulsive shock (ECS) treated rats was also studied in the hippocampus. Repeated ECS was chosen as an active control since prior

electrophysiological experiments showed that it enhanced 5-HT transmission in the hippocampus and they sensitize postsynaptic 5-HT_{1A} receptors, thereby providing an internal standard for both parameters (Chaput *et al.*, 1991; Haddjeri *et al.*, 1998a). Under transient isoflurane anesthesia, these rats received seven ECS, one shock every other day, for 14 days (150 V pulses of 10 msec duration delivered at a frequency of 50 Hz for 1 sec; de Montigny, 1984).

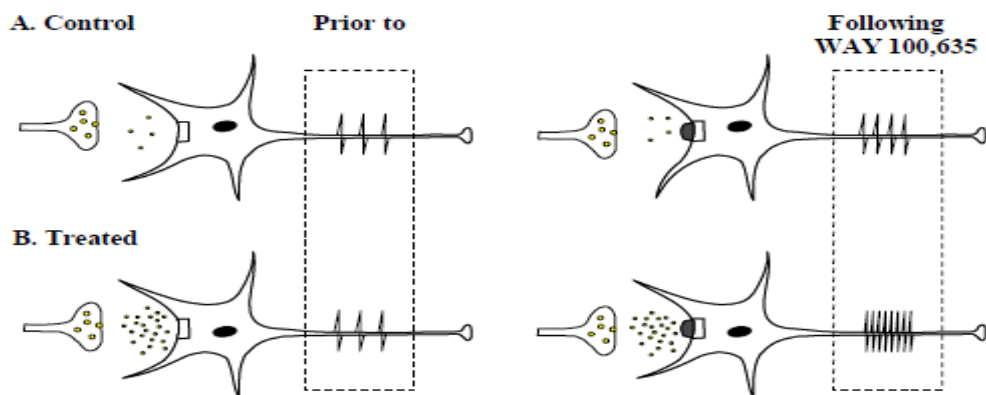


Figure 7. Electrophysiological model representing the assessment of tonic activation of postsynaptic 5-HT_{1A} receptors in rat hippocampus prior to and following systemic administration of 5-HT_{1A} antagonist WAY 100,635. The treated (B) paradigm corresponds to various antidepressant treatments including the tricyclic antidepressant imipramine, the MAOI biefloxatone, mirtazapine, the SSRI paroxetine, the 5-HT_{1A} receptor agonist gepirone, and repeated ECS (Haddjeri *et al.*, 1998a).

3.3.11 Assessment of tonic activation of hippocampus α_2 -, α_1 -adrenoceptors

In paper III, the degree of tonic activation of postsynaptic α_2 - and α_1 -adrenoceptors was assessed using the selective antagonists idazoxan and prazosin, respectively. Indeed, after lowering and obtaining a steady firing baseline idazoxan (1000 μ g/kg) and prazosin (100 μ g/kg) were systemically administered to determine the disinhibitory effects in rats treated with saline or bupropion for 14 days. The above-mentioned doses of the antagonists were administered since such doses were shown to have physiological effects in electrophysiological settings (Curet and de Montigny, 1988a). This approach stems from the model for the assessment of tonic activation of postsynaptic 5-HT_{1A} receptors in hippocampus (Fig. 7).

3.3.12 Stimulation of the ascending 5-HT pathway

The ascending 5-HT pathway was electrically stimulated using a bipolar electrode (NE-100, David Kopf, Tujunga, CA, USA). The electrode was implanted 1 mm anterior to lambda on the midline with a 10° backward angle in the ventromedial tagmentum and 8.0 ± 0.2 mm below the surface of the brain. Two hundred square pulses of 0.5 msec in duration were delivered by a stimulator (S48, Grass Instruments, West Warwick, RI, USA) at an intensity of 300 μ A and a frequency of 1 or 5 Hz. The effects of stimulation of 5-HT pathway were assessed using 1 and 5 Hz for the same neuron. The different frequencies were used to determine the function of terminal 5-HT_{1B} autoreceptors (Chaput *et al.*, 1986a). This approach is based on the evidence that when the frequency is increased to 5 Hz, more 5-HT is released in the extracellular cleft, which consequently exerts a greater negative feedback on the 5-HT release *via* the terminal 5-HT_{1B} autoreceptors (Fig. 8, Chaput *et al.*, 1986a). Therefore, the release of 5-HT is inhibited quickly during the 5 Hz stimulation leading to a smaller release of transmitter in the synapse for each action potential reaching the terminal. The stimulation pulses and the firing activity were analyzed by computer using Spike 2 (Cambridge Electronic Design Limited, UK). Peristimulus time histograms of hippocampal pyramidal neurons were generated to determine the suppression of firing measured in absolute silence (SIL) value in msec. The SIL represents the duration of a total suppression of the hippocampal neuron. This parameter was calculated by computer by dividing the total number of events, suppressed by the stimulation, by the frequency of firing of the recorded neuron.

Moreover, the effects of 1 Hz stimulations of the ascending 5-HT fibers were assessed prior to and following the *i.v.* injections of the α_2 -adrenoceptor agonist clonidine 10 and 400 μ k/kg while recording from the same neuron. The low and high doses of clonidine were used to assess sensitivity of the α_2 -adrenergic auto- and heteroreceptors, respectively. Previous studies showed that clonidine is 10-fold more potent at α_2 -adrenergic autoreceptors than the α_2 -adrenergic heteroreceptors (Frankhuijzen and Mulder 1982; Maura *et al.*, 1985). The low dose of clonidine (10 μ k/kg) potentiates the effect of stimulation of 5-HT pathway by stimulating the α_2 -adrenergic autoreceptors that are present on the NE terminals, leading to inhibition of NE firing and disinhibition of 5-HT terminals (Lacroix *et al.*, 1991). Indeed, the effect of the low, but not the high, dose of

clonidine was abolished when the NE neurons were lesioned (Mongeau *et al.*, 1993). On the other hand, the high dose of clonidine (400 $\mu\text{k}/\text{kg}$) inhibits the effect of 5-HT stimulation by acting on α_2 -adrenergic heteroceptors, located on the 5-HT terminals, leading to inhibition of 5-HT release. Therefore, 1 Hz stimulations of 5-HT bundle result in a greater 5-HT release and increased SIL value after the *i.v.* injection of the low clonidine dose, and a smaller 5-HT release, and a shorter inhibition of pyramidal firing (smaller SIL) following a high dose of clonidine.

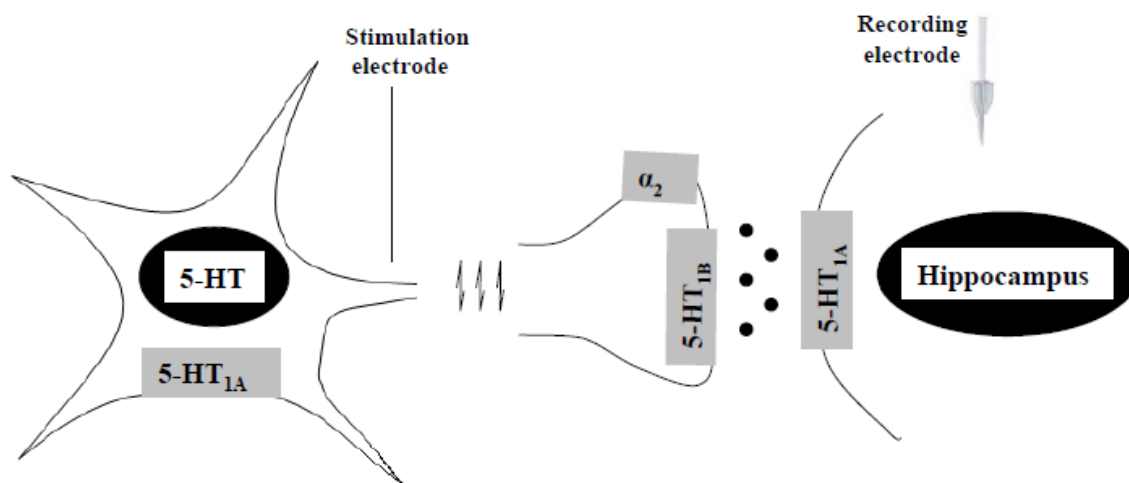


Figure 8. A diagram illustrating electrical stimulation of the ascending DRN 5-HT projection to hippocampus, and microiontophoresis in the postsynaptic region.

3.3.13 Stimulation of the ascending NE pathway

In order to stimulate the ascending NE pathway, a bipolar electrode (NE-100, David Kopf, Tujunga, CA, USA) was positioned with a 10° backward angle in the LC at 1.1 mm posterior to the lambda and 1.1 mm lateral to the midline at a depth of 5.8 ± 0.2 mm from the surface of the brain. Electrical stimulation of NE neurons consisted of 200 square pulses of a duration of 0.5 msec delivered by a stimulator (S48, Grass Instruments, West Warwick, RI, USA) at a current of 800 μA and a frequency of 1 or 5 Hz. The 800 μA current was used since stimulation at this intensity generates consistent SIL values on the firing activity of hippocampal pyramidal neurons (Curet and de Montigny, 1989). The sensitivity of terminal NE α_2 -adrenergic autoreceptors was assessed using the efficacy of stimulation of NE pathway delivered at 1 and 5 Hz on the same neuron. Stimulation of NE pathway at 1 Hz leads to release of NE in the synaptic cleft which suppresses the firing

activity of pyramidal neurons *via* activation of postsynaptic inhibitory α_1 -adrenergic receptors (Fig. 9, Curet and de Montigny, 1988a). However, increasing the frequency to 5 Hz reduces the efficacy of stimulation due to enhanced activation of terminal α_2 -adrenergic autoreceptors. Indeed, administration of α_2 -adrenergic antagonist idazoxan enhanced the efficacy of NE stimulation at 5 Hz (Curet and de Montigny, 1989). The degree of suppression of firing activity of hippocampal pyramidal neurons was determined using SIL values as described above (Chaput *et al.*, 1986a; Curet and de Montigny, 1988a). Curet and de Montigny (1988a) demonstrated that stimulation of the LC pathway generated a period of suppression followed by an activation period. Indeed the latter effect was due to the involvement of β -adrenoceptors since systemic administration of β -adrenoceptor antagonist propranolol selectively diminished the activation period (Curet and de Montigny, 1988a). Thus, the degree of activation of β -adrenoceptors was assessed by determining the duration of activation period (ACT in msec). This value represents the duration of activation relative to the firing activity of the recorded neuron. It was calculated as the time interval initiated by a 50% increase in the firing activity, relative to prestimulation, and terminated by its return to 110% of the prestimulation firing (Curet and de Montigny, 1988a).

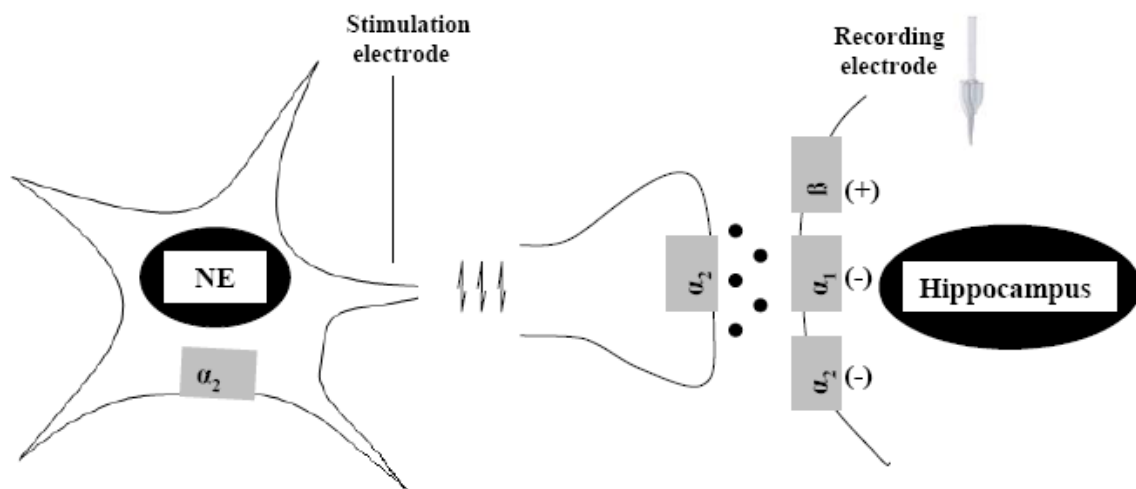


Figure 9. A diagram illustrating electrical stimulation of the ascending LC NE projection to hippocampus, and microiontophoresis in the postsynaptic region. The plus and minus signs in the parentheses represent an excitatory and an inhibitory action, respectively.

3.3.14 *In vivo* determination of 5-HT uptake

In order to assess the relative degree to which trazodone blocks the 5-HTT, the RT₅₀ values were determined after microiontophoretic application of 5-HT in hippocampus CA₃ region. The RT₅₀ values correspond to the time in seconds elapsed from the cessation of microiontophoretic application of 5-HT to obtain a 50% recovery of the initial firing rate (de Montigny *et al.*, 1980). It is a reliable index of 5-HT reuptake process *in vivo*. Indeed, previous experiments showed that acute systemic injection of the SSRI paroxetine significantly increased RT₅₀ values. Furthermore, this phenomenon was also observed in rats after the lesion of 5-HT neurons, thereby eliminating 5-HTT (Piñeyro *et al.*, 1994).

3.3.15 *In vitro* determination of [³H]5-HT uptake

To determine the degree of 5-HT reuptake inhibition in hippocampus, following a 2-day administration of trazodone (5, 10 and 20 mg/kg/day) and the SSRI escitalopram (10 mg/kg/day; used as a positive control), hippocampal slices were incubated in oxygenated Krebs' solution at 37°C to determine *in vitro* [³H]5-HT uptake. After a 3-minute stabilization period, the slices were incubated with 20 nM of [³H]5-HT for 3 minutes. The uptake process was stopped by transferring the slices into 5 ml ice-cold Krebs' solution. The slices were then solubilized in 0.5 ml of Soluene 350. A parallel experiment was carried out at 0°C as a control for passive diffusion. The radioactivity in the media and tissue was determined by liquid scintillation spectrometry (Beckman Coulter, CA, USA). The reuptake activity was assessed by determining the tissue-medium ratio of radioactivity, using the following formula: $(R_C - R_T)/(R_C - R_0)$, where R_C is the ratio of tissue to medium for the control slice, R_T is the ratio of tissue for the treated slice, and R₀ is the ratio of tissue to medium for the control slice at 0°C. For the *in vitro* determination of [³H]5-HT uptake, each set of experiment consisted of 5 test tubes each containing 2 hippocampus slices from a single rat, and a total of 3 – 6 rats were used per group.

3.3.16 Quantification of trazodone and mCPP in the brain

Standards preparation

Trazodone, mCPP and the internal standard domperidone were initially prepared at 1 mg/mL in DMSO and subsequently diluted in acetonitrile. For analysis in brain, trazodone and mCPP were added to 100 mg/mL control brain homogenate (5 – 2500 pg/mg tissue)

and extracted as described below. Domperidone was prepared at 1 µg/mL in acetonitrile to be used as an internal standard.

Sample preparation

Tissue samples were homogenized at 100 mg/mL in deionized water and 100 µL of tissue homogenate was used for the extraction. To each sample, 10 ng (10 µL of 1 µg/mL) of domperidone was added followed by 1 mL of ethyl acetate containing 0.1% (v/v) ammonium hydroxide (30%, w/v) and extraction by vortexing for 10 min. Organic and aqueous layers were separated by centrifugation (10 min, 20,000 x g) and the organic layer removed, evaporated by vacuum centrifugation at room temperature and reconstituted in 200 µL of beginning mobile phase (20% acetonitrile: 80% 0.1% formic acid in water) for LC/MS/MS analysis.

Mass spectrometry

Positive ion electrospray ionization (ESI) mass spectra were obtained with an AB Sciex 3200 QTRAPTM triple quadrupole mass spectrometer (Foster City, CA) with a turbo VTM ion source interfaced to a Shimadzu HPLC system. Samples were chromatographed with a Waters Sunfire C18, 2.5 µm, 50mm×4.6 mm column (Milford, MA). The LC was a gradient elution utilizing 100% acetonitrile as the organic phase and 0.1% formic acid in water as the aqueous as follows: 20% acetonitrile for 0.1 min, linearly ramp to 90% acetonitrile at 1.5 min, hold at 90% for 30 sec, return to 20% acetonitrile over 30 sec and equilibrate column for 30 sec at 20% acetonitrile. The flow rate was 1 ml/min and sample injection volume of 60 µL. The analysis time was 3 min. The mass spectrometer settings were: temperature, 550°C; spray needle, 5500V; curtain gas, 10; collision gas, N₂ (CAD), 3; ion source gas 1 and 2; 55 and 45, respectively. The compound dependent settings for trazodone, mCPP and domperidone were as follows, respectively: declustering potential, 57, 24 and 63; excitation potential, 4, 10, and 9; collision cell entrance potential, 21, 26, and 19; collision energy, 33, 26 and 36; and collision cell exit potential, 3, 3 and 2. Samples were quantified by the internal standard reference method in the MRM mode by monitoring the transition m/z 372→176 for trazodone, m/z 197→154 for mCPP, m/z 426→175 for the internal standard domperidone. Each ion transition was integrated for

250 msec. Quantitation of trazodone and mCPP in the brain were based on standard curves in spiked matrix using the ratio of either analyte peak area to domperidone peak area using $1/x^2$ weighting for both analytes.

3.4 Drugs

Psychotropic drugs

Bupropion HCl	Biovail, Canada
Escitalopram	Lundbeck, Copenhagen, DK
Asenapine	Schering-Plough Corporation, Newhouse, Motherwell, UK
Trazodone HCl	Labopharm, Montreal, QC, Canada

Bupropion HCl and Escitalopram were dissolved in physiological saline. Asenapine was dissolved in distilled water. Hydroxy propyl-beta-cyclodextrin 20% was used to dissolve trazodone HCl.

Other drugs

LSD (Health Canada), 8-OH-DPAT, WAY 100,635, clonidine hydrochloride, idazoxan hydrochloride, DOI, apomorphine, 5-HT creatinine sulfate, (\pm)-NE bitartrate and quisqualic acid were purchased from Sigma, St. Louis, MO, USA, and dissolved in distilled water. [3 H]5-HT was purchased from PerkinElmer Life Sciences (Boston, MA, USA). For quantification experiments in paper IV, trazodone, mCPP and domperidone were obtained from Sigma (St. Louis, MO, USA). All other chemicals and solvents were of reagent or higher grade and obtained from Fisher Scientific (Pittsburgh, PA).

Anaesthetics

Chloral hydrate and isoflurane were purchased from sigma (St. Louis, MO, USA) and Benson (River Edge, NJ, USA), respectively.

3.5 Statistical analysis

All the results are expressed as mean \pm SEM. Data were obtained from 3 to 8 rats per experimental group unless otherwise stated. The n represents the number of neurons tested for a given group. Statistical comparisons for the firing rates were carried out using a one-way analysis of variance (treatment as the main factor) and multiple comparison procedures using Fisher's PLSD *post hoc* test (StatView software). The dose-response curves were constructed using linear regression analysis (GraphPad Prism software Inc, La Jolla, CA). All the obtained ED₅₀ values are also expressed as \pm SEM. In the trazodone study, statistical comparisons were carried out using a one-way analysis of variance (treatment as the main factor) and the Bonferroni *post hoc* analysis was conducted when significant ANOVA results were obtained (GraphPad Software Inc, La Jolla, CA). Statistical comparisons were carried out using the two-tailed Student's *t* test when a parameter was studied in control and treated rats. For the stimulation experiments, the effects of changing the frequency of stimulation from 1 to 5 Hz on the SIL value of the same neuron were assessed using the paired Student's *t* test. Analysis of covariance was used to assess statistical significance of the difference in the degree of reduction in the response of hippocampus neurons when the frequency of stimulation was increased from 1 to 5 Hz in control and trazodone-treated rats.

In all data analysis, statistical significance was taken as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4 RESULTS AND DISCUSSION

CHAPTER I:

AIM: To determine the effects of co-administration of escitalopram and bupropion on DRN 5-HT and LC NE neurons in the rat brain

4.1 Rationale:

As mentioned previously, although the pathophysiology of depression is not precisely understood, more than four decades of research using experimental models and clinical studies have established that the enhancement of 5-HT neurotransmission plays a key role in treatment of this burdensome illness. A great body of literature implicates crucial effects of SSRIs on 5-HT neurons.

Despite the fact that SSRIs are the first line approach in treating depression, there are limitations with respect to their therapeutic effects. One of these limitations is the degree of effectiveness of SSRIs: only 50 to 60% of depressed patients experience marked improvement from such a first antidepressant treatment (DeBattista, 2006). Most importantly, the remission rate of most antidepressant drugs is just 25 to 45% (Thase *et al.*, 2001). Another major limitation of SSRIs is their delayed therapeutic effects. It has been shown that SSRIs promptly inhibit 5-HTT. However, it takes at least 2 to 3 weeks to obtain an antidepressant response. In addition, the lack of effectiveness of SSRIs during the lag phase is sometimes coupled with various side effects, such as nausea and increased anxiety (Trivedi *et al.*, 2006). In contrast, some antidepressant strategies, such as electroconvulsive therapy and sleep deprivation exert their therapeutic effects faster than others, thus the antidepressant response is not necessarily endowed with significant inertia (Daly *et al.*, 2001). Consequently, there have been various attempts, using different strategies, to increase and accelerate the effectiveness of SSRIs and the antidepressant response.

Bupropion, an aminoketone antidepressant, has been the most frequently used adjunctive strategy in North America for the treatment of resistant depression (Mischoulon *et al.*, 2000). Indeed, addition of bupropion to SSRIs enhances effectiveness of antidepressant

response in treatment-resistant patients (Trivedi *et al.*, 2006). Moreover, its efficacy is combined with low risk of sexual dysfunction, weight gain and sedation (DeBattista, 2006) since bupropion has no appreciable affinity for 5-HTT and 5-HT receptors. With respect to the mechanism of action, dopamine reuptake inhibition is unlikely since clinically effective doses of bupropion produce no to low occupancy of dopamine reuptake sites (Meyer *et al.*, 2002; Learned-Coughlin *et al.*, 2003; Kugaya *et al.*, 2003; Argyelan *et al.*, 2005). Moreover, a lack of NE reuptake inhibition is indicated by its lack of inhibitory effect on the tyramine pressor response, contrarily to NE reuptake inhibitors (Gobbi *et al.*, 2003). The proposed NE-releasing mechanism of bupropion leads to a robust increase of DRN 5-HT firing rate after a 2-day treatment, unlike NE reuptake inhibitors (Dong and Blier, 2001). This effect of bupropion is no longer present in LC lesioned rats (Dong and Blier, 2001). Consistent with this view, pharmacological agents that increase raphe firing *via* α_1 -adrenoceptor activation, such as phenylephrine and cirazoline, provide support for the excitatory NE modulation of raphe activity (Millan *et al.*, 1994). In contrast, escitalopram produces a sustained decrease of NE neuronal activity (Dremencov *et al.*, 2007a), which could be clinically counter-productive in some patients. Thus, the present experiments, using *in vivo* electrophysiological techniques, were undertaken to explore short-term and long-term effects of co-administration of bupropion with the SSRI escitalopram, as well as their monotherapies, on DRN 5-HT and LC NE neuronal firing. It was hypothesized that bupropion may prevent the inhibitory action of escitalopram on 5-HT and NE neuronal activity.

Results

4.1.1 Effects of 2-day and 14-day administration of escitalopram, bupropion and their combination on DRN 5-HT neuronal firing activity

A two-day treatment with the SSRI escitalopram (10 mg/kg/day) decreased the spontaneous firing rate of DRN 5-HT neurons by 70% ($p < 0.001$), in comparison to the saline-treated rats. Bupropion, injected subcutaneously at 30 mg/kg/day, increased the firing rate of 5-HT neurons by 30%, which was not significant ($p > 0.05$). Unlike the effects of escitalopram or bupropion given alone, the co-administration of the two drugs unexpectedly doubled the firing rate of DRN 5-HT neurons ($p < 0.01$; Fig. 10A).

The firing rate of DRN 5-HT neurons recovered following the 14-day regimen with escitalopram (10 mg/kg/day). Administration of bupropion for 14 days enhanced the firing rate of 5-HT neurons by 50% ($p < 0.05$). The firing rate of DRN 5-HT neurons remained significantly high following co-administration of escitalopram and bupropion for 14 days when compared to the saline-treated rats ($p < 0.01$; Fig. 10B).

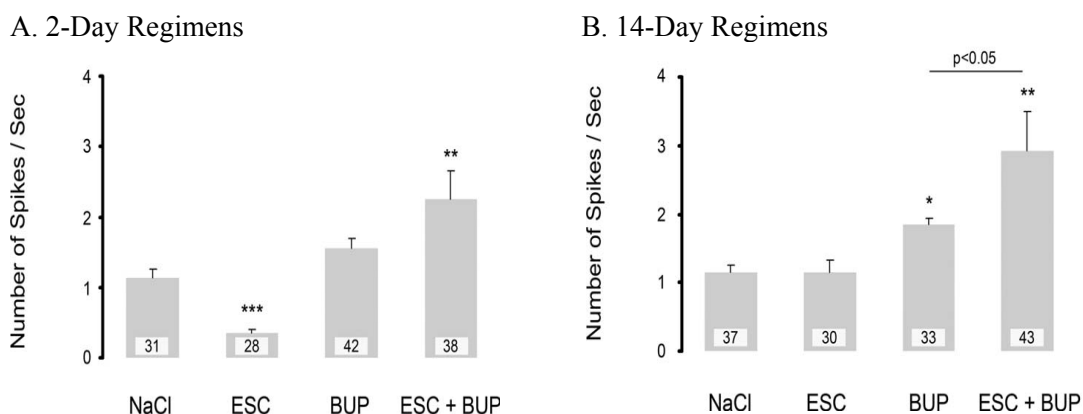


Figure 10: The effects of 2- (A) and 14-day (B) administration of saline, escitalopram (ESC), bupropion (BUP) and the co-administration of the drugs (ESC+BUP) on raphe 5-HT neuronal firing rate. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ when compared to the saline group. The numbers at the bottom of the columns indicate the number of neurons recorded.

Analysis of the number of spontaneously active neurons revealed a significant decrease only in escitalopram-treated rats for 2 days ($p < 0.01$; Table 1). Therefore, the average firing rate for the 2-day escitalopram group is an underestimated value because of the decreased number of spontaneously active 5-HT neurons. Interestingly, bupropion increased percentage of DRN 5-HT neurons discharging in burst mode after both 2- and 14-day regimens ($p < 0.05$ and $p < 0.01$ respectively), whereas the percentage of neurons discharging in burst mode was decreased in the escitalopram-treated rats after the 2-day regimen ($p < 0.001$; Table 2).

Table 1. Average number of serotonergic neurons per electrode descent in the dorsal raphe[§]

Groups	2 days	14 days
NaCl	3.5 ± 0.4 (9)	3.7 ± 0.2 (10)
Escitalopram	2.2 ± 0.3** (13)	3.4 ± 0.3 (9)
Bupropion	3.5 ± 0.3 (12)	3.7 ± 0.3 (9)
Escitalopram + bupropion	3.5 ± 0.4 (11)	3.6 ± 0.2 (12)

[§]A descent consisted of lowering a single-barrelled electrode over a 1 mm distance from the floor of the sylvius aqueduct and recording spontaneously firing 5-HT neurons. The numbers in the parenthesis correspond to the number of descents in each group. ** p < 0.01

Table 2: Percentage of serotonergic neurons displaying burst activity[§]

Groups	2 days	14 days
NaCl	19 ± 2	23 ± 2
Escitalopram	2 ± 1***	20 ± 1
Bupropion	27 ± 4*	36 ± 4**
Escitalopram + bupropion	24 ± 3	23 ± 2

[§]A percentage was first obtained for each rat and then averaged out. The average number of spikes/burst was 2 ± 0.1 in the controls and was not significantly different in any of the other groups. * p < 0.05; ** p < 0.01; *** p < 0.001 when compared to the control.

4.1.2 Assessment of sensitivity of somatodendritic 5-HT_{1A} autoreceptors

The 5-HT autoreceptor agonist LSD was used as a probe to reliably assess responsiveness of 5-HT_{1A} autoreceptor. LSD rapidly and completely inhibited the firing of DRN 5-HT neurons in all control rats at the dose of 10 µg/kg (ED₅₀ = 6.7 ± 1.0 µg/kg). In contrast, the inhibitory effect of this same dose was significantly attenuated on the neuronal activity of 5-HT neurons in rats treated with bupropion for 14 days, and in rats treated with escitalopram plus bupropion for 2 days when compared to the control group (Fig. 11). Indeed, neuronal firing of DRN 5-HT cells in rats given bupropion alone for 14 days and escitalopram plus bupropion for 2 days were completely inhibited at the dose of 20 µg/kg (ED₅₀ = 12.9 ± 1.1 µg/kg, p < 0.05) and 25 µg/kg (ED₅₀ = 14.5 ± 1.1 µg/kg, p < 0.05), respectively (Fig. 12).

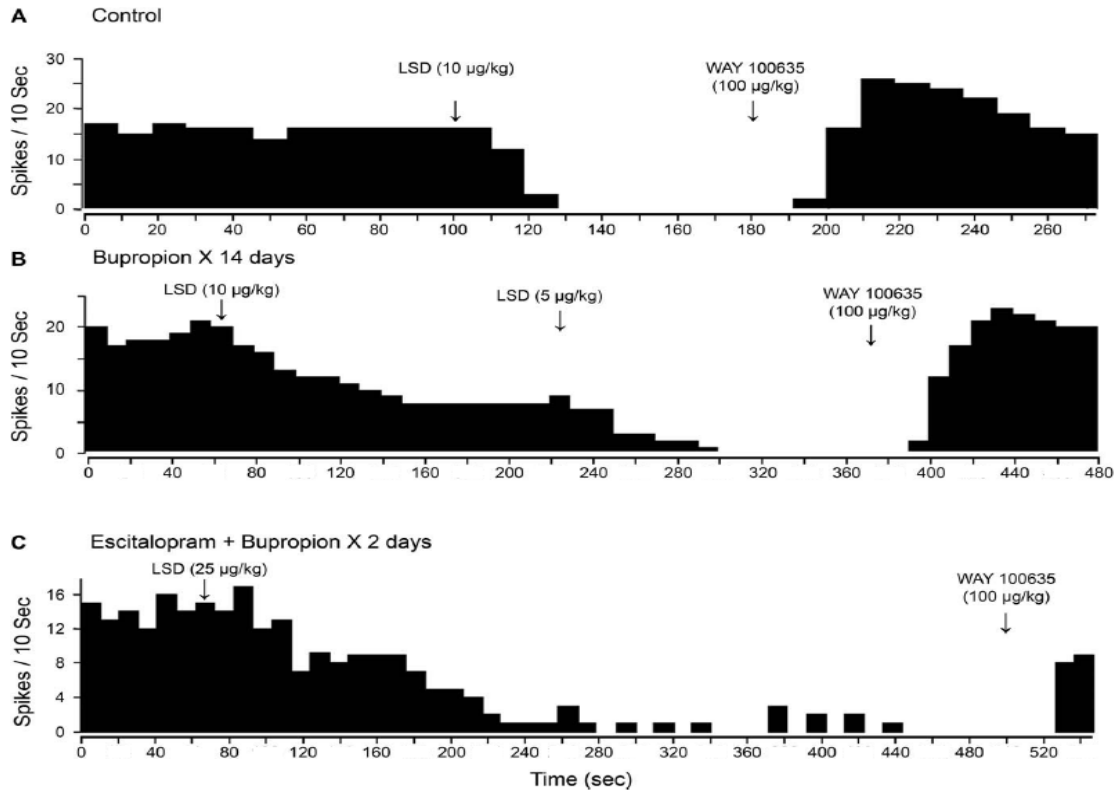


Figure 11: Representative integrated firing rate histograms of dorsal raphe 5-HT neurons illustrating the effect of intravenous administration of 5-HT autoreceptor agonist LSD in suppressing neuronal activity of rats treated with NaCl (A), bupropion (B) for 14 days, and co-administration of escitalopram and bupropion (C) for 2 days.

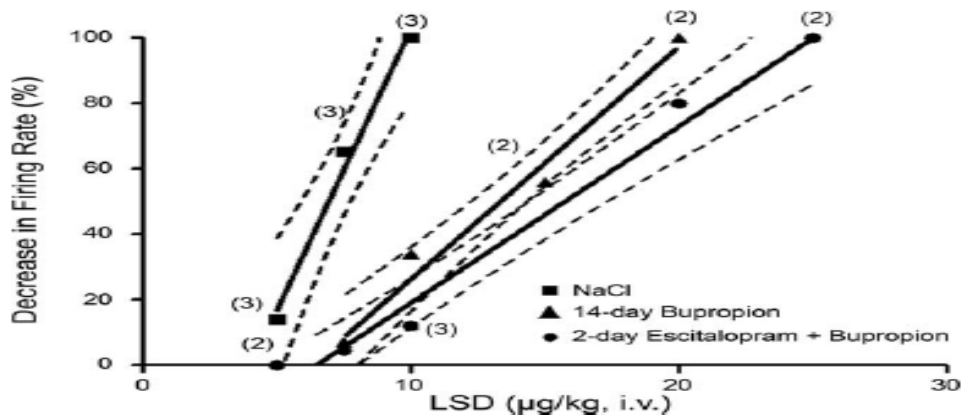


Figure 12: Relationship between the degree of suppression of DRN 5-HT firing activity and doses of LSD administered intravenously in control and treated rats. Only the initial response of a single 5-HT neuron to the first dose of LSD in each rat was used to construct the curves. Outer lines represent the standard error of the regression line. If more than one neuron was tested for a given dose, the number of neurons is illustrated in parentheses.

4.1.3 Effects of 2-day and 14-day administration of escitalopram, bupropion and their combination on LC NE neuronal firing activity

A two-day treatment with bupropion (30 mg/kg/day) reduced the firing rate of LC NE neurons by 55% ($p < 0.001$) when compared to control rats. Escitalopram also decreased the activity of NE neurons by 55% ($p < 0.001$). The co-administration of these drugs decreased the firing rate by 60% ($p < 0.001$; Fig. 13A).

Co-administration of escitalopram and bupropion, for 14 days, prompted a partial recovery of LC NE neuronal firing rate ($p < 0.01$) when compared to the saline group and the 2-day combination data ($p < 0.001$). The mean firing activity of LC NE neurons was back to the baseline after the 14-day treatment with bupropion. Escitalopram, on the other hand, still significantly suppressed firing rate of NE neurons ($p < 0.001$; Fig. 13B).

A. 2-Day Regimens

B. 14-Day Regimens

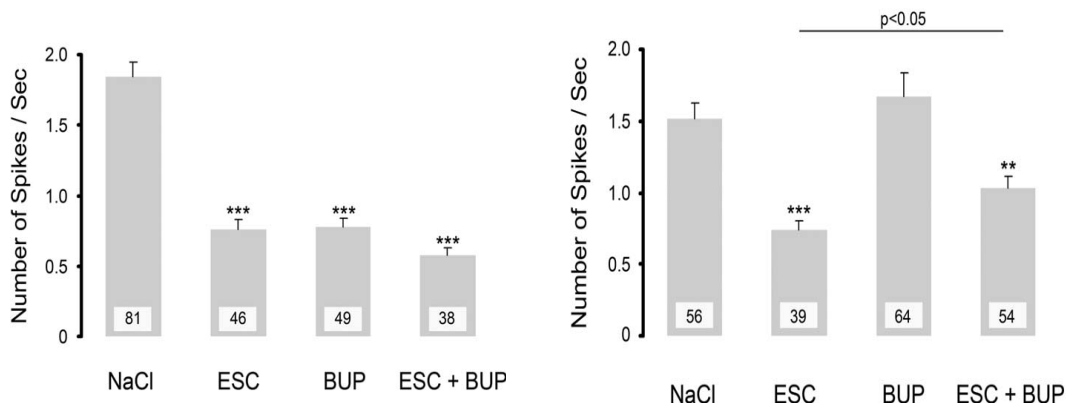


Figure 13: The effects of 2- (A) and 14-day (B) administration of saline, escitalopram (ESC), bupropion (BUP) and the co-administration of the drugs (ESC+BUP) on locus coeruleus NE neuronal firing rate. ** $p < 0.01$; *** $p < 0.001$ when compared to the saline group. The numbers at the bottom of the columns indicate the number of neurons recorded.

It is important to note that the number of spontaneously active NE neurons recorded from the treated rats was lower than that of the control rats after the 2-day regimens in all groups ($p < 0.001$). This parameter was back to normal only in the presence of bupropion after 14 days (Table 3). Therefore, the average firing rate for all the 2-day treated groups and the 14-day ESC group is an underestimated value because of this decreased number of

spontaneously active NE neurons. Burst activity of the LC NE neurons in rats treated with escitalopram, bupropion and the combination was decreased ($p < 0.001$, $p < 0.01$ and $p < 0.001$ respectively) after the 2-day regimens. After 14 days, this parameter was still attenuated in the escitalopram-treated rats ($p < 0.001$) and was increased by 43% only in the bupropion group ($p < 0.01$; Table 4).

Table 3. Average number of norepinephrine neurons per electrode descent in locus coeruleus[§]

Groups	2 days	14 days
NaCl	4.8 ± 0.1 (17)	4.7 ± 0.2 (12)
Escitalopram	2.3 ± 0.2*** (20)	2.2 ± 0.3*** (18)
Bupropion	3.3 ± 0.3*** (15)	4.6 ± 0.3 (14)
Escitalopram + bupropion	2.0 ± 0.2*** (19)	4.2 ± 0.5 (13)

[§]A descent consisted of lowering a single-barrelled electrode over a 500 µm distance from the floor of the fourth ventricle and recording spontaneously firing NE neurons. The numbers in the parenthesis correspond to the number of descents in each group. *** $p < 0.001$

Table 4. Percentage of noradrenergic neurons displaying burst activity[§]

Groups	2 days	14 days
NaCl	32 ± 4	30 ± 3
Escitalopram	12 ± 2***	9 ± 3***
Bupropion	18 ± 3**	43 ± 4**
Escitalopram + bupropion	4 ± 2***	26 ± 2

[§]A percentage was first obtained for each rat and then averaged out. The average number of spikes/burst was 2 ± 0.2 in the controls and was not significantly different in any of the other groups. ** $p < 0.01$; *** $p < 0.001$

4.1.4 Assessment of sensitivity of somatodendritic α_2 -adrenergic autoreceptors

The responsiveness of α_2 -adrenergic autoreceptors was assessed with clonidine. This α_2 -adrenergic autoreceptor agonist completely inhibited the firing of LC NE neurons in control rats at a dose of 5 µg/kg ($ED_{50} = 2.6 \pm 1.0$ µg/kg). However, the inhibitory effect of this dose was significantly attenuated on the neuronal activity of NE neurons in rats treated with escitalopram plus bupropion and rats treated with bupropion for 14 days when compared to the control group (Fig. 14). In fact, neuronal firing of LC NE cells in rats treated with the combination of escitalopram and bupropion, and bupropion alone for 14 days were completely inhibited at the dose of 10 µg/kg ($ED_{50} = 4.8 \pm 1.1$ µg/kg, $p < 0.05$) and 15 µg/kg ($ED_{50} = 6.3 \pm 1.0$ µg/kg, $p < 0.05$), respectively (Fig. 15).

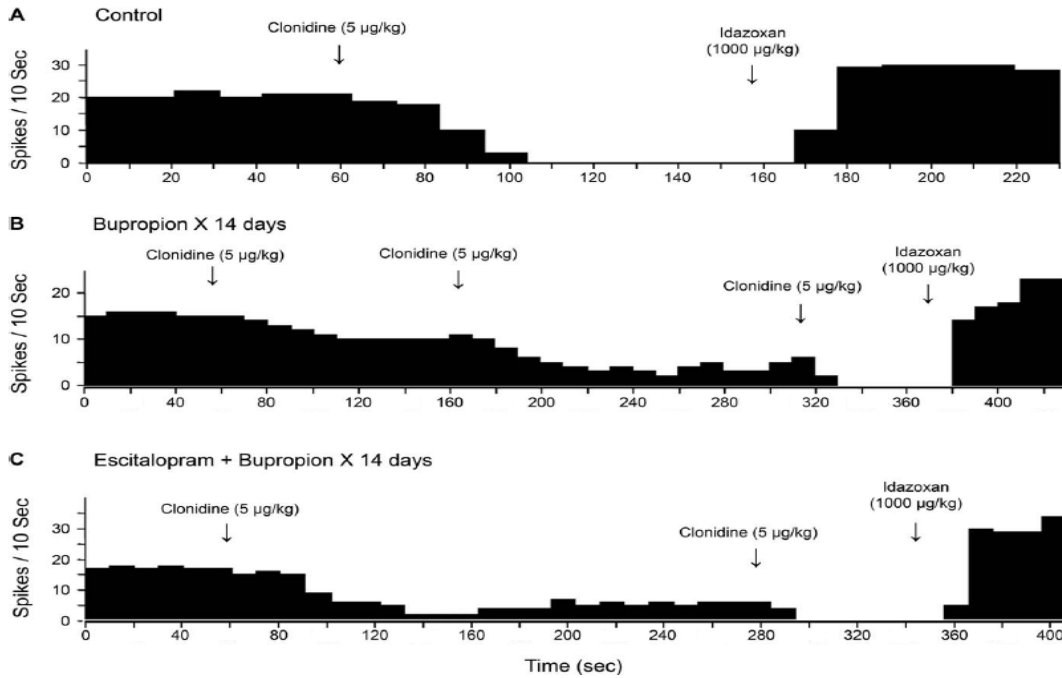


Figure 14: Representative integrated firing rate histograms of locus coeruleus NE neurons illustrating the effect of intravenous administration of α_2 -adrenergic agonist clonidine in suppressing neuronal activity of rats treated with NaCl (A), bupropion (B), and co-administration of escitalopram and bupropion (C) for 14 days.

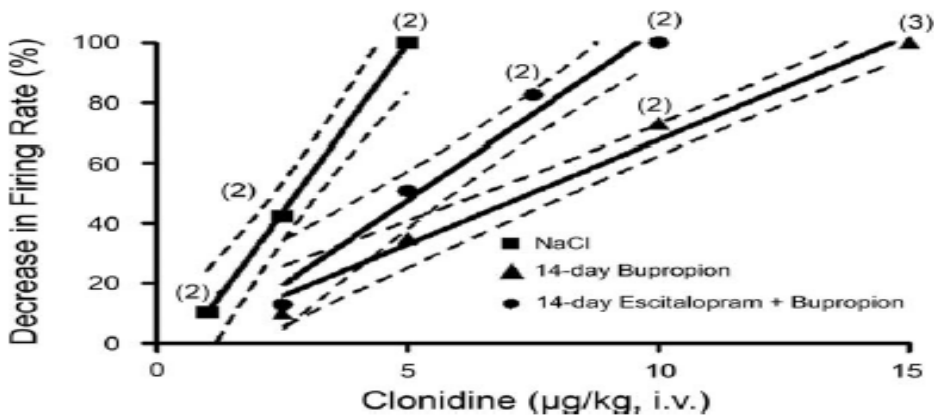


Figure 15: Relationship between the degree of suppression of locus coeruleus NE firing activity and doses of clonidine administered intravenously in control and treated rats. Only the initial response of a single NE neuron to the first dose of clonidine in each rat was used to construct the curves. Outer lines represent the standard error of the regression line. If more than one neuron was tested for a given dose, the number of neurons is illustrated in parentheses.

4.1.5 Discussion

The present electrophysiological results indicate that although repeated 2-day bupropion did not increase firing rate of 5-HT neurons to a statistically significant extent, it robustly enhanced the firing when co-administered with escitalopram after the same period of time. This interaction was also present after 14 days. The robust increase of 5-HT firing likely resulted in part from desensitization of inhibitory 5-HT_{1A} autoreceptors (Fig. 11). Indeed, the dose-response relationship between the degree of suppression of DRN 5-HT firing and doses of LSD showed a significant two-fold shift to the right in combination-treated rats (Fig. 12).

The administration of escitalopram significantly reduced the doublet activity of DRN 5-HT neurons (Table 2). This suppression of firing rate was likely attributable to the excess activation of 5-HT_{1A} autoreceptors by an enhanced concentrations of 5-HT. However, DRN 5-HT firing rate exhibited a full recovery, due to desensitization of 5-HT_{1A} autoreceptors, following a 14-day escitalopram regimen (Fig. 10), as previously reported (El Mansari *et al.*, 2005). Bupropion did not increase the firing rate of DRN 5-HT neurons after 2 days to a significant extent. This parameter was, however, significantly increased after 14-day bupropion regimen, and more neurons were firing in doublets after both treatment periods (Table 2). Doublet activity is believed to increase 5-HT release in postsynaptic regions in comparison to single pulse activity (Gartside *et al.*, 2000). Previous studies showed that 2-day bupropion administration, using minipumps, doubled mean firing of 5-HT neurons (Dong and Blier, 2001). The lesser enhancement observed herein could be due to single daily repeated injections versus prior continuous infusion.

Bupropion is believed to have its potentiating effects on 5-HT neurons *via* LC NE neurons because when these neurons are lesioned, bupropion no longer increases DRN 5-HT firing rate (Dong and Blier, 2001). Lesion studies have documented that NE has an excitatory effect on DRN 5-HT neurons (Svensson *et al.*, 1975). This excitatory effect is mediated *via* α_1 -adrenergic receptors located on the cell body of 5-HT neurons. Stimulatory effect of α_1 -adrenergic receptors has been well established by i.v. injection of the α_1 -adrenergic receptor agonists phenylephrine and cirazoline (Millan *et al.*, 1994). In contrast, systemic

injection of the selective α_1 -adrenoreceptor antagonist prazosin inhibits raphe firing (Marwaha and Aghajanian, 1982).

Two-day co-administration of escitalopram with bupropion resulted in an unexpected robust increase in firing activity of DRN 5-HT neurons (Fig. 10A). This co-administration strategy led to the early desensitization of 5-HT_{1A} autoreceptors (Fig. 12), thus contributing to promptly reverse the inhibitory effect of escitalopram. The NE releasing capacity of bupropion most likely contributed to elevate firing rate of 5-HT neurons above the control baseline value. Microdialysis studies demonstrated that following an acute dose of bupropion, extracellular NE and DA concentration significantly increased in the rat hippocampus (Piacentini *et al.*, 2003), and that bupropion has the ability to increase the extracellular NE and DA concentrations in hypothalamus, nucleus accumbens and frontal cortex of rats (Li *et al.*, 2002). Interestingly, when bupropion was administered with the SSRI fluoxetine, the extracellular NE and DA concentrations were increased eight fold above baseline (Li *et al.*, 2002). It is noteworthy that a dose of 17 mg/kg of bupropion increases extracellular 5-HT levels in hippocampus (Piacentini *et al.*, 2003), but not a dose of 10 mg/kg in the hypothalamus and prefrontal cortex (Li *et al.*, 2002). These results are consistent with our prior observations that bupropion enhanced 5-HT neuronal firing in a dose-dependent manner from 15-30 mg/kg (Dong and Blier, 2001). The presumed increased level of synaptic NE in the DRN by the SSRI/bupropion combination likely stimulated postsynaptic α_1 -adrenergic receptors, resulting in the increased DRN 5-HT firing after the 2-day treatment. Moreover, activation of excitatory D₂ receptors present directly on the cell body of 5-HT neurons also may have contributed to enhance DRN 5-HT firing rate (Aman *et al.*, 2007). Thus, the unexpected substantial increase of DRN 5-HT firing after the 2-day combination treatment could be due to desensitization of 5-HT_{1A} autoreceptors as well as an enhanced activation of excitatory D₂ and α_1 -adrenergic receptors located on 5-HT neurons. Finally, the sustained enhancement of neuronal firing activity of DRN 5-HT after long-term administration of these drugs suggests that there is no adaptation of the excitatory mechanisms driving up this activity.

Short-term administration of bupropion significantly reduced LC NE firing due to overstimulation of α_2 -adrenergic inhibitory autoreceptors located on the cell body of NE neurons (Dong and Blier, 2001). It is worth noting that bupropion has no significant affinity for α_1 -, α_2 -adrenoreceptors or NE transporters (Cusack *et al.*, 1994; Tatsumi *et al.*, 1997). Therefore, the NE released in the cell body area stimulates the α_2 -adrenergic autoreceptors leading to suppression of the firing. Nonetheless, prolonged bupropion administration allowed a complete recovery of NE firing activity, consistent with the desensitization of α_2 -adrenergic autoreceptors reported herein (Fig. 15). This phenomenon was also observed with YM992, a SSRI and a potent 5-HT_{2A} antagonist, which enhances NE release (Szabo and Blier, 2002). It is important to emphasize, however, that when NE reuptake or monoamine oxidase activity is inhibited in a sustained manner, NE firing does not recover due to the lack of desensitization of α_2 -adrenergic cell body autoreceptors (Blier and de Montigny, 1985; Szabo and Blier, 2001a).

Sustained bupropion administration also elevated the percentage of NE neurons firing in burst mode (Table 4), which is believed to increase the amount of NE release for the same number of spikes delivered at regular intervals (Hardebo, 1992). Similar to the short-term effect of bupropion on NE activity, escitalopram also decreased activity of LC NE neurons which contrasts with short-term effects of other SSRIs on NE cells. For instance, long-term, but not short-term, administration of the SSRI paroxetine (Szabo *et al.*, 1999) and fluoxetine (Seager *et al.*, 2004) decreases firing of LC NE neurons. Nevertheless, recent studies have shown that escitalopram is a much more potent SSRI than any of the other SSRIs (El Mansari *et al.*, 2005; Dremencov *et al.*, 2007a; Owens *et al.*, 2001). Indeed, escitalopram is significantly more effective in elevating extracellular 5-HT concentration than racemic citalopram (Mork *et al.*, 2003). The rapid inhibitory action of escitalopram on NE firing is thus likely due to a robust enhancement of 5-HT tone in these terminal areas. This is supported by the observations that this suppressant effect of escitalopram is reversed by the selective 5-HT_{2A} antagonist MDL 100907 (Szabo and Blier, 2001b; Dremencov *et al.*, 2007b). Norepinephrine firing also remained low following prolonged escitalopram administration which is consistent with data obtained with all SSRIs (Seager *et al.*, 2004; Dremencov *et al.*, 2007a).

The mean NE firing had partially recovered following the 14-day combination treatment. This adaptive phenomenon is likely due to desensitization of the cell body α_2 -autoreceptors since twice the dose of clonidine used in control rats was needed to completely inhibit NE firing of combination-treated rats (Figs. 14, 15). However, the inhibitory effect of escitalopram appeared to have prevented the complete recovery of the mean NE firing and an increase in burst activity, as seen in the 14-day bupropion group (Fig. 13B and Table 4). This puts into evidence the robust 5-HT inhibitory tone on NE neurons that cannot be overcome by desensitization of the cell body α_2 -adrenergic autoreceptors.

Since the mechanism of action of bupropion is not fully understood, there has been a debate on whether bupropion exerts its antidepressant effect by blocking NE and DA transporters or by its release enhancing action of these neurotransmitters. In contrast to bupropion, sustained administration of NE blockers, such as desipramine and reboxetine, do not increase firing activity of DRN 5-HT neurons, (Szabo and Blier, 2000). Moreover, the antidepressant effect of bupropion is achieved at doses of 150-300 mg per day in humans, and interestingly only 15% of dopamine reuptake sites are occupied with such clinically effective regimen (Meyer *et al.*, 2002), a value which is in the test-retest variability of the method. Considering that the potency of bupropion for NE reuptake sites is 100 times less than that of DA transporters (Tatsumi *et al.*, 1997), it is unlikely that bupropion exerts its antidepressant effects *via* the blockade of NE reuptake. Furthermore, clinical data indicate drugs that have the ability to block the NE transporter result in attenuation of the increase of systolic blood pressure since entry of intravenously infused tyramine into NE terminals is prevented *via* the NE transporter (Gobbi *et al.*, 2003). However, subjects treated with 150-300 mg per day of bupropion did not exhibit any attenuation in the increase of systolic blood pressure produced by tyramine, whereas the NE reuptake inhibitor nortriptyline abolished this response (Gobbi *et al.*, 2003).

This study suggests that bupropion administered in combination with escitalopram accelerates and potentiates the recovery of DRN 5-HT, and that the inhibitory action of escitalopram on LC NE cells is partially offset by prolonged administration of bupropion.

This study may provide biological basis for the combination of bupropion and SSRIs in the treatment of MDD. Indeed, we are currently investigating the effectiveness of these two antidepressant medications, with complementary mechanisms of action, used from treatment initiation to hasten the therapeutic response in depression.

CHAPTER II:

AIM: To investigate the effects of prolonged administration of bupropion on 5-HT and NE neurotransmission in the rat hippocampus.

4.2 Rationale

Bupropion acts primarily on LC NE neurons (Cooper *et al.*, 1994; Ascher *et al.*, 1995; Dong and Blier, 2001). As discussed previously, a 2-day bupropion regimen attenuates the firing rate of LC NE neurons, which is followed by a complete recovery to the baseline after 2 weeks (El Mansari *et al.*, 2008; Ghanbari *et al.*, 2010). This gradual recovery was attributed to desensitization of α_2 -adrenergic autoreceptors (El Mansari *et al.*, 2008; Ghanbari *et al.*, 2010). Moreover, bupropion doubled DRN 5-HT firing rate after 2 and 14 days due to diminished inhibitory action of 5-HT_{1A} autoreceptors (El Mansari *et al.*, 2008; Ghanbari *et al.*, 2010). The LC-modulated action of bupropion drives its robust 5-HT effect since lesioning the LC NE neurons abolished the increase in 5-HT firing (Dong and Blier, 2001).

Bupropion and its metabolites do not have significant affinities for 5-HT, α - and β -adrenoceptors (Ferris and Beaman, 1983; Cusack *et al.*, 1994). It is a weak DA reuptake blocker. As discussed before the low occupancy of bupropion at DA reuptake sites has been put into evidence in clinical studies (Meyer *et al.*, 2002; Learned-Coughlin *et al.*, 2003; Kugaya *et al.*, 2003; Argyelan *et al.*, 2005). Moreover, such a weak blockade action of bupropion at DA transporters was shown by the lack of effect of bupropion on the firing rate of VTA DA neurons after both 2 and 14 days (Dong and Blier, 2001; El Mansari *et al.*, 2008). The affinity of bupropion for NE transporters is considerably less than that of the DA transporters (Ferris *et al.*, 1981; Tatsumi *et al.*, 1997). In line with this, only high doses of bupropion exhibited blocking effect at NE uptake sites (Ferris *et al.*, 1981). Moreover, it has been shown that all NE reuptake inhibitors impede the increase of systolic blood pressure response to systemic injection of tyramine (Seppala *et al.*, 1981; Turcotte *et al.*, 2001; Debonnel *et al.*, 2007). However, attenuation in the elevation of systolic blood pressure was not observed in individuals treated with bupropion (Gobbi *et al.*, 2003). Given

the weak blocking effect of bupropion at DA and NE uptake sites, both *in vivo* and *in vitro*, it appears that its antidepressant activity may not be attributed to reuptake inhibition.

In vivo experiments indicated that bupropion alone or in combination with SSRIs enhances the extracellular levels of NE and DA in rat nucleus accumbens, hypothalamus and prefrontal cortex (Nomikos *et al.*, 1992; Li *et al.*, 2002). Interestingly, a single injection of bupropion increased NE, DA and 5-HT concentrations in hippocampus of freely moving rats (Piacentini *et al.*, 2003). As mentioned above, previous experiments in our laboratory showed that bupropion exerts robust modulatory actions on 5-HT and NE firing rate that may account for the enhanced release of the neurotransmitters at the postsynaptic regions. Enhancement of 5-HT and NE neurotransmission is believed to play a crucial role in the treatment of depression. Thus, in complement to the previous electrophysiological studies, the present study was undertaken to assess the function of neuronal elements of 5-HT and NE systems in the hippocampus, a brain structure consistently reported to be atrophied in major depression and important in the antidepressant-like response in animals (Santarelli *et al.*, 2003; Pittenger and Duman, 2008).

Results

4.2.1 Assessment of sensitivity of postsynaptic 5-HT_{1A} and α_2 -adrenoceptors

As shown in the integrated firing rate histogram presented in figure 16A, local application of 5-HT and NE suppressed the firing rate of hippocampal pyramidal neurons in a current-dependent manner in the control rats. This paradigm was also applied to rats treated with bupropion or electroconvulsive shock (ECS) for 14 days. Microiontophoretic application of 5-HT in bupropion-treated rats revealed that the number of spikes suppressed/nA was not significantly different from that of the control group (Fig. 16B). However, application of repeated ECSs, as an active control, significantly enhanced the responsiveness of pyramidal neurons to local application of 5-HT ($p < 0.05$; Fig. 16B). On the other hand, the suppressant effect of locally-applied NE on hippocampal firing rate was not modified in any of the treated groups (Fig. 16C).

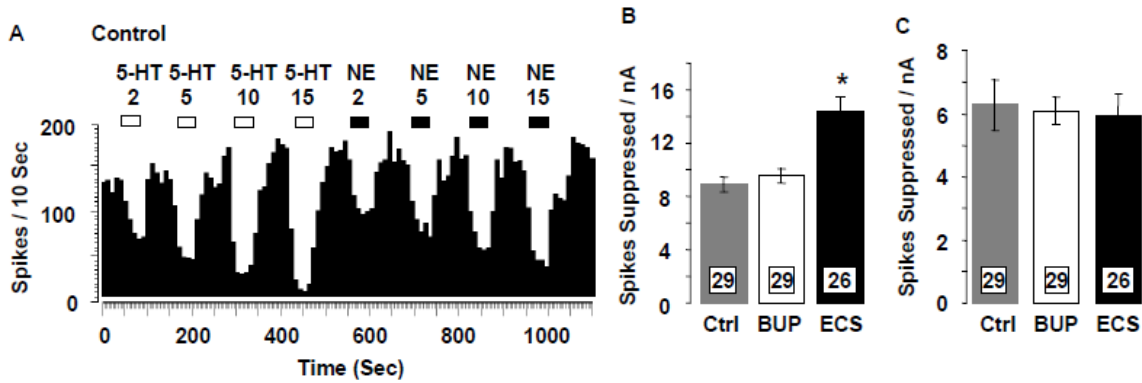


Figure 16. Integrated firing rate histogram of dorsal hippocampus CA₃ pyramidal neuron illustrating microiontophoretic application of 5-HT and NE in control rats (A), and responses of dorsal hippocampus CA₃ pyramidal neurons to microiontophoretic application of 5-HT (B) and NE (C) in rats treated with bupropion (BUP) and electroconvulsive shock (ECS). The number above each bar, 50-sec local application of 5-HT or NE, corresponds to the ejection current in nA. The number of neurons recorded is displayed in each box. * $p < 0.05$

4.2.2 Tonic activation of hippocampus 5-HT_{1A} receptors following sustained administration of bupropion

Systemic administration of 5-HT_{1A} antagonist WAY 100,635 did not modify the firing rate of hippocampal pyramidal neurons in control group ($p > 0.44$, $n = 6$; Fig. 17A). Although systemic injection of WAY 100,635 significantly enhanced the firing rate of pyramidal neurons after 2 days by about 45% ($n = 5$, $p < 0.05$, Fig. 17B), this increase was greater (by 100%) following 14-day administration of bupropion ($n = 6$, $p < 0.01$, Fig. 17C and E). Moreover, in the active control group, administration of WAY 100,635 markedly increased firing rate of hippocampal neurons by 65% in rats given ECS ($n = 6$, $p < 0.05$; Fig. 17D). It is important to note that the inhibitory effect of microiontophoretic application of 5-HT was significantly blocked in all groups following the systemic injection of 5-HT_{1A} antagonist WAY 100,635 (100 $\mu\text{g}/\text{kg}$), confirming that the inhibitory action was mediated by 5-HT_{1A} receptors (Fig. 17).

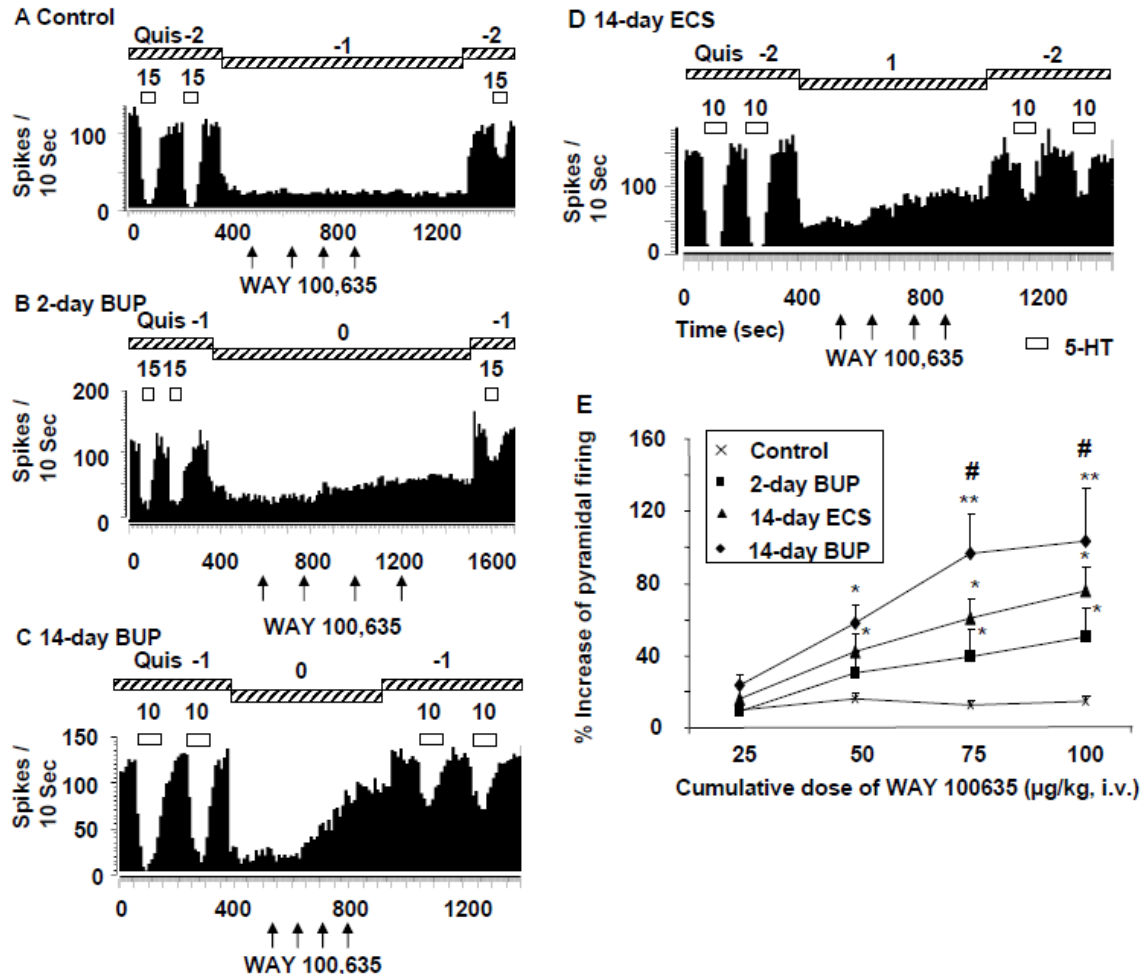


Figure 17. Integrated firing rate histograms of dorsal hippocampus CA₃ pyramidal neurons illustrating systemic administration of incremental doses of 25 µg/kg of WAY 100,635 in control (A), 2-day bupropion (BUP, B), 14-day bupropion (BUP, C) and electroconvulsive shock (ECS, D) treated rats. Each arrow indicates a single injection of 25 µg/kg of WAY 100,635. The overall effect of systemic administration of WAY 100,635 on basal firing in control and treated rats (E). The number above each bar, 50-sec local application of 5-HT, corresponds to the ejection current in nA. * p < 0.05, ** p < 0.01 indicate significant increase in the firing rate induced by WAY 100,635 in the treated rats vs the corresponding dosages in the control group. # indicates significantly larger increase induced by WAY 100,635 in 14-day vs 2-day bupropion-treated rats.

4.2.3 Tonic activation of postsynaptic α_2 - and α_1 -adrenoceptors following sustained administration of bupropion

Systemic application of idazoxan (1000 µg/kg) and prazosin (100 µg/kg) did not markedly modify the firing activity of pyramidal neurons in control rats (n = 5, Fig. 18A). However, such consecutive *i.v.* injections of the selective α_2 - and α_1 -adrenoceptor antagonists idazoxan and prazosin, respectively, enhanced the firing activity of CA3 pyramidal neurons

by 110% ($p < 0.05$) and 50% ($p < 0.05$), respectively, following 14 days of bupropion (Fig. 18).

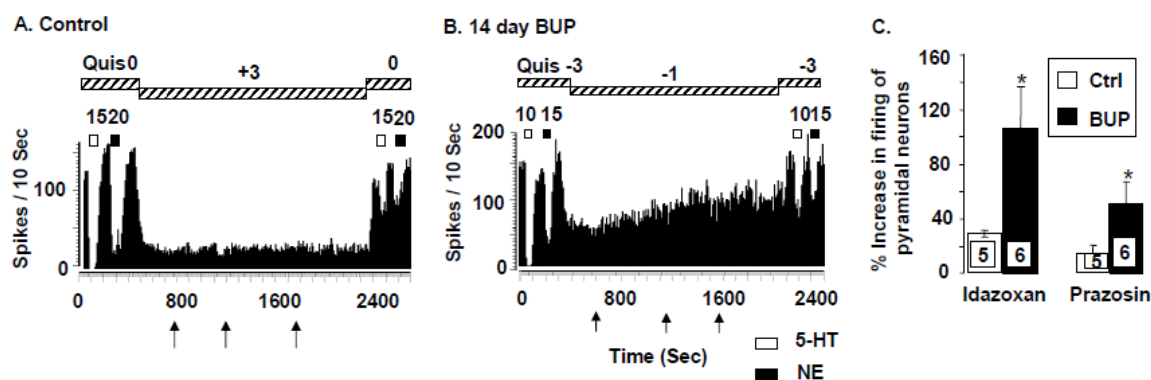


Figure 18. Integrated firing rate histograms of dorsal hippocampus CA₃ pyramidal neurons illustrating systemic administration of idazoxan (1000 $\mu\text{g}/\text{kg}$), prazosin (100 $\mu\text{g}/\text{kg}$) and WAY 100,635 (100 $\mu\text{g}/\text{kg}$) in control (A) and 14-day bupropion (BUP, B) treated rats. The arrows represent the consecutive injections of idazoxan, prazosin and WAY 100,635, respectively. Overall changes of firing activity of pyramidal neurons after the systemic injections of idazoxan and prazosin in control and bupropion-treated rats (C). The numbers in the columns correspond to the number of recorded neurons. The number above each bar, 50-sec local application of 5-HT or NE, corresponds to the ejection current in nA. * $p < 0.05$

4.2.4 Effects of administration of bupropion for 14 days on the responsiveness of hippocampal neurons to synaptically released endogenous 5-HT

The ascending 5-HT pathway was stimulated to determine whether 14-day administration of bupropion had the ability to modulate the endogenous release of 5-HT in the synaptic cleft. The effectiveness of stimulation of the 5-HT bundle at 1 Hz in decreasing the firing rate of CA₃ pyramidal neurons, SIL value, was similar to that of the bupropion-treated group ($p > 0.8$, Fig. 19). Enhancing the frequency of stimulation from 1 to 5 Hz decreased the SIL value by 33% and 32% in control ($p < 0.05$) and bupropion ($p < 0.05$) groups, respectively. The stimulation-induced efficacy in bupropion-treated rats following the increase of frequency to 5 Hz was similar to that of the control group ($p > 0.6$), indicating unaltered function of terminal 5-HT_{1B} autoreceptors (Fig. 19). Systemic administration of the low dose of clonidine (10 $\mu\text{g}/\text{kg}$) significantly enhanced suppression of the firing rate of hippocampal neurons by 30% in the control rats ($p < 0.05$, Fig. 19D). However, administration of the high dose of clonidine (400 $\mu\text{g}/\text{kg}$) reversed the low dose clonidine-induced increase, and significantly decreased the SIL value below the stimulation value in control rats ($p < 0.05$, Fig. 19D). The low dose of clonidine still significantly increased the

suppression of CA₃ pyramidal neurons in 14-day bupropion administrated rats ($p < 0.05$, Fig. 19D), although to a much lesser extent than the control group ($p < 0.05$), thus suggesting a diminished function of α_2 -adrenergic autoreceptors on NE terminals. Following 14-day administration of bupropion, the high dose of clonidine, on the other hand, reversed the enhancing action of 10 $\mu\text{g}/\text{kg}$ of clonidine when compared to the pre-clonidine SIL value ($p > 0.8$), and to a lesser degree from that of the control stimulation value ($p = 0.044$), indicating diminished function of α_2 -adrenergic receptors on 5-HT terminals (Fig. 19D).

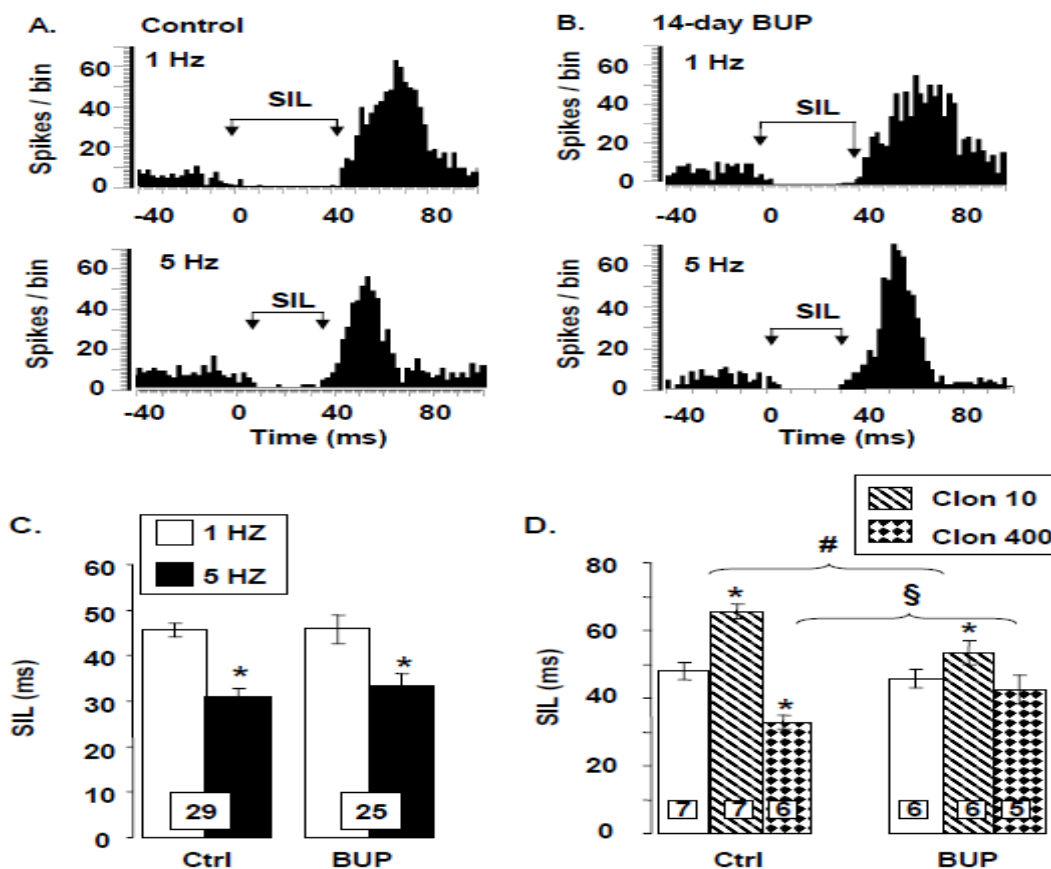


Figure 19. Integrated firing rate histograms illustrating effects of stimulation of the ascending 5-HT pathway, with 1 and 5 Hz, on the firing activity of CA₃ pyramidal neurons in control (A) and 14-day bupropion-treated rats (BUP, B). Suppressing effect of 5-HT pathway stimulations (1 and 5 Hz) on the firing activity of hippocampal neurons in rats administered with NaCl and bupropion (C), and effect of 5-HT pathway stimulation at 1 Hz prior to and following systemic administration of 10 and 400 $\mu\text{g}/\text{kg}$ clonidine. The numbers in the columns correspond to the number of recorded neurons. # indicates $p < 0.05$, comparing the following clonidine (10 $\mu\text{g}/\text{kg}$) SIL value of the bupropion group to the corresponding value of the control group by analysis of covariance, the prior to clonidine values being used as the regressors. § Indicates $p < 0.05$, comparing the following clonidine (400 $\mu\text{g}/\text{kg}$) SIL value of the bupropion group to that of the control group by analysis of covariance, the prior to clonidine values being used as the regressors. SIL: absolute silence

4.2.5 Effects of administration of bupropion for 14 days on the responsiveness of hippocampal neurons to synaptically released endogenous NE

The ascending NE pathway was stimulated to directly assess the sensitivity of terminal α_2 -adrenergic autoreceptors. The effectiveness of electrical stimulation of the NE pathway at 1 Hz in suppressing the firing rate of CA₃ pyramidal neurons was not significantly changed in the 14-day administrated rats when compared to the control group ($p > 0.2$, Fig. 20). The NE stimulation-induced suppression of pyramidal firing rate, SIL value, was significantly reduced by 52% when frequency of stimulation was increased from 1 to 5 Hz in control rats ($p < 0.05$, Figs. 20A and C). Such stimulation-induced reduction of suppression of pyramidal firing following 5 Hz is attributed to greater activation of terminal α_2 -adrenergic autoreceptors (Curet and de Montigny, 1989). However, enhancing the frequency of stimulation of the ascending NE pathway from 1 to 5 Hz produced a lesser degree of suppression (22%, $p < 0.05$) in rats administered bupropion for 14 days than the control group ($p < 0.05$), indicating attenuation of sensitivity of NE terminal α_2 -adrenergic autoreceptors (Figs. 20B and C). Moreover, duration of the activation period (ACT) was significantly reduced in rats administered bupropion for 14 days when compared to the control group, suggesting diminished function of β -adrenoceptors in bupropion-treated rats ($p < 0.05$, Fig. 20C).

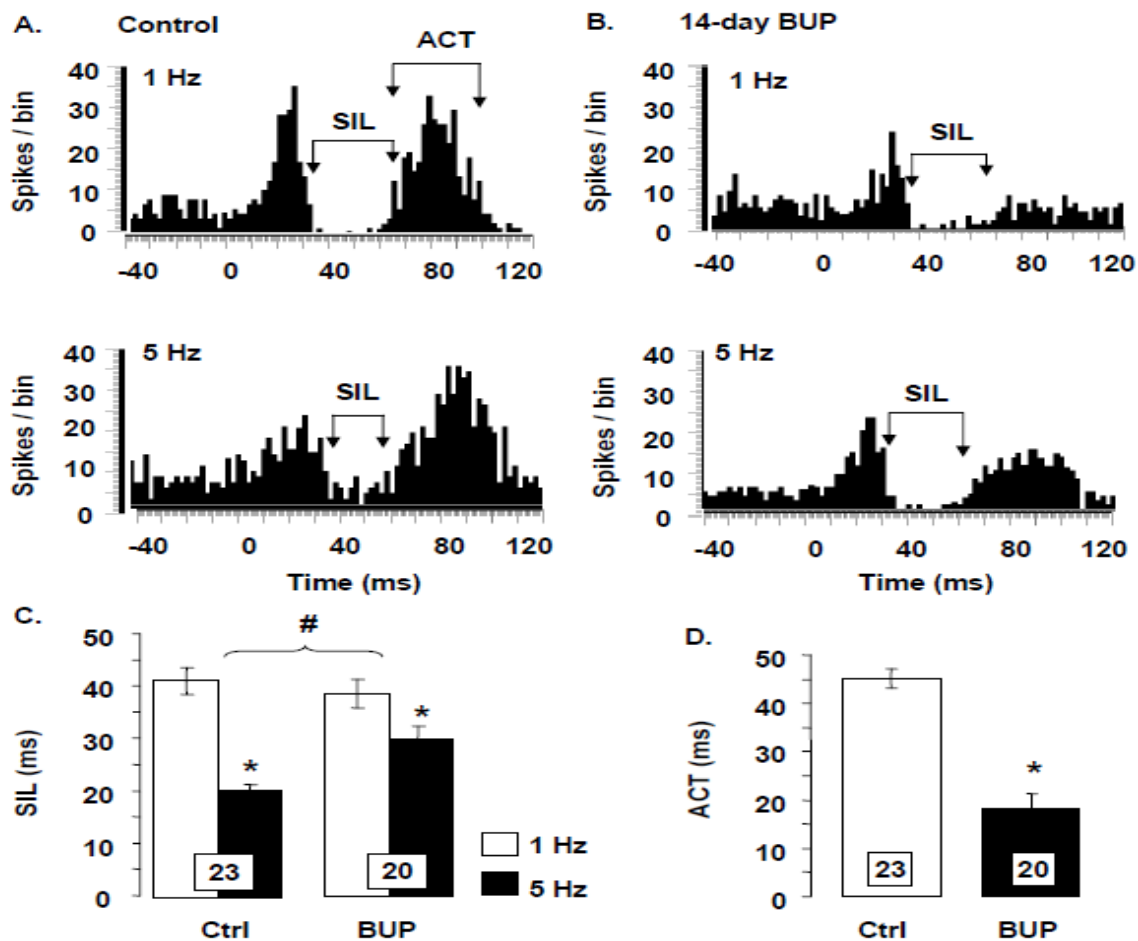


Figure 20. Integrated firing rate histograms illustrating effects of stimulation of the ascending NE pathway, with 1 and 5 Hz, on the firing activity of CA₃ pyramidal neurons in control (A) and 14-day bupropion (BUP, B) treated rats, the suppressing effect of NE pathway stimulations (1 and 5 Hz) on the firing activity (C) and activation (ACT) period (D) of hippocampal neurons in rats administered with NaCl and bupropion. The numbers in the columns correspond to the number of recorded neurons. # indicates $p < 0.05$, comparing the mean 5 Hz SIL value of the bupropion group to that of the corresponding value in the control group by analysis of covariance, the 1 Hz values being used as the regressors. SIL: absolute silence

4.2.5 Discussion

The present electrophysiological data indicate that sustained administration of bupropion for 14 days increased the synaptic levels of 5-HT and NE in the hippocampus. The increase in 5-HT transmission resulted from a previously documented increase in 5-HT neuronal firing in the presence of an attenuated function of α_2 -adrenergic heteroreceptors on 5-HT terminals, thereby relieving 5-HT release from their inhibitory action after long-term administration of bupropion. The increase in inhibitory NE transmission was attributed to the diminished inhibitory action of terminal α_2 -adrenergic autoreceptors, in the presence of

a normalized firing frequency of NE neurons, and an attenuated excitatory action mediated by desensitized postsynaptic β -adrenoceptors.

In parallel to long-term administration of different classes of antidepressant treatments (Haddjeri *et al.*, 1998a), bupropion enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors in hippocampus, as indicated by disinhibition of neuronal activity by WAY 100,635. Indeed, administration of the potent and selective 5-HT_{1A} antagonist WAY 100,635 increased the firing rate of CA₃ pyramidal neurons in rats treated with bupropion for 14 days, but not in controls. Although to a lesser degree, this phenomenon was also present after 2 days (Fig. 17). This early increase was probably due to an increase of the firing rate of DR 5-HT neurons after both 2- and 14-day administration of bupropion (El Mansari *et al.*, 2008; Ghanbari *et al.*, 2010). Although most antidepressants exert their therapeutic effects following prolonged administration, it is not unusual to observe enhancement of the 5-HT tone in hippocampus after only 2 days. It is noteworthy that steady state levels of antidepressants are achieved much faster in rats than in humans due to much faster half lives in rodents. In line with this, microdialysis experiments showed that a single injection of bupropion elevated 5-HT levels in hippocampus (Piacentini *et al.*, 2003). The dual 5-HT and NE reuptake blocker duloxetine and α_2 -adrenoreceptor antagonist mirtazapine also enhanced the activation of postsynaptic 5-HT_{1A} receptors only after 2 days, indicating greater 5-HT transmission in hippocampus, but still to a greater extent after 21 days (Rueter *et al.*, 1998; Besson *et al.*, 2000).

Given the markedly increased 5-HT tone in the hippocampus after 14 days of bupropion administration, it was important to determine the mechanism of such an enhancement. The 5-HT pathway was electrically stimulated to assess the amount of 5-HT released per impulse and allowing to determine the sensitivity of terminal 5-HT_{1B} autoreceptors as well. The 5-HT_{1B} autoreceptors exert an inhibitory role on the release of 5-HT since administration of the terminal 5-HT autoreceptor antagonist methiothepin significantly increased suppression of the firing activity of pyramidal neurons (Chaput *et al.*, 1986a). In addition, when the frequency of stimulation is increased from 1 Hz to 5 Hz, the suppression of the firing is significantly reduced due to greater activation of the presynaptic 5-HT_{1B}

autoreceptors on 5-HT terminals. The responsiveness of terminal 5-HT_{1B} autoreceptors was similar to that in the control group when the frequency was increased to 5 Hz in the bupropion-treated rats (Fig. 19), suggesting that the terminal autoreceptors were not at play in enhancing the 5-HT tone. Therefore, each electrical impulse mimicking action potentials triggered the same amount of synaptically-released 5-HT in the hippocampus in controls and bupropion-treated rats.

The α_2 -adrenoceptor agonist clonidine was then used to probe sensitivity of the α_2 -adrenergic auto- and heteroreceptors. The α_2 -adrenergic heteroreceptors, present on 5-HT terminals modulate the evoked-release of 5-HT from hippocampal terminals (Starke and Montel, 1973; Göthert *et al.*, 1981; Maura *et al.*, 1982). These regulatory receptors are under the inhibitory influence of endogenous NE, which is regulated by α_2 -adrenergic autoreceptors present on the NE terminals. Electrophysiological experiments showed that a low dose of clonidine (10 $\mu\text{g}/\text{kg}$) increases 5-HT transmission through the activation of α_2 -adrenergic autoreceptors. This results in decreasing NE release, thus, removing the inhibitory effect of NE on α_2 -adrenergic heteroreceptors on 5-HT terminals (Mongeau *et al.*, 1993). Indeed, lesioning the LC NE neurons increases 5-HT transmission in hippocampus (Mongeau *et al.*, 1993). High doses of clonidine (100 - 400 $\mu\text{g}/\text{kg}$), on the other hand, activate α_2 -adrenergic heteroreceptors, located on 5-HT terminals, causing a direct reduction in 5-HT transmission (Mongeau *et al.*, 1993). Systemic administration of the low dose of clonidine after 14-day administration of bupropion enhanced the suppression of CA₃ pyramidal neurons, although not to the same extent as of the control group (Fig. 19D), presumably due to desensitization of inhibitory action of α_2 -adrenergic autoreceptors located on NE terminals. Furthermore, the high dose of clonidine did not reduce suppression of the firing rate of hippocampal neurons below the control value, suggesting a diminished function of α_2 -adrenergic heteroreceptors (Fig. 19D). Thus, the enhanced 5-HT tone following the long-term bupropion administration may be due in part to desensitization of 5-HT terminal α_2 -adrenergic heteroreceptors, but not the 5-HT_{1B} autoreceptors. The diminished function of α_2 -adrenergic heteroreceptors on 5-HT terminals may appear inconsistent with the unaltered SIL value of bupropion-treated rats at 1 Hz when compared to that of the control group (Fig 19C). This observation is nevertheless

consistent with the results of previous experiments showing that modulation of 5-HT release by α_2 -adrenergic heteroreceptors is frequency-independent, unlike for terminal 5-HT_{1B} autoreceptors (Blier *et al.*, 1989). A desensitization of α_2 -adrenergic heteroreceptors has also been reported after prolonged administration of other antidepressants that increase the synaptic NE concentration, as for NE reuptake inhibitors and monoamine oxidase inhibitors (Blier and Bouchard, 1994; Mongeau *et al.*, 1994; Rueter *et al.*, 1998).

With respect to NE transmission, systemic administration of α_2 - and α_1 -adrenergic antagonist idazoxan and prazosin, respectively, increased the firing activity of CA₃ pyramidal neurons revealing an enhanced tonic activation of postsynaptic α_2 - and α_1 -adrenergic receptors after prolonged administration of bupropion (Fig. 18). The enhanced synaptic NE availability prompted us to directly assess the sensitivity of terminal α_2 -adrenergic autoreceptors by means of stimulating the NE bundle at 1 and 5 Hz while recording the same pyramidal neuron. It is important to note that suppression of firing induced by stimulation-released endogenous NE is mediated by postsynaptic α_1 -adrenoceptors (Curet and de Montigny 1988a), whereas the inhibitory action of microiontophoretic-applied NE is mediated by postsynaptic α_2 -adrenoceptors (Curet and de Montigny 1988b). Increasing the frequency of stimulation from 1 to 5 Hz markedly reduced the duration of suppression of pyramidal firing in control rats, due to greater activation of terminal α_2 -adrenergic autoreceptors. In rats administered bupropion for 14 days, however, increasing the frequency of stimulation, from 1 to 5 Hz, produced a lesser degree of suppression than in the control group (Fig. 20). This adaptive change indicates attenuation of sensitivity of α_2 -adrenergic autoreceptors, as also suggested by the low dose of clonidine reported herein (Fig. 19D). In addition to the terminal α_2 -adrenergic autoreceptors, previous electrophysiological experiments reported a desensitization of somatodendritic α_2 -adrenergic autoreceptors, which allowed a complete recovery of the initially suppressed firing rate of LC NE neurons after 14-day bupropion administration (El Mansari *et al.*, 2008; Ghanbari *et al.*, 2010). The recovery of NE firing rate was concurrent with an increase in the burst-firing mode (Ghanbari *et al.*, 2010), which induces a greater release of NE in postsynaptic regions (Hardebo, 1992). Furthermore, the β -adrenoceptor-mediated activation period was reduced in rats administered bupropion for 14 days,

indicating diminished function of excitatory β -adrenoceptors (Fig. 20D). This observation is consistent to the previous reports that prolonged administration of bupropion induced a significant down-regulation of β -adrenoceptor binding sites in rat frontal cortex (Gandolfi *et al.*, 1983; Perumal *et al.*, 1986). Down-regulation/desensitization of postsynaptic β -adrenoceptors is a common postsynaptic effect among antidepressants. Such an effect is believed to be an adaptive change in response to the enhanced NE levels in the synaptic cleft. Indeed, lesioning NE neurons prevented down-regulation of β -adrenergic receptors following prolonged administration of antidepressants (Wolfe *et al.*, 1978). The present electrophysiological experiments therefore showed that long-term administration of bupropion resulted in a net increase in inhibitory NE transmission in the hippocampus. This was the result of increased inhibitory transmission through postsynaptic α_1 - and α_2 -adrenergic receptors and diminished excitatory transmission through postsynaptic β -adrenergic receptors.

It is noteworthy that the responsiveness of postsynaptic α_2 -adrenoceptors present on hippocampus pyramidal neurons was not altered following long-term bupropion administration (Fig. 16), unlike that of their presynaptic counterparts on 5-HT and NE neurons (Figs. 19 and 20, respectively). Thus far, we have not observed a desensitization of these postsynaptic α_2 -adrenoceptors in the hippocampus using a variety of antidepressant treatments. In contrast, the postsynaptic α_2 -adrenoceptors in the amygdala are actually sensitized by tricyclic antidepressants (Wang and Aghajanian, 1980; Freedman and Aghajanian, 1985). These results emphasize the heterogeneity of the adaptive properties of α_2 -adrenoceptors in various brain structures.

This study revealed the biological actions of prolonged administration of bupropion on 5-HT and NE neuronal elements in hippocampus. Interestingly, pre-treatment with the catecholamine synthesis inhibitor α -methyl- p -tyrosine blocked the antidepressant-like activity of bupropion (Kwon *et al.*, 2010). In line with this, Cryan and colleagues (2001, 2004) reported that the antidepressant-like effect of bupropion is primarily attributable to the NE system. Furthermore, although tryptophan depletion did not induce relapses in bupropion-remitted patients with depression, certain mood measures were decreased

(Evans *et al.*, 2002). The time-dependent enhancement of 5-HT and NE transmission due to reduced responsiveness of terminal α_2 -adrenergic hetero- and autoreceptors, respectively, may contribute to the therapeutic action of bupropion in major depression.

CHAPTER III:

AIM: To understand the effects of sustained administration of trazodone on 5-HT neurotransmission in DRN and hippocampus

4.3 Rationale

Since its introduction in the 1980's trazodone hydrochloride, a triazolopyridine derivative, has been used in the treatment of major depression (Cunningham *et al.*, 1994), and as a bedtime sedative. The structure of trazodone, unrelated to those of other major classes of antidepressants, results in a low toxicity profile (Al-Yassiri *et al.*, 1981). Its mechanism of action in the treatment of depression has not been fully elucidated, largely in part to its affinity for a variety of receptors which may contribute to its clinical actions (Table 5). Although the antidepressant action of trazodone is partially attributed to blockade of 5-HT reuptake sites, its potency to inhibit 5-HTT is considerably less than that of SSRIs such as citalopram and fluoxetine (Owens *et al.*, 1997). The action of SSRIs on 5-HT neurotransmission has been established over the years, as described previously.

Table 5: Affinities (K_i , nM) of trazodone at various binding sites

	5-HT _{2A}	H ₁	α_1	5-HT _{1A}	5-HTT	5-HT _{2C}
Rat	20 ^a	32 ^{a, e}	40 ^a	42 ^b	126 ^a	251 ^a
Human	25 ^c	1100 ^c	42 ^c	96 ^c	160 ^d	N.D.

^a Millan, 2006; ^b Owens *et al.*, 1997; ^c Cusack *et al.*, 1994; ^d Tatsumi *et al.*, 1997. ^e guinea pig. N.D. not determined.

In addition to its inhibiting action at 5-HTT, trazodone shows moderate potency at blocking 5-HT_{2A/2C} receptors (Table 5) which may contribute to the mechanism of action of trazodone. Microdialysis studies have consistently shown that 5-HT_{2C} antagonists enhance DA concentration in prefrontal cortex (Di Matteo *et al.*, 1999; Gobert and Millan, 1999), while the effect of 5-HT_{2A} antagonists on DA levels remains controversial (Schmidt and Fadayel, 1995; Gobert and Millan, 1999). The active metabolite of trazodone meta-chlorophenyl piperazine (mCPP) is, however, a potent 5-HT_{2C} agonist which would tend to

counteract 5-HT_{2C} antagonism of the parent compound. More importantly, pharmacological experiments have shown that blockade of 5-HT_{2A} receptors restores inhibited LC NE neuronal activity produced by SSRI (Dremencov *et al.*, 2007b). Given the interactions between the monoaminergic neurons *in vivo*, the antagonism of trazodone at 5-HT_{2A} receptors may help compensate for a lower 5-HT inhibitory action at 5-HTT.

Another interesting feature of trazodone is its moderate affinity at 5-HT_{1A} receptors (Table 5). Indeed, Odagaki and colleagues (2005) showed that trazodone acts as an agonist at human 5-HT_{1A} receptors. The agonistic action of trazodone at 5-HT_{1A} receptors may contribute to the modulation of the 5-HT system, in addition to the inhibition of 5-HT uptake. An *in vivo* electrophysiological study showed that systemic administration of trazodone potently suppressed the firing rate of DR 5-HT neurons (Scuvée-Moreau and Dresse, 1982), although this effect could be due to the inhibition of 5-HTT or its potent α_1 -adrenoceptor antagonist action (Table 5). Nevertheless, it is expected that the combined action of trazodone at 5-HTT and 5-HT_{1A} receptors may enhance 5-HT neurotransmission. In line with this, previous studies have shown that 5-HT_{1A} agonists enhance the tonic activation of postsynaptic 5-HT_{1A} receptors due to desensitization of somatodendritic 5-HT_{1A} autoreceptors as well as direct activation of normosensitive postsynaptic 5-HT_{1A} receptors in hippocampus (Blier and de Montigny, 1990).

The various 5-HT agonist-antagonist properties of trazodone may, therefore, contribute to the net therapeutic benefits of this antidepressant. The present *in vivo* electrophysiological experiments were thus undertaken to examine the effects of sustained administration of trazodone on 5-HT neuronal elements in the DR and hippocampus of the rat brain.

Results

4.3.1 Effects of 2- and 14-day administration of trazodone on the firing rate of DRN 5-HT neurons

In comparison to the vehicle group, the 2-day administration of trazodone at the dose of 10 and 20, but not 5, mg/kg/day significantly decreased the spontaneous firing rate of DRN 5-

HT neurons by about 40% and 37%, respectively (vehicle: 1.11 ± 0.08 Hz, $p < 0.01$ for both trazodone 10 and 20 mg/kg/day; Fig. 21). Although trazodone at the dose of 20 mg/kg/day inhibited the 5-HT firing rate to the extent of 10 mg/kg/day, it significantly reduced the number of spontaneously active 5-HT neurons by about 65% after 2 days once compared to the vehicle group ($p < 0.01$; Table 6). Therefore, the average firing rate for the 2-day trazodone 20 mg/kg/day is an underestimated value since this regimen markedly decreased the number of spontaneously active 5-HT neurons. Interestingly, systemic administration of the potent and selective 5-HT_{1A} receptor antagonist WAY 100,635 (100 μ g/kg) normalized the 5-HT firing-inhibition induced by trazodone 10 and 20 mg/kg/day (Figs. 21A and B), and restored the number of spontaneously active neurons in rats treated with trazodone 20 mg/kg/day (Table 6). It is important to note that previous studies in our laboratory as well as others have shown that systemic administration of WAY 100,635 does not significantly alter the average spontaneous firing rate of DRN 5-HT neurons in naive rats (Forster *et al.*, 1995; Gartside *et al.*, 1995; Lejeune and Millan, 1998; Johnson *et al.*, 2002; Haddjeri *et al.*, 2004).

Since trazodone at the dose of 10 mg/kg/day induced a marked physiological action on 5-HT neurons, this dose was used to study the effects of 14-day administration of trazodone on the 5-HT system. In contrast to the 2-day regimen, the firing rate of DRN 5-HT neurons completely recovered to the baseline following 14 days of trazodone administration (10 mg/kg/day; $p > 0.05$; Fig. 21C).

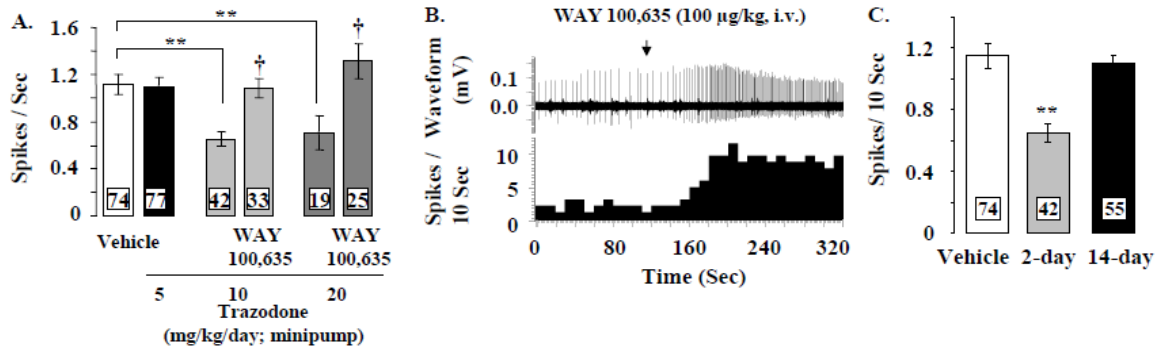


Figure 21. The effects of 2-day administration of vehicle, trazodone 5, 10 and 20 (mg/kg/day) on the firing rate of DRN 5-HT neurons, and the effects of systemic administration of 5-HT_{1A} antagonist WAY 100,635 (100 µg/kg) on the 5-HT firing-inhibition induced by trazodone 10 and 20 (mg/kg/day; A). The lower panel represents the integrated histogram of the firing activity of a 5-HT neuron (upper panel), that was inhibited by 2-day administration of trazodone (10 mg/kg/day), in response to the systemic administration WAY 100,635 (B). The effects of 2- and 14-day administration of vehicle and trazodone 10 (mg/kg/day) on the firing rate of DRN 5-HT neurons (C). ** p < 0.01 when compared to the vehicle group. † Indicates statistical significance compared to prior i.v. injection of WAY 100,635. The numbers at the bottom of each column indicates the number of neurons recorded for the given group.

Table 6. Average number of serotonergic neurons per electrode descent in the dorsal raphe[£]

Groups	2 Days	Post-WAY100,635
Control	4.9 ± 0.5 (15)	
Trazodone (5 mg/kg)	5.5 ± 0.6 (14)	
Trazodone (10 mg/kg)	4.2 ± 0.7 (10)	4.7 ± 0.7 (7)
Trazodone (20 mg/kg)	1.7 ± 0.3* (11)	4.2 ± 0.8 (6)

£ A descent consisted of lowering a single-barrelled electrode over a 1 mm distance from the floor of the sylvius aqueduct and recording spontaneously firing 5-HT neurons. The numbers in the parenthesis correspond to the number of descents in each group. * p < 0.01

4.3.2 Assessment of sensitivity of somatodendritic 5-HT_{1A} autoreceptors

The responsiveness of somatodendritic 5-HT_{1A} autoreceptor, following 14-day trazodone (10 mg/kg/day) regimen, was assessed using the 5-HT autoreceptor agonist LSD probe (Blier *et al.*, 1987; Blier and de Montigny, 1987). The firing of DRN 5-HT neurons in all the naive rats were completely inhibited at the dose of 20 µg/kg (ED₅₀ = 9.0 ± 1.0 µg/kg; Fig. 22). In contrast, the effect of LSD at this dose was markedly attenuated in suppressing the neuronal activity of 5-HT neurons in rats treated with trazodone for 14 days. In fact, higher doses of the LSD were needed to induce a complete suppression of the 5-HT firing

($ED_{50} = 16.0 \pm 1.0 \mu\text{g/kg}$; Fig. 22C). A complete dose-response relationship between the suppression of DRN 5-HT firing rate and different doses of LSD showed a significant two-fold shift to the right in the 14-day trazodone treated rats. In addition to the altered ED_{50} values, the slopes of the two dose-response curves were significantly different (5.2 ± 0.5 versus 2.3 ± 0.1 for the vehicle and trazodone groups, respectively; $F(1, 18) = 5.46$, $p < 0.05$), indicating that the long-term trazodone regimen resulted in 5-HT_{1A} autoreceptor desensitization.

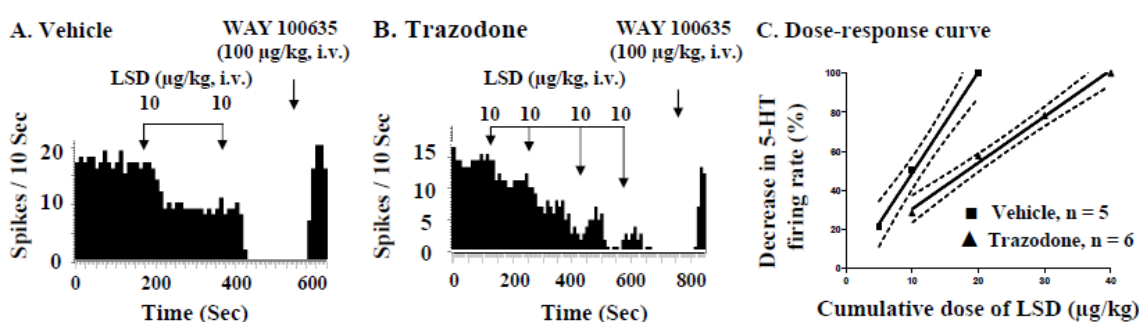


Figure 22. Representative integrated firing rate histograms of DRN 5-HT neurons illustrating the effect of intravenous administration of 5-HT autoreceptor agonist LSD in suppressing neuronal activity of rats treated with vehicle (A) and trazodone (10 mg/kg/day; B) for 14 days, and the relationship between the degree of suppression of DRN 5-HT firing activity and doses of LSD administered intravenously in vehicle and trazodone-treated rats. Outer lines represent the standard error of the regression line (C).

4.3.3 Brain concentration of trazodone and m-CPP following 2-day regimen

The steady-state concentrations of trazodone and its major metabolite m-CPP were 25 ± 2.7 ng/ml and 4 ± 0.25 ng/ml, respectively, following 2-day administration of trazodone (10 mg/kg). These levels are in the same range as those reported by DeVane *et al* (1999).

4.3.4 Effects of sustained administration of trazodone on the responsiveness of dorsal hippocampus CA₃ pyramidal neurons to exogenous 5-HT and NE

Microiontophoretic application of 5-HT and NE suppressed the firing rate of dorsal hippocampal CA₃ pyramidal neurons in the vehicle and trazodone-treated rats (Fig. 23). The inhibitory action of 5-HT and NE is mediated *via* postsynaptic 5-HT_{1A} and α_2 -adrenergic receptors, respectively, since administration of 5-HT_{1A} antagonist WAY 100,635 and α_2 -adrenoceptor antagonist idazoxan readily blocks the suppressant action of

the agonists (Chaput and de Montigny, 1988; Curet and de Montigny, 1988b). Microiontophoretic application of 5-HT and NE in the dorsal hippocampus of rats administered trazodone for 14 days revealed that the number of spikes suppressed/nA was not significantly different from that of the vehicle group ($p > 0.05$ for both 5-HT and NE; Fig. 23), suggesting that 5-HT_{1A} and α_2 -adrenergic receptors in the CA₃ region remain normosensitive following prolonged administration of trazodone.

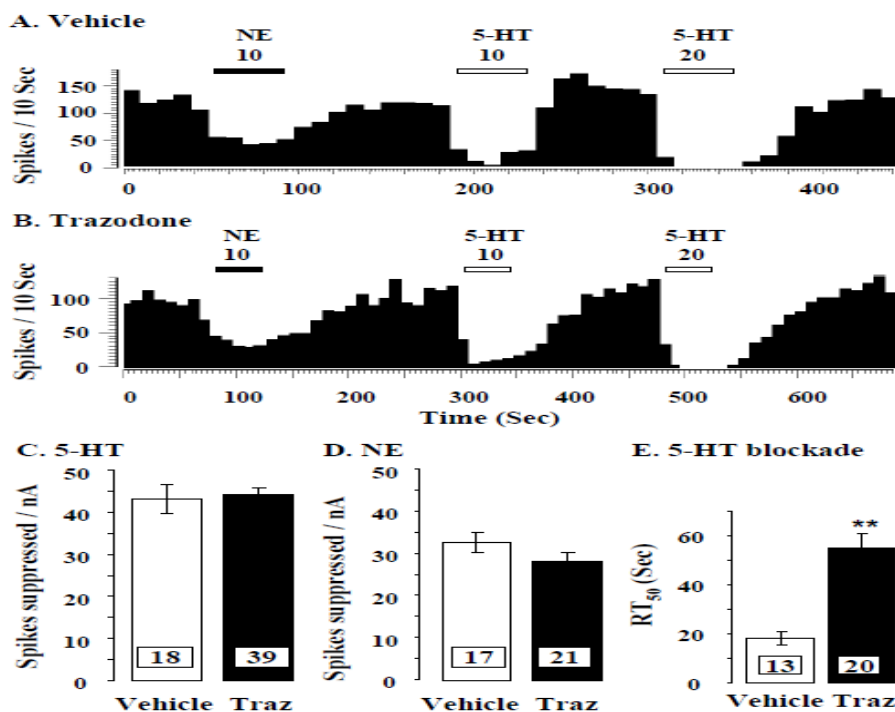


Figure 23. Integrated firing rate histogram of CA₃ pyramidal neuron illustrating microiontophoretic application of 5-HT and NE in rats treated with vehicle (A) and trazodone (Traz, 10 mg/kg/day; B), and responses of the pyramidal neurons to local application of 5-HT (C), NE (D), and the RT₅₀ of pyramidal neurons from microiontophoretic application of 5-HT following 14-day regimens (E). Each bar corresponds to 50-sec application of the agonists, and the number above each bar represents the ejection current in nA. The number of neurons recorded is displayed in each box. ** $p < 0.01$

4.3.5 *In vivo* assessment of the degree of 5-HT reuptake inhibition by trazodone

The function of hippocampus 5-HTT was determined using the recovery time (RT₅₀) of firing rate of pyramidal neurons following a complete suppression of the firing activity induced by local application of 5-HT. Sustained administration of trazodone (10 mg/kg/day) for 14 days significantly increased the RT₅₀ value compared to that of the vehicle group ($p < 0.01$; Fig. 23E), indicating that trazodone blocks 5-HT reuptake process *in vivo*.

4.3.6 *In vitro* determination of [³H]5-HT uptake by trazodone

Following the 2-day regimens, the *ex vivo* experiments from hippocampal slices revealed that trazodone, in a dose-dependent manner, at the dose of 5 and 10 mg/kg/day significantly blocked the 5-HT reuptake process by 57% ($p < 0.05$) and 62% ($p < 0.01$), respectively. It is worth noting that trazodone at the dose of 20 mg/kg/day also markedly inhibited 5-HTT in hippocampus by 70% ($p < 0.01$). The SSRI escitalopram, used as a positive control, at the dose of 10 mg/kg/day significantly inhibited the 5-HT reuptake process by 81% ($p < 0.001$; Fig. 24).

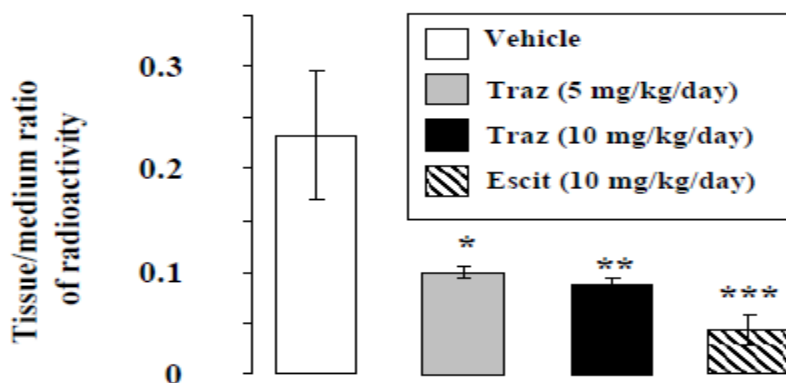


Figure 24. The tissue/medium ratios of radioactivity illustrating the effects of 2-day administration of vehicle, trazodone (Traz, 5 and 10 mg/kg/day) and escitalopram (Escit, 10 mg/kg/day) on the inhibition of hippocampus 5-HTT *ex vivo*. Data were obtained from 3 to 6 rats per experimental group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4.3.7 Assessment of the serotonergic tone determined with the tonic activation of postsynaptic 5-HT_{1A} receptors

As illustrated in figure 25, cumulative administration of 5-HT_{1A} antagonist WAY 100,635 failed to modify the firing activity of dorsal hippocampus CA₃ pyramidal neurons in the vehicle group ($p > 0.05$). On the other hand, the second dose and subsequent doses of systemic injection of WAY 100,635 markedly enhanced the firing activity in rats administered with trazodone for 14 days ($p < 0.05$ for all doses except the first one; Fig. 25C). Indeed, the final injection of WAY 100,635 increased the neuronal activity in the CA₃ region by about 160% compared to control rats. The marked increment in the firing activity of pyramidal neurons in the trazodone-treated rats reflects the degree to which

WAY 100,635 disinhibits neuronal activity due to the tonic activation of postsynaptic 5-HT_{1A} receptors by extracellular 5-HT in the hippocampus. It is important to emphasize that the inhibitory effect of microiontophoretic application of 5-HT was significantly blocked in all rats after the last injection of WAY 100,635 (vehicle: pre-WAY 100,635: 55 ± 5 spikes suppressed/nA, post-WAY 100,635: 29 ± 4 spikes suppressed/nA, n = 6, p < 0.001; 14-day trazodone: pre-WAY 100,635: 46 ± 3, post-WAY 100,635: 12 ± 2, n = 8, p < 0.001), confirming that the inhibitory action was mediated by 5-HT_{1A} receptors.

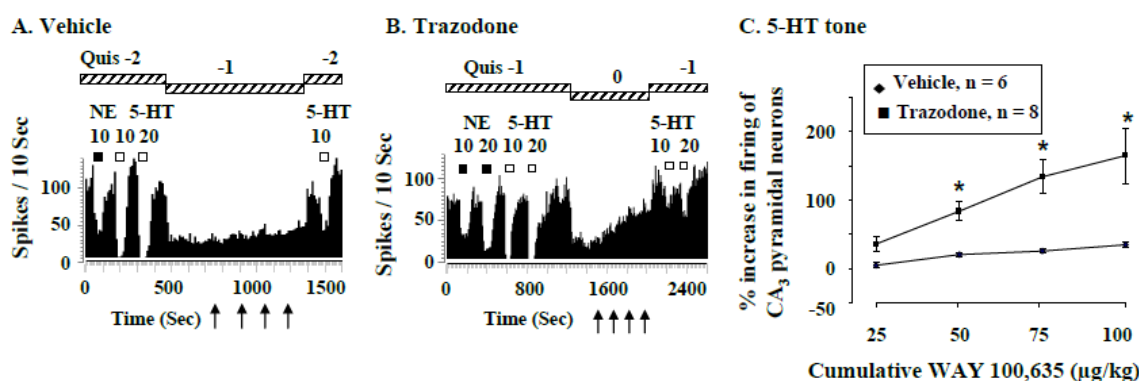


Figure 25. Integrated firing rate histograms of hippocampus CA₃ pyramidal neurons illustrating systemic administration of incremental doses of 25 µg/kg of WAY 100,635 in vehicle (A) and 14-day trazodone (10 mg/kg/day; B), and the overall effect of systemic administration of WAY 100,635 on baseline firing in vehicle and trazodone-treated rats (E). Each bar corresponds to 50-sec application of the agonists, and the number above each bar represents the ejection current in nA. Each arrow indicates a single injection of 25 µg/kg of WAY 100,635. * p < 0.05

4.3.8 Effects of sustained administration of trazodone on the efficacy of the electrical stimulation of the 5-HT afferent fibers to the hippocampus

In order to assess the amount of 5-HT released per electrical impulse in the 5-HT pathway, the 5-HT afferent fibers to the hippocampus were electrically stimulated. The responsiveness of terminal 5-HT_{1B} autoreceptors was also evaluated by increasing the frequency of stimulation from 1 to 5 Hz on the same neuron. The stimulation of the 5-HT pathway at 1 Hz enhanced the period of suppression in rats administered with trazodone for 14 days by 20% once compared to the corresponding SIL value in vehicle group (p < 0.01; Fig. 26). Increasing the frequency of stimulation from 1 to 5 Hz significantly reduced the period of suppression in the vehicle group by 25% (p < 0.01; Fig. 26), an effect due to greater degree of activation of terminal 5-HT_{1B} autoreceptors (Chaput *et al.*, 1986b).

Furthermore, contrary to the vehicle group, the decremental effect obtained by enhancing the frequency of stimulation from 1 to 5 Hz was abolished in rats treated with trazodone and the period of suppression at 5 Hz was greater than the corresponding value in the vehicle group ($p < 0.01$; Fig. 26), indicating that the inhibitory function of terminal autoreceptor was diminished.

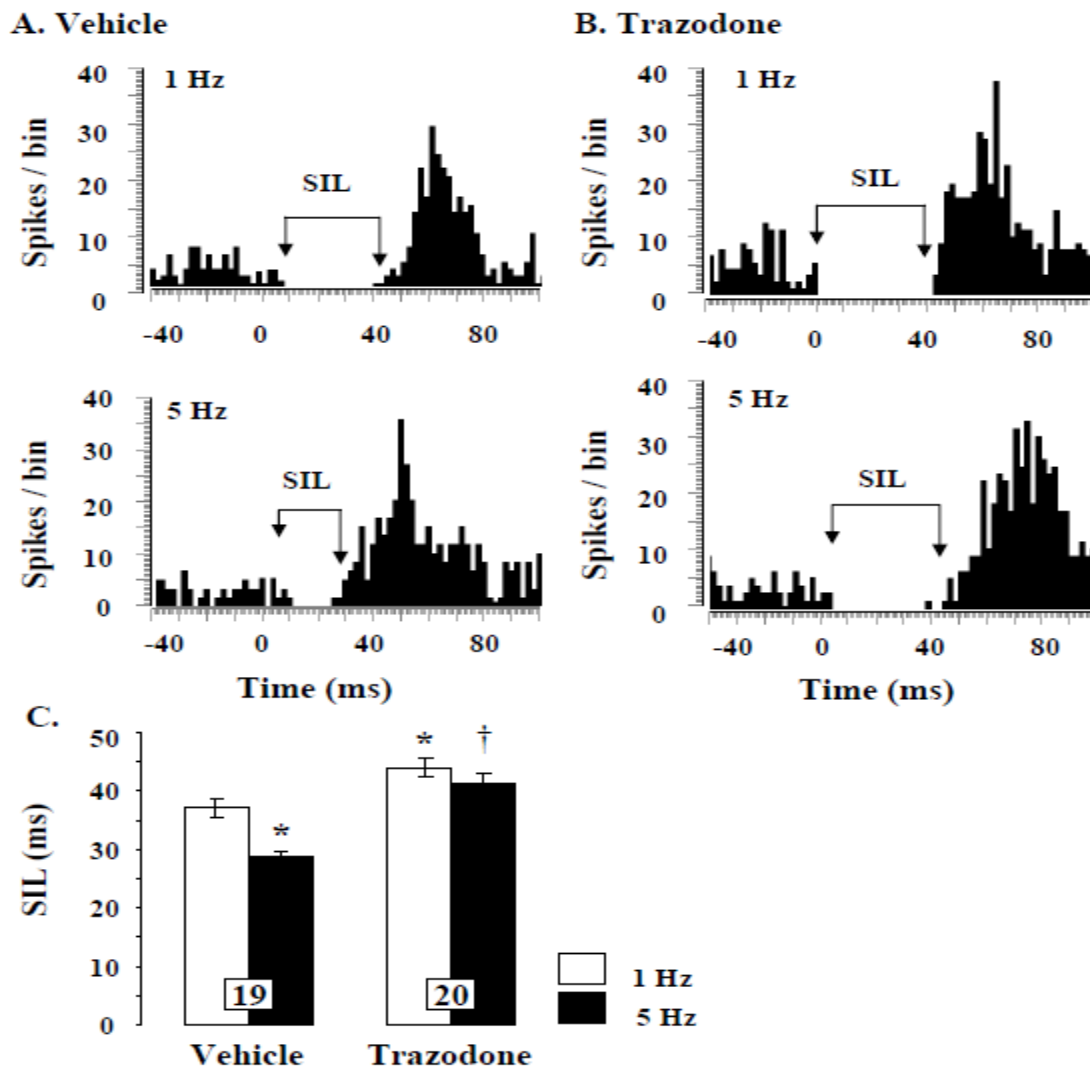


Figure 26. Integrated firing rate histograms illustrating effects of stimulation of the ascending 5-HT pathway, with 1 and 5 Hz, on the firing activity of CA₃ pyramidal neurons in vehicle (A) and 14-day trazodone-treated rats (B), and the suppressing effect of 5-HT pathway stimulations (1 and 5 Hz) on the firing activity of hippocampal neurons in rats administered with vehicle and trazodone (C). The numbers in the columns correspond to the number of recorded neurons. * Indicates $p < 0.01$, comparing the mean SIL value to that of the corresponding value obtained at 1 Hz in the vehicle group. † Indicates statistical significance comparing the mean SIL values obtained at 5 Hz of the trazodone group to that of the corresponding value in the vehicle group by analysis of covariance.

4.3.9 Effects of microiontophoretic application of trazodone on hippocampus CA₃ pyramidal neurons

Trazodone was locally applied in the dorsal hippocampus CA₃ region to characterize its effects at postsynaptic 5-HT_{1A} receptors. The firing activity of pyramidal neurons was markedly reduced by local application of 5-HT (Figs. 27A and B). Microiontophoretic application of trazodone also markedly inhibited the CA₃ neuronal activity (Figs 27A and B). The inhibitory effects of both 5-HT and trazodone were significantly blocked following the systemic administration of WAY 100,635 (100 µg/kg), indicating that these inhibitory actions were mediated by 5-HT_{1A} receptors ($p < 0.01$; Fig. 27). The agonistic action of trazodone at postsynaptic 5-HT_{1A} receptors was further characterized as being full because it did not attenuate the inhibitory action of the endogenous agonist 5-HT when co-applied by microiontophoresis in hippocampus ($p > 0.05$, Fig. 27).

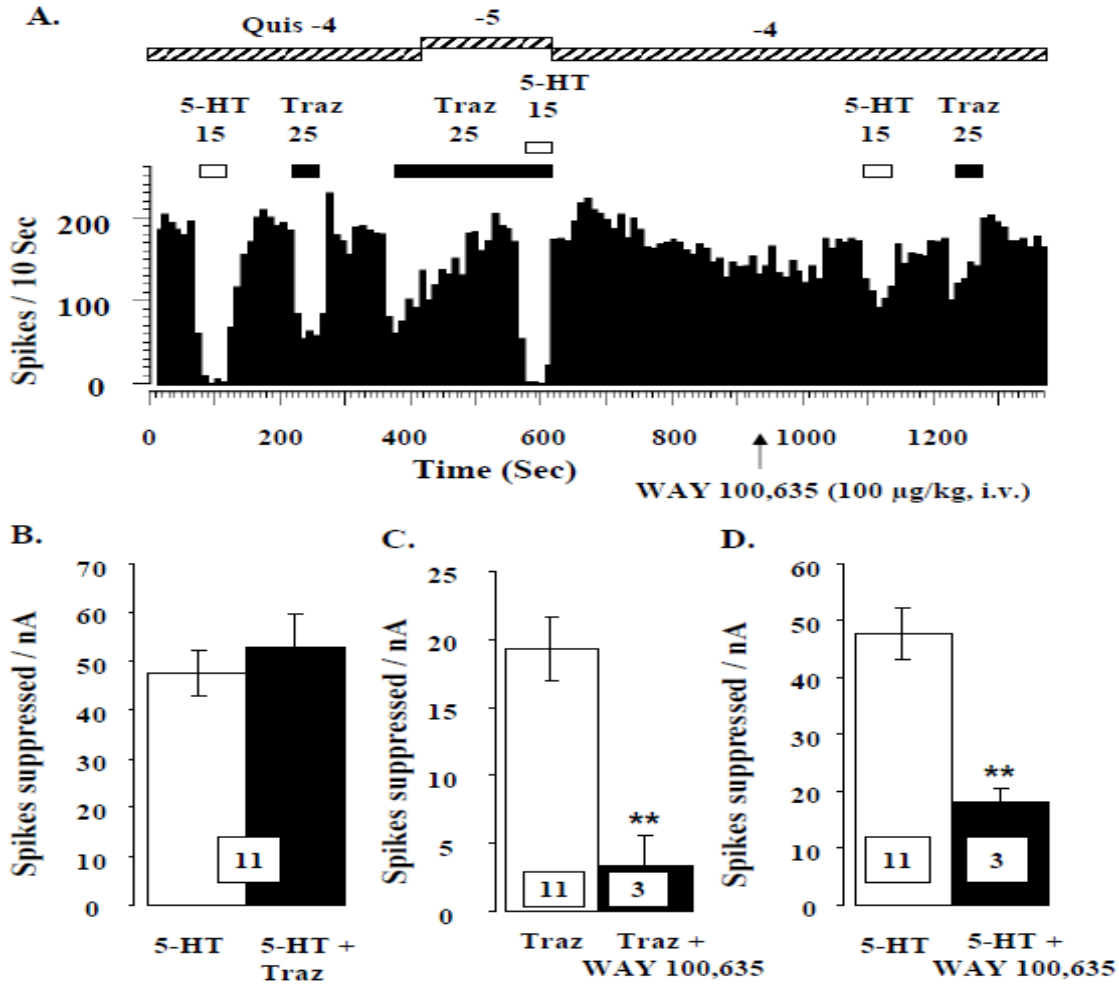


Figure 27. Integrated firing rate histogram of dorsal hippocampus CA₃ pyramidal neuron illustrating microiontophoretic application of trazodone (Traz) and 5-HT (A), and responses of dorsal hippocampus CA₃ pyramidal neurons to microiontophoretic co-application of 5-HT and trazodone (B), trazodone (C), and 5-HT (D) prior to and following *i.v.* injection of 5-HT_{1A} antagonist WAY 100,635. The number above each bar corresponds to the ejection current in nA. The number of neurons recorded is displayed in each box. ** $p < 0.01$

4.3.10 Discussion

These electrophysiological experiments showed that trazodone potently suppressed the firing rate of DR 5-HT neurons after 2 days of sustained administration. This firing rate, however, recovered to baseline following administration of trazodone for 14 days. The complete recovery of 5-HT firing is attributable to decreased sensitivity of 5-HT_{1A} autoreceptors since the suppressant effect of the 5-HT autoreceptor agonist LSD was decreased by the long-term trazodone regimen. With respect to 5-HT neurotransmission in

the forebrain, although the sensitivity of postsynaptic 5-HT_{1A} and α_2 -adrenergic receptors was not changed following its prolonged administration, trazodone increased tonic activation of postsynaptic 5-HT_{1A} receptors in hippocampus, as indicated by disinhibition of neuronal activity by WAY 100,635. This study, therefore, indicates that sustained administration of trazodone enhances 5-HT neurotransmission, at least in part, by desensitizing the inhibitory function of 5-HT_{1A} and 5-HT_{1B} autoreceptors that are present on the cell body and terminals, respectively, in the presence of 5-HT reuptake inhibition.

The *in vitro* data showed that trazodone inhibited 5-HTT in a dose-dependent manner in the hippocampus; a phenomenon that was also observed with the SSRI escitalopram (Fig. 24), suggesting that trazodone modulates the 5-HT system, in part, by inhibiting 5-HT reuptake sites. Although trazodone at the dose of 5 mg/kg/day significantly blocked the 5-HTT, it did not alter the firing rate of 5-HT neurons after 2 days (Fig. 21), indicating that the degree of blockade was below the threshold to induce a net physiological action. This result is akin to what has been reported in humans: an 80% occupancy of 5-HTT is required to obtain an antidepressant effect with a variety of reuptake inhibitors (Meyer *et al.*, 2004). Trazodone 10 and 20 mg/kg/day, on the other hand, suppressed the firing rate of 5-HT neurons following a 2-day administration. In parallel to the 2-day trazodone regimen, acute administration of trazodone dose-dependently reduced the firing rate of 5-HT neurons (Scuvée-Moreau and Dresse, 1982), perhaps due to its blocking property at 5-HTT and/or α_1 -adrenoceptors (Table 5). Although the 2-day administration of trazodone at the dose of 20 mg/kg/day reduced the firing rate of 5-HT neurons to the same extent as 10 mg/kg/day, the number of spontaneously active 5-HT neurons was only reduced by the highest dose. It is well established that short-term administration of SSRI potently suppresses 5-HT neurons due to activation of somatodendritic 5-HT_{1A} autoreceptors by enhancing 5-HT levels in the vicinity of the cell body. In line with this, systemic administration of 5-HT_{1A} antagonist WAY 100,635 reversed the trazodone-induced suppression of 5-HT firing rate to their baseline (Fig. 21), and normalized the number of spontaneously active 5-HT neurons (Table 6), indicating that the suppressant effect may be mediated *via* 5-HT_{1A} receptors and not by blocking α_1 -adrenoceptors. In contrast to the 2-day effect, the firing rate of 5-HT neurons showed a full recovery following 14 days of trazodone

administration. The recovery of 5-HT firing activity was concurrent with desensitization of 5-HT_{1A} autoreceptors (Fig. 22) which may account for firing normalization, a physiological adaptation that was consistently observed with long-term SSRI administration (Piñeyro and Blier, 1999).

Even though long-term administration of trazodone did not alter the sensitivity of postsynaptic 5-HT_{1A} and α_2 -adrenergic receptors (Fig. 23), it induced a robust tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus after 14 days, as revealed by disinhibition of the neuronal firing activity induced by WAY 100,635 (Fig. 25). Enhanced tonic activation is consistent with previous microdialysis experiments showing increased extracellular concentration of 5-HT in terminal brain areas following administration of antidepressants with serotonergic action (Romero *et al.*, 1996). It has been shown that the presumed enhanced 5-HT levels in hippocampus may partially be due to the inhibition of 5-HTT by prolonged, but not a 2-day, administration of the SSRI paroxetine (Besson *et al.*, 2000). The observation that prolonged administration of trazodone considerably increased the overall net effect of 5-HT in hippocampus suggests similar mechanism of action as the SSRIs. In line with this, microdialysis experiments showed that administration of trazodone markedly enhanced the extracellular 5-HT concentration in frontal cortex of freely moving rats (Pazzagli *et al.*, 1999), an effect that was higher than that of the SSRI sertraline (Garrone *et al.*, 2009). Vilazodone, which like trazodone is a SSRI/5-HT_{1A} agonist, enhances extracellular 5-HT concentration in the rat frontal cortex more than the SSRI paroxetine alone (Hughes *et al.*, 2005).

Previous studies have reported that long-term administration of agents that potently inhibit 5-HTT, such as citalopram (Chaput *et al.*, 1986a), fluoxetine (Blier *et al.*, 1988), paroxetine (Chaput *et al.*, 1991) and fluvoxamine (Dong *et al.*, 1999), induce desensitization of 5-HT_{1B} autoreceptors on 5-HT terminals (Piñeyro and Blier, 1999). Thus, it was deemed crucial to assess the sensitivity of terminal autoreceptors since these autoreceptors exert an inhibitory role on the 5-HT release (Chaput *et al.*, 1986a). When compared to the vehicle group, stimulation of the 5-HT bundle at 1 Hz increased the effectiveness of stimulation in rats administered with trazodone for 14 days, as for the abovementioned SSRIs. Increasing

the frequency of stimulation from 1 to 5 Hz reduces the period of suppression, in the vehicle group, due to greater activation of terminal 5-HT_{1B} autoreceptors, as reported previously (Chaput *et al.*, 1986a). In trazodone-treated rats, however, this decremental effect was abolished when the frequency of stimulation was increased from 1 to 5 Hz, suggesting that the inhibitory action of terminal autoreceptors was diminished (Fig. 26). In addition, long-term administration of YM992, which like trazodone blocks 5-HTT and 5-HT_{2A} receptors, desensitized terminal 5-HT autoreceptors (Dong *et al.*, 1999). Desensitization of terminal 5-HT_{1B} autoreceptors may thus be the driving force for the enhanced 5-HT release in the synapse following 14-day administration of trazodone. Indeed, 5-HT reuptake blockade *per se* could not explain the increased 5-HT tone in hippocampus since acute administration of the SSRIs citalopram or fluoxetine does not enhance the effectiveness of stimulation (Piñeyro and Blier, 1999). Furthermore, Groß and colleagues (1987) showed that trazodone increased the electrically-induced [³H]5-HT release from cortical slices, an effect that was independent of 5-HT reuptake inhibition. Moreover, it is worth mentioning that desensitization of terminal 5-HT_{1B} autoreceptor following long-term administration of trazodone is not likely due to the agonistic action of its major metabolite m-CPP on these receptors nor to its potent antagonistic effect at 5-HT₃ receptors, since its concentration is low in the brain, as previously reported at the steady-state level (DeVane *et al.*, 1999).

Microiontophoretic application of trazodone showed that, similar to 5-HT, it acts as an agonist at postsynaptic 5-HT_{1A} receptors in the hippocampus CA₃ region since administration of the 5-HT_{1A} receptor antagonist WAY 100,635 markedly blocked the inhibitory actions of both 5-HT and trazodone (Fig. 27). The agonistic action of trazodone at 5-HT_{1A} receptors was further characterized as being full because it did not antagonize the inhibitory action of the endogenous agonist 5-HT when co-applied by microiontophoresis in hippocampus (Fig. 27). In addition, when trazodone was administered for 14 days and the responsiveness to 5-HT was examined with the minipump in the animal delivering the drug, there was no alteration in the sensitivity of the pyramidal neurons to 5-HT (Fig. 23). On the other hand, the 5-HT_{1A} partial agonists gepirone (Blier and de Montigny, 1990) and asenapine (Ghanbari *et al.*, 2009) significantly offset the inhibitory effect of 5-HT on the

hippocampus firing rate when co-applied locally. Thus far, BAY x 3702 is the only 5-HT_{1A} agonist that acts as a full agonist, like trazodone, at 5-HT_{1A} receptors in the CA₃ region (Dong *et al.*, 1998). Even though both agents behave as full agonists at these postsynaptic 5-HT_{1A} receptors, they did not alter the sensitivity of these receptors following prolonged administration, in contrast to that of the somatodendritic autoreceptors. Furthermore, previous *in vitro* studies showed that trazodone has moderate affinity at 5-HT_{1A} receptors in the frontal cortex of human brain as well as rat hippocampus (Cusack *et al.*, 1994; Owens *et al.*, 1997), and that it acts as an agonist at human 5-HT_{1A} receptors (Odagaki *et al.*, 2005). The agonistic action of trazodone at 5-HT_{1A} receptors, in addition to its modulatory action on 5-HT neurotransmission, may also contribute to enhance DA release in medial prefrontal cortex (Chung *et al.*, 2004).

Enhanced 5-HT transmission by prolonged administration of trazodone may be clinically relevant. Indeed, trazodone similar to other classes of antidepressants including tricyclics, MAOIs, SSRIs, mirtazapine, gepirone, and electroconvulsive shocks shares the property of enhancing the overall 5-HT neurotransmission in hippocampus following long-term administration (Haddjeri *et al.*, 1998a). The therapeutic effect of trazodone in the treatment of major depression may thus be, at least in part, due to the enhancement of 5-HT neurotransmission *via* 5-HTT inhibition and 5-HT_{1A} receptor agonism. This medication however, has not been used as first-line treatment for depression because of its daytime sedative action. The latter effect is likely due to a combination of H₁, 5-HT_{2A} and α_1 -adrenergic receptor antagonism (Table 5), properties that can individually contribute to hypnotic and/or sedative effects. The development of a slow release preparation of trazodone thereby eliminating sharp plasma/brain peaks has, however, helped diminish day-time drowsiness, while maintaining the therapeutic benefit on depressed mood (Sheehan *et al.*, 2009).

CHAPTER IV:

AIM: To characterize the novel drug asenapine at 5-HT_{1A}, 5-HT_{2A}, α_2 -adrenergic and D₂ receptors in the rat brain

4.4 Rationale

Schizophrenia is a complex disorder characterized by positive and negative symptoms as well as cognitive impairment. Conventional antipsychotic medications have been the backbone of the pharmacotherapy of this debilitating disease for decades (Kapur and Mamo, 2003). Despite the fact that classical DA D₂ receptor antagonists, such as haloperidol, dampen the symptoms of schizophrenia, their clinical use is associated with major drawbacks such as extrapyramidal symptoms (EPS), tardive dyskinesia, and low efficacy against negative symptoms (Meltzer, 1995). The new generation of atypical antipsychotics has a combination of pharmacological activities at DA, 5-HT, and noradrenergic receptors (Newman-Tancredi *et al.*, 1996; Schotte *et al.*, 1996; Shahid *et al.*, 2009). Indeed, the pharmacological features of atypical antipsychotics produce advantages over the typical agents in managing schizophrenia (Kane *et al.*, 1988; Davis *et al.*, 2003). Thus, the atypical antipsychotics have become the first-line treatment of schizophrenia (Conley and Kelly, 2002; Kane *et al.*, 2003). Interestingly, a growing body of evidence over the last decade has introduced the clinical use of atypicals in the treatment of mood disorders. Indeed, greater beneficial effect was achieved when atypicals were added to antidepressants in unipolar and bipolar depression (Shelton *et al.*, 2001; Dunner *et al.*, 2007; Tohen *et al.*, 2003; Nelson and Papakostas, 2009). The fact that clinical efficacy of atypicals in the treatment of mood disorders is achieved at subtherapeutic dosage for the treatment of schizophrenia makes this class of drugs as excellent adjuncts. The clinical efficacy of atypicals in the treatment of mood disorders is not surprising considering that these agents are endowed with a unique neurochemical profiles for the monoaminergic receptors. Some of these properties are discussed below.

A unique characteristic of atypical antipsychotics is their superior antagonistic effect at 5-HT_{2A} than at D₂ receptors. The antagonistic effect of these drugs at 5-HT_{2A} receptors is also important in the treatment of patients with unipolar and bipolar depression (Blier, 2005).

This pharmacological feature leads to the reversal of the inhibitory action of SSRIs on LC NE firing (Szabo *et al.*, 1999; Dremencov *et al.*, 2007a) and release of NE (Hatanaka *et al.*, 2000; Kawahara *et al.*, 2007). Indeed, the selective 5-HT_{2A} receptor antagonist MDL 100907 significantly increases NE release in the rat forebrain in the presence of 5-HT reuptake inhibition (Hatanaka *et al.*, 2000). Another important pharmacological characteristic of certain atypical antipsychotics such as clozapine is 5-HT_{1A} receptor agonism (Newman-Tancredi *et al.*, 1996), which enhances DA neurotransmission. Consistent with this view, administration of 5-HT_{1A} agonists enhanced DA and acetylcholine release in rat mPFC (Ichikawa *et al.*, 2001; Diaz-Mataix *et al.*, 2005), whereas the 5-HT_{1A} antagonist WAY 100,635 abolished the enhanced cortical DA release of 5-HT_{1A} agonists (Ichikawa *et al.*, 2001) and that of clozapine (Rollema *et al.*, 1997).

Some atypical antipsychotics, such as clozapine, risperidone and quetiapine, are also potent α_2 -adrenergic receptor antagonists (Schotte *et al.*, 1996). This feature may have clinical relevance since antagonism of α_2 -adrenergic autoreceptors enhances LC NE tone. The α_2 -adrenergic autoreceptors, located on the cell body and terminals of NE neurons, exert an inhibitory role on the firing rate of NE neurons and release of NE, respectively. Indeed, the selective α_2 -adrenoreceptor antagonist idazoxan attenuates the inhibitory effect of these autoreceptors, resulting in an increase in the firing rate of LC NE neurons and NE release in postsynaptic areas (Freedman and Aghajanian, 1984). Interestingly, these observations supported the finding that clozapine increases plasma NE in patients with schizophrenia (Pickar *et al.*, 1992). Moreover, Litman and colleagues showed that addition of idazoxan to the typical antipsychotic fluphenazine significantly improved the positive and negative symptoms of schizophrenic treatment-resistant patients (Litman *et al.*, 1996).

Atypical antipsychotics in current use have significant limitations in regard to efficacy as well as serious side effects, such as prolactin elevation, increased blood lipids, weight gain, and sedation or activation which complicate their clinical utility and may be a strong driver for high rate treatment discontinuation and switching in schizophrenia patients. Thus, new therapeutic agents with improved efficacy and side effect profiles are still desired. Asenapine is a psychopharmacologic agent being developed for the treatment of

schizophrenia (Potkin *et al.* 2007) and bipolar disorder (McIntyre *et al.* 2008). Its distinctive receptor binding profile shows a unique combination of very high affinity for 5-HT, DA and adrenergic receptors (Shahid *et al.*, 2009). The affinity of asenapine for most of these receptors is in the subnanomolar range, suggesting that it may be endowed with effectiveness in schizophrenia and mood disorders. To date there are limited data from *in vivo* models on the functional effects of asenapine on specific receptor subtypes. The present experiments were thus undertaken to explore the *in vivo* electrophysiological effects of asenapine at 5-HT_{1A}, 5-HT_{2A}, α_2 -adrenergic and D₂ receptors in the rat brain.

Results

4.4.1 Assessment of intrinsic effect of systemically-administered asenapine on DRN 5-HT, LC NE and VTA DA spontaneous neuronal firing

Consecutive i.v. injections of asenapine (1, 10, 100 and 1000 $\mu\text{g}/\text{kg}$) did not significantly alter the spontaneous firing rate of DRN 5-HT neurons when compared to the basal firing rate (1.2 ± 0.6 Hz, $n = 5$; $F [4, 20] = 0.09$, $p > 0.05$; Figs. 28 and 29). Furthermore, the same paradigm of consecutive i.v. injections of asenapine did not prevent the inhibitory effect of 5-HT_{1A} autoreceptor agonist 8-OH-DPAT (10 $\mu\text{g}/\text{kg}$, $n = 5$; Fig. 28A).

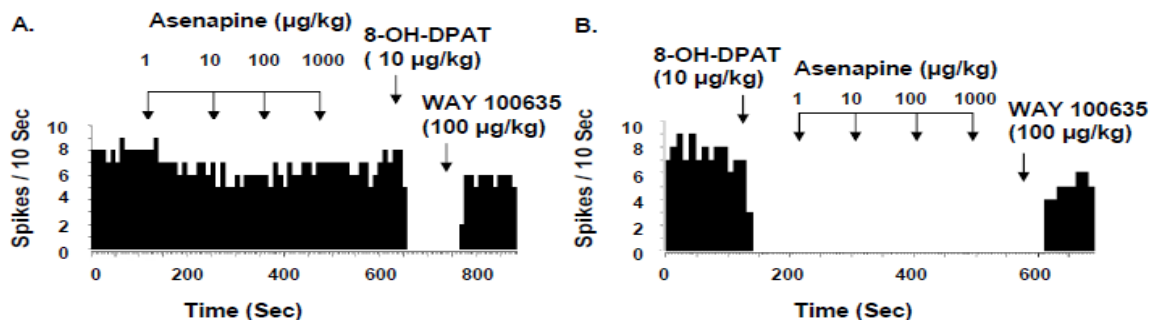


Figure 28. Representative integrated firing rate histogram illustrating the effect of pretreatment with asenapine on the inhibitory effect of 8-OH-DPAT (A), and the effect of asenapine on the 8-OH-DPAT-induced suppression of DRN 5-HT neurons (B).

The consecutive systemic injection of asenapine increased the basal spontaneous firing of NE neurons (1.5 ± 0.7 Hz, $n = 6$) by only 5% at the high dose of 1000 $\mu\text{g}/\text{kg}$, which did not reach statistical significance ($F [5, 24] = 2.52$, $p > 0.05$; Figs. 29A and 30A). The basal firing rate of DA neurons (4.0 ± 0.6 Hz, $n = 5$) was significantly increased following the

systemic administration of 100 $\mu\text{g}/\text{kg}$ and 1000 $\mu\text{g}/\text{kg}$ of asenapine ($F [4, 20] = 4.29, p < 0.05$; Figs. 29A and 32A).

Furthermore, in DRN and LC, systemic administration of asenapine (1, 10, 100, 1000 $\mu\text{g}/\text{kg}$) did not significantly alter the percentage of bursts of 5-HT and NE neurons when compared to that of prior asenapine administration ($F [4, 20] = 0.49, p > 0.05$ and $F [4, 20] = 0.20, p > 0.05$, respectively; Fig. 29B). However, VTA DA neurons exhibited, a significant increase in the number of bursts following the systemic administration of 100 $\mu\text{g}/\text{kg}$ and 1000 $\mu\text{g}/\text{kg}$ of asenapine ($F [4, 20] = 3.66, p < 0.05$; Fig. 29B).

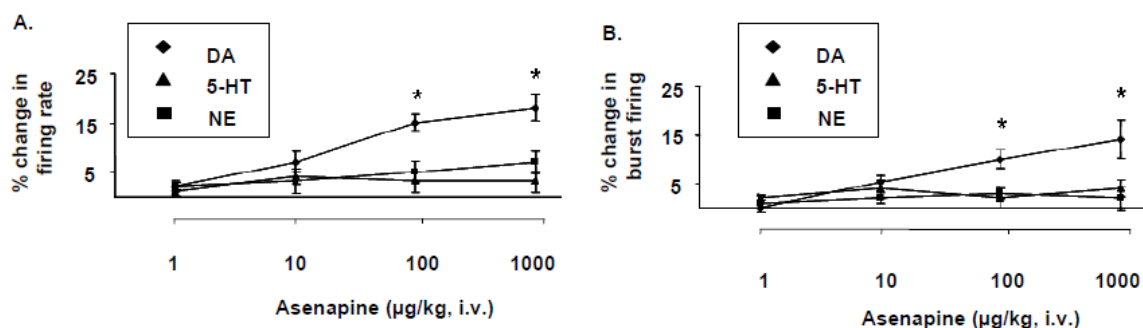


Figure 29. Effects of consecutive systemic administration of asenapine on VTA DA, DRN 5-HT and LC NE neuronal firing rate (A) and burst firing (B) when compared to the prior *i.v.* injection of asenapine. * $p < 0.05$

Although systemic administration of asenapine did not significantly enhance the firing rate of LC NE neurons, it blocked the inhibitory effect of both the α_2 -adrenergic agonist clonidine (10 $\mu\text{g}/\text{kg}$, $n = 4$; Fig. 30A) and the 5-HT_{2A} agonist DOI (50 $\mu\text{g}/\text{kg}$, $n = 4$; Fig. 31A). It is important to note that systemic injections of clonidine and DOI at these doses induce a sustained inhibition of LC NE neuronal firing rate in naive rats. Moreover, the *i.v.* injection of asenapine blocked the inhibitory effect of the D₂ autoreceptor agonist apomorphine (100 $\mu\text{g}/\text{kg}$, $n = 4$; Fig. 32A).

4.4.2 Determination of the potency of systemically-administered asenapine on LC α_2 -adrenergic autoreceptors

In LC, the sustained inhibition of neuronal firing induced by the selective α_2 -adrenergic agonist clonidine (10 $\mu\text{g}/\text{kg}$, *i.v.*) was reversed by asenapine in a dose-dependent manner

starting at a dose of 10 $\mu\text{g}/\text{kg}$. After administration of 100 $\mu\text{g}/\text{kg}$ and 1000 $\mu\text{g}/\text{kg}$ of asenapine, consecutive injections of clonidine (10 $\mu\text{g}/\text{kg}$) did not completely inhibit the NE neuronal firing, thereby indicating that the recovery of NE firing was attributable to α_2 -adrenoceptor antagonism (Fig. 30B). The ED_{50} value for reversing the clonidine-induced inhibition (10 $\mu\text{g}/\text{kg}$) was $85 \pm 2 \mu\text{g}/\text{kg}$ ($n = 12$; Fig. 30C).

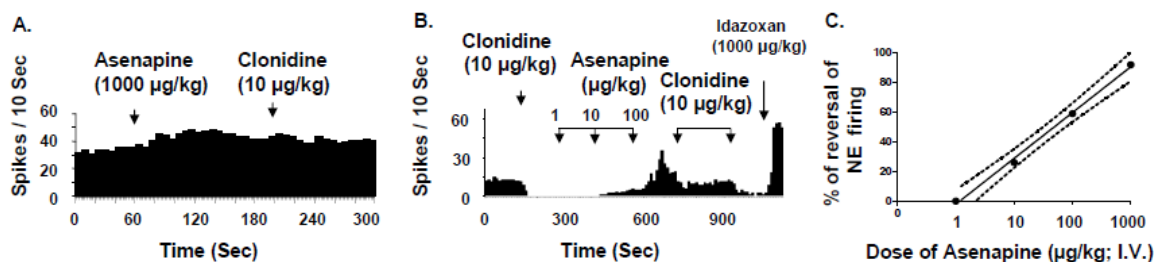


Figure 30. Representative integrated firing rate histogram illustrating the effect of pretreatment with asenapine on the inhibitory effect of clonidine (A), the effect of asenapine on the clonidine-induced suppression of LC NE neurons (B), and relationship between the degree of reversal of LC NE clonidine-induced suppression and doses of asenapine administered intravenously (C).

4.4.3 Determination of the potency of systemically-administered asenapine on 5HT_{2A} receptors using LC neuronal firing

Asenapine dose-dependently reversed and antagonized the inhibitory effect of the 5-HT_{2A} agonist DOI (50 $\mu\text{g}/\text{kg}$, *i.v.*) starting at a dose of 10 $\mu\text{g}/\text{kg}$. Consecutive injections of DOI failed to inhibit NE neuronal firing following the reversal of NE firing by asenapine, thereby indicating that the recovery of the firing was due to 5-HT_{2A} antagonism (Fig. 31B). The ED_{50} value for antagonizing DOI-induced inhibition (50 $\mu\text{g}/\text{kg}$) was $75 \pm 2 \mu\text{g}/\text{kg}$, $n = 8$; Fig. 31C).

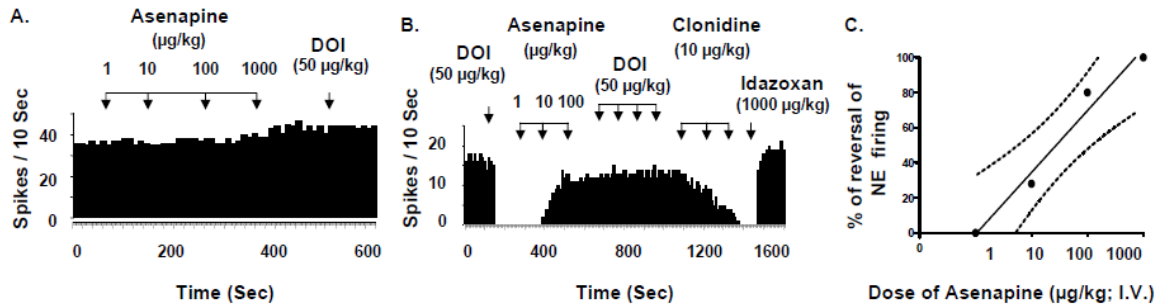


Figure 31. Representative integrated firing rate histogram illustrating the effect of pretreatment with asenapine on the inhibitory effect of DOI (A), the effect of asenapine on the DOI-induced suppression of LC NE neurons (B), and relationship between the degree of reversal of LC NE DOI-induced suppression and doses of asenapine administered intravenously (C).

4.4.4 Determination of the potency of systemically-administered asenapine on VTA D_2 autoreceptors

Asenapine dose-dependently reversed the inhibition of DA neuronal firing, produced by the D_2 agonist apomorphine (40 µg/kg), starting at a dose of 10 µg/kg. Following the injection of asenapine 1000 µg/kg, the inhibitory effect of apomorphine was fully blocked as several consecutive injections of apomorphine 40 µg/kg did not reduce the DA neuronal firing any further (Fig. 32B). The ED_{50} value for reversing apomorphine-induced inhibition (40 µg/kg) was 40 ± 2 µg/kg ($n = 7$; Fig. 32C).

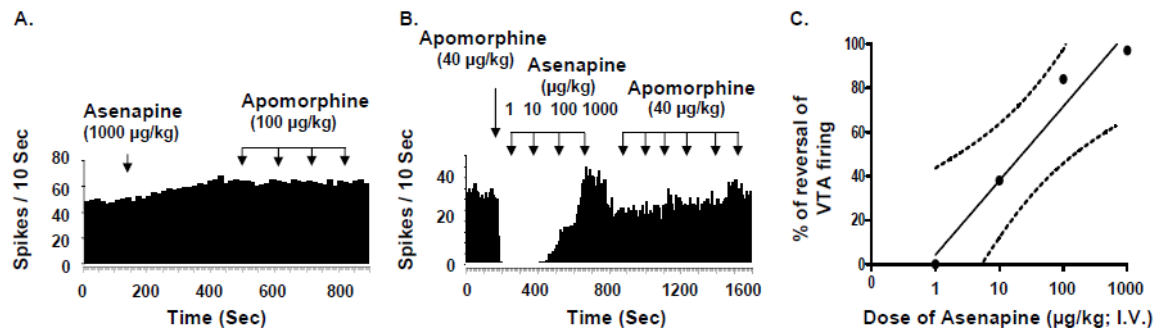


Figure 32. Representative integrated firing rate histogram illustrating the effect of pretreatment with asenapine on the inhibitory effect of apomorphine (A), the effect of asenapine on the apomorphine-induced suppression of VTA DA neurons (B), and relationship between the degree of reversal of VTA DA apomorphine-induced suppression and doses of asenapine administered intravenously (C).

4.4.5 Assessment of the effects of microiontophoretic applications of asenapine on CA_3 hippocampal 5-HT neurons

CA_3 pyramidal neuronal activity was suppressed by microiontophoretic-application of 5-HT (Figs. 33A and B). Microiontophoretic-application of asenapine also markedly

inhibited the CA₃ neuronal activity (Figs. 33A and B). The inhibitory effects of both asenapine and 5-HT were significantly blocked following the systemic injection of WAY 100,635 (50 µg/kg), confirming that this inhibitory action was mediated by 5-HT_{1A} receptors (F [1, 4] = 8.52, p < 0.05; Fig. 33B, and F [1, 4] = 9.50, p < 0.05; Fig. 33C, respectively). However, when asenapine and 5-HT were co-administered microiontophoretically, asenapine partially and significantly offset the inhibitory effect of 5-HT on the neuronal firing, indicating that asenapine acted as a partial 5-HT_{1A} agonist (F [1, 18] = 6.94, p < 0.05; Figs. 33A and B).

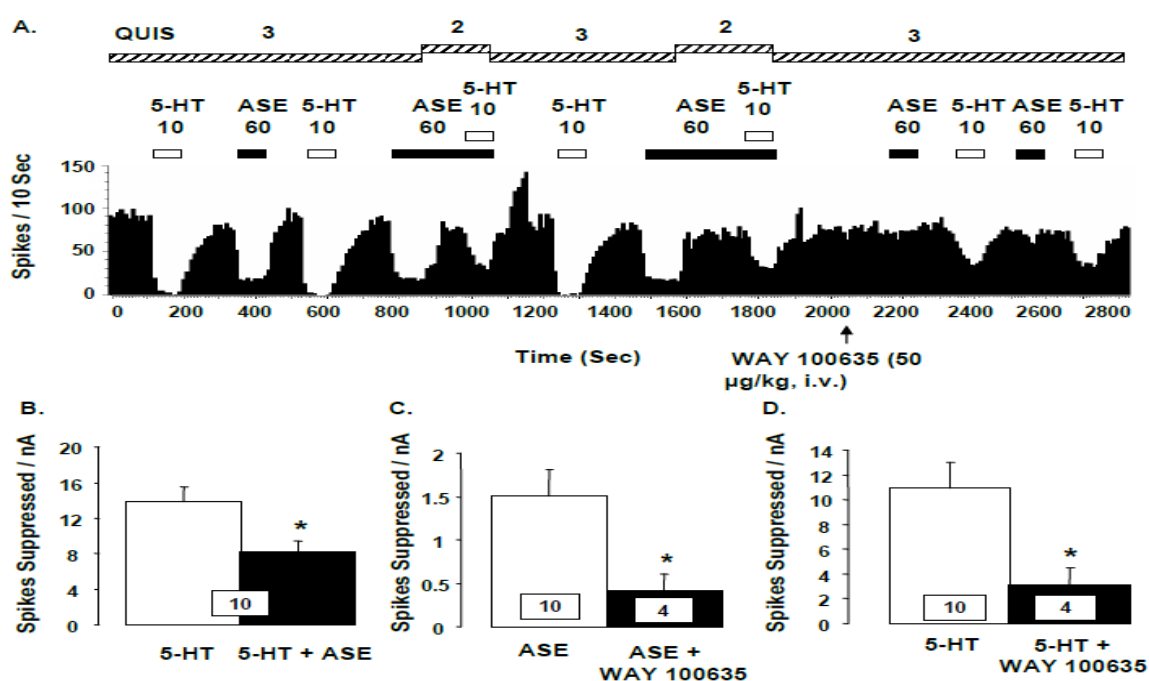


Figure 33. Integrated firing rate histogram of dorsal hippocampus CA₃ pyramidal neuron illustrating microiontophoretic application of asenapine (ASE) and 5-HT (A), and responses of dorsal hippocampus CA₃ pyramidal neurons to microiontophoretic co-application of 5-HT and asenapine (ASE) (B), asenapine (ASE) (C), and 5-HT (D) prior to and following *i.v.* injection of WAY 100635. The number of neurons recorded is displayed in each box.

4.4.6 Assessment of the effects of microiontophoretic applications of asenapine on DRN 5-HT neurons

Asenapine was applied locally in DRN because its systemic administration neither significantly altered the DRN 5-HT firing rate nor reversed the suppressant effect of 5-HT_{1A} autoreceptor agonist 8-OH-DPAT (10 µg/kg; Fig. 28). In DRN, spontaneous activity

of 5-HT neurons was markedly inhibited with the local application of 10 nA of 5-HT (Fig. 34). Microiontophoretic ejection of 25 nA of asenapine also significantly reduced the 5-HT neuronal activity (Fig. 34). The 5-HT_{1A} antagonist WAY 100635 (100 µg/kg), significantly blocked the inhibitory effect of both asenapine and 5-HT, thus confirming that 5-HT_{1A} receptors mediated this inhibitory action (F [1, 4] = 18.49, p < 0.05; Fig. 34C, and F [1, 4] = 8.74, p < 0.05; Fig. 34D, respectively). Asenapine, however, partially offset the inhibitory effect of 5-HT when the two agents were co-administrated microiontophoretically, therefore indicating that asenapine acted as a partial 5-HT_{1A} agonist (F [1, 28] = 6.10, p < 0.05; Figs. 34A and B).

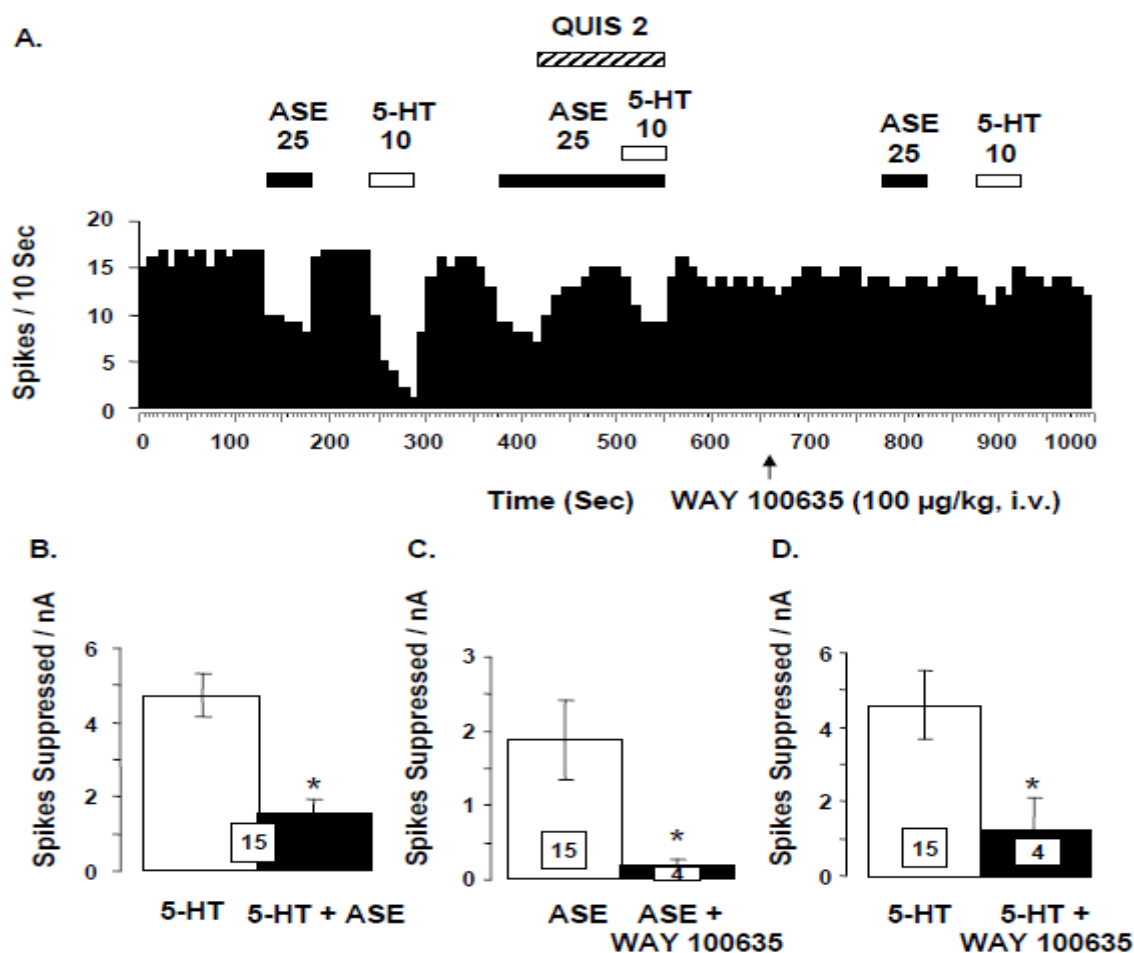


Figure 34. Integrated firing rate histogram of DRN 5-HT neuron illustrating microiontophoretic application of asenapine (ASE) and 5-HT (A), and responses of DRN 5-HT neurons to microiontophoretic co-application of 5-HT and asenapine (ASE) (B), asenapine (ASE) (C), and 5-HT (D) prior to and following *i.v.* injection of WAY 100635. The number of neurons recorded is displayed in each box.

4.4.7 Discussion

The present electrophysiological observations revealed that systemic administration of asenapine increased the spontaneous firing rate of DA neurons in the VTA by 15%, but did not alter the spontaneous firing rates of NE and 5-HT neurons. Asenapine also dose-dependently and potently reversed the inhibitory action of α_2 -adrenergic, D_2 , and 5-HT_{2A} agonists on the firing of catecholamine neurons. Moreover, following the reversal of the monoaminergic neuronal firing, asenapine attenuated the inhibitory action of the above-mentioned agonists. Microiontophoretic applications of asenapine showed that it acted as an agonist on somatodendritic 5-HT_{1A} autoreceptors as well as on postsynaptic 5-HT_{1A} receptors in the hippocampus. The agonistic action of asenapine at 5-HT_{1A} receptors was further characterized as being partial because it partially attenuated the inhibitory action of the full endogenous agonist 5-HT when co-applied by microiontophoresis both in the DRN and hippocampus.

Asenapine is a potent α_2 -adrenergic antagonist as it reversed the inhibitory effect of the α_2 -adrenergic agonist clonidine and subsequently attenuated its suppressant effect (Fig. 30). Despite its potent α_2 -adrenoceptor antagonistic property, asenapine at the dose of 1000 $\mu\text{g}/\text{kg}$ only slightly increased NE neuronal firing and did not alter burst activity. These observations are consistent with previous electrophysiological studies that a 2-day treatment with the α_2 -adrenoceptor antagonist risperidone, which like asenapine has affinity for multiple monoaminergic receptors, did not significantly increase LC NE firing rate (Dremencov *et al.*, 2007b). It is unclear at this time what additional property of asenapine, or current atypical antipsychotics such as risperidone, beyond their α_2 -adrenoceptor antagonism, would prevent them from enhancing the firing of NE neurons, as has been observed with the selective α_2 -adrenergic antagonist idazoxan (Freedman and Aghajanian, 1984; Dong and Blier, 2001).

Asenapine also exhibited a potent antagonistic effect at 5-HT_{2A} receptors (Fig. 31), located on γ -aminobutyric acid (GABA) neurons, controlling the firing rate of LC NE neurons. LC NE neurons are under an inhibitory influence from DRN 5-HT neurons (Aston-Jones *et al.*, 1991). Indeed, abolishing this inhibitory effect by lesioning of 5-HT neurons or inhibiting

5-HT synthesis significantly increases LC NE firing rate (Haddjeri *et al.*, 1997b; Dremencov *et al.*, 2007a). Electrophysiological studies have shown that this inhibitory effect is mediated by 5-HT_{2A} receptors expressed on GABA interneurons (Haddjeri *et al.*, 1997b; Szabo and Blier, 2002). Moreover, chronic administration of SSRIs leads to gradual reduction of LC NE firing rate (Szabo *et al.*, 1999; Dremencov *et al.*, 2007a; Ghanbari *et al.*, 2010). A sustained decrease in the activity of NE neurons could be clinically counterproductive in the treatment of major depression disorder, as it may explain the fatigue and asthenia in some depressed patients following long-term SSRI treatment (Montgomery *et al.*, 1993). Interestingly, NE firing rate is completely recovered following long-term administration of YM992, a SSRI and a potent 5-HT_{2A} antagonist (Szabo and Blier, 2002). These observations are consistent with the results of microdialysis studies showing that coadministration of the selective 5-HT_{2A} receptor antagonist MDL 100907 and the SSRI citalopram, significantly increased extracellular NE levels in the rat frontal cortex (Hatanaka *et al.*, 2000). Thus, it is important to note that the 5-HT_{2A} receptor antagonistic property of the atypical antipsychotics may account for the effectiveness of this class of drugs in treatment-resistant depression (Tohen *et al.*, 2003; McFadden *et al.*, 2004; Papakostas *et al.*, 2005; Nemeroff, 2005; Simon and Nemeroff, 2005).

Microiontophoretic application of asenapine indicated that it is an agonist at DRN and postsynaptic hippocampal 5-HT_{1A} receptors, as indicated by the ability of the 5-HT_{1A} antagonist WAY 100,635 to block its suppressant effects. It was, however, found to be a partial agonist because it attenuated the inhibitory effect of 5-HT (Figs. 33 and 34). To date, all selective 5-HT_{1A} agonists have been shown to be full agonists on the cell body 5-HT_{1A} autoreceptors. These agents inhibit the firing rate of 5-HT neurons, whether they are administered by local microiontophoretic application or systemic injection. It is unlikely, however, that the partial agonistic action of asenapine accounts for its lack of effect on the mean firing rate of 5-HT neurons when administered systemically. Indeed, pindolol which is also a partial 5-HT_{1A} agonist at DRN 5-HT_{1A} receptors can inhibit the firing activity of 5-HT neurons (Haddjeri *et al.*, 2000). The most likely explanation for the lack of effect of asenapine on the spontaneous firing rate of 5-HT neurons is that asenapine enhanced NE release through the antagonism of terminal α_2 -adrenoceptors in the DRN. The enhanced

availability of NE acting on excitatory α_1 -adrenoceptors, located on the cell body of 5-HT neurons, could possibly counterbalance the inhibitory action of asenapine on 5-HT_{1A} receptors. 5-HT neurons would then not be disinhibited after the subsequent administration of WAY 100,635 after asenapine, because this selective 5-HT_{1A} antagonist completely inhibits the firing rate of LC NE neurons (Haddjeri, *et al.*, 1997b). In support of this explanation is the observation that WAY 100,635 can only enhance 5-HT firing when NE neurons are lesioned (Haddjeri *et al.*, 2004).

It is noteworthy that nearly all atypical antipsychotics inhibit the firing rate of 5-HT neurons upon acute systemic injections. Clozapine and olanzapine suppress DRN firing *via* α_1 -adrenoceptor antagonism (Sprouse *et al.*, 1999), ziprazidone and aripiprazole by acting at 5-HT_{1A} autoreceptors (Sprouse *et al.*, 1999; Stark *et al.*, 2007), and risperidone by a combination of both actions (Hertel *et al.*, 1997; Dremencov *et al.*, 2007b). Paliperidone is the only other atypical antipsychotic that does not inhibit firing of 5-HT neurons when administered systemically (Dremencov *et al.*, 2007b). Only the effects of long-term administration of risperidone and paliperidone on DRN firing are known. Risperidone produces a sustained inhibition whereas paliperidone does not (Dremencov *et al.*, 2007b). It will therefore be important to determine if prolonged administration of asenapine will produce a desensitization of the 5-HT_{1A} autoreceptor, as the partial agonist pindolol does (Haddjeri and Blier, 2000), and lead to enhanced levels of firing.

It is important to note that asenapine displays potent *in vitro* antagonistic activity at cloned human 5-HT_{1A} receptors (Shahid *et al.*, 2009). However, the results of the present study indicate that asenapine displays *in vivo* partial agonistic action at 5-HT_{1A} receptors. Interestingly, a microdialysis study in rats put into evidence *in vivo* agonistic action of asenapine at 5-HT_{1A} receptors, as reported herein. Indeed, subcutaneous injection of asenapine increased DA and acetylcholine in rat mPFC and hippocampus *via* 5-HT_{1A} activation because this enhancement was abolished by a small dose of the 5-HT_{1A} antagonist WAY 100,635 (Huang *et al.* 2008). This observation suggests that the enhanced DA and acetylcholine efflux is at least in part mediated by the activation of 5-HT_{1A} receptors by asenapine.

Asenapine exhibited high affinity blockade of D₂ receptors (Fig. 32), which was coupled with a small but significant increase in both firing rate and number of bursts (Fig. 29), as is observed with other D₂ receptor antagonists. The increased burst firing is expected to enhance DA release for the same number of action potentials as single spikes over the same time period of time (Gonon, 1988; Garris *et al.*, 1994). Indeed, Franberg *et al.* (2008) showed that asenapine significantly increased DA release in the projection areas of VTA DA neurons.

With respect to absolute potency of asenapine for D₂ receptors, it is important to mention that when compared to haloperidol, both drugs have similar affinities, similar *in vivo* potency at D₂ autoreceptors in the rat brain, and are both efficacious in schizophrenia in the same dose range (Shahid *et al.*, 2009; Potkin *et al.*, 2007; Chaudhry *et al.*, 2007). Although binding studies of asenapine at cloned human receptors showed that it has a higher affinity for 5-HT_{2A} than D₂ receptors (Shahid *et al.*, 2009), the present study indicated that its potency at VTA D₂ receptors is within the same dose range as for the former receptor. One possible explanation for this discrepancy is the fact that there are reciprocal interactions between monoaminergic neurons (Guiard *et al.*, 2008) and that the high affinity of asenapine for several monoaminergic receptors may be at play. The other possibility is that the 5-HT_{2A} receptors which modulate the firing activity of LC NE neurons have a lower sensitivity than the 5-HT_{2A} receptors expressed *in vitro* in a human cell line.

In conclusion, the present results confirm that the very high *in vitro* affinity of asenapine for 5-HT_{2A}, α_2 -adrenergic, and D₂ receptors leads to potent *in vivo* antagonist activity at these receptors. Furthermore, the current study provides evidence indicating that asenapine has partial agonistic action at the 5-HT_{1A} receptors. Overall the *in vivo* receptor profile of asenapine supports its potential therapeutic utility in the treatment of schizophrenia and mood disorders.

5 CONCLUDING REMARKS

The main part of the thesis was largely focused on the effects of bupropion on the DRN 5-HT and LC NE neuronal activity in combination with the SSRI escitalopram or by itself. The data suggests that bupropion exerts its synergistic effect with escitalopram on DRN 5-HT neurons in a prompt manner and that the inhibitory action of escitalopram on LC NE cells is partially offset by prolonged bupropion administration. Pilot data suggested that escitalopram and bupropion given from treatment initiation produces a rapid and robust antidepressant response (Leuchter *et al.*, 2008; Stewart *et al.*, 2009). In this regard, a controlled clinical study is currently ongoing at Columbia University and in our clinical unit examining this possibility. In line with this, Nelson and colleagues (2004) reported that combination of the SSRI fluoxetine and the NRI desipramine remarkably resulted in greater remission rate than either agent alone. Furthermore, clinical data from our unit have shown that the use of antidepressant combinations from treatment initiation double the remission rate (Blier *et al.*, 2009 and 2010a). These promising findings suggest that greater remission rates can be achieved if two antidepressant drugs with synergistic effects are combined from treatment initiation. This approach provides superior therapeutic effects than consecutive monotherapies, considering that second and subsequent antidepressant drugs are less likely to result in full remission of depressive symptoms (Paykel *et al.*, 1995; Rush *et al.*, 2006; Stahl, 2008). In addition, the results of the postsynaptic experiments showed that prolonged administration of bupropion enhanced both 5-HT and NE transmission in hippocampus due to reduced sensitivity of terminal α_2 -adrenergic hetero- and autoreceptors, respectively (Fig. 35). The increased 5-HT and NE tone may be further enhanced when bupropion is administered with SSRIs, a class of antidepressants that lead to desensitization of terminal 5-HT_{1B} autoreceptors. For instance, prolonged administration of 5-HTT reuptake inhibitor trazodone induced desensitization of terminal 5-HT_{1B} autoreceptors, thereby enhancing 5-HT transmission (Fig. 26). Consequently, the enhancement of 5-HT and NE transmission may contribute to the therapeutic action of antidepressants and their combinations in the treatment of major depression.

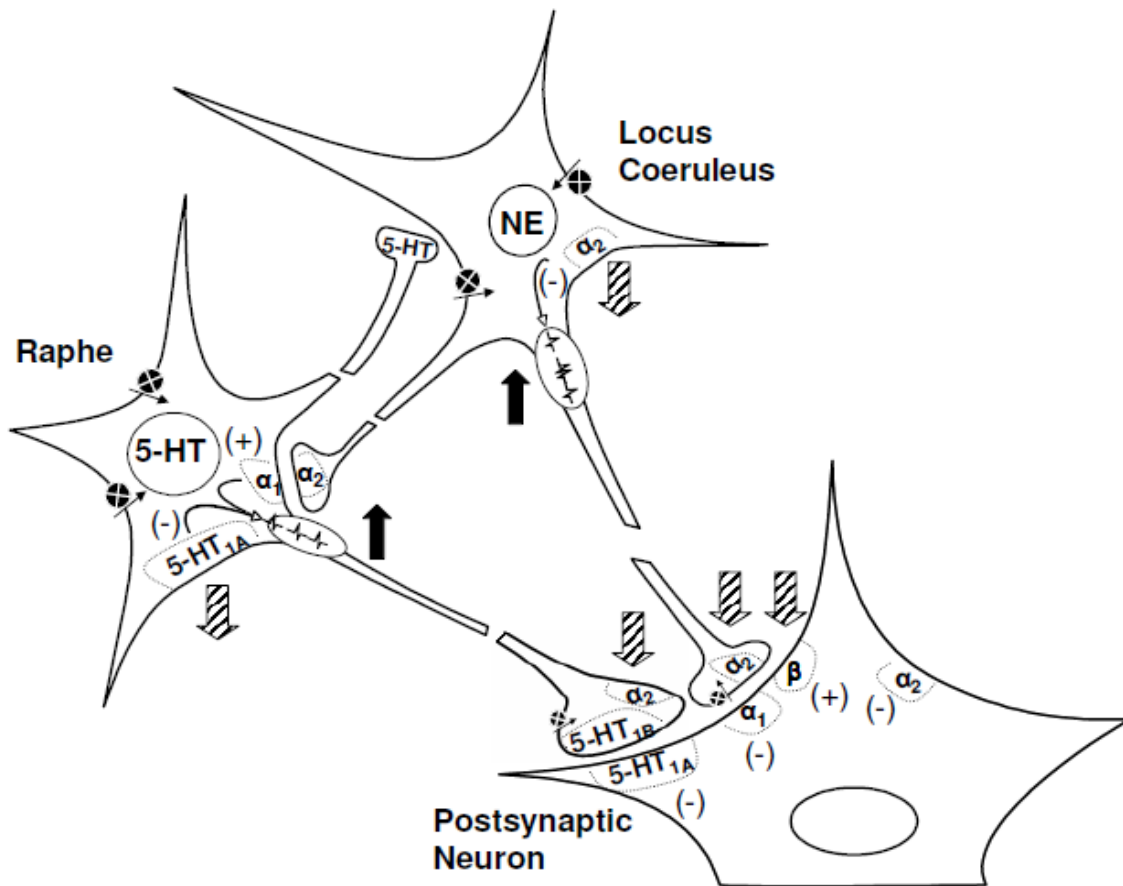


Figure 35. Schema representing the interactions between serotonin (5-HT) and norepinephrine (NE) neurons, and their projections to pyramidal neurons of the hippocampus. The cogwheels represent the monoamine reuptake transporters. The (+) and (-) signs depict, respectively, the excitatory and inhibitory influences of the receptors on the neuronal firing rate. The upward black arrows on the 5-HT and NE axons represent the enhanced firing rate and burst activity of 5-HT and NE neurons, respectively, following long-term administration of bupropion (Ghanbari *et al.*, 2010). The downward hatched arrows on the 5-HT and NE cell bodies correspond to the diminished sensitivity of somatodendritic 5-HT_{1A} and α₂-adrenergic autoreceptors (El Mansari *et al.*, 2008; Ghanbari *et al.*, 2010), presynaptic α₂-adrenergic auto- and heteroreceptors on NE and 5-HT terminals, and the diminished function of postsynaptic β-adrenoceptors on the pyramidal neurons in the hippocampus.

Interestingly, bupropion regimen that altered NE and 5-HT neuronal firing had no effect on the neuronal activity of VTA DA neurons both at 2 or 14 days (El Mansari *et al.*, 2008). Previous studies showed that only very high doses of bupropion suppressed the firing rate of brain stem DA neurons in the rat (Cooper *et al.*, 1994), suggesting that this effect does not constitute a basis for its clinical effectiveness. Whatever the mechanism of action of bupropion on DA system may be, an effect on the firing rate of VTA DA neurons was expected. Indeed, *in vivo* microdialysis studies have demonstrated that after both acute and

prolonged administration of bupropion, there was an enhancement of extracellular DA concentration in the nucleus accumbens and hippocampus regions, but not in the striatum (Nomikos *et al.*, 1992; Piacentini *et al.*, 2003). Together, these results indicate that the increase in DA release may not depend on the firing activity of VTA DA neurons, under not only sub-acute but also prolonged treatment with bupropion, as suggested by electrophysiological experiments (Dong and Blier, 2001; El Mansari *et al.*, 2008). In support of this, local administration of the N-methyl-D-aspartate (NMDA) receptor antagonist ketamine enhanced DA levels in mPFC (Lorrain *et al.*, 2003), suggesting that the DA enhancement in the postsynaptic regions may not be driven by DA neurons in the VTA. Moreover, application of tetrodotoxin to DA neurons in the VTA failed to prevent MK-801-induced DA release in the mPFC (Mathé *et al.*, 1999). It is difficult to dissociate alterations in DA release from neuronal activity of VTA DA neurons. However, *in vivo* studies have shown that bupropion-induced sensitization is rather due to an increase in the ability of bupropion to release DA (Nomikos *et al.*, 1990; Stamford *et al.*, 1989). Nevertheless, unlike bupropion, the DA reuptake inhibitor GBR12909, also known for increasing extracellular levels of DA in the cortex (Caruana *et al.*, 2006), induced a significant decrease in both the firing and burst activity of VTA DA neurons following 2-day administration (Katz *et al.*, 2008), supporting the notion that bupropion is not an effective dopamine reuptake inhibitor (Ascher *et al.*, 1995; Horst and Preskorn, 1998; Sanchez and Hyttel, 1999). Taken together these results indicate that bupropion differentially affects DA release in various brain structures.

Despite the clinical use of trazodone in treating depression for more than two decades, its mechanisms of action were not fully understood since it has affinity for a variety of 5-HT receptors and for the 5-HT transporter. We showed that the effects of trazodone on the 5-HT system, in the DRN and hippocampus, resemble that of the SSRIs. The overall enhancement of 5-HT transmission, by prolonged administration of trazodone, was in part due to its blocking property at 5-HTT, as reported herein both *in vitro* and *in vivo*. Furthermore, prolonged administration of trazodone induced desensitization of 5-HT_{1A} and 5-HT_{1B} autoreceptors, resulting in normalization of firing rate of DRN 5-HT neurons and in enhancement of the neurotransmitter release in hippocampus. In addition,

microiontophoretic experiments showed that trazodone acts as a full agonist at postsynaptic 5-HT_{1A} receptors in hippocampus, a feature that may contribute to the enhanced 5-HT levels in this region, as for 5-HT_{1A} agonist gepirone (Haddjeri *et al.*, 1998a). Although trazodone is an effective tool in the armamentarium of antidepressants, its clinical use has fallen out of favour due to its daytime sedative action. This property likely stems from a combination of H₁, 5-HT_{2A} and α_1 -adrenergic receptor antagonism. The introduction of the slow release formulation has, however, diminished the daytime sedative nature of trazodone while maintaining its antidepressant properties (Sheehan *et al.*, 2009). This may indeed promote its clinical use in the treatment of depression since it acts in part similar to the first-line strategy, without the adverse effects, associated with the SSRI class, such as sexual dysfunction. In line with this, it is absolutely essential to examine the potential effects of short- and long-term administration of trazodone on the firing of catecholamines.

We demonstrated that the novel psychopharmacological agent asenapine, developed for the treatment of schizophrenia and bipolar illness, has potent antagonistic activity at 5-HT_{2A}, D₂, and α_2 -adrenoceptors *in vivo*. In addition, asenapine acted as a partial agonist at 5-HT_{1A} receptors in DRN and hippocampus. The multi-receptor profile of asenapine, *in vivo*, supports its potential therapeutic utility in the treatment of mood disorders, including major depression. Indeed, various studies over the last decade have put into evidence the beneficial effect of atypical antipsychotic addition in treatment-resistant patients (Shelton *et al.*, 2001; Dunner *et al.*, 2007). The antidepressant response induced by the atypical antipsychotics may stem from physiological actions on the monoaminergic systems. In support of this, the typical antipsychotic aripiprazole markedly increased the firing rate of DRN 5-HT neurons at day 2 and 14, and induced an early desensitization of somatodendritic 5-HT_{1A} autoreceptors following 2-day regimen (Chernoloz *et al.*, 2009b). Interestingly, the inhibitory action of the SSRI escitalopram on the firing rate of DRN 5-HT neurons was abolished when aripiprazole was used as an adjunct to the SSRI (Chernoloz *et al.*, 2009b). The *in vivo* receptor profile of asenapine, hence, suggests that it may be an effective option in the treatment of major depression. Thus, it is of interest to study the potential effects of sustained administration of asenapine on the monoaminergic systems.

Taken together, the present data provides some of the underlying neurobiological mechanisms for various medications and/or their add-on strategies in the treatment of MDD.

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