Alterations of monoaminergic systems by sustained triple reuptake inhibition

Jojo Jiang
University of Ottawa, Institute of Mental Health Research
Department of Cellular and Molecular Medicine, Neuroscience Program
Faculty of Medicine
University of Ottawa

This thesis is submitted as a partial fulfillment of the M.Sc. program in Neuroscience.

Submitted on August 14, 2012.

ABSTRACT

Recent approaches in depression therapeutics include triple reuptake inhibitors, drugs that target three monoamine systems. Using *in vivo* electrophysiological and microdialysis techniques, the effects of 2- and 14-day treatments of escitalopram, nomifensine and the co-administration of these two drugs (TRI) were examined in male Sprague-Dawley rats. Short- and long-term TRI administration decreased NE firing and had no effect on DA neurons. Normal 5-HT firing rates were maintained after 2-day TRI administration compared to the robust inhibitory action of selective serotonin reuptake inhibitors (SSRIs). Escitalopram treatment enhanced the tonic activation of the 5-HT_{1A} receptors given the increase in firing observed following WAY100635 administration. Nomifensine treatment enhanced tonic activation of the α_2–adrenoceptors following idazoxan administration. TRI treatment caused a robust increase in extracellular DA levels that was in part mediated by a serotonergic contribution. Therapeutic effects of the drugs examined in this study may be due to the enhancement of 5-HT, NE and/or DA neurotransmission.

TABLE OF CONTENTS

Abstract ii
List of Tables v
List of Figures vi
List of Abbreviations vii
Acknowledgements x
1. Introduction 1
1.1. Major depressive disorder 1
1.2. Monoamine Hypothesis 1
1.2.1. Serotonin system 2
1.2.1.1. Localization 2
1.2.1.2. Synthesis, storage, release, and uptake 3
1.2.1.3. Receptors 4
1.2.2. Norepinephrine system 6
1.2.2.1. Localization 6
1.2.2.2. Synthesis, storage, release, and uptake 7
1.2.2.3. Receptors 7
1.2.3. Dopamine system 8
1.2.3.1. Localization 8
1.2.3.2. Synthesis, storage, release, and uptake 9
1.2.3.3. Receptors 10
1.3. Structural and functional interactions between the monoaminergic systems 11
1.3.1. Serotonin-norepinephrine interactions 12
1.3.2. Serotonin-dopamine interactions 13
1.3.3. Norepinephrine-dopamine interactions 14
1.4. Pharmacological treatments for depression 15
1.4.1. Tricyclic antidepressants (TCAs) 15
1.4.2. Monoamine oxidase inhibitors (MAOIs) 16
1.4.3. Selective serotonin reuptake inhibitors (SSRIs) 16
1.4.3.1. Escitalopram 17
1.4.4. Selective norepinephrine reuptake inhibitors (NRIs) 18
1.4.5. Serotonin-norepinephrine reuptake inhibitors (SNRIs) 18
1.4.6. Norepinephrine-dopamine reuptake inhibitors (NDRIs) 19
1.4.6.1. Bupropion 19
1.4.6.2. Nomifensine 20
1.4.7. Triple reuptake inhibitors (TRIs) 21
1.5. Aims 22
1.5.1. Subacute effects of the combination of escitalopram and nomifensine on the cell bodies of the three monoaminergic systems 22
1.5.2. Sustained effects of the combination of escitalopram and nomifensine on the cell bodies of the three monoaminergic systems 23
1.5.3. Sustained effects of escitalopram and/or nomifensine on the forebrain structures of the three monoaminergic systems 23
2. Materials and Methods 24
2.1. Animals 24

2.2. Experimental preparations 24
2.3. Electrophysiological recording of DRN 5-HT neurons 25
2.4. Electrophysiological recording of LC NE neurons 26
2.5. Electrophysiological recording of VTA DA neurons 27
2.6. Extracellular recording and microiontophoresis of dorsal hippocampus CA3 pyramidal neurons 28
2.7. Tonic activation of postsynaptic 5-HT_{1A} receptors following 14-day administration of escitalopram and/or nomifensine 29
2.8. Tonic activation of postsynaptic α_2- and α_1-adrenoceptors following 14-day administration of escitalopram and/or nomifensine 29
2.9. Microdialysis 30
2.10. Drugs 31
2.11. Statistical analysis 31
3. Results 32
3.1. Sub-acute administration of escitalopram, nomifensine and triple reuptake inhibition 32
3.1.1. Dorsal raphe nucleus (DRN) 32
3.1.2. Locus coeruleus (LC) 33
3.1.3. Ventral tegmental area (VTA) 33
3.2 Sustained administration of escitalopram, nomifensine and triple reuptake inhibition 33
3.2.1. 5-HT 35
3.2.2. NE 35
3.2.3. DA 37
4. Discussion 39
5. References 50

LIST OF TABLES

TABLE 1. Affinity of escitalopram to human serotonin, norepinephrine, and dopamine transporters.

TABLE 2. Affinity of nomifensine to rat serotonin, norepinephrine, and dopamine transporters.

TABLE 3. Effect of triple reuptake inhibition (TRI) administration on the firing pattern of norepinephrine (NE) neurons in the locus coeruleus (LC).

LIST OF FIGURES

FIGURE 1. Location of serotonergic cell bodies and pathways in the rat brain.

FIGURE 2. Location of adrenergic cell bodies and pathways in the rat brain.

FIGURE 3. Location of dopaminergic cell bodies and pathways in the rat brain.

FIGURE 4. The reciprocal interactions between the cell bodies of 5-HT, NE and DA neurons.

FIGURE 5. Example of an electrophysiological recording of a 5-HT neuron in the DRN.

FIGURE 6. Example of an electrophysiological recording of a NE neuron in the LC.

FIGURE 7. Example of an electrophysiological recording of a DA neuron in the VTA.

FIGURE 8. Example of a single action potential from a recording of a CA3 pyramidal neuron in the hippocampus.

FIGURE 9. Subacute administration of escitalopram (Esc), nomifensine (Nomi) and triple reuptake inhibition (TRI).

FIGURE 10. Chronic treatment of Esc, Nomi and TRI on the 5-HT system.

FIGURE 11. Chronic treatment of Esc, Nomi and TRI on the NE system.

FIGURE 12. Chronic treatment of Esc, Nomi and TRI on the DA system.

FIGURE 13. Interactions between DRN 5-HT neurons and LC NE neurons.

LIST OF ABBREVIATIONS

β-OH	hydroxypropyl-β-cyclodextrin
5-HIAA	5-hydroxyindoleacetic
5-HT	5-hydroxytryptamine (serotonin)
5-HTT	serotonin transporter
6-OHDA	6-hydroxydopamine
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
AMPT	*α*-methyl-*p*-tyrosine
ANOVA	analysis of variance
AP	anterior-posterior
cAMP	cyclic adenosine phosphate
DA	dopamine
DALY	disability adjusted life year
DAT	dopamine transporter
DBH	dopamine β-hydroxylase
DOPA	dihydroxyphenylalanine
DRN	dorsal raphe nucleus
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders 4th Edition Text Revision
DSP-4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride
DV	dorsal-ventral
GABA	γ -aminobutyric acid
Esc	escitalopram

HAM-D	Hamilton Rating Scale for Depression
i.p.	intraperitonial
i.v.	intravenous
LC	locus coeruleus
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder
ML	medial-lateral
NAc	nucleus accumbens
NDRI	norepinephrine-dopamine reuptake inhibitor
NE	norepinephrine
NET	norepinephrine transporter
Nomi	nomifensine
OBX	olfactory bulbectomy
PCPA	*para*-chlorophenylalanine
s.c.	subcutaneous
S.E.M.	standard error of mean
SNRI	serotonin-norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
TH	tyrosine hydroxylase
TPH	tryptophan hydroxylase
TRI	triple reuptake inhibition

VMAT	vesicular monoamine transporter
VTA	ventral tegmental area
WHO	World Health Organization

ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to my supervisor, Dr. Pierre Blier, for his continual guidance and mentorship. Working in his lab the last few years has introduced me to the exciting world of neuropsychopharmacology and led to unparalleled opportunities to better myself, both as a person and as a scientist.

I am also indebted to Dr. Mostafa El Mansari for his invaluable advice, expertise and patience throughout my studies. As well, I would like to thank Richard Belanger for not only taking care of my rats but also for all our talks that were always welcomed during long experimental days. My sincere thanks goes out to Maria da Silva for being the "lab mom" to us students on top of dealing with all the administrative hurdles that always seem to arise.

Special thanks go out to my advisory committee members, Dr. Jean-Claude Béïque and Dr. Richard Bergeron for their guidance and suggestions for my research.

To all my colleagues at the IMHR, I will forever remember our lunchtime conversations and the endless entertainment you provided. In particular, Daphne and Stacey have made these years unforgettable and I'll always treasure our friendship.

Lastly, to my parents and Jenny: thanks for all these years of unconditional love and support. I would not be the person I am today without you.

1. INTRODUCTION

1.1. Major depressive disorder

Major depressive disorder (MDD) is characterized by depressed mood and/or loss of pleasure along with significant weight changes, sleep disturbances, psychomotor retardation, fatigue, feelings of worthlessness or guilt and suicidal ideation (American Psychiatric Association & American Psychiatric Association Task Force on DSM-IV, 2000). According to the World Health Organization (WHO, 2012), major depression is one of the leading causes of disability, affecting more than 120 million people worldwide. Unipolar depressive disorders are the third leading cause of burden of disease measured by disability adjusted life years (DALY) globally and is the leading cause in middle- to high-income countries (WHO, 2008). In fact, WHO predicts that depression will become the leading cause of burden of disease worldwide by 2030 (WHO, 2008). Studies have shown that depression is often comorbid with other chronic medical conditions such as cardiovascular disorders and diabetes (Player & Peterson, 2011; Renn et al., 2011). Not only does depression comorbid with other diseases adversely affect prognosis, but it also causes substantial social and economic burdens. Greenberg et al. (2003) found that in 2000 in the United States alone, over $83 billion dollars was spent on direct costs (in-patient, out-patient, and pharmaceutical), suicidal-related costs, and workplace absenteeism costs.

1.2. Monoamine Hypothesis

Monoamine neurotransmitter deficiency has been linked to depression from as early as the 1960s (Schildkraut, 1965). The monoaminergic systems, including serotonin

(5-HT), norepinephrine (NE), and dopamine (DA), were implicated in the pathophysiology of depression when tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), 2 classes of drugs that increase synaptic neurotransmitter levels (see Section 1.4.1 and 1.4.2, respectively), were observed to alleviate depressive mood (Ban, 2006). Furthermore, clinical studies have also shown that depleting these same monoamines causes a relapse in depressive symptoms (Bell et al., 2001; Delgado, 2006). Patients who responded to selective serotonin uptake inhibitors (SSRIs) were much more likely to relapse compared to healthy controls and patients who responded to norepinephrine reuptake inhibitors (NRIs) following tryptophan depletion, a paradigm that depletes brain serotonin. Conversely, catecholamine depletion with *α*-methyl-*p*-tyrosine (AMPT) led to 81% of patients who responded to NRI treatment to relapse compared to 14% of healthy controls and 19% of patients who responded to SSRI administration (Delgado, 2000). Due to the importance of the monoaminergic systems in the etiology of depression, the aim of many antidepressants is to enhance their neurotransmission.

1.2.1. Serotonin system

1.2.1.1. Localization

Serotonin (5-HT) was first identified in blood serum (Rapport et al., 1948) and shortly after that, it was discovered to be present in the central nervous system as well (Bogdanski et al., 1956). Most 5-HT neurons are localized along the midline of the brain stem called raphe nuclei; in fact, Dahlström and Fuxe (1964) described nine 5-HT clusters located in the raphe nuclei and the reticular region of the lower brain stem. The largest cluster of serotonergic neurons (165,000 cells in humans) is found in the dorsal

raphe nucleus (DRN; Tork, 1990). Serotonergic neurons from the raphe project to virtually all regions in the brain, including the limbic system and the cortex (Figure 1).

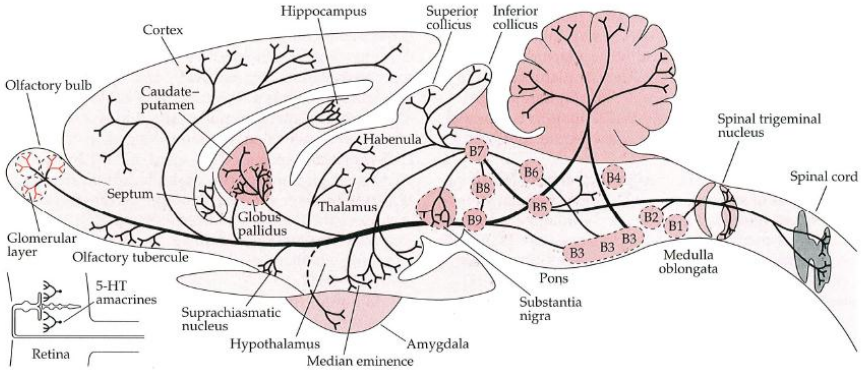

FIGURE 1. LOCATION OF SEROTONERGIC CELL BODIES AND PATHWAYS IN THE RAT BRAIN. From Feldman et al. (1997).

1.2.1.2. Synthesis, storage, release, and uptake

5-HT is synthesized from the amino acid tryptophan in two steps, catalyzed by the enzymes tryptophan hydroxylase (TPH) and aromatic amino acid decarboxylase. There are two distinct TPH isoforms: TPH1 is expressed in the periphery while TPH2 is found in the brain (Walther & Bader, 2003). TPH is the rate-limiting step and this enzyme is located only in serotonergic cells, making it an ideal marker for 5-HT neurons. Although 5-HT synthesis occurs in the axon terminals, TPH is synthesized in the serotonergic cell bodies and transported to the terminals (Meek & Neff, 1972). Several drugs inhibit TPH, but the classic inhibitor is *para*-chlorophenylalanine (PCPA). Koe and Weissman (1966) determined that PCPA is a selective and potent depletor of brain 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in mice, rats and dogs. A single dose of PCPA (300 mg/kg) can decrease brain 5-HT levels by 80% to 90% and last for up to two weeks (Feldman et al., 1997).

Although some 5-HT is present in the cytoplasm, most of the synthesized 5-HT is stored in vesicles. Two different pools of 5-HT is believed to exist (Morot-Gaudry et al., 1981; Tracqui et al., 1983). The majority of the brain 5-HT is present in the large pool; however, this is thought to be a storage pool. The functional, smaller pool only contains 10% to 25% of the total 5-HT neuronal content and it preferentially releases 5-HT. 5-HT is released into the synapse through exocytosis in a Ca^{2+}-dependent manner (Sanders-Bush & Martin, 1982).

5-HT present in the synapse is recycled back into the neuron by serotonin transporters (5-HTT) located in the plasma membrane of serotonergic neurons. This process is mediated by Na^{+} and Cl^{-} ions (Marcusson & Ross, 1990). This uptake process is the target of many antidepressants (see section 1.4.3.).

1.2.1.3. Receptors

Twenty structurally and pharmacologically distinct 5-HT receptor subtypes belonging to seven families of 5-HT receptors have been cloned (5-HT_{1-7}) (Nichols & Nichols, 2008) and with the exception of 5-HT_3 receptor, all these receptors are seven transmembrane, G-protein coupled metabotropic receptors. The 5-HT_3 receptor is unique not only among the 5-HT receptors, but also other monoamines in that it is a ligand-gated ion channel.

The 5-HT_1 receptors are all coupled negatively to adenylate cyclase thereby having an inhibitory mechanism of action. Within the raphe nuclei, the 5-HT_{1A} autoreceptor subtype is located on the somatodendrites of 5-HT neurons and suppresses neuronal firing (Sharp & Hjorth, 1990). There is also a high density of this receptor located in post-synaptic limbic brain regions including the hippocampus and cortical

areas; however, these receptors are located on non-5-HT neurons (heteroreceptors) (Barnes & Sharp, 1999). A review by de Montigny and Blier (1992) outline the different characteristics between 5-HT_{1A} autoreceptors located in the DRN and 5-HT_{1A} heteroreceptors found in the dorsal hippocampus. The first difference between the two 5-HT_{1A} receptors is the difference in potencies of 5-HT and 5-HT_{1A} agonists; while 5-HT displays more activity in the dorsal hippocampus than in the DRN, 5-HT_{1A} agonists such as 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), gepirone, and ipsapirone are more active in the DRN (Blier & de Montigny, 1987b, 1990; Chaput & de Montigny, 1988; Sprouse & Aghajanian, 1988). Another distinction between the two receptors is the fact that 5-HT_{1A} autoreceptors desensitize following prolonged 5-HT_{1A} agonist treatment (gepirone, flesinoxan, and tandospirone) while 5-HT_{1A} heteroreceptors have unaltered sensitivity (Blier & de Montigny, 1987b; Godbout et al., 1991; Hadrava et al., 1995). This is not due to the fact that these drugs act as partial agonists in the dorsal hippocampus and full agonists in the DRN since BAY x 3702 is a full agonist at both the 5-HT_{1A} auto- and heteroreceptor (Dong & Blier, 1998). The highest densities of 5-HT_{1B} receptors are found in the substantia nigra and globus pallidus, with lower levels found in the caudate nucleus, putamen, and nucleus accumbens (NAc) (Bonaventure et al., 1997; Sari et al., 1999). Interestingly, the highest mRNA expression of 5-HT_{1B} receptors is not located in the above areas, but rather in the raphe nuclei, striatum, cortex, and lateral geniculate nucleus (Varnas et al., 2005). Thus, it seems that these receptors are located in the nerve terminals of both 5-HT and non-5-HT neurons. Indeed, Chaput et al. (1986) found that terminal 5-HT autoreceptors (later characterized as the 5-HT_{1B} autoreceptors) modulate 5-HT neurotransmission by regulating 5-HT release.

Unlike 5-HT_1 receptors which are inhibitory, the 5-HT_{2A} receptors are generally excitatory although some are inhibitory (Rueter et al., 2000). Using autoradiography, Lopez-Gimenez et al. (1997) found 5-HT_{2A} receptors in the caudate putamen, NAc, hippocampus, and the motor cortex. 5-HT_{2A} receptor mRNA is found in the above areas but not in the raphe nuclei, cerebellum, substantia nigra or striatum (Burnet et al., 1995). Of further interest is the fact that 5-HT_{2A} receptors are not only found in pyramidal neurons in the hippocampus, but they are also present in GABAergic and glutamatergic interneurons (Jakab & Goldman-Rakic, 1998; Martin-Ruiz et al., 2001). Furthermore, Miner et al. (2003) found that over 70% of the 5-HT_{2A} receptors they labeled were located on either dendritic shafts or spines on postsynaptic neurons whereas 24% of the receptors were found on presynaptic axons and the remainder of the receptors are on glial cells.

1.2.2. Norepinephrine system

1.2.2.1. Localization

Similar to the 5-HT system, Dahlström and Fuxe (1964) identified seven clusters of NE neurons in the brain. These clusters are divided into three groups: the locus coeruleus (LC)

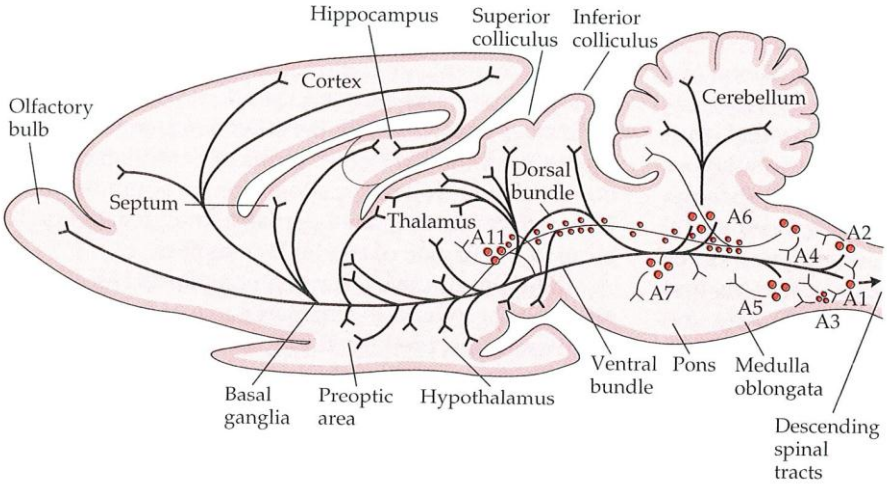

FIGURE 2. LOCATION OF ADRENERGIC CELL BODIES AND PATHWAYS IN THE RAT BRAIN. From Feldman et al. (1997).

complex, the lateral tegmental system, and the dorsal medullary group (Lindvall & Björklund, 1983). Of these three groups, the LC complex is the most important and contains fewer than 50,000 neurons in humans (Sharma et al., 2010). Noradrenergic neurons project to the cerebellum, spinal cord and more importantly, parts of the telencephalon and diencephalon, including the neocortex, hippocampus, amygdala, septum, thalamus, and hypothalamus (Figure 2).

1.2.2.2. Synthesis, storage, release, and uptake

NE is synthesized from the amino acid tyrosine. The first step is the rate-limiting step in which tyrosine is converted into dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase (TH). Aromatic amino acid decarboxylase then converts DOPA into dopamine (DA). DA is the precursor to NE and this conversion is mediated by dopamine β-hydroxylase (DBH).

Once NE is synthesized in the nerve terminals, it is packaged in vesicles by the vesicular monoamine transporter (VMAT). There are two mechanisms of release for NE. The main mechanism is described by Thureson-Klein (1983) in which exocytosis of NE is caused by a stimulus in a Ca^{2+}-dependent manner. NE can also be pumped out through membrane transport proteins independent of Ca^{2+} (Raiteri et al., 1979). The main method of clearing NE from the synapse is through the action of the norepinephrine transporter (NET).

1.2.2.3. Receptors

All the adrenergic receptors are G-protein coupled receptors and are divided into α- and β-adrenergic receptors. Due to the different pharmacological characteristics between pre- and post-synaptic α receptors, Langer (1974) proposed that the post-

synaptic α-receptors should be referred to as α_1 and the pre-synaptic autoreceptor be known as α_2. When α_2 receptors were found post-synaptically as well, classification of α-receptors was based on pharmacological properties instead of location. The suppressant effect of exogenous (microiontophoretically applied) NE on CA3 pyramidal neurons was blocked by systemic administration of idazoxan, a selective α_2 receptor antagonist (Curet & de Montigny, 1988). Conversely, the suppressant effect of endogenous NE (released by electrical stimulation of the LC on hippocampal neurons) is mediated by α_1-adrenoceptors as the initial inhibitory effect of LC stimulation is blocked by prazosin, a selective α_1 receptor antagonist. These two observations indicated that the intrasynaptic adrenoceptors are α_1 and the extrasynaptic receptors are α_2.

1.2.3. Dopamine system

1.2.3.1. Localization

Following the nomenclature system they developed for NE cell groups, Dahlström and Fuxe (1964) assigned eight clusters of DA neurons. According to current nomenclature, A1 to A7 are noradrenergic and A8 to A15 are dopaminergic. There are approximately 450,000 DA neurons in the midbrain including the ventral tegmental area (VTA) and the substantia nigra (German et al., 1983). Four major DA pathways have been identified: mesolimbic, mesocortical, nigrostriatal, and tuberoinfundibular. Of particular importance to psychiatric disorders are the mesolimbic and mesocortical pathways. DA projects from the VTA to the limbic system, in particular the NAc in the mesolimbic system. The mesocortical pathway transmits DA from the VTA to the cortex (Figure 3).

1.2.3.2. Synthesis, storage, release, and uptake

As mentioned for NE synthesis (see section 1.2.2.2), DA is synthesized from tyrosine via the enzymes TH and aromatic amino acid decarboxylase in the nerve terminals. DA is then packaged into vesicles by VMAT. Dopaminergic neurons contain two different pools of DA. The smaller pool (containing only 5% to 20% of the total DA content) is located near the presynaptic membrane and is designed for rapid release. The larger pool is meant for storage of DA neurotransmitter.

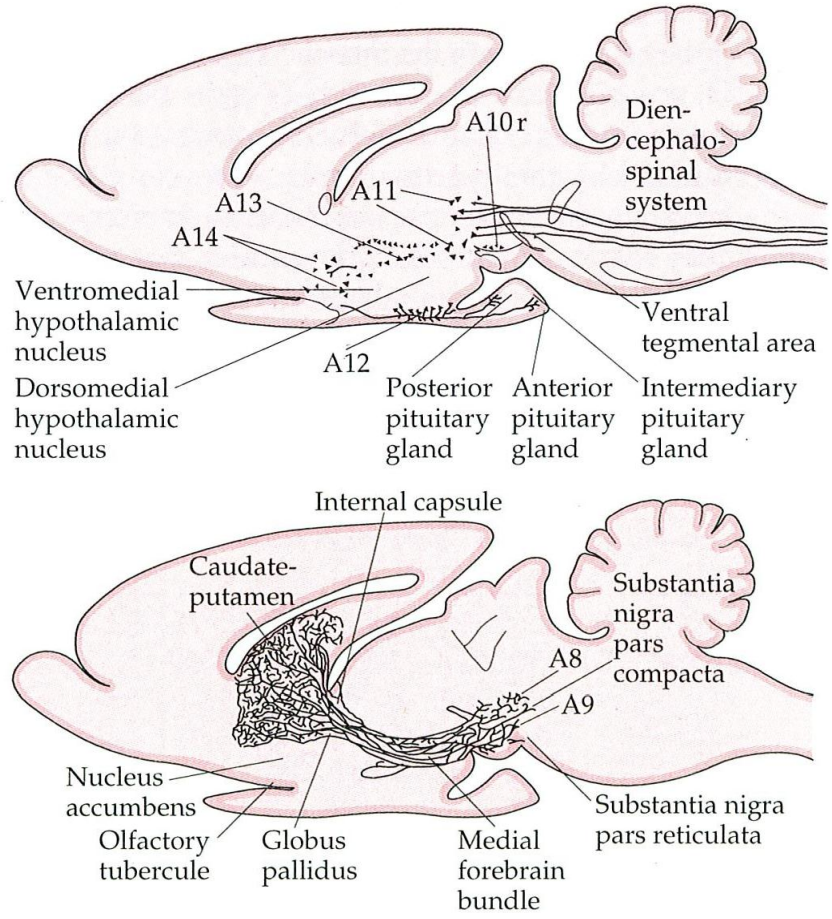

FIGURE 3. LOCATION OF DOPAMINERGIC CELL BODIES AND PATHWAYS IN THE RAT BRAIN. From Feldman et al. (1997).

DA release is mediated through both Ca^{2+}-dependent and Ca^{2+}-independent exocytosis (Raiteri et al., 1979). The extent of DA release depends on both the rate and pattern of DA firing. Although DA neurons exhibit single-spike firing, it is thought that the burst firing mode of DA neurons are more important in DA-mediated synaptic transmission because the amount of DA released per spike is much greater for bursts than

single-spikes (Gonon et al, 1991). Furthermore, recent evidence has shown that DA may be released from NE nerve terminals and this release is mediated by α_2-adrenoceptors (Devoto et al., 2001; 2003; 2004). Administration of clonidine, an α_2-adrenoceptor agonist significantly reduced extracellular DA levels in the prefrontal cortex while the selective α_2-adrenoceptor antagonist, idazoxan, increased DA levels (Devoto et al., 2001) In addition, the atypical antipsychotic, clozapine, increased DA levels in both the prefrontal cortex and occipital cortex and this effect was reversed with clonidine administration, but not the D2 agonist, quinpirole (Devoto et al., 2003).

As for 5-HT and NE, the main mechanism of inactivating synaptic DA is to remove it from the synapse via the dopamine transporter (DAT). Although dopaminergic and noradrenergic neurons only express the gene for their own transporter (Amara & Kuhar, 1993), the transporters themselves show a low specificity for their own transmitters. When the selective NE reuptake inhibitor desipramine was administered, the inhibitory effects of microiontophoretically applied DA in the hippocampus were prolonged, indicating that DA is in part taken up by the NET (Guiard et al., 2008). This is in line with the observation that DA has a greater affinity for the NET than its own transporter (Giros et al., 1994).

1.2.3.3. Receptors

There are two main types of DA receptors: D1 and D2. D2 receptors activate adenylyl cyclase and increase cyclic adenosine monophosphate (cAMP); conversely, D2 receptors inhibit adenylyl cyclase and decrease cAMP. Both D1 and D2 receptor mRNA expression are found in the NAc, caudate-putamen, olfactory tubercle, hypothalamus and cortex. However, only D2 mRNA was identified in the VTA and substantia nigra,

indicating that the autoreceptors on DA neurons in these regions are exclusively D2 (Meador-Woodruff et al., 1991)

1.3. Structural and functional interactions between the monoaminergic systems

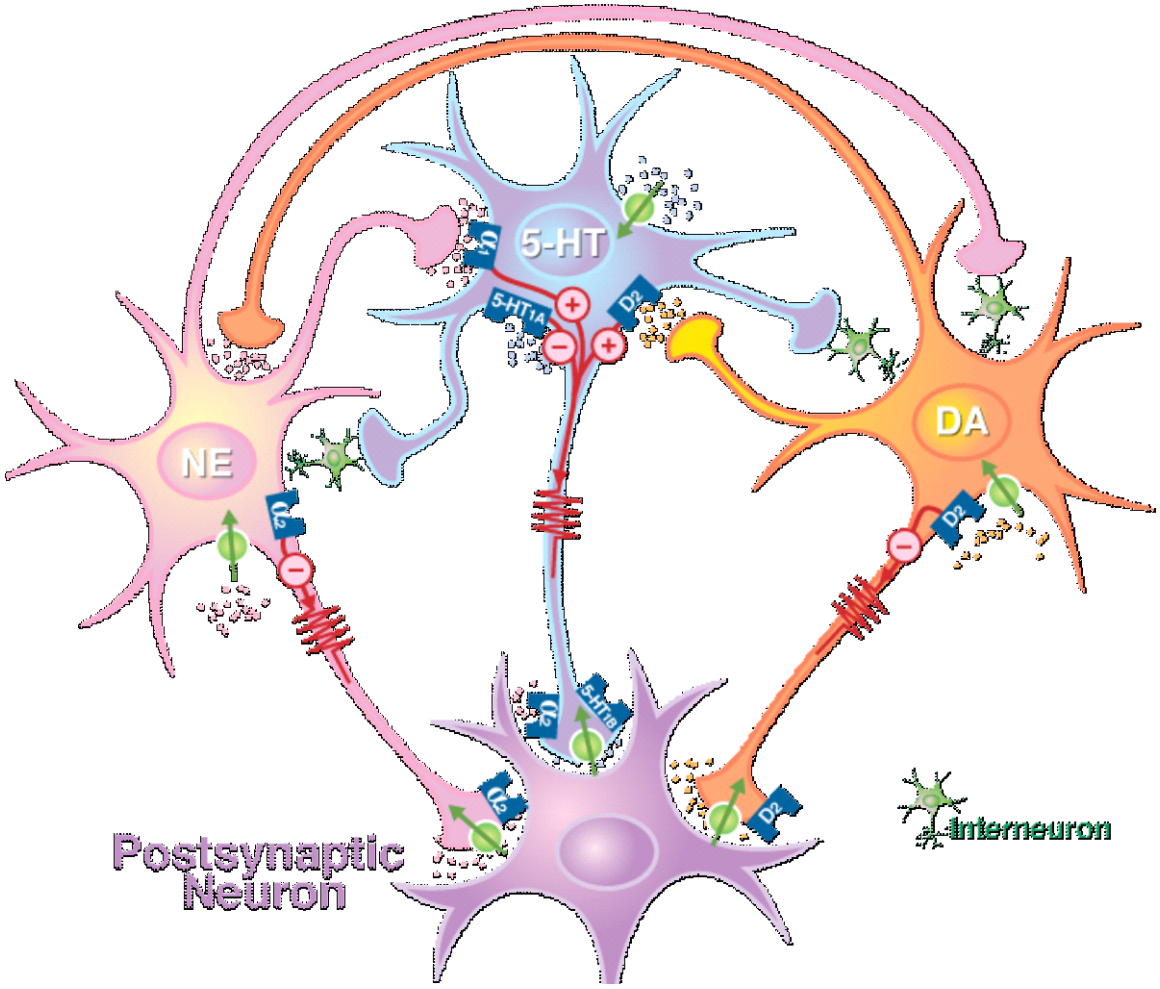

FIGURE 4. THE RECIPROCAL INTERACTIONS BETWEEN THE CELL BODIES OF 5-HT, NE AND DA NEURONS. A positive sign (+) indicates an agonism or a stimulatory effect and a negative sign (-) indicates an antagonism or an inhibitory effect. Modified from Trivedi et al. (2008).

Recent studies have established the existence of reciprocal relationships between the 5-HT, NE and DA systems in the brain (Figure 4). The interactions between the three monoamine systems are complex since acting on one monoaminergic system may

reverberate on the other two. Thus, all three systems must be considered when considering the mechanism of action of antidepressant drugs.

1.3.1. Serotonin-norepinephrine interactions

Depletion of 5-HT by PCPA not only caused decreased levels of 5-HT in the neocortex, but it also lowered NE levels (Reader et al., 1986). Electrophysiologically, NE neurons have about a 70% increase in their firing rates when 5-HT neurons are lesioned (Haddjeri et al., 1997). In addition, 8-OH-DPAT, a 5-HT_{1A} and 5-HT_7 agonist, increases the firing of LC NE neurons and this increase is reversed with WAY 100635, a selective 5-HT_{1A} antagonist, confirming that the enhancement of 8-OH-DPAT on NE neurons is via the 5-HT_{1A} receptor (Szabo et al., 2000). Taken together, this suggests that 5-HT inhibits NE neurons.

NE neurons innervate 5-HT cells in the DRN (Baraban & Aghajanian, 1981). Clonidine is an α_2-adrenergic agonist and local microiontophoretic ejections of clonidine inhibit the firing of NE neurons in the LC. Furthermore, local application of clonidine has no effect on the firing of 5-HT neurons in the DRN; however, systemic *i.v.* injections of clonidine do inhibit 5-HT neuronal firing for a short time (Svensson et al., 1975). Additionally, Svensson et al. (1975) found that when NE neurons were lesioned with 6-hydroxydopamine (6-OHDA), a DA and NE neurotoxin, 5-HT neuronal firing in the DRN was initially abnormally slow. There was no difference in the spontaneous firing rates of 6-OHDA lesioned rats that after 1 week compared to control rats and the inhibitory effect of clonidine injection was abolished. Thus, this indicates an excitatory role of NE input on DRN 5-HT neurons. Interestingly, NE was shown to have an inhibitory effect on 5-HT activity in the median raphe nucleus (Cassano et al., 2009). NE

depletion with DSP-4 caused a significant increase in 5-HT levels in the LC and Cassano et al. (2009) hypothesized that this increase is due to projections from the median raphe nucleus for two reasons. First, 5-HT levels remained unchanged in the prefrontal cortex and hippocampus, two areas which receive innervations mainly from the DRN. Second, 5-HT synthesis is enhanced in the median raphe nucleus, but not the DRN, when NE transmission is attenuated in the LC (Saavedra et al., 1976).

1.3.2. Serotonin-dopamine interactions

Lesioning 5-HT neurons enhanced VTA DA neuron firing by 36% (Guiard et al., 2008). In addition, when Lejeune and Millan (1998) gave flesinoxan, a potent pre- and post-synaptic 5-HT_{1A} receptor agonist, firing rates of DRN 5-HT neurons were decreased while firing rates of VTA DA neurons increased. An earlier study conducted by the same group found that 8-OH-DPAT, a 5-HT_{1A} agonist, also enhanced VTA DA neuronal firing (Lejeune et al., 1997). However, no observable effects were seen when 8-OH-DPAT was applied locally in the VTA (Prisco et al., 1994). Furthermore, 5-HT_{1B} receptors have also been shown to affect DA transmission. The selective 5-HT_{1B} receptor antagonist, SB 216641, was able to reverse the suppressant effects of ethanol on VTA DA neuronal activity while the selective 5-HT_{1B} receptor agonist, CP 94253, enhanced the activity (Yan et al., 2005). Thus, the overall effect of 5-HT on DA seems to be inhibitory.

D2-like DA receptors are expressed on the cell bodies of 5-HT neurons (Mansour et al., 1990; Suzuki et al., 1998), suggesting that DA modulates the 5-HT neuron. Haj-Dahmane (2001) demonstrated that DA activates D2 receptors on DRN 5-HT neurons and induces membrane depolarization, thereby enhancing 5-HT excitability and enabling its release. Additionally, subcutaneous (*s.c.*) injections of apomorphine, a D1 and D2

agonist, caused increased 5-HT transmission which was prevented with SCH 23390 and raclopride, a D1 and D2 antagonist, respectively (Martin-Ruiz et al., 2001). Direct perfusion of apomorphine into the DRN did not affect 5-HT transmission. Furthermore, selective lesion of DA neurons decreased 5-HT firing by 60% (Guiard et al., 2008). All these results signify that DA receptors exert an excitatory effect on DRN 5-HT neurons.

1.3.3. Norepinephrine-dopamine interactions

LC NE neurons innervate the VTA (Simon et al., 1979) and α_2-adrenergic receptors are also found in the VTA (Lee et al., 1998). When NE neurons in the LC are lesioned, VTA DA neuronal firing is enhanced by 70% (Guiard et al., 2008). Conversely, when NE is applied locally in the VTA, DA neuronal firing is suppressed (Guiard et al., 2008). This suppressant effect is blocked after idazoxan, a selective α_2-adrenergic receptor antagonist, treatment. From these data, NE appears to have an inhibitory action on DA neurons.

Using autoradiography techniques, D2 receptors were found to be present in the LC (Yokoyama et al., 1994). Lesioning of DA neurons by 6-OHDA increased LC NE neuron firing by 47% (Guiard et al., 2008). When DA was ejected locally in the LC, NE neuronal firing was inhibited in a current-dependent manner (Guiard et al., 2008). However, unlike the other modulatory effects, the inhibitory action of DA on NE does not seem to be caused by a dopamine receptor, specifically the D2 receptor. When the D2-like receptor agonist quinpirole was given, only a weak inhibitory effect was observed in NE neuronal firing and this effect was current-independent. In addition, raclopride, a D2 receptor agonist, did not affect the inhibitory action of DA on NE firing.

In contrast, idazoxan did block this inhibitory action indicating that the DA modulation of the NE system is due to α_2–adrenergic receptors.

1.4. Pharmacological treatments for depression

1.4.1. Tricyclic antidepressants (TCAs)

Since its discovery in the 1950s, TCAs have been used as a first line treatment for depression (Boyce & Judd, 1999). However, with the emergence of newer classes of antidepressants with better safety and side effect profiles, TCAs have been suggested to be used as a second line choice.

Most TCAs act as a serotonin-norepinephrine reuptake inhibitor (SNRI) by blocking the 5-HTT and NET. However, not all TCAs have the same pharmacological profile as some have a higher affinity for 5-HTT (imipramine, amitriptyline) while others have a higher affinity for NET (nortriptyline, desipramine, protriptyline, northiaden, lofepramine) (Gillman, 2007; Sanchez & Hyttel, 1999). Furthermore, some TCAs such as iprindole and trimipramine do not block either 5-HT or NE reuptake (Tatsumi et al., 1997).

Although TCAs block 5-HT and/or NE transporters much like selective serotonin reuptake inhibitors (SSRIs), SNRIs and norepinephrine reuptake inhibitors (NRIs), it seems to have a different mechanism of action. Unlike with SSRIs, chronic administration of TCAs did not alter either 5-HT neuronal firing or the sensitivity of the 5-HT_{1A} autoreceptor (Blier & de Montigny, 1980). Thus it seems unlikely that a pre-synaptic mechanism of action is responsible for the efficacy of TCAs. Rather, chronic treatment of TCAs sensitizes certain post-synaptic 5-HT and NE receptors in forebrain neurons (Blier et al., 1987; de Montigny & Aghajanian, 1978; Gallager & Bunney, 1979).

The time needed for this enhanced responsiveness would explain the delayed antidepressant effect of TCAs.

1.4.2. Monoamine oxidase inhibitors (MAOIs)

MAOIs were discovered before TCAs and were used as antidepressants. Monoamine oxidase (MAO) is an enzyme responsible for the intracellular breakdown of monoamine transmitters, such as 5-HT, NE, and DA. Thus, MAOIs increase monoamine transmitter levels by preventing the activity of MAO. There are two isoforms of MAO: MAO-A and MAO-B. MAO-A preferentially acts on the 5-HT and NE system while DA is metabolized by both isoforms (Hall et al., 1969; Yang & Neff, 1974).

MAOI administration initially decreases the firing rate of 5-HT neurons due to the increased 5-HT transmitter in the synapse activating 5-HT_{1A} autoreceptors. However, long-term treatment of MAOI causes a desensitization of these 5-HT_{1A} autoreceptors leading to an overall increase in 5-HT transmission (Blier & de Montigny, 1985, 1987a).

Unlike 5-HT neurons, α_2-adrenergic autoreceptors do not undergo desensitization following prolonged MAOI administration (Blier & de Montigny, 1985). The effect of MAOIs on DA neuronal firing was studied by Chenu et al. (2009). Administration of MAOIs that acted on MAOI-A (clorgyline and phenelzine) had no effect sub-acutely but prolonged treatment decreased the firing of DA neurons. Deprenyl, a selective MAOI-B, had no effect either sub-acutely or chronically.

1.4.3. Selective serotonin reuptake inhibitors (SSRIs)

Unlike TCAs and MAOIs which were discovered by serendipity, SSRIs were designed to be antidepressants. The goal was to have the antidepressant effects of TCAs

and MAOIs, but without many of the undesirable side effects. SSRIs block the 5-HTT and this blockade leads to an increase in the synaptic availability of 5-HT.

The administration of SSRIs cause an increase in the levels of 5-HT in the synapse which in turn activates the 5-HT_{1A} autoreceptors and the 5-HT_{1B} autoreceptors located on the cell bodies and terminals of 5-HT neurons, respectively. 5-HT_{1A} autoreceptors inhibit the firing of the 5-HT neurons while 5-HT_{1B} autoreceptors decrease the amount of 5-HT released per action potential thus; the initial consequence of SSRI administration is a decrease in 5-HT neuronal firing. However, long-term administration of SSRIs causes a desensitization of the inhibitory 5-HT_{1A} and 5-HT_{1B} autoreceptors and 5-HT neuronal firing recovers back to a normal level (Pineyro & Blier, 1999). The sensitivity of post-synaptic 5-HT_{1A} receptors is unaltered with prolonged SSRI treatment and taken together with the pre-synaptic results, chronic SSRI administration leads to an enhanced 5-HT neurotransmission (Blier & de Montigny, 1983). Due to the inhibitory action of 5-HT on the catecholamines, prolonged SSRI treatment produces a decrease in the spontaneous firing rates of NE and DA neurons (Dremencov et al., 2009; Ghanbari et al., 2010).

1.4.3.1. Escitalopram

Escitalopram is a highly selective and potent inhibitor of the 5-HT transporter (Garnock-Jones & McCormack, 2010; Owens et al., 2001) (Table 1).

TABLE 1. AFFINITY OF ESCITALOPRAM TO HUMAN SEROTONIN, NOREPINEPHRINE, AND DOPAMINE TRANSPORTERS.

Monoamine	K_i ± SEM (nM)
Serotonin	1.1 ± 0.1
Norepinephrine	7,841 ± 998
Dopamine	27,410 ± 3,106

Owens et al. (2001)

A 7-day regimen of escitalopram significantly decreases 5-HT neuronal firing in the DRN; however, 5-HT firing recovers back to control level after 14 days of escitalopram (El Mansari et al., 2005). Furthermore, an enhanced tonic activation of the post-synaptic 5-HT_{1A} receptor located on hippocampal CA3 pyramidal neurons was observed (Chernoloz et al., 2012a). In addition, Ghanbari et al. (2010) observed a significant decrease in LC NE neuronal firing after both 2- and 14-day administration of escitalopram. Dremencov et al. (2009) examined the effect of escitalopram on the DA neurons in the VTA and found a significant decrease in neuronal firing after both 2 and 14 days of treatment.

1.4.4. Selective norepinephrine reuptake inhibitors (NRIs)

The NE system has also been implicated in the etiology of depression thus; blockers of NET were developed as a new class of antidepressants. One such drug is reboxetine. Reboxetine dose-dependently decreases the firing rate of LC NE neurons after 2 days of treatment and this decrease is sustained after 21 days of administration, indicating that the α_2-adrenergic autoreceptor sensitivity is not impacted with sustained NRI treatment (Szabo & Blier, 2001a). In contrast, the α_2-adrenergic heteroreceptors located on 5-HT terminals in the hippocampus are desensitized following long-term reboxetine administration and this desensitization causes increased levels of 5-HT in the hippocampus (Szabo & Blier, 2001b).

1.4.5. Serotonin-norepinephrine reuptake inhibitors (SNRIs)

The most widely prescribed SNRI to be prescribed has been venlafaxine. However, at its minimal effective dose in depression (75 mg/day), venlafaxine acts as a SSRI and only at higher doses (225 and 375 mg/day) does it act as a SNRI (Debonnel et

al., 2007). Venlafaxine has been shown to be superior to SSRIs as a whole in major depression (Nemeroff et al., 2008).

Like SSRIs, venlafaxine causes an initial decrease in the spontaneous firing rates of DRN 5-HT neurons but this firing recovers back to control levels after 21 days (Béïque et al., 2000). Additionally, venlafaxine causes a dose-dependent decrease in the firing of LC NE neurons and this decrease is sustained after prolonged treatment.

1.4.6. Norepinephrine-dopamine reuptake inhibitors (NDRIs)

1.4.6.1. Bupropion

The only NDRI approved by the FDA is bupropion. Although it is marketed as a blocker of DA and NE transporters, recent evidence demonstrates that bupropion has relatively low affinity for all three monoamine transporters and the average DAT occupancy by bupropion is 14% to 26% in clinical trials (Argyelan et al., 2005; Learned-Coughlin et al., 2003; Meyer et al., 2002; Tatsumi et al., 1997). Bupropion was shown to have a 100-fold lower affinity for the NET than DAT (Tatsumi et al., 1997). Thus, with such a low DAT occupancy, along with the fact that it is derived from diethylpropion (a norepinephrine releaser) (Arias et al., 2009; GlaxoSmithKline, 2008), bupropion has been proposed to be a releaser of NE (Dong & Blier, 2001).

In vivo microdialysis studies have shown that bupropion increases extracellular levels of DA and NE in the hippocampus, hypothamalus, prefrontal cortex, and NAc (Li et al., 2002; Piacentini et al., 2003). Using electrophysiology, Dong and Blier (2001) found that the spontaneous firing rates of LC NE neurons were dramatically decreased following 2-day bupropion administration which was prevented with the blockade of the α_2-adrenergic receptor antagonist, idazoxan. This change in the NE system is postulated

to be responsible for the doubling of the firing rate of 5-HT neurons in the DRN with bupropion administration as the lesion of NE neurons completely negates this 5-HT neuronal firing increase. In addition, bupropion has no effect on the firing rate of VTA DA neurons. These 2-day results were confirmed by El Mansari et al. (2008) who characterized the effects of chronic administration of bupropion. NE neuronal firing recovered to control levels after 14 days of bupropion treatment, indicating a desensitization of the α_2-autoreceptor. This recovery in LC NE neuronal firing is also observed with YM992, a SSRI and potent 5-HT_{2A} antagonist (Szabo & Blier, 2002). Furthermore, YM992 induces a significant increase in extracellular NE levels in the rat prefrontal cortex, paralleling the NE releasing properties of bupropion (Hatanaka et al., 2000). In addition to the recovery of NE neuronal firing rate, sustained bupropion administration also increased the number of NE neurons firing in burst mode (Ghanbari et al., 2010). Action potentials discharging in burst mode rather than single-spike firing are associated with increased neurotransmitter release (Hardebo, 1992), leading support to the theory that bupropion acts as a NE releaser.

1.4.6.2. Nomifensine

Nomifensine, an antidepressant with potent NE and DA reuptake inhibiting properties (Table 2), produces an increase in extracellular levels of NE and DA while only minimally affecting their release (McKillop & Bradford, 1981; Schacht & Heptner, 1974; Tatsumi et al., 1997). Nomifensine was efficacious; however, it was removed from the market due to its hematopoietic toxicity, unrelated to its reuptake inhibitory actions.

TABLE 2. AFFINITY OF NOMIFENSINE TO RAT SEROTONIN, NOREPINEPHRINE, AND DOPAMINE TRANSPORTERS.

Monoamine	K_i ± SEM (nM)
Serotonin	1280 ± 80
Norepinephrine	5.0 ± 0.4
Dopamine	51 ± 8

Richelson and Pfenning (1984)

Nomifensine administration for 2 days produced a significant increase in DRN 5-HT neuron firing that remained elevated by 32% after 14 days compared to controls (Katz et al., 2010). This implies a synergistic effect of the NE and DA reuptake action of nomifensine since reboxetine and GBR 12909 (a potent and selective DA reuptake inhibitor), by themselves, did not produce this effect (Szabo & Blier, 2001a). Two-day administration of nomifensine caused a marked decrease in the firing rate of LC NE neurons and remained decreased after 14 days (Katz et al., 2010). This sustained decrease is likely due to the lack of desensitization of the α_2-adrenergic autoreceptors. In the VTA DA neurons, a significant decrease was observed after 2 days of treatment in firing rate compared to control; however, a complete recovery in firing rate of DA neurons to control level was seen after 14-day administration of nomifensine because the D2 autoreceptors were desensitized.

1.4.7. Triple reuptake inhibitors (TRIs)

Given the reciprocal interactions between the three monoamine systems, enhancing DA neurotransmission may have beneficial effects on 5-HT and NE neurotransmission as well. Therefore, TRIs are currently in development as a possible class of antidepressants with a greater efficacy than SSRIs or SNRIs. One such compound is DOV 216303. DOV 216303 has equal potency for 5-HT and NE reuptake and is about four times less potent for DA reuptake (Skolnick et al., 2006). DOV 216303

was shown to have antidepressant effects in a rat depression model and unlike SSRIs, it did not display any sexual side effects (Breuer et al., 2008). Clinically, DOV 216303 seemed to be as efficacious as citalopram, a SSRI, in reducing the Hamilton Rating Scale for Depression (HAM-D) scores at both weeks 1 and 2 (Skolnick et al., 2006).

Guiard et al. (2011) examined the effects of DOV 216303 and SEP-225289, another novel triple reuptake inhibitor, on monoaminergic neurons. They found that acute injections of both compounds predominantly inhibited the firing rate of LC NE neurons while producing only a partial decrease in VTA DA and DRN 5-HT neuronal firing rate.

1.5. Aims

Given that triple reuptake inhibitors are still being developed (thus limiting access to these drugs), administration of nomifensine plus a SSRI (such as escitalopram) should mimic the effect of triple reuptake inhibitors. The combination of nomifensine and escitalopram treatment will be referred to as a 'triple reuptake inhibitor' (TRI).

The present study is undertaken to assess the effectiveness of a TRI at the cell body of monoaminergic neurons and in forebrain post-synaptic brain regions. This TRI treatment can then be compared to the single and dual reuptake inhibitors to evaluate the effectiveness of TRIs.

1.5.1. Subacute effects of the combination of escitalopram and nomifensine on the cell bodies of the three monoaminergic systems

Short term effects of escitalopram and nomifensine have previously been studied in our lab (Dremencov et al., 2009; Ghanbari et al., 2010; Katz et al., 2010). The subacute effects of the combination of escitalopram and nomifensine on the cell bodies of the three

monoaminergic systems will be examined in this study and compared to the effects seen in single and dual reuptake inhibitors.

1.5.2. Sustained effects of the combination of escitalopram and nomifensine on the cell bodies of the three monoaminergic systems

Prolonged administrations of escitalopram and nomifensine have also been conducted previously (Dremencov et al., 2009; Ghanbari et al., 2010; Katz et al., 2010). The present study will determine the long-term effects of co-administration of escitalopram and nomifensine on the cell bodies of the three monoaminergic systems.

1.5.3. Sustained effects of escitalopram and/or nomifensine on the forebrain structures of the three monoaminergic systems

It is important to emphasize not only the changes in the function of monoaminergic neurons that can underlie the effectiveness of drug treatment but also the responsiveness of postsynaptic neurotransmitter receptors that may contribute to, as well as alter, overall neurotransmission. Indeed, the desensitization of receptors on forebrain postsynaptic areas may dampen the impact of enhanced levels of neurotransmitters (El Mansari & Blier, 1997).

Although the effects of sustained escitalopram administration on hippocampal CA3 pyramidal neurons have been studied by Chernoloz et al. (2012a), postsynaptic effects of nomifensine and the combination of escitalopram and nomifensine are still unknown; this study will determine these effects.

2. MATERIALS AND METHODS

2.1. Animals

Adult male Sprague-Dawley rats (Charles River, Saint-Constant, QC, Canada) weighing 250-350 g at the time of the experiments were used. Animals were housed 2 per cage at standard experimental conditions (12 h light/dark cycle, with lights on at 7:00 am; temperature 21±1°C, 40-50% relative humidity) with access to food and water *ad libitum*. The rats were allowed to acclimatize to their new environment for 1 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.

2.2. Experimental preparations

Rats were anesthetized with an inhalant mixture of isofluorane and oxygen. Subcutaneously implanted osmotic minipumps (Alzet, Durect Corporation, Cupertino, CA) were preloaded with nomifensine (5 mg/kg/day), escitalopram (10 mg/kg/day) or the vehicle, 20% hydroxypropyl-beta-cyclodextrin (β-OH) which provided delivery of each drug for 2 or 14 days. The nomifensine dosage of 5 mg/kg/day was selected as this dose led to a desensitization of the D2 autoreceptors following 14 days of treatment. Escitalopram given at 10 mg/kg/day was selected since this dose produced similar plasma levels in humans taking this drug. The TRI (nomifensine plus escitalopram) was administered using two minipumps. Minipumps remained *in situ* throughout the recordings in order to ensure the steady-state levels of the drug were maintained, thereby

mimicking clinical conditions since these antidepressants have much shorter half-lives in rats than in humans.

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, USA). 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. 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 pharmacological agents. Electrodes were lowered using a hydraulic micropositioner (Kopf Instruments), and all neuronal activity were recorded in real-time using Spike2 software (Cambridge Electronic Design, Cambridge, UK), which were used to analyze neurons offline.

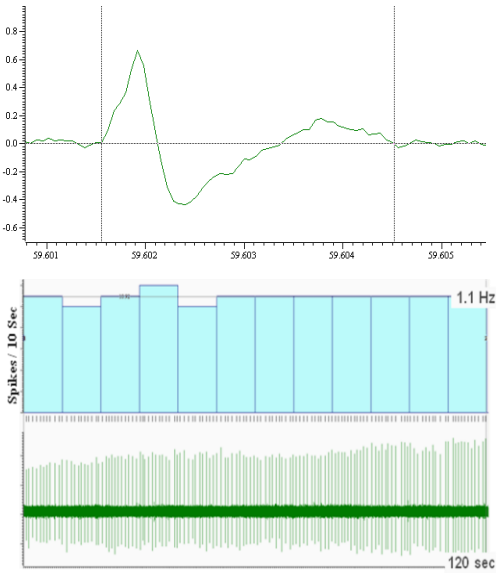

FIGURE 5. EXAMPLE OF AN ELECTROPHYSIOLOGICAL RECORDING OF A 5-HT NEURON IN THE DRN. (Upper panel) A single action potential. Action potentials appear as spikes (lower panel) and display regular firing (middle panel).

2.3. Electrophysiological recording of DRN 5-HT neurons

In order to record 5-HT neurons in the DRN, a burr hole was drilled to descend the single-barreled glass micropipette at the following coordinates (in mm relative to the interaural line): AP + 1 to + 1.2, ML 0, DV 5 to 7. 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 to 2.5 Hz), regular firing rate and long duration (~ 2 ms) positive action potential (Aghajanian & Vandermaelen, 1982) (Figure 5).

On the day of recording, several tracks of DRN 5-HT neurons were recorded to determine the effects of WAY 100635, a selective 5-HT_{1A} antagonist, on the firing activity of 5-HT neurons in control and 2-day TRI treated rats. The recordings were then carried out in the same rat after administration of WAY 100635 (100 µg/kg, *i.v.*), and a subsequent recording of DRN 5-HT neurons from several more tracks.

2.4. Electrophysiological recording of LC NE neurons

LC NE neurons were recorded with single-barreled glass micropipette positioned at (in mm relative to the interaural line): AP – 1.0 to – 1.2, ML 1.0 to 1.3, DV 5 to 7. The presumed NE neurons were identified by their regular firing rate (0.5 to 5 Hz), a biphasic action potential of long duration (~ 2 ms), and a characteristic burst discharge followed by a quiescent period in response to a nociceptive pinch of the contralateral hind paw (Marwaha & Aghajanian, 1982) (Figure 6).

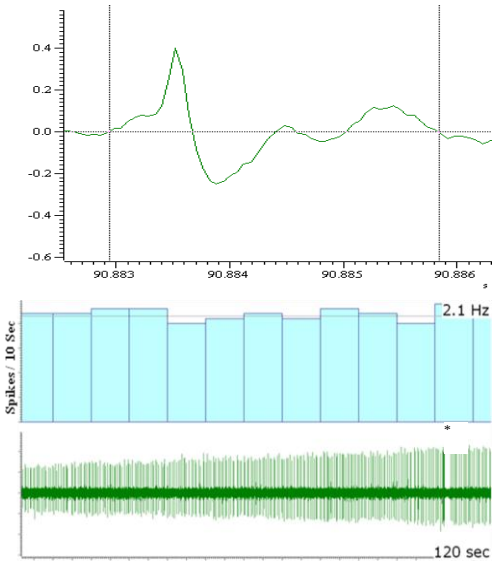

FIGURE 6. EXAMPLE OF AN ELECTROPHYSIOLOGICAL RECORDING OF A NE NEURON IN THE LC. (Upper panel) A single action potential. Neurons display regular firing (middle panel) and action potentials appear as spikes and a pinch to the contralateral hind paw induces a burst discharge followed by a brief quiescent period (lower panel).

Studies have shown that paired or multiple impulses enhance the release of NE

neurotransmitters for the same number of single action potentials over the same period of time (Hardebo, 1992). Thus, the changes in the burst firing of NE neurons were analyzed by interspike interval burst analysis (Grace & Bunney, 1984). The onset of a burst for the 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; Grace & Bunney, 1983). In order to obtain the firing rate of a given neuron, the neuron was recorded for 2 min. The firing rate of the neuron was then obtained by dividing the number of action potentials per second. Thus, each action potential contributed to the firing rate of that particular neuron. In other words, if there was a burst in a given neuron, each action potential of the given burst (i.e. doublet or triplet) contributed to the firing rate.

2.5. Electrophysiological recording of VTA DA neurons

The following coordinates were used for single-barreled glass micropipette descent into the VTA (in mm relative to the interaural line): AP + 3.2 to + 3.6, ML + 0.9 to + 1.1, DV 7 to 9. The presumed DA neurons were identified by well-established electrophysiological criteria (Freeman et al., 1985) including: 1) spontaneous firing rate between 0.5 and 9 Hz, exhibiting bursting activity or irregular firing; 2) biphasic or triphasic waveforms, with an initial positive

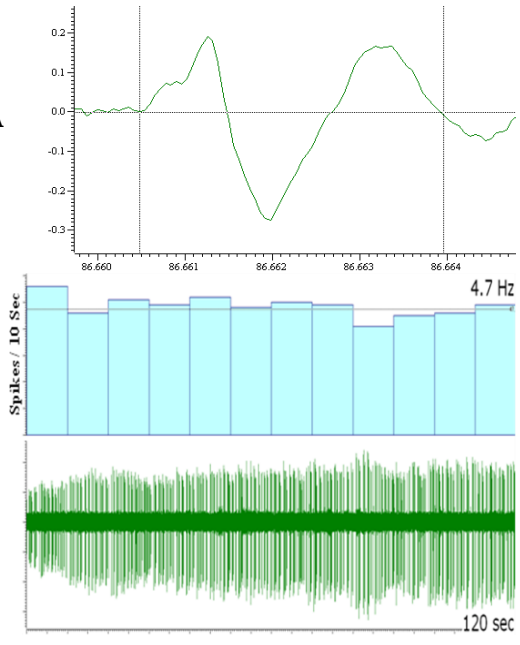

FIGURE 7. EXAMPLE OF AN ELECTROPHYSIOLOGICAL RECORDING OF A DA NEURON IN THE VTA. (Upper panel) A single action potential. Neurons display regular firing (middle panel) in both single-spike and burst-firing mode (lower panel).

deflection (usually notched) followed by a prominent negative phase; 3) long duration action potentials (2.5 to 4 ms) and 4) low pitch sound when monitored by an audioamplifier (Figure 7).

2.6. Extracellular recording and microiontophoresis of dorsal hippocampus CA3 pyramidal neurons

Extracellular recording and microiontophoresis of CA3 pyramidal neurons were carried out with five-barreled glass micropipettes. The central barrel used for the unitary recording was filled with a 2M NaCl solution, and the impedance of these electrodes ranged from 2 to 4 MΩ. The four side barrels were filled with the following solutions: 5-HT creatinine sulfate (15 mM in 200 mM NaCl, pH 4), NE bitartrate (10 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 CA3 region of the hippocampus using the following coordinates: 4 mm anterior to lambda and 4.2 mm lateral (Paxinos & Watson, 1998). CA3 pyramidal neurons were found at a depth of 4.0 ± 0.5 mm below the surface of the brain. Since the pyramidal neurons do not discharge spontaneously in chloral hydrate anesthetized rats, a small current of quisqualate +1 to –4 nanoampere (nA) was used to activate them within their physiological firing range (10 to 15 Hz) (Ranck, 1975). Pyramidal neurons were identified by their large amplitude (0.5 to 1.2 mV) and long-duration (0.8 to 1.2 ms)

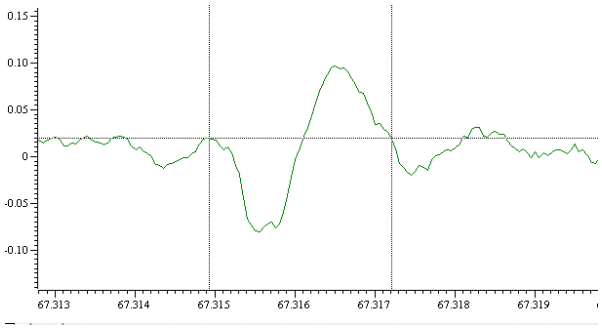

FIGURE 8. EXAMPLE OF A SINGLE ACTION POTENTIAL FROM A RECORDING OF A CA3 PYRAMIDAL NEURON IN THE HIPPOCAMPUS.

simple action potentials, alternating with complex spike discharges (Kandel & Spencer, 1961) (Figure 8).

2.7. Tonic activation of postsynaptic 5-HT_{1A} receptors following 14-day administration of escitalopram and/or nomifensine

In order to assess the degree of activation of the postsynaptic 5-HT_{1A} receptors exerting an inhibitory influence on the firing activity of CA3 pyramidal neurons, the selective 5-HT_{1A} receptor antagonist WAY 100635 was administered intravenously to disinhibit the hippocampal neurons resulting in an increase of their firing activity. The disinhibition is best determined when the neurons are not firing at a high rate; therefore their firing rate was decreased to about 2 – 3 Hz by reducing the ejection current of quisqualate after which WAY 100635 (100 µg/kg) was injected intravenously (Haddjeri & Blier, 2001; Haddjeri et al., 1998). WAY 100635 was administered in incremental doses of 25 µg/kg at 2 minute time intervals. To avoid residual drug effects, only one cell was studied in each rat. Any changes in the firing activity of hippocampus pyramidal neurons reflect an increased level in the tonic activation of the postsynaptic 5-HT_{1A} receptors. It is important to note that WAY 100635, administered intravenously, does not modify the firing rate of 5-HT neurons in the DRN of anesthetized rats (Haddjeri et al., 2004; Lejeune & Millan, 1998).

2.8. Tonic activation of postsynaptic α_2- and α_1-adrenoceptors following 14-day administration of escitalopram and/or nomifensine

The degree of tonic activation of postsynaptic α_2- and α_1-adrenoceptors was assessed using the selective receptor antagonists idazoxan and prazosin, respectively (Ghanbari et al., 2011). Assessment of the disinhibition of the neuronal firing is best

determined when the firing rate is low. Thus, a low stable firing baseline was obtained by lowering the ejection current of quisqualate. Following a steady baseline firing, idazoxan (1000 μg/kg) and prazosin (100 μg/kg) were systemically administered, always in this order, to assess the percentages of changes in the firing activity in rats administered β-OH, escitalopram and/or nomifensine for 14 days. Such doses of the antagonists were chosen since these dosages were shown to have physiological effects in electrophysiological paradigms (Curet & de Montigny, 1988). In order to avoid residual drug effects, only one neuron was studied in each rat.

2.9. Microdialysis

The microdialysis probe was implanted into the nucleus accumbens at the following coordinates relative to the interaural line: AP +11 mm, L +1.5 mm, and DV -8 mm. The active length of the dialysis membrane was 2 mm. The probe was continuously perfused, at a flow rate of 1 μl/min, with the following artificial cerebrospinal fluid: 147 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$, adjusted to pH 7.4 with 2 mM sodium phosphate buffer. After a stabilization period of 120 minutes, dialysate fractions (30 μl) were collected every 30 minutes for 3 hours for measuring dialysate neurotransmitter concentrations. Locations of dialysis probes were confirmed histologically on serial coronal brain sections at the end of each experiment.

PCPA (300 mg/kg) was administered *i.p.* 72, 48 and 24 hours before microdialysis experiments (Dailly et al., 2006) for both the control rats and the rats receiving 14 days of TRI treatment.

2.10. Drugs

Escitalopram (provided by Lundbeck, Copenhagen, Denmark), and nomifensine (Sigma-Aldrich Canada, Oakville, ON, Canada) were dissolved in a 20% (2 g / 10 ml distilled H_2O) solution of hydroxypropyl-beta-cyclodextrin and sonicated until completely dissolved. Idazoxan hydrochloride, prazosin hydrochloride, WAY 100635, and *para*-chlorophenylalanine (PCPA) methyl ester hydrochloride (Sigma-Aldrich), were all dissolved in distilled H_2O.

2.11. Statistical analysis

The data are presented as mean values ± SEM. The n values represent the number of neurons recorded, unless otherwise indicated. Statistical comparisons were carried out using a one-way analysis of variance (ANOVA) (treatment as the main factor) followed by the Bonferroni *post hoc* analysis using GraphPad Prism 5 software (GraphPad Software Inc, La Jolla, CA). Statistical comparisons were performed using the Fisher's exact test when burst parameters were studied in control and TRI treated rats. Statistical significance was taken as $p < 0.05$.

3. RESULTS

3.1. Sub-acute administration of escitalopram, nomifensine and triple reuptake inhibition

Sub-acute administration of escitalopram (10 mg/kg/day) and/or nomifensine (5 mg/kg/day) was examined in all three monoaminergic neuronal systems using subcutaneously implanted osmotic minipumps for 2 days.

3.1.1. Dorsal raphe nucleus (DRN)

Significant changes were observed following 2 days of drug administration. While nomifensine increased ($p < 0.001$) and escitalopram decreased ($p < 0.001$) DRN 5-HT firing, 2-day administration of TRI had no effect compared to control levels (Figure 9A). Thus, the addition of nomifensine to the treatment regimen seems to overcome the initial inhibition in 5-HT firing rate caused by escitalopram. In addition,

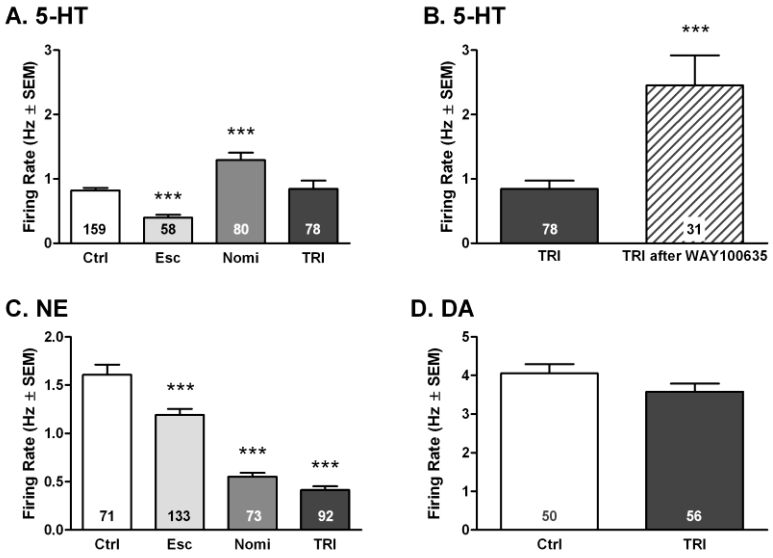

FIGURE 9. SUBACUTE ADMINISTRATION OF ESCITALOPRAM (ESC), NOMIFENSINE (NOMI) AND TRIPLE REUPTAKE INHIBITION (TRI). Effect of Esc, Nomi and TRI on the average firing rate of (A) 5-HT neurons in the dorsal raphe nucleus, (C) NE neurons in the locus coeruleus, and (D) DA neurons in the ventral tegmental area. (B) Acute injections of WAY 1000635 significantly increased the firing rate of 5-HT neurons by three-fold. The number at the bottom of each column represents the number of neurons. ***$p < 0.001$.

acute injection of the selective 5-HT_{1A} receptor antagonist WAY 100635 increased the firing rate of 5-HT neurons after 2 days of TRI by almost 200% ($p < 0.001$) (Figure 9B).

3.1.2. Locus coeruleus (LC)

Two days of escitalopram, nomifensine, and TRI administration resulted in a significant 26%, 66% and 74% decrease, respectively, in firing rates of noradrenergic neurons compared to controls ($p < 0.001$) (Figure 9C). Additionally, the firing patterns of NE neurons changed from 25% of the neurons discharging in bursts in control rats to 7% ($p < 0.01$) and 5% ($p < 0.001$) in rats treated with TRI for 2 days and 14 days, respectively (Table 3).

TABLE 3. EFFECT OF TRIPLE REUPTAKE INHIBITION (TRI) ADMINISTRATION ON THE FIRING PATTERN OF NOREPINEPHRINE (NE) NEURONS IN THE LOCUS COERULEUS (LC).

	NE neurons	
Group	Total, #	Displaying bursts, # (%)
Control 2 days	71	18 (25)
TRI 2 days	92	6 (7)**
Control 14 days	102	25 (25)
TRI 14 days	111	5 (5)***

Significance was determined using Fisher's exact test comparing the TRI group to the respective control group. **$p < 0.01$, ***$p < 0.001$.

3.1.3. Ventral tegmental area (VTA)

A two-day administration of TRI had no significant effect on the firing rates of dopaminergic neurons in the VTA (Figure 9D).

3.2 Sustained administration of escitalopram, nomifensine and triple reuptake inhibition

Sustained administration of escitalopram (10 mg/kg/day) and/or nomifensine (5 mg/kg/day) was examined in all three monoaminergic neuronal systems using

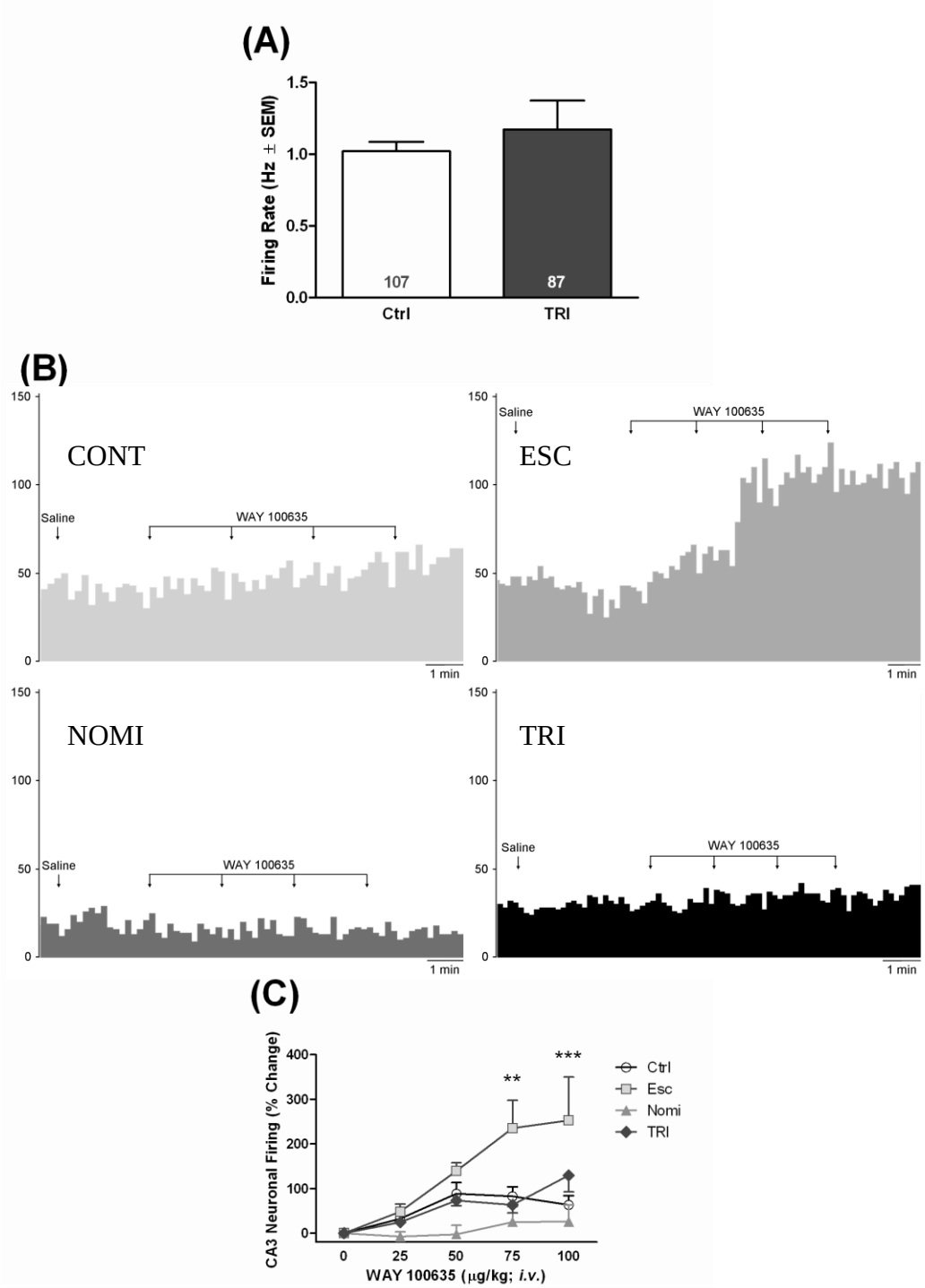

FIGURE 10. CHRONIC TREATMENT OF ESC, NOMI AND TRI ON THE 5-HT SYSTEM. (A) Effect of 14-day treatment of triple reuptake inhibition (TRI) on the average firing rate of 5-HT neurons in the dorsal raphe nucleus. The number at the bottom of each column represents the number of neurons. (B) Integrated firing rate histograms of dorsal hippocampal CA3 pyramidal neurons illustrating systemic injections of saline and WAY 100635 in 4 incremental doses of 25 µg/kg each in a control rat (upper left panel), 14-day treated escitalopram rat (upper right panel), nomifensine rat (lower left panel) and TRI rat (lower right panel). (C) The overall increase of the firing rate of CA3 pyramidal neurons after the systemic injection of WAY 100635 (100 µg/kg) in control and treated rats. $^{**}p < 0.01$, $^{***}p < 0.001$.

subcutaneously implanted osmotic minipumps for 14 days. The cell body experiments were conducted in the same brain areas mentioned previously (DRN, LC, VTA). The forebrain post-synaptic experiments were conducted in the hippocampal CA3 region for 5-HT and NE receptors while DA neurotransmitter levels were assessed in the nucleus accumbens.

3.2.1. 5-HT

The firing rate of the 5-HT neurons remained at control levels after 14 days of TRI administration (Figure 10A). Blockade of inhibitory 5-HT_{1A} receptors located on CA3 pyramidal neurons, achieved with the systemic administration of the selective 5-HT_{1A} receptor antagonist WAY 100635, led to a 53%, 135%, 8%, and 58% increase in firing rates of control (n = 6), escitalopram (n = 5), nomifensine (n = 8) and TRI treated rats (n = 7), respectively (Figure 10C). A two-way repeated measures ANOVA showed significant main effects for both drug treatment ($F_{(3, 129)} = 5.68$, $p < 0.01$), as well as WAY 100635 dosage ($F_{(4, 129)} = 16.97$, $p < 0.001$), while also eliciting a significant drug x WAY 100635 dosage interaction ($F_{(12, 129)} = 3.64$, $p < 0.001$). *Post-hoc* analyses revealed significant differences between the control group and the escitalopram treated group at WAY 100635 dosages of 75 μg/kg ($p < 0.01$) and 100 μg/kg ($p < 0.001$) only (Figure 10C). Only chronic treatment of escitalopram produced an enhanced tonic activation of the post-synaptic 5-HT_{1A} receptors.

3.2.2. NE

The firing rate of the NE neurons remained significantly decreased (by 24%, 51%, and 55%) compared to control levels after 14 days of escitalopram ($p < 0.01$), nomifensine ($p < 0.001$), and TRI administration ($p < 0.001$), respectively (Figure 11A).

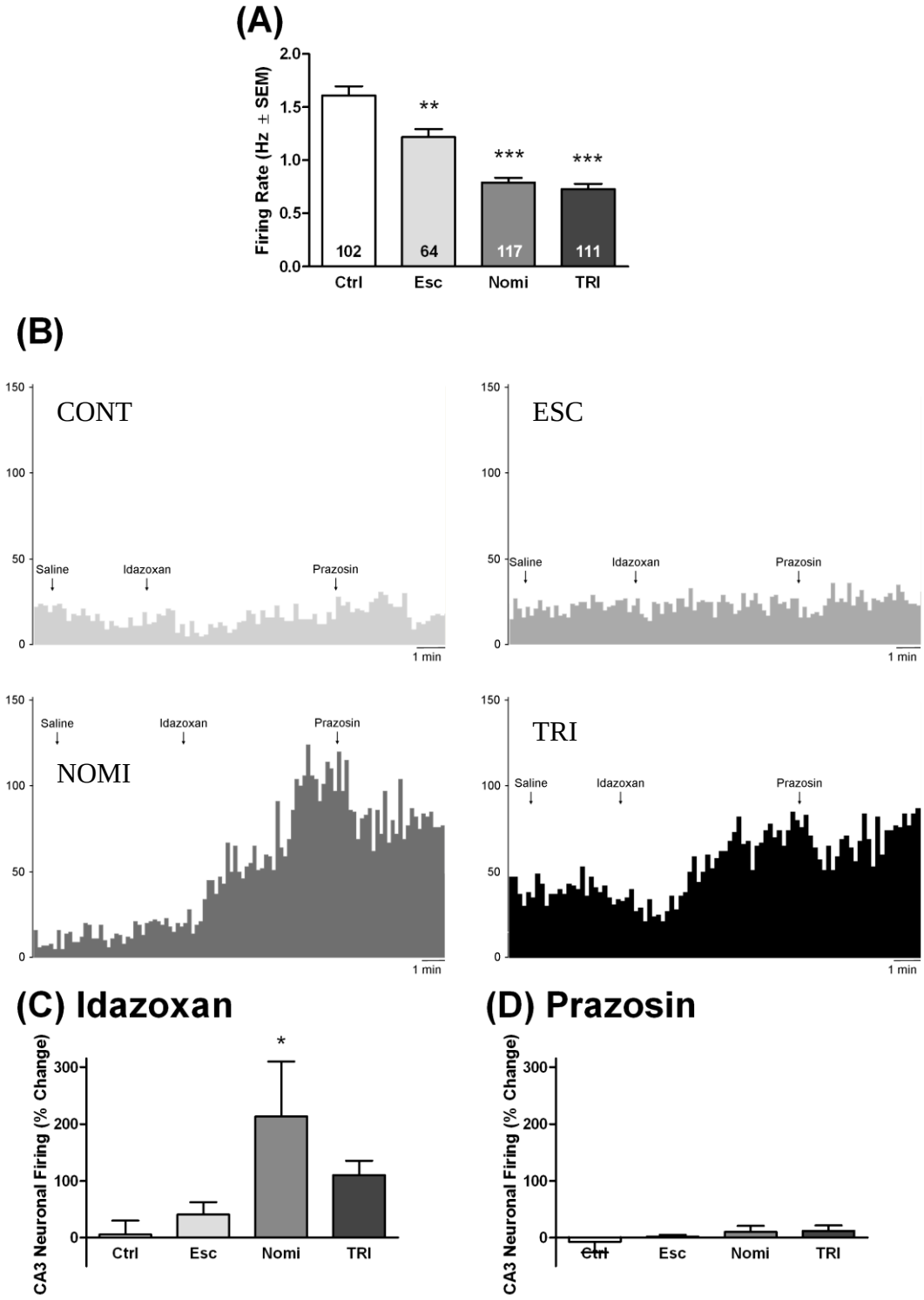

FIGURE 11. CHRONIC TREATMENT OF ESC, NOMI AND TRI ON THE NE SYSTEM. (A) Effect of 14-day treatment of escitalopram (Esc), nomifensine (Nomi) and triple reuptake inhibition (TRI) on the average firing rate of NE neurons in the locus coeruleus. The number at the bottom of each column represents the number of neurons. (B) Integrated firing rate histograms of dorsal hippocampal CA3 pyramidal neurons illustrating systemic injections of saline, idazoxan (1000 µg/kg), and prazosin (100 µg/kg) in a control rat (upper left panel), 14-day treated escitalopram rat (upper right panel), nomifensine rat (lower left panel) and TRI rat (lower right panel). The overall changes of the firing rate of CA3 pyramidal neurons after the systemic injections of idazoxan (C) or prazosin (D) in control and treated rats. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

In order to assess the degree of tonic activation of postsynaptic α_2- and α_1-adrenoceptors in hippocampus, the selective antagonists idazoxan and prazosin, respectively, were used (Curet & de Montigny, 1988; Ghanbari et al., 2011). Consecutive injections of idazoxan and prazosin, respectively, did not alter the firing rate of CA3 pyramidal neurons in the control group (n=5; Figure 11B, C). Systemic injection of idazoxan, but not prazosin, however, significantly increased the firing rate of CA3 pyramidal neurons in rats administered nomifensine for 14 days when compared with the control group, indicating an enhanced tonic activation of postsynaptic α_2-adrenoceptors (n = 6, $p < 0.05$) (Figure 11C, D). Escitalopram (n = 6) treated rats did not show a significant change in firing rate of CA3 pyramidal neurons (Figure 11C, D). While a 14 day treatment of TRI (n = 7) was not significant in a one-way ANOVA ($F_{(3, 46)} = 1.488$, $p > 0.05$), an unpaired *t*-test between the control group and TRI group led to a *p* value of 0.009. Thus, long-term nomifensine administration causes an enhanced tonic activation of the post-synaptic α_2-adrenoceptors.

3.2.3. DA

Fourteen days of escitalopram administration significantly decreased DA firing ($p < 0.05$) while 14 days of nomifensine and TRI treatment did not change the firing (Figure 12A). Sustained escitalopram treatment did not alter DA levels in the NAc, while sustained nomifensine and TRI administration increased DA levels by 67% and 359%, respectively ($p < 0.001$) (Figure 12B). A two-way ANOVA showed significant main effects for both drug treatment ($F_{(1, 166)} = 488.749$, $p < 0.001$), as well as PCPA ($F_{(1, 166)} = 8.299$, $p < 0.01$), while also eliciting a significant drug x PCPA interaction ($F_{(1, 166)} = 4.231$, $p < 0.05$). *Post-hoc* analyses revealed that depletion of serotonin by PCPA caused

a significant decrease in dopamine neurotransmitter levels in TRI treated rats ($p < 0.001$), but no change in control rats ($p = 0.56$; Figure 12C). Therefore, acting on all three monoaminergic systems cause a synergistic increase in DA transmitter levels.

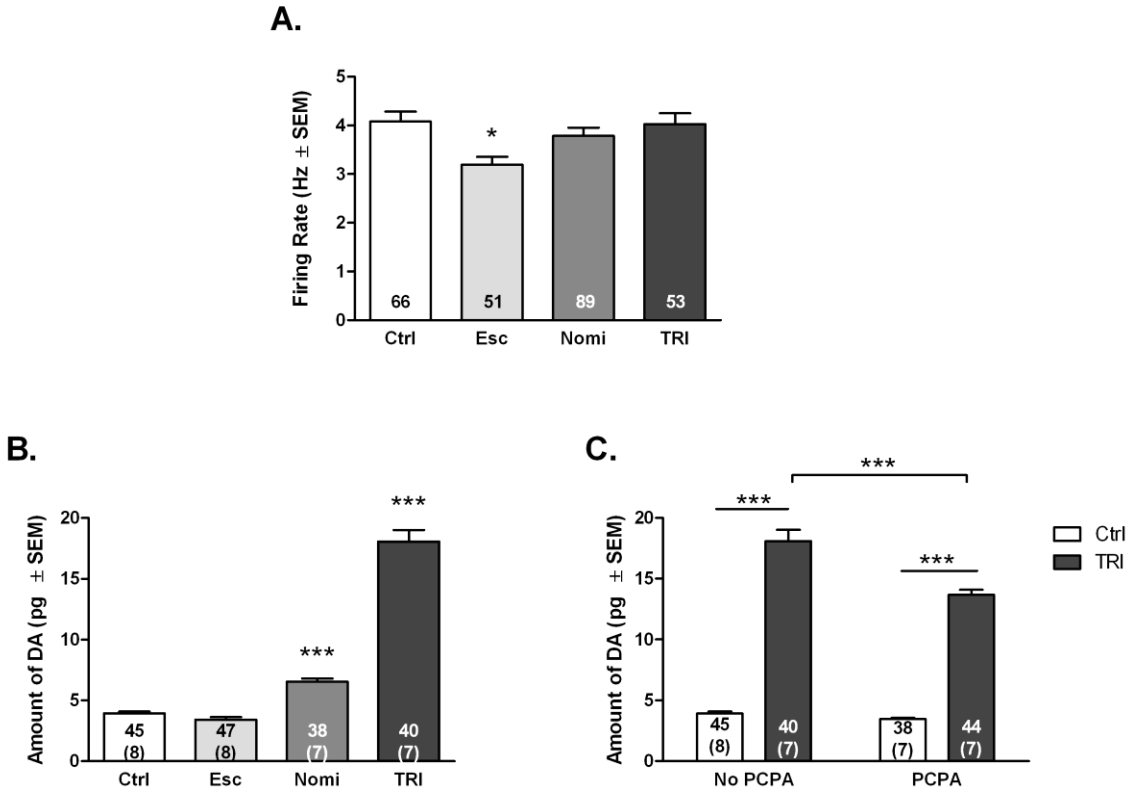

Figure 12. CHRONIC TREATMENT OF ESC, NOMI AND TRI ON THE DA SYSTEM. (A) Effect of 14-day treatment of escitalopram (Esc), nomifensine (Nomi) and triple reuptake inhibition (TRI) on the average firing rate of DA neurons in the ventral tegmental area. The number at the bottom of each column represents the number of neurons. (B) Effect of 14 days of Esc, Nomi and TRI administration on the DA neurotransmitter levels in the nucleus accumbens. (C) Effect of PCPA (300 mg/kg, *i.p.*) on DA neurotransmitter levels in control and 14-day TRI treated rats. The number at the bottom of each column represents the number of samples and the number of rats is indicated in parentheses. *$p < 0.05$, ***$p < 0.001$.

4. DISCUSSION

The present study examined the effects of subacute and prolonged administration of the SSRI escitalopram, the NDRI nomifensine, and the combination of the two drugs (TRI) on the 5-HT, NE, and DA systems.[1] With regards to the 5-HT system, subacute TRI treatment overcame the initial decrease in firing rates observed with escitalopram administration and remained at control levels after sustained TRI treatment. In addition, the results from this study are in line with a review that found many antidepressant treatments decrease NE firing activity (Szabo & Blier, 2001d). TRI treatment, similar to nomifensine treatment, did not enhance the tonic activation of the post-synaptic 5-HT_{1A} receptors observed with long-term escitalopram administration. While escitalopram and TRI administration did not alter the tonic activation of adrenergic receptors, prolonged nomifensine administration caused an enhancement in the tonic activation of the α_2-adrenergic receptor. DA firing rates were unchanged with short- and long-term TRI treatment; however, chronic TRI administration caused a robust increase in DA neurotransmitter levels in the NAc.

All SSRIs cause an initial decrease in the firing rates of 5-HT neurons that recover back to control levels after sustained treatment. The time required to desensitize the inhibitory 5-HT_{1A} autoreceptors is similar to the delayed onset of action observed with SSRIs (Blier, 2001; Pineyro & Blier, 1999). Conversely, Katz et al. (2010) determined

[1] The forebrain post-synaptic results were presented at the 67th Annual Society of Biological Psychiatry Meeting and won the Top Posters Award (35 posters selected out of 900).

that only 2 days of nomifensine treatment was required to desensitize 5-HT_{1A} autoreceptors. Since 2 days of TRI administration resulted in unaltered DRN 5-HT firing rates, it is hypothesized that desensitization of the 5-HT_{1A} autoreceptors occurred rapidly as well. Interestingly, co-administration of escitalopram and bupropion doubled the firing rate of 5-HT neurons after 2 days and the firing rates remained elevated after 14 days of treatment (Ghanbari et al., 2010). This substantial increase may in part be due to a desensitization of the 5-HT_{1A} autoreceptor, but the NE and DA systems also have a contribution. Bupropion increases the level of synaptic NE and this stimulates the post-synaptic α_1-adrenoceptors on 5-HT neurons leading to an increase in 5-HT firing. Added together, the robust 5-HT neuronal firing observed with the co-administration of bupropion and escitalopram may be due to the desensitization of the 5-HT_{1A} autoreceptors in addition to the enhanced activation of the excitatory α_1-adrenoceptors on 5-HT neurons. The mechanism explaining the increase in the 5-HT system with co-administration of escitalopram and bupropion could also explain the results observed in this

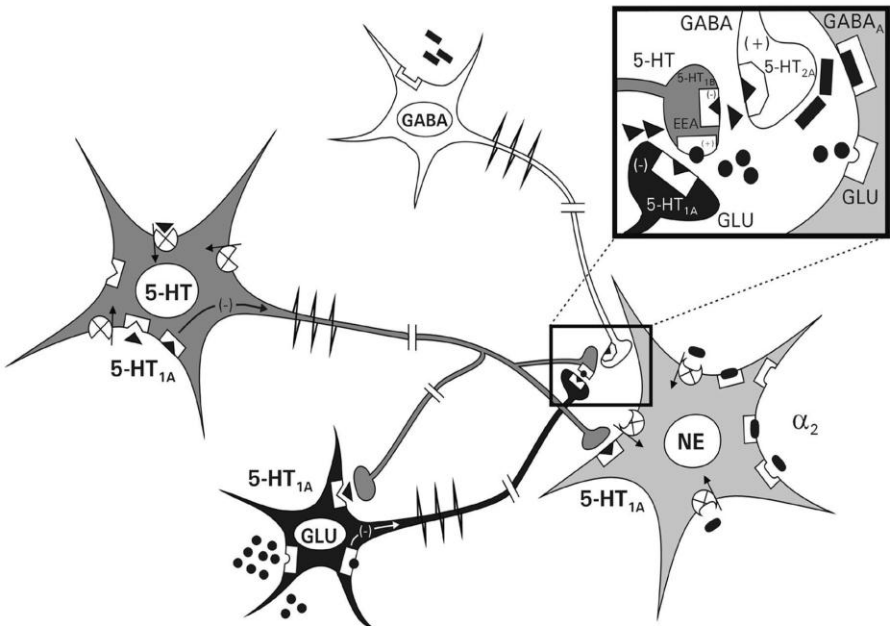

FIGURE 13. INTERACTIONS BETWEEN DRN 5-HT NEURONS AND LC NE NEURONS. From Szabo and Blier (2001c).

experiment.

The effects of *i.v.* WAY 100635 injection on DRN 5-HT neuronal firing has previously been documented by (Haddjeri et al., 2004). WAY 100635 did not alter the firing of 5-HT neurons in naïve animals; however, when NE neurons were lesioned with either 6-OHDA or N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4), WAY 100635 increased the firing of DRN 5-HT neurons by 70 to 80%. This effect can be explained when considering the interactions between the 5-HT and NE systems. Since WAY 100635 is a selective 5-HT_{1A} antagonist, administration of this drug should increase 5-HT neuronal firing. However, systemic injection of WAY 100635 has been shown to suppress LC NE firing activity. Administration of WAY 100635 blocks the inhibitory 5-HT_{1A} receptors on glutamate neurons thereby releasing more glutamate acting on a kainate receptor on 5-HT terminals. This would activate the inhibitory 5-HT_{2A} receptors located on GABA neurons. These GABA neurons project to the LC NE neurons thus inhibiting NE neuronal firing (Szabo & Blier, 2001c, 2001e; Figure 13). As mentioned previously, NE neurons excite DRN 5-HT firing through an α_1-adrenoceptor thus, inhibiting NE neuronal firing with WAY 100635 decreases DRN 5-HT firing. Consequently, the net result of WAY 100635 injections is unaltered firing of DRN 5-HT firing. Lesioning NE neurons remove the attenuated activation of excitatory α_1-adrenoceptors causing the enhanced 5-HT firing. Therefore, the large increase in firing rate observed after *i.v.* injection of WAY 100635 in TRI treated rats may due to a diminished NE input on 5-HT neurons. However, WAY 100635 administration in this study increased the firing of 5-HT neurons by almost 200% while the NE lesion study only produced a 70 to 80% increase (Haddjeri et al., 2004), thus there must be another

factor leading to the increase in 5-HT firing. Indeed, the acute injection of the TRI compound SEP-225289 following WAY 100635 administration increased the firing of DRN 5-HT neurons by approximately 120% (Guiard et al., 2011). The larger increase in 5-HT neuronal firing seen with SEP-225289 and the TRI in this study may be due to a dopaminergic contribution. As mentioned previously, previous studies have shown that DA applied to DRN slices produces a concentration-dependent membrane depolarization of 5-HT neurons (Aman et al., 2007; Haj-Dahmane, 2001). Therefore, the simultaneous activation of the excitatory α_1-adrenergic and D2 receptors may contribute to the robust increase in 5-HT neuronal firing following WAY 100635 administration in TRI rats.

One common and specific characteristic of antidepressant medications is the increased tonic activation of post-synaptic 5-HT_{1A} receptors observed after long-term treatment (Haddjeri et al., 1998). 5-HT_{1A} receptor activation hyperpolarizes and inhibits CA3 pyramidal neurons in the dorsal hippocampus and the injection of the selective 5-HT_{1A} receptor antagonist, WAY 100635, generally does not disinhibit these neurons in anesthetized control rats due to anesthesia. On the other hand, Kasamo et al. (2001) have shown that administration of WAY 100635 in awake rats does increase the firing of CA3 pyramidal neurons (Haddjeri et al., 1998). Long-term antidepressant treatments results in disinhibition and significantly increase the firing activity of CA3 pyramidal neurons upon injection of WAY 100635. Although in this study no increase in the firing activity was seen with either prolonged nomifensine or TRI treatment, escitalopram treatment did cause a significant increase. This increase indicates that the overall 5-HT tone in the hippocampus was enhanced only by long-term escitalopram administration. Unlike all other antidepressant strategies tested thus far, TRI treatment did not enhance 5-HT

transmission in the hippocampus and this could explain why GSK372475, a novel TRI, failed to separate from placebo group in a double-blind study (Learned et al., 2011).

Similar to many antidepressant treatments, escitalopram, nomifensine and TRI administration led to significant decreases in the firing rates of LC NE neurons. In addition, no recovery in firing rates of NE neurons was seen after 14-day administration with all three treatments. This absence of recovery in firing rates is most likely due to a lack of desensitization of the somatodendritic α_2-adrenergic autoreceptors. Furthermore, TRI treatment significantly decreased the burst firing of NE neurons. Interestingly, nomifensine, a drug that acts on the NE and DA system, had little effect on NE burst firing activity (Katz et al., 2010), while previous studies have found that chronic treatment of the SSRIs fluoxetine and escitalopram decrease NE burst firing (Dremencov et al., 2007; Seager et al., 2004; West et al., 2009). Comparing the results obtained in this study with the results of Ghanbari et al. (2010) with escitalopram and bupropion, dissimilarities are apparent. While nomifensine treatment alone caused a significant decrease in LC NE neuronal firing after both 2 and 14 days of treatment, 2-day administration of bupropion caused a decrease in NE neuronal firing but this firing recovered back to baseline after 14 days of treatment. Additionally, both subacute and sustained bupropion treatment led to a significant increase in burst activity of NE neurons while the co-administration of escitalopram and bupropion had no effect on burst activity. The disparity between the results of Ghanbari et al. (2010) and the current results is likely due to the different mechanism of action of bupropion relative to nomifensine, as bupropion is thought to be a NE releaser as opposed to a reuptake inhibitor (Dong & Blier, 2001). Indeed, the administration of the SSRI/5-HT_{2A} antagonist YM992, which

leads to increased NE release, also leads to a recovery of the firing rate of NE neurons and a desensitization of the cell body α_2–adrenergic autoreceptors (Szabo & Blier, 2002).

Similar to assessing the tonic activation of post-synaptic 5-HT_{1A} receptors on hippocampal CA3 pyramidal neurons with WAY 100635, the tonic activation of post-synaptic α_2- and α_1-adrenergic receptors on these same neurons can be tested with idazoxan and prazosin, respectively. While escitalopram treated rats displayed unaltered α_2- and α_1-adrenergic receptor tonic activation, rats treated with nomifensine showed increased firing activity of pyramidal neurons after the *i.v.* injection of idazoxan only, revealing an enhanced tonic activation of the post-synaptic α_2-adrenergic receptors. Bupropion treatment, on the other hand, caused an enhanced tonic activation of both the post-synaptic α_2– and α_1–adrenergic receptors (Ghanbari et al., 2011). Although the co-administration of escitalopram and nomifensine did not reach statistical significance using a one-way ANOVA, an unpaired *t*-test between the TRI and control rats did show that TRI treated rats had significantly higher firing rates following idazoxan administration, indicating that the tonic activation of the post-synaptic α_2-adrenergic receptors may in fact be enhanced.

DA firing rates were unchanged in rats treated with TRI for 2 days. This is surprising considering that both 2-day escitalopram and nomifensine administration resulted in decreased VTA DA firing rates (Dremencov et al., 2009; Katz et al., 2010). Furthermore, 2-day administration of reboxetine, a selective NE reuptake inhibitor, and GBR 12909, a selective DA reuptake inhibitor, also displayed decreased VTA DA neuronal firing (Katz et al., 2010). The discrepancy between TRI treatment and the other antidepressant drugs could in part be due to the considerable decrease in not only the

neuronal firing, but also the firing pattern of NE neurons. Since less NE neurons still displayed burst activity with TRI treatment and burst activity is proportional to the amount of NE neurotransmitters released (Hardebo, 1992), this results in reduced NE available in the synapse. As previous studies have demonstrated that NE exerts an inhibitory action on the DA system (Guiard et al., 2008), having less synaptically available NE would increase the firing rate of VTA DA neurons. However, since TRI treatment blocks the reuptake of DA, which should decrease DA firing, the net effect of TRI treatment on DA neuronal firing rates would be no observable change. Another explanation for the unaltered DA firing rate could be the fact that the somatodentritic D2 receptors underwent a rapid desensitization. Indeed, sustained administration of nomifensine causes the firing of DA neurons to recover back to baseline and this recovery is due to the desensitization of these D2 autoreceptors (Katz et al., 2010).

Tonic activation of D2 receptors were not assessed in the forebrain areas as there has not been a reliable electrophysiological model developed. A previous study has examined the tonic activation of D2 receptors in the prefrontal cortex (Chernoloz et al., 2012b) but these results are not as clear as the data obtained for 5-HT and NE in the CA3 region of the hippocampus. Instead, the post-synaptic dopaminergic effects of escitalopram, nomifensine and TRI treatment were assessed using *in vivo* microdialysis techniques to determine DA neurotransmitter levels in the NAc. Although long-term escitalopram by itself had no observable effect on DA levels in the NAc, nomifensine administration resulted in an approximate 70% increase in DA transmitter levels. Interestingly, the co-administration of escitalopram and nomifensine resulted in a robust 3-fold increase in DA transmitter levels, indicating a synergistic effect of escitalopram on

nomifensine. In line with these results, Golembiowska et al. (2012) found that acute systemic injection of the novel TRI, amitifadine (formerly DOV 21,947), increased DA neurotransimitter levels by about 160% in the NAc and this increase was sustained for 4 hours before DA levels returned to near baseline. In our study, however, the minipump was in the animal continuously delivering the drug which had achieved steady state. In addition, amitifadine increased 5-HT, NE, and DA levels in the prefrontal cortex by 200% to 400%. To determine whether the large increase in DA transmission seen with TRI treated rats in the present study is in fact due to the addition of the serotonin component to nomifensine, the serotonin depletor PCPA was given. While PCPA did not affect DA neurotransmitter levels in control rats, it did significantly decrease DA transmitter levels in TRI treated rats supporting the fact that serotonin plays a role in the enhancement of DA transmission. Another explanation for the robust increase following TRI adminstration may be due to the fact that DA is taken up by 5-HTT (Daws, 2009). Blocking 5-HTTs with escitalopram alone did not have an observable effect on DA transmitter levels however, there may have been a synergistic effect of blocking all three monoamine transporters with TRI treatment.

Altogether, the results from this study indicate that TRI administration did not exhibit the initial inhibitory effects on the cell bodies of 5-HT and DA neurons seen with escitalopram and/or nomifensine administration. However, TRI treatment actually counteracted the tonic activation of the post-synaptic 5-HT_{1A} and α_2-adrenergic receptors observed with escitalopram and nomifensine treatment, respectively. Thus, TRIs could be used in conjunction with other antidepressant drugs that either enhance 5-HT transmission, as is the case with pindolol (Artigas et al., 2006; Fornal et al., 1999), or NE

transmission. In fact, only DA transmission was significantly enhanced with TRI treatment. Therefore, TRIs could theoretically be used as a treatment strategy where there is a deficit in the DA system, such as with Parkinson's disease (Nemeroff et al., 2008).

Apart from their clinical efficacy, TRIs may have a significant advantage over SSRIs with respect to sexual dysfunction. In fact, Breuer et al. (2008) found that both short- and long-term treatment with the novel TRI DOV 216,303 did not affect the sexual behaviour of rats while paroxetine treatment, a SSRI, significantly inhibited sexual behaviour. This beneficial action may result from its dopaminergic properties. Previous studies have found that apomorphine, a non-selective DA agonist, was able to improve sexual function in both men and women (Bechara et al., 2004; Caruso et al., 2004; Pavone et al., 2004).

A concern with TRI is its abuse potential and/or it may cause increased locomotor activity due to the enhancement of the DA transmission. However, recent evidence suggests otherwise. In a double-blind study with addicted individuals, no significant differences were observed with tesofensine, a TRI, and placebo and both these groups separated from the positive control group for dopaminergic/stimulant effects who were given D-amphetamine (Schoede et al., 2010). Additionally, there was no difference in locomotor activity with rats treated with the novel TRIs amitifadine or JZAD-IV-22 (Caldarone et al., 2010; Golembiowska et al., 2012).

A common animal model for depression is removal of the olfactory bulbs. Olfactory bulbectomy (OBX) is the preferred animal model to predict antidepessant activity since OBX rats respond to chronic, but not acute, antidepressant treatment (Grecksch et al., 1997; Song & Leonard, 2005; Uzunova et al., 2004). Although a study

by Breuer et al. (2008) found that chronic DOV 216,303 administration normalized the hyperactivity of OBX-induced rats, a subsequent study by that same group was not able to reproduce the results (Prins et al., 2011). The difference between the two studies was that in the earlier one, DOV 216,303 was given 30 minutes prior to the behaviour testing while the latter study examined the behavioural effects 24 hours after the last drug treatment. Further analysis revealed that at the time of the behavioural testing, DOV 216,303 was no longer present in either the plasma or the brain, indicating that this antidepressant drug loses its antidepressant-like effects in the absence of the drug in the brain.

In terms of the clinical viability of TRIs, multiple studies have been conducted to determine their efficacy, safety, and tolerability. Conflicting results have been obtained with some studies reporting that TRIs such as DOV 216,303 is well tolerated and patients had a decrease in HAM-D scores similar to patients in the citalopram group (Beer et al., 2004; Skolnick et al., 2006). Similarly, the TRI amitifadine was also well tolerated and efficacious (Tran et al., 2012) but MDD patients could not tolerate the TRI GSK372475 and the GSK372475 group did not separate from the placebo group (Learned et al., 2011). Prehaps the difference in clinical viability of TRIs is due to the different binding affinities for the different transporters. Both DOV 216,303 and amitifadine have the highest affinity for 5-HTT, followed by NET and much less for DAT (Lengyel et al., 2008) while GSK372475 has approximately equipotent inhibition of 5-HTT, NET, and DAT (Learned et al., 2011). These studies indicate that TRI are still a viable class of antidepressant medication, but more research must be conducted.

The results from the present study provide more insight of the mechanism of action of drugs that act on one, two and all three monoamine systems. Understanding the principles underlying antidepressant action could provide better therapeutic strategies in the treatment of depression.

5. REFERENCES

Aghajanian, G. K., & Vandermaelen, C. P. (1982). Intracellular recordings from serotonergic dorsal raphe neurons: pacemaker potentials and the effect of LSD. *Brain Res, 238*(2), 463-469.

Aman, T. K., Shen, R. Y., & Haj-Dahmane, S. (2007). D2-like dopamine receptors depolarize dorsal raphe serotonin neurons through the activation of nonselective cationic conductance. *J Pharmacol Exp Ther, 320*(1), 376-385.

Amara, S. G., & Kuhar, M. J. (1993). Neurotransmitter transporters: recent progress. *Annu Rev Neurosci, 16*, 73-93.

American Psychiatric Association, & American Psychiatric Association Task Force on DSM-IV. (2000). *Diagnostic and statistical manual of mental disorders : DSM-IV-TR* (4th ed.). Washington, DC: American Psychiatric Association.

Argyelán, M., Szabó, Z., Kanyó, B., Tanács, A., Kovács, Z., Janka, Z., Pávics, L. (2005). Dopamine transporter availability in medication free and in bupropion treated depression: a 99mTc-TRODAT-1 SPECT study. *J Affect Disord, 89*(1-3), 115-23.

Arias, H. R., Santamaria, A., & Ali, S. F. (2009). Pharmacological and neurotoxicological actions mediated by bupropion and diethylpropion. *Int Rev Neurobiol, 88*, 223-255.

Artigas, F., Adell, A., & Celada, P. (2006). Pindolol augmentation of antidepressant response. *Curr Drug Targets, 7*(2), 139-147.

Ban, T. A. (2006). The role of serendipity in drug discovery. *Dialogues Clin Neurosci, 8*(3), 335-344.

Baraban, J. M., & Aghajanian, G. K. (1981). Noradrenergic innervation of serotonergic neurons in the dorsal raphe: demonstration by electron microscopic autoradiography. *Brain Res, 204*(1), 1-11.

Barnes, N. M., & Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology, 38*(8), 1083-1152.

Bechara, A., Bertolino, M. V., Casabe, A., & Fredotovich, N. (2004). A double-blind randomized placebo control study comparing the objective and subjective changes in female sexual response using sublingual apomorphine. *J Sex Med, 1*(2), 209-214.

Beer, B., Stark, J., Krieter, P., Czobor, P., Beer, G., Lippa, A., & Skolnick, P. (2004). DOV 216,303, a "triple" reuptake inhibitor: safety, tolerability, and pharmacokinetic profile. *J Clin Pharmacol, 44*(12), 1360-1367.

Béïque, J., de Montigny, C., Blier, P., & Debonnel, G. (2000). Effects of sustained administration of the serotonin and norepinephrine reuptake inhibitor venlafaxine: I. in vivo electrophysiological studies in the rat. *Neuropharmacology, 39*(10), 1800-1812.

Bell, C., Abrams, J., & Nutt, D. (2001). Tryptophan depletion and its implications for psychiatry. *Br J Psychiatry, 178*, 399-405.

Blier, P. (2001). Pharmacology of rapid-onset antidepressant treatment strategies. *J Clin Psychiatry, 62 Suppl 15*, 12-17.

Blier, P., & de Montigny, C. (1980). Effect of chronic tricylic antidepressant treatment on the serotoninergic autoreceptor: a microiontophoretic study in the rat. *Naunyn Schmiedebergs Arch Pharmacol, 314*(2), 123-128.

Blier, P., & de Montigny, C. (1983). Electrophysiological investigations on the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. *J Neurosci, 3*(6), 1270-1278.

Blier, P., & de Montigny, C. (1985). Serotoninergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. *Neuroscience, 16*(4), 949-955.

Blier, P., & de Montigny, C. (1987a). Antidepressant monoamine oxidase inhibitors enhance serotonin but not norepinephrine neurotransmission. *Psychopharmacol Ser, 3*, 127-134.

Blier, P., & de Montigny, C. (1987b). Modification of 5-HT neuron properties by sustained administration of the 5-HT1A agonist gepirone: electrophysiological studies in the rat brain. *Synapse, 1*(5), 470-480.

Blier, P., & de Montigny, C. (1990). Differential effect of gepirone on presynaptic and postsynaptic serotonin receptors: single-cell recording studies. *J Clin Psychopharmacol, 10*(3 Suppl), 13S-20S.

Blier, P., de Montigny, C., & Chaput, Y. (1997). Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol, 340*(2-3), 249-58.

Bogdanski, D. F., Pletscher, A., Brodie, B. B., & Undenfriend, S. (1956). Identification and assay of serotonin in brain. *J Pharmacol Exp Ther, 117*(1), 82-88.

Bonaventure, P., Schotte, A., Cras, P., & Leysen, J. E. (1997). Autoradiographic mapping of 5-HT1B- and 5-HT1D receptors in human brain using [3H]alniditan, a new radioligand. *Receptors Channels, 5*(3-4), 225-230.

Boyce, P., & Judd, F. (1999). The place for the tricyclic antidepressants in the treatment of depression. *Aust N Z J Psychiatry, 33*(3), 323-327.

Breuer, M. E., Chan, J. S., Oosting, R. S., Groenink, L., Korte, S. M., Campbell, U., . . . Olivier, B. (2008). The triple monoaminergic reuptake inhibitor DOV 216,303 has antidepressant effects in the rat olfactory bulbectomy model and lacks sexual side effects. *Eur Neuropsychopharmacol, 18*(12), 908-916.

Burnet, P. W., Eastwood, S. L., Lacey, K., & Harrison, P. J. (1995). The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. *Brain Res, 676*(1), 157-168.

Caldarone, B. J., Paterson, N. E., Zhou, J., Brunner, D., Kozikowski, A. P., Westphal, K. G., . . . Ghavami, A. (2010). The novel triple reuptake inhibitor JZAD-IV-22 exhibits an antidepressant pharmacological profile without locomotor stimulant or sensitization properties. *J Pharmacol Exp Ther, 335*(3), 762-770.

Caruso, S., Agnello, C., Intelisano, G., Farina, M., Di Mari, L., & Cianci, A. (2004). Placebo-controlled study on efficacy and safety of daily apomorphine SL intake in premenopausal women affected by hypoactive sexual desire disorder and sexual arousal disorder. *Urology, 63*(5), 955-959.

Cassano, T., Gaetani, S., Morgese M. G., Macheda, T., Laconca, L., Dipasquale, P., Taltavull, J., Shippenberg, T.S., Cuomo, V., & Gobbi, G. (2009). Monoaminergic changes in locus coeruleus and dorsal raphe nucleus following noradrenaline depletion. *Neurochem Res, 34*(8), 1417-1426.

Chaput, Y., Blier, P., & de Montigny, C. (1986). In vivo electrophysiological evidence for the regulatory role of autoreceptors on serotonergic terminals. *J Neurosci, 6*(10), 2796-2801.

Chaput, Y., & de Montigny, C. (1988). Effects of the 5-hydroxytryptamine receptor antagonist, BMY 7378, on 5-hydroxytryptamine neurotransmission: electrophysiological studies in the rat central nervous system. *J Pharmacol Exp Ther, 246*(1), 359-370.

Chenu, F., El Mansari, M., & Blier, P. (2009). Long-term administration of monoamine oxidase inhibitors alters the firing rate and pattern of dopamine neurons in the ventral tegmental area. *Int J Neuropsychopharmacol, 12*(4), 475-485.

Chernoloz, O., El Mansari, M., & Blier, P. (2012a). Effects of Sustained Administration of Quetiapine Alone and in Combination with a Serotonin Reuptake Inhibitor on Norepinephrine and Serotonin Transmission. *Neuropsychopharmacology*.

Chernoloz, O., El Mansari, M., & Blier, P. (2012b). Long-term administration of the dopamine D3/2 receptor agonist pramipexole increases dopamine and serotonin neurotransmission in the male rat forebrain. *J Psychiatry Neurosci, 37*(2), 113-121.

Clerc, G. E., Ruimy, P., & Verdeau-Palles, J. (1994). A double-blind comparison of venlafaxine and fluoxetine in patients hospitalized for major depression and melancholia. The Venlafaxine French Inpatient Study Group. *Int Clin Psychopharmacol, 9*(3), 139-143.

Curet, O., & de Montigny, C. (1988). Electrophysiological characterization of adrenoceptors in the rat dorsal hippocampus. II. Receptors mediating the effect of synaptically released norepinephrine. *Brain Res, 475*(1), 47-57.

Dahlström, A., & Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. *Acta Physiol Scand, 62*(Suppl. 232), 1-55.

Dailly, E., Chenu, F., Petit-Demouliere, B., & Bourin, M. (2006). Specificity and efficacy of noradrenaline, serotonin depletion in discrete brain areas of Swiss mice by neurotoxins. *J Neurosci Methods, 150*(1), 111-115.

Dawe, G. S., Huff, K. D., Vandergriff, J. L., Sharp, T., O'Neill, M. J., & Rasmussen, K. (2001). Olanzapine activates the rat locus coeruleus: in vivo electrophysiology and c-Fos immunoreactivity. *Biol Psychiatry, 50*(7), 510-520.

Daws, L. C. (2009). Unfaithful neurotransmitter transporters: focus on serotonin uptake and implications for antidepressant efficacy. *Pharmacol Ther, 121*(1), 89-99.

de Montigny, C., & Aghajanian, G. K. (1978). Tricyclic antidepressants: long-term treatment increases responsivity of rat forebrain neurons to serotonin. *Science, 202*(4374), 1303-1306.

de Montigny, C., & Blier, P. (1992). Electrophysiological evidence for the distinct properties of presynaptic and postsynaptic 5-HT1A receptors: Possible clinical relevance. In S. Z. Langer, B. N., G. Racagni & J. Mendlewicz (Eds.), *Serotonin Receptor Subtypes: Pharmacological Significance and Clinical Implications* (pp. 80-88). Basel: Karger.

Debonnel, G., Saint-Andre, E., Hebert, C., de Montigny, C., Lavoie, N., & Blier, P. (2007). Differential physiological effects of a low dose and high doses of venlafaxine in major depression. *Int J Neuropsychopharmacol, 10*(1), 51-61.

Delgado, P. L. (2000). Depression: the case for a monoamine deficiency. *J Clin Psychiatry, 61 Suppl 6*, 7-11.

Delgado, P. L. (2006). Monoamine depletion studies: implications for antidepressant discontinuation syndrome. *J Clin Psychiatry, 67 Suppl 4*, 22-26.

Devoto, P., Flore, G., Pani, L., & Gessa, G. L. (2001). Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. *Mol Psychiatry, 6*(6), 657-664.

Devoto, P., Flore, G., Vacca G., Pira, L., Arca, A., Casu, M. A., Pani, L., & Gessa, G. L. (2003). Co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex induced by clozapine, the prototype atypical antipsychotic. *Psychopharmacology (Berl), 167*(1): 79-84.

Devoto, P., Flore, G., Pira, L., Longu, G., & Gessa, G. L. (2004). Alpha2-adrenoceptor medicated co-releaseof dopamine and noradrenaline from noradrenergic neurons in the cerebral cortex. *J Neurochem, 88*(4), 1003-1009.

Dong, J., & Blier, P. (1998). Full agonistic properties of BAY x 3702 on presynaptic and postsynaptic 5-HT1A receptors electrophysiological studies in the rat hippocampus and dorsal raphe. *J Pharmacol Exp Ther, 286*(3),1239-47.

Dong, J., & Blier, P. (2001). Modification of norepinephrine and serotonin, but not dopamine, neuron firing by sustained bupropion treatment. *Psychopharmacology (Berl), 155*(1), 52-57.

Dremencov, E., El Mansari, M., & Blier, P. (2007). Noradrenergic augmentation of escitalopram response by risperidone: electrophysiologic studies in the rat brain. [Research Support, Non-U.S. Gov't]. *Biol Psychiatry, 61*(5), 671-678.

Dremencov, E., El Mansari, M., & Blier, P. (2009). Effects of sustained serotonin reuptake inhibition on the firing of dopamine neurons in the rat ventral tegmental area. *J Psychiatry Neurosci, 34*(3), 223-229.

El Mansari, M., & Blier, P. (1997). In vivo electrophysiological characterization of 5-HT receptors in the guinea pig head of caudate nucleus and orbitofrontal cortex. *Neuropharmacology, 36*(4-5), 577-588.

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), 1191-1198.

El Mansari, M., Sanchez, C., Chouvet, G., Renaud, B., & Haddjeri, N. (2005). Effects of acute and long-term administration of escitalopram and citalopram on serotonin neurotransmission: an in vivo electrophysiological study in rat brain. *Neuropsychopharmacology, 30*(7), 1269-1277.

Feldman, R. S., Meyer, J. S., & Quenzer, L. F. (1997). *Principles of neuropsychopharmacology*. Sunderland, Mass.: Sinauer Associates.

Fornal, C. A., Martin, F. J., Mendlin, A., Metzler, C. W., Bjorvatn, B., & Jacobs, B. L. (1999). Pindolol increases extracellular 5-HT while inhibiting serotonergic neuronal activity. *Eur J Pharmacol, 377*(2-3), 187-191.

Freeman, A. S., Meltzer, L. T., & Bunney, B. S. (1985). Firing properties of substantia nigra dopaminergic neurons in freely moving rats. *Life Sci, 36*(20), 1983-1994.

Gallager, D. W., & Bunney, W. E., Jr. (1979). Failure of chronic lithium treatment to block tricyclic antidepressant-induced 5-HT supersensitivity. *Naunyn Schmiedebergs Arch Pharmacol, 307*(2), 129-133.

Garnock-Jones, K. P., & McCormack, P. L. (2010). Escitalopram: a review of its use in the management of major depressive disorder in adults. *CNS Drugs, 24*(9), 769-796.

German, D. C., Schlusselberg, D. S., & Woodward, D. J. (1983). Three-dimensional computer reconstruction of midbrain dopaminergic neuronal populations: from mouse to man. *J Neural Transm, 57*(4), 243-254.

Ghanbari, R., El Mansari, M., & Blier, P. (2010). Electrophysiological effects of the co-administration of escitalopram and bupropion on rat serotonin and norepinephrine neurons. *J Psychopharmacol, 24*(1), 39-50.

Ghanbari, R., El Mansari, M., & Blier, P. (2011). Enhancement of serotonergic and noradrenergic neurotransmission in the rat hippocampus by sustained administration of bupropion. *Psychopharmacology (Berl), 217*(1), 61-73.

Gillman, P. K. (2007). Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. *Br J Pharmacol, 151*(6), 737-748.

Giros, B., Wang, Y. M., Suter, S., McLeskey, S. B., Pifl, C., & Caron, M. G. (1994). Delineation of discrete domains for substrate, cocaine, and tricyclic antidepressant interactions using chimeric dopamine-norepinephrine transporters. *J Biol Chem, 269*(23), 15985-15988.

GlaxoSmithKline. (2008). Wellbutrin XL (Bupropion hydrochloride extended-release tablets). Retrieved from http://us.gsk.com/products/assets/us_wellbutrinXL.pdf

Godbout, R., Chaput, Y., Blier, P., & de Montigny, C. (1991). Tandospirone and its metabolite, 1-(2-pyrimidinyl)-piperazine--I. Effects of acute and long-term administration of tandospirone on serotonin neurotransmission. *Neuropharmacology, 30*(7), 679-690.

Golembiowska, K., Kowalska, M., & Bymaster, F. P. (2012). Effects of the triple reuptake inhibitor amitifadine on extracellular levels of monoamines in rat brain regions and on locomotor activity. *Synapse, 66*(5), 435-444.

Gonon, F. G., Suaud-Chagny, M. F., Mermet, C. C., & Buda, M. (1991). Relation between impulse flow and extracellular catecholamine levels as studied by in vivo electrochemistry in CNS. In K. Fuxe & L. F. Agnati (Eds.), *Volume transmission in the brain: novel mechanisms for neural transmission* (pp. 337-350). New York: Raven Press.

Grace, A. A., & Bunney, B. S. (1983). Intracellular and extracellular electrophysiology of nigral dopaminergic neurons--1. Identification and characterization. *Neuroscience, 10*(2), 301-315.

Grace, A. A., & Bunney, B. S. (1984). The control of firing pattern in nigral dopamine neurons: burst firing. *J Neurosci, 4*(11), 2877-2890.

Grecksch, G., Zhou, D., Franke, C., Schroder, U., Sabel, B., Becker, A., & Huether, G. (1997). Influence of olfactory bulbectomy and subsequent imipramine treatment on 5-hydroxytryptaminergic presynapses in the rat frontal cortex: behavioural correlates. *Br J Pharmacol, 122*(8), 1725-1731.

Greenberg, P. E., Kessler, R. C., Birnbaum, H. G., Leong, S. A., Lowe, S. W., Berglund, P. A., & Corey-Lisle, P. K. (2003). The economic burden of depression in the United States: how did it change between 1990 and 2000? *J Clin Psychiatry, 64*(12), 1465-1475.

Guiard, B. P., Chenu, F., El Mansari, M., & Blier, P. (2011). Characterization of the electrophysiological properties of triple reuptake inhibitors on monoaminergic neurons. *Int J Neuropsychopharmacol, 14*(2), 211-223.

Guiard, B. P., El Mansari, M., & Blier, P. (2008). Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. *Mol Pharmacol, 74*(5), 1463-1475.

Guiard, B. P., El Mansari, M., Merali, Z., & Blier, P. (2008). Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions. *Int J Neuropsychopharmacol, 11*(5), 625-639.

Haddjeri, N., & Blier, P. (2001). Sustained blockade of neurokinin-1 receptors enhances serotonin neurotransmission. *Biol Psychiatry, 50*(3), 191-199.

Haddjeri, N., Blier, P., & de Montigny, C. (1998). Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT1A receptors. *J Neurosci, 18*(23), 10150-10156.

Haddjeri, N., de Montigny, C., & Blier, P. (1997). Modulation of the firing activity of noradrenergic neurones in the rat locus coeruleus by the 5-hydroxtryptamine system. *Br J Pharmacol, 120*(5), 865-875.

Haddjeri, N., Lavoie, N., & Blier, P. (2004). Electrophysiological evidence for the tonic activation of 5-HT(1A) autoreceptors in the rat dorsal raphe nucleus. *Neuropsychopharmacology, 29*(10), 1800-1806.

Hadrava, V., Blier, P., Dennis, T., Ortemann, C., & de Montigny, C. (1995). Characterization of 5-hydroxytryptamine1A properties of flesinoxan: in vivo electrophysiology and hypothermia study. *Neuropharmacology, 34*(10), 1311-1326.

Haj-Dahmane, S. (2001). D2-like dopamine receptor activation excites rat dorsal raphe 5-HT neurons in vitro. *Eur J Neurosci, 14*(1), 125-134.

Hall, D. W., Logan, B. W., & Parsons, G. H. (1969). Further studies on the inhibition of monoamine oxidase by M and B 9302 (clorgyline). I. Substrate specificity in various mammalian species. *Biochem Pharmacol, 18*(6), 1447-1454.

Hardebo, J. E. (1992). Influence of impulse pattern on noradrenaline release from sympathetic nerves in cerebral and some peripheral vessels. *Acta Physiol Scand, 144*(3), 333-339.

Hatanaka, K., Yatsugi, S., & Yamaguchi, T. (2000). Effect of acute treatment with YM992 on extracellular norepinephrine levels in the rat frontal cortex. *Eur J Pharmacol, 395*(1), 31-36.

Hoyer, D., Clarke, D. E., Fozard, J. R., Hartig, P. R., Martin, G. R., Mylecharane, E. J., . . . Humphrey, P. P. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev, 46*(2), 157-203.

Jakab, R. L., & Goldman-Rakic, P. S. (1998). 5-Hydroxytryptamine2A serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc Natl Acad Sci U S A, 95*(2), 735-740.

Kandel, E. R., & Spencer, W. A. (1961). Electrophysiology of hippocampal neurons. II. After-potentials and repetitive firing. *J Neurophysiol, 24*, 243-259.

Kasamo, K., Suzuki, T., Tada, K., Ueda, N., Matsuda, E., Ishikawa, K., & Kojima, T. (2001). Endogenous 5-HT tonically inhibits spontaneous firing activity of dorsal hippocampus CA1 pyramidal neurons through stimulation of 5-HT(1A) receptors

in quiet awake rats: in vivo electrophysiological evidence. *Neuropsychopharmacology, 24*(2), 141-151.

Katz, N. S., Guiard, B. P., El Mansari, M., & Blier, P. (2010). Effects of acute and sustained administration of the catecholamine reuptake inhibitor nomifensine on the firing activity of monoaminergic neurons. *J Psychopharmacol, 24*(8), 1223-1235.

Koe, B. K., & Weissman, A. (1966). p-Chlorophenylalanine: a specific depletor of brain serotonin. *J Pharmacol Exp Ther, 154*(3), 499-516.

Langer, S. Z. (1974). Presynaptic regulation of catecholamine release. *Biochem Pharmacol, 23*(13), 1793-1800.

Learned-Coughlin, S. M., Bergstrom, M., Savitcheva, I., Ascher, J., Schmith, V. D., & Langstrom, B. (2003). In vivo activity of bupropion at the human dopamine transporter as measured by positron emission tomography. *Biol Psychiatry, 54*(8), 800-805.

Learned, S., Graff, O., Roychowdhury, S., Moate, R., Krishnan, K. R., Archer, G., . . . Ratti, E. (2011). Efficacy, safety, and tolerability of a triple reuptake inhibitor GSK372475 in the treatment of patients with major depressive disorder: two randomized, placebo- and active-controlled clinical trials. *J Psychopharmacol*.

Lee, A., Wissekerke, A. E., Rosin, D. L., & Lynch, K. R. (1998). Localization of alpha2C-adrenergic receptor immunoreactivity in catecholaminergic neurons in the rat central nervous system. *Neuroscience, 84*(4), 1085-1096.

Lejeune, F., & Millan, M. J. (1998). Induction of burst firing in ventral tegmental area dopaminergic neurons by activation of serotonin (5-HT)1A receptors: WAY 100,635-reversible actions of the highly selective ligands, flesinoxan and S 15535. *Synapse, 30*(2), 172-180.

Lejeune, F., Newman-Tancredi, A., Audinot, V., & Millan, M. J. (1997). Interactions of (+)- and (-)-8- and 7-hydroxy-2-(di-n-propylamino)tetralin at human (h)D3, hD2 and h serotonin1A receptors and their modulation of the activity of serotoninergic and dopaminergic neurones in rats. *J Pharmacol Exp Ther, 280*(3), 1241-1249.

Lengyel, K., Pieschl, R., Strong, T., Molski, T., Mattson, G., Lodge N. J., & Li, Y. (2008). Ex vivo assessment of binding site occupancy of monoamine reuptake inhibitors: Methodology and biological significance. *Neuropharmacol, 55*(1), 63-70.

Li, S. X., Perry, K. W., & Wong, D. T. (2002). Influence of fluoxetine on the ability of bupropion to modulate extracellular dopamine and norepinephrine concentrations in three mesocorticolimbic areas of rats. *Neuropharmacology, 42*(2), 181-190.

Lindvall, O., & Björklund, A. (1983). Dopamine and norepinephrine containing neuron nystems: Their anatomy in the rat brain. In P. C. Emson (Ed.), *Chemical neuroanatomy* (pp. 229-255). New York: Raven Press.

Lopez-Gimenez, J. F., Mengod, G., Palacios, J. M., & Vilaro, M. T. (1997). Selective visualization of rat brain 5-HT2A receptors by autoradiography with [3H]MDL 100,907. *Naunyn Schmiedebergs Arch Pharmacol, 356*(4), 446-454.

Mansour, A., Meador-Woodruff, J. H., Bunzow, J. R., Civelli, O., Akil, H., & Watson, S. J. (1990). Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an in situ hybridization-receptor autoradiographic analysis. *J Neurosci, 10*(8), 2587-2600.

Marcusson, J. O., & Ross, S. B. (1990). Binding of some antidepressants to the 5-hydroxytryptamine transporter in brain and platelets. *Psychopharmacology (Berl), 102*(2), 145-155.

Martin-Ruiz, R., Puig, M. V., Celada, P., Shapiro, D. A., Roth, B. L., Mengod, G., & Artigas, F. (2001). Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. *J Neurosci, 21*(24), 9856-9866.

Martin-Ruiz, R., Ugedo, L., Honrubia, M. A., Mengod, G., & Artigas, F. (2001). Control of serotonergic neurons in rat brain by dopaminergic receptors outside the dorsal raphe nucleus. *J Neurochem, 77*(3), 762-775.

Marwaha, J., & Aghajanian, G. K. (1982). Relative potencies of alpha-1 and alpha-2 antagonists in the locus ceruleus, dorsal raphe and dorsal lateral geniculate nuclei: an electrophysiological study. *J Pharmacol Exp Ther, 222*(2), 287-293.

McKillop, D., & Bradford, H. F. (1981). Comparative effects of benztropine and nomifensine on dopamine uptake and release from striatal synaptosomes. *Biochem Pharmacol, 30*(20), 2753-2758.

Meador-Woodruff, J. H., Mansour, A., Healy, D. J., Kuehn, R., Zhou, Q. Y., Bunzow, J. R., . . . Watson, S. J., Jr. (1991). Comparison of the distributions of D1 and D2 dopamine receptor mRNAs in rat brain. *Neuropsychopharmacology, 5*(4), 231-242.

Meek, J. L., & Neff, N. H. (1972). Tryptophan 5-hydroxylase: approximation of half-life and rate of axonal transport. *J Neurochem, 19*(6), 1519-1525.

Meyer, J. H., Goulding, V. S., Wilson, A. A., Hussey, D., Christensen, B. K., & Houle, S. (2002). Bupropion occupancy of the dopamine transporter is low during clinical treatment. *Psychopharmacology (Berl), 163*(1), 102-105.

Miner, L. A., Backstrom, J. R., Sanders-Bush, E., & Sesack, S. R. (2003). Ultrastructural localization of serotonin2A receptors in the middle layers of the rat prelimbic prefrontal cortex. *Neuroscience, 116*(1), 107-117.

Morot-Gaudry, Y., Bourgoin, S., & Hamon, M. (1981). Kinetic characteristics of newly synthesized 3H-5-HT in the brain of control and reserpinized mice. Evidence for the heterogeneous distribution of 5-HT in serotoninergic neurons. *Naunyn Schmiedebergs Arch Pharmacol, 316*(4), 311-316.

Nemeroff, C.B., Entsuah, R., Benattia, I., Demitrack, M., Sloan, D.M., & Thase, M.E. (2008). Comprehensive analysis of remission (COMPARE) with venlafaxine versus SSRIs. *Biol Psychiatry*, *63*(4), 424-34.

Nichols, DE., & Nichols CD. (2008) Serotonin receptors. *Chem Rev, 108*(5), 1614-41.

Owens, M. J., Knight, D. L., & Nemeroff, C. B. (2001). Second-generation SSRIs: human monoamine transporter binding profile of escitalopram and R-fluoxetine. *Biol Psychiatry, 50*(5), 345-350.

Pavone, C., Curto, F., Anello, G., Serretta, V., Almasio, P. L., & Pavone-Macaluso, M. (2004). Prospective, randomized, crossover comparison of sublingual apomorphine (3 mg) with oral sildenafil (50 mg) for male erectile dysfunction. *J Urol, 172*(6 Pt 1), 2347-2349.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). San Diego: Academic Press.

Piacentini, M. F., Clinckers, R., Meeusen, R., Sarre, S., Ebinger, G., & Michotte, Y. (2003). Effect of bupropion on hippocampal neurotransmitters and on peripheral hormonal concentrations in the rat. *J Appl Physiol, 95*(2), 652-656.

Pineyro, G., & Blier, P. (1999). Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacol Rev, 51*(3), 533-591.

Player, M. S., & Peterson, L. E. (2011). Anxiety disorders, hypertension, and cardiovascular risk: a review. *Int J Psychiatry Med, 41*(4), 365-377.

Prins, J., Westphal, K. G., Korte-Bouws, G. A., Quinton, M. S., Schreiber, R., Olivier, B., & Korte, S. M. (2011). The potential and limitations of DOV 216,303 as a triple reuptake inhibitor for the treatment of major depression: a microdialysis study in olfactory bulbectomized rats. *Pharmacol Biochem Behav, 97*(3), 444-452.

Prisco, S., Pagannone, S., & Esposito, E. (1994). Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. *J Pharmacol Exp Ther, 271*(1), 83-90.

Raiteri, M., Cerrito, F., Cervoni, A. M., & Levi, G. (1979). Dopamine can be released by two mechanisms differentially affected by the dopamine transport inhibitor nomifensine. *J Pharmacol Exp Ther, 208*(2), 195-202.

Ranck, J. B. (1975). Behavioral correlates and firing repertoires of neurons in the dorsal hippocampal formation and septum of unrestrained rats. In R. L. a. P. Isaacson, Karl H. (Ed.), *The hippocampus: Neurophysiology and behavior* (pp. 207-244): Plenum Press.

Rapport, M. M., Green, A. A., & Page, I. H. (1948). Crystalline Serotonin. *Science, 108*(2804), 329-330.

Reader, T. A., Brière, R., Grondin, L., & Ferron, A. (1986). Effects of p-chlorophenylalanine on cortical monoamines and on the activity of noradrenergic neurons. *Neurochem Res, 11*(7), 1025-1035.

Renn, B. N., Feliciano, L., & Segal, D. L. (2011). The bidirectional relationship of depression and diabetes: a systematic review. *Clin Psychol Rev, 31*(8), 1239-1246.

Richelson, E., & Pfenning, M. (1984). Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: most antidepressants selectively block norepinephrine uptake. *Eur J Pharmacol, 104*(3-4), 277-286.

Rueter, L.E., Tecott, L.H., & Blier, P. (2000). In vivo electrophysiological examination of 5-HT2 responses in 5-HT2C receptor mutant mice. *Naunyn Schmiedebergs Arch Pharmacol, 361*(5), 484-91

Saavedra, J. M., Grobecker, H., & Zivin, J. (1976). Catecholamines in the raphe nuclei of the rat. *Brain Res, 114*(2): 337-345.

Sanchez, C., & Hyttel, J. (1999). Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cell Mol Neurobiol, 19*(4), 467-489.

Sanders-Bush, E., & Martin, L. L. (1982). Storage and release of serotonin. In N. N. Osborne (Ed.), *Biology of Serotonergic Transmission*: Wiley.

Sari, Y., Miquel, M. C., Brisorgueil, M. J., Ruiz, G., Doucet, E., Hamon, M., & Verge, D. (1999). Cellular and subcellular localization of 5-hydroxytryptamine1B receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. *Neuroscience, 88*(3), 899-915.

Schacht, U., & Heptner, W. (1974). Effect of nomifensine (HOE 984), a new antidepressant, on uptake of noradrenaline and serotonin and on release of noradrenaline in rat brain synaptosomes. *Biochem Pharmacol, 23*(24), 3413-3422.

Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Am J Psychiatry, 122*(5), 509-522.

Schoedel, K. A., Meier, D., Chakraborty, B., Manniche, P. M., & Sellers, E. M. (2010). Subjective and objective effects of the novel triple reuptake inhibitor tesofensine in recreational stimulant users. *Clin Pharmacol Ther, 88*(1), 69-78.

Seager, M. A., Huff, K. D., Barth, V. N., Phebus, L. A., & Rasmussen, K. (2004). Fluoxetine administration potentiates the effect of olanzapine on locus coeruleus neuronal activity. *Biol Psychiatry, 55*(11), 1103-1109.

Sharma, Y., Xu, T., Graf, W. M., Fobbs, A., Sherwood, C. C., Hof, P. R., . . . Manaye, K. F. (2010). Comparative anatomy of the locus coeruleus in humans and nonhuman primates. *J Comp Neurol, 518*(7), 963-971.

Sharp, T., & Hjorth, S. (1990). Application of brain microdialysis to study the pharmacology of the 5-HT1A autoreceptor. *J Neurosci Methods, 34*(1-3), 83-90.

Simon, H., Le Moal, M., Stinus, L., & Calas, A. (1979). Anatomical relationships between the ventral mesencephalic tegmentum--a 10 region and the locus coeruleus as demonstrated by anterograde and retrograde tracing techniques. *J Neural Transm, 44*(1-2), 77-86.

Skolnick, P., Krieter, P., Tizzano, J., Basile, A., Popik, P., Czobor, P., & Lippa, A. (2006). Preclinical and clinical pharmacology of DOV 216,303, a "triple" reuptake inhibitor. *CNS Drug Rev, 12*(2), 123-134.

Song, C., & Leonard, B. E. (2005). The olfactory bulbectomised rat as a model of depression. [Review]. *Neurosci Biobehav Rev, 29*(4-5), 627-647.

Sprouse, J. S., & Aghajanian, G. K. (1988). Responses of hippocampal pyramidal cells to putative serotonin 5-HT1A and 5-HT1B agonists: a comparative study with dorsal raphe neurons. *Neuropharmacology, 27*(7), 707-715.

Suzuki, M., Hurd, Y. L., Sokoloff, P., Schwartz, J. C., & Sedvall, G. (1998). D3 dopamine receptor mRNA is widely expressed in the human brain. *Brain Res, 779*(1-2), 58-74.

Svensson, T. H., Bunney, B. S., & Aghajanian, G. K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res, 92*(2), 291-306.

Szabo, S. T., & Blier, P. (2001a). Effect of the selective noradrenergic reuptake inhibitor reboxetine on the firing activity of noradrenaline and serotonin neurons. *Eur J Neurosci, 13*(11), 2077-2087.

Szabo, S. T., & Blier, P. (2001b). Effects of the selective norepinephrine reuptake inhibitor reboxetine on norepinephrine and serotonin transmission in the rat hippocampus. *Neuropsychopharmacology, 25*(6), 845-857.

Szabo, S. T., & Blier, P. (2001c). Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons. *Brain Res, 922*(1), 9-20.

Szabo, S. T., & Blier, P. (2001d). Response of the norepinephrine system to antidepressant drugs. *CNS Spectr, 6*(8), 679-684.

Szabo, S. T., & Blier, P. (2001e). Serotonin (1A) receptor ligands act on norepinephrine neuron firing through excitatory amino acid and GABA(A) receptors: a microiontophoretic study in the rat locus coeruleus. *Synapse, 42*(4), 203-212.

Szabo, S. T., & Blier, P. (2002). Effects of serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibition plus 5-HT(2A) receptor antagonism on the firing activity of norepinephrine neurons. *J Pharmacol Exp Ther, 302*(3), 983-991.

Szabo, S. T., de Montigny, C., & Blier, P. (2000). Progressive attenuation of the firing activity of locus coeruleus noradrenergic neurons by sustained administration of selective serotonin reuptake inhibitors. *Int J Neuropsychopharmacol, 3*(1), 1-11.

Tatsumi, M., Groshan, K., Blakely, R. D., & Richelson, E. (1997). Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol, 340*(2-3), 249-258.

Thureson-Klein, A. (1983). Exocytosis from large and small dense cored vesicles in noradrenergic nerve terminals. *Neuroscience, 10*(2), 245-259.

Tork, I. (1990). Anatomy of the serotonergic system. *Ann N Y Acad Sci, 600*, 9-34; discussion 34-35.

Tracqui, P., Morot-Gaudry, Y., Staub, J. F., Brezillon, P., Perault-Staub, A. M., Bourgoin, S., & Hamon, M. (1983). Model of brain serotonin metabolism. II. Physiological interpretation. *Am J Physiol, 244*(2), R206-215.

Tran, P., Skolnick, P., Czobor, P., Huang, N. Y., Bradshaw, M., McKinney, A., & Fava, M. (2012). Efficacy and tolerability of the novel triple reuptake inhibitor amitifadine in the treatment of patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. *J Psychiatr Res, 46*(1), 64-71.

Trivedi, M. H., Hollander, E., Nutt, D., & Blier, P. (2008). Clinical evidence and potential neurobiological underpinnings of unresolved symptoms of depression. *J Clin Psychiatry, 69*(2), 246-258.

Uzunova, V., Wrynn, A. S., Kinnunen, A., Ceci, M., Kohler, C., & Uzunov, D. P. (2004). Chronic antidepressants reverse cerebrocortical allopregnanolone decline in the olfactory-bulbectomized rat. *Eur J Pharmacol, 486*(1), 31-34.

Varnas, K., Hurd, Y. L., & Hall, H. (2005). Regional expression of 5-HT1B receptor mRNA in the human brain. *Synapse, 56*(1), 21-28.

Walther, D. J., & Bader, M. (2003). A unique central tryptophan hydroxylase isoform. *Biochem Pharmacol, 66*(9), 1673-1680.

West, C. H., Ritchie, J. C., Boss-Williams, K. A., & Weiss, J. M. (2009). Antidepressant drugs with differing pharmacological actions decrease activity of locus coeruleus neurons. *Int J Neuropsychopharmacol, 12*(5), 627-641.

WHO. (2008). The global burden of disease: 2004 update 2008. Retrieved April 16, 2012, from http://www.who.int/healthinfo/global_burden_disease/GBD_ report_2004update_full.pdf

WHO. (2012). Depression Retrieved April 26, 2012, from http://www.who.int/mental_health/management/depression/definition/en/

Yan Q. S., Zheng, S. Z., Feng, M. J., & Yan, S. E. (2005). Involvement of 5-HT1B receptors within the ventral tegmental area in ethanol-induced increases in mesolimbic dopaminergic transmission. *Brain Res, 1060*(1-2), 126-137.

Yang, H. Y., & Neff, N. H. (1974). The monoamine oxidases of brain: selective inhibition with drugs and the consequences for the metabolism of the biogenic amines. *J Pharmacol Exp Ther, 189*(3), 733-740.

Yokoyama, C., Okamura, H., Nakajima, T., Taguchi, J., & Ibata, Y. (1994). Autoradiographic distribution of [3H]YM-09151-2, a high-affinity and selective antagonist ligand for the dopamine D2 receptor group, in the rat brain and spinal cord. *J Comp Neurol, 344*(1), 121-136.