Electrophysiological Investigations on the Role of Selected Serotonin Receptors and the Serotonin Transporter on Serotonin Transmission in the Rat Brain

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ABSTRACT

This study assessed the *in vivo* effects of various serotonin (5-HT) receptor modulators on 5-HT neurotransmission in the rat hippocampus. Vortioxetine, humanized-vortioxetine, and escitalopram blocked the 5-HT transporter, but similar to ipsapirone did not dampen the sensitivity of postsynaptic 5-HT_{1A} receptors. Long-term administration of all treatments increased the tonic activation of postsynaptic 5-HT_{1A} heteroreceptors, an effect common to all antidepressants. Vortioxetine decreased the function of the terminal 5-HT_{1B} autoreceptor under high but not a low degree of activation, thus showing that its partial agonism led to increased 5-HT release and that long-term administration results in the desensitization of terminal 5-HT_{1B} autoreceptors.

Vortioxetine overcame the effects of 5-HT_{1B} and 5-HT_3 receptor agonists. This study was unable to determine the involvement of 5-HT_7 receptor antagonism exerted by vortioxetine affects 5-HT neurotransmission. Therefore, vortioxetine would appear to exert different actions, via transporter and receptor activity, on the serotonergic system in the hippocampus, consistent with its unique pharmacological profile.

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LIST OF ABBREVIATIONS

β-OH	hydroxypropyl-beta-cyclodextrin
5-HT	5-hydroxytryptamine, or serotonin
5-HTT	5-hydroxytryptamine (serotonin) transporter, or SERT
8-OH-DPAT	8-hydroxy-N,N-dipropyl-2-aminotetralin
AADC	aromatic amino acid decarboxylase
AC	adenylyl cyclase
ACh	acetylcholine
AD	antidepressant
ANOVA	analysis of variance
AP	anterior-posterior
APA	American Psychiatric Association
b.i.d	bis in die
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
CA	Cornu Ammonis
cAMP	cyclic adenosine monophosphate
CBT	cognitive behavioural therapy
CNS	central nervous system
CP-93129	3-(1,2,3,6-tetrahydropyridin-4-yl)-1,4-dihydropyrrolo[3,2-b]pyridin-5-one
CP-94253	3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-b]pyridine
CRH	corticotropin-releasing hormone
DA	dopamine
DALY	disability adjusted life year
DBS	deep brain stimulation
DG	dentate gyrus
DOS	duration of silence
DRN	dorsal raphe nucleus
DSM-V	Diagnostic and Statistical Manual of Mental Disorders 5th Edition
ECS	electroconvulsive shocks
ECT	electroconvulsive shock therapy
FST	forced swim test
GABA	γ-aminobutyric acid
GPCR	G-protein coupled receptor
h-vortioxetine	humanized-vortioxetine
HA	histamine
HAM-D	Hamilton Rating Scale for Depression, or HDRS
HPA	hypothalamic-pituitary-adrenal
i.p.	intraperitoneal
IT_{50}	inhibition time for 50 percent
i.v.	intravenous
ICD-10	International Statistical Classifications of Disease and Related Health Problems 10th Revision
LC	locus coeruleus
LGIC	ligand-gated ion channel

LM	lateral-medial
LP-44	4-[2-(methylthio)phenyl]-*N*-(1,2,3,4-tetrahydro-1-naphthalenyl)-1-piperazinehexanamide
Lu AA21004	1-(2-((2,4-dimethylphenyl)thio)phenyl)piperazine, or vortioxetine
MADRS	Montgomery-Åsberg Depression Rating Scale
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder, or unipolar depression, or depression
MDE	major depressive episode
mPFC	medial prefrontal cortex
MRN	median raphe nucleus
NAc	nucleus accumbens
NADPH	nicotinamide adenine dinucleotide phosphate
NE	norepinephrine
NET	norepinephrine transporter
NRI	norepinephrine reuptake inhibitor
PCPA	p-chlorophenylalanine
PSTH	peristimulus time histogram
TMS	transcranial magnetic stimulation
TPH-1	tryptophan hydroxylase-1
TPH-2	tryptophan hydroxylase-2
TST	tail suspension test
rECS	repeated electroconvulsive shocks
RT_{50}	recovery time for 50 percent
SR 57227	1-(6-chloro-2-pyridinyl)-4-piperidinamine
SNRI	serotonin-norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
VNS	vagus nerve stimulation
VTA	ventral tegmental area
TCA	tricyclic antidepressant
VMAT	vesicular monoamine transporter
WAY100635	*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]- *N*-(2-pyridyl)cyclohexanecarboxamide
WHO	World Health Organization
WO	washout
YLD	years lived with disability

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INTRODUCTION

1.0 Major Depressive Disorder

1.1 Background

Major depressive disorder (MDD, depression) is a widespread and disabling disease which affects one in six people, resulting in a reduction in quality of life, productivity and medical health (Kessler et al., 2003; Kessler et al. 2006). MDD is not only devastating to the individual suffering from the illness, but can have a strong impact on society as well. The economic burden of MDD in the United States alone was estimated at approximately $83.1 billion in the year 2000 (Greenberg et al., 2003). MDD-related decreases in workplace productivity have also been reported in Canada resulting in annual lossess of between $6 and $60 billion (Stephens & Joubert, 2001; Stewart et al., 2003). Lifetime prevalence rates vary but have been reported to range between two and nineteen percent in Canadian adults with a lifetime prevalence rate of approximately twelve percent (Government of Canada, 2006; Waraich et al., 2004; Weissman et al., 1996). Furthermore, in middle to high income countries, MDD is the leading cause of disease burden, as measured in disability adjusted life years (DALYs) (Mathers et al., 2008). Globally, the World Health Organization (WHO) has predicted that within the next 20 years, MDD will become the second leading cause of disease burden, surpassed only by ischemic heart disease (Haden & Campanini, 2001; Mathers et al., 2008).

There are currently two classification systems used for the diagnosis of MDD; the Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-V) and the 10th revision of the International Statistical Classification of Disease and Health Related Problems (ICD-10). The diagnostic criterion most used in North America is the

American's Psychiatric Association's (APA) DSM-V. According to the DSM-V, a definitive diagnosis of MDD requires at least one major depressive episode (MDE) that lasts a minimum of two weeks that are characterized by MDD core symptoms of persistent depressed mood and/or anhedonia in addition to at least four other symptoms such as weight loss or gain, fatigue, insomnia or hypersomnia, psychomotor agitation/retardation, difficulties concentrating, inappropriate guilt, and thoughts of suicide (American Psychiatric Association, 2013). The aforementioned symptoms impair cognitive and social functioning resulting in decreased workplace performance, decreased quality of life, and increased mortality rates (Lépine & Briley, 2011). The severity of mental illness, such as MDD, is often determined by answering a series of questions relating to the patients mental state. The two most common clinical assessment scales are the Montgomery-Åsberg Depression Rating Scale (MADRS) and the Hamilton Depression Rating Scale (HDRS, HAM-D) (Montgomery & Asberg, 1979; Hamilton, 1960). While rating scales are not typically used for diagnosing MDD, they have been found to reliably assess symptom severity (Montgomery & Asberg, 1979). Once diagnosis and severity have been determined, treatment, most commonly in the form of antidepressants (AD) can begin.

Effective AD treatment can sometimes result in the patient experiencing a full remission, which is defined as the complete asymptomatic return to their normal life. Although remission is the desired effect of the treatment of MDD, it is often difficult to achieve (Frank et al., 1991). The majority of patients do not experience full remission after their initial AD treatment and require subsequent treatments. Only one third of patients experience full remission following initial AD treatment, with one third

experiencing a response, and the remaining third experiencing no response (Rush et al., 2006; Hirschfeld et al., 2002). A response is defined as a reduction of MDD symptoms to a level below the amount needed to make a diagnosis (Frank et al., 1991). For example, a score on the seventeen item HAM-D of seven or less is considered normal while a score of eighteen or greater is considered of moderate severity (Hamilton, 1960). Following initial AD treatment, two thirds of patients with MDD do not achieve remission. In order to increase remission rates, decrease relapse rates, and reduce adverse side effects additional AD treatments are needed.

1.2 Etiology

The etiology of this mood disorder is unclear due to its complexity and heterogeneity. For instance, a diagnosis of MDD can be made in two patients who may present with mutually exclusive symptoms. Furthermore, factors that contribute to the development and severity of MDD are both environmental and genetic (Caspi et al., 2003; Kendler et al., 2001; Sullivan et al., 2000). Similarly, susceptibility to MDD is also often difficult to predict. The interactions between the environment, stressful life events, and genetic makeup all influence a person's likelihood to develop MDD (Sullivan et al., 2000).

Research examining MDD in relation to genetic factors have shown that genetic makeup can contribute greatly to the onset and severity of MDD. For instance, diathesis-stress models show an individual's sensitivity to stressful events depends on their genetic composition (Costello et al., 2002). In relation to MDD, behavioural genetic studies indicate that susceptibility is increased after stressful life events in individuals who are at

a high genetic risk while not in individuals at low genetic risk (Kendler et al., 1995; Caspi et al., 2003). Furthermore, individuals that are more genetically susceptible to MDD also present with increased stress-inducing behaviours, resulting in greater stressful life events and MDEs (Kendler et al., 1999). A meta-analysis study reviewing the genetic epidemiology of MDD estimates heritability at 37 percent, although this does not account for the increased prevalence of MDD in women (Sullivan et al., 2000; Marcus et al., 2005; Lépine & Briley, 2011). Although a link has been made between external life stressors and an individual's genetic makeup with an increased risk for MDD, not all genetically high risk individuals necessarily present with MDD, suggesting further elements are involved (Kendler et al., 1995).

The severity of many external life stressors varies from person to person. Life stressors can be either dependent or independent. These stressors range from death of a loved one, the acquisition of a serious illness, divorce, job loss, and assault (Kendler et al., 1995). The role of environmental stressors in the development of MDD is supported by twin studies. Often the development of MDD in one twin will not necessarily occur in the virtually genetically identical other twin – suggesting genes are not the sole determinant (Sullivan et al., 2000).

1.3 Challenging issues in the treatment of MDD

Even in developed countries, where treatment is readily available, only a small minority of people suffering from major depression seek or receive treatment (Lépine & Briley, 2011). Seeking treatment is often deterred by the stigma of mental illness, financial difficulties, symptoms of worthlessness, lack of motivation, and excessive guilt

(Haden & Campanini, 2001). For individuals faced with these obstacles, the potential benefits of treatment are unavailable and they continue to suffer.

Many of the challenges in treatment arise from the cause of major depression. Although some correlations have been made, the cause of this disorder remains elusive (Blier & de Montigny, 1994). A combination of its heterogeneity and our limited understanding of the gene-environment interaction make it difficult to eliminate variables in addition to there most likely being a number of pathways that lead to the common endpoint of MDD (Sullivan et al., 2000).

The complexity of this disorder also makes it difficult to create accurate animal models of depression; therefore research must rely on a number of paradigms to determine the efficacy of ADs (Berton & Nestler, 2006). Paradigms are used to assess depressive-like behaviour in relation to alterations in neurotransmitter functions which are involved in depression. Genetic studies of MDD are complex; gene polymorphisms have been linked to the etiology of depression but gene associations remain difficult due to MDD heterogeneity (Hamer, 2002; Caspi et al., 2003). As previously mentioned, depressed patients often do not have common symptoms, indicating the possibility of several etiological pathways for depression. As a result, a single AD will often not produce remission in all patients making alternate strategies essential.

Health care professionals may not have the time or resources to provide the proper treatment in the primary care setting which may result in their failure to recognize the symptoms and follow best practice recommendations (Haden & Campanini, 2001). As aforementioned, following ideal therapeutic treatment regimens only one third of

patients experience full remission after their initial AD treatment (Rush et al., 2006; Hirschfeld et al., 2002).

Furthermore, with each subsequent AD treatment, a patient's chance of remission decrease and risk of relapse increases (Rush et al., 2006). Once a patient has been treated with two or more different classes of ADs and has not shown a favourable response, they can be considered treatment-resistant (Thase, 2003; Fava, 2003). Patients that do not fully remit may experience a response, which alleviates only some of the symptoms of MDD. However, following this partial remittance of symptoms, patients will often discontinue treatment, which in turn may increase the risk of relapse (Frank et al., 1991; Thase, 2003). The delay between treatment initiation and the onset of therapeutic action, as well as the wide spectrum of adverse side effects associated with ADs, can contribute to treatment discontinuation (Masand, 2003; Yerevanian et al., 2004). Discontinuation not only presents a barrier to treatment but can increase symptom severity, in some cases increasing suicidal behaviour by five-fold (Yerevanian et al, 2004). Therefore, for MDD remission, an ideal balance between effective therapeutic strategies and patient compliance, must be achieved.

2.0 Monoamine Hypothesis

2.1 Monoamines and depression

The monoamine hypothesis of depression is one of the most accepted theories underlying the biological etiology of MDD. The monoamine neurotransmitters involved are serotonin (5-hydroxytryptamine or 5-HT), dopamine (DA), and norepinephrine (NE), their neurons arise from the raphe nuclei, the ventral tegmental area (VTA), and the locus

coeruleus (LC), respectively (Guiard et al., 2008; Price & Drevets, 2009). In brief, this hypothesis states that MDD and the symptoms associated with it are a result of a monoamine deficiency (Schildkraut, 1965; Costello et al., 2002; Delgado, 2000). This is indirectly supported by the fact that many ADs alter monoamine neurotransmission by inhibition of reuptake or metabolism, increasing release, and altering ligand-receptor sensitivity (Schildkraut, 1965; Blier & de Montigny, 1994; Leyton et al., 2000; Dunlop & Nemeroff, 2007; Feighner, 1999; Delgado, 2000; Elhwuegi, 2004). The resulting enhancement in neurotransmission in one or more of these monoamines is thought to underlie the therapeutic effects of ADs. However, while the three monoamine systems exist as separate entities, they are also intricately connected. Thus, the alteration of one system can impact neurotransmission within the other monoamine systems. For example, if 5-HT neurons of the raphe nuclei are lesioned, the firing rate of VTA DA and LC NE is increased 36 and 70 percent, respectively (Haddjeri et al., 1997; Guiard et al., 2008). Furthermore, not only do the monoamine systems interact with one another but they provide strong input to other regions of the brain, such as the hippocampus (Mongeau et al., 1997). Alterations in neurotransmission in these projection areas may be mediated by modifications in monoamine neurotransmission; this may contribute to the pathophysiology of MDD and perhaps account for some of the symptoms.

2.2 Serotonin system

5-HT is an indolealkylamine that was initially discovered in blood serum and shortly after in the central nervous system (CNS) (Rapport et al., 1948; Bogdanski et al., 1956). The primary source of 5-HT in the brain are from the raphe nuclei (Dahlström &

Fuxe, 1964). The raphe nuclei are located along the midline of the brainstem, most of them being in the reticular formation (Jacobs & Azmitia, 1992; Dahlström & Fuxe, 1964). Although serotonergic neurons are restricted to clusters, 50 to 60 percent are located in the dorsal raphe nucleus (DRN), which in turn innervates nearly every part of the brain (Jacobs & Azmitia, 1992; Dahlström & Fuxe, 1964; Baker et al., 2004; Elhwuegi, 2004). Projection areas that receive strong serotonergic input include the hippocampus, frontal cortex, amygdala, and striatum (Hensler et al., 1991; Mongeau et al., 1997; Artigas, 2013).

Serotonin is involved in sleep, appetite, emotion, and mood regulation (Walther & Bader, 2003; Belmaker & Agam, 2008; aan het Rot et al., 2009). Impairment of this neurotransmitter system is associated with anxiety, dementia, and depression (Coppen, 1967; Serretti et al., 2007). Serotonin depletion studies provide evidence for the implication of 5-HT with respect to mood and anxiety disorders, as patients in these studies experience reoccurrence of depressive symptoms, despite having previously responded to serotonergic AD treatment (Delgado et al., 1994; Miller et al., 1992).

The precursor for 5-HT synthesis is the amino acid L-tryptophan which is primarily derived from dietary sources. The first step in 5-HT synthesis in the brain is the transport of L-tryptophan from the blood through the blood-brain barrier (BBB) into the brain (Fernstrom, 1977; Curzon, 1981). Once tryptophan has been transported into the brain, serotonergic neurons convert tryptophan into 5-hydroxytryptophan via the enzyme tryptophan hydroxylase 2 (TPH-2). Another isoform of this enzyme, tryptophan hydroxylase 1 (TPH-1), is found in non-neuronal serotonergic cells (Walther & Bader, 2003). The conversion of tryptophan to 5-hydroxytryptophan is the rate limiting step in

the synthesis of 5-HT (Walther & Bader, 2003). Aromatic L-amino acid decarboxylase (AADC) then converts 5-hydroxytryptophan into 5-hydroxytryptamine (5-HT, serotonin). In serotonergic neurons 5-HT is produced primarily in axon terminals even though TPH-2 is synthesized in the soma and transported to the terminal (Meek & Neff, 2006). The most commonly used enzyme inhibitor to prevent 5-HT synthesis is p-chlorophenylalanine (PCPA) which *in vivo* irreversibly binds and incorporates itself into TPH effectively inactivating the enzyme (Koe & Weissman, 1966; Gál, 1972). 5-HT is degraded by monoamine oxidase (MAO) and aldehyde dehydrogenase into 5-hydroxyindoleacetic acid (Vetulani & Napela, 2000). There are two isoforms of MAO; MAO-A and MAO-B. Both isoforms are located in neurons but are not exclusive to the CNS (Mann et al., 1989).

After 5-HT is synthesized in the cytoplasm it is concentrated into vesicles by a vesicular monoamine transporter (VMAT). There are two isoforms of VMAT; VMAT-1 and VMAT-2 which are located in peripheral organs and the CNS, respectively (Masson et al., 1999). Three distinct pools of synaptic vesicles have been identified as the "ready releasable pool", "proximal pool", and "the reserve pool" which vary in probability of content release (Boehm & Kubista, 2002). Once an action potential reaches the nerve terminal it causes the membrane to depolarize opening voltage-gated Ca^{2+} channels and the exocytosis of vesicular 5-HT into the synapse (Boehm & Kubista, 2002). 5-HT release is modulated by presynaptic receptors (Chaput et al., 1986).

The 5-HT transporter (SERT, 5-HTT) is located on the plasma membrane of the serotonergic neurons and is responsible for the uptake of 5-HT from the synapse back into the neuron (Piñeyro et al., 1994). The reuptake process is mediated by Na^+ and Cl^-

and the function of this reuptake system is voltage-dependent (Sonders et al., 1997; Lin et al., 1996). A wide variety of ADs, such as fluoxetine and escitalopram, target the 5-HTT in order to elicit an AD effect. ADs that exert their therapeutic effect via 5-HTT blockade typically require a high transporter occupancy of approximately 80 percent and cause changes in receptor sensitivity (Blier & de Montigny, 1994; El Mansari et al., 2005; Belmaker & Agam, 2008). Currently selective serotonin reuptake inhibitors (SSRIs) are the first-line treatment for patients with MDD worldwide because of their high efficacy and tolerability (Blier, 2010). These changes in transporter activity and receptor sensitivity enhance 5-HT neurotransmission – which is thought to, at least in part, underlie the AD effect.

5-HT receptors can be either G-protein coupled receptors (GPCRs) or ligand-gated ion channels (LGICs) and are located in both the peripheral and CNS. There are at least fourteen different subtypes of mammalian 5-HT receptors which belong to seven 5-HT receptor families denoted as $5\text{-HT}_{1\text{-}7}$ (Martin & Humphrey, 1994; Hoyer et al., 1994). Each of these receptors are activated to different extents by 5-HT and differences in neuroanatomical location, signal transduction mechanisms, as well as affinities for synthetic drugs generate opportunities for new drugs and make each 5-HT receptor subtype a potential target to contribute to AD response, as shown in figure 1 below (Blier & El Mansari, 2013; Blier & de Montigny, 1990).

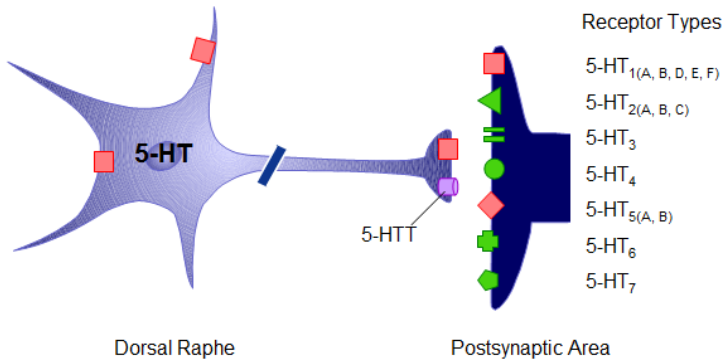


Figure 1 -The distribution of 5-HT receptors in the mammalian brain and some of their basic characteristics. 5-HT_{1A} autoreceptors are located on the cell body and dendrites while 5-HT_{1B} autoreceptors are located on the axon terminal of 5-HT neurons. Green receptors indicate they exert an excitatory effect; Red receptors indicate they exert an inhibitory effect.

The 5-HT_1 class of receptors are GPCRs that can be divided into five receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}). These receptors are coupled to a G_i protein that inhibits adenylyl cyclase (AC) activity resulting in the inhibition of cyclic adenosine monophosphate (cAMP) or to the regulation of K^+ or Ca^{2+} channels (Bard et al., 1993). The regulation of the 5-HT_{1A} receptor is thought to be essential to the AD response (Blier & Ward, 2003). Electrophysiological studies have shown that the administration of many ADs result in the desensitization of 5-HT_{1A} and/or 5-HT_{1B} autoreceptors (Ghanbari et al., 2011; Belmaker & Agam, 2008). 5-HT_{1A} receptor activation results in membrane hyperpolarization and a suppression in neuron firing via the opening of the inwardly rectifying K^+ channel causing an outward K^+ conductance (Aghajanian & Lakoski, 1984). 5-HT_{1A} receptors localized on serotonergic cell bodies and dendrites in the DRN are called somatodentritic autoreceptors while 5-HT_{1B} receptors located on the axon terminal of 5-HT neurons are called terminal autoreceptors. 5-HT_{1A} receptors found on non-serotonergic cells, such as in projection areas of the hippocampus, are called heteroreceptors (Hensler et al., 1991). Both 5-HT_{1A} and 5-HT_{1B}

autoreceptors modulate 5-HT release, 5-HT_{1A} receptors by altering 5-HT neuron firing and 5-HT_{1B} receptors by altering amount of 5-HT released per action potential (Blier & Ward, 2003; Chaput et al., 1986).

There are distinct differences between the 5-HT_{1A} receptors found in the DRN and those in the dorsal hippocampus: 5-HT and 5-HT_{1A} receptor agonist potency differences and desensitization differences. In the dorsal hippocampus 5-HT is more effective than in the DRN, while 5-HT_{1A} receptor agonists such as flesinoxan, ipsapirone, gepirone, and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) are more effective in the DRN (Sprouse & Aghajanian, 1987; Blier & de Montigny; 1987b; Blier & de Montigny, 1990; Chaput & de Montigny, 1988). In addition, 5-HT_{1A} autoreceptors in the DRN desensitize after long-term administration of 5-HT_{1A} receptor agonists and following most AD treatments while those in the hippocampus typically do not (Blier & de Montigny, 1990). A potential explanation for this difference may be attributed to 5-HT_{1A} receptor agonists acting as partial agonists in the hippocampus while 5-HT_{1A} receptors act as full agonists in the DRN (Blier & de Montigny, 1987b; Hadrava et al., 1995). However, the selective 5-HT_{1A} receptor agonist BAY 3702 is a full agonist at 5-HT_{1A} receptors in the hippocampus and prolonged administration does not desensitize these postsynaptic 5-HT_{1A} receptors. In contrast, some AD treatments such as electroconvulsive shocks (ECS) and tricyclic antidepressants (TCAs), like imipramine, have been shown to sensitize 5-HT_{1A} receptors in the hippocampus (Haddjeri et al., 1998; Ishihara & Sasa, 1999; Dong et al., 1998).

Placebo-controlled studies with the 5-HT_{1A} receptor agonist gepirone, buspirone, and ipsapirone have produced positive results for anxiety disorders and/or MDD (Blier &

Ward, 2003). 5-HT_{1A} receptor sensitization is beneficial in the limbic regions. In fact, 5-HT_{1A} receptor activation exerts an inhibitory effect on neurons where limbic areas are typically hyperactive in depressed patients (Price & Drevets, 2009; Murrough et al., 2011). Although 5-HT_{1B} receptors are found in low numbers on pyramidal neurons in the hippocampus, they modulate pyramidal neuron firing by regulating 5-HT release on 5-HT neuron terminals at the hippocampus. Electrophysiological studies have demonstrated that terminal 5-HT_{1B} receptor antagonists enhance the efficacy of 5-HT neurotransmission (Chaput et al., 1986). 5-HT_{1B} receptor knockout mice have been reported to show AD-like effects in addition to augment the effects of SSRIs when co-administered (Murrough & Neumeister, 2011).

The 5-HT_3 receptor is the sole ionotropic monoamine receptor. 5-HT_3 receptors are excitatory ligand-gated $Na^+/K^+/Ca^{2+}$ channels which depolarize the cell membrane to mediate their effect (Rajkumar & Mahesh, 2010). These receptors are found primarily on neuron terminals where they mediate or modulate neurotransmitter release (Rajkumar & Mahesh, 2010). These receptors are also located postsynaptically where they are involved with rapid excitatory neurotransmission (van Hooft & Vijverberg, 2000). 5-HT_3 receptors have also been found on subpopulations of γ-aminobutyric acid (GABA)-ergic interneurons causing a reduction in NE and acetylcholine (ACh) release in the forebrain – making them a new target for ADs (Bétry et al., 2011). Several studies have shown that both acute and chronic 5-HT_3 receptor antagonist administration decreased immobility time in the forced swim test (FST) and tail suspension test (TST) , indicating a possible AD therapeutic activity (Mahesh et al., 2007; Bourin et al., 1996; Kos et al., 2006; Bravo & Maswood, 2006; Ramamoorthy et al., 2008; Bétry et al., 2011). In olfactory

bulbectomized rats, an accepted animal model of depression, the administration of a 5-HT_3 receptor antagonist reversed depressive-like behaviour (Ramamoorthy et al., 2008; Mahesh et al., 2007). Furthermore, 5-HT_3 receptor antagonists may have therapeutic efficacy as an augmentation strategy. For instance, co-administration of an SSRI and the 5-HT_3 receptor antagonist, ondansetron, further decreased FST immobility times in comparison with individual compound regimens (Redrobe & Bourin, 1997).

5-HT_7 receptors increase intracellular cAMP, via a G_s-coupled protein, in order to exert an excitatory effect (Bard et al., 1993; Raut, 1993). In the CNS these receptors are primarily expressed in the cortex, thalamus, hypothalamus and hippocampus (Matthys et al., 2011). Activation of 5-HT_7 receptors inhibits 5-HT neuron firing in the DRN, as shown using intravenous injection (i.v.) of a 5-HT_7 receptor agonist, AS-19 (Mnie-Filali et al., 2009). 5-HT_7 receptors in the DRN and MRN are not localized on serotonergic neurons and therefore do not act as autoreceptors (Duncan et al., 2004). Since 5-HT_7 receptors are excitatory but inhibit 5-HT neuron firing in the DRN, it is reasonable to assume that they are not localized on 5-HT neurons themselves but on GABAergic interneurons (Roberts et al., 2004). 5-HT_7 receptor activation on these neurons would result in the release of GABA on 5-HT neurons in the DRN exerting an inhibitory effect thus reducing firing rate. In several measures of depressive-like behaviour, such as the FST and TST, 5-HT_7 receptor antagonists induced AD-like effects, indicating that 5-HT_7 receptor blockade may be a target for MDD treatment strategies (Guscott et al., 2005; Hedlund et al., 2005; Gupta et al., 2011). As well, 5-HT_7 receptor knockout mice show decreased mobility in both the FST and the TST (Guscott et al., 2005; Hedlund et al., 2005). Furthermore, most AD treatments have been shown to promote neurogenesis

which appears to be important in the AD response. 5-HT_7 receptor antagonism appears to have a robust effect on neurogenesis in comparison to some current ADs. For instance, cell proliferation in the CA3 of the hippocampus is enhanced after 2-3 weeks of fluoxetine treatment while cell proliferation enhancement is observed within 1 week after the administration of the 5-HT_7 receptor antagonist SB-269970 (Mnie-Filali et al., 2009). These properties support the role of 5-HT_7 antagonism as a rapid-acting AD strategy.

Receptor desensitization is an adaptive change that can occur in response to AD treatments. For instance, following a two-day administration of the AD escitalopram a reduction in firing is observed in 5-HT neurons of the DRN. However, after fourteen day administration this effect is no longer observed due to 5-HT_{1A} autoreceptor desensitization (El Mansari et al., 2005). Most of the 5-HT receptors are GPCRs that can desensitize via three main processes; uncoupling (seconds), endocytosis (minutes), and down-regulation and degradation (hours-days) (Ferguson, 2001; Albert & Lemonde, 2004). 5-HT binding to the high affinity state of a receptor results in receptor activation and activation of corresponding G-proteins. G-protein activation results in the activation or inhibition, depending on the receptor subtype, of effectors AC or ion channels which in turn activate appropriate kinases that phosphorylate the receptor initiating uncoupling (Albert & Lemonde, 2004). This causes the receptor to shift into a low affinity-state, 5-HT dissociation, receptor dephosphorylation and receptor resensitization (Albert & Lemonde, 2004). However, if high concentrations of 5-HT are present, a receptor internalization cascade is initiated and the surface receptor is internalized into an intracellular membranous compartment (Ferguson, 2001). If the receptor does not become dephosphorylated and internalization is prolonged, a degradation pathway is

initiated and gene expression may be reduced (Ferguson, 2001; Albert & Lemonde, 2004).

2.3 Projection regions

2.3.1 Overview of postsynaptic regions implicated in MDD

The broad spectrum of symptoms associated MDD suggests that several brain regions are involved (Berton & Nestler, 2006). The monoamine system, which is heavily implicated in the treatment of depression, innervates several brain regions which must also be studied to gain a comprehensive understanding of MDD (Blier & El Mansari, 2013). For instance, the VTA provides strong dopaminergic input to the nucleus accumbens (NAc), a bilateral structure strongly involved in reward pathways (Berton & Nestler, 2006; Dunlop & Nemeroff, 2007). The LC and DRN innervate each other (with NE and 5-HT, respectively) in addition to the NAc, amygdala, frontal cortex, and the hippocampus (Mongeau et al., 1997; Price & Drevets, 2009).

Human brain imaging studies assessing blood flow, or related measures, have detected changes in the hippocampus, amygdala, striatum, thalamus, prefrontal and cingulated cortex in MDD patients (Drevets, 2001; Rajkowska, 2003; Price & Drevets, 2009; Murrough et al., 2011). Decreased glucose metabolism in response to chronic AD treatment has been observed by functional neuroimaging studies in the subgenual anterior cingulate cortex and the hippocampus (Mayberg et al., 2000). However, glucose metabolism remained elevated in depressed patients who did not respond to AD treatment indicating that perhaps lowered glucose metabolism is essential for remission (Mayberg et al., 2000).

Cognitive aspects of depression such as feelings of doom, extreme guilt, worthlessness, hopelessness, and suicidality may be mediated by the hippocampus and frontal cortex (Berton & Nestler, 2006). The striatum, NAc, and the amygdala are important in mediating aversive and reward responses. Abnormalities in these responses may contribute to anxiety, decreased motivation, and anhedonia in patients with depression (Dunlop & Nemeroff, 2007). Alterations in hypothalamic functions are thought to contribute to depression-related changes in sleep, appetite, increased blood cortisol, and loss of interest in sex (Berton & Nestler, 2006; Belmaker & Agam, 2008). Patients with MDD have impaired control of higher cortical regions of the brain such as the medial prefrontal cortex (mPFC), dorsal cingulate cortex, and the dorsomedial prefrontral cortex. This in turn results in impaired working memory, concentration, and executive function and decreased cognitive control of emotion (Murrough et al., 2011). As well, there is typically hyperactivity of the amygdala, hippocampus, insula, and hypothalamus (Murrough et al., 2011; Price & Drevets, 2009; Berton & Nestler, 2006). Increased hypothalamic activity results in an increase of corticotrophin-releasing hormone (CRH) and subsequent cortisol release, and decreased brain-derived neurotrophic factor (BDNF) production – causing a reduction in hippocampal neurogenesis (Belmaker & Agam, 2008; Murrough et al., 2011). It has been shown that the blockade of hippocampal neurogenesis is sufficient to increase hypothalamic-pituitary-adrenal (HPA) axis activity – thus producing a detrimental positive feedback loop (Schloesser et al., 2009). As mentioned previously, several AD therapies target neurogenesis, indicating its importance in the etiology of MDD (Santarelli et al., 2003; Berton & Nestler, 2006; Belmaker & Agam, 2008).

In addition to HPA axis activity, depressed patients also exhibit hyperactive limbic structures (Sapolsky, 2000; Murrough et al., 2011). Increased release of glutamate at the hippocampus by neighbouring hyperactive regions of the brain exert a excitotoxic effect via excess intracellular Ca^{2+} and overactivation of Ca^{2+}-dependent enzymes resulting in cell atrophy or cell death (Sapolsky, 2000). Cell atrophy and death can result in reduced hippocampus volume, a common attribute of depressed patients, which appears to be correlated to the length and severity of MDD (Sheline et al., 1999; Campbell & MacQueen, 2004). A common AD mechanism to reduce hyperactivity and excitotoxicity is by elevating 5-HT neurotransmission in these postsynaptic regions. Increased amounts of 5-HT neurotransmission will increase the activation of postsynaptic 5-HT_{1A} heteroceptors which exert an inhibitory effect on pyramidal neuron firing possibly resulting in reduced excitotoxic effects. As a result, brain activity is restored to normal in most regions of the brain after AD treatment and remission is achieved (Price & Drevets, 2009; Mayberg et al., 2000; Drevets et al., 1992).

2.3.2 Hippocampal circuitry and implication in MDD

The hippocampus is part of the limbic system which not only plays an important role in learning and memory but also in fear and emotion (Zigmond et al., 1999). The perforant path is the main input path to the hippocampus – linking the input from the entorhinal cortex to granule cells of the dentate gyrus (DG). From the DG, the mossy fibers composed of axons arising from granule cells form synapses with pyramidal neurons in the Cornu Ammonis (CA) 3 region where connections are made via the ipsilateral Schaeffers collaterals and the contralateral commissural fibers to the CA1

where most of the hippocampal output is channelled. Information can also be relayed to the subiculum directly from the entorinal cortex (Mongeau et al., 1997; Lavenex & Amaral, 2000). The relay of information through the trisynaptic loop (DG-CA3-CA1) is strongly modulated by cholinergic fibers of the septum, NE fibers of the LC, and 5-HT fibers of the DRN and median raphe nucleus (MRN) (Mongeau et al., 1997).

As aforementioned, monoamine dysfunction is commonly associated with MDD and hippocampus is strongly innervated by 5-HT and NE, making the impact of this dysfunction important for normal hippocampal function and understanding this mood disorder (Mongeau et al., 1997). Patients with MDD can experience a variety of hippocampal abnormalities, ranging from decreased hippocampal gray matter volume to increased glucose metabolism (Price & Drevets, 2009). Therefore it is important to assess hippocampal changes as well as causation and reversal mechanisms, as this may provide information related to lowering AD aversive side effects and increasing remission rates (Mayberg et al., 2000; Price & Drevets, 2009). Furthermore, several studies have demonstrated that most hippocampal deficits are restored after AD treatment (Drevets et al., 1992; Santarelli et al., 2003). For these reasons the hippocampus remains an area of interest in depression research.

2.4 Factors unaccounted for by the monoamine hypothesis

Although the monoamine hypothesis remains the most accept theory of depression there remains several caviets that have yet to be fully explained. Firstly, many drugs alter monoamine function, such as cocaine and amphetamines, but so far they are not effective clinically as ADs (Elhwuegi, 2004). However, these agents may allievate

some symptoms of MDD they do not act around the clock and they may not be able to treat all symptoms of MDD. For instance, patients with MDD may have difficulties sleeping – making such drugs so far clinically ineffective. As the monoamine hypothesis assumes that a monoamine deficiency underlies the etiology of MDD, depletion of 5-HT or NE should theoretically induce MDD symptoms in healthy volunteers. However, monoamine depletion studies have not found this to be true (Elhwuegi, 2004). Nevertheless, an acute tryptophan depletion although decreasing 5-HT synthesis by 90 percent, it does likely not produce a complete decrease in synaptic 5-HT. Moreover, long-term 5-HT depletion studies have not been carried out in humans. Monoamine depletion slightly lowered mood in healthy volunteers who previously responded to a monoaminergic AD treatment or with a family history of MDD (Delgado et al., 1990; Ruhé et al., 2007). Even though the monoamine hypothesis could still be valid, in no way does it rule out other pathophysiological mechanisms.

3.0 Antidepressant treatment

3.1 Overview of antidepressant strategies

MDD is a common yet difficult disorder to treat. The three main categories of MDD treatment are behavioural, physical, and pharmacological (Vetulani & Nalepa, 2000; Scott et al., 1997). The most common behavioural therapy for the treatment of MDD is cognitive behavioural therapy (CBT). CBT focuses on impaired emotions, maladaptive behaviours and cognitive processes and contents usually through a number of goal-oriented exercises that will alter their thought processes. CBT is often used in combination with other AD treatments (Goldapple et al., 2004; Birmaher et al., 1998).

Electroconvulsive shock therapy (ECT), transcranial magnetic stimulation (TMS), vagus nerve stimulation (VNS), deep brain stimulation (DBS) are physical treatments for MDD that are typically administered to treatment-resistant depressed patients where other more conventional ADs have been ineffective (Manta, El Mansari, Debonnel, & Blier, 2012; Grunhaus et al. 2000; Mayberg et al., 2005). Pharmacological treatments of MDD relies on the administration of AD drugs to alter receptor, transporter, or enzyme activity to achieve remission and are the most common form of depression treatment (Boyce & Judd, 1999; Murdoch & Keam, 2005).

3.2 Pharmacological treatment of MDD

Although ADs have been previously discussed, the following will provide a more comprehensive overview. In the 1950s iproniazid, a monoamine oxidase inhibitor (MAOI), was one of the first effective antidepressants drugs to be identified (Vetulani & Nalepa, 2000). Once their clinical AD effectiveness was recognized, a series of new compounds with similar mechanisms of action were introduced. The mechanism of action for these AD drugs is the inhibition of MAO – the enzyme responsible for the degradation of monoamines – thereby increasing monoamine levels. Although initially limited, further research and drug development have resulted in great advances for this class of AD. Initially MAOIs were irreversible blockers of both MAO-A and MAO-B (Vetulani & Nalepa, 2000). The inhibition of the MAO-B isoform has been shown to exert no therapeutic effect, whereas the irreversible and selective type-A MAOI clorgyline was shown to be an AD (Mann et al., 1989). Importantly, these selective MAO-A inhibitors may still be an effective treatment. Finally, the reversible and MAO-A

selective MAOI moclobemide was introduced (Vetulani & Nalepa, 2000). MAOI administration initially decreases 5-HT neuron firing rate due to increased synaptic 5-HT activating 5-HT_{1A} autoreceptors. These 5-HT_{1A} autoreceptors desensitize after chronic administration of MAOIs causing 5-HT neuron firing to return to normal. This results in an overall increase in 5-HT neurotransmission (Blier & de Montigny, 1985; Blier & de Montigny 1987a). Unlike 5-HT_{1A} receptors, long-term administration of MAOIs has not been found to desensitize terminal 5-HT_{1B} autoreceptors (Blier et al., 1988).

An additional class of AD drugs work on transporters, acting as reuptake inhibitors. These ADs fall into either TCAs or SSRIs. Initially, TCAs such as imipramine were used as a first-line treatment for MDD (Boyce & Judd, 1999). Most TCAs block the 5-HTT and the norepinephrine transporter (NET) with varying activity. Some TCAs have a higher affinity for the 5-HTT, like amitriptyline, while others have a higher affinity for NET, such as desipramine (Sánchez & Hyttel, 1999; Gillman, 2009). Nevertheless, tertiary TCAs are metabolized into potent NET inhibitors. 5-HTT or NET blockade causes increased levels of 5-HT and NE, respectively, and receptor sensitivity changes in many regions of the brain implicated in depression. Some TCAs, such as trimipramine and iprindole, are not 5-HTT or NET inhibitors and work by modulating receptor activity (Tatsumi et al., 1997). Long-term administration of TCAs and ECT sensitizes postsynaptic 5-HT_{1A} heteroreceptors in the forebrain regions like the hippocampus (de Montigny & Aghajanian, 1978; Gallager & Bunney, 1979; de Montigny, 1984). These observations suggest that a postsynaptic mechanism of action is important for the efficacy of TCAs. The time between AD administration and observed therapeutic effect is consistent with the amount of time for these adaptive changes in receptor sensitivity to

occur (Blier & de Montigny, 1980; El Mansari et al., 2005; Artigas, 2013). However, TCAs present several safety and tolerability concerns with fatalities due to overdose much less common with SSRIs than TCAs (Buckley & McManus, 2002).

As our understanding regarding the pathophysiology of MDD grew, newer classes of ADs were developed. SSRIs such as fluoxetine, serotonin-norepinephrine reuptake inhibitors (SNRIs) such as venlafaxine, and norepinephrine reuptake inhibitors (NRIs) such as reboxetine followed the introduction of TCAs and were presented as safer and more tolerable class of ADs. All SSRIs have been found as effective ADs via blocking the 5-HTT and enhancing 5-HT transmission, while having no other properties in common (Blier & de Montigny, 1994). Currently, 5-HTT blockade has become the first-line option in the management of patients with MDD (Murdoch & Keam, 2005). Although several TCAs block monoamine reuptake, albeit to a lesser extent except for clomipramine, like SSRIs their mechanism of action appears to differ. SSRIs, along with most other antidepressants, alter 5-HT_{1A} and 5-HT_{1B} autoreceptor sensitivity and 5-HT neuron firing while TCAs do not (Blier & Montigny, 1980). In order for SSRIs to exert a therapeutic effect, they must have a transporter occupancy of 80% (Belmaker & Agam, 2008). SSRI administration results in desensitization of both 5-HT_{1A} and 5-HT_{1B} autoreceptors in the DRN initially causing 5-HT neurons to reduce firing rates and the amount of 5-HT released per action potential which later returns to normal while postsynaptic 5-HT_{1A} heteroreceptor sensitivity remains unaltered (Blier et al., 1988; Piñeyro & Blier, 1999; Blier & de Montigny, 1983).

Clinically, SSRIs are considered an effective treatment for MDD in adults, as evidenced by significant improvements versus placebo on multiple measures of

depression, including the MADRS and the 17-item Ham-D in 6-8-week, randomized, double-blind studies (Frampton, 2011). The function of the reuptake transporter is continually inhibited by the SSRI, eventually resulting in an increase in 5-HT concentrations in forebrain synapses which in turn increases 5-HT neurotransmission (Piñeyro & Blier, 1999). It has been shown that the administration of SSRIs increases extracellular 5-HT concentrations in various regions of the brain implicated in depression such as the hippocampus (Guiard et al., 2012).

3.3 Pharmacological antidepressant augmentation strategies

As previously mentioned, only one third of MDD patients experience remission with first-line treatment, thus often further strategies are necessary (Hirschfeld et al., 2002; Blier & Bergeron, 1995; Chernoloz et al., 2009). One such strategy involves co-administration of ADs, which aims to target several monoamine elements in order to alleviate MDD symptoms. For example, often compounds which inhibit the 5-HT transporter and display receptor activity are co-administered, such as aripiprazole and escitalopram, to achieve greater enhancements in neurotransmission (Chernoloz et al., 2009). With aripiprazole and escitalopram, the combination of this atypical antipsychotic and SSRI resulted in enhanced monoamine activity compared to SSRI administration alone (Chernoloz et al., 2009). In addition, it has been reported that the combined administration of fluoxetine, an SSRI, and pindolol, a 5-HT_{1A} receptor antagonist, produces a faster onset of action and/or higher remission rates than fluoxetine alone (Portella et al., 2009). A five-week regime of this combined therapy produced a significantly greater response in the treatment of treatment-resistant depressed patients

than fluoxetine alone. Similar effects were also observed with paroxetine, an SSRI, augmented with pindolol (Blier & Bergeron, 1995; Zanardi et al., 1997). The enhanced response to these augmentation strategies is thought to enhance 5-HT transmission by altering autoreceptor function (Blier & Ward, 2003).

The effects of SSRIs can also be potentiated by acting on other receptors. For example, the efficacy of fluoxetine is also enhanced by the 5-HT_{2A} and α_2-adrenergic receptor antagonist mianserin similarly to that of pindolol (Maes et al., 1999). As mentioned in section 2.2, other receptor modulation has shown in animal and human trials that 5-HT_{1B} receptor partial agonism, 5-HT_3 and 5-HT_7 receptor antagonism can work synergistically with SSRIs to produce AD-like effects. New compounds are under development that utilize more than one mode of action to alter 5-HT neurotransmission. For example, vilazodone, is an SSRI and a potent 5-HT_{1A} receptor agonist (Blier & El Mansari, 2013). Perhaps novel multimodal compounds, such as vortioxetine or vilazodone, may represent a new generation of ADs that could act on different symptom domains of MDD.

3.4 Vortioxetine (Lu AA21004)

Vortioxetine or Lu AA21004 (1-[2-(2, 4-dimethyl-phenylsulfanyl)-phenyl]-piperazine) is structurally different from all current ADs and acts on both reuptake inhibition and receptor activity. Vortioxetine is a 5-HT_3 and 5-HT_7 receptor antagonist, a 5-HT_{1B} receptor partial agonist, a full 5-HT_{1A} receptor agonist, and a 5-HTT inhibitor *in vitro* with nM affinities of 3.7, 19, 33, 15, and 1.6, respectively (Bang-Anderson et al., 2011). Species differences regarding vortioxetine affinity between rats and humans have

been found at the 5-HT_{1A} receptor with an affinity of 15 nM in humans and 230 nM in rats (Bang-Anderson et al., 2011).

Vortioxetine has been shown to be efficacious at doses of 5 and 10 mg/day (Alvarez et al., 2012). Therapeutic-like effects have been observed in rat brain slices treated with vortioxetine at a dose that equates to a 5-HTT occupancy level of approximately only 43 percent, suggesting that the therapeutic effects observed by vortioxetine are attributed at least in part to its other modes of action (Alvarez et al., 2012; Areberg et al., 2012). Electrophysiological recordings have shown that rat DRN neurons, after sustained administration of vortioxetine, produce an initial decrease in firing rate that rapidly returns to normal (Etiévant et al., 2011). This type of activity observed with SSRIs suggests that vortioxetine may possess rapid-acting AD properties (Etiévant et al., 2011). Unlike fluoxetine, vortioxetine suppressed 5-HT neuron firing while not saturating the 5-HTT (Bétry et al., 2013). 5-HT neuron firing recovery rate has also been shown to be much faster with vortioxetine administration than fluoxetine (Bétry et al., 2013). These observations suggest that the effects of vortioxetine are in part mediated not only by reuptake blockade but also by receptor modulation. Rat microdialysis studies have shown an increase in extracellular ACh, histamine (HA), 5-HT, NE, and DA in the mPFC and an increase in all monoamines in the ventral hippocampus which is common to many ADs (Pehrson et al., 2012; Mørk et al., 2013). Increases in ACh and HA in the mPFC caused by vortioxetine administration have demonstrated enhanced contextual and episodic memory in rats (Mørk et al., 2013). The multimodal activity of vortioxetine may contribute to enhancing cognitive deficits commonly associated with MDD (Katona et al., 2012; Mørk et al., 2013). Subchronic

treatment of vortioxetine has also been shown to increase neurogenesis in the hippocampus – a feature common to most AD treatments (Haddjeri et al., 2012).

The mean elimination half-life of vortioxetine after achievement of steady-state and single dose is 57 hours with 90 percent steady-state achieved after 188 hours in humans (Areberg et al., 2012). In vitro studies have determined that the metabolism of vortioxetine is primarily achieved by cytochrome P450 enzymes that results in the production of four phase one metabolites: a sulphoxide, a N-hydroxylated piperazine, a 4-hydroxyphenyl, and a benzoic acid (Hvenegaard et al., 2012). The most prominent but inactive metabolite of vortioxetine metabolism is the benzoic acid (Lu AA34443) and is unable to cross the BBB in healthy volunteers (Areberg et al., 2012). Virtually all metabolites of vortioxetine formed are dependent on the presence of nicotinamide adenine dinucleotide phosphate (NADPH) with the exception of the conversion of the benzylic hydroxide to the benzoic acid (Hvenegaard et al., 2012). The formation of the benzoic acid from the oxidation of benzylic oxide is the rate-limiting step (Hvenegaard et al., 2012).

Based on strong basic evidence indicating the effectiveness of vortioxetine as an AD, several clinical studies have been initiated. It is suggested that a dose of 20-30mg of vortioxetine is needed in order to occupy the transporter in a clinical relevant manner (i.e. $\geq$ 80 percent) (Stenkrona et al., 2013). However, a double-blind, randomized placebo-controlled, venlafaxine-referenced study of vortioxetine showed that a six-week treatment with 5 and 10 mg of vortioxetine was efficacious and well tolerated in patients with MDD (Alvarez et al., 2012). Vortioxetine was found to be safe and tolerable following an open-label 12 month treatment of MDD (Baldwin et al., 2012). A similar study

comparing the efficacy of placebo, duloxetine, and vortioxetine in elderly patients with MDD determined that both vortioxetine and duloxetine separate from placebo in all measures of response and remission (Katona et al., 2012). A common problem with the treatment of patients with MDD is the high frequency of relapse. Vortioxetine is effective at treating patients with MDD but like all other ADs studies have demonstrated that it is also effective at preventing relapse in patients with MDD, as well as patients with generalized anxiety disorder, with relatively few side effects (Boulenger et al., 2012; Baldwin et al., 2012). However, while the majority of studies present vortioxetine as an effective treatment strategy for MDD, some clinical studies have indicated that vortioxetine does not significantly reduce depression symptoms compared to placebo (Baldwin et al., 2011; Jain et al., 2013; Mahableshwarkar et al., 2013). Lower average scores on 24-item HAM-D and MADRS scales at baseline, insufficient power to detect changes, and inflated placebo responses alone or combined may explain these failed studies. Overall, both basic and clinical studies indicate vortioxetine exerts an AD-like effect.

4.0 Non-monoaminergic antidepressants

As the monoamine hypothesis is considered the central theory with respect to the etiology of depression, virtually all AD drugs work by altering monoamines in some manner. Non-monoaminergic ADs are uncommon and present many difficulties in terms of research and development. For instance, many monoaminergic ADs are developed using animal models of depression; it is unknown whether many of these models would be effective in terms of non-monoaminergic drug assessments (Berton & Nestler, 2006).

Furthermore, antidepressant studies are extremely expensive, involve chronic treatment for hundreds of patients, and carry with them a high level of risk investment (Berton & Nestler, 2006). As a result pharmaceutical and biotechnology companies are not encouraged to pursue research and development with regards to non-monoaminergic based mechanisms. As well, the effectiveness of most non-monoaminergic drugs is often measured by assessing changes in monoamines. Thus, while this may produce novel ADs, it does not provide novel mechanisms of action (Berton & Nestler, 2006). Lastly, the financial incentive for pharmaceutical companies to invest in risky potential non-monoaminergic ADs is not high, as profits of monoaminegic ADs remain robust (Berton & Nestler, 2006). In addition to the risky finical investment, there is also a lack of novel non-monoaminergic targets and high failure rate amoungst clinical trials at late stage testing (Khan et al., 2000; Berton & Nestler, 2006).

5.0 Aim

5.1 Aim overview

AD-like effects have been reported separately with all of the receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_3, and 5-HT_7) and the 5-HTT that vortioxetine has a high affinity for. By acting on all of these properties simultaneously, this agent may produce remission in a greater proportion of patients and/or be effective in SSRI-resistant patients.

5.2 Acute

It has been shown that *in vitro* vortioxetine possesses a high affinity for 5-HT_{1B}, 5-HT_3 and 5-HT_7 receptor types all of which have demonstrated to have AD-like effects.

One aim of this study is to apply selective receptor agents, determine their effects, and determine if vortioxetine is able to reverse them *in vivo*. In addition, given that theoretically 5-HT_{1B} receptor partial agonism should enhance 5-HT neurotransmission, the effect of acute vortioxetine on the effectiveness of electrical stimulation of the 5-HT pathway on the firing activity of 5-HT neurons in CA3 pyramidal neurons was assessed.

5.3 Long-term

Since AD treatments are administered chronically, it is important to assess the changes in neurotransmission that will occur over the course of administration to better understand the mechanism of action. This study is also aimed at determining if the sensitivity of 5-HT_{1A} heteroreceptors has changed and if they become more tonically active after long-term administration of vortioxetine. Since SSRIs are effective ADs and act by blocking 5-HTT function, the 5-HT reuptake blockade properties of vortioxetine was also investigated.

5.4 Hypothesis

That vortioxetine may exert unique antidepressant effects by occupying the 5-HTT less and acting on several of the 5-HT receptors.

MATERIALS AND METHODS

6.0 Animals

Adult male Sprague-Dawley rats (Charles River, Saint-Constant, QC, Canada) weighing 250-350 g at the time of the experiment were used. Animals were housed 2 per cage at standard laboratory conditions (12 h light/dark cycle, with lights off at 1900; temperature 21±1°C, 40-50% relative humidity) with *ad libitum* access to food and water. Animals were handled in accordance to the guidelines established by the Canadian Council on Animal Care.

7.0 Drug administration

7.1 Drugs

Vortioxetine was provided by H. Lundbeck A/S and Takeda Pharmaceutical Company Ltd. (Copenhagen, Denmark) and dissolved in a 20% hydroxypropyl-beta-cyclodextrin (β-OH) solution and sonicated until completely dissolved (Etiévant et al., 2011). Escitalopram was provided by H. Lundbeck (Copenhagen, Denmark) and dissolved in a 0.9% NaCl solution and sonicated until completely dissolved (El Mansari et al., 2005). Ipsapirone was purchased from Tocris Bioscience (Burlington, ON, Canada) and dissolved in 20% β-OH and sonicated until completely dissolved (Dong, de Montigny, Blier, 1997). LP-44 and CP-94253 were purchased from Tocris Bioscience (Burlington, ON, Canada) and dissolved in a 20% β-OH solution. SR 57227 and ondansetron were purchased from Tocris Bioscience (Burlington, ON, Canada) and dissolved in distilled H_2O. WAY 100635 was purchased from Sigma-Aldrich (Canada)

and dissolved in distilled H_2O. Chloral hydrate was purchased from Sigma-Aldrich (Canada) and dissolved in a 0.9% NaCl solution to 400 mg/kg.

7.2 Acute drug administration

Vortioxetine, LP-44, and CP-94253 were administered i.v. at doses of 4, 4, and 2 mg/kg respectively. SR 57227, ondansetron, and WAY 100635 were administered via i.v. at doses of 50, 500, and 100 μg/kg respectively. The lateral tail vien was the site for i.v. injections. Chloral hydrate was administered i.p. at a dose of 400 mg/kg.

7.3 Long-term drug administration

Rats were anesthetised with isoflurane to implant subcutaneous osmotic Alzet minipumps (Palo Alto, CA, USA) in the back region. The combination of ipsapirone with vortioxetine will be referred to as humanized-vortioxetine (h-vortioxetine) and was be used to account for a much lower affinity at the 5-HT_{1A} receptor of rats versus humans. Minipumps delivered either vortioxetine or escitalopram at a dose of 5 mg/kg/day for 14 days, based on previous 14 day electrophysiological studies. Ipsapirone was administered via i.v. at a dose of 7.5 mg/kg *bis in die* (b.i.d), intraperitoneal (i.p.) for 14 days. Minipumps remained *in situ* during electrophysiological recording to maintain steady-state concentrations of the drug. In order to determine 5-HT_{1B} receptor sensitivity minipumps were removed on day 14 and the electrophysiological experiment was performed 24 hours later. The washout period ensured that true 5-HT_{1B} autoreceptor desensitization occurred instead of 5-HT_{1B} autoreceptor blockade. During electrophysiological recordings the rats were anesthetised with choral hydrate (400 mg/kg

i.p.). Supplemental doses were given to prevent any reaction to a tail pinch and maintain constant anaesthesia.

8.0 Microiontophoresis and extracellular recording of CA3 dorsal hippocampal pyramidal neurons

Extracellular single cell recording and microiontophoresis of CA3 pyramidal neurons was carried out with a five-barreled glass micropipette with a tip broken back to 10-12 μm. The central barrel used for the unitary recording was filled with a 2 M NaCl solution, and the impedance of these electrodes ranged from 2 to 4 MΩ. The four side barrels were filled with the following solutions: quisqualic acid (1.5 mM in 200 mM NaCl, pH 8), 5-HT creatinine sulfate (10 mM in 200 mM NaCl, pH 4), and the last barrel was filled with a 2 M NaCl solution for automatic current balancing. The micropipette was then lowered into the dorsal CA3 region of the hippocampus using the following coordinates; 4.2 mm anterior to lambda and 4.2 mm lateral (Paxinos, & Watson, 2007). Hippocampal CA3 pyramidal neurons are found at a depth of 4.0 ± 0.5 mm below the surface of the brain. Since pyramidal neurons do not discharge spontaneously in chloral hydrate anesthetised rats, a small current of quisqualate +2 to –5 nanoampere (nA) was applied to activate them within their physiological firing range (10 to 15 Hz) as shown in figure 2 (Ranck, 1976). Pyramidal neurons are identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple action potentials, alternating with complex spike discharges (Kandel et al., 1961). Pyramidal neuron responsiveness to 5-HT is another characteristic of pyramidal neurons which was determined by the reduction

in firing rate upon the ejection of microiontophoretically applied 5-HT for 50 seconds resulting in activation of 5-HT_{1A} heteroreceptors located on pyramidal neurons.

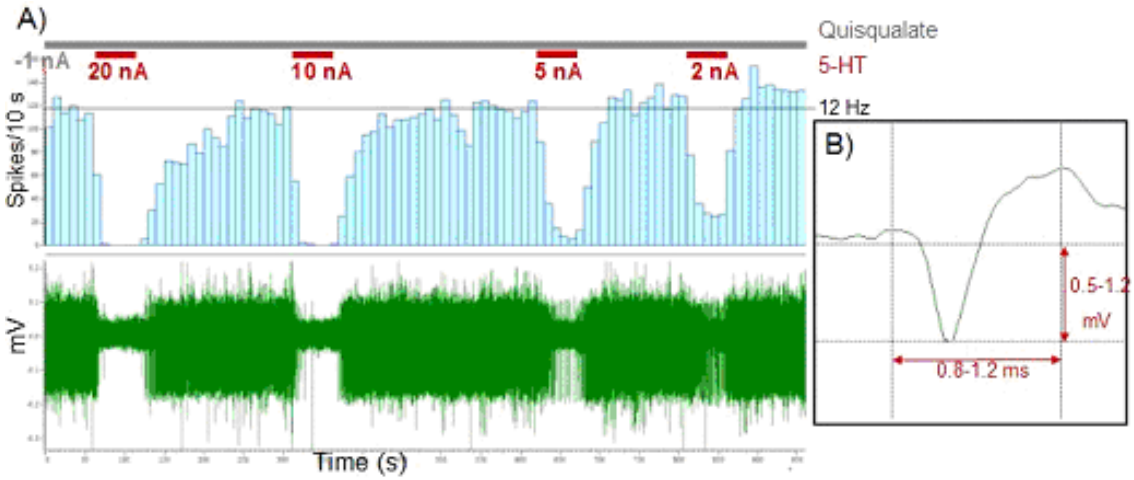


Figure 2 – Example of hippocampal CA3 pyramidal neuron extracellular electrophysiological recording. A) CA3 pyramidal neuron firing histogram shown in 10 s bins with an average firing rate of 12 Hz (top). Electrophysiological recording of the corresponding action potentials to the histogram (bottom). B) A single action potential of a CA3 pyramidal neuron. Quisqualate was microiontophoretically applied to activate pyramidal neurons. The microiontophoretic application of 5-HT at various currents inhibited pyramidal neuron firing via 5-HT_{1A} heteroreceptors.

9.0 5-HT transporter blockade assessment

In order to assess the relative degree to which vortioxetine blocks the 5-HTT, the recovery period (RT_{50}) was determined after the microiontophoretic application of 5-HT in the CA3 region of the hippocampus. RT_{50} is defined as the time, in seconds, elapsed from the termination of microiontophoretic application of 5-HT to obtain a 50% recovery of the initial firing rate (Piñeyro et al., 1994). It is a reliable index of 5-HT reuptake process *in vivo*. Previous experiments have shown that acute systemic injection of paroxetine, an SSRI, will significantly increases RT_{50} values. In support, this occurrence was again observed in 5-HT neuron lesioned rats – thus eliminating the role of the 5-HTT (Piñeyro et al., 1994).

10.0 Tonic activation of 5-HT_{1A} heteroceptor assessment

After long-term treatments, the degree of tonic activation of hippocampus CA3 5-HT_{1A} receptors was assessed using systemic injections of the selective 5-HT_{1A} receptor antagonist WAY 100635 (Haddjeri et al., 1998). Such disinhibition of the neuronal activity is best assessed when the firing rate is low. Indeed, a low stable firing baseline was first obtained by lowering the ejection current of quisqualate. The baseline firing will be recorded for at least 2 minutes before the administration of WAY 100635. WAY 100,635 (100 μg/kg) will be injected i.v. in incremental doses of 25 μg/kg at time intervals of 2 minutes to detect changes in the firing activity of hippocampus pyramidal neurons in rats administered with vehicle, vortioxetine, h-vortioxetine, ipsapirone, or escitalopram for 14 days. Such curves represent stable changes in the firing rate of CA3 pyramidal neurons as percentages of baseline firing following each dose of the antagonist. In order to avoid residual drug effects, only one neuron in each rat will be studied. Importantly, it has been shown that the systemic administration of WAY 100635 does not alter DRN 5-HT neuron firing activity and that an elevation in hippocampal pyramidal neuron firing activity reflects the level of tonic activation of the postsynaptic 5-HT_{1A} heteroreceptor.

11.0 Assessment of postsynaptic 5-HT_{1A} heteroreceptor sensitivity

After the long-term administration of a treatment, the responsiveness of postsynaptic 5-HT_{1A} heteroreceptors was determined by microiontophoretically applying 5-HT on CA3 pyramidal neurons for a 50 second period. Postsynaptic 5-HT_{1A} receptor sensitivity was measured using IT_{50} values. The IT_{50} value is determined by multiplying

current used to microiontophortically apply 5-HT by the time required to achieve a 50% reduction in firing – producing an index of pyramidal neuron responsiveness to 5-HT.

12.0 Electrical stimulation of the 5-HT pathway and 5-HT release

Extensive serotonergic projections from both the MRN and DRN extend to the CA3 region of hippocampus. In order to electrically stimulate the ascending 5-HT pathway a bipolar electrode (NE-100, David Kopf, Tujunga, CA, USA) was implanted 1 mm anterior to lambda on the midline with a 10° backward angle in the ventromedial tegmentum and 8.0 ± 0.2 mm below the surface of the brain. Two hundred square pulses with a duration of 0.5 ms were delivered by a stimulator (S48, Grass Instruments, West Warwick, RI, USA) at an intensity of 300 μA and a frequency of 1 and 5 Hz. The stimulation of the 5-HT pathway induces a brief suppressant period due to the release of 5-HT in the synapse. The effects of stimulation of ascending 5-HT pathway are assessed using 1 and 5 Hz, on the same neuron, to determine the function of terminal 5-HT_{1B} autoreceptors (Chaput et al., 1986).

The two series of stimulations, 1 and 5 Hz, were carried out based on previous electrophysiological studies demonstrating that 5-HT_{1B} autoreceptor activation decreases the 5-HT release in the terminal areas and that increasing the frequency of stimulation from 1 to 5 Hz induces a greater activation of 5-HT_{1B} autoreceptors (Belmaker & Agam, 2008). The increased 5-HT_{1B} autoreceptor activation, induced by the 5 Hz stimulation, results in a greater negative feedback on 5-HT release (Chaput et al., 1986). Since less 5-HT is released into the synapse, obtained at 5 Hz stimulation, a smaller period of suppression is present compared to the 1 Hz stimulation. The stimulation pulses and the

firing activity were analyzed by computer using Spike 2 (Cambridge Electronic Design Limited, UK). Peristimulus time histograms (PSTHs) of CA3 pyramidal neurons will be produced to determine the suppression of firing measured as a duration of silence (DOS) value in ms. The DOS value corresponds to the duration of a total suppression of the hippocampal CA3 pyramidal neuron(Chaput et al., 1986).

For the acute studies, PSTHs of hippocampal pyramidal neuron firing activity will be generated before and after the i.v. administration of 5-HT_{1B}, 5-HT_3 and 5-HT_7 receptor agonists, antagonists, and vortioxetine to measure their effects on terminal 5-HT release resulting in the suppression of pyramidal neuron firing. PSTHs will be produced by the stimulation before and after the i.v. administration of each selective agent.

The effect of vortioxetine on the sensitivity of the terminal 5-HT_{1B} autoreceptor was assessed by comparing the effectiveness of 200 stimulations delivered at 1 Hz and 5 Hz. The faster stimulations produce a greater degree of activation of the terminal 5-HT autoreceptor, thereby decreasing 5-HT release for each impulse reaching 5-HT terminals. If the terminal autoreceptor is desensitized, the difference between the effectiveness of the two rates of stimulation will be significantly decreased. To determine whether the response to vortioxetine is due to true desensitization of terminal 5-HT_{1B} autoreceptors, or to a competition of vortioxetine at the autoreceptor sites, the efficacy of electrical stimulation was examined after a washout period (minipumps were taken out under isoflurane anesthesia and electrophysiological recordings were carried out 24 h later).

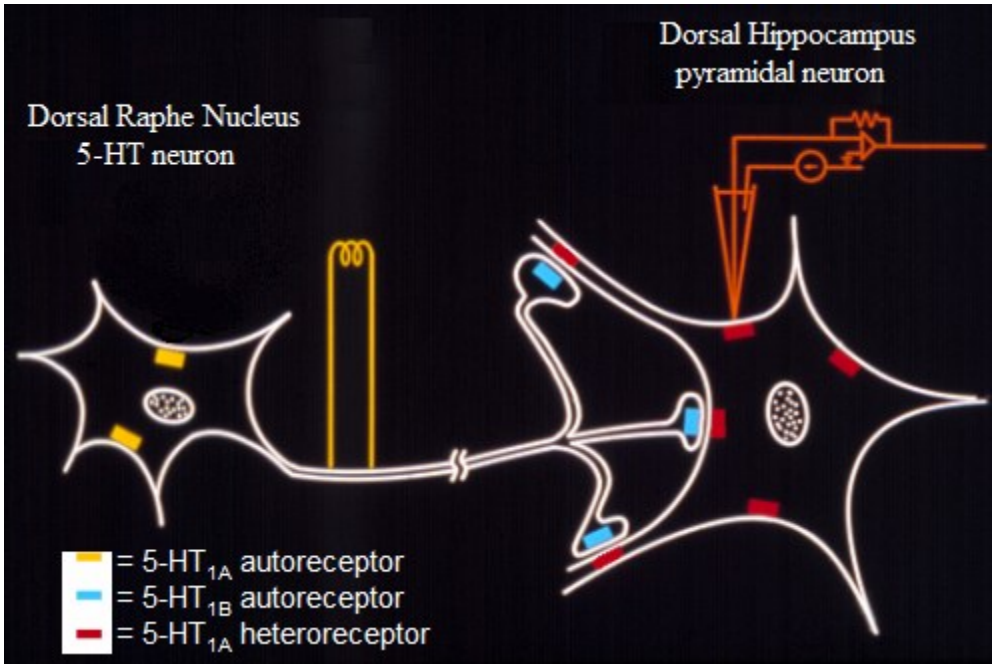


Figure 3 – Electrophysiological paradigm used to study the brain 5-HT system in laboratory animals *in vivo*. Fibres originating from the DRN and the MRN are electrically stimulated in the ventromedial tegmentum (AP: 4,2 & LM: 4.2). The effectiveness and responsiveness of postsynaptic 5-HT receptors can be assessed by recording in the pyramidal layer of the hippocampus. 5-HT_{1B} autoreceptor responsiveness can be altered by modifying the frequency of electrical stimulation. The degree of tonic activation of the 5-HT_{1A} heteroreceptor can also be assessed by the systemic administration of WAY 100635 (a potent 5-HT_{1A} receptor antagonist) and observing an increase in pyramidal neuron firing rate – translating to an increase in 5-HT neurotransmission (Haddjeri et al., 1998).

13.0 Statistical Analysis

All results are reported as mean values ± SEM. Statistical comparisons were carried out using the two-tailed Student's paired/unpaired *t* test, Fisher's exact test and 2-way Repeated Measures Analysis of Variance (ANOVA) when a parameter was studied in vehicle and treated rats. Statistical significance was taken as $p < 0.05$.

RESULTS

14.0 Effect of acute administration of:

14.1 The 5-HT_{1B} receptor agonist, CP-94253, and vortioxetine on the activation of 5-HT_{1B} autoreceptors

The 5-HT fibres from the raphe nuclei projecting to the hippocampus were electrically stimulated to determine the amount of 5-HT released per stimulus. The frequency of the stimulation was increased from 1 to 5 Hz on the same neuron to assess the function of the terminal 5-HT_{1B} autoreceptors. Again, in control rats, the DOS for the 5 Hz stimulation was significantly smaller than that of the 1 Hz stimulation. Once 1 and 5 Hz stimulations were carried out, an injection of 2 mg/kg CP-94253 was administered followed by a 6 mg/kg injection of vortioxetine with stimulations repeated after each injection. The DOS at 1 and 5 Hz following the administration of CP-94253 were significantly smaller compared to controls. This effect was reversed by the subsequent administration of 6 mg/kg of vortioxetine as shown below. However, unlike in control and following the CP-94253 injection the 1 and 5 Hz stimulations did not significantly differ – suggesting that vortioxetine acts as a 5-HT_{1B} receptor partial agonist and is able to reverse the effects of a potent 5-HT_{1B} receptor agonist (figure 4).

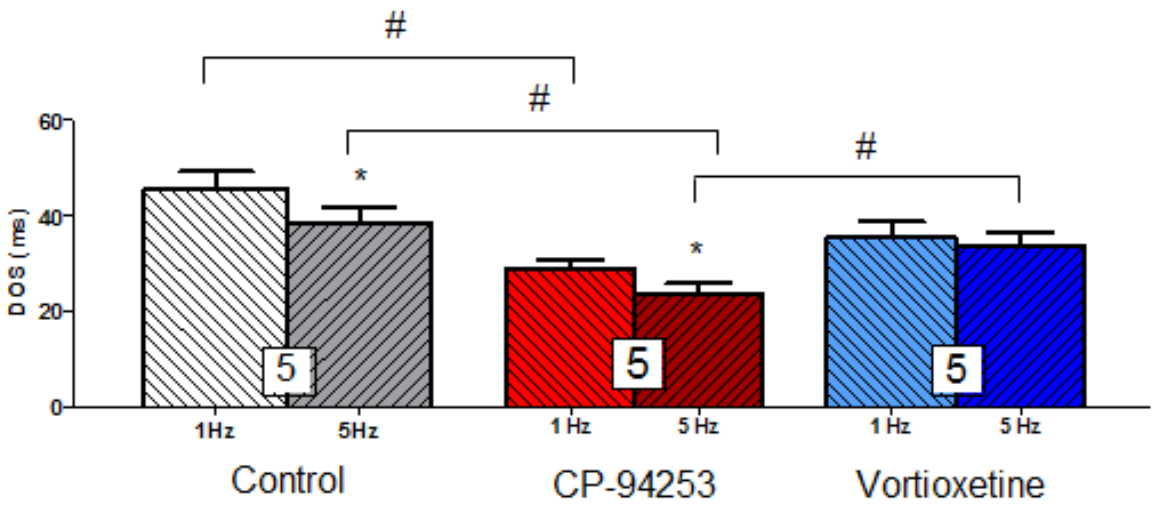


Figure 4 – The effect of electrical stimulation of the 5-HT pathway on CA3 pyramidal neurons after the administration of a 5-HT_{1B} receptor agonist followed by the reversal with vortioxetine. In control animals, there is a significantly shorter DOS during the 5 Hz stimulation compared to the 1 Hz. After the systemic administration (i.v.) of 2 mg/kg of CP-94253 the DOS at both 1 Hz and 5 Hz was completed this resulted in a shorter mean DOS at 1 and 5 Hz. Following the systemic administration of CP-94253, 6mg/kg of vortioxetine was systemically administered (i.v.) where both 1 Hz and 5 Hz stimulations were completed. Vortioxetine was able to reverse the effects of CP-94253. After vortioxetine administration the DOS at 1 Hz is no longer significantly different from that of 5 Hz.

14.2 Vortioxetine alone on the efficacy of electrical stimulation of the ascending 5-HT pathway on CA3 pyramidal neurons

The 5-HT fibres from the raphe nuclei projecting to the hippocampus were electrically stimulated to determine the amount of 5-HT released per stimulus. The frequency of the stimulation was increased from 1 to 5 Hz on the same neuron to assess the overall acute effect vortioxetine on 5-HT release. In control rats, the DOS for the 5 Hz stimulation was 25% smaller ($p=0.0006$) than that of the 1 Hz stimulation. Once 1 and 5 Hz stimulations were carried out on the same neuron, an injection of 6 mg/kg of vortioxetine was administered. Following vortioxetine injection both 1 Hz and 5 Hz stimulations were carried out. After vortioxetine injection the DOS between 1 and 5 Hz was no longer

significant (p=0.7). The results shown in figure 5 indicate that the electrically-evoked release of 5-HT was enhanced by the administration of vortioxetine and that vortioxetine is acting as a 5-HT_{1B} receptor partial agonist.

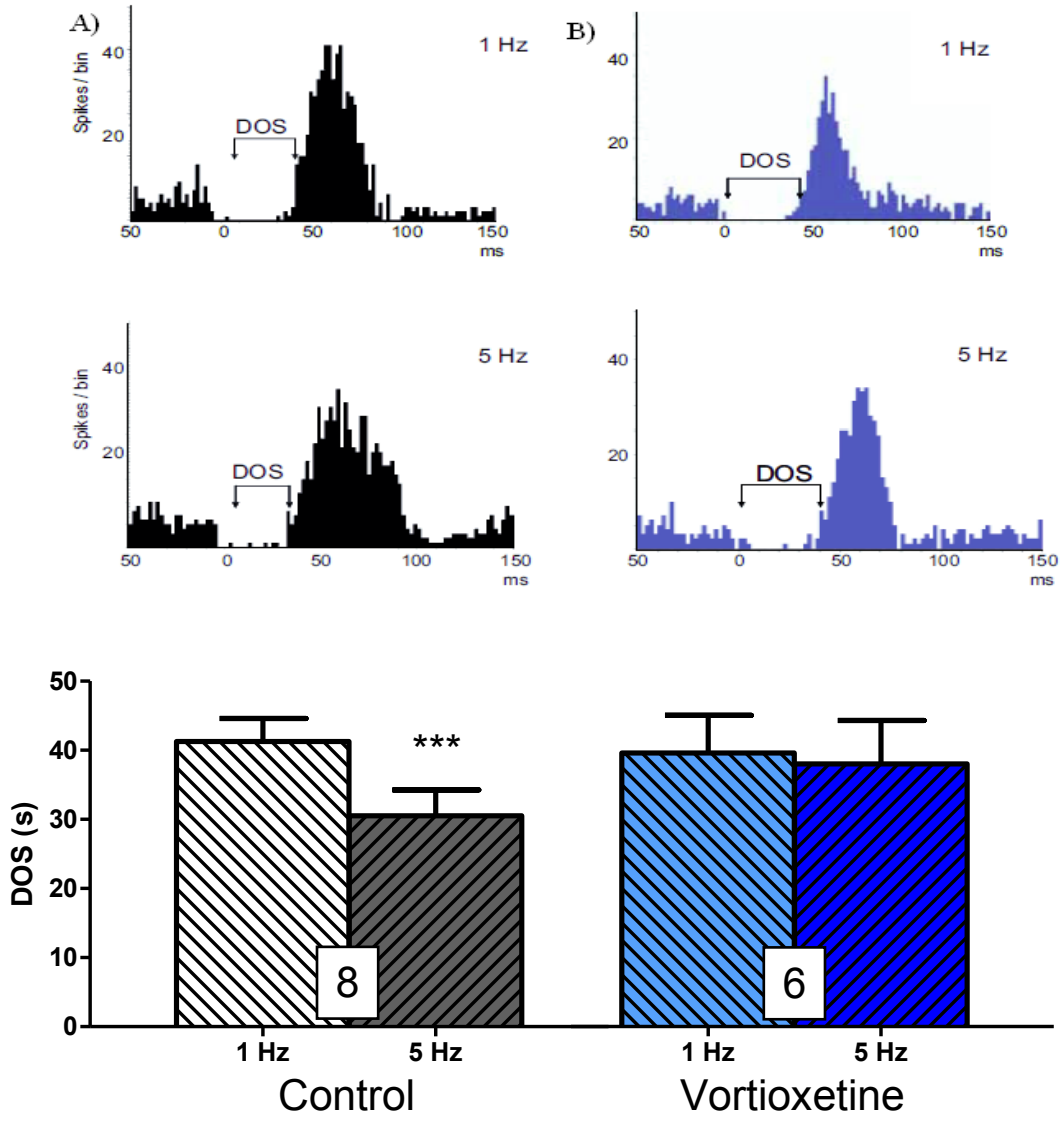


Figure 5 – The effect of electrical stimulation of the 5-HT pathway on CA3 pyramidal neurons before and after the acute administration of vortioxetine. (A) In control animals, there is significantly shorter DOS during the 5 Hz stimulation compared to the 1 Hz. The increased activation of the presynaptic 5-HT_{1B} terminal autoreceptor results in decreased release of 5-HT per stimulus and a decreased activation of the postsynaptic 5-HT_{1A} heteroreceptor. (B) After the systemic administration (i.v.) of 6 mg/kg of vortioxetine the DOS at 1 Hz is no longer significantly different from that of 5 Hz. A comparison of the DOS at 1 Hz pre- and post- administration of 6 mg/kg vortioxetine yielded no significant difference.

14.3 The 5-HT_3 receptor agonist SR 57227 and the 5-HT_3 receptor antagonist ondansetron on the efficacy of electrical stimulation on the ascending 5-HT pathway.

In control rats, the DOS for the 5 Hz stimulation was significantly smaller than that of the 1 Hz stimulation. Once 1 and 5 Hz stimulations were carried out an injection of 50 µg/kg of SR 57227 was administered followed by a 500 µg/kg injection of ondansetron with 1 and 5 Hz stimulations repeated after each injection. The 5-HT_3 receptor agonist, SR 57227, decreased the electrically-evoked release of 5-HT and ondansetron altered the effects induced by SR 57227 suggesting 5-HT_3 receptor antagonistic effects (figure 6).

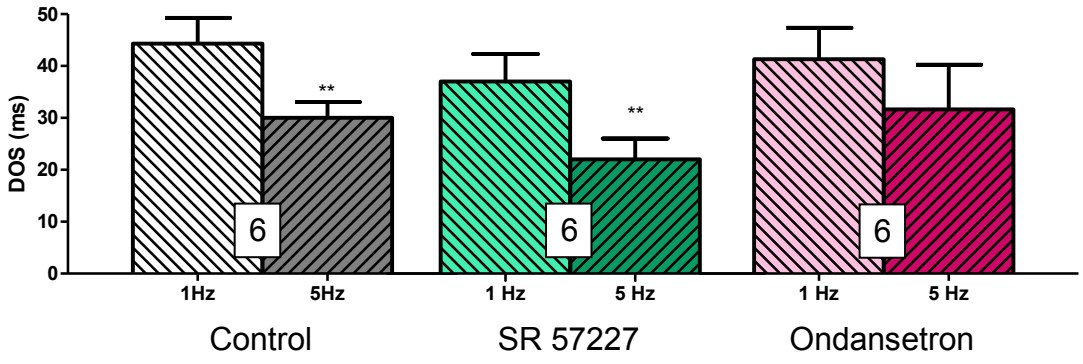


Figure 6 – The effect of electrical stimulation of the 5-HT pathway on CA3 pyramidal neurons. In control animals, there is a significantly shorter DOS during the 5 Hz stimulation compared to the 1 Hz. After the systemic administration (i.v.) of 50 µg/kg of SR 57227 the DOS at both 1 Hz and 5 Hz was completed this resulted in a shorter mean DOS at 1 and 5 Hz. Following the systemic administration of SR 57227, 500 µg/kg of ondanstron was systemically administered (i.v.) where both 1 Hz and 5 Hz stimulations were completed.

14.4 The 5-HT_3 receptor agonist, SR 57227, and vortioxetine on the efficacy of electrical stimulation on the ascending 5-HT pathway

In control rats, the DOS for the 5 Hz stimulation was significantly smaller than that of the 1 Hz stimulation. Once 1 and 5 Hz stimulations were carried out an injection of 50 µg/kg of SR 57227 was administered followed by a 6 mg/kg injection of vortioxetine with 1 and 5 Hz stimulations repeated after each injection. The 5-HT_3 receptor agonist, SR 57227, decreased the electrically-evoked release of 5-HT and vortioxetine reversed the effects induced by SR 57227 suggesting 5-HT_3 receptor antagonistic effects (figure 7).

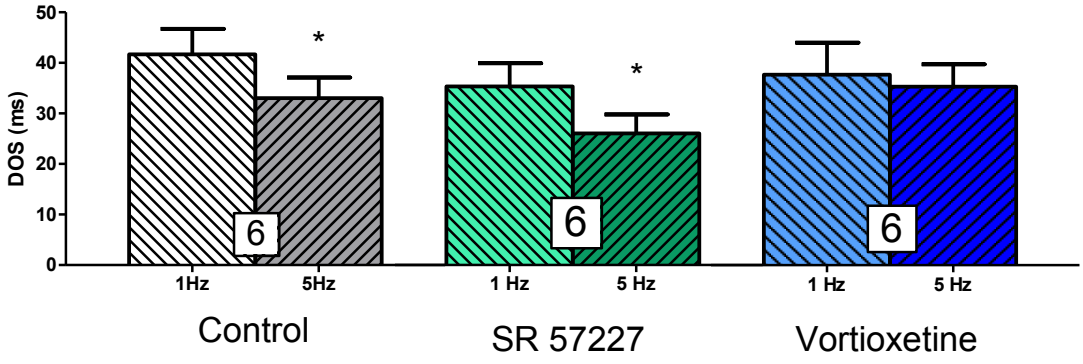


Figure 7 – The effect of electrical stimulation of the 5-HT pathway on CA3 pyramidal neurons. In control animals, there is a significantly shorter DOS during the 5 Hz stimulation compared to the 1 Hz. After the systemic administration (i.v.) of 50 µg/kg of SR 57227 the DOS at both 1 Hz and 5 Hz was completed this resulted in a shorter mean DOS at 1 and 5 Hz. Following the systemic administration of SR 57227, 6 mg/kg of vortioxetine was systemically administered (i.v.) where both 1 Hz and 5 Hz stimulations were completed. Vortioxetine was able to reverse the effects of SR 57227. After vortioxetine administration the DOS at 1 Hz is no longer significantly different from that of 5 Hz.

14.5 The 5-HT_7 receptor agonist LP-44 and vortioxetine on the efficacy of electrical stimulation of the ascending 5-HT pathway

In control rats, the DOS for the 5 Hz stimulation was significantly smaller than that of the 1 Hz stimulation. Once 1 and 5 Hz stimulations were carried out an injection

of 4 mg/kg of LP-44 was administered followed by a 6 mg/kg injection of vortioxetine with 1 and 5 Hz stimulations repeated after each injection. The 5-HT_7 receptor agonist, LP-44, prevented the decrease of the electrically evoked release of 5-HT at 5 Hz and vortioxetine reversed the effects induced by LP-44 suggesting 5-HT_7 receptor antagonistic effects (figure 8).

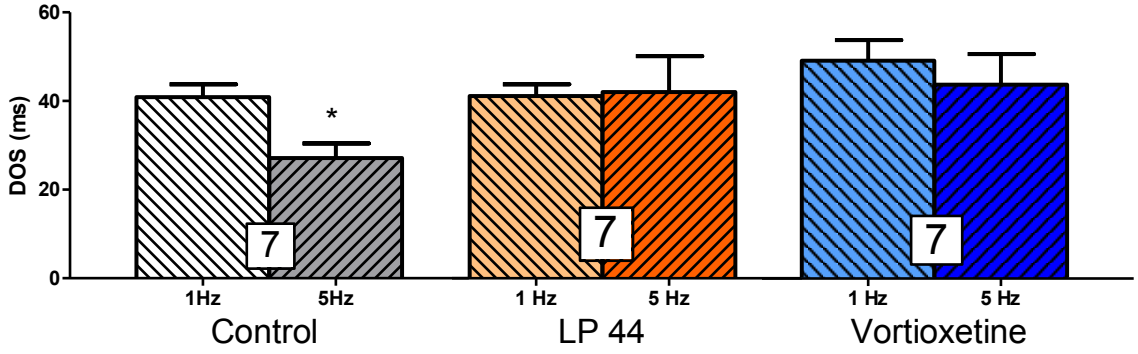


Figure 8 - The effect of electrical stimulation of the 5-HT pathway on CA3 pyramidal neurons. In control animals, there is a significantly shorter DOS during the 5 Hz stimulation compared to the 1 Hz. After the systemic administration (i.v.) of 4 mg/kg of LP-44 the DOS at both 1 Hz and 5 Hz was completed this resulted in a longer DOS at 5 Hz. This indicates that 5-HT_7 receptor agonism enhances 5-HT neurotransmission. Following the administration of LP-44, 6 mg/kg of vortioxetine was systemically administered (i.v.) where both 1 Hz and 5 Hz stimulations were completed. Since 5-HT_7 receptor agonism affected electrical stimulation in a similarly way as vortioxetine does, we were unable to determine the role of 5-HT_7 receptor antagonism that vortioxetine has.

15.0 Long-term Study

15.1 Postsynaptic 5-HT_{1A} receptor sensitivity in the CA3 of the hippocampus

IT_{50} values were determined as a measure of postsynaptic 5-HT_{1A} receptor sensitivity. There was no postsynaptic 5-HT_{1A} receptor alterations at 5 nA and 20 nA microiontophoretically applied currents of 5-HT to the pyramidal neuron. IT_{50} values of CA3 neuron firing were 49 ± 7 nC, 52 ± 4 nC, 59 ± 22 nC, 56 ± 25 nC in 14-day vehicle, vortioxetine, h-vortioxetine, ipsapirone administered rats, respectively for 5 nA. As shown in figure 9, IT_{50} values of CA3 neuron firing were 48 ± 5 nC, 60 ± 7 nC, 42 ± 8

nC, 51 ± 15 nC, 45 ± 7 nC in 14-day vehicle, vortioxetine, h-vortioxetine, ipsapirone, and escitalopram administered rats, respectively for 20 nA. These results are consistent with prior results obtained with SSRIs and 5-HT_{1A} receptor agonists (Mongeau et al., 1997).

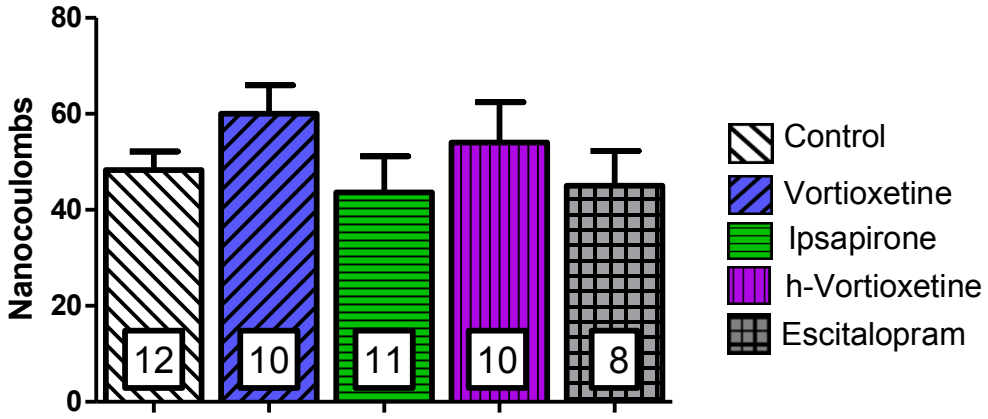


Figure 9 – Postsynaptic 5-HT_{1A} receptor sensitivity assessment. IT_{50} values of 14 day administered vehicle (20% β-OH s.c. x 14-day), vortioxetine (5 mg/kg/day s.c.), h-vortioxetine (5 mg/kg/day vortioxetine and 7.5 mg/kg b.i.d, i.p.), ipsapirone (7.5 mg/kg b.i.d i.p.), and escitalopram (5 mg/kg/d s.c.). The microiontophoretic application of 10 mM 5-HT 20 nA was preformed on CA pyramidal neurons. IT_{50} values were obtained by multiplying the time required to inhibit baseline firing rate by 50 percent by the nA applied to induce inhibition. There was no significant change in IT_{50} values indicating that there is no change in postsynaptic 5-HT_{1A} receptor sensitivity.

15.2 5-HTT blockade properties

In order to assess reuptake inhibition by vortioxetine and h-vortioxetine, the RT_{50} value was measured. RT_{50} values provide insight as to how well the 5-HTT is working. The reuptake blockade properties of ipsapirone were assessed in order to determine if ipsapirone alone possesses and reuptake blockade properties. Escitalopram was used to assess at a dose (5 mg/kg/day) that occupies the transporter to the same extent as vortioxetine as a positive control (i.e. about 60%). After a 14-day administration of vortioxetine, h-vortioxetine, escitalopram the 5-HT reuptake in the CA3 region of the

hippocampus is decreased. As shown in figure 10, all groups that received 14-day administration of an agent that blocks the 5-HTT significantly and increased RT_{50} values at a current of 20 nA of microiontophoretically applied 5-HT.

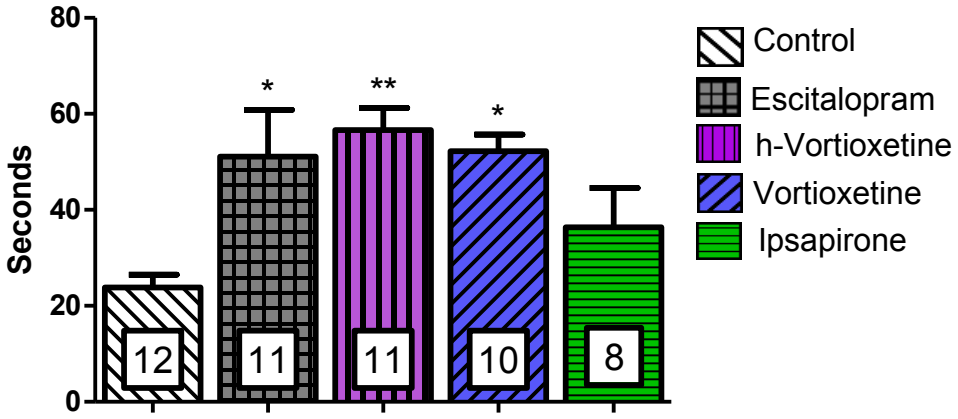
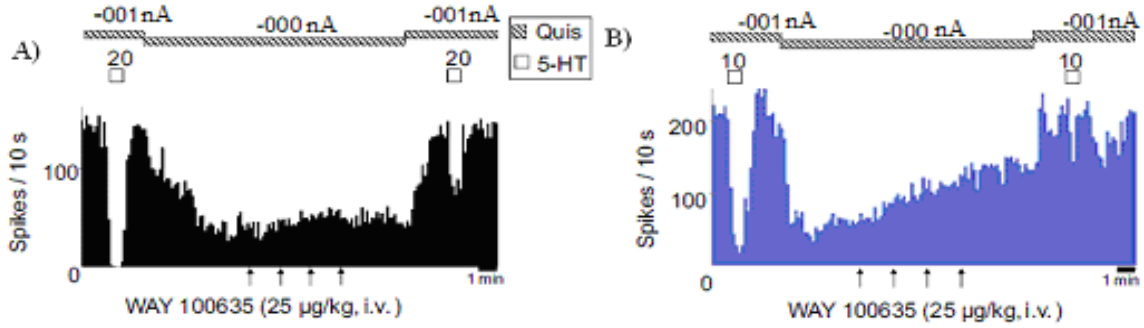


Figure 10 – A comparison of RT_{50} values from 14-day control, vortioxetine, h-vortioxetine administered rats. The microiontophoretic application of 10 mM 5-HT at 20 nA induced 100% inhibition. The amount of time taken for neuronal firing rate to return to 50% of the baseline was determined. A significant increase in RT_{50} values was found after the 14-day administration of escitalopram (5 mg/kg s.c.), vortioxetine (5 mg/kg s.c.), h-vortioxetine (vortioxetine 5 mg/kg s.c. and ipsapirone 7.5 mg/kg b.i.d i.p.), and ipsapirone (7.5 mg/kg b.i.d i.p.) – indicating an increase in 5-HTT blockade by agents that block the 5-HTT.

15.3 Tonic activation of the postsynaptic 5-HT_{1A} receptor

The 14-day administration of vortioxetine, h-vortioxetine, ipsapirone, and escitalopram demonstrated that there is an enhanced tonic activation of the postsynaptic 5-HT_{1A} receptors. This was demonstrated by the increased firing rate of pyramidal neurons in the CA3 in 14-day treated rats after injecting the 5-HT_{1A} receptor antagonist WAY 100635. The increase in pyramidal neuron firing rate was significantly higher in all treatments relative to controls. As shown in figure 11, the tonic activation after the 14-day administration of vortioxetine, h-vortioxetine, ipsapirone, and escitalopram was

enhanced by 241, 256, 370, and 297 percent, respectively. An enhanced tonic activation is a common characteristic of most AD treatments (El Mansari et al., 2005; Blier & de Montigny, 1990) (n = 6).



C)

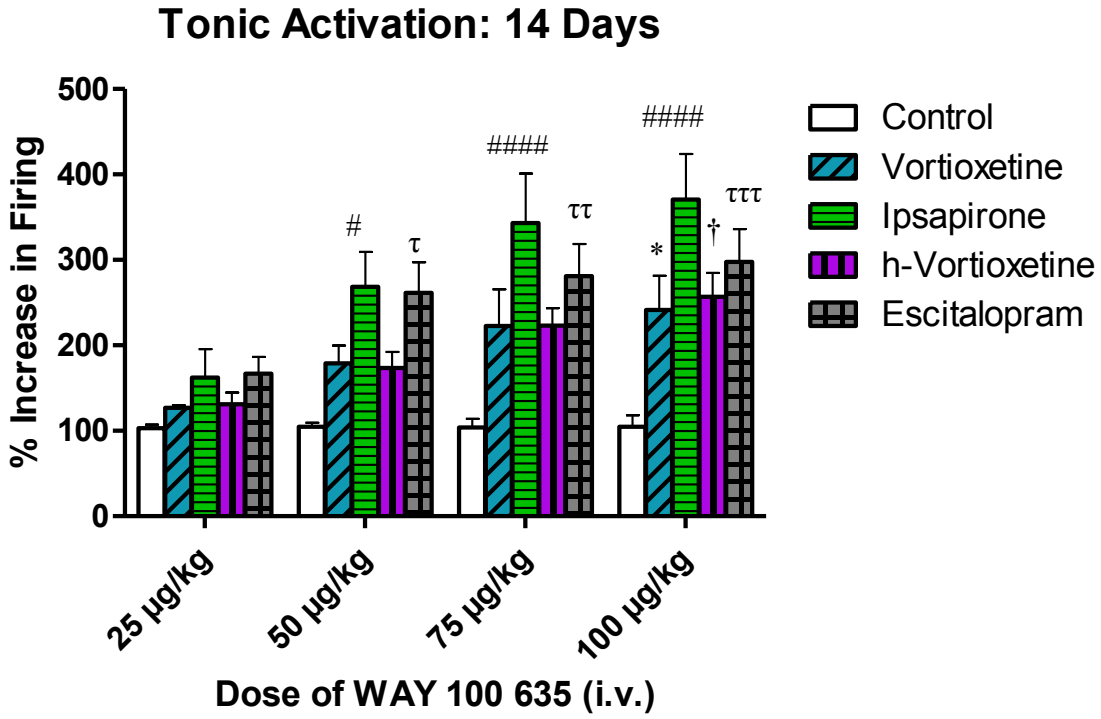


Figure 11 – Tonic activation of the postsynaptic 5-HT_{1A} receptor in the CA3 region of the hippocampus after the 14-day administration of (B) vortioxetine (5 mg/kg/d), h-vortioxetine (vortioxetine 5 mg/kg/d and ipsapirone 7.5 mg/kg b.i.d i.p.), ipsapirone (7.5 mg/kg b.i.d), and escitalopram (5 mg/kg/d s.c.). 100 µg/kg of WAY 100 635 in doses of 25 µg/kg was administered i.v. and neuron firing rates were recorded for 120 s after each injection. All treatments (C) show the postsynaptic 5-HT_{1A} receptors are tonically active relative to control (A).

15.4 Assessment of the sensitivity of terminal 5-HT$_{1B}$ autoreceptors

In order to if long-term administration of vortioxetine alters 5-HT$_{1B}$ receptor sensitivity, rats were administered vortioxetine for 14 days and electrical stimulation of the ascending 5-HT bundle was preformed. Since vortioxetine has a strong affinity for the 5-HT$_{1B}$ receptor, a decreased efficacy of electrical stimulation can be explained by either desensitization of the 5-HT$_{1B}$ autoreceptor or competition between endogenous 5-HT and vortioxetine. For this reason, a 24-hour washout (WO) period was done to discount the second explanation of decreased electrical stimulation efficacy. As shown in figure 12, vortioxetine administered for 14 days in rats with a 24-hour WO period no longer produced a decrease effectiveness of the 5 Hz stimulation. This indicates that 14-day administration of vortioxetine desensitizes the terminal 5-HT$_{1B}$ autoreceptor.

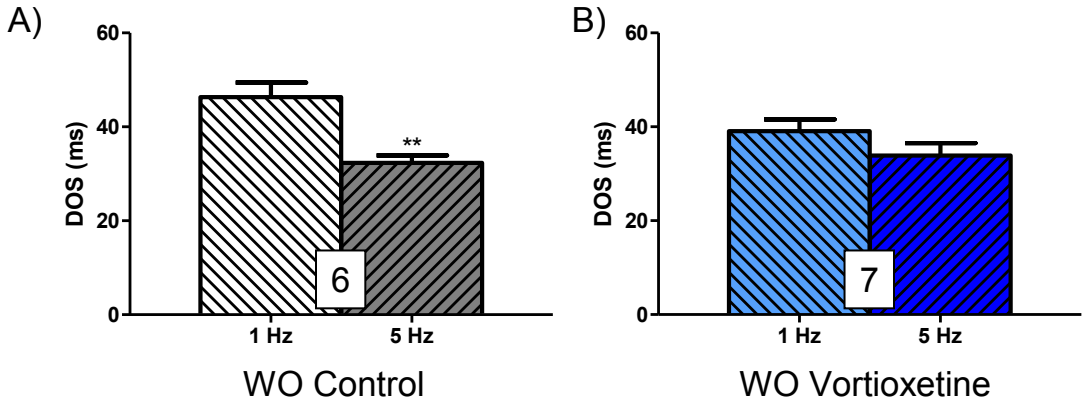


Figure 12 – The effect of electrical stimulation of the 5-HT pathway on CA3 pyramidal neurons in 14-day vortioxetine (5 mg/kg/d) administered rats after a 24 hour WO period. The efficacy of electrical stimulation decreased indicated as no significant difference between the 1 and 5 Hz stimulations suggests that the terminal autoreceptors are desensitized after 14-day administration of vortioxetine.

DISCUSSION

16.0 Study Objectives

This study examined the acute and long-term effects of a multimodal antidepressant on the role of selected 5-HT receptors and the 5-HTT on 5-HT transmission in the rat hippocampus. *In vivo* changes in receptor sensitivity/activation and various pharmacological properties were found to significantly modulate 5-HT neurotransmission in a postsynaptic structure. By understanding the relationship between 5-HT receptor and transporter functions in the hippocampus, new and more effective approaches may be developed in the treatment of MDD.

16.1 Acute impact of various degrees of 5-HT_{1B} receptor partial agonism on electrically-evoked 5-HT release

The acute administration of the 5-HT_{1B} receptor full agonist, CP-94253, was found to decrease hippocampal 5-HT neurotransmission as shown in figure 5. This is not surprising considering, as mentioned earlier, the activation of these autoreceptors exert a negative feedback on 5-HT release. In addition, previous electrophysiological studies have demonstrated that the electrically-evoked release of 5-HT is decreased following the administration of the 5-HT_{1B} receptor agonist CP-93129 - again confirming a decreased release of 5-HT per stimulus in the hippocampus subsequent to the administration of a 5-HT_{1B} receptor agonist (Piñeyro et al., 1995). This data suggests an increased activation of terminal 5-HT_{1B} autoreceptors results increased negative feedback on 5-HT terminals. Furthermore, microdialysis studies have demonstrated decreased concentrations of hippocampal 5-HT following the acute administration of a selective 5-HT_{1B} receptor agonist (Sharp et al., 1989).

The impact of a partial 5-HT_{1B} receptor agonist on the release of 5-HT release in the rat hippocampus had not previously been examined. Interestingly, the acute administration of the multimodal agent vortioxetine, which has 5-HT_{1B} receptor partial agonism, prevented the decrease in the electrically-evoked release of 5-HT at a high frequency of stimulations as shown in figure 4. Considering that vortioxetine has a high affinity for the 5-HT_{1B} receptor and that 5-HT_{1B} receptor modulation has been shown to decrease 5-HT release per stimulus, it is likely that this effect is mediated by 5-HT_{1B} receptor partial agonism exerted by vortioxetine. The 5-HT_{1B} receptor agonist experiments do not support vortioxetine acting as a 5-HT_{1B} receptor full agonist, but as a 5-HT_{1B} receptor partial agonist *in vivo*. This is because vortioxetine, unlike the full agonist CP-94253, did not alter the effectiveness of the low frequency of stimulation. Had vortioxetine acted as a pure antagonist, it would have enhanced the effectiveness of low-frequency stimulations, like the terminal 5-HT autoreceptor antagonist methiothepin (Chaput et al., 1986). However, it is important to note that previous studies did not utilize multimodal compounds, such as vortioxetine, thus synergistic effects may be at play. Although vortioxetine acts as a 5-HT_{1B} receptor partial agonist it might be expected that 5-HT release will be decreased but this was not the case which may suggest other actions are involved such as SSRI activity or 5-HT_3 or 5-HT_7 receptor activity. However, it is likely that the acute enhancement of 5-HT neurotransmission is enhanced as a result of a combination of actions exerted by vortioxetine. Previous studies have demonstrated that combinations of ADs that utilize more than one mode of action potentiate 5-HT neurotransmission in the hippocampus such as the combination of paroxetine and mirtazapine (Bessonet al., 2000). Although different mechanisms underlie the effects of

ADs, similar to vortioxetine, virtually all ADs enhance 5-HT neurotransmission (Blier & de Montigny, 1994; Blier & El Mansari, 2013).

The behavioural implications of 5-HT_{1B} receptor activation are not well understood. For example, one study has shown that the administration of a 5-HT_{1B} receptor agonist reduces exploration in the elevated plus maze – a measure of anxiety – indicating increased anxiolytic behaviour (Lin & Parsons, 2002). In addition, AD-like effects have been produced in mice devoid of 5-HT_{1B} receptors when SSRIs were injected into the striatum and substantia nigra but not the hippocampus or frontal cortex (Chenu et al., 2008). In contrast, decreased immobility times have been reported in a mouse FST after the administration of the 5-HT_{1B} receptor agonist CP-94253. The reduced immobility time exerted by CP-94253 in the FST is mediated by activation of 5-HT_{1B} receptors — most probably located postsynaptically and/or as heteroreceptors, and that the DA and the NE systems are involved in this action (Tatarczynska et al., 2005). These results implicate 5-HT_{1B} heteroreceptors specifically in the mechanisms of AD-like action of 5-HT_{1B} agonists – which are found in high concentrations in the basal ganglia (Murrough & Neumeister, 2011). 5-HT_{1B} receptors may mediate behavioural and AD effects through its impact on DA signalling in the basal ganglia. A reduction in 5-HT_{1B} heteroreceptor function on DA terminals within the ventral pallidum/ventral striatium and NAc would be expected to lead to impaired DA signalling within the VTA-NAc reward pathway (Cao et al., 2010). It has been suggested that vortioxetine regulates the mesocortical and mesolimbic DA tracts differently (Pehrson et al., 2012). By acting on different regions of the brain, the sensitivity of 5-HT_{1B} autoreceptors may change, while the sensitivity of 5-HT_{1B} heteroreceptors may remain unaltered – similar to those of pre-

and postsynaptic 5-HT_{1A} receptors in the DRN and the hippocampus. Given that vortioxetine acts as a 5-HT_{1B} receptor partial agonist, it is likely that 5-HT_{1B} receptor partial agonists are able to exert AD-like effects by producing different effects in different regions of the brain. 5-HT_{1B} receptor partial agonism may also play in important role in the regulation of postsynaptic regions of the brain under different degree of activation. For example, the administration of vortioxetine did not alter 5-HT release in the hippocampus during the 1 Hz stimulation but significantly enhanced it at 5 Hz. This suggests that vortioxetine may act as an agonist in less 5-HT activated regions of the brain, while acting more as an antagonist in other more 5-HT activated regions of the brain. 5-HT_{1B} receptor agonism has a complex functional role across multiple brain regions that have divergent functions for autoreceptors and heteroreceptors (Murrough & Neumeister, 2011).

Given that 5-HT_{1B} receptor agonism is able to elevate DA levels in the NAc, perhaps 5-HT_{1B} receptor agonism exerted by vortioxetine plays an important role in enhancing neurotransmission in different regions of the brain (Benloucif et al., 1993; Boulenguez et al., 1997). For example, as mentioned earlier vortioxetine is able to increase extracellular ACh, 5-HT, NE, and DA in the mPFC and hippocampus – indicating that 5-HT_{1B} receptor partial agonism may play an important role in increasing levels of these transmitters (Pehrson et al., 2012; Mørk et al., 2013). However, previous microdialysis experiments have demonstrated that vortioxetine does not increase NAc DA outflow providing indirect evidence that vortioxetine does not act as a 5-HT_{1B} receptor full agonist *in vivo* (Pehrson et al., 2012). The findings from the present study, in addition to those of the previous study just mentioned, supports that vortioxetine is able

to enhance neurotransmission in selective postsynaptic regions of the brain and that it is not acting as a full 5-HT_{1B} receptor agonist, such as CP-94253, but as a partial agonist.

Although 5-HT release was enhanced at 5 Hz, the 5 Hz stimulation did not exceed the 1 Hz stimulation again indicating that although vortioxetine can show some antagonistic properties and that it is not acting as a full antagonist *in vivo*. The administration of a 5-HT_{1B} receptor antagonist has been shown to increase extracellular 5-HT concentrations in the hippocampus (Malagié et al., 2001). The co-administration of 5-HT_{1B} receptor antagonists with SSRIs has also been shown to further increase extracellular 5-HT neurotransmission (Malagié et al., 2001). Therefore, it is not surprising that the SSRI and 5-HT_{1B} receptor activity of vortioxetine is able to enhance 5-HT neurotransmission in the rat hippocampus. Vortioxetine is not acting as a 5-HT_{1B} receptor antagonist nor agonist but a 5-HT_{1B} receptor partial agonist *in* vivo and the 5-HT_{1B} receptor partial agonism in addition to other modes of action is able to potentiate 5-HT neurotransmission in the rat hippocampus. The enhanced hippocampal 5-HT neurotransmission is common to most ADs in addition it may mitigate the hyperactivity commonly observed in this region of the brain in patients with MDD (Alvarez et al., 2012; El Mansari et al., 2005; Blier & de Montigny, 1987b; Price & Drevets, 2009; Murrough et al., 2011).

16.2 Acute impact of the 5-HT_3 receptor on the electrically-evoked 5-HT release

The present study demonstrated that electrical stimulation of the ascending 5-HT pathway following the administration of the 5-HT_3 receptor agonist and antagonist, SR 57227, ondansetron, respectively, produced no significant change in the amount of 5-HT release per stimulus in the rat hippocampus as shown in figure 7. In support of the present

study, no extracellular 5-HT concentration changes were detected following the administration of SR 57227 in the rat hippocampus (Mørk et al., 2012). In contrast, it has been reported that the 5-HT_3 receptor agonist 2-methyl 5-HT enhanced the electrically-evoked release of 5-HT in brain slices (Haddjeri & Blier, 1995). 5-HT_3 receptor antagonists have no effect on basal 5-HT release indicating that they are not tonically active (Haddjeri & Blier, 1995). However, unlike 2-methyl 5-HT, SR 57227 may act differently *in vivo* than in brain slices and more potently on different subtypes of 5-HT_3 receptors that are localized on specific subpopulations of neurons that exert an inhibitory effect on 5-HT release. This has been seen with other serotonergic receptor families. For example, most 5-HT_{1A} receptor full agonists in the DRN act as 5-HT_{1A} receptor partial agonist in the hippocampus (Blier & de Montigny, 1987b; Hadrava et al., 1995). 5-HT_3 receptor antagonists eliminate the inhibitory tonus on the release of NE and ACh differently in the forebrain via heteroreceptors localized on GABAergic interneurons which may also contribute to their effect *in vivo* (Giovannini et al., 1998; Matsumoto et al., 1995). Similar to 5-HT_3 receptor antagonists, vortioxetine has been found to enhance NE and ACh in the hippocampus (Bang-Anderson et al., 2011; Pehrson et al., 2012; Mørk et al., 2012).

In the present study following the administration of the 5-HT_3 receptor antagonist ondansetron, there was no significant change in electrically-evoked release of 5-HT in the rat hippocampus. In support of the present study, previous microdialysis studies have shown no extracellular changes in 5-HT concentrations were observed in the rat hippocampus after the administration of ondansetron (Mørk et al., 2012). This was important to determine if 5-HT_3 receptors played a key role in the release of 5-HT in the

hippocampus and to determine if vortioxetine is able to reverse the effects of SR 57227 similar to ondansetron. However, upon closer examination with Student's paired t test a small but significant decrease in the electrically-evoked release of 5-HT was observed at 1 Hz after the administration of SR 57227 which was reversed by ondansetron in the rat hippocampus. These results indicate a trend that SR 57227 decreased 5-HT neurotransmission in the hippocampus that was reversed by both ondansetron and vortioxetine. Although the mechanism of action is not fully understood, the effects observed may be mediated by an indirect pathway. 5-HT_3 receptor-mediated membrane depolarizations have been observed on heterogeneous populations of hippocampal cells, however not at the pyramidal neuron level (Yakel & Jackson, 1988; Andrade & Nicoll, 1987; Colino & Halliwell, 1987). The activation of 5-HT_3 receptors localized on GABAergic interneurons may exert some negative feedback on 5-HT terminals. Similarly, 5-HT_3 receptors on GABAergic interneurons become excited and exert a negative tone on the hippocampus which may explain in part the decrease found after the administration of SR 57227 and the reversal after ondansetron and vortioxetine administration (Mørk et al., 2012; Passani et al., 1994).

The subsequent administration of vortioxetine following SR 57227 enhanced the electrically-evoked release of 5-HT. A student's paired t test revealed a trend that the effects of SR 57227 were reversed suggesting that vortioxetine is acting as a 5-HT_3 receptor antagonist *in vivo* since vortioxetine alone does not alter 5-HT transmission at 1 Hz. The *in vivo* functional action of vortioxetine has again been confirmed as a 5-HT_3 antagonist by microdialysis and adenylyl cyclase activation experiments (Mørk et al., 2012). These experiments have demonstrated that 5-HT levels are enhanced in the mPFC

and hippocampus after the co-administration of an SSRI and a 5-HT_3 receptor antagonist. The mechanism is not fully understood but it is thought that the activation of 5-HT_3 receptors localized on GABAergic interneurons projecting back to the 5-HT terminal would excite these interneurons and result in the release of GABA onto the presynaptic 5-HT terminal – acting as negative feedback mechanism modulating terminal 5-HT release. Another mode 5-HT_3 receptor antagonism exerted by vortioxetine may work through is by increasing the availability of 5-HT for other 5-HT receptors such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C} receptors therefore elevating serotonergic neurotransmission similar to mirtazapine (Rajkumar & Mahesh, 2010).

5-HT_3 receptor antagonism may not be tonically active or increase extracellular 5-HT in the hippocampus alone, but the co-administration with SSRIs have been repeatedly found to potentiate the effects of SSRIs (Mørk et al., 2009; Mørk et al., 2012; Ramamoorthy et al., 2008). Recent data has shown that vortioxetine produced a markedly faster recovery of 5-HT neuronal firing in the DRN, and it has been suggested that 5-HT_3 antagonism played a role (Bétry et al., 2013). A clinically relevant additional benefit of 5-HT_3 receptor antagonism for the treatment of MDD is the use of 5-HT_3 receptor antagonists such as ondansetron in the treatment of emesis during cancer chemotherapy – as nausea and emesis is a common side effect of many ADs (Herrstedt & Dombernowsky, 2007; Alvarez et al., 2012). 5-HT_3 receptor antagonism possesses antiemetic properties – with nausea and vomiting being side effects common to AD medications that contribute to discontinuation and relapse. Interestingly, the initially described SSRI litoxetine with antiemetic properties was later found to possess moderate 5-HT_3 receptor antagonism properties (Angel et al., 1993). These findings indicate that

either the co-administration of an SSRI in addition to a 5-HT_3 receptor antagonist or a multimodal agent that acts as a SSRI and 5-HT_3 receptor, such as vortioxetine, could enhance 5-HT neurotransmission to produce AD and antiemesis effects, similar to that of litoxetine, after long-term administration.

16.3 Acute impact of the 5-HT_7 receptor on the 5-HT system

The 5-HT_7 receptor agonist LP 44 increased the electrically-evoked release of 5-HT at 5 Hz but did not alter 5-HT release at 1 Hz. This finding indicated that 5-HT_7 receptors only modulate hippocampal 5-HT release under high frequencies of stimulation. As shown in figure 8, following the injection of LP 44, the administration of vortioxetine did not produce any further alteration. Therefore, we could not make any conclusion about the potential antagonism of 5-HT_7 receptors by vortioxetine. Furthermore, vortioxetine has a lower affinity for the rat 5-HT_7 receptor than for its human counterpart (200 nM versus 19 nM), thus likely underestimating the involvement of 5-HT_7 receptor antagonism in the present study (Bang-Anderson et al., 2011). Nevertheless, sustained 5-HT_7 receptor antagonism for one week has also been shown to produce a tonic activation of the postsynaptic 5-HT_{1A} heteroreceptor – indicating enhanced hippocamal 5-HT neurotransmission (Mnie-Filali et al., 2011). In addition, cell proliferation in the CA3 of the hippocampus is enhanced after 2-3 weeks of fluoxetine treatment while cell proliferation enhancement is observed within 1 week after the administration of the 5-HT_7 receptor antagonist SB-269970 – indicating that 5-HT_7 receptor antagonists exert a more rapid cell proliferation effect than the SSRIs (Mnie-Filali et al., 2009). Thus, these studies provide indirect evidence that 5-HT_7 antagonism enhances 5-HT neurotransmission and neurogenesis in the hippocampus. However, the

present study was unable to determine the extent to which 5-HT_7 receptor antagonism affects 5-HT release per stimulus.

In the DRN 5-HT_7 receptor agonism results in the inhibition of 5-HT neuron firing. Since these receptors are stimulatory, but result in the inhibition of 5-HT neurons, it is likely that they are mediating their effect via GABAergic interneurons (Mnie-Filali et al., 2011; Bard et al., 1993). This suggests that the enhancement in 5-HT evoked release exerted by LP 44 in the hippocampus at a high frequency of stimulation may be a result of the activation on 5-HT_7 receptors on GABergic interneurons in the hippocampus as well. 5-HT_7 receptor antagonism has been shown to produce AD-like effects in the mouse FST and TST (Hedlund et al., 2005). The 5-HT_7 receptor has been associated with sleep disturbances that are commonly seen in depression. It has been suggested that 5-HT_7 receptor antagonism decreases the time in rapid eye movement (REM) sleep in an AD-like manner in mice (Hedlund et al., 2005).

The 5-HT system is intricately connected to the hypothalamo–pituitary–adrenal-(HPA) axis where an abundance of 5-HT_7 receptors are localized (Ruat et al., 1993; Mnie-Filali et al., 2009). The activation of 5-HT_7 receptors may stimulate the release of glucocorticoids in the hippocampus. As previously mentioned, glucocorticoid elevation, decreases hippocampal neurogenesis and occurs during stress and is implicated strongly in the aetiology of depression (Kendler et al., 1999; Nandam et al., 2007; Schloesser et al., 2009). Hippocampal glucocorticoid receptor expression is mediated by the 5-HT system via 5-HT_7 receptors but not essential in producing an AD-like effect because mice with genetically-enhanced neurogenesis do not present an active phenotype (Laplante et al., 2002). Therefore the deactivation or desensitization of these receptors decrease

glucocorticoid release and enhance neurogenesis. Neurogenesis is important to elicit and AD-like response in behavioural models of AD activity (Nandam et al., 2007; Santarelli et al., 2003; Schloesser et al., 2009). It has previously been shown that after the long term administration of SSRIs results in the desensitization of 5-HT_7 receptors (Mnie-Filali et al., 2011). This desensitization may result in decreased glucocorticoid release in the hippocampus and increase neurogenesis – supporting a role for 5-HT_7 receptor antagonism in the treatment of MDD. Clinical trials examining the use of vortioxetine for the treatment MDD have indeed demonstrated that vortioxetine separates from placebo, it is comparable to venlafaxine but with less aversive side effects, and it is effective at preventing relapse (Jain et al., 2013; Alvarez et al., 2012; Boulenger et al., 2012). AD effects may also be a result of a desensitization of 5-HT_7 receptors in the HPA axis resulting in decreased release of glucocorticoids into the hippocampus and therefore promoting neurogenesis in the DG. In addition, by blocking the 5-HTT and acting as a 5-HT_7 receptor antagonist, in addition to several other receptors, multimodal 5-HT agents such as vortioxetine likely produces AD-like effects by enhancing 5-HT release in postsynaptic regions while altering receptor sensitivity and tonically activating hippocampal 5-HT_{1A} heteroreceptors, common characteristics of ADs.

Vortioxetine has previously been shown to improve various cognitive tests of speed of processing, verbal learning and memory. Both vortioxetine and the SNRI duloxetine improved Rey Auditory Verbal Learning Test (RAVLT) acquisition by 71 percent and 68 percent and delayed recall, respectively (Baker et al., 2004). Duloxetine and vortioxetine also improved delayed recall by 66 percent and 72 percent, respectively. However, vortioxetine had a direct effect on the Digit Symbol Substitution Test (DSST) by

significantly increasing scores by 83 percent while duloxetine did not (Baker et al., 2004). Both vortioxetine and duloxetine separated from placebo in all measures of response and remission for depression. These results suggest that vortioxetine may improve cognitive dysfunction beyond verbal learning and memory while having aversive effects related to sexual dysfunction (Baker et al., 2004).

16.4 Impact of low occupancy of the 5-HTT on the 5-HT system

There was a significant increase in RT_{50} value following a 14-day administration of vortioxetine, h-vortioxetine and the SSRI escitalopram as shown in figure 10. Since vortioxetine has a low occupancy for the 5-HTT, escitalopram was used at a dose of 5 mg/kg in order to better mimic the effects of low occupancy alone. It was not surprising that each of the treatment groups, except ipsapirone, produced a significant increase in RT_{50} considering all other SSRIs, such as citalopram, escitalopram, and paroxetine, have been found to produce a prolonged 5-HT reuptake process *in vivo* (Gobbi et al., 2001; El Mansari et al., 2005). SSRIs have been found to produce a tonic activation of the $5\text{-}HT_{1A}$ receptor in the hippocampus and increases in extracellular 5-HT various projections of the brain such as the hippocampus and the mPFC (El Mansari et al., 2005; Pehrson et al., 2012). Although SSRIs produce a tonic activation of $5\text{-}HT_{1A}$ receptors, none of these groups altered the sensitivity of postsynaptic $5\text{-}HT_{1A}$ receptors. The blockade of the 5-HTT results in increased extracellular 5-HT which typically translates to enhanced 5-HT neurotransmission. Previous studies have demonstrated that escitalopram and vortioxetine increases extracellular 5-HT in the hippocampus, mPFC and NAc (Pehrson et al., 2012; Mørk et al., 2012). Increased 5-HT neurotransmission in these regions are common actions of ADs (Matsumoto et al., 1995; Blier & de Montigny, 1994; Mørk et

al., 2012; Pehrson et al., 2012). Since 5-HTT activity is decreased and results in increased extracellular 5-HT, it indicates that escitalopram and vortioxetine are functioning as an SSRI *in vivo*. They both doubled the RT_{50} values when compared to control. It is however important to emphasize that escitalopram at a regimen twice higher than that used in the study increases the RT_{50} value five fold (10 mg/kg/day; El Mansari et al., 2005). The later regimen of escitalopram produces in rats similar plasma levels as those obtained in humans using therapeutic doses. These *in vivo* experiments support the *in vitro* experiments indicating that vortioxetine has SSRI properties. As with any chronic treatment with SSRIs, it is likely that 5-HT transmission is enhanced not only because of decreased 5-HTT function but also presynaptic 5-HT_{1A} and 5-HT_{1B} receptor desensitization (Bétry et al., 2013).

16.5 Effect on 5-HT_{1A} receptor sensitivity in the hippocampus

The sensitivity of postsynaptic 5-HT_{1A} receptors was assessed after the 14-day administration of vortioxetine, h-vortioxetine, ipsapirone, and escitalopram by determining the IT_{50} value for each. As shown in figure 9, after 14 days the IT_{50} values in all administered groups did not significantly differ from controls. Consistently with these results, 5-HT_{1A} receptor agonists such as gepirone and ipsapirone resulted in the desensitization of only the presynaptic 5-HT_{1A} autoreceptors but not postsynaptic 5-HT_{1A} heteroreceptors in the hippocampus (Blier et al., 1988; Blier & de Montigny, 1990; Dong et al., 1997). Furthermore, previous electrophysiological studies have shown that vortioxetine desensitizes presynaptic 5-HT_{1A} receptors in the DRN to a degree much faster than fluoxetine – indicating that vortioxetine acts more potently on the 5-HT system (Etiévant et al., 2011). However, the present study demonstrated that like

gepirone and ipsapirone, h-vortioxetine and vortioxetine did not alter 5-HT_{1A} receptors sensitivity in the hippocampus – a trait common to most ADs (El Mansari et al., 2005; Ghanbari et al., 2011). This selective desensitization enables ADs and vortioxetine to reduce negative feedback on 5-HT neurons in the DRN and increase their excitability resulting in increased 5-HT in the hippocampus activating postsynaptic 5-HT_{1A} heteroreceptors more and exerting more of a negative tone on the hippocampus.

16.6 Effect of vortioxetine on 5-HT tonic activation in the hippocampus

Although there was no change in postsynaptic 5-HT_{1A} receptor sensitivity in any of the four groups of drugs administered groups, a 14-day administration of vortioxetine, h-vortioxetine, ipsapirone, and escitalopram all produced a tonic activation of these receptors in the CA3 hippocampus. A tonic activation of the postsynaptic 5-HT_{1A} heteroreceptors is an indicator that 5-HT neurotransmission is enhanced in the hippocampus. In support of these treatments other AD agents with similar modes of action, such as citalopram and mirtazapine, have been found to produce a tonic activation of 5-HT_{1A} receptors in the hippocampus (Haddjeri et al., 1998; Etiévant et al., 2011; El Mansari et al., 2005; Ghanbari et al., 2011). For example, a 14-day administration of ipsapirone produced a tonic activation of the 5-HT_{1A} receptor which is typical of most other 5-HT_{1A} receptor agonists such as gepirone (Haddjeri et al., 1998). However, it was unexpected to see that the combination of vortioxetine and ipsapirone (h-vortioxetine) resulted in a tonic activation of the postsynaptic 5-HT_{1A} receptors that was inferior to that of ipsapirone alone. It was unexpected since lithium combinations with a variety of antidepressants and the SSRI paroxetine plus mirtazapine acted in synergy to produce a greater tonic activation of postsynaptic 5-HT_{1A} heteroreceptors. Given that h-vortioxetine

produces a lesser tonic activation than ipsapirone alone it does not necessarily mean that it will be less effective as an AD. For example, in the context of repeated ECS (rECS), previous studies have demonstrated that a lesser, but still significant, tonic activation was produced than other medications, whereas ECT is considered as a strategy of greater efficacy (Haddjeri et al., 1998). The greater efficacy exerted by rECS in the treatment of MDD may result from additional action(s) of rECS on other neurotransmitter systems, leading to an enhanced AD response. Furthermore, the combination of the 5-HT_{1A} receptor agonist with vortioxetine does not mimic the full profile of vortioxetine in humans as the present study is still missing the 5-HT_7 receptor antagonism in rats (Bang-Anderson et al., 2011). As mention earlier, 5-HT_7 receptor antagonism alone after one week can enhance such tonic activation of 5-HT_{1A} receptors (Mnie-Filali et al., 2011). Therefore it is likely that the present study underestimates the expected degree of tonic activation produced by vortioxetine in humans. Taken together these results indicate that all treatment groups (vortioxetine, h-vortioxetine, ipsapirone and a dose of 5 mg/kg of escitalopram) are able to enhance hippocampal 5-HT neurotransmission in rats and that these agents possess a characteristic common to other ADs.

16.7 Impact on 5-HT_{1B} receptor sensitivity after long-term administration

After the long-term administration of vortioxetine and vehicle the sensitivity of the terminal 5-HT_{1B} autoreceptor was assessed. The efficacy of the electrical stimulation of the ascending 5-HT bundle in control animals with a 24 h (washout) WO period demonstrated that terminal 5-HT_{1B} autoreceptor sensitivity was unaltered. No change in the efficacy in the electrically-evoked release of 5-HT in control animals with a 24 h WO period indicates that no change in 5-HT_{1B} receptor sensitivity occurred. However, after

long-term administration of vortioxetine and a 24 h WO period the efficacy of electrical stimulation was decreased. This indicates that presynaptic terminal 5-HT_{1B} autoreceptors are desensitized. It is unclear why there was no increase in DOS at 1 Hz after 14-day administration of vortioxetine after a 24 hour WO considering the 5-HT_{1B} receptors were desensitized and it was expected that more 5-HT release would occur per stimuli. Perhaps like the desensitization process, happening within hours for 5-HT_{1A} receptors in the DRN, the resensitization of terminal 5-HT_{1B} receptors occurs over a much shorter time translating to no increase in DOS at one Hz as shown in figure in figure 12. The desensitization of terminal 5-HT_{1B} autoreceptors decreases negative feedback modulating 5-HT release and enhances 5-HT neurotransmission in the hippocampus. This effect is common to several other antidepressants. For example, other after the long-term administration of SSRIs, such as citalopram or fluoxetine, resulted in a decreased efficacy in the electrically-evoked release of 5-HT in the rat hippocampus (Chaput et al., 1988). The increased hippocampal extracellular 5-HT elicited by vortioxetine, similar to citalopram and fluoxetine, results in the prolonged activation of 5-HT_{1B} receptors and initiates the desensitization cascade. However, desensitization may either be due to a down regulation of terminal 5-HT_{1B} autoreceptors and/or an uncoupling from G-proteins (Blier et al., 1988; Aghajanian & Lakoski, 1984). The desensitization of terminal 5-HT_{1B} autoreceptors results in increased 5-HT release per stimulus enhancing 5-HT neurotransmission in the hippocampus. In support, 5-HT_{1B} receptor KO mice have been shown to have enhanced 5-HT neurotransmission in the hippocampus and mPFC (Piñeyro et al., 1995). Another study demonstrated that 5-HT_{1B} autoreceptors limit the effects of SSRIs in the mouse frontal cortex and hippocampus suggesting that the SSRI

effects exerted by vortioxetine may be potentiated by 5-HT_{1B} receptor desensitization (Malagié et al., 2001). Therefore, the desensitization of terminal 5-HT_{1B} autoreceptors is thought to play an important role in the long-term enhancement of 5-HT neurotransmission in the hippocampus and may underlie some of the AD-like effects reported in previous studies by the long-term administration of ADs and vortioxetine.

16.8 Conclusion

In summary, the administration of vortioxetine alone enhanced the efficacy of the electrically-evoked release of 5-HT as a 5-HT_{1B} partial agonist and was able to reverse the effects of a 5-HT_{1B} receptor agonist. The 5-HT_3 receptor agonist and antagonist did not significantly alter the electrically-evoked 5-HT, however, a trend indicated that 5-HT_3 receptor agonism decreases the electrically-evoked release of 5-HT which was reversed by ondansetron and by vortioxetine. This study demonstrated that 5-HT_7 receptor agonism enhances the electrically-evoked release of 5-HT in the hippocampus however, the extent of 5-HT_7 receptor antagonism exerted by vortioxetine to affect electrically-evoked release of 5-HT was not possible to be determined. Vortioxetine, h-vortioxetine, and a dose of 5mg/kg of escitalopram blocks the 5-HTT, but vortioxetine, h-vortioxetine, ipsapirone do not dampen the sensitivity of postsynaptic 5-HT_{1A} receptors. Long-term administration increases the tonic activation of postsynaptic 5-HT_{1A} heteroreceptors in the hippocampus, an effect common to all antidepressants. In addition, acute administration of CP-94253 decreased the electrically-evoked release of 5-HT while vortioxetine was able to overcome the effects of a potent 5-HT_{1B} receptor agonist and decreased the function of the terminal 5-HT_{1B} autoreceptor under high but not a low degree of activation, thus showing that its partial agonism led to increased 5-HT release.

The long-term administration of vortioxetine, similar to the SSRI citalopram, results in enhanced 5-HT neurotransmission via the desensitization of terminal 5-HT_{1B} autoreceptors in the hippocampus. This study has demonstrated that selective 5-HT agents alone, such as escitalopram, and multimodal agents, such as vortioxetine, alter 5-HT neurotransmission through different receptors and exert different actions, via transporter and/or receptor activity, on the serotonergic system in the hippocampus consistent with other antidepressant strategies and with a unique pharmacological profile.

Many of the actions that vortioxetine exerts on the hippocampus is consistent with the actions of many ADs and supports both preclinical and clinical evidence in favour of vortioxetine enhancing 5-HT neurotransmission which acts as an AD. As augmentation strategies are effective after failure of monotherapy, it is conceivable that vortioxetine may provide additional benefits in some patients. Without necessarily exhibiting an overall superior effectiveness over medications, vortioxetine may exert additional benefits in some symptom domains. For instance, the melatonin receptor agonist and 5-$HT_{2B/C}$ antagonist agomelatine, while producing exactly the same improvements on the Hamilton depression and anxiety scales as the AD venlafaxine, had a greater effect in reducing significantly anhedonia (Martinotti et al., 2012). Similarly vortioxetine, while being as effective in reducing overall depression scores as duloxetine, had a superior effect in some cognitive tests in a population of elderly patients with MDD (Katona et al. 2012). Since MDD is known to be heterogeneous in symptom features, ADs with differential effects on specific symptom domains may advance the efficacy of the pharmacological treatment of this disorder. With the multimodal mechanisms of action vortioxetine exerts it is expected to be more beneficial than current treatments and

therefore increasing remission rates, decreasing relapse rates and becoming more tolerable in patients with MDD.

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