Alterations of the monoaminergic systems in the rat brain by sustained administration of carisbamate and lamotrigine

Stacey Shim
University of Ottawa, Institute of Mental Health Research
Department of Cellular and Molecular Medicine, Neuroscience Program
Ottawa, ON, Canada

Supervised by Dr. Pierre Blier, M.D., Ph.D.

This thesis is submitted as a partial fulfillment of the M. Sc. program in Neuroscience.
Submitted on October 25th, 2012

ABSTRACT

Carisbamate (CRS) and lamotrigine (LTG) are anticonvulsants which act mainly on neuronal voltage-gated sodium channels, that have been shown to have antidepressant-like effects in animal models of depression. *In vivo* electrophysiological recordings were carried out following 2 and 14 days of CRS or LTG administration. Overall firing activity in the dorsal raphe, locus coeruleus and ventral tegmental area were decreased with CRS. Similarly, a decrease in the dorsal raphe was also observed with LTG. Despite these presynaptic decreases in firing activity, both anticonvulsants exhibited significant enhancement of serotonergic transmission in the hippocampus as demonstrated by increased tonic activation of postsynaptic 5-HT_{1A} receptors. This may be attributed to the observed desensitization of the terminal 5-HT_{1B} autoreceptors. This study suggests that the enhanced serotonergic effect may be associated with an antiglutamatergic effect, and may contribute to the antidepressant-like effect of CRS in the forced swim test and the antidepressant properties of LTG.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine, serotonin
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
AC	anticonvulsant
AD	antidepressant
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	analysis of variance
AP	anterior-posterior
APA	American Psychiatric Association
BPD	bipolar disorder
CNS	central nervous system
CRS	carisbamate
DA	dopamine
DOI	(±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride
DOS	duration of suppression
DRN	dorsal raphe nucleus
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders 4th Edition Text Revision
DV	dorsal-ventral
EAAT	excitatory amino acid transporter
ECS	electroconvulsive shocks
ED_{50}	50% effective dose
FST	forced swim test
GABA	gamma-aminobutyric acid
HAM-D	Hamilton Depression Rating Scale
i.p.	intraperitoneal
i.v.	intravenous
ISI	interspike interval
LC	locus coeruleus
LTG	lamotrigine

MADRS	Montgomery-Åsberg Depression Rating Scale
MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder, unipolar depression, clinical depression
MDE	major depressive episode
mGluR	metabotropic glutamate receptor
ML	medial-lateral
mTOR	mammalian target of rapamycin
NAc	nucleus accumbens
NE	norepinephrine
NMDA	*N*-methyl-D-aspartate
NRI	norepinephrine reuptake inhibitor
PFC	prefrontal cortex
RT_{50}	50% recovery time
s.c.	subcutaneous
SEM	standard error of the mean
SNRI	serotonin norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
TPH2	tryptophan hydroxylase 2
VMAT	vesicular monoamine transporter
VTA	ventral tegmental area
WHO	World Health Organization

ACKNOWLEDGMENTS

There are many people to whom I owe my gratitude to that have contributed to my success in my graduate studies. First and foremost I would sincerely like to thank Dr. Pierre Blier for giving me this great opportunity to work under his supervision and also to Dr. Mostafa El Mansari for his guidance in the lab, as I have learned so much from them throughout this experience. My fellow colleagues I've met through the lab, in particular Jojo Jiang, Daphne Kamino, and Agnes Crnic have also been an integral part of my journey and it would not have been the same without them.

I would like to extend a warm thank you to the members of my graduate advisory committee, Dr. Paul Albert and Dr. Verner Knott. I sincerely appreciate the time they've taken to give me feedback and helpful suggestions towards my research. I would also like to thank Richard Belanger for taking such great care of my rats, as well as Ortho-McNeil Janssen Scientific Affairs, LLC for supporting a part of my study.

Lastly, I owe a huge thank you to my family and friends, particularly my parents and my sisters who have been there for me every step of the way and for constantly giving me their support. My warmest thank you goes to both of my grandmothers that always hoped I would do my best in the field. It truly would not have been possible without any of them.

INTRODUCTION

1. Major depressive disorder

1.1. Background

Major depressive disorder (MDD) is a common and serious psychiatric illness with a high prevalence affecting over 98 million people worldwide (WHO, 2008). According to the World Health Organization (WHO) it is already ranked as the number one leading cause of burden of disease, as measured by disability-adjusted life years, in middle- to high-income countries worldwide (WHO, 2008).

According to the American Psychiatric Association's (APA) Diagnostic and Statistical Manual of Mental Disorders 4th Edition Text Revision (DSM-IV-TR; APA, 2000), the established criteria for diagnosing MDD is characterized by at least one major depressive episode (MDE) of a minimum length of 2 weeks of depressed mood and/or anhedonia in association with at least four other depressive symptoms, such as changes in weight, sleep, psychomotor activity, or diminished energy, concentration, self-worth, or suicidal ideation. These depressive symptoms interfere significantly with the individual's normal, daily functioning of the social, emotional and occupational aspects of their life (Parikh et al., 2001). The severity of the disorder is typically rated by two scales of MDD that are often used in clinical practice, the Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960) and the Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery and Åsberg, 1979).

The ultimate goal in the treatment of MDD is remission, which is defined as the complete asymptomatic return to all aspects of their normal life (Frank et al., 1991). There are many effective antidepressant (AD) treatments to date, however, only one-third of

treated patients will achieve full remission within 8 weeks of therapy (Thase, 2003). Those who do not fully remit may experience a response, which is defined as the significant improvement of symptoms such that the individual is only just below the criteria for MDD diagnosis, where difficulties can arise in adherence to treatments as the individual begins to experience some symptomatic relief, thus running a higher risk of relapse (Frank et al, 1991; Thase, 2003). In addition, the long time it takes to improve symptoms may also lead to patients discontinuing their treatment thus resulting in a 5-fold increase in suicidal behaviour (Yerevanian et al., 2004). When patients require more AD treatment steps, their risk of relapse is increased and remission rates are reduced (Rush et al., 2006). Indeed, patients who do not exhibit a response following two or more alternate trials with different classes of ADs are diagnosed as treatment resistant (Fava, 2003; Thase, 2003). Adverse side effects are also a continuing problem with conventional ADs. Thus, there is still a need for new and effective ADs.

1.2. Etiology

Due to the multifactorial nature of the disorder, our current understanding of the etiology of MDD is very limited and its occurrence may be predicted based on a combination of both genetic and environmental risk factors (Caspi et al., 2003; Karg et al., 2011). According to a recent meta-analysis, the heritability of the disorder is 37%, with the genetic component of the disease increasing with certain forms of the disorder (Sullivan et al., 2000). Not only can genetic susceptibility predispose an individual to have a natural propensity towards stressful life events as well as to MDEs, but the experience of external life stressors can have a causal effect on the incidence of MDEs (Kendler et al., 1999). However, the current lack of knowledge of which genes and external factors increase

susceptibility renders it difficult to create ideal animal models of depression, therefore we must rely on paradigms to determine the efficacy of ADs (Berton and Nestler, 2006). *In vivo* electrophysiology is one of the less ambiguous methods, as it provides a direct measure of the changes in neurotransmitter function implicated in depression.

Polymorphisms in genes related to the pathophysiology of depression have been identified (Hamer, 2002). However, due to the heterogeneous nature of depression, it is difficult to use marker genes for association studies, as there is no single set of symptoms that is common for all depressed patients since it is likely that multiple pathways are convergent to depression (Rush, 2007). Therefore, where a treatment may be effective in one individual it may not have any effect in another, thus there is still a need for alternate treatment strategies acting on different targets.

2. Bipolar disorder

Bipolar disorder (BPD) is a mood disorder that is characterized not only by depressive episodes but is distinguished from MDD by the occurrence of hypomanic or manic episodes. In bipolar I disorder the depressive phase is predominant and can last for over a year in as many as one fifth of bipolar patients (Judd et al., 2002; Keller et al., 1986). Thus, difficulties can arise in differentiating MDD from bipolar depression which often results in misdiagnosis and suboptimal treatments. Indeed, conventional ADs prescribed for the treatment of BPD are not only associated with poor response rates but can worsen the outcome of the illness and precipitate rapid cycling and mania (Ghaemi et al., 2000). Therefore, alternate therapies have been developed for treatment of both spectrums of the disorder. There is a high prevalence of BPD and MDD in patients with seizures and thus

many anticonvulsants such as lamotrigine are prescribed as mood stabilizers. Lamotrigine works on mania to a degree but is more typically used for treating depressive episodes and has in fact shown a well-tolerated and significant AD efficacy in both the HAM-D and the MADRS (Calabrese et al., 1999).

The monoaminergic systems have been implicated in both MDD and BPD. The depressed phase of BPD is associated with reduced serotonergic activity, similar to MDD albeit to a lesser extent (Mahmood and Silverstone, 2001). Dopamine and norepinephrine are involved in mania as catecholamine depletion with alpha-methyl-paratyrosine has been shown to attenuate manic symptoms (Bunney WE Jr et al., 1971). Thus, it is important to study the effects on the monoaminergic systems when determining the efficacy of potential treatments for mood disorders.

3. The monoaminergic systems

The heterogeneity of depression makes it difficult to ascertain which of the main brain areas implicated in mediating mood, emotion and cognitive responses contribute to MDD, as the regions involved would vary between individuals depending on their symptoms (Rush, 2007; Berton and Nestler, 2006). However, the main brain regions that are thought to be involved in producing the depressive symptoms, such as the prefrontal cortex (PFC), hippocampus, amygdala, nucleus accumbens (NAc) and hypothalamus, all have substantial innervations coming from the dorsal raphe nucleus (DRN), locus coeruleus (LC) and the ventral tegmental area (VTA) which have been shown to be rich in the monoamines serotonin (5-HT), norepinephrine (NE) and dopamine (DA), respectively, indicating that they may play important modulatory roles on the aforementioned forebrain areas (Celada et

al., 2001; Berridge and Waterhouse, 2003; Albanese et al., 1986; Berton and Nestler, 2006). Indeed, much focus has been given to the 5-HT, NE and DA monoaminergic systems as they have been shown to have overlapping roles in regulating mood, emotion and cognitive function (Stahl, 2000). Thus, dysregulation of any of the systems may be contributing factors to depression.

A study by Guiard et al., (2008a) highlighted the importance of reciprocal functional interactions between these three key systems (Figure 1). Given these interactions, it is important to regard these three systems as a whole when deducing the mechanism of action of ADs since any effect on one system may result in subsequent alterations in the other two systems.

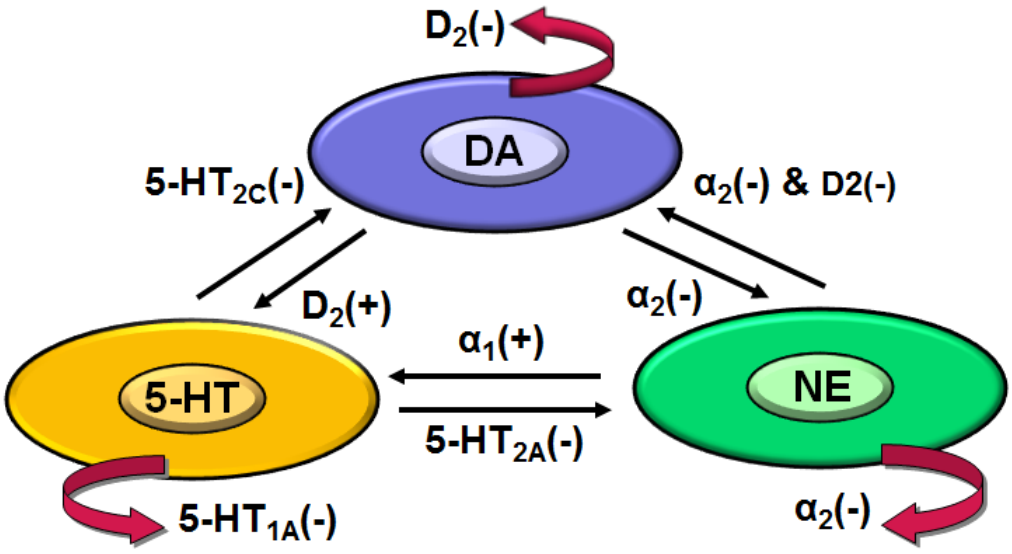

Figure 1. Schematic representation of the reciprocal interactions between the cell bodies of DA, NE, and 5-HT neurons. A (+) sign indicates an agonism or a stimulatory effect and a (-) sign indicates an antagonism or an inhibitory effect on firing activity. The inhibitory autoreceptors are indicated by the curved arrows. Adapted from: Guiard et al. (2008a; 2008b)

3.1. The serotonin system

Serotonin, also known as 5-hydroxytryptamine, is an indolamine neurotransmitter that is located in abundance in the DRN, containing approximately 50-60% of the CNS's 5-HT neurons in humans (Baker et al., 1990). This system is implicated in emotion and mood and thus, dysregulation of this system is thought to be involved in MDD and anxiety disorders. Evidence of its involvement is observed in acute tryptophan depletion studies where a brief recurrence of depressive symptoms is observed in patients that had previously shown a response to serotonergic-related ADs such as selective serotonin reuptake inhibitors (SSRIs; Delgado et al., 1994; Miller et al., 1992).

5-HT is synthesized inside the serotonergic nerve terminal from its precursor tryptophan, and its conversion to 5-hydroxytryptophan is mediated by the rate-limiting enzyme tryptophan hydroxylase 2 (TPH2; Fuller et al., 1980; Walther et al., 2003). Following synthesis, 5-HT is stored into vesicles via the vesicular monoamine transporter (VMAT) until they are exocytosed into the synapse where they can then act either on the presynaptic or postsynaptic receptors. Excess 5-HT is then removed from the synapse and is returned to the nerve terminal through the 5-HT transporters. Once in the nerve terminal 5-HT is either taken up by the vesicles or metabolized by monoamine oxidase into its metabolite 5-hydroxyindoleacetic acid (Szabo et al., 2009).

Somatodendritic 5-HT_{1A} receptors implement a negative feedback regulatory mechanism such that 5-HT activates the $G_{\alpha i3}$ proteins of these receptors, thus binding of 5-HT to these receptors causes a dampening of neuronal firing activity through inhibition of adenylyl cyclase and reduces the amount of available 5-HT in the postsynaptic cleft (Mannoury la Cour et al., 2006; De Vivo and Mayaani, 1986). A study by Stockmeier et al.

(1998) demonstrated an increase in these auto-inhibitory 5-HT_{1A} receptors in the DRN in depressed suicide victims, which may contribute to a decrease in serotonergic activity. Other presynaptic regulatory receptors are the terminal 5-HT_{1B} autoreceptors and α_2-adrenergic heteroceptors, the latter which are coupled to inhibitory G-proteins ($G_{\alpha i}$) that regulate vesicular exocytosis through inactivation of the terminal calcium channels.

Postsynaptic 5-HT_{1A} receptors are also $G_{\alpha o}$-protein coupled receptors, which directly results in the opening of potassium channels, thus hyperpolarizing the cell (Mannoury la Cour et al., 2006; Oleskevich, 1995). Both postmortem in situ hybridization and PET imaging studies have shown that there is a reduction in 5-HT_{1A} receptor levels in postsynaptic regions in unmedicated, depressives and depressed suicide victims which is also in line with decreased serotonergic activity and thus a diminished inhibitory effect of the postsynaptic receptors (Drevets et al., 1999; López et al., 1998; Lopez-Figueroa et al., 2004). Therefore, in determining the effect of AD drug regimens on the monoamine systems, it is important to regard the postsynaptic effects, in particular the tonic activation of postsynaptic receptors caused by long-term AD treatments. A study by Haddjeri et al. (1998) demonstrated enhanced serotonergic neurotransmission incurred by AD drugs and electroconvulsive shocks (ECS) is due to enhanced activation or sensitivity of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus CA3 pyramidal neurons, which can be measured in terms of the degree of disinhibition in the presence of the selective 5-HT_{1A} antagonist WAY 100635. Thus, it is important to determine the responsiveness of 5-HT_{1A} receptors in these neurons when assessing the efficacy of AD treatments.

3.2. The dopaminergic system

The mesolimbic, mesocortical, nigrostriatal and incertohypothalamic pathways are four pathways of the dopaminergic system that can be implicated in depression (Dunlop and Nemeroff, 2007; Szabo et al., 2009). Dopaminergic cell bodies of the mesolimbic pathway, also known as the reward pathway, originate in the VTA and project to many important limbic areas such as the NAc, hippocampus and the amygdala, and play a key role in reward and motivation (Swanson, 1982; Albanese et al., 1986). The mesocortical pathway also originates in the VTA and projects largely to the PFC, and is an important mediator of executive functions as well as playing a part in concentration. The nigrostriatal pathway arises from the substantia nigra pars compacta and projects to the striatum, and has been shown to be important in regulating motor activity. The incertohypothalamic pathway projects from the medial portion of the zone incerta to the amygdaloid and hypothalamic nuclei that play a role in sexual behaviour. Thus, dysregulation in any of these systems may be implicated in anhedonia, diminished motivation, inability to think or concentrate, altered psychomotor activity as well as sexual dysfunction, which are some of the major endophenotypes of MDD.

DA is a catecholamine that is synthesized in the nerve terminal from its precursor tyrosine, which is mediated by the rate-limiting enzyme tyrosine hydroxylase (Szabo et al, 2009). Storage, release, reuptake and metabolism of DA is the same as with 5-HT, however reuptake is through the DA transporter and in some cases through the NE transporter, as DA and NE are structurally similar. DA is able to exert an effect on the postsynaptic neuron through action on either the dopamine 1 (D_1) or 2 (D_2) family of receptors. The firing

activity and synaptic release of DA neurons is regulated through the $G_{i/o}$-protein coupled D_2 somatodendritic and terminal autoreceptors, respectively.

3.3. The noradrenergic system

The noradrenergic system is very widespread throughout the CNS, as about 90% of the NE projections to various forebrain regions such as the amygdala, PFC and NAc, are from the LC (Foote et al., 1983). The noradrenergic system is particularly important in dealing with emotional pain and other stressful stimuli by initiation of the fight or flight response through the hypothalamic-pituitary-adrenal axis via the dorsal medial PFC (Radley et al., 2008). Evidence of this is apparent in electrophysiological studies, where a brief excitation of noradrenergic firing activity is observed in response to a noxious stimuli, such as the nociceptive pinch of the contralateral hindpaw (Aghajanian and Vandermaelen, 1982b). Thus, an imbalanced system would result in the inability to adequately respond to external stressors which is one of the key characteristics of MDD (Szabo et al., 2009).

Structurally similar to DA, noradrenaline is a catecholamine which shares the same synthetic pathway, as it is synthesized directly from DA which is mediated by the enzyme dopamine-β-hydroxylase (Iverson, 1991). In the same way as 5-HT and DA, NE is stored into vesicles by VMAT then released into the synapse where the excess is taken up by the NET for reuse or subsequent degradation by monoamine oxidase (Szabo et al., 2009). Or NE can act on presynaptic inhibitory $G_{i/o}$-protein coupled α_2-adrenoceptors located both at the cell body to inhibit firing and also at the terminals to block release. Reduced NE neurotransmission in MDD has been indicated in postmortem studies where unmedicated, depressed suicide victims display an upregulation of the inhibitory α_2-adrenergic receptors in the LC (Ordway et al., 2003). Indeed, clinical studies of catecholamine depletion via the

tyrosine hydroxylase inhibitor alpha-methyl-paratyrosine have shown that there is a relapse of depressive symptoms in patients who had remitted with noradrenergic-related ADs such as norepinephrine reuptake inhibitors (NRIs; Miller et al., 1996).

A recent study showed that bupropion increased the tonic activation of postsynaptic α_2- and α_1-adrenoceptors by endogenous NE, and both trazodone and quetiapine also enhanced tonic activation of the α_2-adrenergic receptors (Ghanbari et al., 2011; Ghanbari et al., 2012; Chernoloz et al., 2012). Thus, as with postsynaptic 5-HT_{1A} receptors, it is important to determine the responsiveness of these receptors when determining the mechanism of action of ADs.

4. Monoamine hypothesis of depression & current ADs

The monoamine hypothesis of depression states that deficiencies of the central monoamine levels underlie the pathophysiology of depression (Krishnan and Nestler, 2008). Thus, most of the current available ADs have been geared towards enhancing monoamine levels. ADs such as the tricyclics, the SSRIs, NRIs and the 5-HT and NE reuptake inhibitors (SNRIs) act to immediately and transiently enhance monoamine levels by blocking reuptake through the respective transporters (Berton and Nestler, 2006). This effect is also achieved by preventing monoamine degradation with the use of monoamine oxidase inhibitors (MAOIs). The transient increase in 5-HT levels that occurs around the cell bodies and dendrites is not observed in the postsynaptic areas due to a negative feedback mechanism via activation of the somatodendritic 5-HT_{1A} autoreceptors, thus firing activity is reduced (Bel and Artigas, 1992). Following chronic AD treatment, firing activity is returned to

normal and postsynaptic 5-HT levels are elevated due to desensitization of the 5-HT_{1A} autoreceptors (Bel and Artigas, 1993).

ADs such as the reuptake inhibitors and the MAOIs do not exhibit any therapeutic effect until after a couple of weeks of treatment due in part to the time it takes for desensitization of the 5-HT_{1A} autoreceptors to occur (Piñeyro and Blier, 1999). However, auto-inhibition in response to ADs may not necessarily be related to treatment resistance, there may be other long-term changes required for an AD effect, thus the necessity for different treatment strategies (Artigas et al., 2006). Furthermore, tryptophan depletion studies indicate that a simple deficiency of 5-HT in itself is not enough to trigger MDEs in healthy volunteers or exacerbate depressive symptoms in untreated depression (Salomon et al., 1997; Delgado et al., 1994). In addition, catecholamine and combined tryptophan depletion in untreated depressed patients also does not yield any mood changes (Berman et al., 2002). Therefore, these studies support the idea that other brain areas that are modulated by the monoamines, may be contributing to the disorder. Thus, there is a need for alternate AD treatments acting on different targets.

5. The glutamatergic system and MDD

5.1. Background

Studies have indicated that enhancement of monoamine neurotransmission may require alternate mechanisms to produce an AD effect and may also require secondary transcriptional and translational changes in downstream regions, ultimately altering neuroplasticity and in turn, cognitive function (Krishnan and Nestler, 2008; Charney and Manji, 2004). Therefore, systems that play a more integral role in neuroplasticity and cell

survival would be of interest in the treatment and pathophysiology of MDD. The glutamatergic system in particular, has become a potential target for the treatment of MDD as studies have suggested that abnormalities in the system are involved in the pathophysiology (Sanacora et al., 2008; Hashimoto et al., 2007). Indeed, glucose metabolism, and by inference glutamate transmission which accounts for about 85-90% of the glucose metabolic measure, has been shown to be elevated in various cortico-limbic brain structures of patients with MDD (Sibson et al., 1998; Drevets et al., 2004). Functional neuroimaging studies have supported the idea that decreased glucose metabolism in the hippocampus and the subgenual anterior cingulate cortex is associated with a clinical response to long-term treatment with conventional ADs, and conversely increased metabolism was related to non-response, indicating that specific changes in the metabolic activity of the limbic system are essential for depression remission (Mayberg et al., 2000).

Glutamate acts as the cardinal mediator of excitatory neurotransmission at the synapse and is present at higher concentrations than the monoamines in the brain as it is utilized in up to 60% of synapses (Krishnan and Nestler, 2008; Kugaya and Sanacora, 2005; Mathew et al., 2008). It is synthesized from the precursor glutamine via the enzyme glutaminase. Following synthesis, it is packaged into vesicles through the vesicular glutamate transporter and its release is then regulated by voltage-dependent sodium channels. Upon release glutamate is able to act on various receptors, namely the ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptors, as well as the G-protein coupled metabotropic glutamate receptors (mGluRs; Szabo et al., 2009). Since there are no metabolizing enzymes for glutamate at the bouton, all of the excess glutamate is taken up by excitatory amino-acid

transporters (EAATs) which are located predominantly in the adjacent glial cells, where it is then cycled back into glutamine by glutamine synthetase for subsequent transport back to the glutamate neuron which is important for maintaining synaptic glutamate concentrations (Sanacora et al., 2008).

It has been demonstrated in animal models of depression such as in the forced swim test (FST), that local application of glutamate to the NAc results in an increase in the immobility time, which is a measure of despair (Rada et al., 2003). In accordance with this, in diseased states such as in depression there has been an observance of higher plasma levels of glutamate in depressed patients (Altamura et al., 1993; Mauri et al., 1998). This may be attributed to a significant reduction in astrocytic glial cells that has been observed in limbic areas such as the PFC and the amygdala of patients with MDD, and consequently reduced expression levels of EAAT1 and EAAT2 have been shown in the frontal cortex which can disrupt regulation of synaptic glutamate concentrations as there would be reduced removal of excess glutamate from the synapse (Ongür et al., 1998; Altshuler et al., 2010; Miguel-Hidalgo et al., 2010; Bowley et al., 2002). In addition, dysfunctional NMDA receptors have been observed in the frontal cortex of suicide victims (Nowak et al., 1995). Increased presynaptic release of glutamate in the hippocampus would also lead to an excess of glutamate in the synapse which could subsequently become excitotoxic, as it would result in an excess of intracellular calcium and thus an overactivation of calcium-dependent enzymes, eventually leading to cell atrophy and possibly even cell death (Figure 2; Sapolsky, 2000). Cell atrophy and death could result in hippocampal shrinkage, which seems to be a common finding in depressed patients (Campbell et al, 2004). Indeed, reduction in hippocampal volume appears to be correlated with the time span of the illness (Sheline et al., 1999).

Therefore, drugs that are effective in blocking this pathway by reducing excessive glutamate release would have the benefit of enhancing cellular plasticity and cell survival.

5.2. Antidepressants with neuroprotective potential

Consistent with this notion, several classes of ADs have been shown to have beneficial antiglutamatergic effects. The tricyclic AD imipramine has demonstrated a potential neuroprotective effect by reducing potassium-induced glutamate overflow in the rat PFC (Michael-Titus et al., 2000). Similarly, a study by Bonanno et al. (2005) showed that chronic treatment with three ADs (fluoxetine, desipramine, reboxetine) with differing mechanisms reduced depolarization-evoked glutamate release in the hippocampus of rats. Studies have shown that imipramine and fluoxetine inhibit voltage-gated Na^+ channels which may contribute to their antiglutamatergic effects (Nicholson et al., 2002; Lenkey et al., 2006).

In humans, a reduction in glucose metabolism and thus, in glutamate transmission is observed in the amygdala and in the hippocampus following chronic treatment with SSRIs (Drevets et al., 2002; Mayberg et al., 2000). In support of this, a significant reduction in serum glutamate levels has been observed following 5-week subchronic treatment with ADs (Maes et al., 1998). Furthermore, chronic (14-day) treatment with conventional ADs from every principal class (tricyclic ADs, SSRI and ECS) have been shown to downregulate as well as dampen the function of N-methyl-D-aspartate receptor (NMDAR) subunits thus diminishing glutamatergic activity (Paul et al., 1994; Skolnick et al., 1996). This evidence suggests that the therapeutic effect of ADs may be related to a neuroprotective role through the reduction of glutamate neurotransmission.

5.3. Antiglutamatergic drugs with antidepressant potential

Reduction of glutamate transmission may be one of the final common steps in the mechanism of action in which AD drugs exert their therapeutic effect. Thus, the use of antiglutamatergic drugs as an alternate treatment strategy for MDD has become an area of interest (Sanacora et al., 2012; McCarthy et al., 2012). Various non-competitive NMDA receptor antagonists such as dizolcipine (MK-801) and ketamine have been shown to exert AD-like effects in the FST model of depression (Trullas and Skolnick, 1990; Yilmaz et al., 2002). Indeed, despite a short half-life of only three hours, a single infusion of ketamine has been shown to have a rapid AD effect in treatment-resistant patients with MDD which lasts for approximately one week (aan het Rot et al., 2010; Zarate et al., 2006; Berman et al., 2000). It is believed that in rats, ketamine exerts its rapid AD-like effects through activation of the mammalian target of rapamycin (mTOR) pathway, resulting in increased synaptogenesis on dendritic spines in the PFC (Li et al., 2010). Thus, further lending support to the idea that long-term neural adaptations are responsible for the AD response.

6. Depression and Epilepsy

Depressive disorders are the most frequent psychiatric comorbidity in patients with epilepsy as it is easier to manage seizures when depression is under control (Cramer et al., 2003). Animal models have demonstrated depressive-like deficits in the FST, as well as anhedonia-like symptoms in the saccharin solution preference test following induced status epilepticus, which were also associated with reduced 5-HT release and concentration in the hippocampus following raphe stimulation, as well as enhanced function of presynaptic and diminished function of postsynaptic 5-HT_{1A} receptors (Mazarati et al., 2008; Pineda et al.,

2011). Conversely, monoamine deficits have been shown to be correlated with an increased susceptibility to seizures (Shouse et al., 2001). In addition, both disorders have been associated with neuronal hyperexcitability in select forebrain regions as both have been shown to exhibit hippocampal atrophy, thus it is possible that one affliction may be promoting the other (Campbell et al, 2004; Salmenperä et al., 2001). Therefore, it is likely that both disorders share a similar pathogenic mechanism involving reduced serotonergic transmission and excessive glutamate transmission.

6.1. Overlapping function of anticonvulsant and antidepressant drugs

Due to the shared pathogenic similarities between depression and epilepsy, it is not surprising that there would be overlapping functions between ADs and anticonvulsant (AC) drugs. ACs are known to act as mood stabilizers in BPD and have also been shown to have beneficial AD effects in a clinical setting. For example, vagus nerve stimulation is effective in both epilepsy and treatment-resistant depression, presumably through an enhancement of postsynaptic serotonergic and noradrenergic transmission (Schlaepfer et al., 2008; Manta et al., 2009). An important mechanism of action of some ACs is through inhibition of the voltage-dependent sodium channels, as it would inhibit the excessive firing of neurons that is characteristic of seizures, thus blocking the release of excitatory amino acids such as glutamate, and subsequently altering synaptic plasticity.

As mentioned in the previous section, this neuroprotective mechanism also appears to be an effect caused by the conventional ADs fluoxetine, desipramine and imipramine, which consequently have also been shown to produce AC effects (Dailey et al., 1992; Jobe et al., 1983). Animal models of epilepsy have shown that administration of the SSRI fluoxetine to epileptic rats reversed the excitability caused by status epilepticus induction

(Mazarati et al., 2008). Furthermore, it was demonstrated that fluoxetine reduced the ED_{50} (50% effective dose) values of the ACs carbamazepine and phenytoin in the maximal electroshock test and it was also shown to reduce seizure frequency in epileptic patients when used as an adjunctive therapy, which suggests that enhancing 5-HT has beneficial AC effects (Leander, 1992; Albano et al., 2006).

Conversely, a study by Barbosa et al. (2003) showed that in treatment-resistant patients, augmentation of fluoxetine with the AC lamotrigine had a numerically superior AD effect, but missing the 0.05 value than to fluoxetine alone. Normann et al. (2002) demonstrated that lamotrigine administered in addition to the SSRI paroxetine resulted in an improvement of depressive symptoms as early as the 7^{th} day of treatment. This additive effect suggests that the rapid antiglutamatergic effect of AC drugs has the potential to speed up the onset of action of ADs by acting on a pathway that may be one of the final steps in the mechanism of action of ADs (Sanacora et al., 2008). A recent preclinical study by Bourin and others (2009) demonstrated that the AD-like effect of the ACs lamotrigine, phenytoin and topiramate in the FST was reversed by veratrine, the Na^+ channel opener that non-selectively releases glutamate, while the AD-like effect of the conventional ADs (i.e. paroxetine, desipramine, imipramine) was not reversed. These observations support the idea that the AD-like effect of ACs may be due in part to a reduction in glutamatergic transmission putatively via blockade of voltage-dependent sodium channels.

6.2. Carisbamate

Carisbamate (CRS; RWJ-333369; (S)-2-*O*-carbamoyl-1-*o*-chlorophenyl-ethanol) is a phenyl monocarbamate that has been shown to block presynaptic voltage-gated Na^+ channels and repetitive action potential firing in a use-dependent manner in rat hippocampal

neurons which may underlie some of its AC activity (Liu et al., 2009; Lee et al., 2011). CRS was shown to inhibit the $Na_v1.2$ isoform of voltage-gated sodium channels, which is highly expressed in the hippocampus (Liu et al., 2009; Goldin, 2001). Its mechanism of action is related to an antiglutamatergic effect as selective reductions in excitatory, and not inhibitory transmission have been observed at clinically effective doses for epilepsy in the granule cell of the dentate gyrus (Lee et al., 2011). Indeed, it has been shown to display neuroprotective properties as it dose-dependently reduced neuronal loss in the hippocampus, amygdala, and the thalamus following status epilepticus insult at doses of 60-120 mg/kg (i.p.) in rats (François et al., 2011). This neuroprotective effect may be related to the reestablishment of calcium homeostasis following overactivity (Deshpande et al., 2008).

Randomized, double-blind, placebo-controlled phase II clinical trials were completed for the use of CRS in adjunctive treatment of partial onset seizures in patients with refractory epilepsy, which showed that the effective dose ranged from 300-1600 mg/day with doses of 200-400 mg/day displaying high tolerability, while doses exceeding 1000 mg/day were associated with transient adverse effects such as nausea, dizziness, headache and drowsiness (Novak et al., 2007). However, CRS failed to display consistent efficacy data across the dose range vs. the placebo for the treatment of partial onset seizures in phase III trials, thus the clinical program for using CRS as an antiepileptic was discontinued (Bialer et al., 2010). However, its efficacy as a potential mood stabilizer or an AD remains to be studied.

In preclinical rodent models of generalized and partial epilepsy, CRS has a broad spectrum of AC activity with ED_{50} values ranging from 5-60 mg/kg in important limbic brain areas (Novak et al., 2007). Organ toxicity in both juvenile and adult rats has only been

observed at doses of 80 and 160 mg/kg, and the maximum tolerated oral dose ranged between 360-600 mg/kg with adverse effects such as sedation, ataxia, as well as prostration (Novak et al., 2007). Personal communications with another research group demonstrated that CRS (p.o.) was shown to have an antimania-like effect in dominant rats at 3 mg/kg twice daily for 5 weeks: it reduced their competitiveness, however, at ten times the dose (30 mg/kg) it enhanced competitiveness in both dominant and submissive rats. They also observed anxiolytic activity in the mouse four-plate test at 60 mg/kg as well as reduced headshakes in the DOI headshake model. They also showed that CRS indeed had an AD-like effect in the FST model of depression in both mice (10 mg/kg; p.o.) and rat (30 mg/kg) models. Furthermore, the research group determined there was no significant affinity of the drug for 5-HT, DA, GABA, NMDA, cannabinoid, and opioid receptors. Therefore, it appears to be a drug with promising potential as an AD, perhaps through an antiglutamatergic mechanism via sodium channel blockade (Figure 2).

6.3. Lamotrigine

Lamotrigine (LTG; 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) which is known in the clinic as Lamictal®, is a phenyltriazine derivative which first came out as an AC in 1994, but has been shown to have beneficial AD effects in the depressed phase of BPD, notably in more severely depressed patients (Geddes et al., 2009). LTG is currently not indicated in MDD but in BPD maintenance. However, it has demonstrated some benefits in treatment-resistant unipolar depression, particularly in both a well-tolerated improved response, as well as shortening of the time to response when used as an augmentation strategy (Ivković et al., 2009; Barbosa et al., 2003; Normann et al., 2002).

With a principal mechanism of action similar to carisbamate, LTG is also thought to inhibit glutamate release by use- and voltage-dependent blockade of the $Na_v1.2$ isoform in rats as well as the human brain type IIA of sodium channels, which are prominent in hippocampal neurons, particularly during repetitive action potential firing (Liu et al., 2006; Xie et al., 2001; Kuo and Lu, 1997). Its mechanism of action is shown to be related to an antiglutamatergic effect, as a decrease in excitatory transmission has been observed in granule cells of the dentate gyrus (Lee et al., 2008). In accordance with this, microdialysis studies have demonstrated dose-dependent reductions in both basal and veratridine-induced increases in extracellular glutamate levels in the hippocampus of freely moving rats in response to LTG (Ahmad et al., 2005). Indeed, LTG has been shown to block the release of glutamate induced by veratrine, as well as interfere with the release of harmful oxygen radicals such as nitric oxide in forebrain regions, thus reducing excitotoxicity (Figure 2; Leach et al., 1986; Lizasoain et al., 1995). It should be noted that veratrine is not specific for glutamate neurons, however *in vivo* microdialysis studies have shown that under basal conditons, acute LTG reduces extracellular glutamate levels without affecting that of 5-HT, DA or GABA (Ahmad et al., 2005). Therefore, it appears that its use as an antiglutamatergic drug would have considerable neuroprotective potential.

As mentioned previously, LTG has an AD-like effect in the FST which was reversed by veratrine. Codagnone et al. (2007) demonstrated that LTG at a dose of 20 mg/kg exerted this AD-like effect which was shown to be related to a serotonergic effect in the modified FST that was dependent on its sodium channel blocking activity, and thus may be linked to its inhibition of glutamate release. This may also be linked to its clinical AD effect in humans as it is thought that the AD properties of antiglutamatergic drugs are mediated

through the reduction of glutamate transmission and nitric oxide levels in excitotoxicity of postsynaptic regions, as well as an increase in neurotransmission of 5-HT (Calabrese et al., 1999; Prica et al., 2008; Sapolsky, 2000). Postsynaptic 5-HT_{1A} receptors may be involved in LTG's AD-like effect in the FST as its anti-immobility effect was shown to be potentiated by the selective 5-HT_{1A} receptor agonist 8-OH-DPAT, the latter which is unaffected by *para*-chlorophenylalanine-induced 5-HT depletion indicating its action is through the postsynaptic receptors (Bourin et al., 2005; Luscombe at al., 1993). In addition, local activation of these inhibitory receptors by 8-OH-DPAT in the hippocampus has been shown to inhibit limbic seizures induced by kainic acid in rats, as well as improve the depressive-like impairments of epileptic rats in the FST (Gariboldi et al., 1996; Pineda et al., 2011). This further lends support to the idea that there is a shared common mechanism involved in ADs and AC drugs, perhaps mediated through postsynaptic 5-HT_{1A} receptors.

Therefore, we hypothesized that the effectiveness of these ACs in animal models may be due in part to an alteration of monoaminergic transmission (s) on glutamate neurons. Thus, the present electrophysiological study was aimed at determining the effects of both the short- and long-term administration of the sodium channel blocker CRS on monoaminergic transmission in both presynaptic and postsynaptic regions of the brain, in relation to its AD-like properties. Following characterization, a comparison of CRS with LTG was carried out to determine if its efficacy in animal models could be translated to a potential AD effect in a clinical setting.

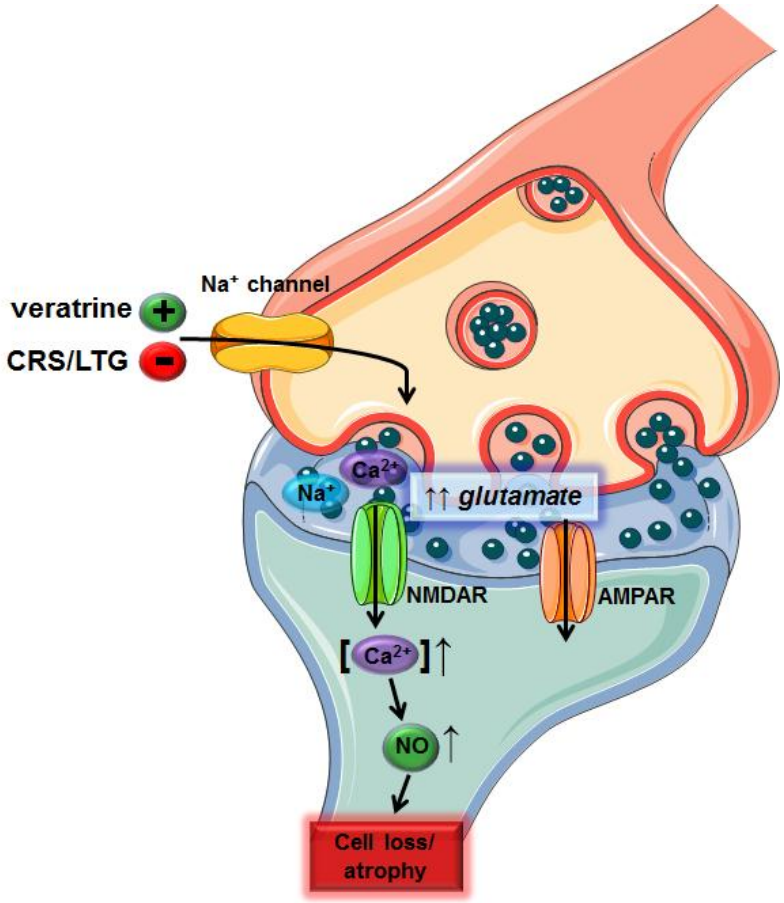

Figure 2. The glutamatergic system in depression. Excessive glutamate transmission leads to an excess of intracellular calcium and a subsequent increase in nitric oxide levels via activation of nitric oxide synthase, thus resulting in cytotoxicity. Veratrine is a sodium channel opener (+) which non-selectively facilitates glutamate release. Putative action of CRS and LTG is through sodium channel blockade (-) and subsequent neuroprotective activity.

MATERIALS AND METHODS

1. Animals

Adult male Sprague-Dawley rats (Charles River, Saint-Constant, QC, Canada) weighing 250-300 g at the time of the experiments were used. Animals were housed 2 per cage under standard laboratory conditions (12:12 h light/dark cycle; light cycle start at 7:00 am; temperature 21±1°C, 40-50% relative humidity) with access to food and water *ad libitum.* All animals were handled in accordance with the guidelines of the Canadian Council on Animal Care and the local animal care committee of the University of Ottawa, Institute of Mental Health Research (Ottawa, Canada).

2. Sustained Treatments

For short and long-term treatments, CRS was administered via subcutaneously (s.c.) implanted osmotic minipumps (Alzet, Durect Corporation, Cupertino, CA) for 2 and 14 days at a dose of 60 mg/kg/day, while control rats received the vehicle (40% propylene glycol: 30% ethanol). The minipumps remained *in situ* throughout the recordings to ensure steady-state levels of the drug in order to mimic clinical conditions. LTG was administered via intraperitoneal (i.p.) injections for 2 and 14 days at a dose of 25 mg/kg/day as it could not be dissolved in the 2 mL volume of the minipumps, while control rats received the vehicle (35% propylene glycol: 7% ethanol).

3. *In vivo* Electrophysiological Experiments

The extracellular unitary recordings of the monoaminergic neurons were carried out using a single-unit glass micropipette filled with 2 M NaCl solution and impedance range between 2 and 4 MΩ. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Supplemental doses of chloral hydrate (50-100 mg/kg, i.p.) were given to prevent any nociceptive reaction to tail or hindpaw pinch. A burr hole was drilled at specific stereotaxic coordinates for each defined region for recordings, and neurons were identified by their spike shape, duration and frequency. Neuronal activity was recorded in real-time using the Spike2 software (Cambridge Electronic Design, Cambridge, UK), which was also used for analysis of neurons offline. Body temperature was maintained at 37°C throughout the experiments using a thermistor-controlled heating pad (Seabrook Medical Instruments, Saint-Hyacinthe, Quebec, Canada). A catheter was inserted in the lateral tail vein for systemic i.v. injections of pharmacological agents.

3.1. Recording dorsal raphe nucleus (DRN) 5-HT neurons

5-HT neurons are recorded reliably at the following coordinates (in mm from lambda): anterior-posterior (AP) 1.0 to 1.2, medial-lateral (ML) 0, dorsal-ventral (DV) 5.0 to 7.0. They are identified by their slow, regular firing rate (0.5-2.5 Hz), and their long spike duration which is between 2-5 ms (Figure 3; Aghajanian and Vandermaelen, 1982a). Studies by Crawford et al. (2010) have suggested that 5-HT neurons may also display a variety of basal firing activity including low or no firing, however it should be noted that these studies

were carried out *in vitro* in slices so the effect of the noradrenergic input is cut, and they are giving phenylephrine to restore the firing activity thus their results are showing differences in excitability rather than the absolute *in vivo* effects.

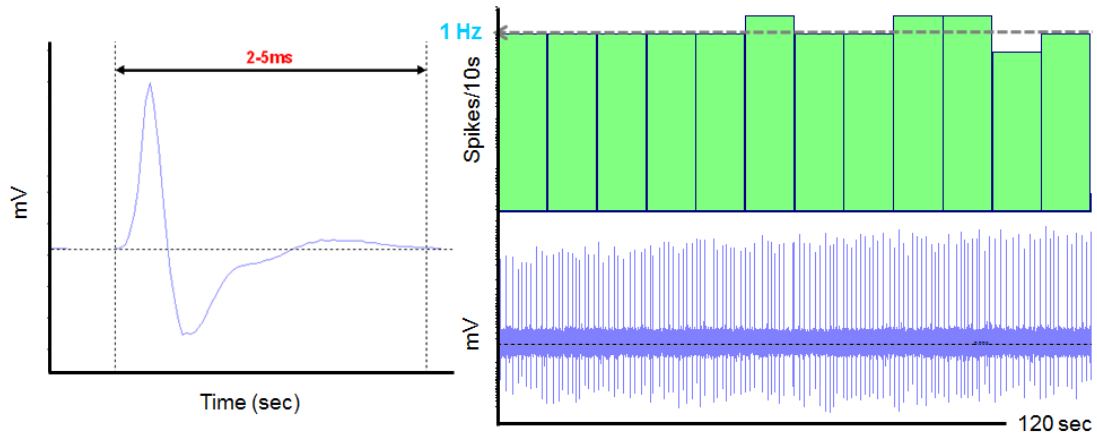

Figure 3. Example of electrophysiological recording of DRN 5-HT neuron. Single action potential (left) appears as uniform spikes (bottom, right). Frequency of firing activity is slow and regular (upper, right).

3.2. Recording locus coeruleus (LC) NE neurons

NE neurons are reliably recorded at the following coordinates (in mm from lambda): AP -1.0 to -1.2, ML 1.0 to 1.3, DV 5.0 to 7.0. They are identified by their regular firing rate (1.0-5.0 Hz), and their positive action-potential duration which is between 0.8-1.2 ms. They can also be identified by a brief excitation to a nociceptive pinch of the contralateral hindpaw followed by a short period of inhibition (Figure 4; Aghajanian and Vandermaelen, 1982b).

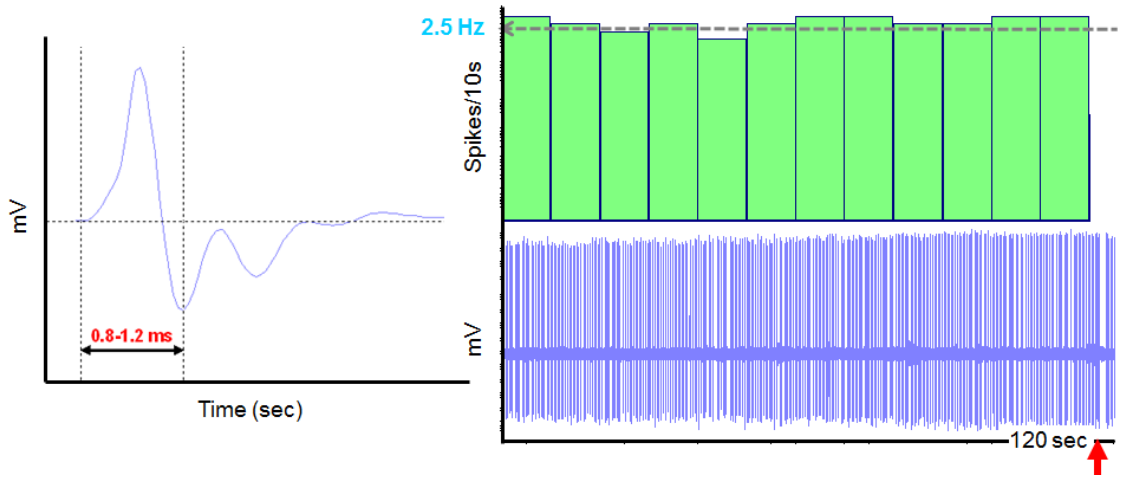

Figure 4. Example of electrophysiological recording of LC NE neuron. Single action potential (left) appears as uniform spikes (bottom, right). Frequency of firing activity is regular (upper, right). Identified by pinch of the contralateral hindpaw which induces a rapid firing of action potentials (arrow) followed by a period of silence.

3.3 Recording ventral tegmental area (VTA) DA neurons

DA neurons are reliably recorded at the following coordinates (in mm from lambda): AP 3.2 to 3.6, ML 0.9 to 1.1, DV 7.0 to 9.0. They are identified by their biphasic spikes (usually with an inflection point in the rising phase) with a large negative phase, irregular firing rate (2.0-9.0 Hz), their long spike duration which is between 2.0-4.0 ms. They often exhibit bursts of 3-10 spikes and decreasing amplitude (Grace and Bunney, 1983). A duration of >1.1 ms from the start of the action potential to the center of the negative trough will also be used to determine the DA neuron identity (Figure 5; Ungless et al., 2004).

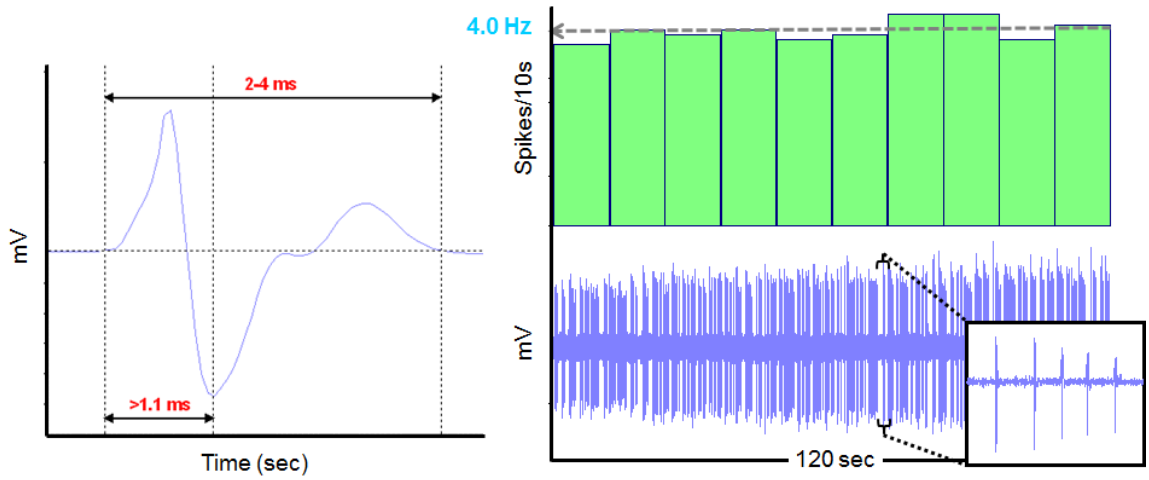

Figure 5. Example of electrophysiological recording of VTA DA neuron. Single action potential (left) appears as irregular spikes (bottom, right). Frequency of firing activity is fast (upper, right) with elicited burst activity of decreasing amplitude (inset).

4. Extracellular recordings and microiontophoresis of dorsal hippocampus CA3 pyramidal neurons

Microiontophoresis with a five-barreled micropipette was used to record dorsal hippocampus CA3 pyramidal neurons at AP 3.8 to 4.5, ML 4 to 4.2, DV 3 to 4.5. They are identified by their large amplitude (0.5-1.2 mV) with long duration (0.8-1.2 ms) single action potentials, alternating with complex spike discharge (Figure 6; Kandel and Spencer, 1961). The central barrel of the micropipette was used for extracellular unitary recording and filled with 2 M NaCl. Side barrels were loaded with 5-HT creatinine sulfate (15 mM in 200 mM NaCl, pH 4) , NE bitartrate (10 mM in 200 mM NaCl, pH 4), 2 M saline (automatic current balancing) and quisqualic acid (AMPA and group I metabotropic glutamate receptors agonist; 1.5 mM in 200 mM NaCl, pH 8) to activate the pyramidal neurons.

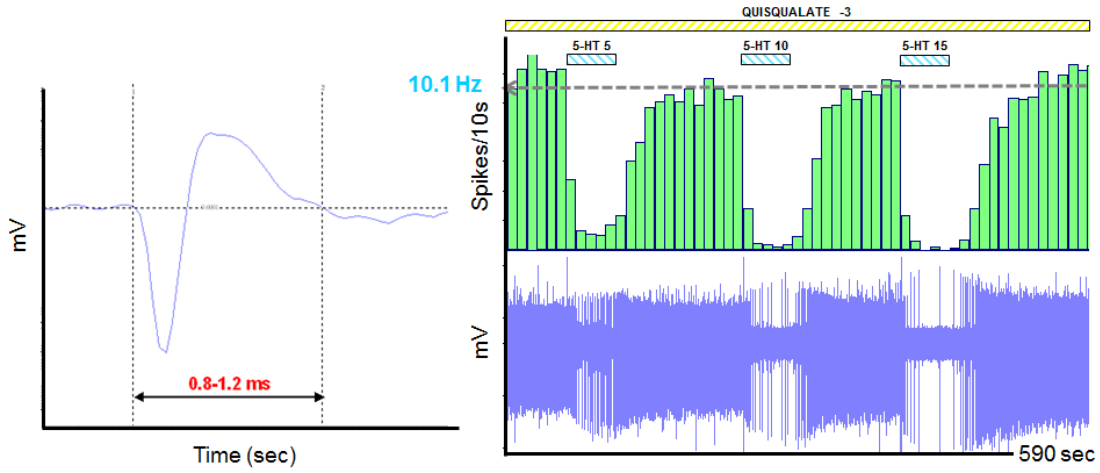

Figure 6. Example of electrophysiological recording of hippocampus CA3 pyramidal neuron. Single action potential (left) appears as irregular spikes (bottom, right). Frequency of firing activity is activated by ejection currents (nA) of quisqualate and inhibited by 5-HT via inhibitory postsynaptic 5-HT_{1A} receptors (upper, right).

Neuronal responsiveness of the CA3 pyramidal neurons was determined by 50 s microiontophoretic applications of 5-HT and NE, and measured by the number of spikes suppressed within the 50 s ejection period divided by the current used (spikes suppressed/nA). To determine the relative degree to which the 5-HT or NE transporters were blocked, the 50% recovery time (RT_{50}) was measured by determining the time (in s) following a 50 s ejection period of 5-HT or NE to recover 50% of the initial firing rate (de Montigny et al., 1980).

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

Following the long-term regimen, in order to assess the degree of activation of the postsynaptic 5-HT_{1A} receptors exerting an inhibitory effect on the firing activity of CA_3 pyramidal neurons, WAY 100635 was administered (i.v.) to disinhibit the hippocampal neurons resulting in enhanced firing activity. It is most desirable to determine the disinhibition when the neurons are not firing at a high rate, therefore their firing rate was

reduced to about 10-20 Hz by decreasing the ejection current of quisqualate and the selective 5-HT_{1A} receptor antagonist WAY 100635 (25-100 μg/kg) was then injected intravenously (i.v.; Haddjeri et al., 1998) in incremental doses of 25 μg/kg at time intervals of 2 minutes. In order to avoid residual drug effects, only one cell is studied per rat. Any changes in the firing activity of hippocampus pyramidal neurons reflect an increased level in the tonic activation of the postsynaptic 5-HT_{1A} receptors. It is noteworthy to mention that WAY 100635 administered i.v., does not alter the firing rate of 5-HT neurons in the DRN of anesthetized rats (Lejeune and Millan, 1998; Haddjeri et al., 2004).

4.2. Tonic activation of postsynaptic α-adrenergic receptors

The degree of tonic activation of postsynaptic α_2- and α_1-adrenoceptors was assessed using the selective antagonists idazoxan and prazosin, respectively. After reducing and maintaining a steady firing baseline, idazoxan (1 mg/kg) and prazosin (100 μg/kg) were systematically administered (i.v.) to determine the disinhibition effects in control rats (vehicle-treated) and in rats treated with CRS or LTG for 14 days. In a recent study, bupropion was shown to increase the tonic activation of postsynaptic α_2- and α_1-adrenoceptors by endogenous NE (Ghanbari et al., 2011).

5. Sensitivity of terminal 5-HT_{1B} autoreceptors

The CA3 region of the hippocampus receives extensive projections from the median and DRN 5-HT neurons. The ascending 5-HT pathway afferent to the hippocampus was electrically stimulated by a bipolar electrode (NE-100, David Kopf, Tujunga, CA, USA) which 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 cortical surface of the brain. 200 square pulses with duration of 0.5 ms were delivered by a stimulator (S48, Grass Instruments, West Warwick, RI, USA) at an intensity of 300 μA and at frequencies of 1 Hz and 5 Hz. Stimulation of the 5-HT pathway induces a brief period of suppression due to the release of 5-HT in the synapse, exerting an inhibitory effect on the CA3 hippocampal pyramidal neurons, which corresponds to the duration of suppression (DOS) value. Peristimulus time histograms of CA3 pyramidal neurons were generated to determine the suppression of firing measured as the DOS (in ms) which is defined as the time interval initiated by a 50% decrease in the number of spikes per bin from the mean prestimulation probability of firing, to the time it returned to 90% of that same prestimulation value (Chaput et al., 1986). Stimulations of 1 Hz and 5 Hz on the same neuron, were used to determine the function of terminal 5-HT_{1B} autoreceptors (Chaput et al., 1986). These two series of stimulations were used because previous studies showed that activation of terminal 5-HT_{1B} autoreceptors decreases the 5-HT release in the terminal areas and that increasing the frequency of stimulation from 1 to 5 Hz induces a greater degree of activation of the autoreceptors, thus resulting in a greater negative feedback on the release of 5-HT (Chaput et al., 1986). As a result, the smaller release of neurotransmitter in the synapse obtained at 5 Hz, induces a smaller DOS value compared to that of the 1 Hz stimulation. If the autoreceptor is desensitized, the difference between the effectiveness of the two rates of stimulation will be significantly reduced.

The stimulation pulses and the firing activity were analyzed by computer using Spike 2 (Cambridge Electronic Design Limited, UK).

6. Statistical Analysis

All results are reported as mean values ± SEM. Statistical comparisons were carried out using the two-tailed Student's *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 $p < 0.05$.

The firing patterns of the monoaminergic neurons were analyzed by interspike interval (ISI) burst analysis. The onset of a burst was signified by the occurrence of two spikes with ISI < 0.08 seconds for NE and DA, and ISI < 0.01 s for 5-HT. The termination of a burst was defined as an ISI > 0.16 seconds for NE and DA, and ISI > 0.01 s for 5-HT.

7. Drugs

Carisbamate was provided by Ortho-McNeil Janssen Scientific Affairs, LLC (Titusville, NJ, USA) and dissolved in 40% propylene glycol: 30% ethanol. Lamotrigine was purchased from LKT Laboratories, Inc (St. Paul, MN, USA) and dissolved in 35% propylene glycol: 7% ethanol. WAY 100635 was purchased from Sigma (St. Louis, MO, USA) and dissolved in distilled water.

RESULTS

1. Carisbamate

1.1. Effects of 2- and 14-day administration of carisbamate on the firing activity of DRN 5-HT, LC NE and VTA DA neurons

In comparison to the vehicle group, the 2-day administration of CRS resulted in a significant reduction of the DRN firing rate by 28% ($p < 0.001$; Figure 7 A). This may be attributed to either a significant decrease in the number of bursting vs. non-bursting neurons by 14% ($p < 0.05$), or to a significant decrease in the burst rate by 47% ($p < 0.05$; Table 1). The significant reduction in firing activity persisted after the 14-day CRS administration (Figure 7 B) to a similar degree of 24% ($p < 0.01$), but with no changes in the burst activity (Table 1).

The 2-day administration of CRS yielded no changes in the firing activity (Figure 7 C) or in the burst activity of the LC NE neurons (Table 1). The 14-day CRS administration resulted in a significant reduction in the LC firing rate by 34% ($p < 0.001$; (Figure 7 D). This may be due to a significant decrease in the number of bursting vs. non-bursting neurons by 19% ($p < 0.01$; Table 1).

The 2-day administration of CRS yielded no changes in the firing activity (Figure 7 E) or in the burst activity of the VTA DA neurons. The 14-day carisbamate administration significantly decreased the firing activity of VTA DA neurons by 26% ($p < 0.001$; Figure 7 F). This may be attributed to either a significant reduction in the burst rate by 27% ($p < 0.05$), or to a significant decrease in the mean spikes per burst by 15% ($p < 0.05$; Table 1).

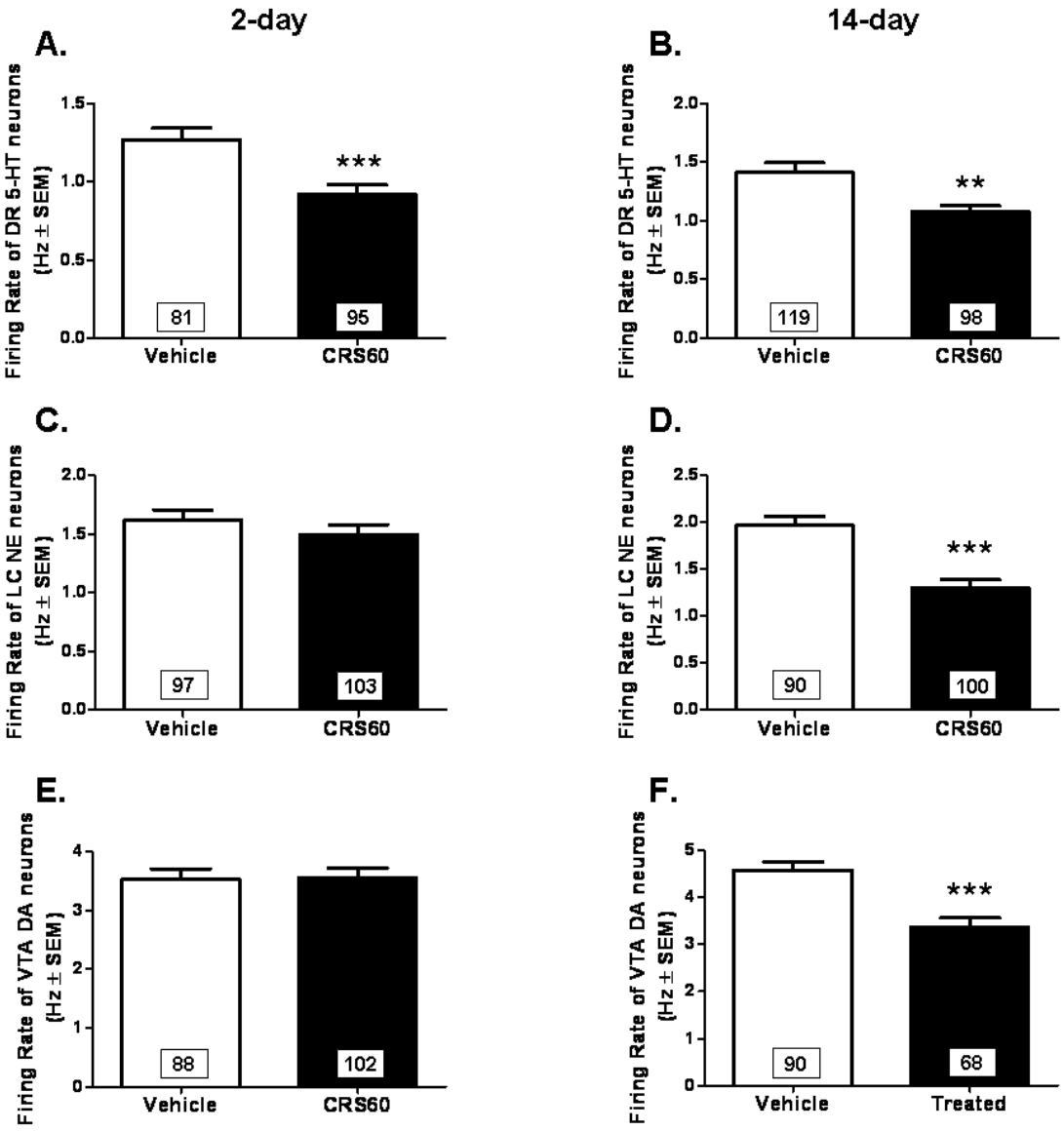

Figure 7. Effects of 2- and 14-day CRS administration on monoaminergic firing activity. Mean (± SEM) of the firing rate of DRN 5-HT (A and B), LC NE (C and D), VTA DA (E and F) neurons in vehicle rats and rats treated with CRS (60 mg/kg/day; s.c.) for 2 days (A, C and E) and 14 days (B, D and F). The numbers in the boxes correspond to the number of neurons recorded (4 to 8 rats tested per group). ***$p < 0.001$ in (A, D and F) and **$p < 0.01$ in (B) using unpaired Student's *t*-test.

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

			Firing activity (Hz ± SEM)		**bursting vs. non-bursting neurons (%)**		**Burst rate (bursts/minute ± SEM)**		**Mean # spikes/burst ± SEM**	
Drug	Area	TX	***Veh***	***TX***	***Veh***	***TX***	***Veh***	***TX***	***Veh***	***TX***
CRS	**DRN 5-HT**	2-day	1.3 ± 0.07	0.9 ± 0.06 ***	37	23 *	15.8 ± 2.52	8.4 ± 1.74 *	2.1 ± 0.06	2.1 ± 0.05
		14-day	1.4 ± 0.08	1.1 ± 0.05 **	28	28	13.4 ± 2.92	12.1 ± 2.86	2.1 ± 0.04	2.1 ± 0.07
	LC NE	2-day	1.6 ± 0.09	1.5 ± 0.08	21	28	3.0 ± 0.62	2.6 ± 1.19	2.1 ± 0.05	2.0 ± 0.03
		14-day	2.0 ± 0.09	1.3 ± 0.09 ***	40	21 **	2.3 ± 0.35	3.2 ± 0.69	2.1 ± 0.06	2.1 ± 0.05
	VTA DA	2-day	3.5 ± 0.18	3.6 ± 0.16	87	94	18.3 ± 2.29	18.5 ± 1.83	2.7 ± 0.13	2.9 ± 0.15
		14-day	4.6 ± 0.17	3.4 ± 0.19 ***	99	96	30.9 ± 2.35	22.7 ± 2.41 *	3.3 ± 0.16	2.8 ± 0.12 *
LTG	**DRN 5-HT**	2-day	1.1 ± 0.06	0.9 ± 0.05 **	34	29	11.7 ± 1.44	11.6 ± 1.91	2.1 ± 0.05	2.0 ± 0.04
		14-day	1.0 ± 0.06	0.8 ± 0.05 **	39	23	15.7 ± 3.19	11.5 ± 2.74	2.0 ± 0.03	2.1 ± 0.05

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

1.2. Assessment of the overall serotonergic tone following 14-day carisbamate administration on postsynaptic 5-HT$_{1A}$ receptors on dorsal hippocampus CA3 pyramidal neurons

As illustrated in Figure 8 A, cumulative systemic injections of WAY 100,635 does not significantly alter the firing activity of dorsal hippocampus CA3 pyramidal neurons in the vehicle group. The 14-day CRS administered group however, displayed enhanced tonic activation of the postsynaptic 5-HT$_{1A}$ receptors by extracellular 5-HT in the hippocampus, as shown by a robust disinhibition of the neuronal firing rate (Figure 8 B) in response to WAY 100635 at 50 μg/kg (321% of baseline), 75 μg/kg (391%) and 100 μg/kg (396%) cumulative doses ($p < 0.001$ for the three doses; Figure 8 C). Indeed, the last injection of WAY 100635 enhanced the pyramidal neuron firing activity by approximately 3.8-fold compared with vehicle rats, indicating that there is enhanced serotonergic transmission in the postsynaptic region. Microiontophoretic application of exogenous 5-HT resulted in no changes in the sensitivity of the postsynaptic 5-HT$_{1A}$ receptors as shown by a lack of significant difference in the number of spikes suppressed per nanoampere (Figure 9 A). There was no change in the RT$_{50}$, indicating a lack of effect at the 5-HT transporters (Figure 9 B).

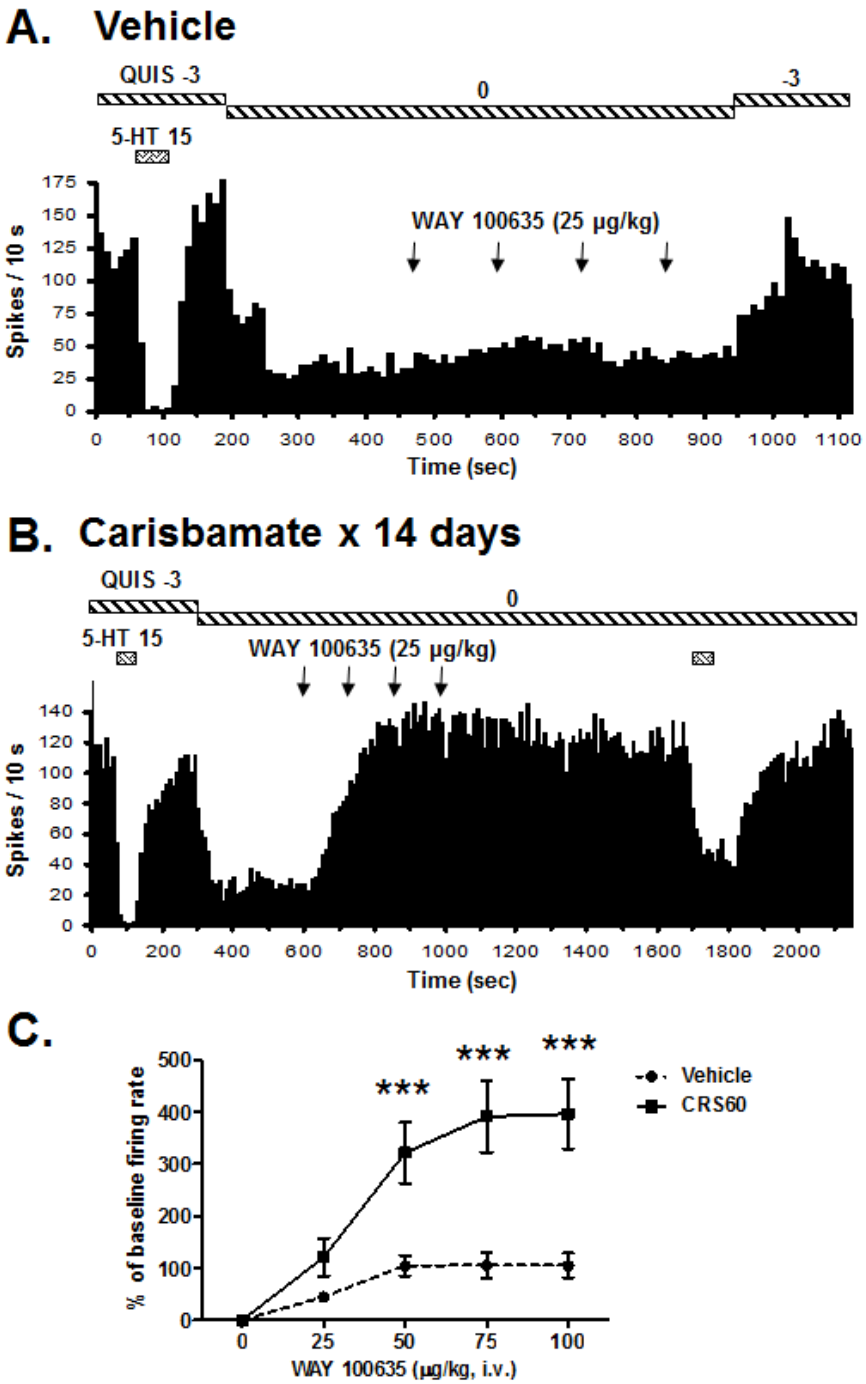

Figure 8. Effect of long-term CRS administration on tonic activation of 5-HT$_{1A}$ receptors. Integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons showing the effects of systemic administration of 4 incremental doses (shown by arrows) of WAY 100635 (25 µg/kg) in vehicle (A) and CRS (60 mg/kg/day; B) treated rats. Each bar corresponds to the application of quisqualate or 5-HT, and the number above each bar represents the ejection current in nA. The overall changes in the % of baseline firing rate of dorsal hippocampus CA3 pyramidal neurons in vehicle rats and rats treated with CRS for 14 days (C). (6 to 7 rats tested per group). ***$p < 0.001$ at 50, 75 and 100 µg/kg cumulative doses using Two-way Repeated Measures ANOVA.

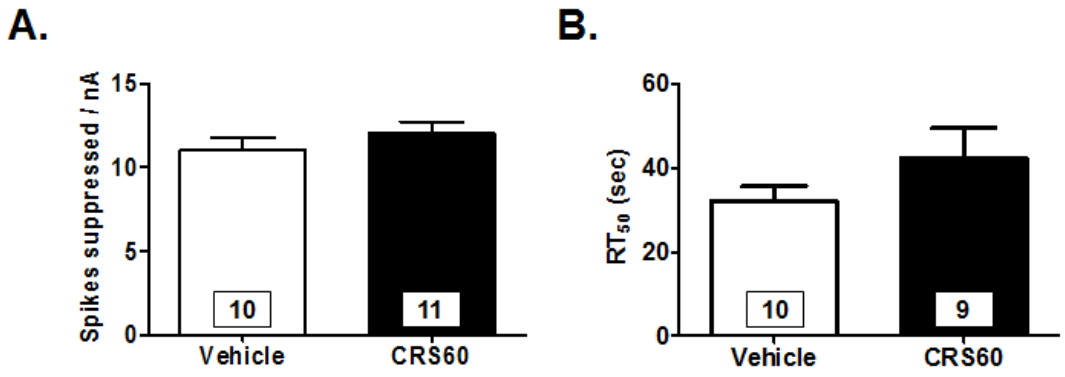

Figure 9. Lack of effect of long-term CRS administration on sensitivity of postsynaptic 5-HT$_{1A}$ receptors and the 5-HT reuptake transporters. Mean (± SEM) number of spikes suppressed/nA (A) and the time to a 50% recovery of firing (RT_{50}) (B) of dorsal hippocampus CA3 pyramidal neurons in response to microiontophoretic application of 5-HT in vehicle rats and rats treated with CRS (60 mg/kg/day; s.c.) for 14 days. The numbers in the boxes correspond to the number of neurons recorded (6 to 7 rats tested per group).

1.3. Assessment of the overall noradrenergic tone following 14-day carisbamate administration on postsynaptic α-adrenergic receptors on dorsal hippocampus CA3 pyramidal neurons

The long-term administration of CRS resulted in no changes in the tonic activation of the α_2- and α_1-adrenergic receptors as shown by a lack of significant disinhibition of the neuronal firing rate in response to the selective antagonists idazoxan and prazosin, respectively. (Figure 10). Microiontophoretic application of exogenous NE resulted in no changes in the sensitivity of the postsynaptic α-adrenergic receptors as shown by a lack of significant difference in the number of spikes suppressed per nanoampere (Figure 11 A). There was also no change in the RT_{50}, indicating a lack of effect at the NE transporters (Figure 11 B).

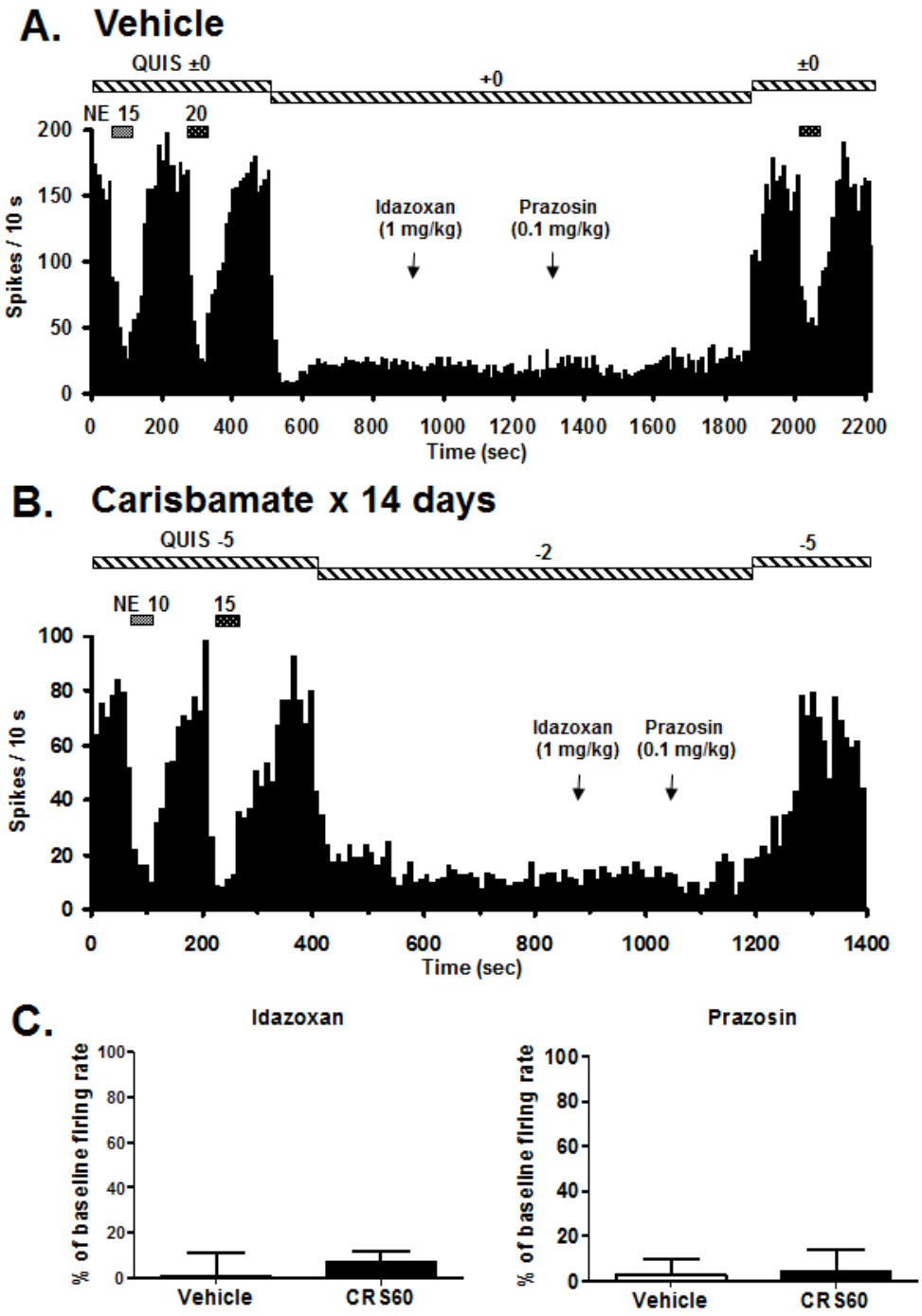

Figure 10. Lack of effect of long-term CRS administration on tonic activation of α-adrenergic receptors. Integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons showing the effects of systemic administration (shown by arrows) of idazoxan (1 mg/kg) and prazosin (0.1 mg/kg) in vehicle (A) and CRS (60 mg/kg/day; B) treated rats. Each bar corresponds to the application of quisqualate or NE, and the number above each bar represents the ejection current in nA. The overall changes in the % of baseline firing rate of dorsal hippocampus CA3 pyramidal neurons in vehicle rats and rats treated with CRS for 14 days (C). (6 to 7 rats tested per group).

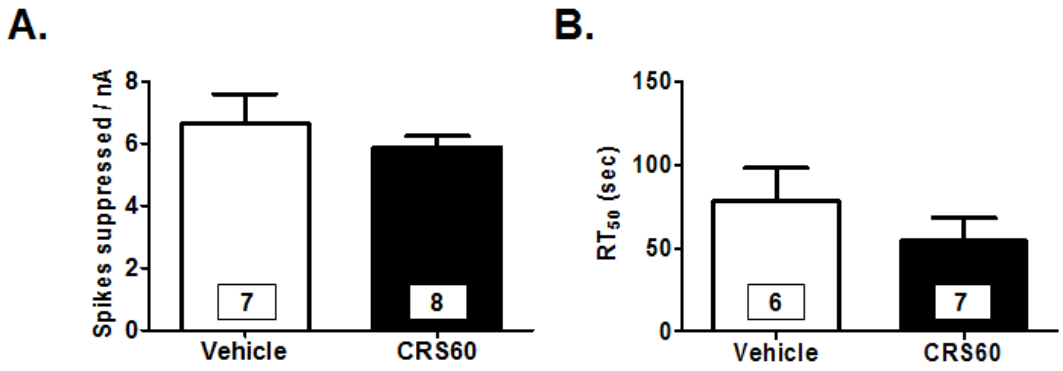

Figure 11. Lack of effect of long-term CRS administration on sensitivity of postsynaptic α_2-adrenergic receptors and the NE reuptake transporters. Mean (± SEM) number of spikes suppressed/nA (A) and the time to a 50% recovery of firing (RT_{50}) (B) of dorsal hippocampus CA3 pyramidal neurons in response to microiontophoretic application of NE in vehicle rats and rats treated with CRS (60 mg/kg/day; s.c.) for 14 days. The numbers in the boxes correspond to the number of neurons recorded (6 to 7 rats tested per group).

1.4. Assessment of the effects of 14-day carisbamate administration on the function of terminal 5-HT_{1B} autoreceptors

The 5-HT fibers afferent to the hippocampus were electrically stimulated to determine the amount of 5-HT released per impulse. 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. This resulted in a significantly reduced duration of suppression (DOS) in the vehicle group by 20% ($p < 0.001$; Figures 12 A, B, E) due to a greater degree of activation of the autoreceptors, thus resulting in a greater negative feedback on the release of 5-HT at 5 Hz (Chaput et al., 1986). Conversely, the rats treated with CRS reduced the difference between the effectiveness of the two stimulation frequencies, indicating that the autoinhibitory effect of the terminal 5-HT_{1B} receptor was dampened (Figures 12 C, D, E).

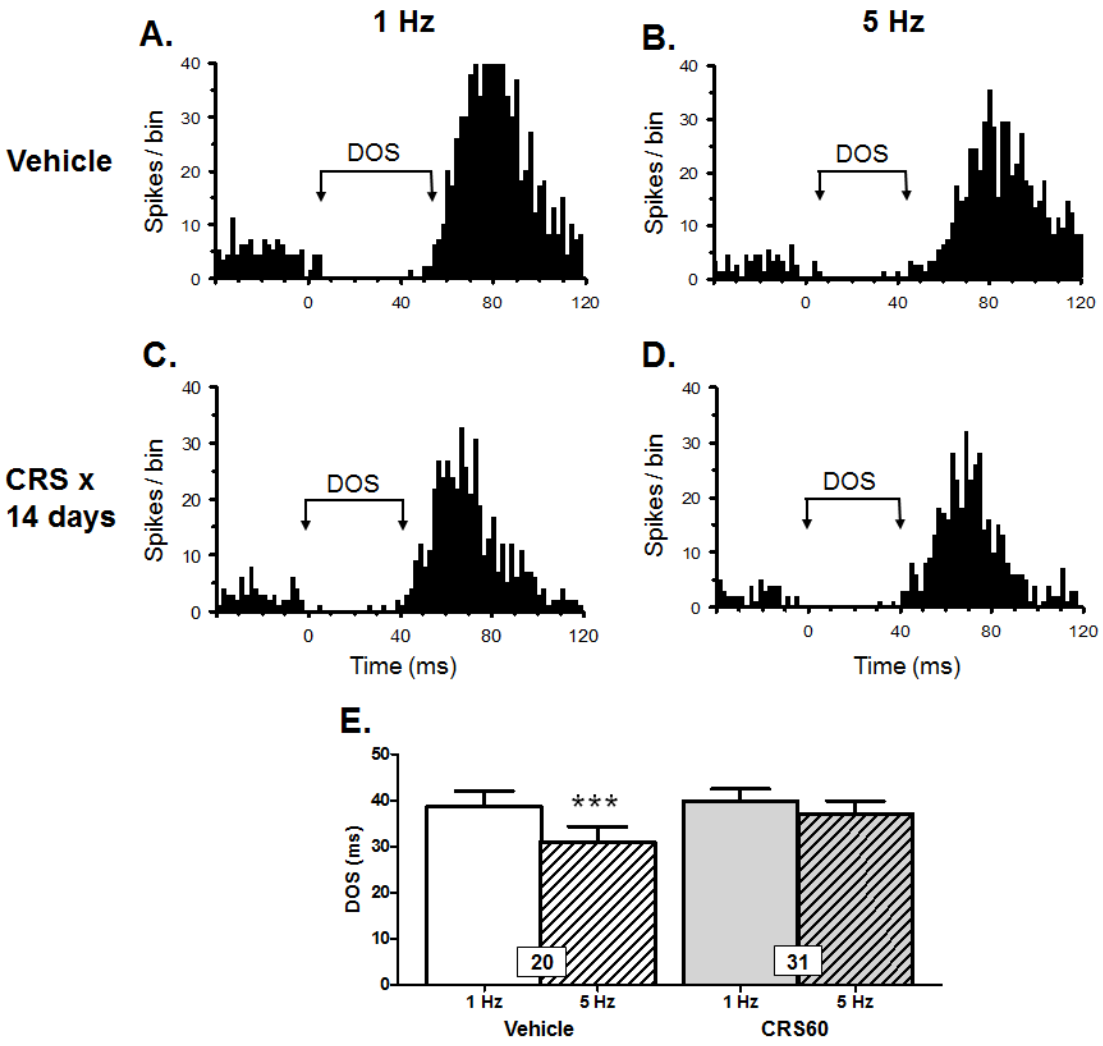

Figure 12. Effect of long-term CRS administration on sensitivity of terminal 5-HT$_{1B}$ autoreceptors. Peristimulus time histograms showing the effects of stimulation of the ascending 5-HT pathway on the firing activity of CA3 pyramidal neurons at frequencies of 1 and 5 Hz (vehicle: A and B, CRS: C and D, respectively). The overall effect of the stimulations at both frequencies on vehicle and CRS 14-day treated rats (E). The numbers in the boxes correspond to the number of neurons recorded (11 to 14 rats tested per group). ***$p < 0.001$ (E) using paired Student's *t*-test.

2. Lamotrigine

2.1. Effects of 2- and 14-day administration of lamotrigine on the firing activity of DRN 5-HT neurons

In comparison to the vehicle group, the 2-day administration of LTG resulted in a significant decrease in the DRN firing rate by 24% ($p < 0.01$; Figure 13 A), but with no changes in the burst activity (Table 1). Similar to CRS, this significant reduction in the firing rate also persisted after the 14-day LTG administration (Figure 13 B) to a similar degree of 22% ($p < 0.01$), which was accompanied with a significant decrease in the number of bursting vs. non-bursting neurons by 16% ($p < 0.05$; Table 1).

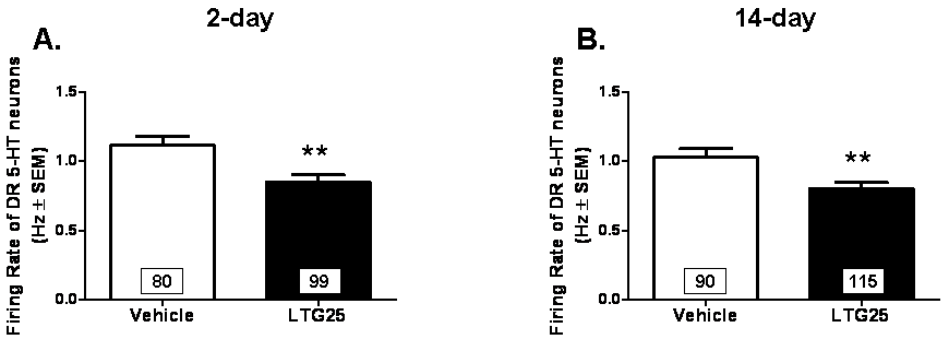

Figure 13. Effects of 2- and 14-day LTG administration on serotonergic firing activity. Mean (± SEM) of the firing rate of DRN 5-HT neurons in vehicle rats and rats treated with LTG (25 mg/kg/day; i.p.) for 2 days (A) and 14 days (B). The numbers in the boxes correspond to the number of neurons recorded (4 to 5 rats tested per group). ***p* < 0.01 in (A and B) using unpaired Student's *t*-test.

2.2. Assessment of the overall serotonergic tone following 14-day lamotrigine administration on postsynaptic 5-HT$_{1A}$ receptors on dorsal hippocampus CA3 pyramidal neurons

As illustrated in Figure 14 A, cumulative systemic injections of WAY 100635 did not significantly change the firing activity of dorsal hippocampus CA3 pyramidal neurons in the vehicle group. Similar to CRS, the 14-day LTG administered group displayed enhanced tonic activation of the postsynaptic 5-HT$_{1A}$ receptors as shown by a significant disinhibition of the neuronal firing rate (Figure 14 B) in response to WAY 100635 at 75 μg/kg (364% of baseline) and 100 μg/kg (395%) cumulative doses ($p < 0.001$ for both doses; Figure 14 C). Indeed, the last injection of WAY 100,635 enhanced the pyramidal neuron firing activity by approximately 3.1-fold compared with vehicle rats. This indicates that there is enhanced serotonergic transmission in this postsynaptic region. Microiontophoretic application of exogenous 5-HT resulted in no significant differences in the sensitivity of the postsynaptic 5-HT$_{1A}$ receptors as shown by a lack of change in the number of spikes suppressed per nanoampere (Figure 15 A). There was no change in the RT$_{50}$, indicating a lack of effect at the 5-HT transporters (Figure 15 B).

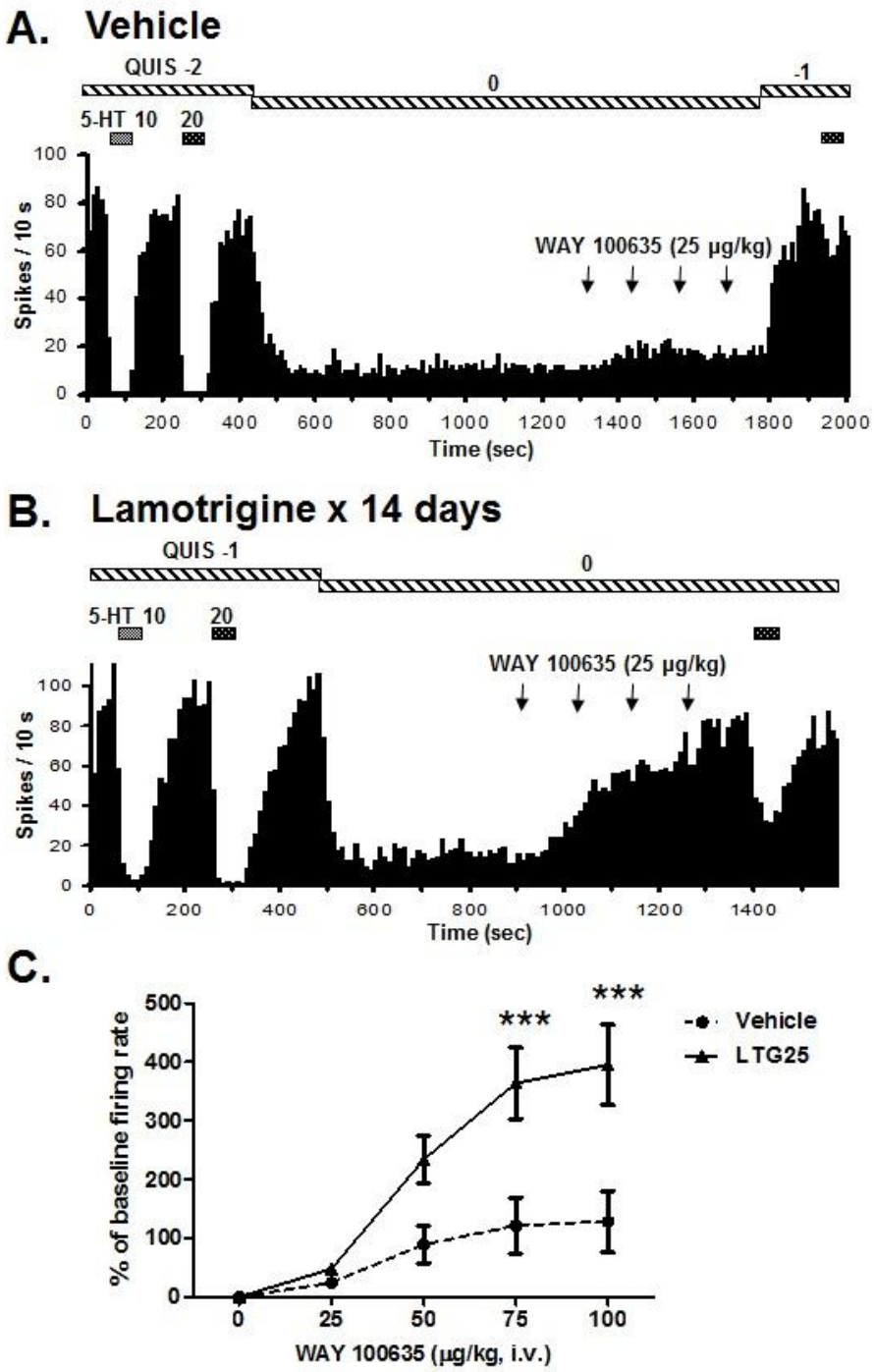

Figure 14. Effect of long-term LTG administration on tonic activation of 5-HT$_{1A}$ receptors. Integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons showing the effects of systemic administration of 4 incremental doses (shown by arrows) of WAY 100635 (25 µg/kg) in vehicle (A) and LTG (25 mg/kg/day; B) treated rats. Each bar corresponds to the application of quisqualate or 5-HT, and the number above each bar represents the ejection current in nA. The overall changes in the % of baseline firing rate of dorsal hippocampus CA3 pyramidal neurons in vehicle rats and rats treated with LTG for 14 days (C). (5 to 6 rats tested per group). ***$p < 0.001$ at 75 and 100 µg/kg cumulative doses using Two-way Repeated Measures ANOVA.

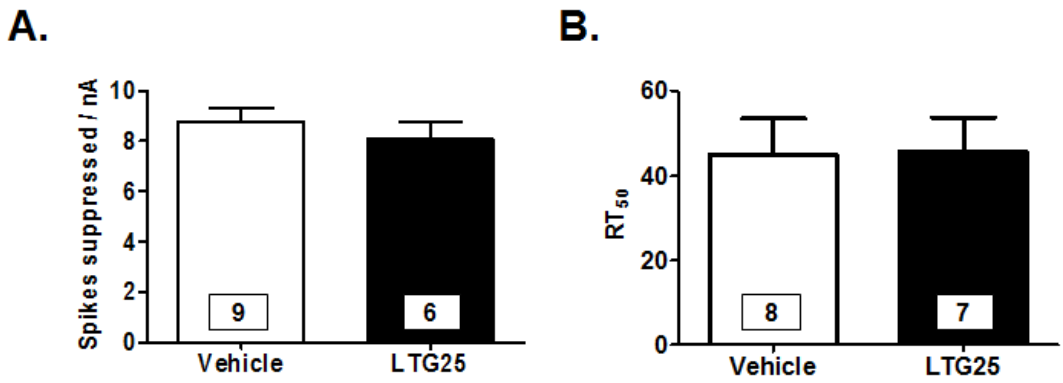

Figure 15. Lack of effect of long-term LTG administration on sensitivity of postsynaptic 5-HT_{1A} receptors and the 5-HT reuptake transporters. Mean (± SEM) number of spikes suppressed/nA (A) and the time to a 50% recovery of firing (RT_{50}) (B) of dorsal hippocampus CA3 pyramidal neurons in response to microiontophoretic application of 5-HT in vehicle rats and rats treated with LTG (25 mg/kg/day; i.p.) for 14 days. The numbers in the boxes correspond to the number of neurons recorded (5 to 6 rats tested per group).

2.3. Assessment of the effects of 14-day lamotrigine administration on the function of terminal 5-HT_{1B} autoreceptors

Increasing the frequency of the stimulation from 1 to 5 Hz resulted in a significantly reduced DOS in the vehicle group by 34% ($p < 0.001$; Figures 16 A, B, E) due to a greater degree of activation of the autoreceptors, thus resulting in less of 5-HT being released at 5 Hz (Chaput et al., 1986). Similar to CRS, the rats treated with LTG reduced the difference between the effectiveness of the two stimulation frequencies, indicating that the autoinhibitory effect of the terminal 5-HT_{1B} receptor was diminished (Figures 16 C, D, E). The DOS at a stimulation frequency of 1 Hz was significantly increased with LTG by 20% compared with the vehicle group suggesting that the spontaneous 5-HT firing activity is increased ($p < 0.05$; Fig. 16 E).

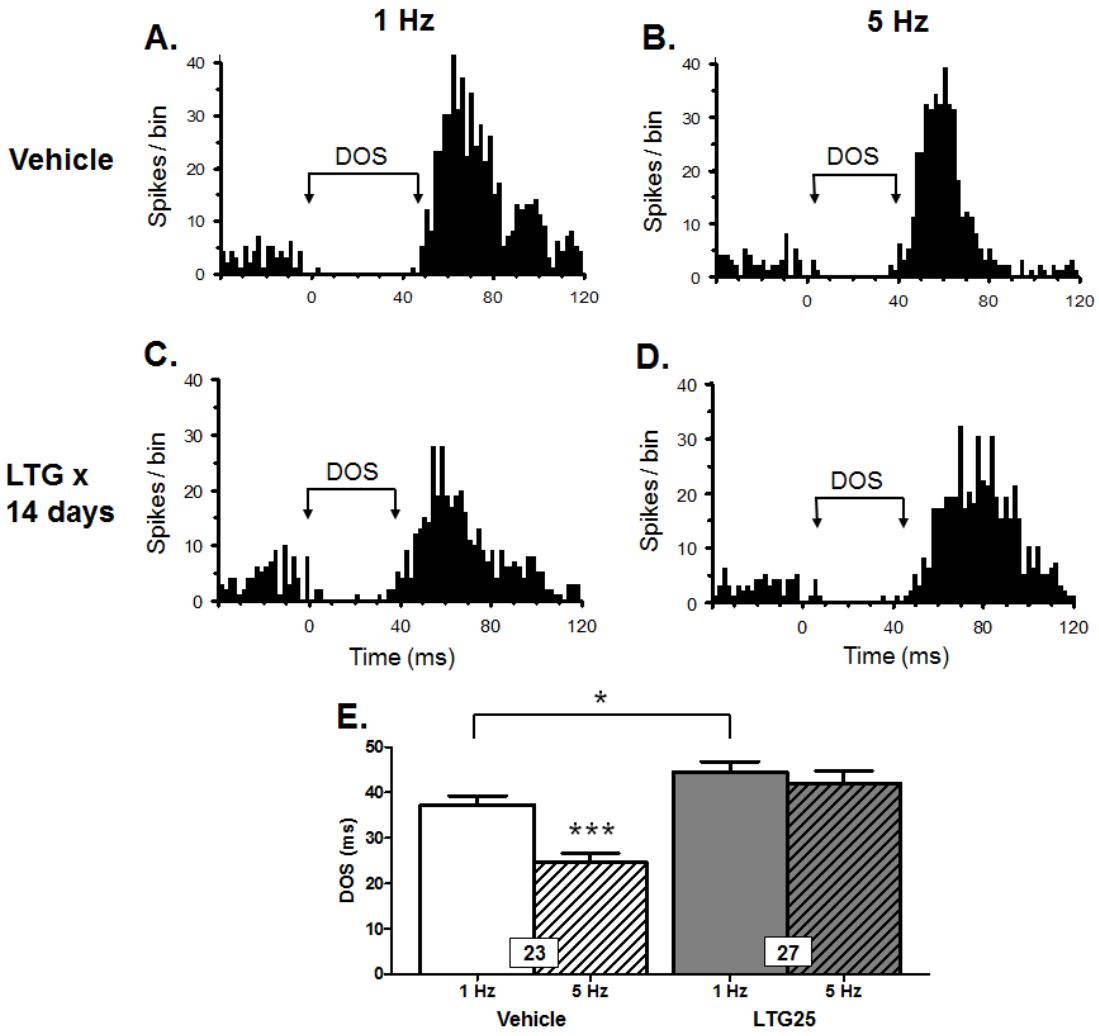

Figure 16. Effect of long-term LTG administration on sensitivity of terminal 5-HT$_{1B}$ autoreceptors. Peristimulus time histograms showing the effects of stimulation of the ascending 5-HT pathway on the firing activity of CA3 pyramidal neurons at frequencies of 1 and 5 Hz (vehicle: A and B, LTG: C and D, respectively). The overall effect of the stimulations at both frequencies on vehicle and LTG 14-day treated rats (E). The numbers in the boxes correspond to the number of neurons recorded. (7 to 8 rats tested per group). ***$p < 0.001$ (E) using paired Student's *t*-test and *$p < 0.05$ using unpaired Student's *t*-test.

DISCUSSION

The present study showed that despite a decrease of presynaptic 5-HT, NE and DA neuronal firing activity, both CRS and LTG enhanced the inhibitory action of 5-HT transmission on postsynaptic glutamate pyramidal neurons in the hippocampus, but with no changes in noradrenergic transmission (Figure 17). The postsynaptic enhancement of 5-HT neurotransmission may be due in part to desensitization of the terminal 5-HT_{1B} autoreceptors (Figure 17). This suggests that these ACs could increase 5-HT transmission and potentially mitigate neuronal hyperexcitability, hence exerting an AD-like effect.

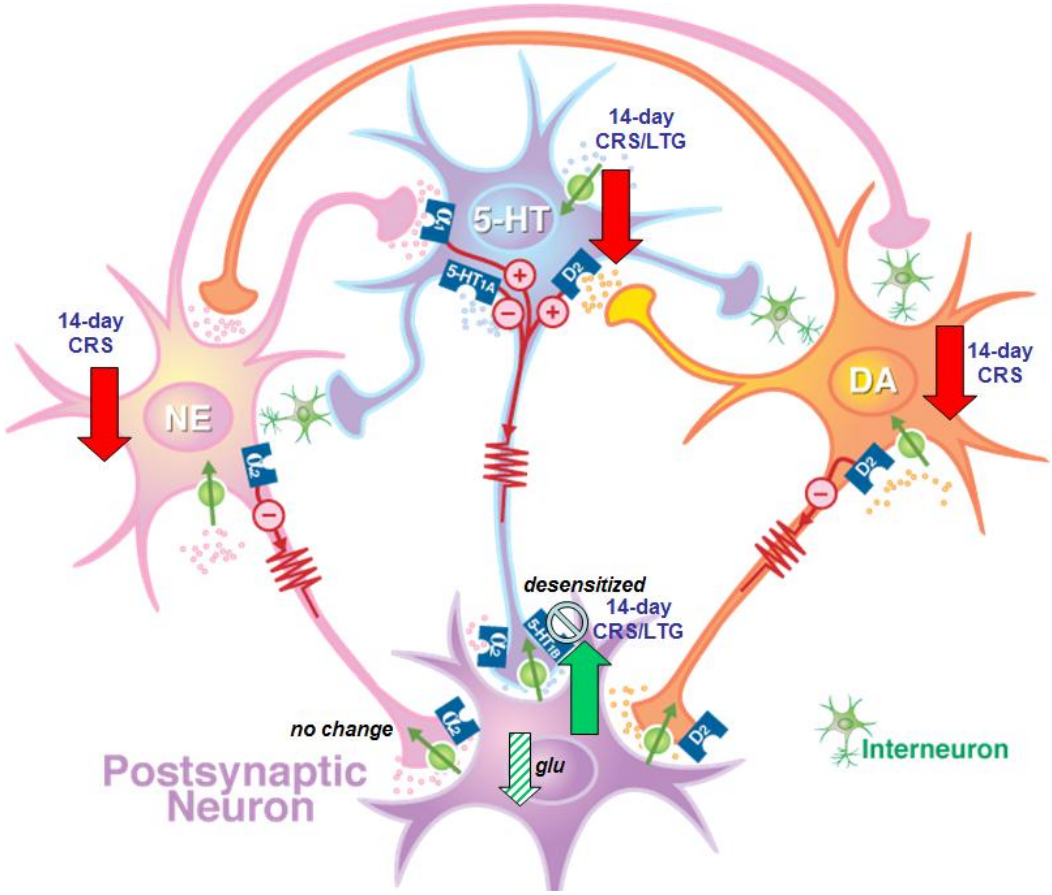

Figure 17. Summary of results. Decreases in firing activity were observed following the long-term treatment with CRS in DRN 5-HT, LC NE and VTA DA neurons (solid red arrows), with no changes in postsynaptic noradrenergic transmission but with enhanced serotonergic transmission in the hippocampus (solid green arrow). Similarly long-term LTG caused a decrease in DRN 5-HT activity, also with enhanced 5-HT activity postsynaptically. Increased 5-HT transmission may reduce glutamatergic activity of postsynaptic neurons (hatched arrow). Long-term treatment with both drugs caused a desensitization of the terminal 5-HT_{1B} autoreceptors.

Given the profuse excitatory innervations to the DRN, it is not surprising that a suppression in the firing activity of 5-HT neurons was observed with both short- and long-term administration of the sodium channel blockers CRS and LTG, as the putative reduction in glutamate transmission would result in reduced excitatory activation of these neurons (Figures 7 and 13; Celada et al., 2001). In support of this, a microdialysis study demonstrated that systemic i.p. injection of LTG at a dose similar to that used in our study, resulted in a decrease in extracellular 5-HT in the DRN (Tanahashi et al., 2012). Attenuated glutamatergic activity influencing monoaminergic neuronal firing activity is also reflected by the concurrent decreases in burst activity that were observed (Table 1), as burst firing of neurons is known to be mediated by the input of glutamatergic drive (Grace and Bunney, 1984). The presynaptic reduction in the NE firing rate following the 14-day CRS administration (Figure 7) is in agreement with other studies that show that the effect of ADs such as the SSRIs are associated with dampened noradrenergic activity following the long-term regimens (Szabo et al., 2000). Similarly, a decrease in dopaminergic firing activity in the VTA (Figure 7) is also seen with sustained 5-HT reuptake inhibition (Dremencov et al., 2009). The apparent delay in the reductions in NE and DA firing relative to 5-HT firing activity, the latter which was decreased as early as 2 days of the CRS administration may be due in part to an effect that is secondary to the postsynaptic enhancement of serotonergic transmission. Given the reciprocal interactions between the monoaminergic systems (Figure 1; Guiard et al., 2008a; 2008b), it is possible that the enhancement of 5-HT transmission acts on the inhibitory 5-HT_{2A} and 5-HT_{2C} receptors to reduce NE and DA firing rates, respectively. These decreases in firing activity of NE and DA lends support to an antimania

effect of CRS for BPD as catecholamine depletion has been shown to attenuate symptoms of mania (Bunney WE Jr et al., 1971).

The present findings did not demonstrate any postsynaptic changes in noradrenergic transmission (Figures 10 and 11), however there was a significant change in serotonergic transmission. The enhancement of tonic activation we observed, as demonstrated by a robust disinhibition of the firing activity in response to the selective 5-HT_{1A} receptor antagonist WAY 100635, following both long-term treatments with CRS and LTG (Figures 8 and 14) indicates that there is increased 5-HT transmission in the hippocampus. These results are similar to other ADs tested as well as the recent MDD intervention, vagus nerve stimulation previously used in epilepsy, that have also been shown to increase tonic activation (Haddjeri et al., 1998; Manta et al., 2009). This is in accordance with microdialysis studies which have shown that chronic treatment with lamotrigine for both 2 and 14 days, increases basal extracellular 5-HT in the hippocampus of freely moving rats by approximately 80% and 100%, respectively at a dose of 10 mg/kg/day (Ahmad et al., 2004). This may explain the 3.1-fold increase in tonic activation we observed following the 14-day LTG treatment which was given at a daily dose 2.5 times more potent than that used by Ahmad and others. Similarly, the AC carbamazepine which has also been shown to have some sodium channel blocking properties, also causes an increase in extracellular hippocampal 5-HT (Okada et al., 1998). Furthermore, an increase in the DOS following long-term LTG administration was observed at 1 Hz (Figure 16 E), indicating that more 5-HT is being released per electrical impulse reaching the terminal. A stimulation frequency of 1 Hz is thought to mimic spontaneous serotonergic firing activity of anesthetized rats, suggesting that more 5-HT is being spontaneously released with chronic LTG in these rats (Chaput et al., 1986). Evidence

that the enhanced 5-HT is related to a potential AD effect was observed in a study by Consoni and others (2006) that demonstrated that the AD-like effect of LTG in the modified FST was associated with an increase in swimming which is a serotonergic-related behaviour (Page et al., 1999; Slattery and Cryan, 2012). This serotonergic effect is dependent on its sodium channel blocking activity since the increase in swimming was inhibited by veratrine (Codagnone et al., 2007). This suggests that the altered serotonergic transmission in postsynaptic brain areas caused by LTG and CRS may be due to their sodium channel blocking properties and by inference, their control of glutamate. Therefore, the present results are in accordance with the hypothesis that antiglutamatergic drugs exert their AD effect partly through suppression of glutamatergic activity and an increase in 5-HT transmission (Consoni et al., 2006).

Supporting the involvement of reduced glutamatergic activity and enhanced 5-HT activity for a potential AD effect is paralleled by studies using NMDAR antagonists. The non-competitive NMDAR antagonist MK-801 was shown to have an AD-like effect in the FST (Trullas and Skolnick, 1990). It was reported that glutamate injected directly into the NAc caused an increase in immobility time through a reduction in swimming, which was reversed by local administration of MK-801, suggesting that glutamate is involved in behavioural depression, thus reinforcing the idea that reducing its transmission is implicated in an AD-like response (Rada et al., 2003). Furthermore, microdialysis studies demonstrated an increase in extracellular 5-HT in the NAc, hippocampus and the striatum in freely moving rats following administration of MK-801 (Yan et al., 1997; Whitton et al., 1992). This suggests that if the enhancement of serotonergic transmission we observed is in fact due to the antiglutamatergic effects of CRS and LTG, then we could expect to see enhanced

5-HT in the NAc and the striatum as well. Given that the NAc receives many excitatory glutamatergic innervations from the hippocampus it is not surprising that similar results were obtained for both of these regions (Cotman and Monaghan, 1986). Elevating 5-HT in these regions would have the potential to ameliorate motivation, learning and memory deficits that are often implicated within these areas in MDD.

Despite a presynaptic decrease in 5-HT neuronal firing activity, we still observed a postsynaptic increase in serotonergic transmission with both of the ACs tested. This contrariety is not uncommon as a similar result was obtained by Besson and others (2000), where a mirtazapine and paroxetine combination yielded a decrease in DRN 5-HT firing activity after a 2-day administration, but with significantly enhanced tonic activation in hippocampus CA3 pyramidal neurons. Furthermore, it was demonstrated by Gariboldi and others (1996) that activation of postsynaptic 5-HT_{1A} receptors by the agonist 8-OH-DPAT through both local intrahippocampal as well as systemic administration, inhibits the onset of limbic seizures induced by kainic acid in rats, which shows that even though it reduces presynaptic 5-HT firing activity it is still able to exert its antiglutamatergic effect through its action on postsynaptic 5-HT_{1A} receptors. In addition, *para*-chlorophenylalanine-induced 5-HT depletion (through the inhibition of tryptophan hydroxylase) or lesioning of 5-HT neurons with 5,7-DHT does not diminish the anti-immobility effect of 8-OH-DPAT in the FST, indicating that the postsynaptic area is more important for AD-like action (Luscombe et al., 1993). A possible explanation for the discrepancy may be due to two recurrent pathways with opposing effects (Figure 18; Hajós et al., 1999; Celada et al., 2001). One is an indirect negative feedback pathway where reduced glutamatergic activity, potentially through a blockade of sodium channels, would result in less activation of GABA exerting an

inhibitory effect on dorsal raphe 5-HT, allowing increased 5-HT transmission in postsynaptic regions and greater activation of the inhibitory 5-HT_{1A} receptors, which could explain the significant enhancement of tonic activation that we observed with CRS and LTG. The other is a direct pathway where dorsal raphe 5-HT neurons have direct glutamatergic innervations, thus reduced glutamatergic activity from both sodium channel blockade and from activation of postsynaptic 5-HT_{1A} receptors would result in reduced excitatory activation of serotonergic firing activity which could account for the persistent decreases we observed at both 2- and 14-day CRS and LTG administrations.

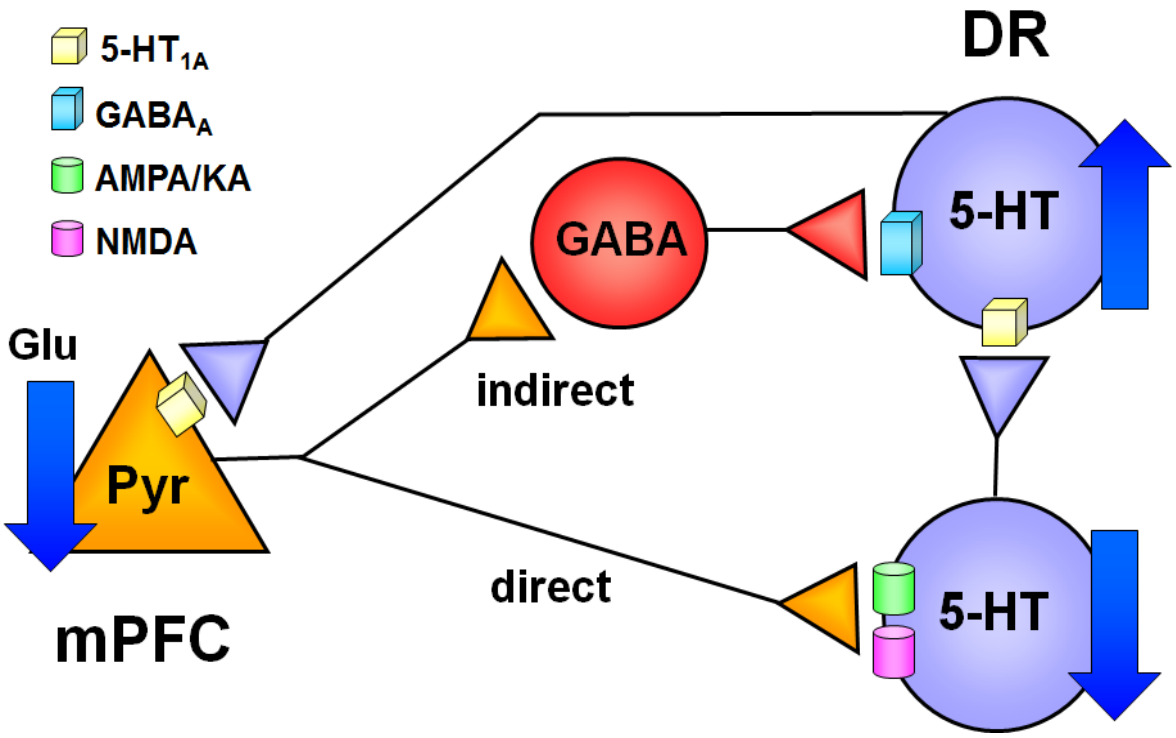

Figure 18. Schematic representation of the recurrent pathway between mPFC pyramidal and DR neurons. Reduction of glutamatergic transmission in forebrain regions has opposing effects depending on the direct or indirect pathway. Recurrent pathway from the DR to the mPFC further reduces glutamatergic activity via the 5-HT_{1A} receptors. Adapted from: Celada et al. (2001).

Electrical stimulation of the 5-HT pathway eliminates the effect of the receptors in the DRN, and increasing the frequency of the stimulation from 1 to 5 Hz, reduces the amount of 5-HT released per action potential due to activation of the terminal 5-HT_{1B} autoreceptors (Chaput et al., 1986). Long-term administration of SSRIs has been shown to result in desensitization of the terminal 5-HT_{1B} autoreceptors, thus reducing the difference between the effectiveness of the two stimulations and increasing the amount of 5-HT released (Chaput et al., 1991). Our study showed that the difference between the effectiveness of the 1 and 5 Hz stimulations was minimized following long-term administration with both CRS and LTG (Figures 12 E and 16 E), which indicates a desensitization of the terminal 5-HT_{1B} autoreceptors hence contributing to the observed enhancement of serotonergic tone. Desensitization would result in more 5-HT being released from the terminals to act on the inhibitory postsynaptic 5-HT_{1A} receptors, thus exerting an antiglutamatergic effect. Pindolol, which has been shown to act at presynaptic 5-HT_{1B} receptors in the FST, was demonstrated to enhance the anti-immobility effect of LTG, thus supporting the potential role of 5-HT_{1B} in an AD-like effect (Bourin et al., 1998; Bourin et al., 2005).

More 5-HT being released from the terminals could lead to the observed enhancement of tonic activation of the postsynaptic 5-HT_{1A} receptors, which supports the idea that the AD response of LTG and CRS is partly mediated by these receptors. As mentioned previously, the AD-like effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT is not affected by 5-HT depletion indicating its effect in the FST is on the postsynaptic receptors (Luscombe et al., 1993). A study by Bourin and others (2005) demonstrated that the anti-immobility effect of LTG was potentiated by 8-OH-DPAT which further highlights the

importance of activation of these receptors. Local activation of postsynaptic 5-HT_{1A} receptors by 8-OH-DPAT has also been implicated in reducing seizure activity in the hippocampus as well as improvement of seizure-induced depressive deficits in animal models (Gariboldi et al., 1996; Pineda et al., 2011). This suggests that the mechanism of action of LTG and CRS is linked to their AD-like effect through activation of the postsynaptic 5-HT_{1A} receptors, which would be beneficial for the treatment of both bi- and unipolar depression where postsynaptic reductions in 5-HT_{1A} receptor density have been observed (Drevets, 2000; Lopez-Figueroa et al., 2004). It is important to note that the net increase in 5-HT transmission may also be due to enhanced responsivity of the postsynaptic 5-HT_{1A} receptors, as tricyclic ADs and ECS have been shown to increase the sensitivity of these receptors to 5-HT (de Montigny and Aghajanian, 1978; de Montigny, 1984). However the present study did not show a change in 5-HT_{1A} receptor sensitivity following CRS and LTG administration to be a contributing factor (Figures 9 A and 15 A). Furthermore, there were no changes in the 5-HT reuptake process with either drug (Figures 9 B and 15 B), indicating that the 5-HT transporters are not involved in the observed enhancement of serotonergic tone.

The indirect attenuation of glutamatergic activity through activation of postsynaptic 5-HT_{1A} receptors via increased 5-HT transmission, in addition with the potential inhibition of glutamate release via sodium channel blockade, would have substantial antiglutamatergic activity. This reduction in glutamatergic activity caused by LTG and CRS would have considerable neuroprotective benefits, especially in pathophysiological states of neuronal hyperexcitability which can be excitotoxic, such as in depression and epilepsy. Indeed, Talarowska and others (2012) reported elevated levels of nitric oxide concentration in the

plasma of patients with recurrent depressive disorder which were shown to be associated with the severity of the symptoms, and also linked to impairments in working and declarative memory which are cognitive processes that rely on the frontal cortex, hippocampus and the amygdala. LTG has been shown to significantly inhibit both seizure- and veratrine-induced production of nitric oxide in rat brain forebrain regions (Bashkatova et al., 2003; Lizasoain et al., 1995). CRS has also displayed neuroprotective properties as it was shown to reduce epileptic-induced neuronal loss in the hippocampus, amygdala, and the thalamus at the same dose that we used in our study (François et al., 2011). This is in support of the hypothesis that states that the AD effect of antiglutamatergic drugs is due in part to a suppression of glutamatergic activity and subsequent decreases in nitric oxide levels (Figure 2; Consoni et al., 2006).

In addition to these effects, reduction of glutamatergic activity also has the benefit of enhancing cellular survival and plasticity by promoting neurotrophic signaling (Charney and Manji, 2004). Reductions in neurotrophic factors important for neuronal survival such as BDNF and TrkB have been observed in suicide victims and also in the hippocampus of rats exposed to restraint stress (Dwivedi, 2009; Smith et al., 1995). These effects were shown to be reversed with chronic AD treatments such as SSRIs, NRIs and repeated ECS (Nibuya et al., 1995). Indeed, BDNF infusion to the midbrain had significant AD-like effects in the FST (Siuciak et al., 1997). A study by Li and others (2011) demonstrated that chronic 14-day LTG administration at a similar dose to that used in our study, improved depressive-like deficits induced by chronic unpredictable stress in the sucrose preference test, novelty-suppressed feeding test and FST models of depression, which was dependent on elevated BDNF levels in the hippocampus and the frontal cortex. This may also be due in part to the

observed enhancement of serotonergic transmission caused by LTG since it was shown that SSRIs elevate BDNF in the hippocampus (Nibuya et al., 1995). This suggests that enhancing BDNF may be the final common step in the mechanism of action of ADs that gives ACs the advantage in potentially advancing the onset of action of ADs, since unlike ADs, directly reducing glutamate transmission does not require second messenger systems in order to act on BDNF and thus enhance cellular survival and plasticity.

Drugs which have AD activity have been shown to enhance tonic activation of the postsynaptic 5-HT_{1A} receptors, for example the SSRI paroxetine, the tricyclic AD imipramine, the MAOI befloxatone, repeated ECS as well as the non-conventional ADs bupropion, quetiapine, trazodone and vague nerve stimulation have all demonstrated this effect (Haddjeri et al., 1998; Ghanbari et al., 2011; Chernoloz et al., 2012; Ghanbari et al., 2010; Manta et al., 2009). This suggests that the postsynaptic enhancement of serotonergic transmission observed with CRS may be related to a potential AD effect in a clinical setting. It also supports LTG's AD effect in the clinic as it is more typically used for treatment of the depressed phase of BPD rather than mania or hypomania which tends to be precipitated by ADs that elevate 5-HT such as the tricyclic ADs, MAOIs and to a lesser extent the SSRIs (Geddes et al., 2009; Salvadore et al., 2010). Furthermore, enhancement of 5-HT transmission with SSRIs has beneficial AC effects in both patients and in animal models (Albano et al., 2006; Leander, 1992; Mazarati et al., 2008). It has been suggested that changes in serotonergic neurotransmission may provide a common link between epilepsy and depression, as augmenting AD treatments with ACs improves AD effects (Jobe et al., 1999; Barbosa et al., 2003; Normann et al., 2002). One way to determine if the therapeutic AD effect of LTG in patients with MDD is indeed due to increased serotonergic

transmission would be to see if tryptophan depletion causes a relapse in depressive symptoms.

In conclusion, the present study reinforces the hypothesis that the potential AD-like effect of CRS and LTG could be due in part to a serotonergic involvement and a direct attenuation of glutamate transmission. The potential of these drugs to rectify the impairments in cellular plasticity and cell survival through their putative antiglutamatergic effects, as well as their positive serotonergic effect in forebrain regions supports the use of CRS and LTG for use as augmentation strategy, or they may in fact represent a monotherapy for unipolar depression in the growing field of glutamate-based treatments.

REFERENCES

aan het Rot M, Collins KA, Murrough JW, Perez AM, Reich DL, Charney DS et al. (2010). Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. *Biological Psychiatry*. **67**: 139-145.

Aghajanian GK and Vandermaelen CP (1982a). Intracellular recordings from serotonergic dorsal raphe neurons: pacemaker potentials and the effect of LSD. *Brain Research.* **238**: 463-469.

Aghajanian GK and Vandermaelen CP (1982b). Alpha 2-adrenoceptor-mediated hyperpolarization of locus coeruleus neurons: intracellular studies in vivo. *Science.* **215**: 1394-1396.

Ahmad S, Fowler LJ, Whitton PS (2004). Effect of acute and chronic lamotrigine on basal and stimulated extracellular 5-hydroxytryptamine and dopamine in the hippocampus of the freely moving rat. *British Journal of Pharmacology*. **142**: 136-142.

Ahmad S, Fowler LJ, Whitton PS (2005). Lamotrigine, carbamazepine and phenytoin differentially alter extracellular levels of 5-hydroxytryptamine, dopamine and amino acids. *Epilepsy Research*. **63**: 141-149.

Albanese A, Altavista MC, Rossi P (1986). Organization of central nervous system dopaminergic pathways. *Journal of Neural Transmission*. **22**: Suppl 3-17.

Albano C, Cupello A, Mainardi P, Scarrone S, Favale E (2006). Successful treatment of epilepsy with serotonin reuptake inhibitors: proposed mechanism. *Neurochemical Research*. **31**: 509-514.

Altamura CA, Mauri MC, Ferrara A, Moro AR, D'Andrea G, Zamberlan F (1993). Plasma and platelet excitatory amino acids in psychiatric disorders. *The American Journal of Psychiatry*. **150**: 1731-1733.

Altshuler LL, Abulseoud OA, Foland-Ross L, Bartzokis G, Chang S, Mintz J et al. (2010). Amygdala astrocyte reduction in subjects with major depressive disorder but not bipolar disorder. *Bipolar Disorder*. **12**: 541-549.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington: American Psychiatric Association. 2000.

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

Baker KG, Halliday GM, Törk I (1990). Cytoarchitecture of the human dorsal raphe nucleus. *Journal of Comparative Neurology*. **301**: 147-161.

Barbosa L, Berk M, Vorster M (2003). A double-blind, randomized, placebo-controlled trial of augmentation with lamotrigine or placebo in patients concomitantly treated with fluoxetine for resistant major depressive episodes. *Journal of Clinical Psychiatry*. **64**: 403-407.

Bashkatova V, Narkevich V, Vitskova G, Vanin A (2003). The influence of anticonvulsant and antioxidant drugs on nitric oxide level and lipid peroxidation in the rat brain during penthylenetetrazole-induced epileptiform model seizures. *Progress in Neuro-Psychopharmacology &* Biological *Psychiatry*. **27**: 487-492.

Bel N and Artigas F (1992). Fluvoxamine preferentially increases extracellular 5-hydroxytryptamine in the raphe nuclei: an in vivo microdialysis study. *European Journal of Pharmacology*. **229**: 101-103.

Bel N and Artigas F (1993). Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not in raphe nuclei. *Synapse*. **15**: 243-345.

Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS et al. (2000). Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*. **47**: 351-354.

Berman RM, Sanacora G, Anand A, Roach LM, Fasula MK, Finkelstein CO et al. (2002). Monoamine depletion in unmedicated depressed subjects. *Biological Psychiatry*. **51**: 469-473.

Berridge CW and Waterhouse BD (2003). The locus coeruleus – noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews*. **42**: 33-84.

Berton O and Nestler EJ (2006). New approaches to antidepressant drug discovery: beyond monoamines. *Nature Reviews: Neuroscience.* **7**: 137-151.

Besson A, Haddjeri N, Blier P, de Montigny C (2000). Effects of the co-administration of mirtazapine and paroxetine on serotonergic neurotransmission in the rat brain. *European Neuropsychpharmacology*. **10**: 177-188.

Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS (2010). Progress report on new antiepileptic drugs: A summary of the Tenth Eilat Conference (EILAT X). *Epilepsy Research*. **92**: 89-124.

Blier P and de Montigny C (1994). Current advances and trends in the treatment of depression. *Trends in Pharmacological Sciences*. **15**: 220-226.

Bonanno G, Giambelli R, Raiteri L, Tiraboschi E, Zappettini S, Musazzi L et al. (2005). Chronic antidepressants reduce depolarization-evoked glutamate release and protein

interactions favoring formation of SNARE complex in hippocampus. *The Journal of Neuroscience*. **25**: 3270-3279.

Bourin M, Redrobe JP, Baker GB (1998). Pindolol does not act only on 5-HT_{1A} receptors in augmenting antidepressant activity in mouse forced swimming test. *Psychopharmacology*. **136**: 226-234.

Bourin M, Masse F, Hascoët M (2005). Evidence for the activity of lamotrigine at 5-HT_{1A} receptors in the mouse forced swimming test. *Journal of Psychiatry & Neuroscience*. **30**: 275-282.

Bourin M, Chenu F, Hascoët M (2009). The role of sodium channels in the mechanism of action of antidepressants and mood stabilizers. *Current Drug Targets*. **10**: 1052-1060.

Bowley MP, Drevets WC, Ongür D, Price JL (2002). Low glial numbers in the amygdala in major depressive disorder. *Biological Psychiatry*. **52**: 404-412.

Bunney WE Jr, Brodie HK, Murphy DL, Goodwin FK (1971). Studies of alpha-methyl-para-tyrosine, L-dopa, L-tryptophan in depression and mania. *The American Journal of Psychiatry*. **127**: 872-881.

Calabrese JR, Bowden CL, Sachs GS, Ascher JA, Monaghan E, Rudd GD (1999). A double-blind placebo-controlled study of lamotrigine monotherapy in outpatients with bipolar I depression. Lamictal 602 study group. *The Journal of Clinical Psychiatry.* **60**: 79-88.

Campbell S, Marriott M, Nahmias C, MacQueen GM (2004). Lower hippocampal volume in patients suffering from depression: a meta-analysis. *The American Journal of Psychiatry*. **161**: 598-607.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*. **301**: 386-9.

Celada P, Puig MV, Casanovas JM, Guillazo G, Artigas F (2001). Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: involvement of serotonin-1A, $GABA_A$, and glutamate receptors. *The Journal of Neuroscience*. **21**: 9917- 9929.

Chaput Y, Blier P, de Montigny C (1986). In vivo electrophysiological evidence for the regulatory role of autoreceptors on serotonergic terminals. *The Journal of Neuroscience*. **6**: 2796-2801.

Chaput Y, de Montigny C, Blier P (1991). Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. *Neuropsychopharmacology*. **5**: 219-229.

Charney DS and Manji HK (2004). Life stress, genes, and depression: multiple pathways lead to increased risk and new opportunities for intervention. *Science STKE*. **225**: re5.

Chernoloz O, El Mansari M, Blier P (2012). Effects of sustained administration of quetiapine alone and in combination with a serotonin reuptake inhibitor on norepinephrine and serotonin transmission. *Neuropsychopharmacology*. **37**: 1717-1728.

Codagnone FT, Consoni FT, Rodrigues AL, Vital MA, Andreatini R (2007). Veratrine blocks the lamotrigine-induced swimming increase and immobility decrease in the modified forced swimming test. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. **31**: 1307-1311.

Consoni FT, Vital MA, Andreatini R (2006). Dual monoamine modulation for the antidepressant-like effect of lamotrigine in the modified forced swimming test. *European Neuropsychopharmacology*. **16**: 451-458.

Cotman CW and Monaghan DT (1986). Anatomical organization of excitatory amino acid receptors and their properties. *Advances in Experimental Medicine and Biology*. **203**: 237-252.

Cramer JA, Blum D, Reed M, Fanning K; Epilepsy Impact Project Group (2003). The influence of comorbid depression on seizure severity. *Epilepsia*. **44**: 1578-1584.

Crawford LK, Craige CP, Beck SG (2010). Increased intrinsic excitability of lateral wing serotonin neurons of the dorsal raphe: a mechanism for selective activation in stress circuits. *Journal of Neurophysiology*. **103**: 2652-2663.

Dailey JW, Yan QS, Mishra PK, Burger RL, Jobe PC (1992). Effects of fluoxetine on convulsions and on brain serotonin as detected by microdialysis in genetically epilepsy-prone rats. *Journal of Pharmacology and Experimental Therapeutics*. **260**: 533-540.

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

de Montigny C, Wang RY, Reader TA, Aghajanian GK (1980). Monoaminergic denervation of the rat hippocampus: microiontophoretic studies on pre- and postsynaptic supersensitivity to norepinephrine and serotonin. *Brain Research*. **200**: 363–376.

de Montigny C (1984). Electroconvulsive treatments enhance responsiveness of forebrain neurons to serotonin. *Journal of Pharmacology and Experimental Therapeutics*. **228**: 230-234.

De Vivo M and Mayani S (1986). Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. *Journal of Pharmacology and Experimental Therapeutics*. **238**: 248-253.

Delgado PL, Price LH, Miller HL, Salomon RM, Aghajanian GK, Heninger GR et al. (1994). Serotonin and the neurobiology of depression: effects of tryptophan depletion in drug-free depressed patients. *Archives of General Psychiatry*. **51**: 865-874.

Deshpande LS, Nagarkatti N, Ziobro JM, Sombati S, DeLorenzo RJ (2008). Carisbamate prevents the development and expression of spontaneous recurrent epileptiform discharges and is neuroprotective in cultured hippocampal neurons. *Epilepsia*. **49**: 1795-1802.

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

Drevets WC, Frank E, Price JC, Kupfer DJ, Holt D, Greer PJ et al. (1999). PET imaging of serotonin 1A receptor binding in depression. *Biological Psychiatry*. **46**: 1375-1387.

Drevets WC (2000). Neuroimaging studies of mood disorders. *Biological Psychiatry*. **48**: 813-829.

Drevets WC, Bogers W, Raichle ME (2002). Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. *European Neuropsychopharmacology*. **12**: 527-544.

Drevets WC (2004). Neuroplasticity in mood disorders. *Dialogues in Clinical Neuroscience*. **6**: 199-216.

Dunlop BW and Nemeroff CB (2007). The role of dopamine in the pathophysiology of depression. *Archives of General Psychiatry*. **64**: 327-337.

Dwivedi Y (2009). Brain-derived neurotrophic factor: role in depression and suicide. *Neuropsychiatric Disease and Treatment*. **5**: 433-449.

Fava M (2003). Diagnosis and definition of treatment-resistant depression. *Biological Psychiatry*. **53**: 649-659.

Foote SL, Bloom FE, Aston-Jones G (1983). Nucleus locus ceruleus : new evidence of anatomical and physiological specificity. *Physiological Reviews*. **63**: 844-914.

François J, Germe K, Ferrandon A, Koning E, Nehlig A (2011). Carisbamate has powerful disease-modifying effects in the lithium-pilocarpine model of temporal lobe epilepsy. *Neuropharmacology*. **61**: 313-328.

Frank E, Prien RF, Jarrett RB, Keller MB, Kupfer DJ, Lavori PW et al. (1991). Conceptualization and rationale for consensus definitions of terms in major depressive disorder. Remission, recovery, relapse, and recurrence. *Archives of General Psychiatry*. 48: 851-855.

Fuller RW (1980). Pharmacology of central serotonin neurons. *Annual Review of Pharmacology and Toxicology*. **20**: 111-127.

Gariboldi M, Tutka P, Samanin R, Vezzani A (1996). Stimulation of 5-HT_{1A} receptors in the dorsal hippocampus and inhibition of limbic seizures induced by kainic acid in rats. *British Journal of Pharmacology*. **119**: 813-818.

Geddes JR, Calabrese JR, Goodwin GM (2009). Lamotrigine for treatment of bipolar depression : independent meta-analysis and meta-regression of individual patient data from five randomised trials. *The British Journal of Psychiatry*. **194**: 4-9.

Ghaemi SN, Boiman EE, Goodwin FK (2000). Diagnosing bipolar disorder and the effect of antidepressants: a naturalistic study. *Journal of Clinical Psychiatry*. **61**: 804-808.

Ghanbari R, El Mansari M, Blier P (2010). Sustained administration of trazodone enhances serotonergic neurotransmission: In vivo electrophysiological study in the rat brain. *The Journal of Pharmacology and Experimental Therapeutics*. **335**: 197-206.

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

Ghanbari R, El Mansari M, Blier P (2012). Electrophysiological impact of trazodone on the dopamine and norepinephrine systems in the rat brain. *European Neuropsychopharmacology*. **22**: 518-526.

Goldin AL (2001). Resurgence of sodium channel research. *Annual Review of Physiology.* **63**: 871-894.

Grace AA and Bunney BS (1983). Intracellular and extracellular electrophysiology of nigral dopaminergic neurons-- 1. Identification and characterization. *Neuroscience*. **10**: 301-315.

Grace AA and Bunney BS (1984). The control of firing pattern in nigral dopamine neurons: burst firing. *The Journal of Neuroscience*. **4**: 2877-2890.

Guiard BP, El Mansari M, Merali Z, Blier P (2008a). Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions. *International Journal of Neuropsychopharmacology*. **11**: 625-639.

Guiard BP, El Mansari M, Blier P (2008b). Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. *Molecular Pharmacology.* **74**: 1463-1475.

Haddjeri N, de Montigny C, Blier P (1997). Modulation of the firing activity of noradrenergic neurones in the rat locus coeruleus by the 5-hydroxytryptamine system. *British Journal of Pharmacology.* **120**: 865-875.

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

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

Hajós M, Hajós-Korcsok E, Sharp T (1999). Role of the medial prefrontal cortex in 5-HT_{1A} receptor-induced inhibition of 5-HT neuronal activity in the rat. *British Journal of Pharmacology*. **126**: 1741-1750.

Hamer D (2002). Genetics. Rethinking behavior genetics. *Science*. **298**: 71-72.

Hamilton M (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery & Psychiatry*. **23**: 56-62.

Hannon J and Hoyer D. (2008). Molecular biology of 5-HT receptors. *Behavioural Brain Research*. **195**: 198-213.

Hashimoto K, Sawa A, Iyo M (2007). Increased levels of glutamate in brains from patients with mood disorders. *Biological Psychiatry.* **62**: 1310-1316.

Heurteaux C, Lucas G, Guy N, El Yacoubi M, Thümmler S, Peng XD et al. (2006). Deletion of the background potassium channel TREK-1 results in a depression-resistant phenotype. *Nature Neuroscience.* **9**: 1134-1141.

Iverson LL. The uptake and storage of noradrenaline in sympathetic nerves. London, England: Cambridge University Press. 1991.

Ivković M, Damjanović A, Jovanović A, Cvetić T, Jasović-Gasić M (2009). Lamotrigine versus lithium augmentation of antidepressant therapy in treatment-resistant depression: efficacy and tolerability. *Psychiatria Danubina*. **21**: 187-193.

Jobe PC, Woods TW, Dailey JW (1983). Proconvulsant and anticonvulsant effects of tricyclic antidepressants in genetically epilepsy-prone rats. *Epilepsy International Symposium*. Abstract. 229.

Jobe PC, Dailey JW, Wernicke JF (1999). A noradrenergic and serotonergic hypothesis of linkage between epilepsy and affective disorders. *Critical Reviews in Neurobiology*. **13**: 317-356.

Judd LL, Akiskal HS, Schettler PJ, Endicott J, Maser J, Solomon DA et al. (2002). The long-term natural history of the weekly symptomatic status of bipolar I disorder. *Archives of General Psychiatry*. **59**: 530-537.

Kandel ER and Spencer WA (1961). Electrophysiology of hippocampal neurons. II. After-potentials and repetitive firing. *Journal of Neurophysiology*. **24**: 243-259.

Karg K, Burmeister M, Shedden K, Sen S (2011). The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited. *Archives of General Psychiatry*. **68**: 444-454.

Keller MB, Lavori PW, Coryell W, Andreasen NC, Endicott J, Clayton PJ et al. (1986). Differential outcome of pure manic, mixed/cycling, and pure depressive episodes in patients with bipolar illness. *The Journal of the American Medical Association*. **255**: 3138-3142.

Kendler KS, Karkowski LM, Prescott CA (1999). Causal relationship between stressful life events and the onset of major depression. *The American Journal of Psychiatry*. **156**: 837-841.

Ketter TA, Manji HK, Post RM (2003). Potential mechanisms of action of lamotrigine in the treatment of bipolar disorders. *Journal of Clinical Psychopharmacology*. **23**: 484-495.

Krishnan V and Nestler EJ (2008). The molecular neurobiology of depression. *Nature*. **455**: 894-902.

Kugaya A and Sanacora G (2005). Beyond monoamines: glutamatergic function in mood disorders. *CNS Spectrums*. **10**: 808-819.

Kuo CC and Lu L (1997). Characterization of lamotrigine inhibition of Na^+ channels in rat hippocampal neurones. *British Journal of Pharmacology*. **121**: 1231-1238.

Leach MJ, Marden CM, Miller AA (1986). Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: II. Neurochemical studies on the mechanism of action. *Epilepsia*. **27**: 490-497.

Leander JD. (1992). Fluoxetine, a selective serotonin-uptake inhibitor, enhances the anticonvulsant effects of phenytoin, carbamazepine, and ameltolide (LY201116). *Epilepsia*. **3**: 573-576.

Lee CY, Fu WM, Chen CC, Su MJ, Liou HH (2008). Lamotrigine inhibits postsynaptic AMPA receptor and glutamate release in the dentate gyrus. *Epilepsia*. **49**: 888-897.

Lee CY, Lee ML, Shih CC, Liou HH (2011). Carisbamate (RWJ-333369) inhibits glutamate transmission in the granule cell of the dentate gyrus. *Neuropharmacology*. **61**: 1239-1247.

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

Lenkey N, Karoly R, Kiss JP, Szasz BK, Vizi ES, Mike A (2006). The mechanism of activity-dependent sodium channel inhibition by the antidepressants fluoxetine and desipramine. *Molecular Pharmacology*. **70**: 2052-2063.

Li N, Lee B, Liu R-J, Banasr M, Dwyer JM, Iwata M et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*. **329**: 959-964.

Li N, He X, Zhang Y, Qi X, Li H, Zhu X et al. (2011). Brain-derived neurotrophic factor signalling mediates antidepressant effects of lamotrigine. *International Journal of Neuropsychopharmacology*. **14**: 1091-1098.

Liu Y, Qin N, Reitz T, Wang Y, Flores CM (2006). Inhibition of the rat brain sodium channel Nav1.2 after prolonged exposure to gabapentin. *Epilepsy Research*. **70**: 263-268.

Liu Y, Yohrling GJ, Wang Y, Hutchinson TL, Brenneman DE, Flores CM et al. (2009). Carisbamate, a novel neuromodulator, inhibits voltage-gated sodium channels and action potential firing of rat hippocampal neurons. *Epilepsy Research.* **83**: 66-72.

Lizasoain I, Knowles RG, Moncada S (1995). Inhibition by lamotrigine of generation of nitric oxide in rat forebrain slices. *Journal of Neurochemistry*. **64**: 636-642.

López JF, Chalmers DT, Little KY, Watson SJ (1998). A.E. Bennett Research Award. Regulation of serotonin$_{1A}$, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. *Biological Psychiatry*. **43**: 547-573.

López-Figueroa AL, Norton CS, López-Figueroa MO, Armellini-Dodel D, Burke S, Akil H et al. (2004). Serotonin 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2A}, receptor mRNA expression in

subjects with major depression, bipolar disorder, and schizophrenia. *Biological Psychiatry*. **55**: 225-233.

Luscombe GP, Martin KF, Hutchins LJ, Gosden J, Heal DJ (1993). Mediation of the antidepressant-like effect of 8-OH-DPAT in mice by postsynaptic 5-HT1A receptors. *British Journal of Pharmacology*. **108**: 669-677.

Maes M, Verkerk R, Vandoolaeghe E, Lin A, Scharpé S (1998). Serum levels of excitatory amino acids, serine, glycine, histidine, threonine, taurine, alanine and arginine in treatment-resistant depression: modulation by treatment with antidepressants and prediction of clinical responsivity. *Acta Psychiatrica Scandinavica*. **97**: 302-308.

Mahmood T and Silverstone T (2001). Serotonin and bipolar disorder. *Journal of Affective Disorders*. **66**: 1-11.

Mannoury la Cour C, El Mestikawy S, Hanoun N, Hamon M, Lanfumey L (2006). Regional differences in the coupling of 5-hydroxytryptamine-1A receptors to G proteins in the rat brain. *Molecular Pharmacology*. **70**: 1013-1021.

Manta S, Dong J, Debonnel G, Blier P (2009). Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *Journal of Psychiatry & Neuroscience*. **34**: 272-280.

Mathew SJ, Manji HK, Charney DS (2008). Novel drugs and therapeutic targets for severe mood disorders. *Neuropsychopharmacology*. **33**: 2080-2092.

Mauri MC, Ferrara A, Boscati L, Bravin S, Zamberlan F, Alecci M et al. (1998). Plasma and platelet amino acid concentrations in patients affected by major depression and under fluvoxamine treatment. *Neuropsychobiology*. **37**: 124-129.

Mayberg HS, Brannan SK, Tekell JL, Silva JA, Mahurin RK, McGinnis S et al. (2000). Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. *Biological Psychiatry*. **48**: 830-843.

Mazarati A, Siddarth P, Baldwin RA, Shin D, Caplan R, Sankar R (2008). Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine. *Brain*. **131**: 2071-2083.

McCarthy DJ, Alexander R, Smith MA, Pathak S, Kanes S, Lee CM et al. (2012). Glutamate-based depression (GBD). *Medical Hypotheses*. **78**: 675-681.

McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A (2003). The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. *Archives of General Psychiatry*. **60**: 497-502.

Michael-Titus AT, Bains S, Jeetle J, Whelpton R et al. (2000). Imipramine and phenelzine decrease glutamate overflow in the prefrontal cortex – a possible mechanism of neuroprotection in major depression? *Neuroscience*. **100**: 681-684.

Miguel-Hidalgo JJ, Waltzer R, Whittom AA, Austin MC, Rajkowska G, Stockmeier CA (2010). Glial and glutamatergic markers in depression, alcoholism, and their comorbidity. *Journal of Affective Disorders*. **127**: 230-240.

Miller HL, Delgado PL, Salomon RM, Licinio J, Barr LC, Charney DS (1992). Acute tryptophan depletion: a method of studying antidepressant action. *Journal of Clinical Psychiatry*. **53**: Suppl 28-35.

Miller HL, Delgado PL, Salomon RM, Berman R, Krystal JH, Heninger GR et al. (1996). Clinical and biochemical effects of catecholamine depletion on antidepressant-induced remission of depression. *Archives of General Psychiatry*. **53**: 117-128.

Mongeau R, Blier P, de Montigny C (1993). In vivo electrophysiological evidence for tonic activation by endogenous noradrenaline of alpha 2-adrenoceptors on 5-hydroxytryptamine terminals in the rat hippocampus. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 347: 266-272.

Mongeau R, de Montigny C, Blier P (1994). Electrophysiologic evidence for desensitization of alpha 2-adrenoceptors on serotonin terminals following long-term treatment with drugs increasing norepinephrine synaptic concentration. *Neuropsychopharmacology*. 10: 41-51.

Montgomery SA and Åsberg M (1979). A new depression scale designed to be sensitive to change. *British Journal of Psychiatry*. **134**: 382-389.

Nibuya M, Morinobu S, Duman RS (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *Journal of Neuroscience*. **15**: 7539-7547.

Nicholson GM, Blanche T, Mansfield K, Tran Y (2002). Differential blockade of neuronal voltage-gated Na^+ and K^+ channels by antidepressant drugs. *European Journal of Pharmacology*. **452**: 35-48.

Normann C, Hummel B, Schärer LO, Hörn M, Grunze H, Walden J (2002). Lamotrigine as adjunct to paroxetine in acute depression: a placebo-controlled, double-blind study. *Journal of Clinical Psychiatry*. **63**: 337-344.

Novak GP, Kelley M, Zannikos P, Klein B (2007). Carisbamate (RWJ-333369). *Neurotherapeutics*. **4**: 106-109.

Nowak G, Ordway GA, Paul IA (1995). Alterations in the *N*-methyl-D-aspartate (NMDA) receptor complex in the frontal cortex of suicide victims. *Brain Research*. **675**: 157-164.

Okada M, Hirano T, Mizuno K, Kawata Y, Wada K, Murakami T et al. (1998). Effects of carbamazepine on hippocampal serotonergic system. *Epilepsy Research*. **31**: 187-198.

Oleskevich S (1995). G alpha o1 decapeptide modulates the hippocampal 5-HT_{1A} potassium current. *Journal of Neurophysiology*. **74**: 2189-2193.

Ongür D, Drevets WC, Price JL (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *PNAS*. **95**: 13290-13295.

Ordway GA, Schenk J, Stockmeier CA, May W, Klimek V (2003). Elevated agonist binding to α_2-adrenoceptors in the locus coeruleus in major depression. *Biological Psychiatry*. **53**: 315-323.

Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I (1999). Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology*. **147**: 162-167.

Parikh SV, Lam RW, CANMAT Depression Work Group (2001). Canadian clinical practice guidelines for the treatment of depressive disorders. *Canadian Journal of Psychiatry*. **46**: Supp1.

Paul IA, Nowak G, Layer RT, Popik P, Skolnick P (1994). Adaptation of the N-methyl-D-aspartate receptor complex following chronic antidepressant treatments. *The Journal of Pharmacology and Experimental Therapeutics*. **269**: 95-102.

Pineda EA, Hensler JG, Sankar R, Shin D, Burke TF, Mazarati AM (2011). Plasticity of presynaptic and postsynaptic serotonin 1A receptors in an animal model of epilepsy – associated with depression. *Neuropsychopharmacology*. **36**: 1305-1316.

Piñeyro and Blier (1999). Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacological Reviews*. **51**: 533-591.

Prica C, Hascoet M, Bourin M (2008). Antidepressant-like effect of lamotrigine is reversed by veratrine: A possible role of sodium channels in bipolar depression. *Behavioural Brain Research*. **191**: 49-54.

Rada P, Moreno SA, Tucci S, Gonzalez LE, Harrison T, Chau DT et al. (2003). Glutamate release in the nucleus accumbens is involved in behavioral depression during the porsolt swim test. *Neuroscience*. **119**: 557-565.

Radley JJ, Williams B, Sawchenko PE (2008). Noradrenergic innervation of the dorsal medial prefrontal cortex modulates the hypothalamo-pituitary-adrenal responses to acute emotional stress. *The Journal of Neuroscience*. **28**: 5806-5816.

Ramamoorthy R, Radhakrishnan M, Borah M (2008). Antidepressant-like effects of serotonin type-3 antagonist, ondansetron: an investigation in behaviour-based rodent models. *Behavioural Pharmacology*. **191**: 29-40.

Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D et al. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *The American Journal of Psychiatry*. **163**: 1905-1917.

Rush AJ (2007). The varied clinical presentations of major depressive disorder. *Journal of Clinical Psychiatry*. **68** [suppl 8]: 4-10.

Salmenperä T, Kälviäinen R, Partanen K, Pitkänen A (2001). Hippocampal and amygdaloid damage in partial epilepsy: A cross-sectional MRI study of 241 patients. *Epilepsy Research*. **46**: 69-82.

Salomon RM, Miller HL, Krystal JH, Heninger GR, Charney DS (1997). Lack of behavioral effects of monoamine depletion in healthy subjects. *Biological Psychiatry*. **41**: 58-64.

Salvadore G, Quiroz JA, Machado-Vieira R, Henter ID, Manji HK, Zarate CA Jr (2010). The neurobiology of the switch process in bipolar disorder: a review. *Journal of Clinical Psychiatry*. **71**: 1488-1501.

Sanacora G, Zarate CA, Krystal JH, Manji HK (2008). Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nature Reviews Drug Discovery*. **75**: 426-437.

Sanacora G, Treccani G, Popoli M (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*. **62**: 63-77.

Sapolsky RM (2000). The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biological Psychiatry*. **48**: 755-765.

Schlaepfer TE, Frick C, Zobel A, Maier W, Heuser I, Bajbouj M et al. (2008). Vagus nerve stimulation for depression: efficacy and safety in a European study. *Psychological Medicine*. **38**: 651-661.

Sheline YI, Sanghavi M, Mintun MA, Gado MH (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *Journal of Neuroscience*. **19**: 5034-5043.

Shouse MN, Staba RJ, Ko PY, Saquib SF, Farber PR (2001). Monoamines and seizures: microdialysis findings in locus ceruleus and amygdala before and during amygdala kindling. *Brain Research*. **892**: 176-192.

Sibson NR, Dhankhar A, Mason GF, Rothman DL, Behar KL, Shulman RG (1998). Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *PNAS*. **95**: 316-321.

Sitges M, Guarneros A, Nekrassov V (2007). Effects of carbamazepine, phenytoin, valproic acid, oxcarbazepine, lamotrigine, topiramate and vinpocetine on the presynaptic Ca^{2+} channel-mediated release of [3H]glutamate: Comparison with the Na^{+} channel-mediated release. *Neuropharmacology*. **53**: 854-862.

Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacology Biochemistry and Behavior*. **56**: 131-137.

Skolnick P, Layer RT, Popik P, Nowak G, Paul IA, Trullas R (1996). Adaptation of N-methyl-D-aspartate receptors following antidepressant treatment: implications for the pharmacotherapy of depression. *Pharmacopsychiatry*. **29**: 23-26.

Slattery DA and Cryan JF (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols*. **7**: 1009-1014.

Smith MA, Makino S, Kvetnansky R, Post RM (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *The Journal of Neuroscience*. **15**: 1768-1777.

Stahl SM. In: *Essential Psychopharmacology: Neuroscientific Basis and Practical Applications*. 2nd ed. Cambridge, UK: Cambridge University Press; 2000:135-197.

Stockmeier CA, Shapiro LA, Dilley GE, Kolli TN, Friedman L, Rajkowska G (1998). Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression – postmortem evidence for decreased serotonin activity. *The Journal of Neuroscience*. **18**: 7394-7401.

Sullivan PF, Neale MC, Kendler KS (2000). Genetic epidemiology of depression: review and meta-analysis. *The American Journal of Psychiatry*. **157**: 1552-1562.

Svensson TH, Bunney BS, Aghajanian GK (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Research*. **92**: 291-306.

Swanson LW (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Research Bulletin*. **9**: 321-353.

Szabo ST, de Montigny C, Blier P (2000). Progressive attenuation of the firing activity of locus coeruleus noradrenergic neurons by sustained administration of selective serotonin reuptake inhibitors. *The International Journal of Neuropsychopharmacology*. **3**: 1-11.

Szabo ST, Gould TD, Manji HK. Introduction to neurotransmitters, receptors, signal transduction, and second messengers. In: *The American Psychiatric Publishing Textbook of Psychopharmacology*, 4th edition, Schatzberg AF, Nemeroff CB editors, American Psychiatric Publishing, Washington, DC: 2009: 3-58.

Talarowska M, Gałecki P, Maes M, Orzechowska A, Chamielec M, Bartosz G et al. (2012). Nitric oxide plasma concentration associated with cognitive impairment in patients with recurrent depressive disorder. *Neuroscience Letters*. **510**: 127-131.

Tanahashi S, Yamamura S, Nakagawa M, Motomura E, Okada M (2012). Effect of lamotrigine and carbamazepine on corticotropin-releasing factor-associated serotonergic transmission in rat dorsal raphe nucleus. *Psychopharmacology*. **220**: 599-610.

Thase ME (2003). Achieving remission and managing relapse in depression. *The Journal of Clinical Psychiatry.* **64** [suppl 18]: 3-7.

Tremblay P and Blier P (2006). Catecholaminergic strategies for the treatment of major depression. *Current Drug Targets*. **7**: 149-158.

Trullas R and Skolnick P (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *European Journal of Pharmacology*. **185**: 1-10.

Ungless MA, Magill PJ, Bolam JP (2004). Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science*. **303**: 2040-2042.

Walther DJ, Peter JU, Bashammakh S, Hörtnagl H, Voits M, Fink H et al. (2003). Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science.* **299**: 76.

Whitton PS, Biggs CS, Pearce BR, Fowler LJ (1992). MK-801 increases extracellular 5-hydroxytryptamine in rat hippocampus and striatum in vivo. *Journal of Neurochemistry*. **58**: 1573-1575.

WHO. (2008). The Global Burden of Disease: 2004 Update. Geneva: WHO Press.

Xie X, Dale TJ, John VH, Cater HL, Peakman TC, Clare JJ (2001). Electrophysiological and pharmacological properties of the human brain type IIA Na^+ channel expressed in a stable mammalian cell line. *Pflügers Archiv: European Journal of Physiology*. **441**: 425-433.

Yan QS, Reith ME, Jobe PC, Dailey JW (1997). Dizolcipine (MK-801) increases not only dopamine but also serotonin and norepinephrine transmissions in the nucleus accumbens as measured by microdialysis in freely moving rats. *Brain Research*. **765**: 149-158.

Yerevanian BI, Koek RJ, Feusner JD, Hwang S, Mintz J (2004). Antidepressants and suicidal behavior in unipolar depression. *Acta psychiatrica Scandinavica.* **110**: 452-458.

Yilmaz A, Schulz D, Aksoy A, Canbeyli R (2002). Prolonged effect of an anesthetic dose of ketamine on behavioral despair. *Pharmacology Biochemistry and Behavior*. **71**: 341-344.

Zarate CA Jr, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA et al. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of General Psychiatry*. **63**: 856-864.