



**Effects of Acute and Sustained Administration of Vilazodone (EMD68843) on
Monoaminergic Systems: an In Vivo Electrophysiological Study**

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ABSTRACT

Vilazodone is a partial 5-HT_{1A} receptor agonist and a selective serotonin reuptake inhibitor (SSRI). Acute administration caused a dose-dependent decrease in dorsal raphe (DR) serotonin (5-HT) neuron firing rates. Vilazodone significantly decreased DR 5-HT neuronal firing following 2-day administration, which was shown to recover completely after 14-day administration. The 2-day administration of vilazodone significantly decreased firing in ventral tegmental area dopamine neurons; this effect persisted after 14-day treatment. The firing rate of norepinephrine neurons in the locus coeruleus was not significantly altered following 2-day treatment but did decrease following 14-day treatment. In the hippocampus, 14-day treatment with vilazodone significantly enhanced tonic activation, while having no effect on 5-HT reuptake. Vilazodone produced effects similar to conventional SSRIs while also inducing alterations in monoaminergic neurons that may be associated with its 5-HT_{1A} properties and may have a role in the field of treatment resistant depression.

TABLE OF CONTENTS

ABSTRACT	i
TABLE OF CONTENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
LIST OF ABBREVIATIONS	vii
ACKNOWLEDGEMENTS	ix
INTRODUCTION	1
1.0 Major depressive disorder	1
1.1 Background	1
1.2 Etiology	5
2.0 Monoamine hypothesis.....	7
2.1 Serotonin	11
2.2 Dopamine	15
2.3 Norepinephrine	18
3.0 Pharmacological treatment for depression.....	20
3.1 Tricyclics (TCAs)	21
3.2 Monoamine Oxidase Inhibitors (MAOIs).....	22
3.3 Selective Serotonin Reuptake Inhibitors (SSRIs)	24
3.4 5-HT _{1A} agonists	25
3.6 Vilazodone.....	29
4.0 Objectives and Hypotheses	32
MATERIALS AND METHODS	36
1.0 Animals.....	36
2.0 Experimental preparations	36
2.1 Experimental preparations for acute treatment.....	36
2.2 Experimental preparations for 2-day and 14-day treatment	37
2.0 Electrophysiological Experiments in the Cell Body.....	37
3.1 DRN 5-HT neurons	38
3.2 VTA DA neurons	39
3.3 LC NE neurons.....	40
4.0 Electrophysiological recording of dorsal hippocampus CA3 pyramidal neurons..	41

4.1 Tonic activation of postsynaptic 5-HT _{1A} receptors.....	42
5.0 Drugs.....	43
6.0 Statistical Analysis	43
RESULTS	44
1.0 Effects of vilazodone on the firing of monoaminergic neurons.....	44
1.1 Effects of vilazodone on acute 5-HT firing	44
1.2 Effects of vilazodone on 5-HT neuron firing (2-day and 14-day)	46
1.3 Effects of vilazodone on VTA neuron firing (2-day and 14-day)	50
1.4 Effects of vilazodone on LC neuron firing (2-day and 14-day).....	58
2.0 Effect of vilazodone on 5-HT _{1A} receptors on dorsal hippocampus CA3 pyramidal neurons.....	61
2.1 Effect of 14-day vilazodone administration on tonic activation of 5-HT _{1A} receptors	61
2.2 Effect of long-term vilazodone administration on spike suppression and RT ₅₀ .	63
DISCUSSION	65
1.0 Effects of vilazodone on the serotonin system	65
2.0 Effects of vilazodone on the dopamine system	68
3.0 Effects of vilazodone on the noradrenaline system	70
4.0 Effects of vilazodone on the hippocampus	73
5.0 Conclusion	76
REFERENCES.....	79

LIST OF TABLES

TABLE 1. Summary table of the effect of 2- and 14-day administration of vilazodone on the firing and burst activity of DRN 5-HT, LC NE, and VTA DA neurons.

LIST OF FIGURES

FIGURE 1. Reciprocal interactions between the cell bodies of DA, NE, and 5-HT neurons.

FIGURE 2. Summary of the two serotonin pathways.

FIGURE 3. Summary of the five dopamine pathways.

FIGURE 4. Summary of the two norepinephrine pathways.

FIGURE 5. Example of electrophysiological recording of DRN 5-HT neuron.

FIGURE 6. Example of electrophysiological recording of VTA DA neuron.

FIGURE 7. Example of electrophysiological recording of LC NE neuron.

FIGURE 8. Effects of acute vilazodone administration on DRN 5-HT neuronal firing.

FIGURE 9. Effects of 2-day and 14-day vilazodone administration on DRN 5-HT neurons – drug delivery comparisons.

FIGURE 10. Effects of 2-day and 14-day vilazodone administration on DRN 5-HT neurons.

FIGURE 11. Effects of 2-day and 14-day vilazodone administration on VTA DA neurons.

FIGURE 12. Effects of 2-day and 14-day vilazodone administration on number of bursts per minute in VTA DA neurons.

FIGURE 13. Effects of 2-day and 14-day vilazodone administration on percent spikes in burst in VTA DA neurons.

FIGURE 14. Effects of 2-day and 14-day vilazodone administration on mean number of spikes in burst in VTA DA neurons.

FIGURE 15. Effects of 2-day and 14-day vilazodone administration on burst duration in VTA DA neurons.

FIGURE 16. Effects of 2-day and 14-day vilazodone administration on LC NE neurons.

FIGURE 17. Effect of long-term vilazodone administration on tonic activation of 5-HT_{1A} receptors.

FIGURE 18. Effect of long-term vilazodone administration on spike suppression and RT₅₀.

FIGURE 19. Effects on an SSRI on the 5-HT_{1A} autoreceptor of DRN 5-HT neurons.

FIGURE 20. Summary of the effects of vilazodone on the presynaptic monoaminergic systems and the hippocampus.

LIST OF ABBREVIATIONS

β -OH	hydroxypropyl- β -cyclodextrin
5-HT	5-hydroxytryptamine (serotonin)
AD	antidepressant
ANOVA	analysis of variance
AP	anterior-posterior
APA	American Psychiatric Association
cAMP	cyclic adenosine phosphate
COMT	Catechol-O-methyltransferase
CSF	cerebrospinal fluid
DA	dopamine
DALY	disability adjusted life year
DAT	dopamine active transporter
DRN	dorsal raphe nucleus
DSM-V-TR	Diagnostic and Statistical Manual of Mental Disorders 5th Edition Text Revision
DV	dorsal-ventral
HAM-D	Hamilton Rating Scale for Depression
IC ₅₀	50% effective dose
<i>i.p.</i>	intraperitoneal
<i>i.v.</i>	intravenous
LC	locus coeruleus
MADRS	Montgomery-Åsberg Depression Rating Scale

MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder
ML	medial-lateral
NE	norepinephrine
NET	norepinephrine transporter
PCPA	<i>para</i> -chlorophenylalanine
RT ₅₀	50% recovery time
<i>s.c.</i>	subcutaneous
S.E.M.	standard error of mean
SERT	serotonin transporter
SNRI	serotonin-norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
VMAT	vesicular monoamine transporter
VTA	ventral tegmental area
WHO	World Health Organization

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INTRODUCTION

1.0 Major depressive disorder

1.1 Background

Major depressive disorder (MDD or depression) is a debilitating psychiatric illness affecting an estimated 120 million individuals worldwide with a lifetime prevalence of 10-15% (Lepine and Briley, 2011). The impact of depression is felt globally, as this condition solidly holds third place with regards to leading disabling conditions (in low, middle and high income countries), overcoming common conditions such as unintentional accidents and cerebrovascular events (Mathers et al, 2008). In middle to high income countries specifically, depression is cited as the leading cause of disease burden, as measured in disability adjusted life years or DALYs, providing further indications of the widespread effects of this disorder (Mathers et al, 2008). Sadly, the prognosis for MDD does not look good; within the next 20 years, it is projected that depression will become the second cause of disease burden globally, outdone only by ischemic heart disease (Mathers et al, 2008). With its widespread prevalence, depression imposes burdens not only at the individual level but on the economic stage as well. In the United States, the economic expense of depression was estimated at \$80 billion due to a combination of suicide-mortality costs, direct medical costs and workplace costs (Greenberg et al, 2003). Similar figures have been calculated for Canada, with predicted monetary losses as high as \$60 billion often cited (Stephens and Joubert, 2001).

The diagnosis of depression is based upon the American's Psychiatric Association's (APA) DSM-V, which states that the patient must experience depressed mood or anhedonia for a minimum of 2 weeks on most days for most of the day; this behaviour must differ from baseline behavioural patterns to qualify for diagnosis (DSM-V; APA, 2013). Furthermore, the individual must demonstrate some level of impaired function, either at the social, occupational or educational level, due to these depressive symptoms (DSM-V; APA, 2013). Mood changes as a result of the physiological effects of an existing medical condition, under the influence of illicit substances or directly following the death of a loved one are excluded from the MDD diagnosis (DSM-V; APA, 2013). Lastly, at least five of the following nine specific symptoms must be present for the majority of the time: daily feelings of depressed mood, anhedonia, significant change in appetite/weight, change in sleep (insomnia or hypersomnia), change in activity level (psychomotor agitation or retardation), fatigue, feelings of guilt or worthlessness, issues with concentration and/or suicidality. Following diagnosis, the severity and extent of depression can also be assessed, typically utilizing self-assessments of mental state and symptom severity. However, this can pose some difficulties, as these assessments are highly subjective, which can result in issues with reliability and objectivity. To standardize severity assessments, two common rating scales are utilized in clinical practice: the Montgomery-Åsberg Depression Rating Scale (MADRS, Montgomery and Åsberg, 1979) and the Hamilton Depression Rating Scale (HAM-D, Hamilton, 1960).

Once diagnosis is reached and severity discerned, treatment of depression can begin, mostly in the form of antidepressant (AD) treatments. A patient is considered to be responsive to treatment if they demonstrate a 50% improvement in their HAM-D or MADRS score (Nierenberg et al, 2003). However, it is important to remember that ‘response’ does not translate to complete disappearance of symptoms, known as remission, and that these individuals often still experience the debilitating effects of MDD (Thase, 2003). In fact, individuals who are considered “responsive” to treatment have been shown to still exhibit subsyndromal depressive symptoms and often face significant psychological burden despite being labelled as responsive to treatment (Thase, 2003).

The primary aim of AD treatment is to fully restore function in individuals by achieving a state of remission, as mentioned above, in which patients are asymptomatic or present with minimal residual symptoms (Frank et al, 1991; Nierenberg et al, 2003). Ideally, remission would indicate full resolution of MDD but this is often not the case as only one third of patients are considered in remission following first line therapy (Thase, 2003; Trivedi and Daly, 2008). Furthermore, even extensive rounds of AD treatment have not been shown to be fully effective as only two thirds of patients were shown to be in remission following four trials of therapy (Trivedi and Daly, 2008). Those who do not remain in remission are considered to be relapsing (episode of depression occurring within 6 months following response/remission) or to have recurrence (occurs after 6 months) (Nierenberg et al, 2003). These individuals may require several trials of treatment with varying AD types. Additional treatments steps are often associated with poorer

outcomes, as individuals who require one or more treatments experience lower acute remission rates as well as higher relapse rates (Rush et al, 2006).

As with most medical interventions, AD treatment poses an array of side effects which can have significant impacts on patients. Side effects vary based on the specific AD used and can include tachycardia, sexual dysfunction, insomnia, gastrointestinal upset, weight gain and a variety of others (Goodman et al, 2011). These adverse effects of ADs not only pose a nuisance to patients but have been shown to significantly reduce self-assessments of quality in both adolescents and adults with depression (Cheung et al, 2003). Furthermore, side effects have been linked to lower compliance rates for ADs. Adverse events such as fatigue, sexual dysfunction and weight gain are commonly cited amongst patients who admit to being noncompliant with their AD medications (Ashton et al, 2005). Discontinuation of ADs without proper medical supervision does not only pose issues with effective management of MDD but can have further medical consequences as well as up to 20% of individuals experience “discontinuation syndromes” following the abrupt cessation of AD usage (Warner et al, 2006). These syndromes have been associated with several classes of ADs and produce flu-like symptoms, sensory disturbances, hyperarousal, and a variety of other unpleasant symptoms, which can influence future medication compliance as well as quality of life (Warner et al, 2006). Lastly, discontinuation of AD treatment has been shown to increase the risk for suicidal behavior as much as five fold, indicating that effective management of AD side effects is essential (Yerevanian et al, 2004).

While ADs have revolutionized the treatment of MDD, there is clearly still much work to be done to ensure higher remission rates and effective management of medication side effects. A new rising development in the AD field aims to fulfil this niche: multimodal medications which act on several pharmacological targets synergistically. Early studies have shown some promise for these compounds within the realm of MDD, as some multimodal ADs have been shown to provide significantly higher rates of remission in comparison to more traditional compounds (Thase, 2003). This provides an exciting new direction for MDD and AD treatment strategy research.

1.2 Etiology

Due to the heterogeneous nature of depression, a specific etiology has been difficult to pinpoint and remains elusive (Hasler, 2010). MDD can present differently in many patients, both in terms of expression of symptoms and age of onset, as well as other factors such as interactions with other psychiatric and somatic illnesses, which presents challenges in determining specific causes of illness (Brigitta, 2002). Furthermore, animal models often prove ineffective for the study of depression; clinically this disorder is characterized on the basis of subjective physician assessments and self-assessments which is difficult to recreate with non-human subjects (Duman, 2010). Current animal models focus strongly on the quantifiable measures of depression, such as psychomotor disturbances or appetite changes, often neglecting the more prominent and cardinal subjective symptoms which may decrease the relevance of these models to human patient counterparts (Duman, 2010).

Despite the above mentioned difficulties, research has provided some insight into the disorder as current research has elucidated strong linkages with both environmental factors and genetic factors with regards to the pathophysiology of depression (Brigitta, 2002; Caspi et al, 2003). Population based and twin based studies both suggest a strong genetic link for depression; some studies quote numbers as high as 37% for the heritability of the disorder with strong familial aggregations that cannot be explained by shared environmental influences alone (Sullivan et al, 2000). Genetic studies have even identified specific polymorphisms in the genes coding for the 5-HT transporter (5-HTT) which when present may predispose individuals for depression and other affective disorders, further solidifying the genetic contributions to MDD (Collier et al, 1996; Karg and Sen, 2012).

As with many disorders, MDD commonly does not exist in a vacuum and is subject to the external influences of one's environment and experiences. In fact, research has indicated that the impact of non-genetic factors with respect to depression appear to be much greater than that of other MDD's such as bipolar disorder, indicating both the presence and magnitude of influence environmental factors may have on this illness (Malhi et al, 2000; Brigitta, 2002). It appears as though MDD involves a complex interplay between both environment and genetic factors (Sullivan et al, 2000). For instance, individuals with a specific polymorphism for the 5-HTT gene that is associated with greater risk for MDD were found to respond more severely to traumatic life events indicating the

requirement of both pre-disposing genetic factors and precipitating environmental influences (Caspi et al, 2003).

2.0 Monoamine hypothesis

While the etiology of depression has not been fully elucidated, much of the current research focuses on the involvement of the monoamine systems: serotonin (5-HT) from the dorsal raphe nucleus (DRN), dopamine (DA) from the ventral tegmental area (VTA) and noradrenaline (NE) from the locus coeruleus (LC) (Guiard et al, 2008). This emphasis is warranted, as these three systems have been shown to play a significant role in MDD. Studies from as early as the 1960's noted that compounds with monoamine depleting ability such as the anti-hypertension medication reserpine could induce motor retardation and behavioural changes in both animal models and humans whilst compounds such as iproniazid, which are able to elevate monoamine levels, produced positive effects on mood (Schildkraut, 1965; Delgado 2000; Krishnan and Nestler, 2008). Once the association between monoamine depletion and mood disturbances was detected, more direct depletion studies were initiated to fully assess the effects of depletion on a wide spectrum of individuals. Serotonin depletion studies utilizing tryptophan depletion produced significant depressive mood changes in individuals previously diagnosed with depression (Moreno et al, 1999, Delgado 2000). Depletion of all three monoamines using the tryptophan hydroxylase inhibitor alpha-methyl-p-tyrosine was able to produce significant increases in HAM-D scores in individuals with a history of depression (Berman et al, 1999).

Studies assessing the amount of 5-HT in CSF have also been helpful in further strengthening the linkage between monoamines and MDD's; individuals with depression report lower 5-HT levels than control subjects (Carpenter et al, 1998; Delgado 2000; Hou et al, 2006). Furthermore, CSF levels of monoamines appear to be somewhat linked to severity as well, as patients experiencing more severe MDD and a high likelihood of suicidal intent were found to have even lower CSF 5-HT levels than their depressed but non-suicidal counterparts (Hou et al, 2006). As well, lowered levels of 5-HT were noted in the CSF of healthy individuals with a family history of depression, indicating a possible genetic component (France et al, 2007). Alterations in other monoamines, such as DA and NE have also been detected in depressed patients, implicating not only 5-HT as an important monoamine in MDD (Christensen et al, 1980; Gerner et al, 1984). Based on these observations, the 'monoamine hypothesis' has been postulated, which states simply that decreases in levels of monoamines may be responsible for MDD (Brigitta, 2002; Krishan and Nestler, 2008). This is a rather simplistic view of the situation, as more modern interpretations of the monoamine hypothesis further explore the effects of receptor upregulation, neurotransmitter synthesis and degradation as well as alterations in genetic regulation (Krishnan and Nestler, 2008). Nonetheless, the basic principles remain the same; various alterations of the monoaminergic system may be associated or even the causative factor of MDD.

As with many other neural structures, the monoamine regions share intricate connections and reciprocal interactions and are thus able to alter each other's activity significantly (Refer to Figure 1, adapted from Guiard et al, 2008). For instance, when dorsal raphe nucleus (DRN) 5-HT neurons are lesioned, the firing rate of locus coeruleus (LC) NE neurons increases by 70% while the reverse; a lesion of the NE neurons, induces erratic, low firing of DRN 5-HT neurons (Guiard et al, 2008). Further connections are highlighted in Figure 1 (adapted from Guiard et al, 2008). These reciprocal interactions must be considered when studying the relationship between MDD and the monoaminergic systems, as they can significantly impact activity and firing levels.

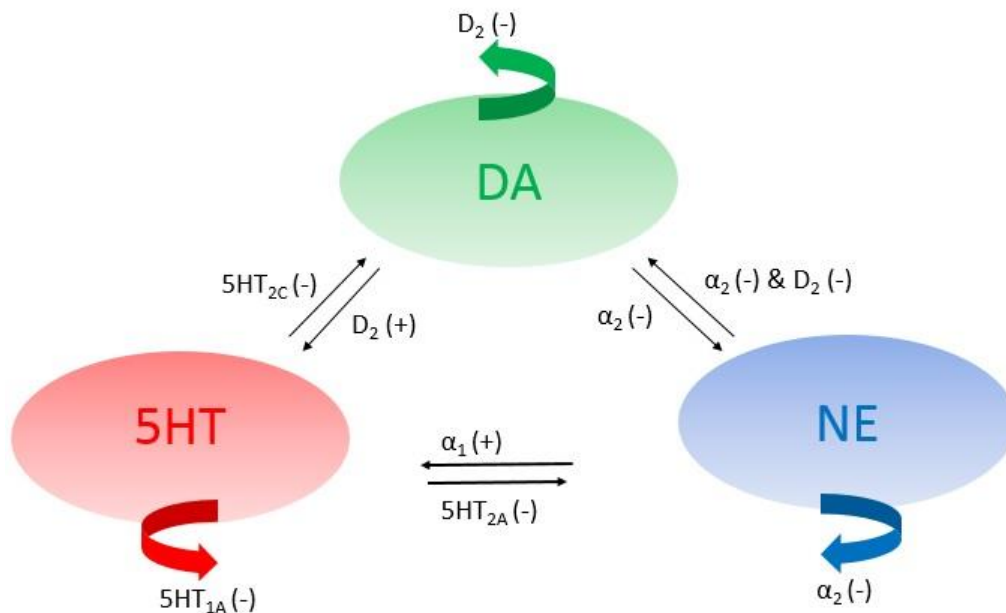


Figure 1. Reciprocal interactions between the cell bodies of DA, NE, and 5-HT neurons. A positive symbol (+) indicates a stimulatory effect while a negative symbol (-) indicates inhibitory effects. Autoreceptors are represented by arrows present on the cell body. Adapted from Guiard et al, 2008.

While the monoamine hypothesis provides some insight into the pathophysiology of depression, it still leaves many questions unanswered. For instance, due to the important role of monoamines in the pathophysiology of MDD,

one would speculate that depressive symptoms could be induced in healthy individuals following monoamine depletion, as this would mimic the brain chemistry of those with MDD (Delgado, 2000). However this was not the case, as most healthy individuals experienced no changes in mood following depletion, even when both 5-HT and NE are depleted (Salomon et al, 1997; Moreno et al, 1999, Delgado, 2000). Interestingly, healthy controls with a significant family history of depression experienced significant alterations in mood following monoamine depletion, indicating possible genetic linkages which may modulate the effects of monoamine alterations on affect (Klaassen et al, 1999). Nonetheless, while depletion studies indicate a strong monoaminergic role with regards to the etiology of depression, these brain structures are not the only ones at play when it comes to MDD.

It is thought that the effects of monoamines may not be solely related to quantifiable changes in concentration, but downstream transmission effects as well, as the monoaminergic systems project to a variety of brain regions, including the amygdala, prefrontal cortex, hippocampus and others (Drevets et al, 2008). For instance, antidepressant medications are able to elevate monoamine levels to significant levels almost immediately, but patients still experience a 3-6 weeks lag in terms of symptomatic relief (Krishnan and Nestler, 2008). It may be that alterations in monoamine levels have lengthier and complex downstream effects on transmission in the above mentioned projection areas, which in turn may alter information processing and result in MDD symptomology (Krishnan and Nestler,

2008). Thus, the monoamine hypothesis serves as only one piece of the complex puzzle and must be integrated with other etiological factors.

2.1 Serotonin

Serotonin (5-HT) has long been associated with MDD. This neurotransmitter plays a role in many mood related functions and has been linked to the regulation of anger, memory, stress and addiction (Berger et al, 2009). 5-HT also plays a role in non-cognitive functions, such as motor control and sleep rhythms (Berger et al, 2009). 5-HT is well dispersed in the brain and has been found to innervate almost all brain nuclei, with the greatest concentrations found along the midline in the raphe (Charnay and Leger, 2010). The main 5-HT pathways can be divided into two branches, which are further explained in Figure 2.

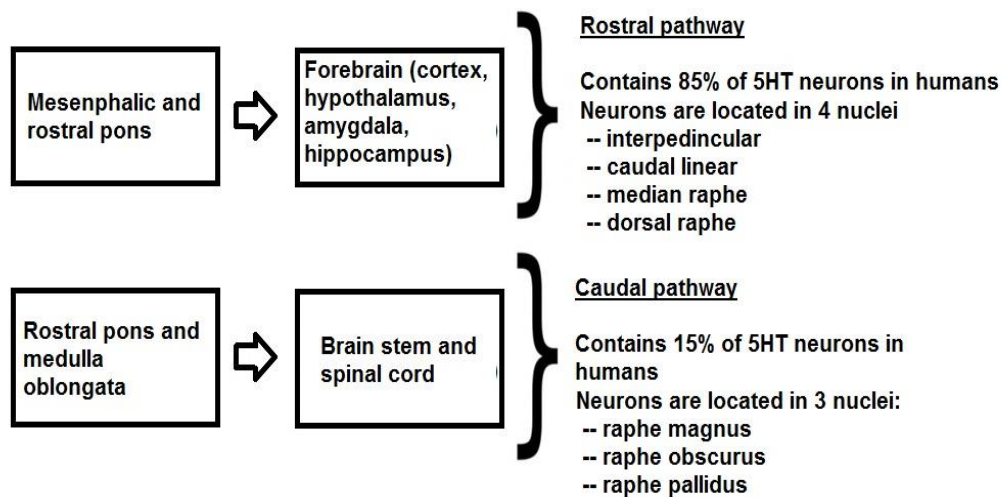


Figure 2. Summary of the two serotonin pathways. Adapted from Charnay and Leger, 2010.

5-HT synthesis requires the presence of tryptophan, with the enzyme tryptophan hydroxylase 2 catalyzing the conversion (Walther and Bader, 2003;

Charnay and Leger, 2010). Genetic variations in this essential enzyme have been associated with mood disorders, indicating its importance with respect to MDD (Matthes et al, 2010). Following synthesis, 5-HT is stored in vesicles, which is facilitated via the vesicular monoamine transporter (VMAT), awaiting a neuronal impulse to stimulate release (Parsons, 2000). Storage is highly regulated and responds to changes in pH as well as thermodynamic changes within the cell (Parsons, 2000). Once released, 5-HT can be re-absorbed back into the neuron via the serotonin reuptake transporter or SERT, which is a predominant drug target in the treatment of MDD (Owens and Nemeroff, 1994; Kroeze and Roth, 1998). Following reuptake, excess 5-HT is metabolized by monoamine oxidase (MAO), which acts in minutes to break down 5-HT, thus lowering its concentration inside the cell (Sirek and Sirek, 1970).

Following release, 5-HT can act on a variety of serotonergic receptors, as this class of receptors contains 14 different sub-types; each class has been found to play a different role (Barnes and Sharp, 1999). For instance, 5-HT_{1B} receptors have are thought to be involved in motor control and attention and have been speculated to be associated with disorders such as attention-deficit hyperactivity disorder (Maroteaux et al 1992; Mill et al, 2005). The 5-HT₂ receptor has been associated with mood disorders, as suicide victims have been found to have increased 5-HT₂ binding sites in comparison to controls (Arango et al, 1990). Specifically, the 5-HT_{2A} receptor has been associated with schizophrenia and cognitive decline whilst the 5-HT_{2B} receptor is more strongly associated with anxiety (Kennett et al, 1996; Umbricht et al, 2003; Doly et al, 2009). The 5-HT₃ receptor shows implications in

a variety of systems, including the gastrointestinal system, the pain system and many others (Farber et al, 2004). The 5-HT₇ receptor has been speculated to be involved in epilepsy and pain, although studies remain unclear on the exact extent of its role (Hedlund, 2009). However, its role in depression shows stronger evidence, as drugs that target this receptor display antidepressant properties in both animal and human studies (Hedlund, 2009).

The main focus with respect to MDD has been on the 5-HT_{1A} receptor. The distribution of this receptor has been shown to be high in several regions of the brain, particularly the limbic areas such as the hippocampus as well as the median and dorsal raphe nuclei (Barnes and Sharp, 1999). Thus, these receptors are located both presynaptically on the 5-HT releasing neurons of the raphe as well as postsynaptically within forebrain regions such as the hippocampus.

The presynaptic 5-HT_{1A} receptor is located on both the soma and dendrites of 5-HT neurons with the dorsal raphe nucleus and are coined as autoreceptors, as they are responsive to the neurotransmitter release from the neuron upon which they are located (Barnes and Sharp, 1999; Mannoury la Cour et al, 2006). When 5-HT binds to these receptors, it activates the G_{ai3} protein which inhibits adenylate cyclase, resulting in decreased 5-HT release as well as a decrease in overall neurotransmission (Sharp and Hjorth, 1990; Raymond et al, 1993; Mannoury la Cour et al, 2006). The postsynaptic 5-HT_{1A} receptor shows similar structural properties as the presynaptic receptor, but differs in pharmacology and location. This receptor is found primarily in forebrain regions, such as the hippocampus and unlike the presynaptic receptor, it appears to utilize second messengers such as

inositol triphosphate for signal conduction purposes (Claustre et al, 1991). Furthermore, this receptor appears to activate a $G_{\alpha o}$ protein, which in turn hyperpolarizes the cell via its ability to directly open potassium channels, thus allowing the efflux of positive ions (Luscher et al, 1997).

Despite these differences, both the presynaptic and postsynaptic 5-HT_{1A} receptors have been strongly implicated in MDD. For instance, PET imaging studies noted 5-HT_{1A} receptor binding was decreased in patients with depression, with the most prominent decreases occurring in the area of the midbrain raphe, indicating perhaps the involvement of the presynaptic 5-HT_{1A} receptors (Drevets et al, 1999). Further studies have determined that suicidal individuals experience decreased binding in the dorsal raphe nucleus specifically when compared to control, thus further implicating the presynaptic receptors (Stockmeier et al, 1998; Boldrini et al, 2008). With respect to postsynaptic 5-HT_{1A} receptors, studies have found significant genetic variability in depressed individuals as they display decreased mRNA expression of the 5-HT_{1A} receptor within the hippocampus (Lopez-Figueroa et al, 2004). Interestingly, long term treatment with common antidepressants such as tricyclics results in changes within the postsynaptic 5-HT_{1A} receptors, further indicating their involvement in MDD. These receptors experience enhanced tonic activation, and as a result enhanced activity and sensitivity, which in turn leads to positive changes in 5-HT neurotransmission (Haddjeri et al, 1998). Due to the involvement of both types of 5-HT_{1A} receptors in MDD, it is important to consider both when assessing for novel antidepressant agents.

2.2 Dopamine

Dopamine is a modulatory neurotransmitter with a variety of systemic effects; it is implicated in sensory detection, motor function, hormone modulation and a variety of other pathways (Sidhu et al, 2003; Bressan et al 2005). With respect to mood, dopamine has been found to be essential in the regulation of reward and pleasure pathways and lowered levels of this neurotransmitter have been associated with anhedonia, a cardinal symptom of MDD (Swanson, 1982; Bressan et al, 2005). Specifically, DA has been found to be associated with MDD in several pre-clinical and clinical studies (Dunlop and Nemeroff, 2007). Furthermore, AD agents which specifically target DA have been shown to produce favourable remission responses, particularly towards the symptoms of depression that can be easily associated with pathological DA alterations such as loss of pleasure or motivation, further implicating this neurotransmitter and MDD (Nutt et al, 2007).

Synthesis of DA occurs much like its fellow catecholamine norepinephrine: it is synthesized from the amino acid tyrosine and L-amino acid decarboxylase in the nerve terminal of dopamine neurons (Dunnett, 2005; Dunlop and Nemeroff, 2007). Following synthesis, the neurotransmitter is packaged into vesicles via VMAT and stored until stimulated for release by an action potential. (Dunnett, 2005; Dunlop and Nemeroff, 2007). The firing in DA neurons can occur either in single spike patterns or in bursts; bursting patterns are of particular significance as they have been associated with the modulation of DA release (Gonon, 1988). Bursting has also been implicated in MDD: animal models of depression were

found to have decreased VTA DA neuron bursting, specifically long bursts associated with DA release, in comparison to controls (Friedman et al, 2008).

Following DA release, the neurotransmitter can either be taken back up into the synapse via DAT in the basal ganglia or NET in the prefrontal cortex (Devoto, 2001; Dunlop and Nemeroff, 2007; Guiard et al, 2008). PET scan studies have found significant reductions in levels of DAT in the bilateral caudate and putamen of depressed patients in comparison to controls, suggesting that MDD is able to down-regulate the expression of this transporter (Meyer et al, 2001a).

Dopaminergic neurons originate primarily from brainstem nuclei, such as the VTA and SN pars compacta and project to a variety of brain regions (Dunlop and Nemeroff, 2007). The five predominant dopamine pathways include the nigrostriatal, mesolimbic, mesocortical, tuberoinfundubular and incertohypothalamic pathways (summarized in Figure 2, Meador-Woodruff et al, 1991; Dunlop and Nemeroff, 2007). The thalamic pathway is only found in humans and has not been found in rats, thus in rats typically only four pathways are observed (Sanchez-Gonzalez et al, 2005).

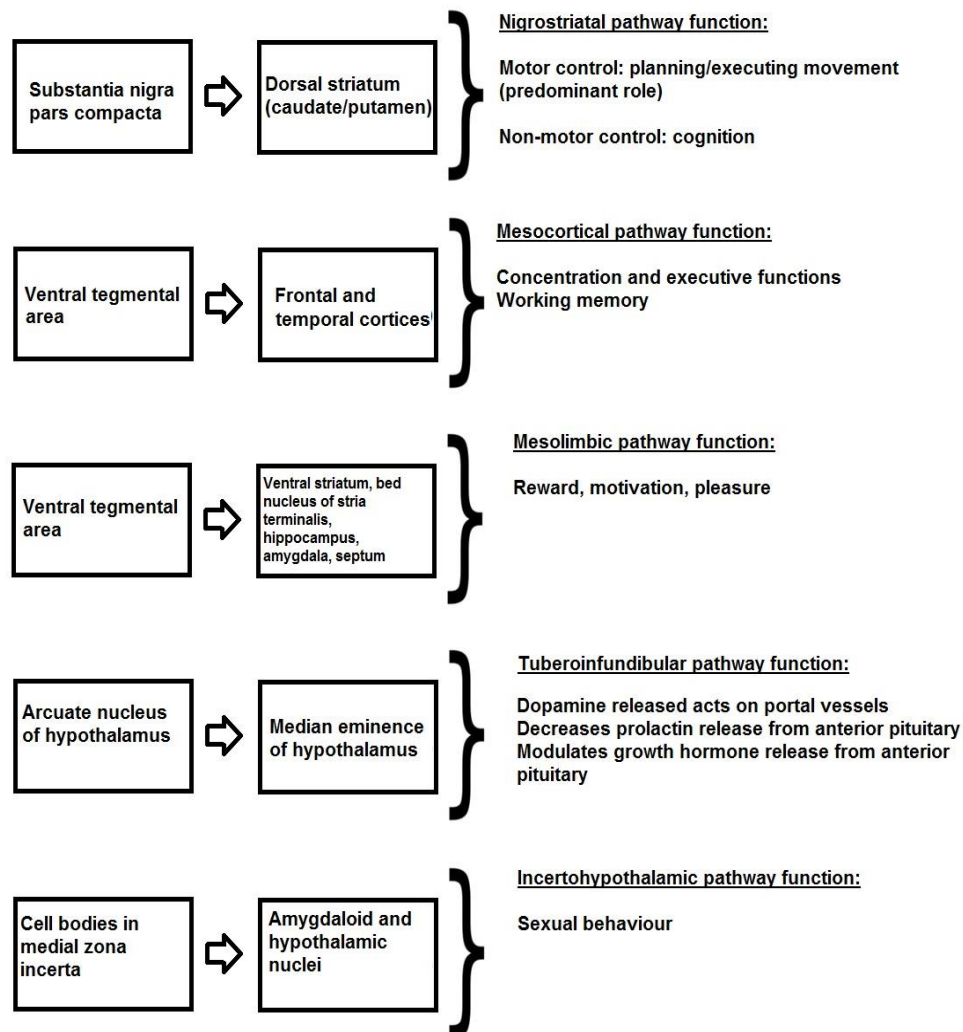


Figure 3. Summary of the five dopamine pathways. Adapted from Dunlop and Nemeroff, 2007.

Two functionally and structurally distinct classes of DA receptors exist: D₁-family and D₂-family receptors, although both are classified as G-protein coupled receptors, specifically G_{sα} and G_{iα} proteins respectively (Siddhu et al, 2003). Each class can further be divided, with the D₁-family incorporating the D₁ and D₅ subtypes and the D₂-family the D₂, D₃ and D₄ subtypes (Dunlop and Nemeroff, 2007). When activated, the D₁ receptors activate adenylate cyclase, which results in increased levels of cAMP, a second messenger which has many downstream

effects (Siddhu et al, 2003). Activation of D₂ has the opposite affect by inhibiting adenylate cyclase and thus lowering cAMP thereby reducing downstream effects (Siddhu et al, 2003). Both receptor subtypes have been previously implicated in MDD. For instance, PET scans conducted on patients with MDD with anger attacks noted lower D₁ binding in both the left and right striatum, indicating possible dopaminergic changes in these individuals (Dougherty et al, 2006). Furthermore, specific polymorphisms in the gene associated with the D₂ receptor have been found to correlate strongly to the presence of depression (Peroutka et al, 1998).

2.3 Norepinephrine

Norepinephrine (NE) is a catecholamine found in the central nervous system and is predominantly responsible for arousal and waking, although it has been implicated in memory and learning as well (Mason, 1981; Berridge and Waterhouse, 2003). The predominant NE pathways are summarized in Figure 2 (adapted from Mason, 1981).

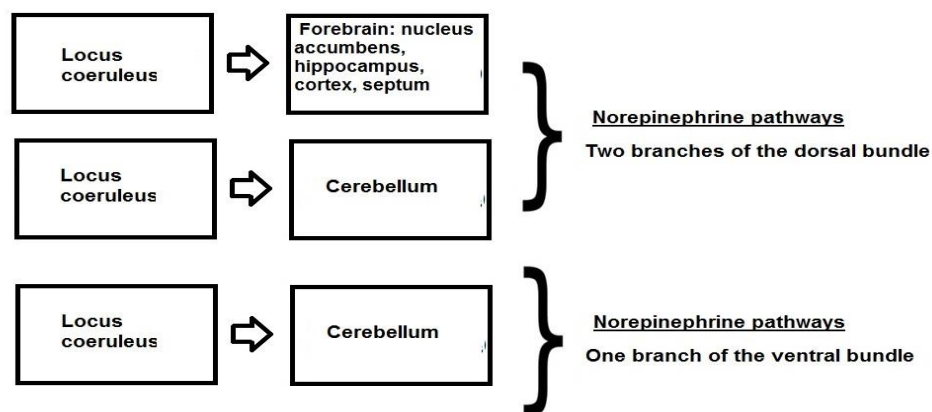


Figure 4. Summary of the two norepinephrine pathways. Adapted from Mason, 1981.

Agents which are able to block NE reuptake, resulting in increased levels of the monoamine synaptically, are able to elicit an antidepressant effect, indicating a connection between NE and MDD (Anand and Charney, 2000). Furthermore, post-mortem analysis of MDD patients indicated significant down-regulation of the NE transporter responsible to reuptake; this is most likely due to the low levels of NE present in these individuals and provides further evidence of the involvement of NE in mood disorders (Klimek et al, 1997). Specifically, it appears as though deficiencies in the NE system are involved in the psychomotor symptoms of depression, such as motor retardation and fatigue (Brunello et al, 2002). Not surprisingly, individuals treated with antidepressants that target the NE system experience improvements in these psychomotor symptoms (Brunello et al, 2002).

Synthesis of NE is based on the amino acid tyrosine, with dopamine acting as an intermediary step, and requires the enzyme tyrosine hydroxylase to catalyze the reaction (Robertson, 2004). NE can also be synthesised from the monoamine dopamine via the enzyme dopamine- β -hydroxylase (Iversen, 1967). Following synthesis, the neurotransmitter is stored in granules located at nerve endings utilizing the VMAT transporter (Robertson, 2004).

Once release of NE is initiated, this versatile neurotransmitter can act on two categories of adrenergic receptors: α -receptors and β -receptors (Furchgott, 1967). These receptors have a variety of effects on many different systems within the body (Furchgott, 1967). Within the α -receptor family, the α_1 -adrenergic receptors are generally excitatory, acting on G_q proteins to stimulate PLC and induce many downstream effects, such as smooth muscle contraction as well as a

variety of other systemic effects (Langer et al, 1985; Cooper et al, 2003). The α_2 -adrenergic receptors are typically inhibitory, acting on a $G_{i/o}$ protein which in turn inhibits adenylate cyclase and allows for many neuronal and organ-specific effects (Cooper et al, 2003). The β -receptors are linked to a G_s protein which stimulates adenylate cyclase which in turn results in several systemic downstream effects (Cooper et al, 2003).

With respect to MDD, the α_2 -adrenergic receptor has been shown to be quite involved. For instance, MDD patients display reduced efficacy with respect to their α_2 -adrenergic receptor; the application of specific agonists in these individuals is unable to induce as robust a response both in heart rate and NE levels as that in control subjects (Siever and Uhde, 1984). These results have been confirmed in animal studies, which indicated that depressed animal models displayed decreased α_2 -adrenergic receptor both in the locus coeruleus and in limbic regions (Simson et al, 1986; Ordway et al, 1999). While current research predominantly focuses on this sub-group of adrenergic receptors, there have been other receptors in this family that have also been implicated in MDD. For instance, the α_1 -adrenergic receptor has been found to be upregulated in unmedicated depressed patients, hinting at a possible relationship with MDD (Ordway et al, 2003).

3.0 Pharmacological treatment for depression

As previously mentioned, the predominant treatment strategy for depression involves the use of pharmacological agents known as antidepressants (ADs). The current classes of ADs can be subdivided into three categories: first, second and third generation ADs (Millan, 2004). The first generation of antidepressants was

introduced in the 1950s and comprised of two medications: TCAs and MAOIs (Coccaro and Siever, 1985). Second generation medications focused largely on targeting monoaminergic reuptake and are comprised of SSRIs and SNRI's (Millan, 2004). Lastly, the newest line of ADs are labelled as third generation and consist of a variety of novel monoaminergic and non-monoaminergic targets (Millan, 2004).

3.1 Tricyclics (TCAs)

The use of TCAs has declined significantly with the invention of newer and more tolerable medications; this class has been primarily relegated to usage in treatment resistant individuals or those with severe depression (Rudorfer and Potter, 1999; Burton, 2006). The most common pharmacological mechanism for these ADs appears to be via blockage of the NET and SERT to prevent the reuptake of NE and 5-HT respectively; thus mainly acting as SNRIs (Blier and de Montigny, 1983; Tatsumi et al, 1997; Pineyro and Blier, 1999). The end result of reuptake blockage is increased levels of NE and 5-HT, which in turn can have downstream effects on neurotransmission. For instance, post-synaptic hippocampal 5-HT_{1A} receptors show significant sensitization following TCA treatment (de Montigny and Aghajanian, 1978; Blier and de Montigny, 1994). Interestingly, TCA does not produce desensitization of 5-HT_{1A} somatic autoreceptors, a common effect of many other ADs (Blier and de Montigny, 1980; Pineyro and Blier, 1999). TCA treatment was not found to be able to significantly alter the genetic expression of 5-HT receptors, thus this sensitization may occur as a result of post-translational changes rather than changes in mRNA expression (Yau et al, 1999). As well, TCAs have been shown to induce increased responsiveness to NE in the amygdala and other

brain regions, which has been shown to have positive effects with respect to mediating MDD (Blier and de Montigny, 1994).

However, TCAs show considerable pharmacological variability; for instance the TCA imipramine shows considerably higher SERT binding while desipramine, which is also classified as a TCA, binds more favourably to NET (Tatsumi et al, 1997; Gillman, 2007). Further pharmacological effects include blockage of the α_1 -adrenergic receptor, histamine receptor and cholinergic receptors; these widespread pharmacological effects can result in a variety of side effects associated with TCAs (Goodman et al, 2011). Patients may experience dry mouth, constipation, vision issues and cognitive issues due to the anti-cholinergic effects (Rudorfer and Potter, 1999). Cardiovascular effects can be quite severe as they include arrhythmias and alterations in heart conduction (Rudorfer and Potter, 1999). Due to these side effects, TCAs are no longer considered a first line treatment for depression (Burton, 2006).

3.2 Monoamine Oxidase Inhibitors (MAOIs)

The second AD in the first generation AD category is the MAOI compound (Millan, 2004). These compounds work by inhibiting the enzyme MAO, which is found systemically, resulting in an increase in monoamine levels, as this enzyme is responsible for their breakdown (Fiedorowicz and Swartz, 2004; Yamada and Yasuhara, 2004). The two MAO subtypes known as MAO-A and MAO-B both present with different pharmacological properties. MAO-A is predominantly involved in the reuptake of 5-HT and NE while both isoforms are able to induce reuptake of DA (Hall et al., 1969; Yang and Neff, 1974; Fiedorowicz and Swartz,

2004). With respect to ADs, most MAOIs act on the MAO-A isoform while the MAO-B isoform shows no significant therapeutic benefit (Mann et al, 1989; Blier and de Montigny, 1994).

The main mechanism of action with this AD is similar to that of TCAs: due to inhibition of monoamine degradation, increased levels of neurotransmitter are present in the synapse, specifically 5-HT and NE. MAOIs have been found to increase monoamine levels in both the DRN as well as projection areas such as the hippocampus (Celada and Artigas, 1993). The increased levels of 5-HT activate the 5-HT_{1A} autoreceptor, resulting in decreased firing following acute treatment. However, long term treatment induces a desensitization in the autoreceptor, allowing firing levels to recover completely, thus maintaining 5-HT release (Blier et de Montigny, 1985). The postsynaptic hippocampal 5-HT_{1A} receptor is also altered following long term MAOI treatment, showing enhanced tonic activation and as a result, enhanced 5-HT neurotransmission (Haddjeri et al, 1998). While NE firing in the LC does decrease significantly following MAOI treatment, unlike for 5-HT neurons there is no effect on somatic autoreceptor sensitivity, thus this decrease is sustained and there is no recovery of firing following long term treatment (Blier et de Montigny, 1985). Long term MAOI treatment does however produce desensitization of the α_2 -adrenoceptor, which in turn results in decreased NE-mediated inhibition of 5-HT release from CA3 pyramidal hippocampal neurons, producing an overall increase in 5-HT neurotransmission (Mongeau et al, 1994). MAOIs are also able to alter DA transmission, decreasing both firing and bursting activity, most likely through the 5-HT₃ receptor (Chenu et al, 2009).

With the advent of new classes of ADs, MAOIs are not as commonly prescribed as they once were, as they bear significant side effects particularly with respect to cardiovascular events and hypertension (Yamada and Yasuhara, 2004; Goodman et al, 2011). Nonetheless, these compounds still play a significant role in the treatment of depression and may be best suited for certain populations of patients, such as those who are identified as treatment resistant (Amsterdam and Shults, 2005).

3.3 Selective Serotonin Reuptake Inhibitors (SSRIs)

The introduction of SSRIs in the 1980's significantly revolutionized the AD world and this medication remains the current first line treatment for MDD (Slattery et al, 2004; Gelenberg, 2010). The mechanism of action of these compounds revolves around the effect of increased 5-HT levels, as with many ADs. SSRIs block SERT, resulting in increased levels of 5-HT synaptically (Blier and de Montigny, 1994). Acutely, this increase produce a decrease in firing, as the 5-HT can act upon inhibitory 5-HT_{1A} autoreceptors. However, much like with MAOI's, these autoreceptors become desensitized and firing recovers following long term treatment, resulting in increase in 5-HT levels and neurotransmission (Blier and de Montigny, 1994). While this effect is seen in the DRN 5-HT_{1A} autoreceptor, no such desensitization is observed in the hippocampal post-synaptic 5-HT_{1A} heteroreceptor following long term treatment (Chaput et al, 1991). However, SSRIs do appear to be able to increase 5-HT transmission in the forebrain, including the hippocampus, as they are able to increase tonic activation and thus neurotransmission (Haddjeri et al 1998).

The effects of SSRI are not solely limited to the 5-HT_{1A} receptor. In fact, SSRIs have been shown to activate the 5-HT₇ receptor as well, which can induce increases in 5-HT firing when the inhibitory effects of the 5-HT_{1A} receptor are blocked (Bosker et al, 2009; Mnie-Filali et al, 2011). Long term administration of SSRIs is able to affect other monoaminergic systems as well and has been shown to cause decreases in NE firing and both DA firing and bursting (Szabo et al, 1999; Dremencov et al, 2009).

The success of SSRIs is based on both its efficacy at alleviating MDD symptoms as well as its general tolerability (Stahl, 1998). The most common side effects of SSRIs include insomnia, nausea and sexual dysfunction, although there is some evidence of adaptation to these some of these effects (Stahl, 1998; Goodman et al, 2011). Nevertheless, SSRIs are better tolerated than other ADs such as TCAs and also show better patient compliance (Anderson and Tomenson, 1995; Brambilla et al, 2005). The success of SSRIs has led to the creation of other monoaminergic reuptake inhibitors, such as SNRI's and triple reuptake inhibitors, which may provide further strategies for MDD treatment (Blier, 2006; Guiard et al 2009).

3.4 5-HT_{1A} agonists

The 5-HT_{1A} receptor is often discussed with respect to MDD and ADs. For instance, the 3-6 week time lag between treatment initiation and symptom relief experienced by patients undergoing SSRI is thought to be due to the presynaptic desensitization process of this receptor (Blier et al, 1990; Blier and de Montigny, 1994). Furthermore, imaging studies have found functional differences in the 5-

HT_{1A} receptor of depressed patients in comparison to controls; depressed individuals experience a robust and significant decrease in the binding potential of 5-HT_{1A} receptors in both the dorsal raphe and mesotemporal cortex (Drevets et al, 2008). Thus, it is not surprising that during the development of third generation of ADs, a heavy focus was placed on this receptor and its agonistic agents (Millan, 2004).

Following the administration of a 5-HT_{1A} receptor agonist, effects similar to those of SSRIs are observed, indicating that these compounds may also be able to positively alter neurotransmission. In presynaptic areas, such as the dorsal raphe, firing initially decreases, as the agonist acts on inhibitory autoreceptors. However, following long term treatment, the receptor desensitizes and firing rates recover (Blier and de Montigny, 1987; Blier and Ward, 2003). In postsynaptic areas such as the hippocampus, no such desensitization is seen, although enhanced tonic activation does occur (Blier and de Montigny, 1987; Haddjeri et al, 1998).

Preclinical trials have also elucidated that the usage of 5-HT_{1A} agonists may have beneficial effects, as treatment with 5-HT_{1A} agonists has successfully reduced behavioural deficits in animal models of depression (Kennett et al 1987). As well, the benefit of 5-HT_{1A} agonists during the forced swim test, a common stressor used in the study of depression, were comparable to that of other antidepressants such as tricyclics; 5-HT_{1A} agonists were equally effective in decreasing immobility times during the testing (Wieland and Lucki, 1990). Clinically, 5-HT_{1A} agonists have been found to be successful in treating depression in patients and may even be associated with fewer side effects, given that patients reported fewer complaints of

sedation and sexual dysfunction (Robinson et al, 1990; Feiger et al, 2003). However, while 5-HT_{1A} agonists do appear to have promising results, they show no clear indications for becoming a first-line treatment strategy (Heiser and Wilcox, 1998). The most significant role for these medications appears to be as an augmentation strategy, in particular in combination with SSRIs and for the treatment of resistant or severe MDD (Artigas et al, 1996; Landen et al, 1998; Trivedi et al, 2006).

The role of 5-HT_{1A} agonists with respect to augmentation involves the ability of the 5-HT_{1A} autoreceptor to desensitize. Typically, with SSRIs, the time course for this desensitization results in a 3-6 week time lag between medication administration and effective treatment control (Blier et al, 1990). The addition of a 5-HT_{1A} agonist could reduce this delay because, as these agents could immediately stimulate the 5-HT_{1A} receptor, thus promoting faster desensitization and enhancement of transmission than that of an SSRI alone (Blier et al, 1997). Preclinical studies have provided some evidence for this. For instance, low doses of the 5-HT_{1A} agonist buspirone in combination with SSRIs have been shown to produce more robust changes in immobility during forced swim tests than SSRI treatment alone (Redrobe and Bourin, 1998). A rapid and robust antidepressant effect of augmentation has been detected in MDD patients when blocking the 5-HT_{1A} autoreceptor with pindolol and concurrently administering the 5-HT_{1A} agonist buspirone, thus turning the latter into a selective postsynaptic 5-HT_{1A} agonist (Blier et al, 1997).

Augmentation strategies are not solely limited to 5-HT_{1A} agonists. Studies have shown that presynaptic 5-HT_{1A} receptor antagonists may also have beneficial effects with respect to increased neurotransmission in forebrain regions (Gartside et al, 1995; Artigas et al, 1996). The 5-HT_{1A} antagonist is able to block the inhibitory effects on 5-HT firing that are induced by SSRIs, thus mimicking 5-HT_{1A} desensitization and allowing increased levels of extracellular 5-HT, which in turn can increase neurotransmission (Gartside et al, 1995; Artigas et al, 1996). These effects occur predominantly at the presynaptic 5-HT_{1A} autoreceptor due to the fact that the 5-HT_{1A} heteroreceptor is immune to desensitization using a SSRI, thus its blockade does not produce significant benefits (Artigas et al, 1996). Augmentation with SSRIs and a 5-HT_{1A} antagonist has been used clinically and has been shown to produce beneficial results in as early as 1 week in both in individuals with typical depression as well as those with drug-resistant depression (Artigas et al, 1996; Blier and Bergeron, 1995). Further meta-analysis studies have confirmed that this effect is prevalent within several MDD patient populations thus providing evidence that augmentation with 5-HT_{1A} antagonists can in fact enhance the effectiveness of SSRI treatment (Portella et al, 2011).

3.5 Multimodal agents

Multimodal therapy has long been used to treat a variety of medical conditions, including stroke, infection and cancer (Gupta et al, 2006; Shoskes et al, 2003; Kudo-Saito et al, 2005). Within the realm of depression, multimodal therapy is also of use, as it can address several monoaminergic targets simultaneously (Blier, 2002). Indeed, current research has indicated success with several

multimodal medications with respect to MDD. For instance, a six week double-blind randomized study noted that combination treatments of SSRIs/mirtazapine and venlafaxine/mirtazapine and bupropion/mirtazapine all produced significantly improvements in HAM-D scores as well as greater remission rates than SSRI monotherapy (Blier et al, 2009; Blier et al, 2010). Furthermore, combination treatments were well tolerated and compliance was comparable to that expected of monotherapy (Blier et al, 2010). However, combination therapy does not always have to come in the form of multiple pills; some single pill compounds are able to target several monoaminergic receptors simultaneously. For example, the novel AD vortioxetine (LUAA21004) displays 5-HT₃ and 5-HT₇ receptor antagonism, 5-HT_{1B} receptor partial agonism, 5-HT_{1A} receptor agonism, 5-HT_{1D} receptor antagonism and SERT blockage, all in one pill (Pehrson et al, 2013). Preclinically, the compound was found to significantly increase levels of all three monoamines implicated in depression in the hippocampus and prefrontal cortex and upon behavioural testing, was found to show significant antidepressant-like effects (Pehrson et al, 2013; Mork et al, 2012). Clinically, vortioxetine has been shown to be both effective and tolerable in the treatment of MDD (Baldwin et al, 2012; Alvarez et al, 2012). The success of this compound supports the use of similar combination therapies, such as the novel AD vilazodone.

3.6 Vilazodone

Vilazodone (EMD68843) is a partial 5-HT_{1A} receptor agonist (IC₅₀ of 0.2 nM in vitro) as well as a selective serotonin reuptake inhibitor with significant blockage of the SERT (IC₅₀ of 0.5 nM) (Bartoszyk et al, 1997). This dual action

antidepressant was approved for the treatment of MDD by the FDA in January of 2011 (Cruz, 2012). The 5-HT_{1A} agonism of vilazodone was confirmed in vitro using [³⁵S]GTPγS binding studies in Sf9 cells as well as in rat hippocampal membranes (Page et al, 2002; Hughes et al, 2005). Further in vitro studies were also able to confirm the effects of vilazodone on the 5-HT reuptake transporter, indicating that this compound can inhibit reuptake similarly to the conventional SSRIs (Dawson and Watson, 2009). For instance, at an acute dose of 10 mg/kg, vilazodone was shown to exhibit a 100% occupancy of the 5-HT transporter in the hippocampus and the cortex, as measured by [³H]DASB displacement studies (Hughes et al, 2005).

The neurochemical effects of vilazodone have also been assessed using in vivo microdialysis studies and have indicated that this compound can effectively alter extracellular 5-HT levels. Studies performed in the rat medial prefrontal cortex and ventral hippocampus indicated dose-dependent significant increases in extracellular 5-HT levels following a single vilazodone administration (Page et al, 2002; Hughes et al, 2005). Moreover, these increases were substantially greater than those produced with the use of the conventional SSRI fluoxetine, displaying a 527% vs 165% increase in the frontal cortex and 558% vs 274% increase in the ventral hippocampus (Page et al, 2002). Similar responses were witnessed in the dorsal lateral frontal cortex, where vilazodone was once again able to increase extracellular serotonin levels two-fold in comparison to the SSRI paroxetine alone (Hughes et al, 2005). An analogous response as that seen with vilazodone was reproduced when paroxetine was combined with the 5-HT_{1A} antagonist

WAY100635, indicating that the mechanism of action behind the ability of vilazodone to potentially increase extracellular 5-HT levels may be closely linked with its affinity for both the SERT and the 5-HT_{1A} receptor (Hughes et al, 2005). While vilazodone is able to significantly alter 5-HT levels at an acute timeline, the other main monoamines remain unaffected, as neither DA nor NE levels show significant changes in response to acute vilazodone administration (Hughes et al, 2005).

The efficacy of the ability of vilazodone to alter behaviour, both depressive and anxiolytic, has been studied in rat animal models. Vilazodone given at doses as low as 1.0 mg/kg was found to reduce the amount of time spent immobile and increase swimming behaviour during the forced swim test in both rats and mice (Page et al, 2002). Almost all currently accepted classes of ADs are able to effectively modify immobility measured during this test, thus vilazodone demonstrated that it also falls in this category (Dawson and Watson, 2009). Furthermore, when vilazodone was administered at high doses to rats prophylactically in predator stress studies, it was shown to effectively block stress potentiation of startle, indicating potential anxiolytic benefits (Adamec et al, 2004). Further anxiolytic-like effects of vilazodone were confirmed in studies utilizing the shock-probe test, in which dose-dependent administration of vilazodone was able to reduce burying, a behaviour indicative of anxiety (Treit et al, 2001). In addition, vilazodone has been shown to significantly reduce ultrasonic vocalizations in rats following foot shocks, further indicating its anxiolytic potential (Bartoszyk et al, 1997).

Randomized, double-blind, placebo controlled studies have indicated a place for vilazodone in the clinical setting as well. Patients treated for 8 weeks with vilazodone (titrated to 40 mg/day following a 2 week period) experienced significant improvements as measured by MADRS scores (Rickels et al, 2009; Khan et al, 2011). Side effects consisted of mostly mild to moderate gastrointestinal upset (diarrhea and nausea) and prompted only 5.1% of patients to discontinue treatment, indicating that the drug appears well tolerated (Khan et al, 2011). Vilazodone was also shown to display anxiolytic effects, improving Hamilton Anxiety Rating Scale scores as well when compared to placebo (Khan et al, 2011; Khan et al, 2014). Initial studies indicated that remission rates were higher with vilazodone (25.4% with vilazodone vs 18.1% with placebo), however the improvement was not significantly relevant; this may have been attributed to the relatively short study period of 8 weeks (Khan et al, 2011). Nonetheless, more recent studies found that vilazodone differed significantly both in response rates and remission rates in comparison to placebo, further indicating its effectiveness and clinical relevancy (Khan et al, 2014). To date, no comparative studies of vilazodone and other ADs have been completed; following the completion of randomized double-blind trials the clinical significance of vilazodone will be better elucidated.

4.0 Objectives and Hypotheses

The objectives of this study are:

1. To determine the effects of acute vilazodone administration on 5-HT neuron firing.
2. To determine the effects of sustained administration (2-day and 14-day) of vilazodone on the three monoaminergic systems:
 - a. Serotonin
 - b. Dopamine
 - c. Norepinephrine
3. To determine the effects of chronic vilazodone administration (14-day) on hippocampal neuronal elements.

The associated hypotheses of these are:

1. Much like other 5-HT_{1A} agonists and SSRIs tested in this paradigm, it is expected that vilazodone will induce a dose-dependent inhibition of firing in the 5-HT neuron when administered acutely, due to indirect and direct activation of inhibitory 5-HT_{1A} autoreceptors (Blier and de Montigny, 1983).
2. The effects of sustained administration are expected to vary in each of the three monoaminergic systems tested:
 - a. Serotonin: After 2 days, vilazodone is expected to significantly reduce firing due to the activation of inhibitory 5-HT_{1A} autoreceptors. This is expected to recover following 14-day vilazodone administration due to the desensitization of these

receptors (Blier and de Montigny, 1983; Blier and de Montigny, 1990a)

- b. Dopamine: After 2 days, vilazodone is expected to significantly reduce firing. It is expected that this firing reduction will persist following 14-day administration. This has been previously documented with the SSRI escitalopram (Dremencov et al, 2009). However, 5-HT_{1A} agonists have been shown to produce significant elevations in DA firing which may potentiate this response and produced alterations in DA neuron firing (Arborelius et al, 1993)
- c. Norepinephrine: After 2 days, vilazodone is not expected to induce a significant inhibitory effect. Following 14 days, the firing of NE neurons is expected to be significantly reduced. This response is expected as NE neurons have been shown to produce this response following SSRI treatment (Szabo et al, 2000). However, 5-HT_{1A} agonists have been shown to produce the opposite response: an increase in 5-HT firing (Lejeune and Millan, 2000). This may also modulate the firing effects of vilazodone.

- 3. Following a 14-day regimen, vilazodone is expected to induce a significant increase in 5-HT neurotransmission within the hippocampus. As well, vilazodone is expected to produce an increase in the RT₅₀ measure, indicating significant blockage of the SERT. No change in the sensitivity of the post-synaptic 5-HT_{1A} receptor is expected following vilazodone treatment. These effects have been observed in previous

electrophysiological experiments with both SSRIs and 5-HT_{1A} agonists
(Haddjeri et al, 1998)

MATERIALS AND METHODS

1.0 Animals

Adult male Sprague-Dawley rats (Charles River, Saint-Constant, QC, Canada) were used. Rats weighed 250-350 g at the time of the experiments. Two animals were kept per cage and were housed in standard laboratory conditions (12:12 h light/dark cycle; light cycle start at 7:00 am; temperature $21\pm 1^{\circ}\text{C}$, 40-50% relative humidity). Food and water were provided ad libitum. All animals were handled in accordance with the guidelines of the Canadian Council on Animal Care and the local animal care committee of the University of Ottawa, Institute of Mental Health Research (Ottawa, Canada).

2.0 Experimental preparations

2.1 Experimental preparations for acute treatment

Acute experiments were aimed to detect the dose at which vilazodone was able to induce full inhibition of 5-HT neuron firing. Vilazodone was administered intravenously at varying doses of 200 $\mu\text{g/kg}$, 300 $\mu\text{g/kg}$ and 400 $\mu\text{g/kg}$ and the resulting change in firing rate was assessed. Once firing activity was inhibited or began to stabilize the 5-HT_{1A} receptor antagonist WAY100635 was administered at a dose of 100 $\mu\text{g/kg}$ to reverse the initial drug effects.

Rats were also treated with the synthetic serotonin depletory para-chlorophenylalanine (PCPA) to examine whether vilazodone inhibits neuronal firing directly via its ability to block 5-HT reuptake or indirectly via its 5-HT_{1A} agonism (Cunningham and Lakoski, 1990). Rats were treated for 3 days with a 300

mg/kg intraperitoneal (i.p.) injections of PCPA given daily. The same procedure as described above was utilized to assess the ability of vilazodone to inhibit 5-HT firing following PCPA treatment.

2.2 Experimental preparations for 2-day and 14-day treatment

For both 2-day and 14-day treatments, vilazodone was administered via intraperitoneal (i.p.) injections at a dose of 5 mg/kg. Control rats received an equivalent injection of 40% hydroxylpropyl-beta-cyclodextrin to mimic handling and injection stress. Injections were performed at the same time every day to ensure ideal drug delivery.

The 5 mg/kg dosage of vilazodone and intraperitoneal (i.p.) injection route was selected following extensive dosage trials. With an increased vilazodone dose, such as 10 mg/kg, the level of 5-HT inhibition was too great to be able to assess the effects of the drug. At this dose, rats experienced significant signs of toxicity, including weight loss and agitation upon handling. As well, while the use of subcutaneous osmotic minipumps would have been ideal to standardize drug delivery, vilazodone proved to be very difficult to dissolve in small volumes and thus this method of drug delivery was not reliable for extended periods of time.

2.0 Electrophysiological Experiments in the Cell Body

Single-unit, extracellular recordings of monoaminergic neurons were achieved utilizing single-barreled glass electrodes filled with 2 M NaCl solution (impedance ranging from 2.5 – 5 M Ω). Experiments were performed in rats anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Additional doses of

chloral hydrate were given (50 – 100 mg/kg, i.p.) to ensure animals showed no nociceptive response to physical pressure as induced by tail or paw pinch. Body temperature was maintained at 37°C using a thermistor-controlled heating pad (Seabrook Medical Instruments, Saint-Hyacinthe, Quebec, Canada).

Specific predefined co-ordinates based on the suture lines of the skull were utilized to correctly localize the monoaminergic regions of interest. Once identified, these regions were accessed via a burr hole drilled into the skull. Neurons were individually identified based on firing characteristics such as spike shape, duration and frequency and recorded in real-time using Spike2 software (Cambridge Electronic Design, Cambridge, UK). Drugs were administered systemically to the animal intravenously via a catheter inserted into the lateral tail vein prior to the start of recording.

3.1 DRN 5-HT neurons

The DRN was localized using the following co-ordinates (in mm from lambda): AP ± 1 to ± 1.2 , ML 0, DV 5.0 to 7.0. 5-HT neurons were identified by their slow, regular firing rate typically ranging from 0.5 to 2.5 Hz, as well as their long duration action potential which ranges from 0.8-1.2 ms (Aghajanian and Vandermaelen, 1982). An example recording is shown in Figure 5.

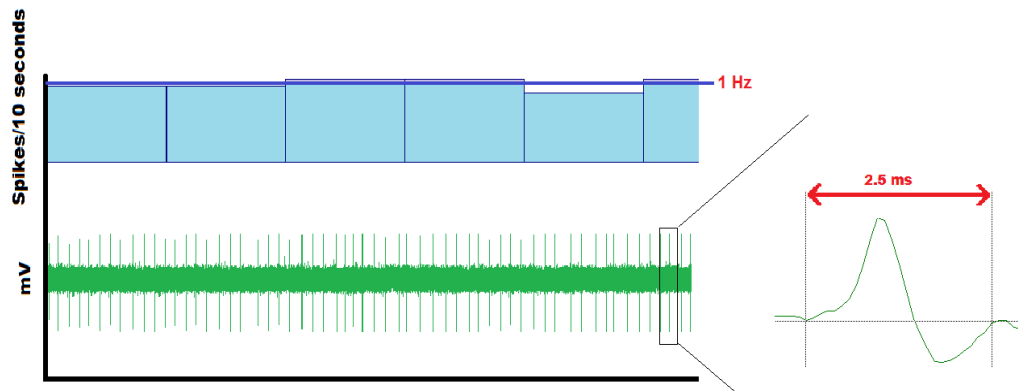


Figure 5. Example of electrophysiological recording of DRN 5-HT neuron.

3.2 VTA DA neurons

The VTA was localized using the following co-ordinates (in mm from lambda): AP 3.2 to 3.6, ML 0.9 to 1.1, DV 7.0 to 9.0. DA neurons in this region were identified by their biphasic spikes, which typically display an inflection point in the rising phase, known as a “notch”. As well, these neurons exhibit a large negative phase, irregular firing rate (2.0-9.0 Hz) and long spike duration (2.0-4.0 ms). In addition to displaying tonic firing, DA neurons also undergo phasic firing or bursting. Typically, these bursts contain 3-10 spikes and demonstrate decreasing amplitude (Grace and Bunney, 1983). An additional measure of DA neurons is the duration from the start of the spike to the center of the negative trough, which in DA neurons is typically greater than 1.1 ms (Ungless et al., 2004). An example recording is shown in Figure 6.

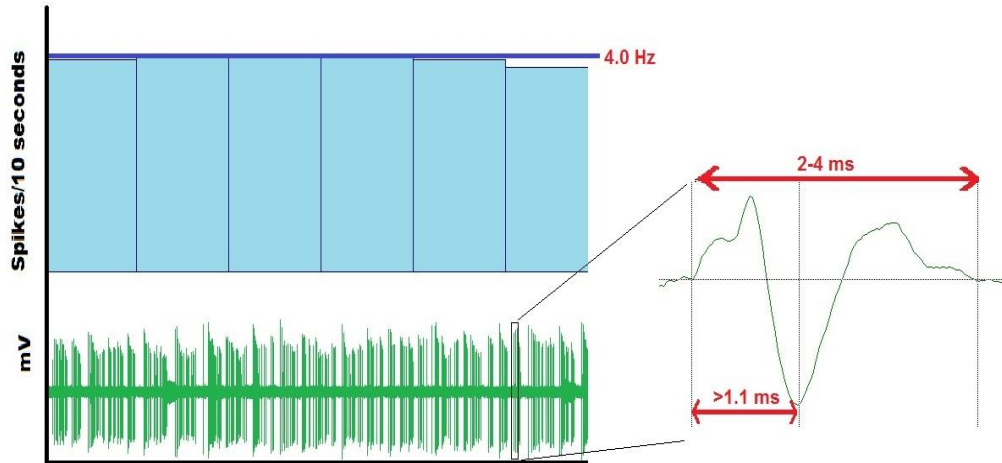


Figure 6. Example of electrophysiological recording of VTA DA neuron.

3.3 LC NE neurons

The LC was localized using the following co-ordinates (in mm from lambda): AP – 1.0 to -1.2, ML 01.0 to 1.3, DV 5.0 to 7.0. NE neurons in this regions where identified by their biphasic spikes, lasting typically 2-3 ms. These neurons also possess regular firing rates (0.5-5.0 Hz) as well as characteristic burst discharge following a nociceptive pinch of the contralateral hind paw. This burst discharge is typically followed by a period of quiescence, making this an easily identifiable pattern (Marwaha and Aghajanian, 1982). An example recording is shown in Figure 7.

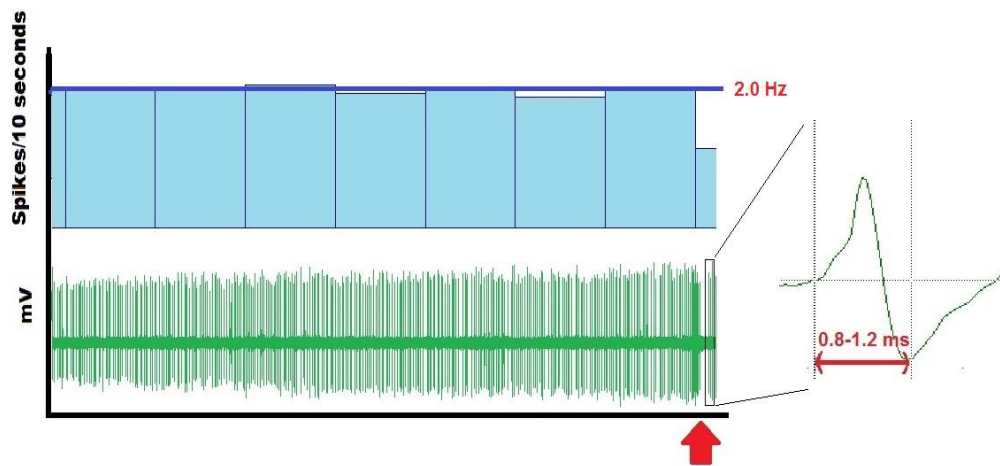


Figure 7. Example of electrophysiological recording of LC NE neuron.

4.0 Electrophysiological recording of dorsal hippocampus CA3 pyramidal neurons

Extracellular recordings of CA3 pyramidal neurons were completed using a five-barreled glass micropipette. The central barrel contained 2M NaCl solution and an impedance measuring between 2 to 4 MΩ. This main barrel was utilized for unitary recordings. The four remaining barrels were filled with: 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 2 M NaCl solution (used for automatic current balancing). The dorsal CA3 region of the hippocampus was approximated using the following stereotaxic co-ordinates: 4 mm anterior to lambda and 4.2 mm lateral with a depth of 4.0 ± 0.5 mm (Paxinos and Watson, 2005). Due to the quiescent nature of pyramidal neurons in chloral hydrate anesthetized rats, a +1 to – 4 nA current of quisqualate was used to activate these neurons so that they could be recorded. The current used was altered to cause

activations in the physiological firing range (10 to 15 Hz) for these neurons (Ranck, 1975)

Identification of pyramidal neurons was based on their characteristic action potentials: large amplitude (0.5-1.2 mV), long duration (0.8 -1.2 ms) and alternating simple and complex spike discharges (Kandel and Spencer, 1961).

4.1 Tonic activation of postsynaptic 5-HT_{1A} receptors

Following 14-day administration of vilazodone, the degree of activation of post-synaptic 5-HT_{1A} receptors and the resulting inhibition of CA3 pyramidal neuron activity was assessed. This was done using the selective 5-HT_{1A} receptor antagonist WAY 100635, which was administered intravenously, as this compound results in the disinhibition of hippocampal neurons which in turn results in an increase in firing activity. To be able to detect changes in disinhibition, firing rate was reduced to 2-3 Hz by reducing the ejection current of quisqualate. Once a steady, lowered firing rate was achieved, WAY100635 (100 µg/kg) was administered intravenously at increments of 25 µg/kg at two minute time intervals. (Haddjeri et al, 1998). Alterations in the firing activity of CA3 pyramidal cells would indicate changes in the tonic activation of the postsynaptic 5-HT_{1A} receptors. Only one cell per rat was assessed, as the firing rate of all cells would be compromised post WAY100635 injection and would thus not serve as a good model.

To determine the responsiveness of CA3 pyramidal neurons to 5-HT, 5-HT was microiontophoretically applied for 60 seconds and the number of spikes suppressed during this time was measured. This value was divided by the amount of

current used, as measured in nA (spikes suppressed/nA). The relative degree to which the 5-HT transporter was blocked by the 14-day administration of vilazodone was also measured. This was done by assessing the 50% recovery time (RT₅₀), which was measured by determining the time it takes (in seconds) following a 60 second ejection period of 5-HT for firing to recover to 50% of its initial rate (de Montigny et al., 1980). WAY 100635 does not alter the firing rate of 5-HT neurons in the DRN of anesthetized rats, making it useful in this experimental context (Lejeune and Millan, 1998).

5.0 Drugs

Vilazodone (provided by Forrest Laboratories Inc, New York City, USA) was dissolved in 40% hydroxylpropyl-beta-cyclodextrin (β -OH, 4 g/10 mL distilled H₂O). The solution was sonicated for 10 minutes or until completely dissolved. WAY 100635, and *para*-chlorophenylalanine (PCPA) methyl ester hydrochloride (Sigma-Aldrich) were all dissolved in distilled H₂O.

6.0 Statistical Analysis

All results were reported as mean values $\pm$ SEM. Data was obtained from 5 to 6 rats per experimental group. For the assessment of two groups, statistical comparisons were carried out using the two-tailed Student's *t* test when a parameter was studied in control and treated rats. Statistical significance was taken as $p < 0.05$. For the assessment of three or more groups, values were compared using one-way ANOVA followed by Tukey post-hoc analysis. Statistical significance was taken as $p < 0.05$. All statistical tests were completed utilizing GraphPad Prism 5 software (GraphPad Software Inc, La Jolla, CA).

RESULTS

1.0 Effects of vilazodone on the firing of monoaminergic neurons

1.1 Effects of vilazodone on acute 5-HT firing

Acute administration of vilazodone produced a dose-dependent decrease in DR 5-HT neuron firing. A dose of 400 µg/kg produced a complete inhibition of firing (Figure 8). This inhibition was reversed by a 100 µg/kg injection of the 5-HT_{1A} receptor antagonist WAY100635.

No significant difference was seen with respect to 5-HT neuronal inhibition, when 5-HT was depleted in PCPA treated rats (student's *t* test, Figure 8). Sample size is indicated in Figure 8 and is denoted by the brackets located beside the data point.

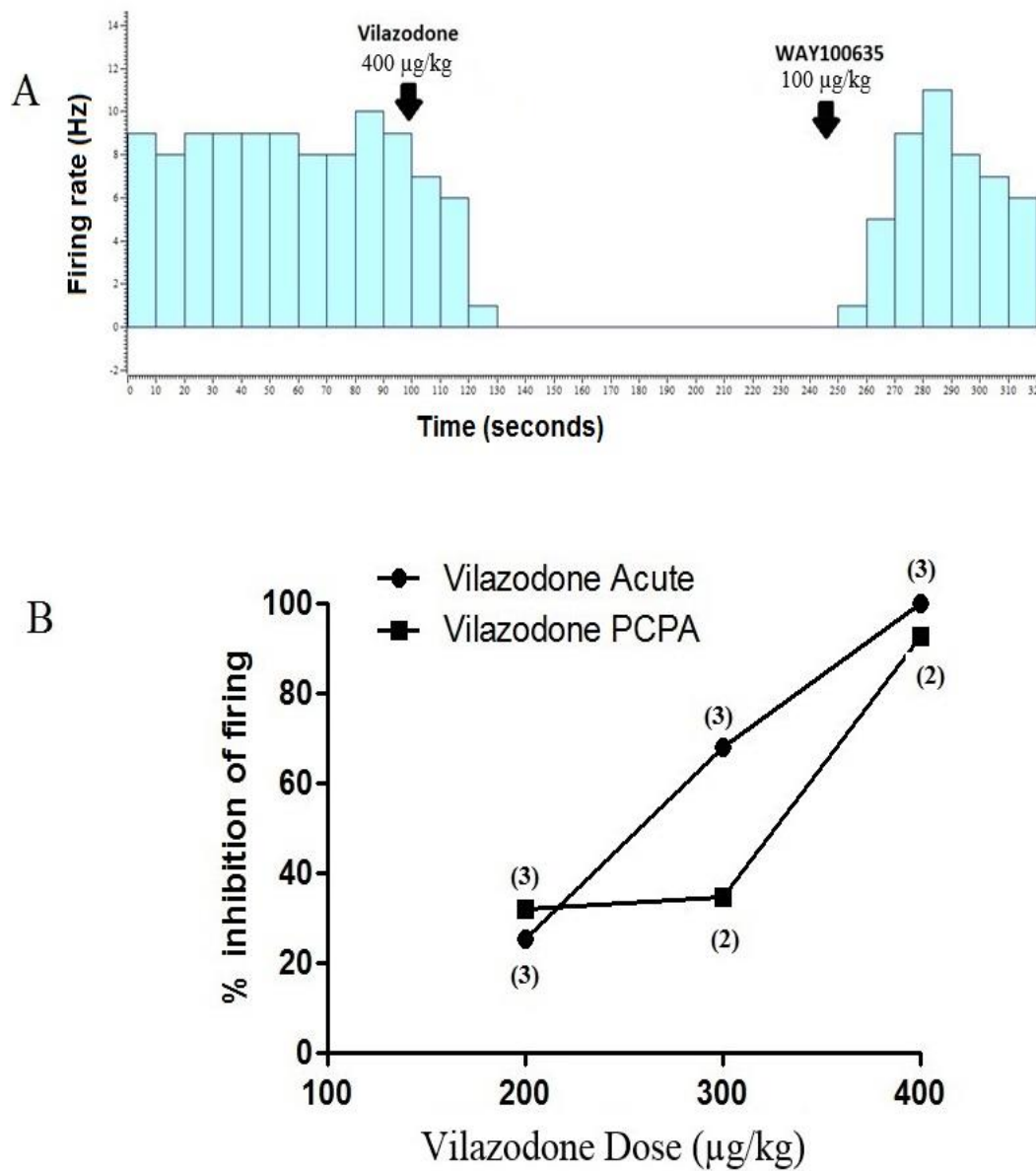


Figure 8. Effects of acute vilazodone administration on DRN 5-HT neuronal firing. Vilazodone administered intravenously at a dose of 400 µg/kg produced full inhibition of 5-HT firing, which was reversible with 100 µg/kg of WAY100635 (A). PCPA treated rats showed no significant difference in 5-HT inhibition upon vilazodone administration in comparison (B).

1.2 Effects of vilazodone on 5-HT neuron firing (2-day and 14-day)

Vilazodone was first administered via subcutaneous minipumps at a dosage of 10 mg/kg for a 2-day period. A significant decrease (***) $p < 0.001$, Student's t -test; Figure 9) of 39.7% in comparison to control was witnessed with respect to 5-HT firing rate (5 rats, 95 neurons; Figure 9). However, during drug preparation, it was noted that the compound was difficult to dissolve in the small volumes required for the usage of minipumps. Therefore, a different method of drug delivery was utilized, intraperitoneal injection, which allowed for larger volumes to properly dissolve the compound. Using this route, at 10 mg/kg, a 90% inhibition of firing was recorded in vilazodone administered animals compared to controls (3 rats, 20 neurons, *** $p < 0.001$, student's t test; Figure 9). This indicated that the minipumps did not efficiently deliver the drug to the circulatory system, thus the i.p. method of injection was utilized for all further experiments. However, the 10 mg/kg dose of vilazodone produced severe physical detriments as rats began to quickly lose weight, demonstrated a failure to thrive and became easily agitated. This indicated that the drug may possibly be toxic when administered at 10 mg/kg via intraperitoneal injection. Furthermore, the 10 mg/kg dose produced such a robust inhibition of firing that analysis was difficult to conduct due to the low number of spikes per recording. As such, a dose of 5 mg/kg was selected for further experiments. At this dose, rats did not experience any negative physical effects from the drug and recordings were effectively analyzed.

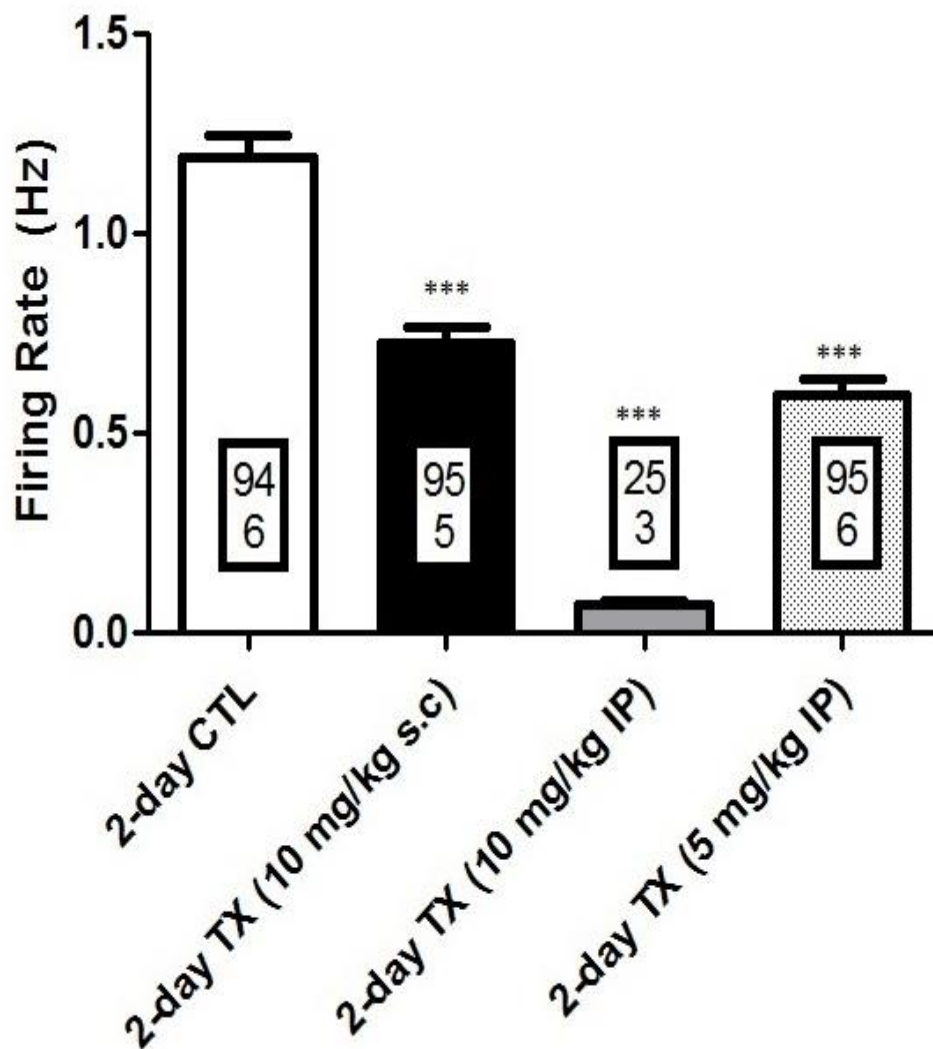


Figure 9. Effects of 2-day and 14-day vilazodone administration on DRN 5-HT neurons – drug delivery comparisons. Mean ($\pm$ SEM) of the firing rate of DRN 5-HT neurons in control rats and rats treated with vilazodone (varying doses and injection methods as mentioned above) for 2-day and 14-day. The numbers in the boxes indicate the number of neurons and number of rats recorded, respectively. *** $p < 0.001$ using student's T-test.

Administration of 5 mg/kg vilazodone via i.p. injection resulted in a robust decrease of 50% (***) $p < 0.001$ one-way ANOVA, *** $p < 0.001$ Tukey post-hoc; Figure 10) in 5-HT neuron firing with respect to controls. Following 14-day administration of vilazodone (5 mg/kg, i.p.), no significant changes (Figure 10) in firing rate were observed when comparing treated and control animals. Significant changes were observed between 2-day treated 5-HT neuron firing rates and 14-day treated firing rates; a 45% increase in firing was observed at 14-days (***) $p < 0.001$ one-way ANOVA, *** $p < 0.001$ Tukey post-hoc; Figure 10). Therefore, the firing decrease observed in 2-day treated animals was shown to have recovered at 14-days.

However, no significant changes were witnessed in bursting activity nor neurons per track in both 2 and 14-day treated animals (Table 1).

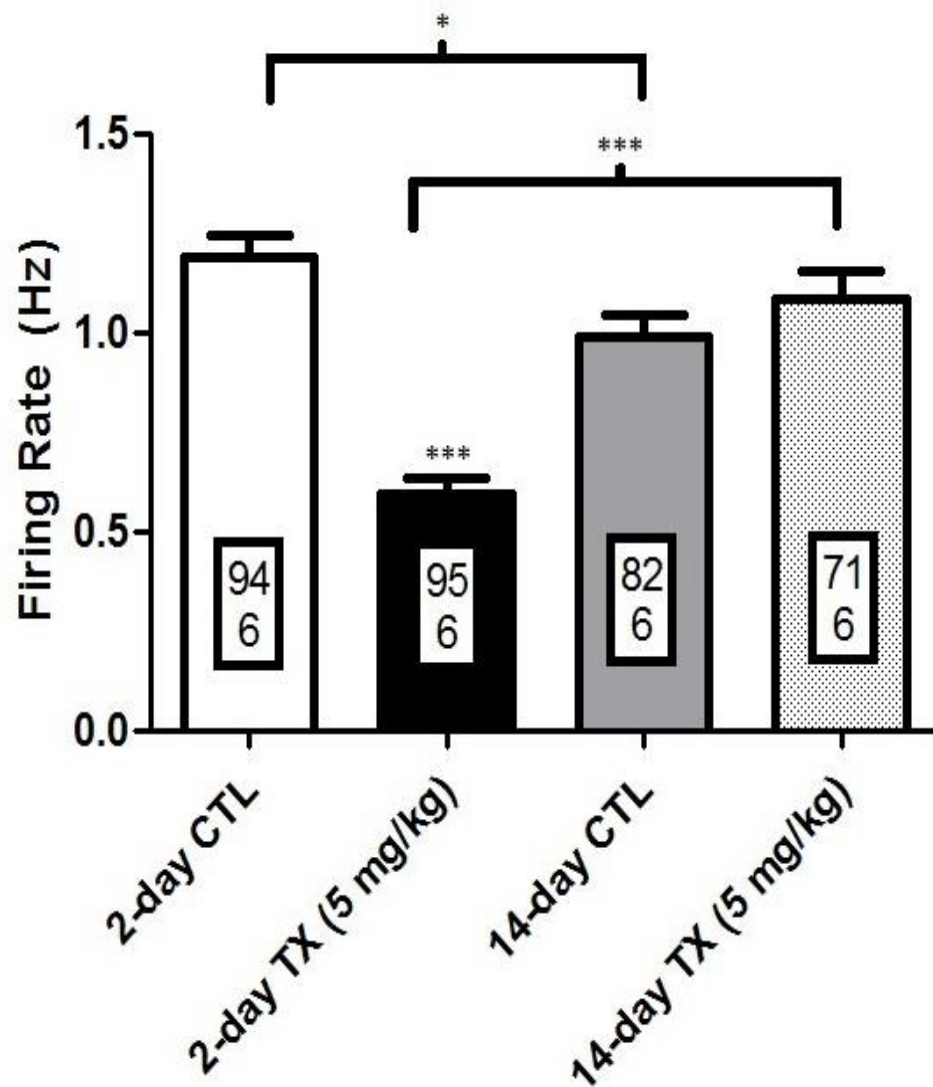


Figure 10. Effects of 2-day and 14-day vilazodone administration on DRN 5-HT neurons. Mean ($\pm$ SEM) of the firing rate of DRN 5-HT neurons in control rats and rats treated with vilazodone (5 mg/kg/day; i.p.) for 2-day and 14-day. The numbers in the boxes indicate the number of neurons and number of rats recorded, respectively. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ using one-way ANOVA and Tukey post-hoc test.

1.3 Effects of vilazodone on VTA neuron firing (2-day and 14-day)

The 2-day administration of vilazodone also induced changes in DA firing, decreasing firing by 35% (*** $p < 0.001$ one-way ANOVA, *** $p < 0.001$ Tukey post-hoc; Figure 11) in comparison to control animals. Following 14-day treatment, there is a non-significant decrease in firing of 19% (*** $p < 0.001$ one-way ANOVA, $p > 0.05$ Tukey post-hoc; Figure 11). This may indicate a partial recovery of DA firing rate following long term treatment of vilazodone. No significant changes between 2 and 14-day treated animals were noted.

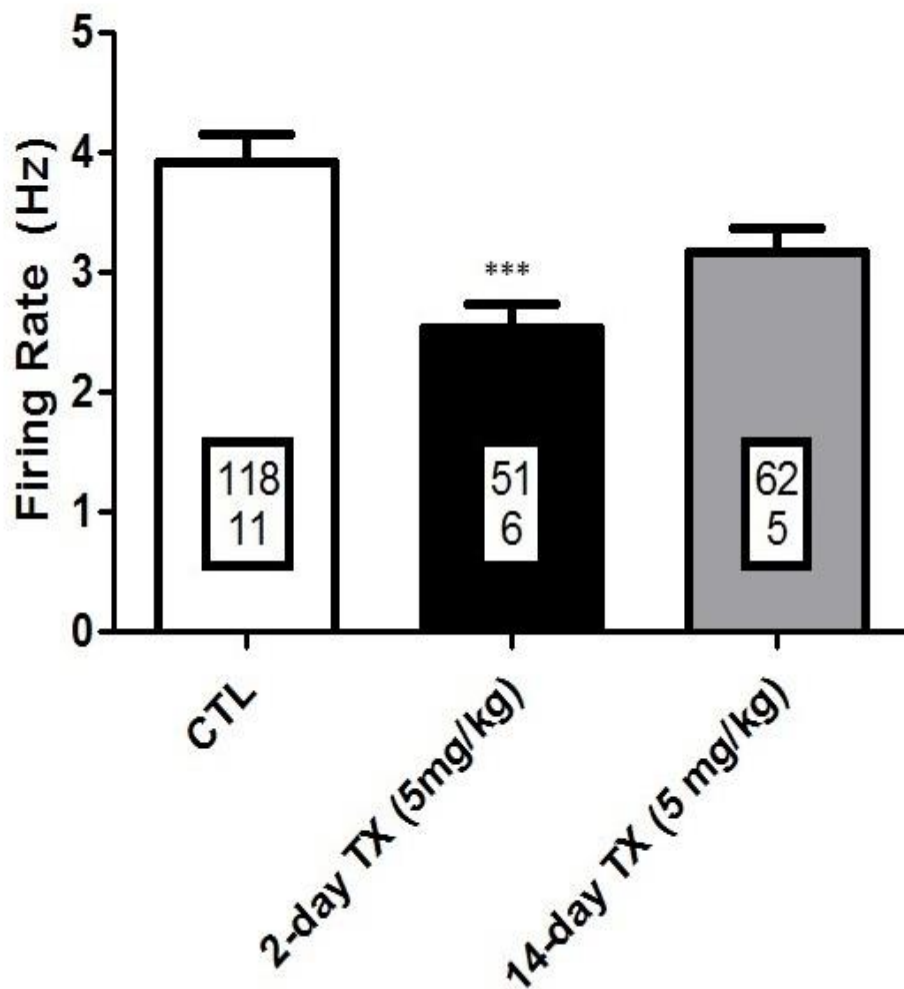


Figure 11. Effects of 2-day and 14-day vilazodone administration on VTA DA neurons. Mean ($\pm$ SEM) of the firing rate of VTA DA neurons in control rats (2 and 14 day pooled) and rats treated with vilazodone (5 mg/kg/day; i.p.) for 2-day and 14-day. The numbers in the boxes indicate the number of neurons and number of rats recorded, respectively. *** $p < 0.001$ using one-way ANOVA and Tukey post-hoc test.

The 2-day administration of vilazodone also altered the burst activity of DA neurons. Bursts per minute decreased by 69% ($***p < 0.001$ one-way ANOVA, $*** p < 0.001$ Tukey post-hoc; Figure 12) in comparison to controls. Following 14-day treatment, the number of bursts per minute in treated animals decreased significantly by 43%, ($*** p < 0.001$ one-way ANOVA, $*p < 0.05$ Tukey post-hoc; Figure 12). This indicates a partial recovery of burst activity in comparison to 2-days treated animals.

Following 2-day administration of vilazodone, percent spikes per burst was found to decrease by 59% ($***p < 0.001$ one-way ANOVA, $*** p < 0.001$ Tukey post-hoc; Figure 13) in comparison to controls. Following 14-day treatment, the number of percent spikes per burst continued to experience a robust decrease of 53% ($***p < 0.001$ one-way ANOVA, $*** p < 0.001$ Tukey post-hoc; Figure 13).

Following 2-day administration of vilazodone, a significant change in mean number of spikes per burst was also detected ($*p < 0.05$ one way ANOVA; Figure 14) however post-hoc analysis determined no significance between groups. The Tukey test is quite conservative and thus may be too stringent to detect borderline significance. 2-day administrated demonstrated a decrease of 31% was witnessed ($*p < 0.05$ one way ANOVA, $p > 0.05$ Tukey post-hoc; Figure 14) in treated animals in comparison to controls with respect to mean number of spikes in burst. Following 14-day administration, a significant

decrease of 29% occurred ($*p < 0.05$ one way ANOVA, $p > 0.05$ Tukey post-hoc; Figure 14).

The 2-day administration of vilazodone resulted in a significant decrease of 37% in burst duration in treated animals ($***p < 0.001$ one-way ANOVA, $**p < 0.01$ Tukey post-hoc; Figure 15) with respect to control. Following 14-day administration, a further, significant 23% decrease in burst duration was observed ($***p < 0.001$ one-way ANOVA, $*p < 0.05$ Tukey post-hoc; Figure 15).

No significant changes were witnessed in neurons per track in both 2 and 14-day treated animals.

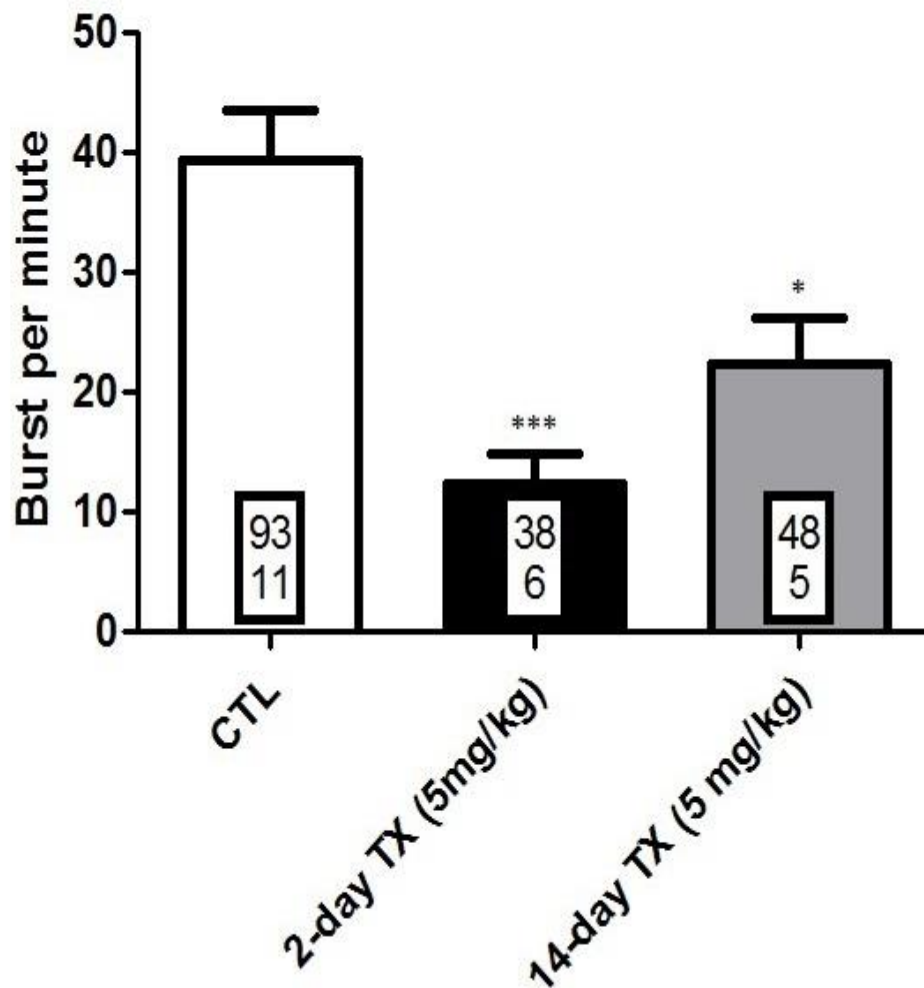


Figure 12. Effects of 2-day and 14-day vilazodone administration on number of bursts per minute in VTA DA neurons. Mean ($\pm$ SEM) of the number of bursts per minute of VTA DA neurons in control rats (2 and 14 day pooled) and rats treated with vilazodone (5 mg/kg/day; i.p.) for 2-day and 14-day. The numbers in the boxes indicate the number of neurons and number of rats recorded, respectively. * $p < 0.05$ and *** $p < 0.001$ using one-way ANOVA and Tukey post-hoc test.

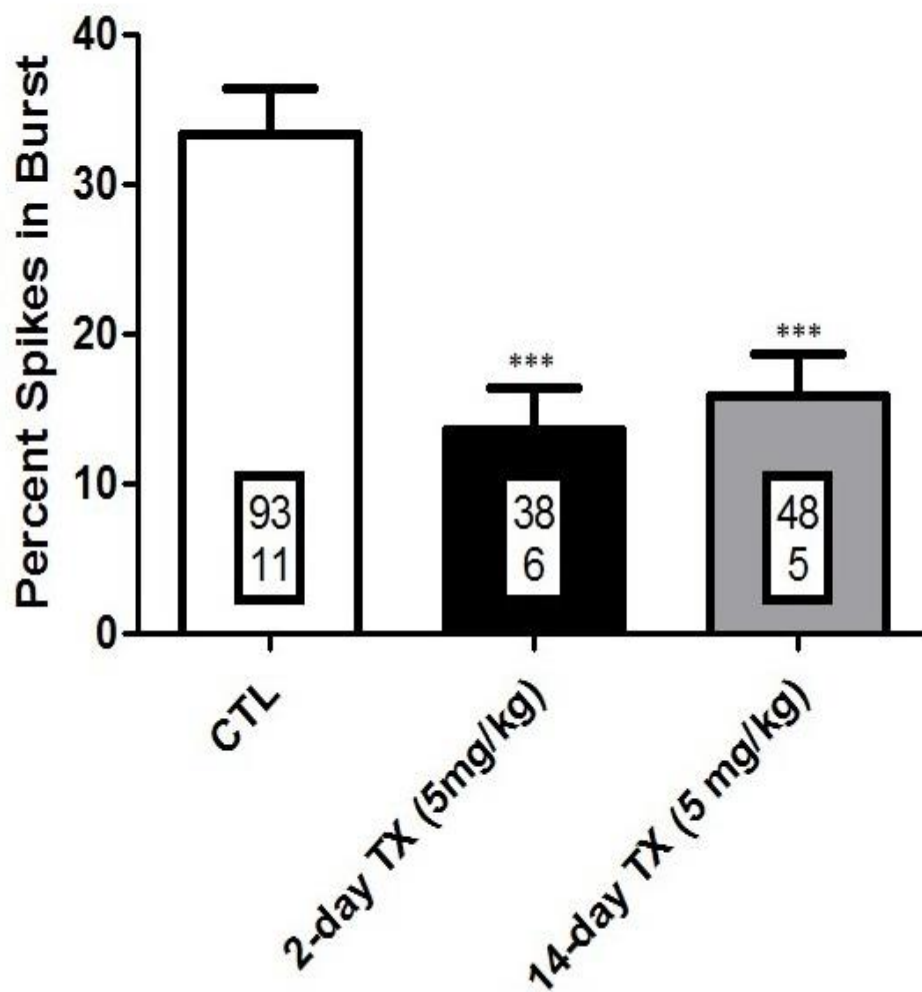


Figure 13. Effects of 2-day and 14-day vilazodone administration on percent spikes in burst in VTA DA neurons. Mean ($\pm$ SEM) of the percent spikes in burst of VTA DA neurons in control rats (2 and 14 day pooled) and rats treated with vilazodone (5 mg/kg/day; i.p.) for 2-day and 14-day. The numbers in the boxes indicate the number of neurons and number of rats recorded, respectively. *** $p < 0.001$ using one-way ANOVA and Tukey post-hoc test.

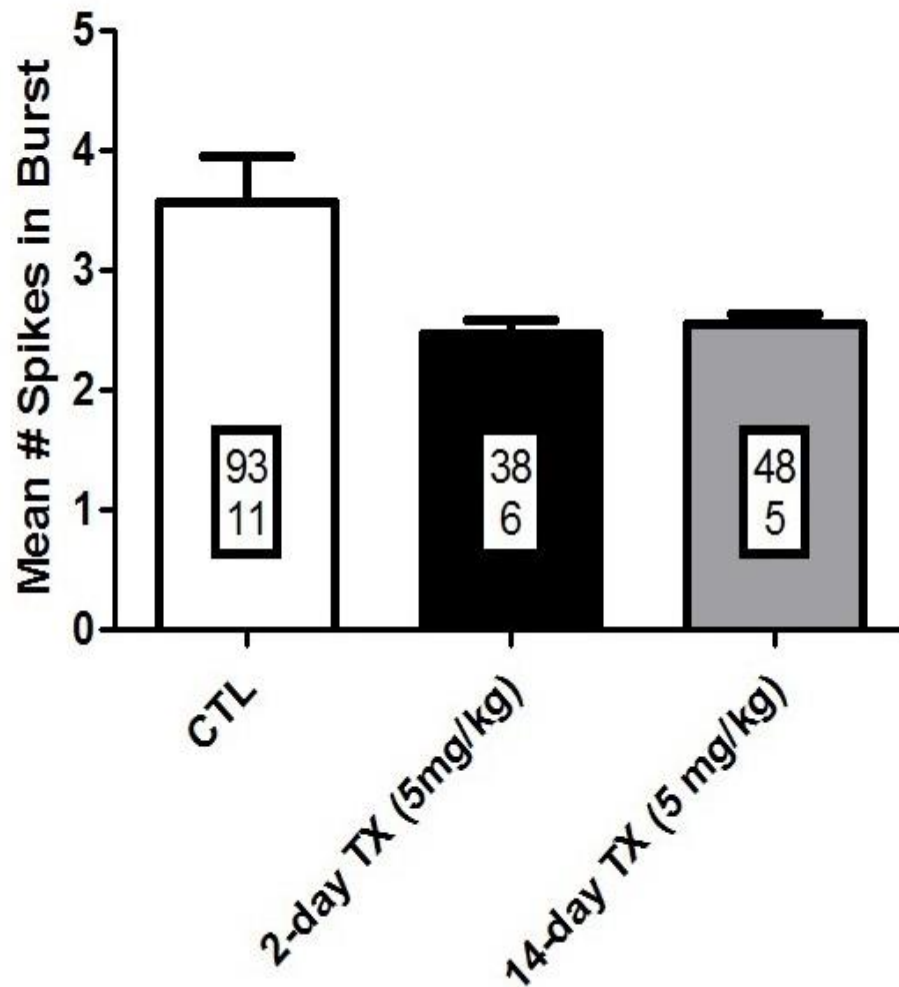


Figure 14. Effects of 2-day and 14-day vilazodone administration on mean number of spikes in burst in VTA DA neurons. Mean ($\pm$ SEM) of the mean number of spikes in bursts in VTA DA neurons in control rats (2 and 14 day pooled) and rats treated with vilazodone (5 mg/kg/day; i.p.) for 2-day and 14-day. The numbers in the boxes indicate the number of neurons and number of rats recorded, respectively.

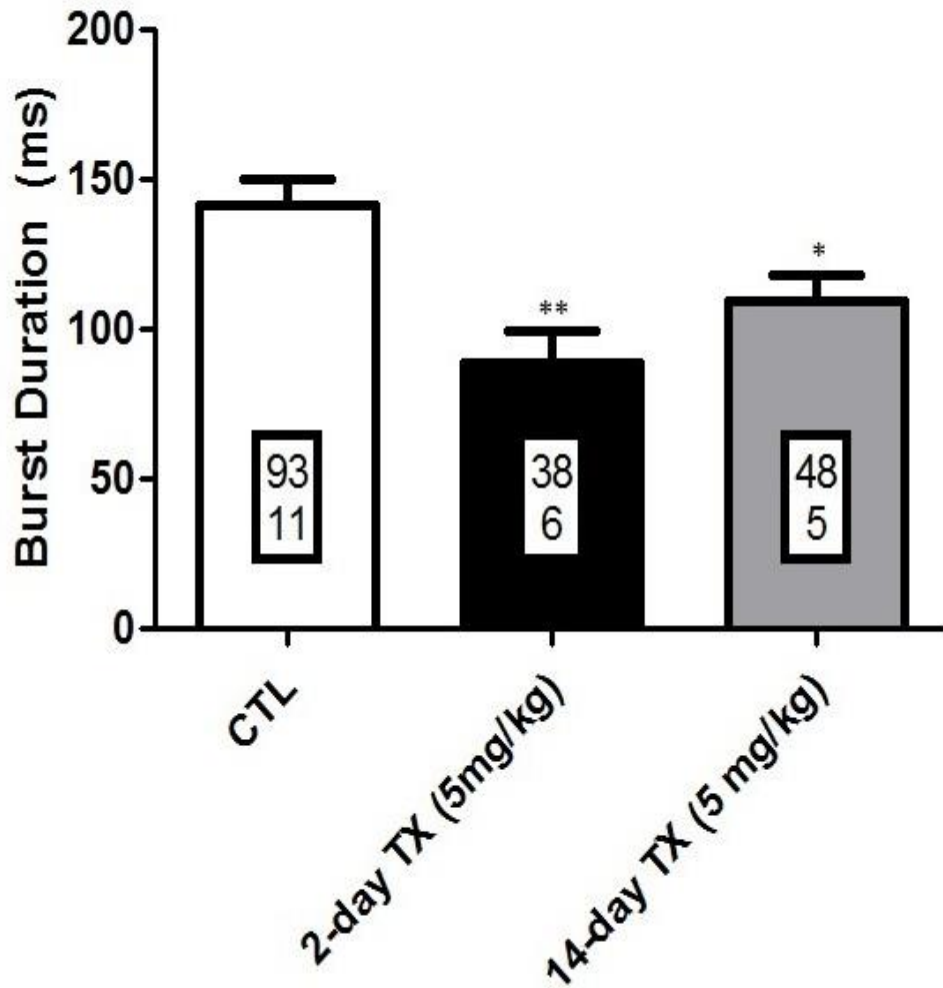


Figure 15. Effects of 2-day and 14-day vilazodone administration on burst duration in VTA DA neurons. Mean ($\pm$ SEM) of burst duration of VTA DA neurons in control rats (2 and 14 day pooled) and rats treated with vilazodone (5 mg/kg/day; i.p.) for 2-day and 14-day. The numbers in the boxes indicate the number of neurons and number of rats recorded, respectively. * $p < 0.05$ and ** $p < 0.01$ using one-way ANOVA and Tukey post-hoc test.

1.4 Effects of vilazodone on LC neuron firing (2-day and 14-day)

The 2-day administration of vilazodone caused a small, non-significant decrease in LC firing by 6% ($p > 0.05$ one-way ANOVA; Figure 16) in comparison to control animals. Following 14-day treatment, there is a significant larger decrease in firing of 31% (*** $p < 0.001$ one-way ANOVA, *** $p < 0.001$ Tukey post-hoc; Figure 16). Furthermore, significant changes between 2 and 14-day treated animals were noted (*** $p < 0.001$ one-way ANOVA, ** $p < 0.01$ Tukey post-hoc; Figure 16).

No significant changes were witnessed in neurons per track nor bursting parameters in both 2 and 14-day treated animals (Figure 12, Appendix 1).

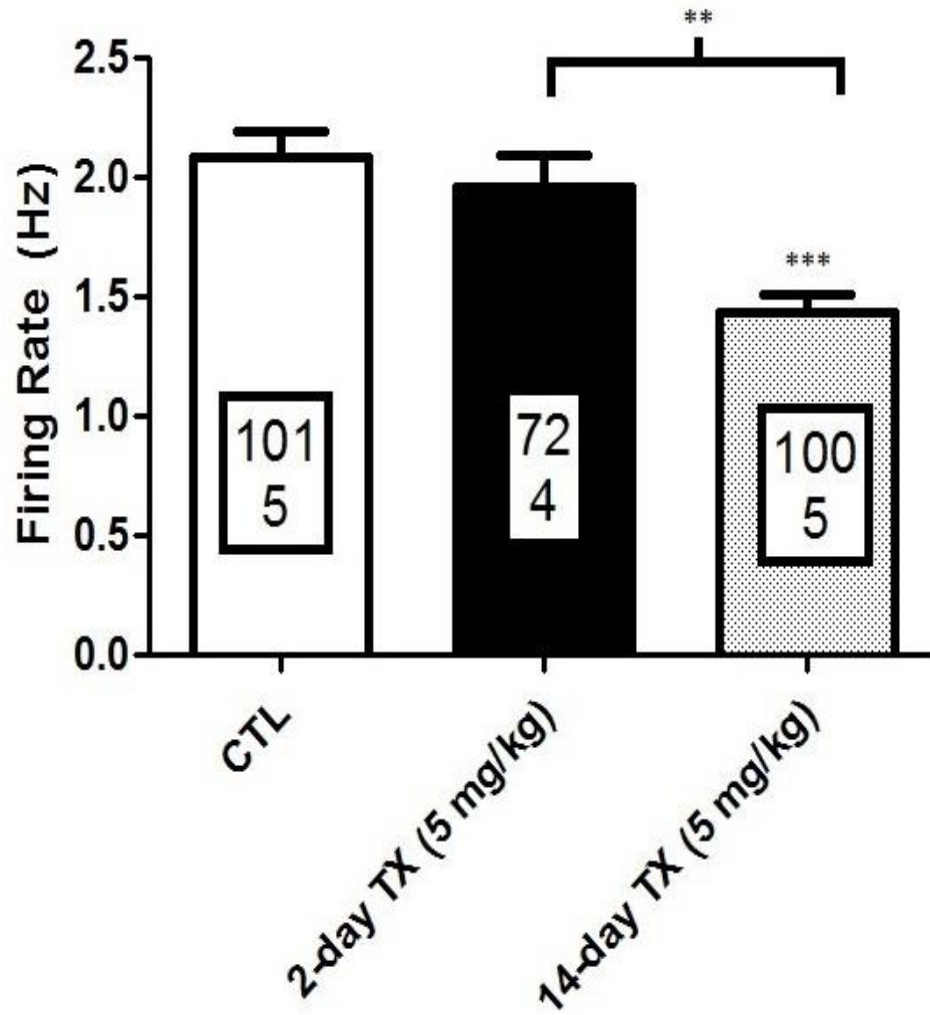


Figure 16. Effects of 2-day and 14-day vilazodone administration on LC NE neurons. Mean ($\pm$ SEM) of the firing rate of LC NE neurons in control rats (2 and 14 day pooled) and rats treated with vilazodone (5 mg/kg/day; i.p.) for 2-day and 14-day. The numbers in the boxes indicate the number of neurons and number of rats recorded, respectively. ** $p < 0.01$ and *** $p < 0.001$ using one-way ANOVA and Tukey post-hoc test.

Table 1. Summary table of the effect of 2- and 14-day administration of vilazodone on the firing and burst activity of DRN 5-HT, LC NE, and VTA DA neurons.

Area	TX	Firing activity (Hz $\pm$ SEM)		Bursting vs. non-bursting neurons (%)		Burst rate (Bursts/minute $\pm$ SEM)		Mean # spikes/burst $\pm$ SEM	
		Control	TX	Control	TX	Control	TX	Control	TX
DRN 5-HT	2-day	1.2 $\pm$ 0.06	0.6 $\pm$ 0.04***	18	17	4.6 $\pm$ 1.28	3.1 $\pm$ 0.90	2.0 $\pm$ 0.01	2.6 $\pm$ 0.45
	14-day	1.0 $\pm$ 0.05	1.1 $\pm$ 0.07	18	25	6.6 $\pm$ 2.73	5.7 $\pm$ 1.82	2.1 $\pm$ 0.07	2.1 $\pm$ 0.04
LC NE	2-day	2.1 $\pm$ 0.11	2.0 $\pm$ 0.13	35	43	5.4 $\pm$ 1.02	6.9 $\pm$ 2.10	2.1 $\pm$ 0.04	2.1 $\pm$ 0.04
	14-day	na ⁺	1.4 $\pm$ 0.07***	na	19	na	2.3 $\pm$ 0.50	na	2.1 $\pm$ 0.03
VTA DA	2-day	3.9 $\pm$ 0.23	2.5 $\pm$ 0.19***	79	75	39.3 $\pm$ 4.11	12.3 $\pm$ 2.48***	3.6 $\pm$ 0.38	2.5 $\pm$ 0.11
	14-day	na	3.2 $\pm$ 0.19	na	77	na	22.4 $\pm$ 3.84*	na	2.5 $\pm$ 0.09

na⁺ - no statistical difference was present between 2-day and 14-day controls thus results were grouped with 2-day controls and no individual values were recorded

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

2.0 Effect of vilazodone on 5-HT_{1A} receptors on dorsal hippocampus CA3 pyramidal neurons

2.1 Effect of 14-day vilazodone administration on tonic activation of 5-HT_{1A} receptors

Systemic injections of the 5-HT_{1A} antagonist WAY100635 did not significantly alter firing of CA3 hippocampus pyramidal neurons in controls animals ($p > 0.05$ one-way ANOVA with repeated measures). Long term vilazodone administration was able to significantly alter firing, resulting in enhanced tonic activation by extracellular 5-HT. This was confirmed with a robust disinhibition of neuronal firing in response to WAY100635 at doses of 25 µg/kg (50% of baseline), 50 µg/kg (110% of baseline), 75 µg/kg (127% of baseline) and 100 µg/kg (125% of baseline) cumulative doses (** $p < 0.01$ one-way ANOVA with two way repeated measures; Figure 17). This 2.2-fold increase in pyramidal neuron firing activity may indicate a net increase in 5-HT transmission following vilazodone treatment.

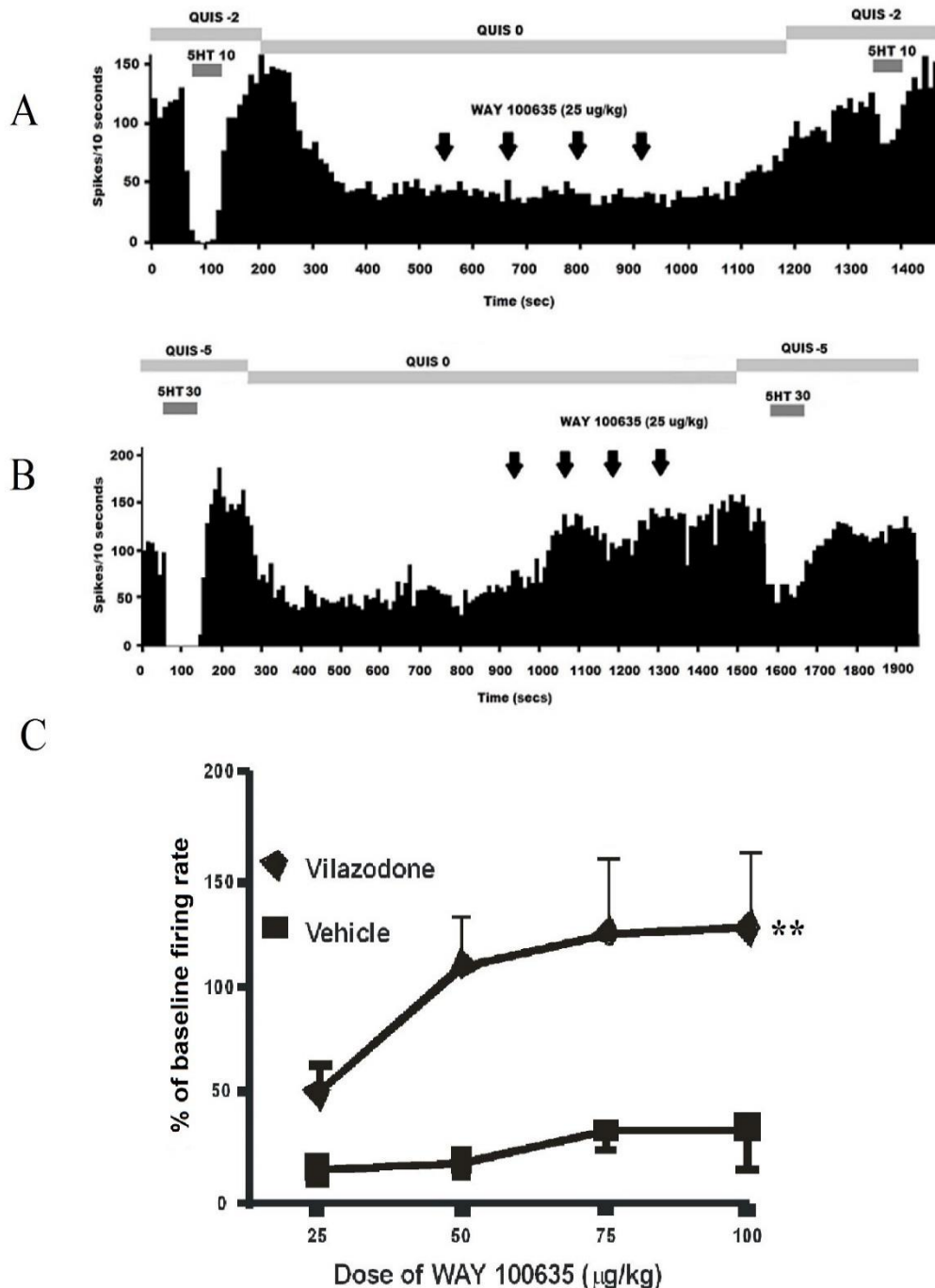


Figure 17. Effect of long-term vilazodone administration on tonic activation of 5-HT_{1A} receptors. Firing rate histograms of dorsal hippocampus CA3 pyramidal neurons. Systemic WAY100635 administration was given in incremental doses of 25 $\mu\text{g/kg}$, as indicated by the arrows. Control animals (A) and 14-day vilazodone treated animals (5 mg/kg) (B) are illustrated. The ejection current of quisqualate is denoted on the histogram. Overall percent changes in the baseline firing rate of hippocampal pyramidal neurons is also denoted (C). ** $p < 0.01$ one-way ANOVA with repeated measures.

2.2 Effect of long-term vilazodone administration on spike suppression and RT_{50} .

Both treated and control animals received microiontophoretic applications of exogenous 5-HT to detect alterations in the sensitivity of the CA3 hippocampal 5-HT_{1A} receptors. No changes were observed, as indicated by the number of spikes suppressed per nano-ampere, which remained insignificant between control and treated animals (Figure 18). Furthermore, there was no significant change in RT_{50} between treated and control animals, indicating that vilazodone had no viable effect on the hippocampal 5-HT transporter as well (Figure 18).

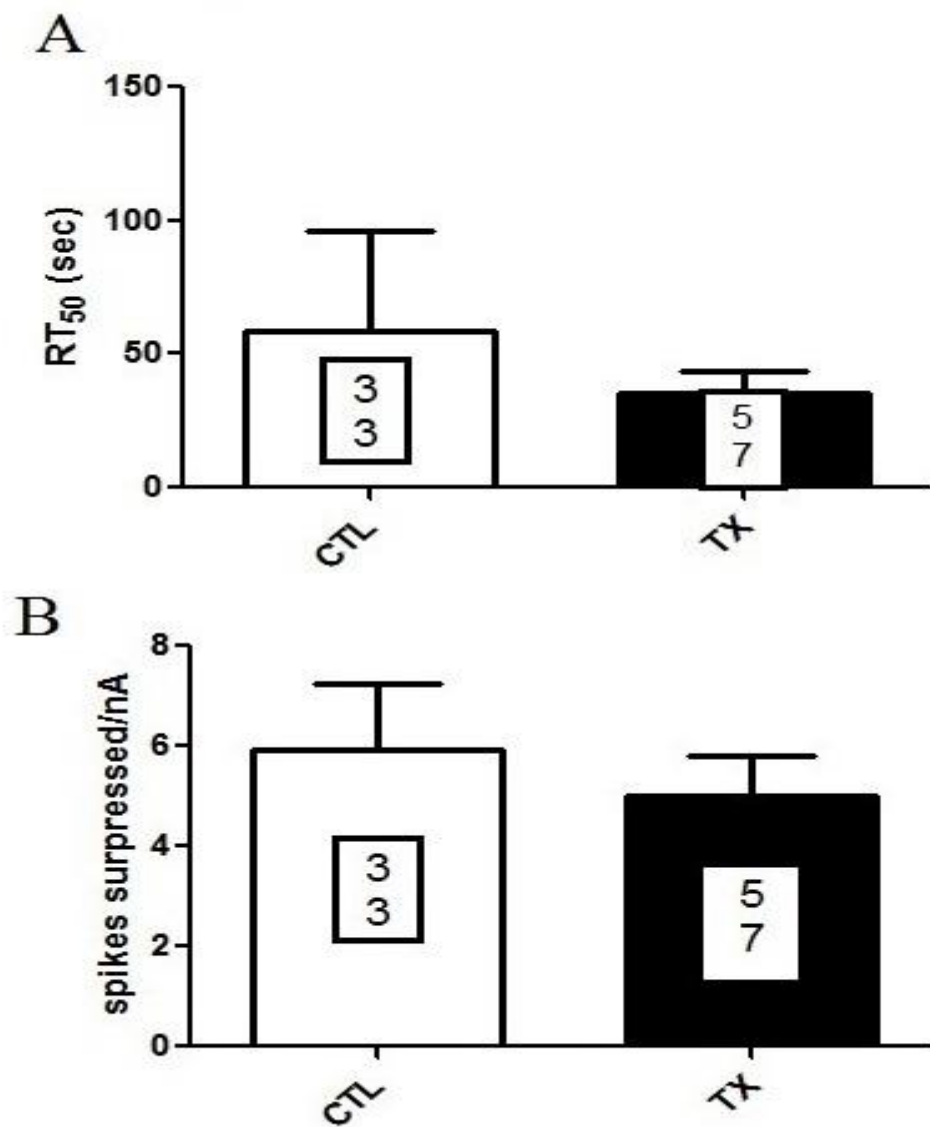


Figure 18. Effect of long-term vilazodone administration on spike suppression and RT₅₀. Mean ($\pm$ SEM) number of spikes suppressed/nA in 14-day control and vilazodone (5 mg/kg) treated rats (A). The time to a 50% recovery of firing (RT₅₀) of dorsal hippocampus CA3 pyramidal neurons following microiontophoretic application of 5-HT in 14-day control and vilazodone (5 mg/kg) treated rats (B). The number in the boxes indicates the number of neurons and rats recorded, respectively.

DISCUSSION

1.0 Effects of vilazodone on the serotonin system

Acute administration of vilazodone produced a robust decrease in serotonin firing, which may have occurred either indirectly, due to the drugs SSRI component or directly via 5-HT_{1A} receptor agonism as the drug is also a partial 5-HT_{1A} receptor agonist as aforementioned (Sheard et al, 1972; Sprouse and Aghajanian 1987; Hughes et al, 2005). To determine which of vilazodone multimodal effects caused the decrease in 5-HT neuron firing rate, 5-HT levels were depleted prior to drug administration via administration of PCPA, an inhibitor of tryptophan hydroxylase which would in turn dampen the SSRI potential of the compound (Feldman et al, 1997). Following PCPA administration, 5-HT firing remained significantly decreased in response to acute vilazodone administration, which indicated that the drug can directly activate 5-HT_{1A} autoreceptors despite significant decreases in 5-HT levels and thus decreased SSRI activity. While vilazodone has been shown to be a partial 5-HT_{1A} agonist with an IC₅₀ of 0.2 nM in vitro, some controversy exists as to its 5-HT_{1A} agonism properties in vivo (Bartoszyk et al, 1997). For instance, voltammetry studies in the guinea pig DRN failed to confirm that vilazodone was a 5-HT_{1A} agonist (Roberts et al, 2005). Further microdialysis studies also postulated that vilazodone may in fact display little to no 5-HT_{1A} agonism (Hughes et al, 2005). However, other studies provide strong evidence of its 5-HT_{1A} agonism properties, such as binding studies conducted in vitro as well as in vivo electrophysiological studies (Page et al, 2002; Ashby et al, 2013). To fully elucidate the 5-HT_{1A} properties of vilazodone further experiments are required; nonetheless

the current PCPA depletion results provide further evidence to the existing literature regarding the effectiveness of vilazodone as an in vivo 5-HT_{1A} agonist.

Many of the presynaptic effects of vilazodone are similar to those seen with other conventional SSRIs, which is expected given that vilazodone has been shown to have an occupancy of 100% for the SERT in some brain regions (at doses of 10 mg/kg, Hughes et al, 2005). The decrease in firing following 2-day administration of vilazodone was most likely due to the activation of 5-HT_{1A} autoreceptors (Blier et al, 1990; Blier and de Montigny, 1994). Following 14-day administration, the 5-HT firing in the DRN returned to baseline, much as is seen with other SSRIs (Blier et al, 1990; Blier and de Montigny, 1994). The proposed mechanism of action is postulated to involve 5-HT_{1A} autoreceptor desensitization and is illustrated in Figure 19 (Blier and Ward, 2002). Administration of a 5-HT_{1A} agonist alone is able to induce decreases in 5-HT firing as well as presynaptic 5-HT_{1A} desensitization, much like an SSRI thus the fact that vilazodone produces similar effects was expected (Blier and de Montigny, 1990a; Blier and de Montigny, 1990b; Kreiss and Lucki, 1997). Previous electrophysiological studies of vilazodone have seen similar effects, with vilazodone even producing 5-HT_{1A} desensitization faster than SSRIs fluoxetine and paroxetine (Ashby et al, 2013). Furthermore, the desensitization was confirmed to be related to the 5-HT_{1A} agonist properties of the drug rather than the SSRI properties, as vilazodone was able to significantly increase the ID₅₀ of 8-OH-DPAT while conventional SSRIs had no effect (Ashby et al, 2013).

An interesting future consideration is the length of time for desensitization to occur. As vilazodone contains two mechanisms of action, perhaps it would be

able to induce desensitization at a faster rate. This has been previously postulated in animal behavioural trials and could be further studied by comparing the effects on 5-HT firing following a 7-day administration of SSRI and vilazodone separately, allowing for more precise detection in changes in desensitization (Redrobe and Bourin, 1998).

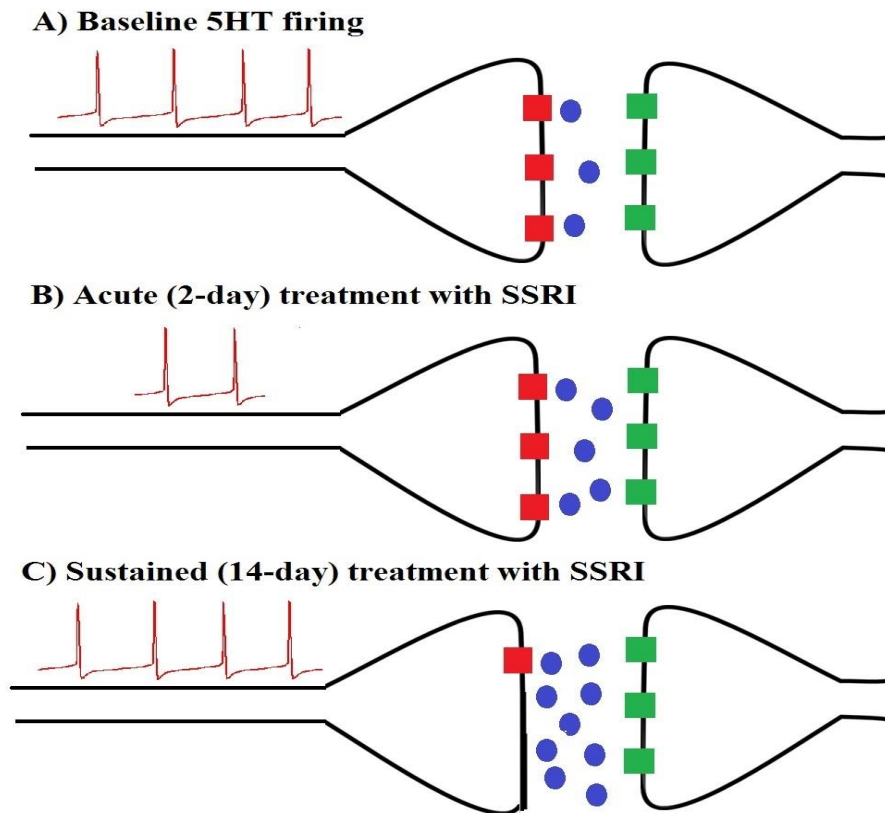


Figure 19. Effects on an SSRI on the 5-HT_{1A} autoreceptor of DRN 5-HT neurons. A) At baseline, the 5-HT neuron is firing. The red squares represent 5-HT_{1A} autoreceptors, the green squares are post-synaptic neuron receptors and the blue circles are 5-HT being released in response to neuron stimuli. B) Following acute SSRI treatment, there is blockage of SERT which causes a buildup of 5-HT endogenously. This build up will in turn activate 5-HT_{1A} autoreceptors which will result in an inhibition of firing. C) Following sustained treatment, the 5-HT_{1A} receptors become desensitized and no longer inhibit firing, allowing the firing rate to recover and return to baseline. (Adapted from Blier and Ward, 2002).

2.0 Effects of vilazodone on the dopamine system

Vilazodone was able to reduced DA firing after 2-day administration, as has been previously witnessed with SSRI administration. Previous studies have indicated that 5-HT is inhibitory to DA firing, as lesions to the DRN produced robust increases of up to 36% with respect to DA neuron firing (Guiard et al, 2008). Administration of an SSRI would thus be expected to produce decreases in DA firing, as it would result in increased synaptic levels of the inhibitory 5-HT neurotransmitter. This has in fact been confirmed with several SSRIs following both acute and sustained administration, including fluoxetine and escitalopram (Prisco et al, 1994; Di Mascio et al, 1998; Dremencov et al, 2009). The effect is thought to be mediated by the activation of 5-HT_{2C} excitatory receptor, located on inhibitory GABAergic interneurons thus producing a decrease in DA neuron firing upon activation (Dremencov et al, 2009). Interestingly, while other SSRIs assessed have been shown to produce DA firing decreases as high as 50% following 2-day administration, vilazodone induced a less robust decrease of 37% (Chernoloz et al, 2009; Dremencov et al, 2009). This effect may be due to the 5-HT_{1A} properties of vilazodone, as 5-HT_{1A} has been found to facilitate DA release in a number of brain regions (Alex and Pehek, 2007). In the VTA specifically, the 5-HT_{1A} receptor agonist 8-OH DPAT has been shown to produce excitatory effects in a subpopulation of neurons when administered at low doses and when administered somatodendritically as well (Arborelius et al, 1993; Prisco et al, 1994). This may also provide some explanation with respect to the effects of vilazodone following

14-day administration, as typical SSRIs were able to produce significant decreases in DA firing up to 50% while vilazodone produced only a non-significant decrease of 22% (Dremencov et al, 2009). The inhibitory effects of the SSRI mode of action of vilazodone may have been dampened by the excitatory properties of 5-HT_{1A} agonism following 14-day administration. Therefore, while vilazodone results in decreased DA firing, the attenuation is not as pronounced as is seen with typical SSRIs, which may be a result of the possible excitatory effects of 5-HT_{1A} agonism which may offset the decrease in firing.

DA neurons in the VTA have been found to exhibit two firing patterns: tonic firing which occurs under normal, non-arousing conditions and burst firing which occurs when an arousing sensory stimulus is present (Cooper, 2002). This burst firing has significant implications to the downstream projection areas of the VTA, such as it can result in increased concentrations of DA in these regions, in particular the striatal regions important in reward and pleasure circuits within the brain (Cooper, 2002). Vilazodone was found to produce significant reductions in the bursting properties of DA neurons including percent spikes in burst, bursts per minute and burst duration. Similar results were seen when the dopaminergic effects of other SSRI compounds were assessed as their administration also resulted in decreased bursting in VTA DA neurons (Dremencov et al, 2009). Previous studies have indicated that 5-HT_{1A} receptor agonists increase DA neuron bursting parameters, however this was not noted with vilazodone, providing some evidence that perhaps the SSRI action of the drug predominates with respect to burst firing inhibition (Arborelius et al, 1993; Diaz-Mataix et al, 2005).

The decrease in bursting noted in response to vilazodone could result in a reduction in DA neurotransmission which may initially seem counterproductive to its successfulness as an AD. However this decrease in DA transmission may be a temporary result of SSRI treatment which may dissipate once the 5-HT_{2C} receptor deemed responsible for the effects develops effective tolerance levels; this postulate is one of the proposed mechanisms for the delay witnessed with SSRIs such as fluoxetine (Dailly et al, 2004). Clinical studies have confirmed this effect, as healthy volunteers treated with SSRIs for 7 days exhibited decreased neural responsiveness to both pleasant and aversive stimuli, an indication of decrease DA transmission following SSRI administration (McCabe et al, 2010). Human studies measuring the CSF concentrations of a DA metabolite in MDD patients indicated similar results, with significant decreases following 6 weeks of SSRI therapy (Sheline et al, 1997).

3.0 Effects of vilazodone on the noradrenaline system

Following 2-day vilazodone administration, LC NE firing rates remained unchanged whilst after 14-day treatment a significant decrease was observed. The inhibitory properties are expected as lesion studies have confirmed that when the DRN is lesioned, NE firing in the LC increases up to 70%, indicating an inhibitory effect of 5-HT (Haddjeri et al, 1997; Guiard et al, 2008). This effect is thought to be mediated through the 5-HT_{2A} receptor which is located on inhibitory GABAergic interneurons (Szabo and Blier, 2002; Seager et al, 2005).

The effects of vilazodone on the LC neurons appears to differ slightly from what has been observed with other SSRIs in previous studies. Studies conducted with the

SSRI escitalopram produced significant decrease in firing at both 2-day and 14-day time points, indicating that both short and sustained treatment was able to induce an effect on firing (Dremencov et al, 2007). However, studies conducted with other SSRIs such as citalopram produced similar changes as those seen with vilazodone: no change in firing at 2-day administration and a significant change at 14-day administration, indicating that this seems to be the predominant effect (Szabo et al, 2000; Dremencov et al, 2007). It is thought that this effect is due to the desensitization of 5-HT_{1A} receptors following 14-day treatment. Typically, the acute administration of a 5-HT_{1A} agonist is able to induce increases in LC NE neuron firing; this effect is abolished following long term SSRI treatment indicating that the desensitization induced by the treatment is able to alter NE firing (Piercey et al, 1994; Szabo et al, 2000). Desensitization of the 5-HT_{1A} receptor would limit its inhibitory capacity, resulting in an increase in 5-HT and in turn producing a decrease in LC NE firing, as is witnessed in SSRI treatment (Szabo et al, 2000). This mechanism of action would account for the results seen with vilazodone, as its 5-HT_{1A} agonist properties in combination with its SSRI characteristics induced 5-HT_{1A} desensitization, as witnessed by the recovery in DRN 5-HT firing following 14-day treatment. The decrease in NE firing induced by SSRIs has often been seen as a detrimental effect in major depression of SSRIs and can in fact be reversed with the use of atypical antipsychotics which act as 5-HT_{2A} antagonists (Szabo and Blier, 2002).

Much like DA neurons, the neurons in the LC exhibit both tonic and burst firing. The burst firing of NE neurons has been shown to elevate NE concentrations in a

variety of brain regions including the PFC and LC, which have both been implicated in MDD (Florin-Lechener et al, 1996; Berridge and Abercrombie, 1999). Following vilazodone administration bursting remained unchanged, which differs from previous SSRI results which show a significant decrease in the bursting parameters of LC NE neurons (Dremencov et al, 2007). Much as with decreases in LC NE firing rate, the effects are thought to be due to the 5-HT_{2A} receptor (Dremencov et al, 2007). The different results with respect to bursting may be due to the 5-HT_{1A} agonist properties of vilazodone. 5-HT_{1A} agonists have been noted to significantly increase NE levels in several brain regions and produce robust increases in LC NE firing, indicating stimulatory effects on the NE system (Done and Sharp, 1994; Lejeune and Millan, 2000). Perhaps the lack of alterations in NE bursting witnessed with vilazodone can be attributed to this property.

Interestingly, previous microdialysis studies indicated that vilazodone was unable to induce any changes in DA or NE levels in the rat frontal cortex (Hughes et al, 2005). However, these microdialysis studies were conducted on acute timelines as short as 240 minutes thus the effects of vilazodone may not have been detectable (Hughes et al, 2005). Further experiments with vilazodone may initiate new microdialysis trials following longer time constants with respect to vilazodone administration to confirm that the noted electrophysiological changes also induced changes in the levels of DA and NE detected in the rat cortex. Furthermore, the lack of effect on NE and DA levels in the rat frontal cortex is was previously utilized to substantiate claims that vilazodone lacked any 5-HT_{1A} agonist properties, as other 5-HT_{1A} such as 8-OH-DPAT were able to induce significant changes in NE and DA

levels (Hughes et al, 2005). As the current study indicated, vilazodone is able to alter NE and DA firing properties, thus putting these claims in question and providing indirect evidence of 5-HT_{1A} activity.

4.0 Effects of vilazodone on the hippocampus

The effects witnessed in the hippocampus further mimic the effects seen not only with SSRIs and 5-HT_{1A} agonists, but with all antidepressants as tonic activation was increased indicating a net increase in 5-HT neurotransmission (Haddjeri et al, 1998). During baseline firing, the application of a 5-HT_{1A} antagonist such as WAY100635 will produce only small increases in CA3 pyramidal firing rates. The antagonist blocks the inhibitory 5-HT_{1A} receptor and produces disinhibition but due to small amount of 5-HT present, baseline firing level are already so low that the effects of this disinhibition are minimal (Haddjeri et al, 1998; Blier and de Montigny, 1999). However, in the presence of an SSRI, 5-HT_{1A} transmission in the hippocampus is significantly higher. This can be confirmed with the administration of a 5-HT_{1A} antagonist, which results in significantly increased activity of CA3 pyramidal neurons in this case due to the higher 5-HT tone (Haddjeri et al, 1998; Blier and de Montigny, 1999). The long-term administration of the 5-HT_{1A} agonist gepirone produced similar results, confirming its ability to induce enhancements in neurotransmission as well (Haddjeri et al, 1998).

Unexpectedly, vilazodone did not to produce any alterations in RT₅₀, which is a measure of the time (in seconds) elapsed from the termination of microiontophoretic application of 5-HT to obtain a 50% recovery of the initial firing rate (Pineyro et al, 1994). This measure allows an approximation of the 5-HT

reuptake process and is typically elevated following acute SSRI treatment as SERT is blocked thus not allowing for effective reuptake (Pineyro et al, 1994). Thus, it is surprising to see no change in RT_{50} following vilazodone administration as it exhibits SSRI action with its effects on the presynaptic monoaminergic regions. The RT_{50} measure has been proven to be very effective in deducing the ability of a drug to block SERT. For instance, hippocampal recordings conducted on rats following 14-day administration of SSRIs escitalopram and citalopram both produced significant increases in RT_{50} (El Mansari et al, 2005). Furthermore, the antidepressant trazodone, known to display considerably less SERT blockade than typical SSRIs, was able also to induce a more than 2 fold increase in RT_{50} using the same recording method in the hippocampus, indicating that the method used is effective at detecting even suboptimal SERT blockade (Ghanbari et al, 2010). As well, the SSRI activity of vilazodone has been confirmed in many in vitro and in vivo models. For instance, studies conducted on LLCPK cells which contained the human SERT transporter indicated that vilazodone was able to inhibit 5-HT reuptake with a potency greater than some typical SSRIs such as fluoxetine (Dawson and Watson, 2009). Further in vitro studies utilizing [3H]DASB ligand binding studies have also similarly confirmed the SSRI property of vilazodone (Hughes et al, 2005). In vivo electrophysiological studies using p-chloroamphetamine, a potent monoamine releaser that utilizes SERT to induce monoamine depletion, also indicated that vilazodone is able to induce SERT blockade as depletion was significantly decreased with both vilazodone and typical SSRI administration (Ashby et al, 2013).

A possible explanation for these controversial results may be related to the dosage used, as the dose of 5 mg/kg utilized may have been too low to induce full SERT blockade in this instance. A study conducted by Hughes et al determined that at 3 mg/kg vilazodone displayed a 60% occupancy of SERT in the hippocampus therefore the dosage of 5 mg/kg used in the current study may have been insufficient (Hughes et al, 2005). Previous [^{11}C] DASB PET studies in humans have concluded that an occupancy of at least 80% is required for SSRIs to demonstrate clinical efficacy (Meyer et al, 2001b). The 5 mg/kg dosage utilized may have not satisfied this requirement, thus the reuptake effects of vilazodone may have been missed. In fact, previous studies state that based on this principle, the dose of 10 mg/kg of vilazodone would be ideal to induce clinical efficacy (Hughes et al, 2005). Furthermore, most previous medications tested using the tonic activation paradigm utilized doses greater than 10 mg/kg, indicating the importance of adequate dose within these experiments (Haddjeri et al, 1998).

However when 10 mg/kg/day was attempted in the current study, a significant deterioration in the rats was witnessed as the higher dose resulted in weight loss, lethargy, and agitation in the animals, indicating toxicity. This could be due to the method of drug administration, as intraperitoneal administration was utilized due to difficulties experienced with dissolving the compound adequately to allow subcutaneous minipumps delivery. Previous studies with vilazodone have successfully administered doses of 10 mg/kg using oral gavage, thus this method could be adopted in future studies to provide more clarification on the dose-specific effects of vilazodone by avoiding a possible irritant effect in the peritoneal cavity

(Hughes et al, 2005). Therefore, while vilazodone has previously been shown to exhibit SERT occupancy, due to issues with drug toxicity in rats, the dosage used was inadequate to provide clear evidence confirming this effect. Nonetheless, further studies by El Mansari et al have shown promising evidence for the SERT activity of vilazodone: when the drug is administered acutely via IV it is able to induce a two-fold increase in RT_{50} at doses of 400 $\mu\text{g/kg}$ (El Mansari et al, 2014 unpublished data). This provides further evidence of dosing issues in the current study and has become the focus of further vilazodone studies.

Dosing may have also played a role in the degree of tonic activation noted with vilazodone administration. With vilazodone being a combination drug with both SSRI and 5-HT_{1A} agonist action, one might question whether it would be able to induce greater levels of tonic activation. However, vilazodone was able to induce an increase of 50-125% in tonic activation while previous studies conducted with escitalopram and citalopram, which exhibit solely SSRI action, resulted in 400-600% increases (El Mansari et al, 2005). The dosage of 5 mg/kg may have been inadequate and thus not induced the degree of tonic activation that would be expected. Further experiments with increased dosages or using oral gavage as a method of drug delivery could aid in further elucidating this discrepancy as well.

5.0 Conclusion

This study determined that vilazodone was able to alter firing presynaptically in all three of the monoaminergic systems studied as well as in the hippocampus (Figure 20).

The clinical benefit of vilazodone remains to be fully elucidated; the best assessment of this would be via double-blind randomized comparisons to other antidepressant medications, which have yet to be conducted. The most likely role for vilazodone will be within subsets of MDD patients, particularly those with treatment resistant depression. Augmentation and combination strategies have long been accepted in the treatment of resistant depression. A variety of different combinations have been utilized, although most commonly the SSRI is the initiating medication with additional compounds such as 5-HT agents, TCA's, atypical antipsychotics and many others added on (Fava, 2000; Nelson, 2003). Furthermore, clinical research has already indicated a degree of success with treatment resistant depressed patients when treated specifically with SSRIs and 5-HT_{1A} medications combined, with more significant advantages to the multimodal treatment seen in more severely affected individuals (Landen et al, 1998; Trivedi et al, 2006). This indicates not only that this strategy is successful but that vilazodone may be of specific benefit in these individuals as well.

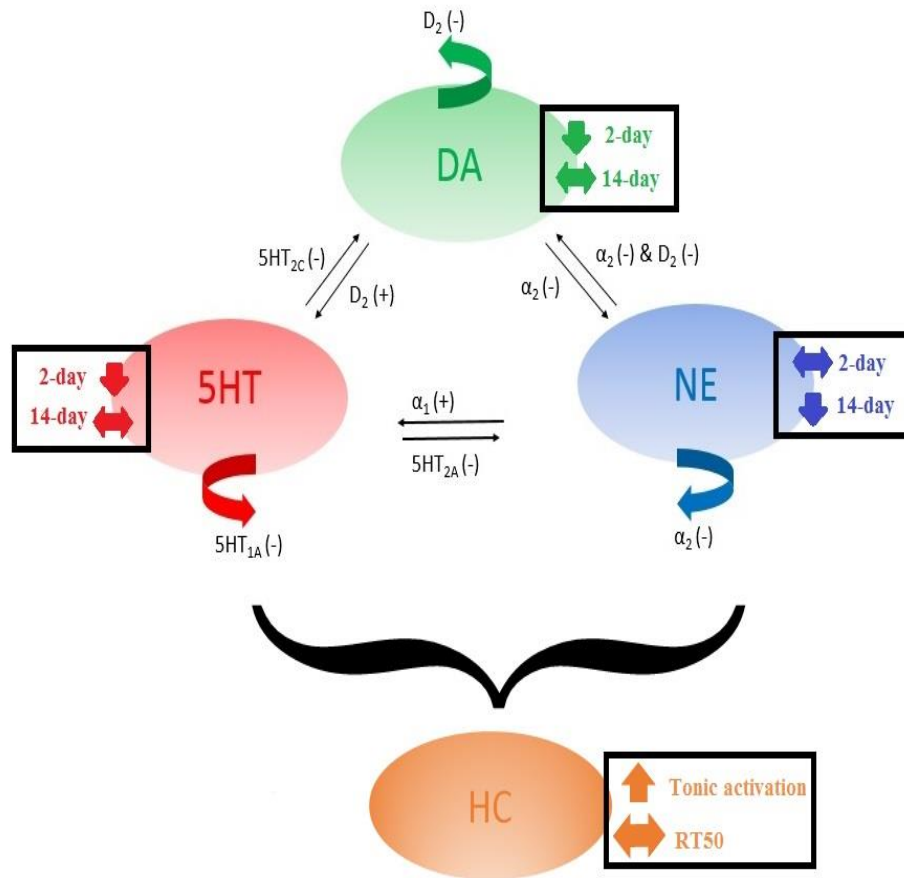


Figure 20. Summary of the effects of vilazodone on the presynaptic monoaminergic systems and the hippocampus. Adapted from Guiard et al, 2008.

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