INVESTIGATING THE POTENTIAL ROLE OF SEROTONIN-2B RECEPTOR ANTAGONISM IN THE NEURONAL ACTIONS OF ADJUNCTIVE ARIPIPRAZOLE

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Abstract

Introduction: Using serotonin-2B (5-HT_{2B}) receptor knockout (KO) mice, previous research has demonstrated that 5-HT_{2B} receptors contribute to the antidepressant-like response. Several serotonin-dopamine partial agonists (i.e. aripiprazole; previously known as atypical antipsychotics) that exhibit high affinity antagonism at the 5-HT_{2B} receptor have been successfully used in combination with selective 5-HT reuptake inhibitors (SSRIs) in difficult to treat major depressive disorder. However, the exact contribution of the antagonistic action of aripiprazole on 5-HT_{2B} receptors in the context of adjunct therapy is not known. **Methods:** In-vivo electrophysiological recordings of ventral tegmental area (VTA) dopamine (DA), prelimbic and infralimbic medial prefrontal cortical (mPFC) pyramidal and dorsal CA3 hippocampal pyramidal neurons were conducted in anaesthetized Sprague-Dawley rats. **Results:** Acute administration of the 5-HT_{2B} receptor agonist BW723c86 (6 mg/kg, intravenously [i.v.]) decreased the firing and bursting activity of DA neurons, which was abolished by pre-administration of the selective 5-HT_{2B} receptor antagonist RS127445 (2 mg/kg, subcutaneously [s.c.]). After two days, an SSRI-induced (escitalopram 10 mg/kg/day, s.c.) decrease in DA firing activity was rescued by co-administration of the selective 5-HT_{2B} receptor antagonist LY266097 (0.6 mg/kg/day, intraperitoneally [i.p.]). Aripiprazole (2 mg/kg/day, s.c.) administered alone or in combination with escitalopram for 14 days increased mPFC neuron firing and bursting activity, however escitalopram alone did not. LY266097 alone or the addition of LY266097 for the last three days of a 14-day escitalopram treatment increased mPFC pyramidal neuron firing and bursting activity more so than escitalopram alone. Finally, acute ejection of BW723c86 via microiontophoresis impaired SSRI binding to the serotonin transporter (5-HTT) in dorsal hippocampal pyramidal neurons. **Conclusions:** 5-HT_{2B} receptor blockade rescues an SSRI-mediated decrease in DA firing activity. The increase in mPFC pyramidal neuron firing and bursting activity mediated by aripiprazole and in combination with escitalopram may be, at least partly, moderated by 5-HT_{2B} receptors expressed in this brain area. Lastly, 5-HT_{2B} receptor agonism may impair SSRI binding to 5-HTT in the hippocampus. Altogether, these results strengthen the view that 5-HT_{2B} receptor antagonism contributes to the therapeutic effect of aripiprazole.

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List of abbreviations

5-HT – Serotonin

5-HTT – Serotonin transporter

AA – Arachidonic acid or antagonistic activity

ABCs – Aripiprazole, brexpiprazole, cariprazine

CACC – Canadian Council on Animal Care

CNS – Central nervous system

DA – Dopamine

DAG - Diacylglycerol

DAT – Dopamine transporter

dlPFC – dorsolateral prefrontal cortex

DoS – Duration of silence

DRN – Dorsal raphe nucleus

EPS – Extrapyramidal side-effects

FST – Forced swim test

GABA – gamma-aminobutyric acid

GI – Gastrointestinal

InFr - Infralimbic

I.P. – Intraperitoneal

IP_3 – Inositol triphosphate

ISI – Interspike interval

I.V. – Intravenous

KO – Knockout

LC – Locus coeruleus

LSD – Lysergic acid diethylamide

MAch – Muscarinic acetylcholine

MADRS – Montgomery Asberg Depression Rating Scale

MAO – Monoamine oxidase

MAOI – Monoamide oxidase inhibitor

MDD – Major depressive disorder

MDE – Major depressive episode

mRNA – messenger ribonucleic acid

MT - Melatonin

mPFC – Medial prefrontal cortex

NADPH - Nicotinamide adenine dinucleotide phosphate

NE – Norepinephrine

NET – Norepinephrine transporter

nM – Nanomolar

NO – Nitric oxide

NRI – Norepinephrine reuptake inhibitor

OR – Odds-ratio

PIP_2 - Phosphatidyl inositol diphosphate

PLA2 – Phospholipase A2

PLC – Phospholipase C

PKG – Protein Kinase G

PrL – Prelimbic

ROS – Reactive oxygen species

SNRI – Serotonin norepinephrine reuptake inhibitor

SRI – Serotonin reuptake inhibitor

SSRI – Selective serotonin reuptake inhibitor

S.C. – Subcutaneous

TACE - TNF-α converting enzyme

TH – Tyrosine hydroxylase

TNF-α - tumor necrosis factor – α

Tph – Tryptophan hydroxylase

TRI – Tricyclic reuptake inhibitor

TST – Tail suspension test

VTA – Ventral tegmental area

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Chapter 1: Introduction

Chapter 1.1: A framework for understanding the antidepressant response

"If a fright or despondency lasts for a long time, it is a melancholic affection" wrote Hippocrates in *Aphorisms*. Melas- meaning black, and kholē- meaning bile, "black bile" is one of the four humors the ancient Greeks thought to compose the human body- and was thought to be in excess during a melancholic affection or depression. Today, depression is defined as a set of symptoms that vary greatly between individuals. To be diagnosed with depression, an individual must exhibit a low mood and/or the inability to feel pleasure (anhedonia) accompanied with three to four of seven other symptoms including: dysregulation of feeding, sleep and psychomotor function, fatigue, feelings of worthlessness or excessive guilt, cognitive dysfunction and/or recurrent suicidal ideation for at least two weeks (American Psychiatric Association, 2013). In Canada, the 1-year incidence rate of depression is about 1.8% and the 1-year prevalence rate of depression is about 5.5%, which have remained unchanged in the last two decades (Patten et al., 2015; Patten et al., 2016). Using current population estimates, approximately 660 000 new cases of depression emerge each year, and about two million individuals in Canada are affected by depression at any one time in a single year. To maintain a steady 1-year prevalence rate, roughly 30% of the depressive population in Canada must theoretically be in remission at any given moment within the year. At least some of these remissions can be attributable to oral pharmacotherapy (Rush et al., 2006) and select classes of psychopharmacologic agents are recommended as first-line treatment for moderate to severe depression (Lam et al., 2016).

The following psychobiological model of depression may provide a framework for the effectiveness of psychopharmacologic agents. Several investigations have shown that in the context of dysphoric mood, individuals recall more negative memories from the past, termed "depressive ruminations" (Nolen-Hoeksema, 2000). Furthermore, depressive ruminations predict the onset of major depressive episodes (MDEs), the presence of depressive disorders and their chronicity (Nolen-Hoeksema, 2000). In general, ruminative individuals exhibit an overactive memory recall and a top-down memory control deficit, functions attributable to the hippocampus and frontal cortex, respectively (Scoville and Milner, 1957; Fawcett et al., 2015). Interestingly, the hippocampus and the dorsolateral prefrontal cortex (dlPFC) display an increased and decreased

reactivity, respectively, in depressed individuals during rumination tasks (Siegle et al., 2007; Mandell et al., 2014). Furthermore, treatment-nonresponsive patients accrue greater hippocampal atrophy overtime (Phillips et al, 2015). Altogether, these investigations suggest an excitotoxic effect of depressive rumination on the hippocampus. In contrast, treatment-responsive patients exhibit reduced glucose metabolism and increased volume in hippocampus over the course of treatment. Whereas in the dlPFC, glucose metabolism is increased (Mayberg et al., 2000; Phillips et al., 2015). Altogether, these studies suggest antidepressant treatments may reduce the tendency for depressive rumination. As will be discussed, antidepressant treatments increase the activity of either one or all the monoamine systems (serotonin [5-HT], norepinephrine [NE], and DA), which have demonstrated inhibitory actions in the rat hippocampus (Sprouse and Aghajanian, 1988; Guiard et al., 2008).

Back-translation is a powerful research technique and begins with clinical remarks as revealed by a physician's careful observation and/or by concentrated efforts of scientists on randomized-controlled trials. These remarks are taken to the bench to uncover mechanistic basis(es) in an attempt to explain important phenomena (Shakhnovich et al., 2018), such as the antidepressant response. Back-translation can lead to two types of discovery; i) drugs with a primary clinical indication might have unforeseen downstream consequences and ii) drugs with an unrelated clinical indication may exert a therapeutic effect on the indication of investigation (Shakhnovich et al., 2018). First, a history of drug discovery in depression will be presented along with their clinical efficacies. Then, these drugs will be discussed in terms of their effects on three neurotransmitter systems: the 5-HT, NE and DA systems. Afterwards, a hypothesis for non-response will be presented as a downstream consequence of the most prescribed class of medications, SSRIs. Then, medication combination for treatment-resistant depression will be spoken on with an emphasis on DA partial agonists (previously known as atypical antipsychotics and initially indicated for schizophrenia). Finally, a case for an understudied drug action will be presented: 5-HT_{2B} receptor antagonism, followed by electrophysiological studies on this receptor.

Chapter 1.2: History of drug discovery in depression

Monoamine oxidase inhibitors (MAOIs)

The first MAOI, iproniazid, was initially synthetized in 1951 to treat patients with tuberculosis (Ban, 2006). However, the treating physicians noticed that the drug caused relative euphoria and over-active behaviours (Selikoff et al., 1952). Subsequent studies revealed that iproniazid was a potent inhibitor of the monoamine oxidase (MAO) enzyme in rat liver (Davidson, 1957) and a group of physicians concluded that iproniazid was a potential "psychic energizer" that was most effective in depressed patients (Loomer et al., 1957). MAOs are expressed as two isoforms: MAO-A and -B and catalyze the degradation of a variety of neurotransmitters including the indoleamines 5-HT and histamine, as well as the catecholamines DA, NE and epinephrine (Youdim et al., 2006). Recently, brain-penetrant and selective radioligands have been developed to target MAO-A or -B in the human brain. These studies have demonstrated that MAO-A binding is elevated by about 30% across brain regions (Meyer et al., 2006), while MAO-B binding is elevated by about 25% in the cortex (Moriguchi et al., 2019) during a MDE. These studies suggest that increased monoamine catabolism may increase susceptibility to a MDE. Theoretically, inhibition of either or both isoforms of MAO would be beneficial for an individual experiencing an MDE. However, first-generation MAOIs bind irreversibly to MAO-A and MAO-B, which tends to generate adverse effects such as hypertensive crisis due to tyramine accumulation and subsequent calcium-independent NE release (Sacher et al., 2011). Since the conception of iproniazid, reversible and selective MAOIs have been developed (Youdim et al., 2006). For example, the reversible and selective MAO-A inhibitor moclobemide has demonstrated better tolerability, yet possibly inferior efficacy, relative to the older irreversible, non-selective inhibitors phenelzine and tranylcypromine (Lotufo-Neto et al., 1999). All three of these compounds are used to treat major depressive disorder (MDD) in Canada today, along with the irreversible, and preferential MAO-B inhibitor selegiline (Kennedy et al., 2016; see table 1).

Compound	Priority[a]	MAO Selectivity[b]	Inhibition Type[b]
Moclobemide	2nd	A	Reversible
Phenelzine	3rd	A and B	Irreversible
Selegiline	2nd	B (low dose)	Irreversible
Tranylcypromine	3rd	A and B	Irreversible

Table 1. MAOIs currently prescribed in Canada, their recommended priority in treatment and associated pharmacodynamic properties. Adapted with modifications with data from [a]Kennedy et al., 2016 [b]Youdim et al., 2006.

Tricyclic re-uptake inhibitors (TRIs)

The discovery of TRIs began with the search for chlorpromazine-like substances that would be effective in schizophrenia (Ban, 2006). A candidate drug, G 22,355, failed to ameliorate schizophrenia symptoms, however, demonstrated efficacy in the treatment of depression (Kuhn, 1957). These findings led to the release of the first TRI for the treatment of depression, imipramine. Small modifications to the chemical structure of these antihistamine derivatives led to the development of several other TRIs with a wide range of pharmacodynamic properties (Domino, 1999; Gillman, 2007). These compounds all inhibit 5-HT and NE reuptake, antagonise histamine-H_1 receptors, NE-α_1 receptors, muscarinic acetylcholine (MACh) receptors and 5-HT_{2A} receptors to some degree, however the clinical relevance of these drug-receptor interactions vary (Gillman, 2007). Gillman (2007) suggests that the clinical relevance for serotonin reuptake inhibition (SRI) would be at a nanomolar (nM) K_i value of 20. This is because amitriptyline (K_i = 20 nM) 150 mg administered to an individual does not cause serotonin toxicity when co-prescribed with the MAOI tranylcypromine. Whereas, clomipramine (K_i = 0.14) administered with tranylcypromine at the same dose, does cause serotonin toxicity (Gillman, 1998). As opposed to Gillman's (2007) observation that nortriptyline and desipramine are selective norepinephrine reuptake inhibitors (NRI) as measured by the tyramine pressor test in humans, all five TRIs listed in table 2 attenuate a tyramine-induced rise in blood pressure (Blier et al., 2007; Hassan et al., 1985; Larochelle et al., 1979; Seppala et al., 1981; Turcotte et al., 2001). These varying actions on the 5-HTT and norepinephrine transporter (NET) have important clinical implications. For example, weak SRI agents such as amitriptyline and nortriptyline may be used more confidently to avoid serotonin syndrome with MAOI co-administration (Gillman, 2007). Furthermore, stronger NRI agents such as nortriptyline and desipramine may be useful in preventing a tyramine-induced hypertensive crisis induced by an MAOI (Gillman, 2007).

Perhaps the most uniform drug action amongst the TRIs is high affinity histamine-H_1 receptor antagonism, which has been linked to sedation and weight-gain (Nicholson, 1983; Kroeze et al., 2003). Furthermore, TRIs are largely anticholinergic and have been linked to an increased risk of mental impairment in especially in the elderly population (Collamati et al., 2016). It is also important to note that NE-α_1 receptor antagonism is a common pharmacotherapy for hypertension (i.e. the selective antagonist prazosin) and may pose a risk for a hypotensive episode in otherwise healthy

individuals. For these reasons, desipramine would be the ideal TRI for the treatment of depression to avoid histamine-H_1 mediated weight-gain/sedation, muscarinic acetylcholine (MACh) receptor mediated mental impairment and NE-α1 receptor mediated hypotension. However, if 5-HT_{2A} receptor antagonism is a desired drug trait to ameliorate the antidepressant response, then other TRIs may be more useful (see chapter 1.4)

Compound	Priority[a]	5-HTT[b]	NET[b]	H_1 ant.[b]	α_1 ant.[b]	MACh ant.[b]	5-HT_{2A} ant.[b]
Amitriptyline	2nd	20	50	1	27	18	29
Clomipramine	2nd	0.14	54	15	32	25	35
Desipramine	2nd	18	.83	110	100	100	280
Imipramine	2nd	7	60	40	32	46	80
Nortriptyline	2nd	100	10	6.3	55	37	44

Table 2. TRIs currently prescribed in Canada, their recommended priority in treatment and associated binding affinities for various transporters and receptors. Adapted with modifications with data from Kennedy et al., 2016[a] and Gillman, 2007[b]. All integers represent K_i values in nM.

Selective-serotonin reuptake inhibitors (SSRIs)

It can be argued that an old-age antihistamine could have been marketed as an SRI medication (Hellbom, 2006). Chlorpheniramine has only two drug actions; it is a histamine-H1 antagonist and weak SRI comparable to desipramine (K_i = 15; Tatsumi et al., 1997). Slight modifications to the structure of chlorpheniramine led to the discovery of the first SSRI zimelidine (Hellbom, 2006). However, it was not until 1987 when fluoxetine (Prozac) was available to the public as the first SSRI medication for the treatment of depression that was devoid of any other pharmacodynamic actions (Wong et al., 1983; Hellbom, 2006). As six of fifteen first-line therapies are SSRIs, they are the most prescribed class of medications for depression (see table 3). However, SSRIs are not without adverse effects, and are mediated by an SRI-dependent increase in synaptic 5-HT at specific 5-HT receptor subtypes in discrete regions of the body where the relevant physiologic processes are regulated (Stahl, 1998). These include a dysregulation of body temperature (hypothalamic 5-HT_{1A} receptors), a decrease in sexual functioning (lumbar spinal 5-HT_2 receptors), and gastrointestinal (GI)-related dysfunction and nausea (area posttrema and gut 5-HT_3 receptors) (Nutt, 1997; Stahl, 1998) that may decrease acceptability and compliance. However, in a large analysis of SSRI efficacy (response rate) and acceptability (drop-out rate) escitalopram demonstrated the best balance between efficacy and acceptability. This psychopharmacologic agent may represent the first choice for a prescribing physician (Cipriani et

al., 2018). However, adverse effects may be mitigated by the addition of a medication. For example, the addition of 5-HT_3 antagonist medications such as cisaparide and ondansetron to an acute dose of a SSRI reduces nausea and GI-related side effects (Bergeron and Blier, 1994; Bailey et al., 1995).

Compound	Priority[a]	5-HTT (K_i [nM])[b]	NET (K_i [nM])[b]	DAT (K_i [nM])[b]	Efficacy OR[c]	Acceptability OR[c]
Citalopram	1st	1.6	6 190	16 540	0.91	1.11
Escitalopram	1st	1.1	7 841	27 410	0.76	1.19
Fluoxetine	1st	1.1	599	3 764	1.00	1.00
Fluvoxamine	1st	2.3	1 427	16 790	1.02	0.82
Paroxetine	1st	0.1	45	268	0.98	0.91
Sertraline	1st	0.26	714	22	0.80	1.14

Table 3. SSRIs currently prescribed in Canada, their recommended priority in treatment and their binding affinities for the monoamine transporters, along with efficacy odds-ratio (OR) and acceptability OR data. Adapted with modifications with data from [a]Kennedy et al., 2016, [b]Owens et al., 2001 and [c]Cipriani et al., 2018. DAT = Dopamine Transporter.

Serotonin-norepinephrine re-uptake inhibitors (SNRIs)

The SNRIs were developed as a structurally novel group of drugs that can inhibit the reuptake of serotonin and norepinephrine yet are devoid of any additional drug actions possessed by the TRIs (Burnett and Dinan, 1998). Four of the fifteen first-line agents are SNRIs, thus being the second most prescribed class of antidepressant medication (Kennedy et al., 2016). Each of the SNRIs have a ratio of 5-HTT:NET reuptake, resulting in preferential inhibition of one transporter over the other. The earlier SNRIs desvenlafaxine, duloxetine and venlafaxine have preference for the 5-HTT, whereas the later SNRIs milnacipran and levomilnacipran have preference for the NET (see table 4). To achieve significant NRI activity in humans, larger doses of the 5-HTT preferring compounds must be administered. For example, 150 mg/day of venlafaxine can inhibit 5-HTT as demonstrated by a decrease in whole-blood 5-HT, whereas 300 mg/d of venlafaxine is not able to supress a tyramine-induced increase in blood pressure in healthy participants (Blier et al., 2007). However, 225 mg/day and 375 mg/day doses of venlafaxine can supress a tyramine-induced increase in blood pressure in depressed patients (Debonnel et al., 2007). These observations are critical for the treating physician, as an alternative treatment strategy should not be sought until a noradrenergic dose is reached.

Compound	Priority[a]	IC_{50} 5-HTT	IC_{50} NET	IC_{50} DAT	(IC_{50} 5-HTT: IC_{50} NET)
Desvenlafaxine (Deecher et al., 2006)	1st	*47*	*531*	*N.D.*	*10:1*
Duloxetine (Vaishnavi et al., 2004)	1st	*3.7*	*20*	*439*	*5:1*
Levomilnacipran (Auclair et al., 2013)	2nd	*19*	*10.5*	*>100 000*	*1:2*
Milnacipran (Vaishnavi et al., 2004)	1st	*151*	*68*	*>100 000*	*1:2*
Venlafaxine (Vaishnavi et al., 2004)	1st	*145*	*1420*	*3070*	*10:1*

Table 4. SNRIs currently prescribed in Canada, their recommended priority in treatment and their associated concentrations (nM) at which they inhibit transporter activity by 50% (IC_{50}). Ratio of 5-HTT:NET reuptake based on IC_{50}. Adapted with modifications from [a]Kennedy et al., 2016 and others.

Serotonin-noradrenaline receptor antagonists

Mianserin and the closely related mirtazapine are the first serotonin-noradrenaline receptor antagonists to be characterized in *vitro* (Peroutka and Snyder, 1981; De Boer et al., 1988). Mianserin binds to the NET, histamine-H_1 receptors, NEα_1 and -α_2 receptors, and 5-$HT_{2A/B/C}$ receptors (Peroutka and Snyder, 1981; Kelder et al., 1997; Wainscott et al., 1996). Mirtazapine has no significant affinities for the monoamine transporters, however binds to histamine-H_1 receptors, adrenergic-α_2 receptors, 5-$HT_{2A/C}$ receptors and to a lesser extent 5-HT_3 receptors (de Boer et al., 1988; Van der Mey et al., 2006). Due to a lack of any significant SRI capabilities and antagonistic actions at 5-HT_2 and 5-HT_3 receptors, these medications are not associated with sexual dysfunction and/or GI-related dysfunction and nausea, respectively (Nutt, 1997; Stahl, 1998). However, mirtazapine lacks significant antagonism at NEα_1 receptors and may be associated with less sedation relative to mianserin (Wakeling, 1983; Nutt, 1997). Both medications are effective and considered first-line treatments for depression (Kennedy et al., 2016).

DA-norepinephrine releaser: Bupropion

Bupropion was primarily characterized as an NE/DA reuptake inhibitor, however is also nicotinic acetylcholine receptor antagonist, and 5-HT_3 receptor negative allosteric modulator (Slemmer et al., 2000; Stahl et al., 2004; Pandhare et al., 2017). There is controversy regarding the property of bupropion to inhibit reuptake of NET and DAT at clinically relevant doses. The 80% rule-of-thumb states that neurological and mental illness arises with loss of approximately 80% of neurotransmitter nuclei (i.e. substantia nigra, nucleus basalis). In parallel, SSRIs exert therapeutic their effect by occupying 80% of 5-HTT (Meyer et al., 2007) and provides the rational that the

monoamine systems are resilient and require significant alterations in their function to produce clinically relevant effects (Blier, 2008). In contrast, therapeutically relevant doses of bupropion occupy 15-25% of DAT and do not alter responses on the tyramine pressor test (a proxy of NET inhibition; Gobbi et al., 2003; Stahl et al., 2004). Thus, bupropion might not be exerting its effects through DAT or NET inhibition but rather acts as a DA and NE releaser (Gobbi et al., 2003; Blier, 2008). Regardless of how the therapeutic effects of bupropion are produced, it is effective and considered first-line for the treatment of MDD (Kennedy et al., 2016).

Melatonin receptor agonist: Agomelatine

Agomelatine is the first melatonin modulating compound developed for the treatment of depression (Yous et al., 1992). In addition to high-affinity agonism at melatonin-1 (MT_1) and -2 (MT_2) receptors, agomelatine is an antagonist at 5-$HT_{2B/C}$ receptors (Millan et al., 2003). As with mianserin and mirtazapine, agomelatine is associated with fewer sexual-related side effects due to lack of SRI properties (Kennedy et al., 2008). Agomelatine is considered first-line for the treatment of MDD (Kennedy et al., 2016).

SRI and serotonin receptor modulator: Vortioxetine

Vortioxetine has a rich pharmacological profile. It is an SRI (K_i = 1.6), 5-HT_{1A} agonist (K_i = 15), 5-HT_{1B} partial agonist (K_i = 33), 5-HT_3 (K_i = 33) and 5-HT_7 antagonist (K_i = 19) (Bang-Anderson et al., 2011). Vortioxetine is unique amongst the drugs with intrinsic antidepressant activity, as it has been reported to improve cognitive functioning irrespective of depressive symptoms (McIntyre et al., 2016), possibly due to 5-HT_3 and 5-HT_7 antagonistic action (Leiser et al., 2014). Vortioxetine is considered first-line for the treatment of MDD (Kennedy et al., 2016).

Chapter 1.3: Neural correlates of the antidepressant response

Neural correlates of the antidepressant response – 5-HT

The most consistent finding amongst long-term antidepressant treatment is the enhancement of 5-HT transmission in the hippocampus (Haddjeri et al., 1998). This enhancement of 5-HT neurotransmission appears to be non-exclusive to 5-HT modulating compounds and occurs via several adaptive changes in the 5-HT system (see table 5; Blier and de Montigny, 1994; Haddjeri et al., 1998; Chernoloz et al., 2009a; Ghanbari et al., 2011). These adaptive changes are considered neural correlates of the antidepressant response and will be discussed in detail below.

Somatodendritic 5-HT_{1A} autoreceptor responsiveness

The inhibitory action of the 5-HT_{1A} autoreceptor were initially characterized by Aghajanian et al., (1972), where iontophoretically ejected 5-HT inhibited the activity of 5-HT neurons in the dorsal raphe nucleus (DRN). These and other 5-HT_1 receptors couple to $G_{i/o}$ proteins that initiate inhibitory intracellular cascades including Gα-mediated inhibition of cAMP formation and Gβ-mediated activation of K^+ channels (Albert and Tiberi, 2001). Alterations in the sensitivity of the 5-HT_{1A} autoreceptor were initially described with the repeated administration of the SSRI zimelidine (Blier and de Montigny, 1983). Zimelidine administration for two to seven days decreased the spontaneous and firing activity of 5-HT neurons. However, zimelidine administration for fourteen days recovered the spontaneous and firing activity of 5-HT neurons (Blier and de Montigny, 1983). An increased dose of the preferential 5-HT_{1A} autoreceptor agonist, lysergic acid diethylamide (LSD), was required to silence 5-HT neurons in pretreated animals and indicated a desensitization of this receptor (Blier and de Montigny, 1983). In general, receptor desensitization occurs via several adaptive mechanisms and the described effect may be mediated by a combination of; i) receptor-effector uncoupling, ii) receptor phosphorylation and subsequent β-arrestin mediated internalization, iii) following long-term activation, receptor ubiquitylation and degradation (Albert, 2004; Riad et al., 2004). However, this prolonged time required for desensitization is much longer than the time-courses of the above processes, indicating that transcriptional regulators of the HTR1A gene may be responsible for the long-term desensitization detected initially (Albert and Vahid-Ansari, 2019). Furthermore, the inhibitory effect of electrically-evoked release of 5-HT on 5-HT_{1A} receptors in the hippocampus was increased following repeated zimelidine administration, while the sensitivity of 5-HT_{1A} receptors was unaltered (Blier and de Montigny, 1983). These results

suggest that a reduction in the tonic inhibitory control of 5-HT neurons, in addition to SRI, is one method by which a therapeutic compound may increase 5-HT neurotransmission. However, compounds with weak SRI capabilities (i.e. imipramine) do not desensitize 5-HT_{1A} receptors, and others with no SRI capabilities (i.e. bupropion) do. These comparisons suggest that other adaptive mechanisms may also increase 5-HT neurotransmission.

Terminal 5-HT_{1B} autoreceptor responsiveness

While 5-HT_{1A} receptor agonists inhibit 5-HT neuron firing, 5-HT_{1B} receptor agonists do not, indicating a predominance of 5-HT_{1A}-mediated effects presynaptically (Middlemiss and Hutson, 1990). However, electrical stimulation of the ascending 5-HT pathway silences the activity of dorsal hippocampal cells and is prolonged by administration of the preferential 5-HT_{1B} receptor antagonist methiothepin (Chaput et al., 1986). The duration of silence (DoS) is decreased when stimulating the 5-HT bundle at 5 Hz relative to 1 Hz. However, the decreased magnitude of DoS at 5 Hz is abolished after the administration of methiothepin and is no longer different from stimulation at 1 Hz. These results suggest that terminal 5-HT_{1B} receptors exert a strong inhibitory control 5-HT release from nerve terminals, actually not through a $G_{i/o}$-dependent mechanism (Chaput et al., 1986; Blier, 1991). Furthermore, desensitization of 5-HT_{1B} receptors due to long-term administration of medications for depression would contribute to a net increase in 5-HT transmission (Blier and de Montigny, 1994). Interestingly, potent SRI compounds (fluoxetine, venlafaxine and vortioxetine) but not weak SRI compounds (TRIs) or selective 5-HT_{1A} agonists are able to desensitize these receptors (see table 5).

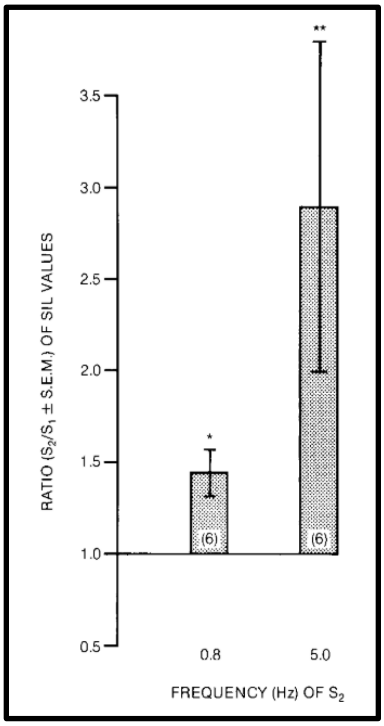

Figure 1. DoS ratio is equivalent to DoS elicited by electrical stimulation of the 5-HT bundle in methiothepin 1 mg/kg-treated animals divided by DoS in control animals. In both stimulation protocols (0.8 and 5.0 Hz), methiothepin produces a significantly greater DoS in hippocampal neurons. Adapted from Chaput et al., 1986.

Compound	5-HT firing activity	Somatodendritic 5-HT_{1A} autoreceptor responsiveness	Terminal 5-HT_{1B} autoreceptor responsiveness	Postsynaptic 5-HT_{1A} receptor responsiveness	Net effect on 5-HT transmission
MAOI	Normalized* (Blier and de Montigny, 1985)	Decrease (Blier and de Montigny, 1985; Blier et al., 1986a)	No effect (Blier et al., 1988)	No effect/ Decrease (Blier et al., 1986a; Blier et al., 1986b; Haddjeri et al., 1998)	Increase (Haddjeri et al., 1998a; Haddjeri et al., 1998b)
TRI	No change (Blier and de Montigny, 1980)	No effect (Blier and de Montigny, 1980)	No change	Increase (de Montigny and Aghajanian, 1978)	Increase (Haddjeri et al., 1998a)
SSRI	Normalized* (Blier and de Montigny, 1983)	Decrease (Blier and de Montigny, 1983)	Decrease (Blier et al., 1988)	No change (de Montigny et al., 1981)	Increase (Haddjeri et al., 1998a)
Gepirone 5-HT_{1A} agonist	Normalized* (Blier and De Montigny, 1987a)	Decrease (Blier and De Montigny, 1987a)	No change (Blier and De Montigny, 1987a)	No change (Blier and De Montigny, 1987a)	Increase (Haddjeri et al., 1998a)
SNRI	Normalized* (Béïque et al., 2000a)	Decrease (Béïque et al., 2000a)	Decrease (Béïque et al., 2000b)	No change (Béïque et al., 2000a)	Increase (Béïque et al., 2000a)
Mirtazapine	Increase (Haddjeri et al., 1997)	Decrease	No change (Haddjeri et al., 1997)	No change (Haddjeri et al., 1998)	Increase (Haddjeri et al., 1998a)
Bupropion	Increase (El Mansari et al., 2008)	Decrease (El Mansari et al., 2008)	No change (Ghanbari et al., 2011)	No change (Ghanbari et al., 2011)	Increase (Ghanbari et al., 2011)
Agomelatine	Increase (Chenu et al., 2013)	No change (Chenu et al., 2013)	N.D.	No change (Chenu et al., 2013)	Increase (Chenu et al., 2013)
Vortioxetine	Normalized* (Betry et al., 2013)	Decrease (Betry et al., 2013)	Decrease (El Mansari et al., 2015)	No change (El Mansari et al., 2015)	Increase (El Mansari et al., 2015)

Table 5. The effects of long-term administration of medications for depression on several parameters related to the activity and function of the 5-HT system. Adapted from Blier and El Mansari, 2013 with modifications.*Refers to an initial decrease in firing activity upon treatment initiation followed by a recovery. N.D. = no data.

Neural correlates of the antidepressant response – NE

Sensitivity of somatodendritic NE-α_2 autoreceptors and NE firing activity

All NE modulating compounds decrease the firing of NE neurons after sustained administration. In contrast to the adaptive response of the 5-HT_{1A} receptor, the tonic activation of somatodendritic NE-α_2 autoreceptors does not result in desensitization in the presence of compounds which increase synaptic NE concentrations by blocking NET or MAO (i.e. phenylzine,

desipramine, venlafaxine and reboxetine; Blier and de Montigny 1985; Lacroix et al., 1991; Béïque et al., 2000b; Szabo et al., 2001a). Administration of the NE-α_2 antagonist piperoxane reverses the inhibition of NE neurons, providing evidence that NE modulating compounds are acting through normosensitive somatodendritic NE-α_2 autoreceptors coupled to $G_{i/o}$ proteins (Blier and de Montigny, 1985; Bylund, 1992; Szabo et al., 2001a). Interestingly, presynaptic inhibition of NE neurons is primarily mediated by the NE-α_{2A} receptor subtype and to a lesser extent the NE-α_{2C} receptor subtype, the former being resistant to internalization (Philipp et al., 2002; Olli-Lahdesmaki et al., 2003). Decreased NE firing activity is hypothesized to contribute to anxiolysis by diminishing NE-mediated stress responses (Szabo et al., 2001b). For example, long-term administration of SSRIs decreases NE firing, decreases NE outflow in the amygdala, and prevents an NE surge after acute stress (Szabo et al., 1999; Kawahara et al., 2007). However, the anxiolytic effects of NE firing reduction may only be relevant in such compounds that exert no additional effect on NE neurons (see table 6). On the other hand, mirtazapine and bupropion have been reported to increase NE firing activity. Moreover, bupropion desensitizes somatodendritic NE-α_2 autoreceptors and increases net NE transmission (see table 6). These two medications may represent an alternate strategy towards achieving a superior antidepressant response in combination with NRIs (Invernizzi and Garattini, 2004; Blier et al., 2010).

Terminal NE-α_2 autoreceptor and heteroreceptor responsiveness

Terminal NE-α_2 autoreceptor responsiveness can be assessed in a similar manner to terminal 5-HT_{1B} autoreceptors. Stimulation of the locus coeruleus (LC) evokes NE release and inhibits hippocampal neurons, and this effect is decreased with 5 Hz stimulation relative to 1 Hz stimulation. However, administration of the NE-α_2 receptor antagonist idazoxan abolishes a 5 Hz - induced inhibition, indicating a negative-feedback mechanism (Curet and de Montigny, 1989). Long-term treatment with desipramine attenuates inhibitory effect of the 5 Hz stimulation, indicating desensitization of this receptor (Lacroix et al., 1991). This may result in an increase in the tonic activation of NE-a_1 receptors expressed on 5-HT neurons, and although this does not result in an increase in 5-HT release *per se*, sufficient activation of NE-a_1 receptors is required for 5-HT release (Bortolozzi and Artigas, 2003). Alternatively, clonidine (an NE-α_2 receptor agonist) at varying doses can be used in conjunction with electrophysiology to assess autoreceptor versus heteroreceptor responsiveness (Mongeau et al., 1994). Low-dose clonidine administration preferentially activates

NE-α_2 autoreceptors on NE terminals, reducing the amount of NE available to supress 5-HT firing via tonic activation of NE-α_2 heteroreceptors on 5-HT neurons. Whereas high-dose clonidine administration directly activates NE-α_2 heteroreceptors on 5-HT neurons and reduces 5-HT release. These effects are reflected by the DoS following electrical stimulation of the 5-HT bundle (see figure 2; Mongeau et al., 1994). All NE modulating compounds, except SNRIs, diminish the effects of high-dose clonidine on the DoS (see table 6) indicating a desensitization of these inhibitory heteroreceptors (Szabo et al., 2001b). As these receptors are located on 5-HT terminals, desensitization may indicate a greater net effect on 5-HT neurotransmission (Blier and de Montigny, 1994).

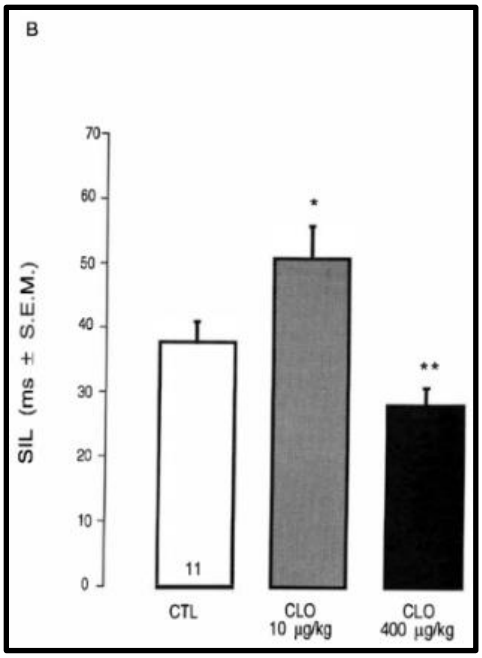

Figure 2. DoS in hippocampal neurons following electrical stimulation of the 5-HT bundle in control, clonidine 10 µg/kg and clonidine 400 µg/kg -treated animals. DoS is significantly increased following clonidine 10 µg/kg (grey bar) and significantly decreased following clonidine 400 µg/kg (black bar). Adapted from Mongeau et al., 1994.

Compound	NE firing activity	Somatodendritic NE-α_2 autoreceptor responsiveness	Terminal NE-α_2 responsiveness	Postsynaptic NE-$\alpha_{1/2}$ receptor responsiveness	Net effect on NE transmission
MAOI	Decrease (Blier and de Montigny, 1985)	No change (Blier and de Montigny, 1985)	Decrease (Mongeau et al., 1994)	No change (Blier et al., 1986a)	Increase (Finberg et al., 1993)
TRI	Decrease (Szabo et al., 2000)	No change (Lacroix et al., 1991)	Decrease (autoreceptor; (Lacroix et al., 1991)	No change/Decrease (Blier et al., 1987b; Lacroix et al., 1991)	Increase (Beyer et al., 2002)
Reboxetine Nisoxetine NRI	Decrease (Szabo et al., 2001a)	No change (Szabo et al., 2001a)	Decrease (Mongeau et al., 1994)	No change/ Decrease (Szabo et al., 2001c; Parini et al., 2005)	Increase (Sachetti et al., 1999)
SSRI	Decrease (Szabo et al., 1999)	N.D.	No change (Mongeau et al., 1994)	N.D.	Decrease (Kawahara et al., 2007)
Venlafaxine SNRI	Decrease (Béïque et al., 2000a)	No change (Béïque et al., 2000b)	No change (Béïque et al., 2000b)	No change (Béïque et al., 2000a)	Increase (Beyer et al., 2002)
Mirtazapine	Increase (Haddjeri et al., 1997)	No change (Haddjeri et al., 1997)	Decrease (Haddjeri et al., 1997)	No change (Haddjeri et al., 1997)	Increase (Kelder et al., 1997)
Bupropion	Normalized* (El Mansari et al., 2008)	Decrease (El Mansari et al., 2008)	Decrease (El Mansari et al., 2008)	No change (Ghanbari et al., 2011)	Increase (Ghanbari et al., 2011)
Agomelatine	No change (Chenu et al., 2013)	N.D.	N.D.	N.D.	Putatively no change (N.D.)
Vortioxetine	Decrease (Ebhrahimzadeh et al., 2018)	N.D.	No change (Ebhrahimzadeh et al., 2018)	No change (Ebhrahimzadeh et al., 2018)	No change (Ebhrahimzadeh et al., 2018)

Table 6. The effects of medications for depression on several parameters related to the activity and function of the NE system. Adapted from Szabo and Blier, 2001b with modifications.*Refers to an initial decrease in firing activity upon treatment initiation followed by a recovery. N.D. = no data.

Neural correlates of the antidepressant response – DA

Sensitivity of the DA-$D_{2/3}$ somatodendritic autoreceptor and DA firing and bursting activity

The mixed DA-$D_{2/3}$ agonist pramipexole administered for two days decreased DA firing and bursting activity in the VTA, while it's administration for fourteen days recovered these parameters (Chernoloz et al., 2009a). Further investigation revealed a relatively higher dose of the DA-$D_{2\text{-like}}$ agonist apomorphine was required to silence DA neurons in pretreated rats, indicating the desensitization of these receptors (Chernoloz et al., 2009a). DA-$D_{2/3}$ receptors are coupled to $G_{i/o}$ proteins leading to the inhibition of cAMP, K^+ and Ca^{2+} currents (Huff, 1996). Presynaptic inhibition

is primarily mediated by the DA-D_{2S} receptor isoform relative to the DA-D_{2L} receptor isoform and is sensitive to desensitization (Morris et al., 2007; Gantz et al., 2015), and so is the DA-D_3 receptor (Min et al., 2013). Interestingly, DA-D_2 or DA-D_3 receptor preferring compounds are both able to elicit significant decreases in DA activity highlighting the importance of both receptor subtypes (Etievant et al., 2009; Delcourte et al., 2018). The desensitization of these receptors was likely responsible for the increased tonic activation of DA receptors expressed on pyramidal neurons of the cortex (Chernoloz et al., 2009a). Agomelatine alone was not able to increase DA firing activity, however, increased DA bursting activity (Chenu et al., 2013). Bursting is a highly relevant electrophysiological parameter that is a function of temporal summation, such that two action potentials initiated at sub-second (0.08-0.16s) time intervals can elicit greater release of DA relative to two action potentials at time points of greater duration (Grace and Bunney, 1984; Gonon, 1988). These two medications may also represent alternative treatment strategies in the treatment of MDD. Furthermore, the hypothesis that bupropion is a DA releaser rather than a DAT inhibitor is indicated by a lack of effect on DA firing at any time-point and increases in DA levels in several brain areas, respectively (Li et al., 2002; El Mansari et al., 2008). Finally, the administration of 5-HT modulating compounds for a sustained period decrease DA firing and bursting activity, which may be averse to the antidepressant response (see chapter 1.4).

Terminal DA-$D_{2/3}$ heteroreceptor activation

Previous studies have demonstrated that DA may increase the activity of 5-HT neurons via the activation of DA-D_2-like heteroreceptors (Haj-Dahmane et al., 2001; Aman et al., 2007). As pramiprexole and agomelatine were able to increase DA transmission, the administration of paliperidone in pretreated rats was used to assess tonic activation of these receptors (Chernoloz et al., 2009a). The DA-D_2 antagonist paliperidone elicits no effect on 5-HT neurons alone (Dremencov et al., 2007), however, after sustained treatment with pramipexole or agomelatine, paliperidone administration decreased the activity of 5-HT neurons. These results indicate that increasing the activation of DA-$D_{2/3}$ heteroreceptors on 5-HT neurons is one additional method to elicit a greater net effect on 5-HT transmission and promotes an antidepressant response (Chernoloz et al., 2009a; Chenu et al., 2013). Indeed, DA-D_{2L} receptor knockout impairs 5-HT_{1A}-mediated signalling in 5-HT neurons and the capacity of 5-HT_{1A} receptor agonists to induce antidepressant-like effects

(Shioda et al., 2019) suggesting a minimal level of DA-D_2 receptor activity is required for serotonin-mediated antidepressant effects, as is the case with NE-α_1 activity.

Compound	DA firing activity	DA bursting activity	Somatodendritic DA-$D_{2/3}$ autoreceptor responsiveness	Terminal DA-$D_{2/3}$ heteroreceptor activation	Net effect on DA transmission
MAOI	Decrease (Chenu et al., 2009)	Decrease (Chenu et al., 2009)	N.D.	N.D.	Increase (Colzi et al., 1992)
SSRI	Decrease (Dremencov et al., 2009)	Decrease (Dremencov et al., 2009)	N.D.	N.D.	Decrease (Smith et al., 2000)
Bupropion	No change (El Mansari et al., 2008)	N.D.	N.D.	N.D.	Increase (Li et al., 2002)
Pramiprexole DA-$D_{2/3}$ agonist	Normalized* (Chernoloz et al., 2009a)	Decrease/No change (Chernoloz et al., 2009a)	Decrease (Chernoloz et al., 2009a)	Increase (Chernoloz et al., 2012a)	Increase (Chernoloz et al., 2012a)
Agomelatine	No change (Chenu et al., 2013)	Increase (Chenu et al., 2013)	N.D.	Increase (Chenu et al., 2013)	Increase (Chenu et al., 2013)
Vortioxetine	Decrease (Ebrahimzadeh et al., 2018)	N.D.	N.D.	N.D.	Putative decrease or no change (N.D.)

Table 7. The effects of medications for depression on several parameters related to the activity and function of the DA system. Adapted from El Mansari et al., 2010 with modifications.*Refers to an initial decrease in firing activity upon treatment initiation followed by a recovery. N.D. = no data.

Chapter 1.4: Strategies for overcoming non-response

Despite the emergence of medications for depression targeting various aspects of monoamine neurotransmission, rates of remission are low (30-50%) (Thase et al., 2005; Rush et al., 2006). Furthermore, a delay in providing effective treatment may reduce the likelihood for remission and full functional recovery, therefore it is of upmost importance to provide early optimized treatment strategies (Oluboka et al., 2018). Once a specific treatment at a specific dose is deemed ineffective, the treating physician should first consider a dose increase to optimize treatment. However, a medication switch should be considered if a higher dose is not tolerated or if no improvement is noticed after 2-4 weeks (Kennedy et al., 2016). Switching to a different class of medication may be deemed appropriate when a patient requires a simpler medication regimen (Kennedy et al., 2016; Oluboka et al., 2018). Alternatively, an adjunctive medication may compliment the initial antidepressant drug while targeting specific side effects and residual symptoms when there is a partial response (Cameron et al., 2014; Kennedy et al., 2016; Oluboka et al., 2018). As SSRIs and SNRIs compose ten of the fifteen first-line agents recommended for the treatment of depression (Kennedy et al., 2016), adjunctive treatment is most common with these classes of medication. TRI, mirtazapine and bupropion addition have demonstrated increase remission rates relative to monotherapy (Rush et al., 2006; Blier et al., 2009; Blier et al., 2010; Rocha et al., 2012). The use of medications initially indicated for schizophrenia, at a low dose, have shown consistent success as adjuncts for difficult to treat depression. Previously known as second-generation or atypical antipsychotics, the DA-D_2 receptor antagonist medications olanzapine, quetiapine and risperidone have shown superiority over placebo when used in addition to SSRI or SNRI therapy, as demonstrated in a meta-analysis of ten clinical trials (Papakostas et al., 2007). These medications display higher affinity antagonism for DA-D_2 and/or 5-$HT_{2A/B/C}$ receptors (see table 8; Schotte et al., 1996; Wainscott et al., 1996). The addition of these medications may lead to greater efficacy, although they may increase the side-effect burden related to DA-D_2 receptor antagonism such as tardive dyskinesia (Kane et al., 2004; Papakostas et al., 2007). Finally, the most recent advancement in adjunct therapy has been the use of DA-$D_{2/3}$ partial agonist medications aripiprazole, brexpiprazole and cariprazine (ABCs). Aripiprazole was the first of these medications to emerge and has been deemed superior over placebo when used in addition to SSRI or SNRI therapy, as demonstrated in a meta-analysis of ten clinical trials (Luan et

al., 2018). Whereas there are ongoing trials to more thoroughly evaluate the effectiveness of the latter two medications.

Compound	Binding Affinities (K_i [nM])			
	D_2	**5-HT_{2A}**	**5-HT_{2B}**	**5-HT_{2C}**
Olanzapine	21-31[a]	2.5[a]	12[b]	>1000[c]
Quetiapine	390-700[a]	96[a]	N.D.	11.7[c]
Risperidone	5.9-6.2[a]	0.52[a]	29[b]	8.9[c]

Table 8. Binding affinities of three DA antagonists successfully used as adjunct therapies. Binding affinity values (Ki) taken from [a]Schotte et al., 1996, [b]Wainscott et al., 1996 and [c]Cross et al., 2016. N.D. = no data.

A neurobiological basis for adjunct therapy

Following the monoamine deficiency hypothesis, depressive symptoms may arise from insufficient levels of one or all the monoamine neurotransmitters (Schildkraut, 1965; Coppen, 1967). Furthermore, the most common symptoms that persist after treatment initiation are fatigue, anhedonia, concentration difficulties and cognitive impairment and are thought to be driven by a lack of NE and/or DA neurotransmission (Trivedi et al., 2008; Blier and Briley, 2011; Conradi et al., 2011). In parallel, investigation of the various medications for depression on the monoamine systems has led to the discovery of unintended downstream consequences. For example, long-term SSRI and SNRI administration consistently increase 5-HT neurotransmission yet decrease NE and DA neurotransmission. The inhibitory actions of serotonin on the latter two monoamine systems may give rise to non-response and/or residual symptoms (Blier and El Mansari, 2013). SSRIs are thought to mediate inhibitory actions on the firing of NE neurons via indirect activation of 5-HT_{2A} receptors expressed on gamma-aminobutyric acid (GABA) interneurons projecting to the LC from neighbouring regions (Szabo and Blier, 2001b). Evidence for this is demonstrated by the inhibitory actions of a preferential 5-HT_{2A} receptor agonist (DOI) on NE neurons, which are reversed by a selective 5-HT_{2A} receptor antagonist (MDL 100,907; Szabo and Blier, 2001d). Furthermore, long-term administration of SSRIs, but not the SRI and 5-HT_{2A} receptor antagonist YM992, decrease NE firing activity (Szabo and Blier, 2000; Szabo and Blier, 2002). Finally, risperidone, quetiapine and aripiprazole all possess 5-HT_{2A} receptor antagonist properties and rescue an SSRI-mediated decrease in NE firing (Dremencov et al., 2007; Chernoloz et al., 2009a; Chernoloz et al., 2012b). SSRIs also mediate inhibitory actions on the firing of DA neurons via indirect activation of the 5-HT_{2C} receptor on GABA interneurons found locally in the VTA

(Dremencov et al., 2009). Evidence for this is demonstrated by the inhibitory actions of RO 60-0175, a preferential 5-HT_{2C} receptor agonist, on VTA neurons, which are reversed by the selective 5-HT_{2C} antagonist SB 242084 (Di Matteo et al., 2000). Furthermore, co-administration of SB 242084 or aripiprazole and an SSRI prevents an SSRI mediated-decrease in DA activity (Dremencov et al., 2009). Altogether, these data identify two potential mechanisms by which the DA antagonist and partial agonist medications are effective adjuncts.

An Overview of Aripiprazole, Brexpiprazole and Cariprazine (ABCs)

Third-generation medications for the treatment of schizophrenia have emerged, called the DA-$D_{2/3}$ partial agonist medications, aripiprazole (Shapiro et al., 2003), brexpiprazole (Maeda et al., 2014) and cariprazine (Kiss et al., 2010; collectively termed the ABCs). These compounds are unique from the second-generation counterparts in a variety of ways. Mainly, partial agonist activity at DA-$D_{2/3}$ receptors results in a greater therapeutic versatility of these compounds due to functional selectivity. As the functional selectivity of a compound is a direct function of endogenous neurotransmitter availability, administration of the ABCs would act as DA-$D_{2/3}$ agonists in brain regions where there is less DA. For example, these medications would act as full agonists in brain regions with elevated MAO-A/B activity (Meyer et al., 2006; Moriguchi et al., 2019). As proof of concept, pramiprexole and other mixed DA-$D_{2/3}$ full agonist and have shown effectiveness in depressive patients (Waehrens et al., 1981; Corrigan et al., 2000; Izumi et al., 2000). Furthermore, the ABCs display high affinity 5-HT_{1A} partial agonism *in-vitro*, a drug action which increases tonic inhibition of the hippocampus and displays antidepressant efficacy on its own (Haddjeri et al., 1998; Blier and Ward, 2003). In addition to these drug actions, the ABCs display antagonistic actions at other receptors also targeted by the TRIs, the serotonin-norepinephrine antagonists and the DA antagonist predecessors that may act together to promote the antidepressant response (see table 9).

Compound	Binding Affinities (K_i [nM])												
	D_2	D_3	5-HT_{1A}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_7	H_1	α1A	α1B	α2A	α2B	α2C
Aripiprazole[a]	0.74-3.3	1.0-9.7	5.6	8.7-35	0.36	22-180	10.3	25	25.7	34.8	74.3	103	37.9
Brexpiprazole[b]	0.30-0.35	1.1	0.09-0.12	0.47	1.9	N.D.	N.D.	19	18 (α1)	0.17	N.D.	N.D.	0.59
Cariprazine[c]	0.48-9.3	0.09-0.70	2.6-4.6	19-55	0.58	135	112	23	132	>1000	>1000	N.D.	N.D.

Table 9. Binding affinities of the ABCs. Data provided by: [a]Shapiro et al., 2003, [b]Maeda et al., 2014 and [c]Kiss et al., 2010. N.D. = no data.

The clinician should not discount the use of the other ABCs if one is not efficacious for an individual, as the ABCs have distinct pharmacological profiles that may contribute to the antidepressant response (see table 10). For example, only brexpiprazole has sub-nanomolar affinity for the NE-α_{2C} receptor, similarly to the efficacious medication mirtazapine in MDD. Even then, *in vitro* binding and accumulation assays may not represent the *in vivo* situation. For this reason, the effects of ABCs in electrophysiological studies will be summarized.

Compound	Drug Actions
Aripiprazole	➢ Highest affinity for 5-HT_{2B}, 5-HT_{2C} and 5-HT_7 ➢ Moderate affinity for D_2, 5-HT_{2A}, α_{1A}, α_{1B} and α_{2C} ➢ Lowest affinity for D_3, 5-HT_{1A} and H_1
Brexpiprazole	➢ Highest affinity for D_2, 5-HT_{1A}, 5-HT_{2A}, α_{1B} and α_{2C} ➢ Moderate affinity for D_3, H_1, α_{1A} ➢ Lowest affinity for 5-HT_{2B}
Cariprazine	➢ Highest affinity for D_3 ➢ Moderate affinity for 5-HT_{1A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_7 and H_1 ➢ Lowest affinity for D_2, 5-HT_{2A}, α_{1A} and α_{1B}

Table 10. Summary table comparing the relative affinities of the ABCs.

Electrophysiological studies evaluating the effects of the ABCs

Effects of the ABCs on 5-HT activity

Aripiprazole administered acutely i.v. completely inhibited the firing activity of 5-HT neurons (Stark et al., 2007; Dahan et al., 2009; Oosterhof et al., 2014). Furthermore, aripiprazole 2 mg/kg/day s.c. for 2 and 14 days increased the firing activity of 5-HT neurons (Chernoloz et al., 2009a). Moreover, aripiprazole 2 mg/kg/day s.c. co-administered with escitalopram for 2 and 14 days restored 5-HT firing to baseline (Chernoloz et al., 2009a). Brexpiprazole administered i.v. acutely also completely inhibited the firing activity of 5-HT neurons and reversed a clonidine-

mediated decrease in DoS mediated by electrically-evoked 5-HT release, a proxy for NE-α_2 heteroreceptor activity on 5-HT terminals (Oosterhof et al., 2014). In contrast to aripiprazole, brexpiprazole 1 mg/kg/day s.c. administration for 2 days increased the firing activity of 5-HT neurons that diminished after 14 days of administration (Oosterhof et al., 2016). Cariprazine administered acutely i.v. also completely inhibited the firing activity of 5-HT neurons (Herman et al., 2018). However, cariprazine 0.6 mg/kg/day s.c. for 2 and 14 days left unaltered 5-HT firing activity and did not rescue a SSRI-mediated decrease in 5-HT firing activity after 2 days of co-administration with escitalopram (Ebrahimzadeh et al., unpublished).

Compound	Parameter				
	5-HT$_{1A}$ efficacy (% E_{max})	**Acute inhibition (ED_{50} [mg/kg] i.v.)**	**Firing 2-day administration s.c.**	**Firing 14-day administration s.c.**	**SSRI firing rescue s.c.**
Aripiprazole	73	0.5-0.7	Increased	Increased	2 and 14 days
Brexpiprazole	60	0.25	Increased	No change	N.D.
Cariprazine	39	ED_{100}: 0.15-0.35	No change	No change	14 days

Table 11. Effects of the ABCs on parameters relevant to 5-HT activity. % E_{max} = maximum efficacy relative to endogenous agonist, ED_{50} = the dose at which 50% of the maximal response is achieved in-vivo. N.D. = no data.

Effects of the ABCs on LC-NE activity

There are no electrophysiological data available on the acute effects of aripiprazole on LC-NE neurons. Aripiprazole 2 mg/kg/day s.c. for 2 and 14 days did not elicit any changes in LC-NE firing activity (Chernoloz et al., 2009a). However, the co-administration of aripiprazole and escitalopram for 2 days partially rescued an SSRI-mediated decrease in LC-NE firing activity, and co-administration for 14 days fully rescued an SSRI-mediated decrease in LC-NE firing activity (Chernoloz et al., 2009a). Acute brexpiprazole administration i.v. was able to block NE-mediated NE-α_{1B} activation in the lateral geniculate nucleus, reverse DOI(5-HT$_{2A}$)-mediated inhibition of LC-NE neurons and was able to increase the firing activity of LC-NE neurons 1-4 hours after i.v. administration. Brexpiprazole lacked any activity at NE-α_2 autoreceptors expressed on LC-NE neurons yet was a potent antagonist at NE-α_2 heteroreceptors (Oosterhof et al., 2014). Brexpiprazole 1 mg/kg/day administered s.c. for 2 and 14 days also increased LC-NE firing activity (Oosterhof et al., 2016). Cariprazine administered acutely was able to reverse DOI(5-HT$_{2A}$)-mediated inhibition of LC-NE neurons but was also devoid of an activity on NE-α_2 autoreceptors

expressed on LC-NE neurons (Herman et al., 2018). Cariprazine 0.6 mg/kg/day administered s.c. for 2 and 14 days also increased LC-NE firing activity (Ebrahimzadeh et al., unpublished). Furthermore, cariprazine co-administered with escitalopram for 2 and 14 days fully rescued an SSRI-mediated decreased in LC-NE activity (Ebrahimzadeh et al., unpublished).

Compound	Parameter				
	5-HT_{2A} AA (IC_{50}[nM])	**DOI reversal (ED_{50} [mg/kg] i.v.)**	**Firing 2-day administration s.c.**	**Firing 14-day administration s.c.**	**SSRI firing rescue**
Aripiprazole	55.2	Not tested	No change	No change	2 (partial) and 14 days
Brexpiprazole	6.5	0.11	Increased	Increased	N.D.
Cariprazine	403	0.066	Increased	Increased	2 and 14 days

Table 12. Effects of the ABCs on parameters relevant to NE activity. AA = antagonist activity. IC_{50} = the concentration at which 50% of the maximal response is achieved in-vitro, ED_{50} = the dose at which 50% of the maximal response is achieved in-vivo. N.D. = no data.

Effects of the ABCs on DA activity

Aripiprazole, when applied directly to DA neurons via iontophoresis, inhibits these cells and the effect is blocked with a DA-D_2 but not -D_1 antagonist and is consistent with binding affinity studies (Momiyama et al., 1996; Shapiro et al., 2003). The partial agonistic action of aripiprazole, however, is clearly demonstrated when the inhibition of DA neurons reaches a plateau at about -15 to -30% of baseline (Bortolozzi et al., 2007; Dahan et al., 2009; Oosterhof et al., 2014). Furthermore, aripiprazole can partially reverse DA-D_2 mediated inhibition by apomorphine (ED_{50}: 0.3 mg/kg i.v.; Dahan et al., 2009). When administered long-term for either 2 or 14 days, aripiprazole 2 mg/kg/day s.c. alone has no effect on DA neurons. However, when co-administered with escitalopram, aripiprazole can rescue an SSRI-mediated decrease in firing activity (Chernoloz et al., 2009a). In contrast, brexpiprazole is not able to inhibit the firing activity of DA neurons but can partially reverse DA-D_2 mediated inhibition by apomorphine more potently than aripiprazole (ED_{50}: 0.065 mg/kg i.v.; Oosterhof et al., 2014). In longer-term regimens (2 or 14 days), brexpiprazole 1 mg/kg/day s.c. had no effect on DA neurons (Oosterhof et al., 2016). Finally, cariprazine may also inhibit DA neurons, and reaches a plateau at about -40% of baseline (Delcourte et al., 2018). Long-term administration regiments of cariprazine 0.6 mg/kg/day s.c. for 2 and 14-days did not modify the firing activity of DA neurons and did not rescue an SSRI-mediated decrease in firing activity (Ebhrahimzadeh et al., unpublished).

Compound	Parameter						
	D_2:D_3 Efficacy (% E_{max})	**Acute inhibition i.v.**	**Apomorphine Reversal (ED_{50} [mg/kg])**	**Firing 2-day administration s.c.**	**Firing 14-day administration s.c.**	**5-HT_{2C} Affinity (K_i)**	**SSRI firing rescue s.c.**
Aripiprazole	61:28	-15 to -30%	0.3	No change	No change	22-180	2 and 14 days
Brexpiprazole	43:15	None	0.065	No change	No change	N.D.	N.D.
Cariprazine	30:71	-40%	Not tested	No change	No change	135	No

Table 13. Effects of the ABCs on parameters relevant to DA activity. % E_{max} = maximum efficacy relative to endogenous agonist, ED_{50} = the dose at which 50% of the maximal response is achieved in-vivo. N.D. = no data.

Effects of the ABCs on 5-HT and NE transmission in the hippocampus

Long-term treatment with brexpiprazole and cariprazine alone elicited an increase in the tonic activation of 5-HT_{1A} receptors in the hippocampus, whereas aripiprazole did not (Oosterhof et al., 2016; Ebhrahimzadeh et al., 2019; Ebhrahimzadeh et al., unpublished). However, the long-term co-administration of aripiprazole and escitalopram elicited a greater increase in the tonic activation of 5-HT_{1A} receptors relative to escitalopram alone, indicating a synergy (Ebhrahimzadeh et al., 2019). Finally, only brexpiprazole elicited an increase in the tonic activation of NE-α_2 receptors in the hippocampus at 2 and 14 days (Oosterhof et al., 2016).

Compound(s)	Net effects on neurotransmission in the hippocampus			
	5-HT		**NE**	
	2 days	**14 days**	**2 days**	**14 days**
Aripiprazole	≈	≈	N.D.	N.D.
Aripiprazole/Escitalopram	≈	↑↑	N.D.	N.D.
Brexpiprazole	↑	↑	↑	↑
Cariprazine	≈	↑	≈	≈
Cariprazine/Escitalopram	≈	≈	≈	≈

Table 14. Effects of the ABCs alone or in combination with the SSRI escitalopram on net 5-HT and NE neurotransmission. ↑ indicates an increase, ↓ indicates a decrease and ≈ indicates no change on neurotransmission in the hippocampus. N.D. = no data.

Trends, similarities and differences of the ABCs

The ED_{50} value for cariprazine could not be determined, although brexipiprazole and cariprazine inhibited 5-HT neurons more potently than aripipirazole, irrespective of intrinsic activity (see table 11). Interestingly, aripiprazole alone elicited an increase in 5-HT firing activity at 2 and 14 days, where brexpiprazole only did so for 2 days and cariprazine did not whatsoever. To reiterate, aripiprazole is the highest efficacy and cariprazine is the lowest efficacy DA- D_2 receptor

partial agonist. Furthermore, DA-D_2 receptor activation has been shown to increase the firing activity of 5-HT neurons (Chernoloz et al., 2009a; Chenu et al., 2013). This factor might explain: i) the capacity of aripiprazole to rescue an SSRI-mediated decrease in 5-HT activity at 2 days, and maintained for 14 days ii) the capacity of brexpiprazole to increase 5-HT neuron firing for only 2 days and iii) the failure of cariprazine to elicit an increase in 5-HT firing or rescue an SSRI-mediated decrease in 5-HT activity at any time point. The superiority of aripiprazole to modulate 5-HT activity, even in the presence of SSRIs, may explain its efficacy as an adjunct.

Cariprazine weakly antagonised the 5-HT_{2A} receptor *in-vitro* yet more potently reversed a DOI-mediated inhibition of NE activity relative to brexpiprazole *in-vivo* (Oosterhof et al., 2014; Herman et al., 2018). This is surprising considering brexpiprazole is a relatively higher affinity antagonist and displays more potent antagonist activity at 5-HT_{2A} receptors (Maeda et al., 2014). Moreover, cariprazine fully rescued an SSRI-mediated decrease in NE activity at 2 days. Whereas, aripiprazole only did so at 14 days. This is probably due to the higher affinity of cariprazine for the 5-HT_{2A} receptor relative to aripiprazole *in-vivo.* Interestingly, 5-HT_{2A} receptor antagonism is thought to diminish extrapyramidal side-effects (EPS) such as akathisia (Tatara et al., 2012). In a pooled-data analysis, cariprazine monotherapy produced an incidence of EPS (17-26%) similarly to aripiprazole adjunctively (25% for akathisia) and relatively higher than brexpiprazole adjunctively (9% for akathisia; Citrome, 2015). Sub-analysis of cariprazine doses in one study of bipolar depression revealed that lower doses of cariprazine (0.75-1.5 mg/day) produced an incidence of akathisia similarly to brexpiprazole (8-12%; Durgam et al., 2016). This suggests that lower doses of cariprazine typically used adjunctively would produce an incidence of akathisia lower than aripiprazole and would be in line with preclinical evidence.

Interestingly, the acute inhibitory effects of aripiprazole are blocked by a selective DA-D_2 antagonist but not by not by a preferential DA-D_3 antagonist (Etievant et al., 2009). In contrast, the acute inhibitory effects of cariprazine are blocked by a selective DA-D_3 antagonist but not by a selective DA-D_2 antagonist (Delcourte et al., 2018). Altogether, these results speak to the preferential efficacies of aripiprazole and cariprazine, however, they are both able to elicit significant decreases in DA firing activity (Oosterhof et al., 2014; Delcourte et al., 2018). Brexpiprazole is weakly efficacious for both DA-D_2 and -D_3 receptors and cannot elicit significant changes in DA firing activity acutely (Maeda et al., 2014; Oosterhof et al., 2014). However,

brexpiprazole potently reverses the effects of a high efficacy DA-D_2 agonist (i.e., apomorphine) due to its slightly higher affinity in addition to low efficacy (Oosterhof et al., 2014). The lack of agonistic activity at these receptors may explain the lower rates of akathisia in those individuals receiving brexpiprazole adjunctively (9%) relative to aripiprazole adjunctively (25%) and cariprazine alone (17-26%) (Citrome, 2015). No changes in overall DA firing rate were detected in any of the ABCs administered alone, although aripiprazole but not cariprazine was able to rescue an SSRI-mediated decrease in DA firing activity (Chernoloz et al., 2009a; Ebrahimzadeh et al., unpublished). This is probably due to the higher affinity of aripiprazole for the 5-HT_{2C} receptor relative to cariprazine *in-vivo.*

When applied by microiontophoresis, brexpiprazole and cariprazine reduced the activity of hippocampal neurons and this effect was blocked after systemic 5-HT_{1A} antagonist administration (Oosterhof et al., 2014; Herman et al., 2018). However, brexpiprazole and cariprazine did not reduce the capacity of 5-HT to inhibit hippocampal neurons, indicating full agonistic activity at these post-synaptic 5-HT_{1A} receptors (Oosterhof et al., 2014; Herman et al., 2018). In line with these acute data, long-term treatment with brexpiprazole and cariprazine, but not aripiprazole, increased 5-HT neurotransmission in the hippocampus indicating that these exogenous agonists can have a summating action with endogenous 5-HT and not attenuate its signal transduction. Theoretically, these medications could be effective as a monotherapy in the clinic because effective monotherapies for depression can increase 5-HT neurotransmission in the hippocampus (Blier et al., 1997; Haddjeri et al., 1998). In contrast, co-administration of escitalopram with cariprazine diminished the prior effect on 5-HT neurotransmission (Ebrahimzadeh et al., unpublished). Whereas, co-administration of escitalopram with aripiprazole synergistically increased 5-HT neurotransmission in the hippocampus more so than escitalopram alone (Ebhrahimzadeh et al., 2019). Finally, brexpiprazole elicited an increase in NE neurotransmission in the hippocampus at 2 and 14 days (Oosterhof et al., 2016).

In summary, the ABCs should be considered as separate treatment strategies due to their differential effects on the monoamine systems. All three of these medications augment 5-HT activity on their own, albeit detected via distinct electrophysiological paradigms. Aripiprazole may be suitable as a DA augmenting strategy because it may prevent an SSRI-induced decrease in DA activity and increases the tonic activation of 5-HT neurons via this mechanism (Chernoloz et al.,

2009). Brexpiprazole may be suitable as a NE augmenting strategy because it may elicit an increase in LC-NE neuron activity alone and in NE neurotransmission to projection areas (Oosterhof et al., 2016). However, further studies evaluating its electrophysiological effects when co-administered an SSRI are needed. Finally, cariprazine may be suitable as a NE augmenting strategy because it may elicit an increase in LC-NE neuron activity alone and prevent an SSRI-induced decrease in LC-NE activity (Ebhrahimzadeh et al., unpublished).

A case for studying the 5-HT_{2B} receptor

In an analysis of eight short-term trials evaluating the antidepressant effect of adjunctive aripiprazole, three studies using doses of 2.5-3 mg/day produced similar remission rates as studies using doses up to 20 mg/day (Luan et al., 2018). A second meta-analysis confirmed this finding, demonstrating similar remission rates between patients receiving low-dose aripiprazole (5 mg/day or less) and high-dose aripiprazole (more than 5 mg/day) (Romeo et al., 2018). Interestingly, Kamijima et al (2013) reported a significant improvement of 5/10 Montgomery Asberg Depression Rating Scale (MADRS) items one week after aripiprazole 3 mg/day administration, that is when the drug had reached only half of its stable plasma concentration. Therefore, the neural effects of aripiprazole at low doses may be correlated with the antidepressant response. Whereas, higher doses may produce more side-effects and decrease tolerability and compliance. Aripiprazole administered at lower doses (0.3-3 mg/kg) increased DA dialysate levels in the mPFC (Li et al., 2004; Zocchi et al., 2005; Bortolozzi et al., 2007; Tanahashi et al., 2012). These results, along with exogenous DA-$D_{2/3}$ receptor agonism, suggest that aripiprazole may be exerting an antidepressant response by increasing mesocortical DA transmission, an effect that would be beneficial for an individual suffering from an MDE plausibly due to increased monoamine catabolism (Meyer et al., 2006; Moriguchi et al., 2019). Whereas, aripiprazole administered at higher doses (10 mg/kg) decreased DA outflow in the mPFC and striatum (Semba et al., 1995; Li et al., 2004; Tanahashi et al., 2012) and are consistent with clinical data demonstrating the efficacy of higher doses of aripiprazole in schizophrenia (Stip and Tourjman, 2010). Furthermore, preclinical data has demonstrated multiple additive and synergistic effects of low dose aripiprazole when combined with SSRIs; aripiprazole administered alone at a low dose (2 mg/kg/day s.c.) increased the activity of 5-HT neurons at 2 and 14 days, and rescued an SSRI-mediated decrease in 5-HT and DA activity at 2 days and all three monoamine systems at 14 days. Long-term aripiprazole and escitalopram (2/5

mg/kg/day) co-administration increased 5-HT neurotransmission in the hippocampus, whereas subthreshold escitalopram, and aripiprazole alone did not (Ebrahimzadeh et al., 2019). The acute concomitant administration of a low-dose of aripiprazole (0.03-0.3 mg/kg i.p.) and a subthreshold dose of an SSRI consistently decreased immobility time in the tail suspension test (TST) or forced swim test (FST; Kamei et al., 2008; Bourin et al., 2009). Importantly, the synergistic effects of aripiprazole (2 mg/kg s.c.) along with a therapeutic dose of an SSRI on the FST were preserved over time and were superior to the antidepressant-like effects of an SSRI alone (Lapointe et al., 2019). Altogether, these data suggest that low-dose aripiprazole is exerting some therapeutic effects via its highest affinity targets; the DA-D_2 receptor and 5-HT_{2B} receptor. However, aripiprazole mediated 5-HT_{2B} receptor antagonism (K_i = 0.36 nM; Shapiro et al., 2003) has never been explored in its capacity to contribute to the antidepressant response the context of adjunct therapy.

Chapter 1.5: 5-HT_{2B} receptors: signalling, expression, and function in the central nervous system (CNS)

5-HT_{2B} receptor signalling

The 5-HT_2 family of receptors include the 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} receptor subtypes (Barnes and Sharpe, 1999). This family of receptors classically couples to the G_q signalling cascade, leading to phospholipase C (PLC) activation, cleavage of phosphatidyl inositol diphosphate (PIP_2) to diacylglycerol (DAG) and inositol triphosphate (IP_3) and the release of intracellular calcium (Barnes and Sharpe, 1999). Phylogenetics has demonstrated that the 5-HT_{2B} receptor is more genetically distant than it's 5-HT_{2A} and 5-HT_{2C} counterparts and may have a distinct physiological role (Barnes and Sharpe, 1999). Indeed, the 5-HT_{2B} receptor has been shown to couple to alternative signalling pathways. In the absence of 5-HT, constitutive phosphorylation of 5-HTT by the 5-HT_{2B} receptor is mediated by the nitric oxide/protein kinase G (NO/PKG) pathway and is associated with maximal 5-HTT transport capacity (Launay et al., 2006). However, 5-HT_{2B} receptor stimulation promotes additional phosphorylation of 5-HTT and the Na^+, K^+-ATPase (Launay et al., 2006). Chronic activation of the 5-HT_{2B} receptor may lead to the hyperphosphorylation of these two targets, reducing both 5-HTT velocity and Na^+ / K^+ exchange (Launay et al., 2006). Furthermore, SSRI medications have an impaired capacity to bind to the hyperphosphorylated 5-HTT (Launay et al., 2006). Alternatively, activation of the 5-HT_{2B} receptor may also initiate the phospholipase-A2/arachidonic acid (PLA2/AA) signalling cascade leading to upregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and subsequent reactive oxygen species (ROS) production (Schneider et al., 2006). Increased ROS signals activation of tumor necrosis factor(TNF)-α converting enzyme (TACE) metalloproteinase, which catalyzes the proteolytic cleavage of pro-TNF-α to soluble TNF-α. Finally, TNF-α increases intraneuronal MAO activity leading to 5-HT catabolism (Schneider et al., 2006). Altogether, these studies demonstrate two pathways by which the 5-HT_{2B} receptor can regulate 5-HT levels. By decreasing reuptake velocity and degrading intraneuronal 5-HT stores, tonic 5-HT_{2B} receptor activation may significantly decrease the amount of 5-HT available for release and may have important implications for the antidepressant response. Paradoxically, SSRI medications also reduce 5-HTT reuptake velocity. As 5-HT_{2B} receptor antagonism is added to an SSRI regimen routinely in clinical practice, reduction of 5-HTT reuptake velocity may not be sufficient in a subset of TRD individuals.

5-HT$_{2B}$ receptor expression in the brain

The first report of the 5-HT$_{2B}$ receptor characterized its role in 5-HT mediated contractions of the rat stomach fundus (Baxter et al., 1994). However, owing to its relatively low expression, there were several conflicting reports regarding the presence of the 5-HT$_{2B}$ receptor messenger ribonucleic acid (mRNA) in the brain (Shmuck et al., 1994; Barnes and Sharpe, 1999). Then, immunohistochemical identification of this receptor in the mouse and rat brains ended this dispute (Doo-Sup Choi and Maroteaux, 1996; Duxon et al., 1997). Interestingly, the mouse, rat and human 5-HT$_{2B}$ receptors are expressed to a similar degree across certain brain regions. For example, the highest expression of 5-HT$_{2B}$ receptors is consistently found in the cerebellum, while the lowest expression is found in the hippocampus (Doo-Sup Choi and Maroteaux, 1996; Duxon et al., 1997; Bevilacqua et al., 2010). Unlike the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors widespread distribution in the CNS, the 5-HT$_{2B}$ receptor appears to be expressed in discrete brain regions and virtually all brainstem nuclei (Duxon et al., 1997; Bonnaventure et al., 2002). These receptors appear to have a somatic localization on frontal cortex pyramidal neurons (Duxon et al., 1997; Niebert et al., 2011). Furthermore, recent advancements in single-cell reverse-transcription polymerase chain reaction has allowed for precision acquisition of mRNA from specific cell-types. Among Pet-1/tryptophan hydroxylase-2(Tph2)/5-HT$_{1A}$ positive neurons in the mouse DRN, 80% expressed 5-HT$_{2B}$ receptor mRNA (Diaz et al., 2012). Belmer et al., (2018) revealed a somatodendritic localization of the 5-HT$_{2B}$ receptor on 5-HT neurons, suggesting a role as an autoreceptor. In addition, Cathala et al., (2019) revealed the expression the 5-HT$_{2B}$ receptor on GABAergic interneurons of the rat DRN and suggests a species-related anatamo-functional difference. However, neither study investigated the opposing cell-type and therefore cannot exclude the possibility of expression on both 5-HT and GABA neurons. In contrast, only 40% of DA-D$_2$/tyrosine hydroxylase (TH) positive neurons in the VTA expressed 5-HT$_{2B}$ receptor mRNA, although the exact localization of the receptor could not be determined. These 5-HT$_{2B}$ receptor expressing mesostriatal DA neurons projected exclusively to the NAc shell (Doly et al., 2017). In summary, 5-HT$_{2B}$ receptors are expressed in brain regions previously shown to be affected in depression and may provide a unique avenue to modulate the antidepressant response.

Mouse (IHC + mRNA)	Rat (IHC + mRNA)	Human (mRNA[g])
Cerebellum, hippocampus (IHC[a]) DRN 5-HT neurons[b], VTA DA neurons[c] (mRNA)	Cerebellum, medial amygdala, lateral septum, dorsal hypothalamic nucleus, frontal cortex, spinal cord, hippocampus (IHC[d]) DRN GABA internenurons[f], LC, Paraventricular nucleus of the hypothalamus, habenula (mRNA[e])	Cerebellum, occipital lobe, frontal lobe, parietal lobe, medulla oblongata, temporal lobe, pituitary gland, nucleus accumbens, pons, olfactory region, diencephalon, hippocampus, thalamus

Table 15. Distribution of 5-HT_{2B} receptor mRNA and protein across mouse, rat and human brain. Data taken from [a]Doo-Sup Choi and Maroteaux, 1996, [b]Diaz et al., 2012, [c]Doly et al., 2017, [d]Duxon et al., 1997, [e]Bonnaventure et al., 2002, [f]Cathala et al., 2019 and [g]Bevilacqua et al., 2010.

Effects of 5-HT_{2B} receptor ligands on neural activity

Studying the role of the 5-HT_{2B} receptor historically has been difficult because of the lack of available selective agonists and antagonists. However, considerable progress has been made in the past two decades. Two compounds, LY266097 and RS127445 were identified as high affinity antagonists with over 100- and 1000-fold selectivity over the other 5-HT_2 subtypes, respectively (Audia et al., 1996; Bonhaus et al., 1999; see Devroye et al., 2018 for a detailed review of ligands). Furthermore, BW723c86 has been identified as a preferential 5-HT_{2B} receptor agonist, with conflicting reports of selectively over the other 5-HT_2 subtypes ranging from 2 to 10-fold (Cussac et al., 2002; Knight et al., 2004; Banas et al., 2011). The effects of these ligands on neural activity and behaviour will be discussed below.

Effects of 5-HT_{2B} receptor ligands on 5-HT activity

There are conflicting lines of evidence as to how the 5-HT_{2B} receptor may alter the activity of 5-HT neurons in mice and rats. In mice, local infusion of BW723c86 into the DRN increased local 5-HT outflow and this effect was blocked by RS127445. Whereas, infusion of RS127445 had no effect on its own (Doly et al., 2008). Furthermore, bath-application of BW723c86 increased the firing rate of 5-HT neurons *ex-vivo* and acute pre-treatment with BW723c86 (5 mg/kg s.c.) reduced the inhibitory effects of a 5-HT_{1A} agonist *in-vivo* (Belmer et al., 2018). Finally, pre-treatment with RS127445 (0.5 mg/kg i.p.) diminished an SSRI-induced increase of 5-HT in the hippocampus (Diaz et al., 2012). Altogether, these results suggest an excitatory effect of 5-HT_{2B} receptor activation on the activity of 5-HT neurons.

In rats, local infusion of RS127445 into the DRN increased local 5-HT outflow and increased 5-HT outflow in the mPFC (Devroye et al., 2017; Cathala et al., 2019). RS127445 (0.16 mg/kg i.p.) increased 5-HT outflow into the DRN into the mPFC as well, whereas, BW723c86 (2.5 mg/kg i.p.) failed to alter 5-HT outflow in the frontal cortex (Gobert et al., 2000; Cathala et al., 2019). These antagonist-mediated effects were abolished by the co-infusion of bicuculline (a $GABA_A$ antagonist) suggesting a disinhibition phenomenon, in which GABAergic interneurons may inhibit 5-HT neurons via the $5\text{-}HT_{2B}$ receptor (Cathala et al., 2019). Furthermore, acute RS127445 (0.16 mg/kg i.p.) administration increased the activity of 5-HT neurons *in-vivo* (Devroye et al., 2017).

These two sets of results are in clear opposition of each other and may be explained by a combination of 1) inter-species differences and 2) differential effects on $5\text{-}HT_{2B}$ receptor expressing versus non-expressing neurons in the DRN (Diaz et al, 2012; Cathala et al., 2019). Clearly, studies using longer-term treatments with these ligands are warranted in order to mimic the clinical condition where patients take medications for long periods of time.

Effects of $5\text{-}HT_{2B}$ receptor ligands on DA activity

Systemic administration of BW723c86 (2.5-3 mg/kg i.p. or s.c.) failed to alter DA outflow in the nucleus accumbens (NAc) and the frontal cortex (Gobert et al., 2000; Auclair et al., 2010). Contrary to these results, systemic administration of RS1277445 (0.16 mg/kg i.p.) or LY266097 (0.63 mg/kg i.p.) decreased and increased DA levels in the NAc and the frontal cortex, respectively (Auclair et al., 2010; Devroye et al., 2016; Devroye et al., 2017). Furthermore, acute RS127445 (0.16 mg/kg i.p.) administration decreased DA neuron firing, whereas long-term LY266097 (0.63 mg/kg i.p.) administration increased DA neuron firing (Chenu et al., 2014; Devroye et al., 2017). In summary, $5\text{-}HT_{2B}$ receptor activation exerts an opposite control on ascending DA pathways (Devroye et al., 2017), although long-term pharmacological inactivation of this receptor may be required to increase overall DA activity.

A possible role for $5\text{-}HT_{2B}$ receptors in the antidepressant response

Several lines of preclinical evidence have demonstrated the involvement of $5\text{-}HT_{2B}$ receptors in the antidepressant-like response (Diaz et al., 2012; Diaz et al., 2016; Belmer et al., 2018). For example, $5\text{-}HT_{2B}$ receptor knockout ($5\text{-}HT_{2B}$ KO) mice display an antidepressant-like

phenotype, such that at baseline, these mice exhibit a reduced latency to feed in the novelty-supressed feeding task, increased sucrose consumption and express increased BDNF mRNA and protein levels in the hippocampus (Diaz et al., 2016). In contrast, selective ablation of 5-HT_{2B} receptors expressed on 5-HT neurons in mice (5-HT_{2B} $KO^{5\text{-}HT}$) prevents the effects of fluoxetine in the FST (Belmer et al., 2018). Whereas, acute BW723c86 (3 or 10 mg/kg) administration decreased immobility time in the FST (Diaz et al., 2011; Diaz et al., 2012). Furthermore, in 5-HT_{2B} $KO^{5\text{-}HT}$ mice or the pharmacological blockade of the 5-HT_{2B} receptor prevents an SSRI-induced increase in 5-HT levels and cell proliferation in the hippocampus, suggesting these receptors are required for the neurogenic effect of SSRIs (Diaz et al., 2012; Belmer et al., 2018). Clinically, agomelatine does not block the 5-HTT yet can produce an antidepressant response as a result of $MT_{1/2}$ activation and 5-$HT_{2B/C}$ receptor antagonism. However, 5-HT_{2B} receptor antagonism was the only drug action able to elicit an increase in DA firing activity and all three drug actions were required to fully mimic the effects of agomelatine on DA neurons (Chenu et al., 2013; 2014).

Rational for the possible involvement of 5-HT_{2B} receptor antagonism in the antidepressant efficacy of adjunct aripiprazole

Objective 1: As previously discussed, 5-HT_{2B} receptor activation exerts an opposite control on ascending DA pathways (Devroye et al., 2017). The first objective of this study is to extensively explore the electrophysiological effects of 5-HT_{2B} receptor ligands on DA activity.

Objective 2: Moreover, the co-administration of aripiprazole and escitalopram, rescues an SSRI-mediated decrease in 5-HT and DA activity at two days (Chernoloz et al., 2009a) and administration of 5-HT_{2B} receptor antagonists increased 5-HT and DA activity (Chenu et al., 2014; Devroye et al., 2017). Therefore, the second objective of this study is to investigate the 5-HT_{2B} receptor antagonistic property of aripiprazole and how it may be mediating these rescue effects on SSRI-mediated suppression of 5-HT and DA activity.

Objective 3: Furthermore, low dose aripiprazole (0.3-3 mg/kg) and 5-HT_{2B} receptor antagonists can elicit an increase in DA outflow into the mPFC (Li et al., 2004; Zocchi et al., 2005; Bortolozzi et al., 2007; Tanahashi et al., 2012; Devroye et al., 2016; Devroye et al., 2017). The rat mPFC is somewhat analogous to the dlPFC (Brodmann area 46) in humans and shows a late increase in metabolic activity in treatment-responsive MDD patients (Mayberg et al., 2000; Uylings

et al., 2003). The third objective of this study is to explore the acute and long-term effects of aripiprazole and 5-HT_{2B} receptor antagonists on mPFC pyramidal cortical activity.

Objective 4: Previous research has demonstrated an interaction between the 5-HTT and 5-HT_{2B} receptor activation *in-vitro,* such that activation of the 5-HT_{2B} receptor impairs 5-HTT transport efficacy via hyperphosphorylation of 5-HTT and the Na+,K+ -ATPase (i.e. slowing re-uptake of 5-HT) (Launay et al., 2006). The fourth objective of this study is thus to explore 5-HTT transport efficacy after microiontophoretic application of a 5-HT_{2B} receptor agonist.

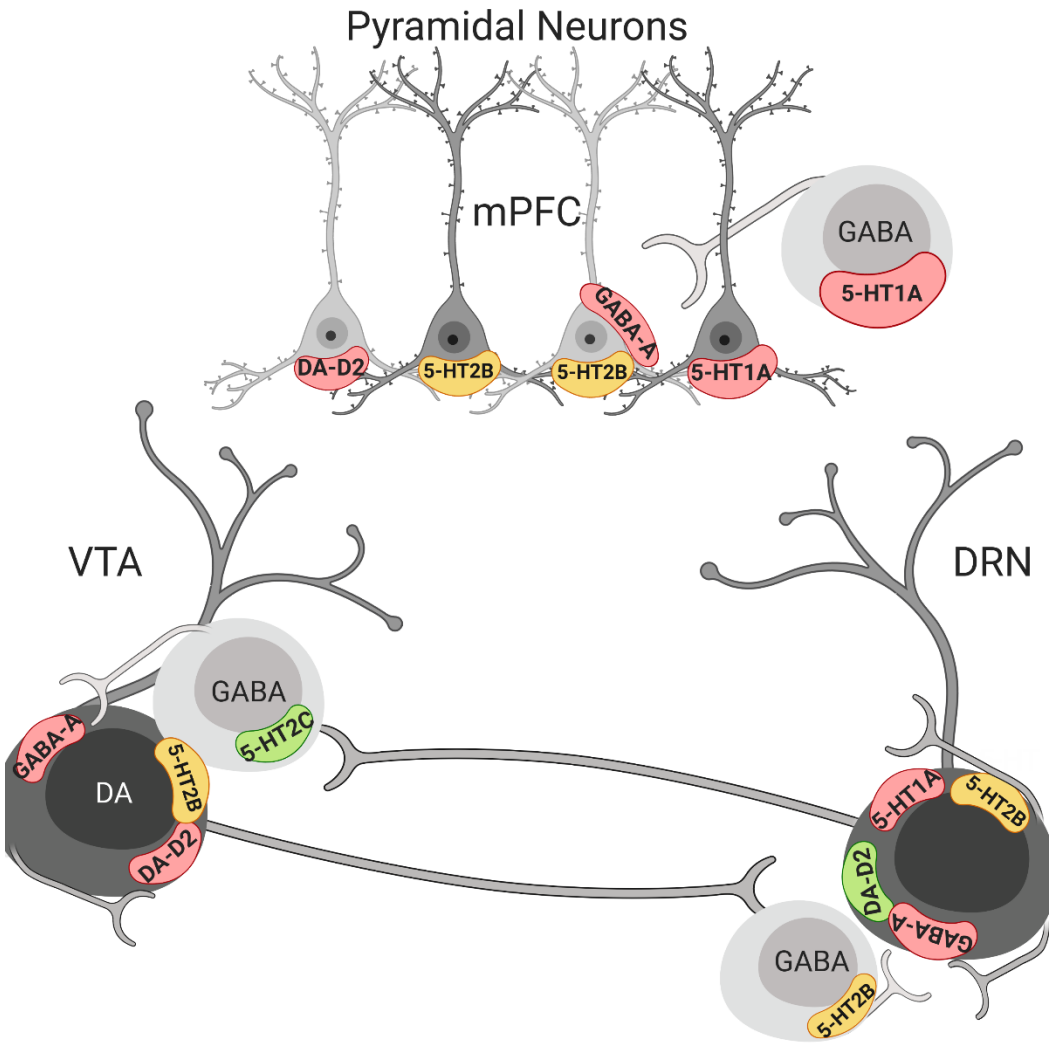

Figure 3. Schematic representation of putative 5-HT_{2B} receptor expression on cortical pyramidal neurons and on monoaminergic nuclei. In-vivo electrophysiology is the ideal technique to assess overall changes elicited by 5-HT_{2B} receptor manipulation and subsequent reciprocal interactions at various heteroreceptors. Red indicates inhibitory activity; green indicates excitatory activity and yellow indicates unknown activity and undergoing investigation.

Chapter 2: Methods and materials

Animals

Male Sprague-Dawley rats (Charles River, St. Constant, Canada) weighing 250-350g were housed under standard laboratory conditions (12 h light/dark cycle), with access to food and water *ad libitum. In-vivo* extracellular recordings were carried out in chloral hydrate anesthetized rats (400 mg/kg i.p.) proceeding fixation of the rodent into the stereotaxic apparatus. These extracellular recordings in the VTA, DRN and mPFC were carried out using single-barrel glass micropipettes (Stoelting, Wood Dale, IL, USA) preloaded with a 2 M sodium chloride solution. The rodent's body temperature was maintained at 37°C throughout the experiment via a water-based heating pad. If applicable, prior to the electrophysiologic recordings, a catheter was inserted into the lateral tail vein for systemic i.v. injection of pharmacologic agents. At the end of the experiments, animals were euthanized with a lethal dose of chloral hydrate. All animals were handled according to the Canadian Council on Animal Care (CACC) guidelines, and all protocols of this study were approved by the local Animal Care Committee (The Royal's Institute of Mental Health Research, Ottawa, Canada).

In-vivo electrophysiological recordings

Recording of VTA DA neurons. Putative DA neurons were recorded by positioning the single-barrel glass micropipettes according to the following coordinates (in millimeters from lambda): A-P, 3.2-3.7; M-L, 0.6-1.0; D-V, 7.0-9.0. At these coordinates, DA neurons were identified according to the following electrophysiological properties: 1) a firing rate of 2-10 Hz, 2) a biphasic or triphasic action potential with a "notch" in the rising phase and a prominent negative inflection and, 3) a spike duration >1.1 ms from spike initiation to the trough of the negative inflection. Furthermore, burst firing in DA neurons was analysed using the following characterization: a series of 2-10 spikes of decreasing amplitude ("spike train"), with a maximal interspike interval (ISI) of 80 ms for initiation of the spike train, and a maximal ISI of 160 ms for the continuation of the spike train (Grace and Bunney, 1984; Ungless and Grace, 2012).

Recording of DRN 5-HT neurons. Putative 5-HT neurons were recorded by positioning the single-barrel glass micropipettes according to the following coordinates (in millimeters from lambda): A-P, 0.8-1.2; M-L, 0; D-V, 5.0-7.0. At these coordinates, 5-HT neurons were identified according to the following electrophysiological properties: 1) a firing rate of 0.5-3 Hz, 2) a biphasic

or triphasic action potential with steady, rhythmic firing and, 3) a spike duration of 1.5-3.0 ms (Vandermaelen and Aghajanian, 1983). Furthermore, burst firing in DRN 5-HT neurons was analysed using the following characterization: a series of 2 or more spikes, with a maximal ISI of 20 ms for initiation and continuation of the spike train (Hajos et al., 2007).

Recording of prelimbic/infralimbic medial prefrontal cortical (mPFC) pyramidal neurons. Putative mPFC pyramidal neurons were recorded by positioning the single-barrel glass micropipettes according to the following coordinates (in millimeters from bregma): A-P, 3.2-3.4; M-L, 0.6-0.8; D-V, 2.5-5.5. mPFC neurons were further characterized based on subregion (Paxinos and Watson, 2007), such that all neurons recorded at a D-V of 2.5-4.3 were classified as prelimbic (PrL) and all neurons recorded at a D-V of 4.3-5.5 were classified infralimbic (InFr). At these coordinates, pyramidal neurons were identified according to the following electrophysiological properties: 1) a firing rate of 0.01-3 Hz, 2) a biphasic or triphasic action potential with highly irregular firing and, 3) a spike with a positive inflection duration greater than 0.36 ms and negative inflection duration greater than 1.08 ms to exclude any fast-spiking interneurons. Furthermore, burst firing in mPFC pyramidal neurons was analysed using the following characterization: a series of 2 or more spikes, with a maximal ISI of 45 ms for the initiation and continuation of the spike train (Laviolette et al., 2005; Riga et al., 2017).

Recording of hippocampal CA3 dorsal hippocampus neurons. Putative hippocampal pyramidal neurons were identified by positioning five-barrel glass micropipettes according to the following neuroanatomical coordinates (in millimeters from lambda): A/P, 4.0-4.2; M/L; 4.0-4.2; D/V, 3.5-4.5. Since pyramidal neurons do not discharge spontaneously under chloral hydrate anesthesia, quisqualic acid was used to activate these neurons within their physiological range (10-15 Hz; Ranck, 1975). At these coordinates, putative pyramidal neurons were identified according to the following electrophysiological properties: 1) large amplitude (0.5-1.2 mV), 2) long duration (0.8-1.2 ms) simple action potentials alternating with 3) complex spike discharges.

Chemical compounds and administration regimens

Acute systemic treatment experiments. BW723c86, a preferential 5-HT_{2B} receptor agonist (1 mg/kg) and RS127445 a selective 5-HT_{2B} receptor antagonist (2 mg/kg) (Tocris; Burlington, Canada) were dissolved in 10% lactic acid. BW723c86 (1 mg/kg) was administered alone i.v. every 90 seconds until the total dose of 6 mg/kg was reached, or five minutes after the administration of

RS127445 (2 mg/kg s.c.). In these same experiments, the full DA agonist apomorphine (40 µg/kg i.v.) and the full DA antagonist haloperidol (200 µg/kg i.v.) (Sigma-Aldrich; Oakville, Canada) were dissolved in distilled water and were administered at the end of DA neuron recordings to confirm their identity pharmacologically. In another set of acute systemic experiments, aripiprazole (0.6 mg/kg i.v.) (LKT Laboratories; St. Paul, USA) was dissolved in 2% lactic acid and was administered after initially recording 5-8 neurons per rat to establish baseline activity.

Short-term (2-day) administration experiments. LY266097, a selective 5-HT_{2B} receptor antagonist (0.6 mg/kg/day i.p.) (Tocris; Burlington, Canada) was dissolved in 20% hydroxypropyl-beta-cyclodextrin as previously described (Chenu et al., 2014). Escitalopram (10 mg/kg/day via osmotic mini-pump) was provided as a gift (Lundbeck) and was dissolved in distilled water. These drugs were administered alone or concomitantly for two days. The appropriate vehicle was used all control animals.

Long-term (14-day) administration treatment experiments. Aripiprazole and escitalopram (10 mg/kg/day via osmotic mini-pump) were administered alone or concomitantly for 14 days. Additionally, LY266097 (0.6 mg/kg/day i.p.) was administered alone in sham operated rats or in conjunction with escitalopram treated rats on the last three days of a 14-day treatment regimen. The appropriate vehicle was used all control animals.

Iontophoretic experiments for in-vivo determination of 5-HT uptake. The following compounds were used to fill the 5-barrel electrode: 10 mM 5-HT in 200 mM NaCl (pH 4), 10 mM BW723c86 in 200 mM NaCl (pH 3.1), 1.5 mM quisqualate in 200 mM NaCl (pH 8), and 2 M NaCl used for automatic current balancing. The fifth barrel is used for recording. Iontophoretic ejection of 5-HT for 50 seconds (s) suppresses the firing activity of pyramidal neurons of the CA3 region of the hippocampus. The inhibited pyramidal neurons gradually regain their initial firing activity after the completion of ejection due to reuptake of 5- HT. To reliably determine in vivo the activity of 5-HT transporter (5-HTT), RT50 index was used. It is defined as the time elapsed from the cessation of iontophoretic application of 5-HT to 50% recovery of the initial firing rate (de Montigny et al., 1980; Piñeyro et al., 1994). RT50 (via the ejection of 5-HT at a current of +10 nA) was determined after the following manipulations recorded in a single neuron, during the continuous ejection of quisqualate at -1 nA: No treatment (baseline), escitalopram 0.2 mg/kg administered i.v., and during 3 minutes of continuous 10 mM BW723c86 ejection at a current of +20 nA.

Data acquisition and statistical analyses

Two-minute recordings of VTA DA neurons and DRN 5-HT neurons, as well as five-minute recordings of mPFC pyramidal neurons were acquired using CED Spike2 data acquisition software. Data were exported to burstiDAtor (www.github.com/nno/burstidator/releases) for firing (spikes/s) and bursting (bursts/min) rate analysis of all neurons according to their varying parameters above. Data are expressed as means ± S.E.M. and were analyzed using SigmaPlot 12.5. The following tests were employed in SigmaPlot: The Shapiro-Wilk test for normality, the Leveα test for equal variance, the paired t-test, the Kruskal-Wallis one-way ANOVA on Ranks, the repeated-measures ANOVA and the two-way repeated-measures ANOVA.

Chapter 3: Results

Chapter 3.1: Acute 5-HT_{2B} receptor activation inhibits the activity of DA neurons in the VTA

To confirm that the inhibitory effect of BW723c86 was mediated through 5-HT_{2B} receptors, the selective 5-HT_{2B} receptor antagonist RS127445 (2 mg/kg, s.c.) was administered five minutes prior to the administration of BW723c86 in another set of experiments. A two-way ANOVA followed by a Holm-Sidak posthoc test on firing activity revealed a significant effect of treatment (RS127445 pre-pre-treatment versus control; $F [1,39] = 5.1$, $p < .05$; two-way repeated measures ANOVA; Holm-Sidak pairwise comparisons set to $p = .05$), a significant effect of the dose of BW723c86 administration and a significant interaction between treatment and the dose of BW723c86. An acute i.v. dose of the preferential 5-HT_{2B} receptor agonist BW723c86 at 4 and 6 mg/kg significantly decreased the firing activity of DA neurons by 10 and 20%, respectively ($F [3,39] = 5.2$, $p < .01$; two-way repeated measures ANOVA; Holm-Sidak pairwise comparisons set to $p = .05$; Figure 4A). In RS127445 pretreated rats, the effects of BW723c86 at 4 and 6 mg/kg were abolished as indicated by the interaction effect ($F [3,39] = 6.0$, $p < .01$; two-way repeated measures ANOVA; Holm-Sidak pairwise comparisons set to $p = .05$; Figure 4A).

A two-way ANOVA followed by a Holm-Sidak posthoc test on bursting activity revealed no significant effect of treatment (RS127445 pre-treatment versus saline; $F [1,39] = 2.0$, $p > .05$; two-way repeated measures ANOVA; Holm-Sidak pairwise comparisons set to $p = .05$), a significant effect of the dose of BW723c86 administration and a significant interaction between treatment and the dose of BW723c86. An acute i.v. dose of BW723c86 at 6 mg/kg inhibited the bursting activity of DA neurons by 40% ($F [3,39] = 8.5$, $p < .001$; two-way repeated measures ANOVA; Holm-Sidak pairwise comparisons set to $p = .05$; Figure 4B). In RS127445 pretreated rats, the effects of BW723c86 at 6 mg/kg were abolished as indicated by the interaction effect ($F [3,39] = 3.8$, $p < .05$; two-way repeated measures ANOVA; Holm-Sidak pairwise comparisons set to $p = .05$; Figure 4B).

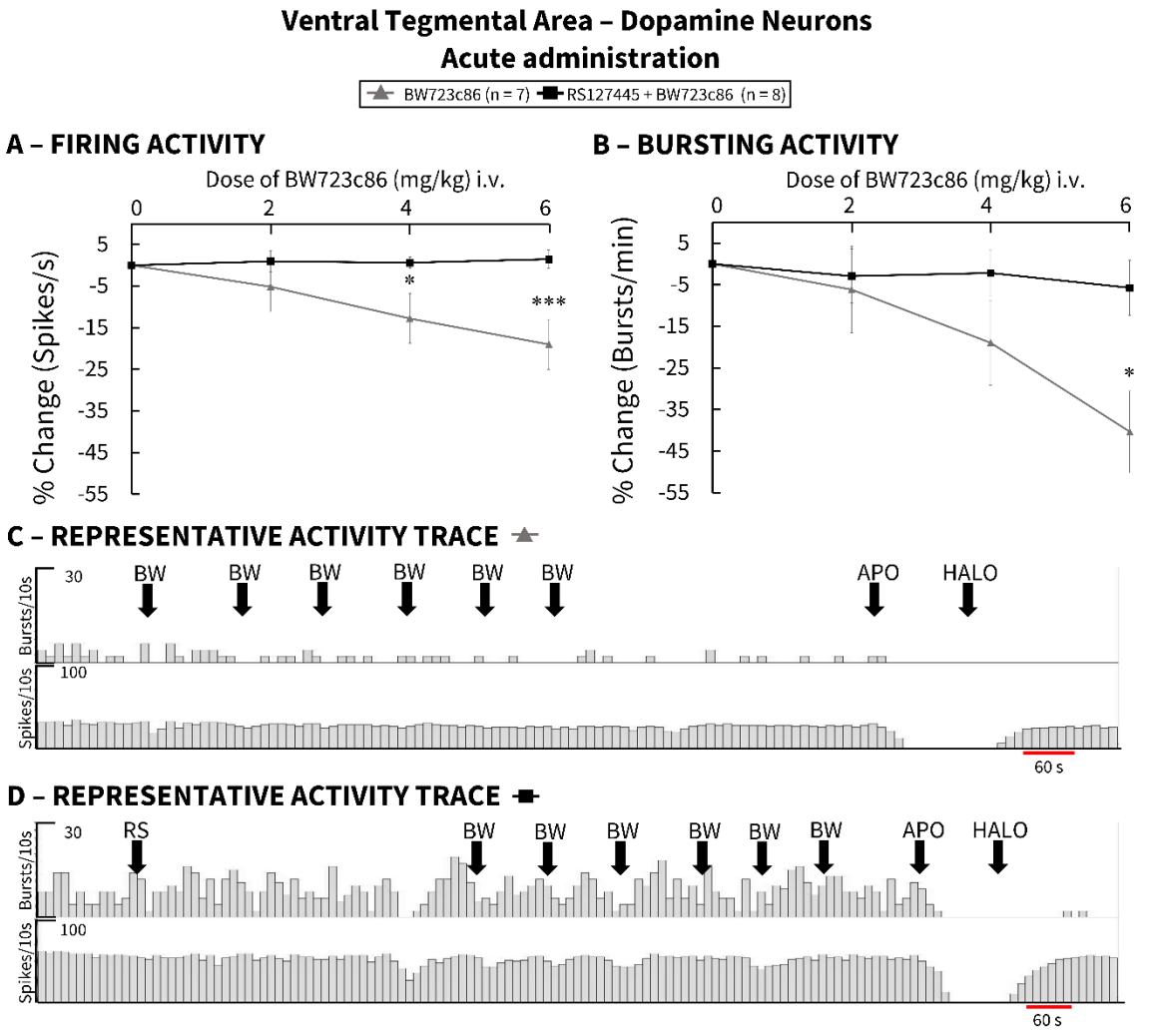

Figure 4. (A) Percent change in the firing rate of ventral tegmental area (VTA) DA neurons in rats administered an acute cumulative dose of the 5-HT_{2B} receptor agonist BW723c86 (6 mg/kg, i.v.; grey lines) or the same cumulative dose after administration of the selective 5-HT_{2B} receptor antagonist RS127445 (2 mg/kg, s.c.; black lines) **(B)** Percent change in the bursting rate of DA neurons in rats administered an acute cumulative dose of the 5-HT_{2B} receptor agonist BW723c86 (6 mg/kg, i.v ; black lines) or the same cumulative dose after administration of the selective 5-HT_{2B} receptor antagonist RS127445 (2 mg/kg, s.c.; black lines). Note that the acute effects of BW723c86 were prevented in DA neurons administered RS127445. Data and are presented as mean ± S.E.M. *p < .05, *** p < .001 relative to baseline. Pairwise comparisons using the Holm-Sidak method. **(C)** Integrated bursting [Bursts/10s; up to 30 bursts/10s] and firing [Spikes/10s; up to 100 spikes/10s] rate histogram of a representative DA neuron, where BW723c86 (BW; 1 mg/kg, i.v.) was administered once every 90 seconds up to 6 mg/kg. At the end of the experiment, the DA-$D_{2\text{-like}}$ agonist apomorphine (APO; 40 µg/kg, i.v.) silenced the DA neuron and this was reversed by the DA-$D_{2\text{-like}}$ antagonist haloperidol (HALO; 200 µg/kg, i.v.). **(D)** Integrated bursting and firing rate histogram of a representative DA neuron undergoing the same administration regimen, however, RS127455 was administered five minutes beforehand.

Chapter 3.2: Subacute (2-day) 5-HT_{2B} receptor antagonism rescues an SSRI-mediated inhibition of DA but not 5-HT neurons

In the VTA, a two-day regimen of escitalopram (10 mg/kg/day, s.c.) significantly decreased the firing activity of DA neurons ($H [3] = 25.64$, $p < .001$; Kruskal-Wallis One-Way ANOVA on Ranks; Dunn's method pairwise comparisons, $p < .05$; Figure 5A), but not their bursting activity in bursts/min ($H [3] = 2.80$, $p > .05$; Kruskal-Wallis One-Way ANOVA on Ranks; Figure 5B) nor their bursting activity in % spikes in burst (%SIB; $H [3] = 3.28$, $p > .05$; Kruskal-Wallis One-Way ANOVA on Ranks; Figure 5C) nor the number of spontaneously active DA neurons per electrode descent (population activity; $H [3] = 1.76$, $p > 0.5$; Kruskal-Wallis One-Way ANOVA on Ranks; Figure 5D). Whereas the administration of the selective 5-HT_{2B} receptor antagonist LY266097 (0.6 mg/kg/day, i.p.) alone, for two days, had no effect on firing and bursting activity, its co-administration counteracted the inhibitory effect of escitalopram on DA neuron firing activity ($p > .05$; Figure 5A/B).

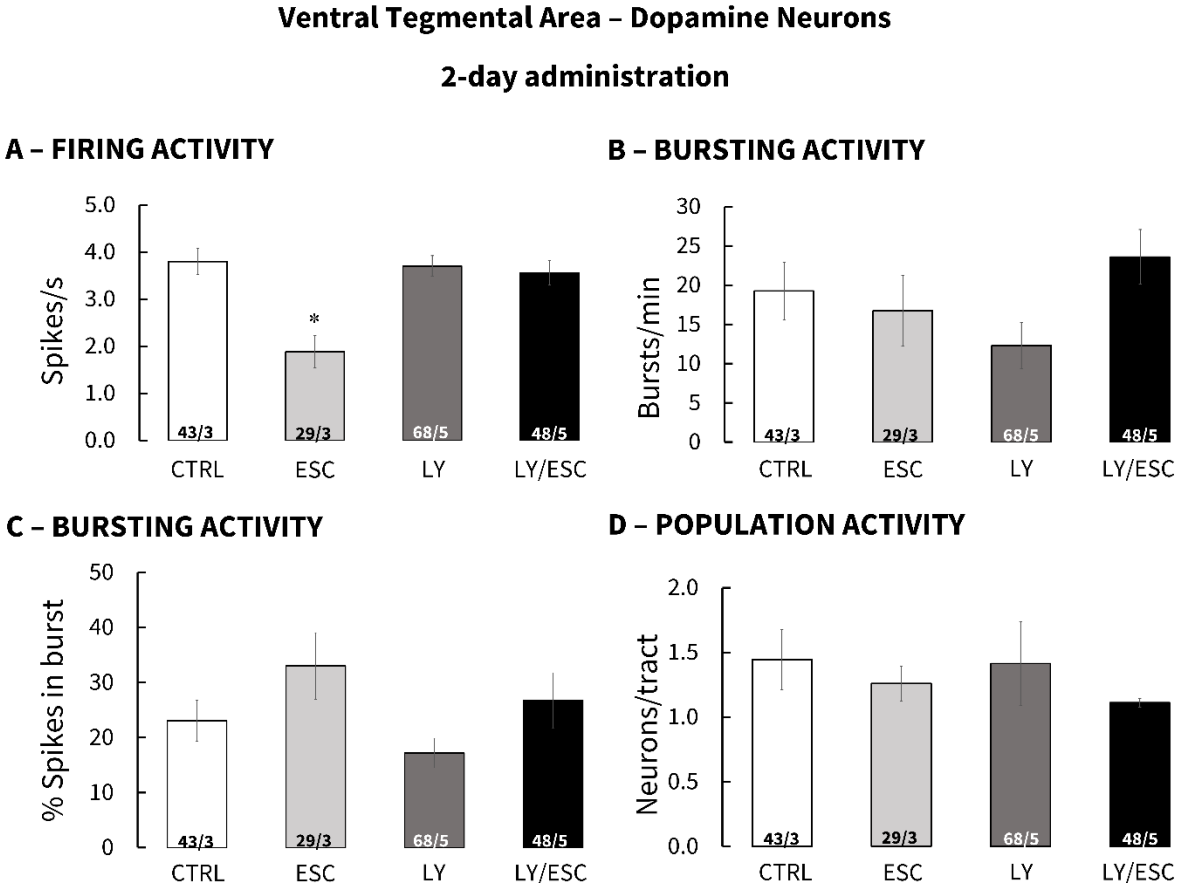

Figure 5. (A) Firing rate of ventral tegmental area (VTA) DA neurons in rats administered vehicle, escitalopram (10 mg/kg/day, s.c.), the 5-HT_{2B} receptor antagonist LY266097 (0.6 mg/kg/day, i.p.) and their combination for two days **(B)** Bursting rate in bursts/min of DA neurons in the same rats administered these same regimens **(C)** Bursting rate in % spikes in burst of DA neurons in the same rats administered these same regimens **(D)** Population activity of DA neurons per electrode descent in the same rats administered these same regimens. Data and are presented as mean ± S.E.M. *p < .05 relative to control group. Pairwise comparisons using Dunn's method. Numerators within the bars represent the total number of neurons recorded, and denominators within the same bars represent the total number of rats used.

In the DRN, a two-day regimen of escitalopram significantly decreased the firing activity of 5-HT neurons (H [3] = 101.99, p < .001; Kruskal-Wallis One-Way ANOVA on Ranks; Dunn's method pairwise comparisons, p < .05; Figure 6A), but not their bursting activity (H [3] = 19.79, p < .001; Kruskal-Wallis One-Way ANOVA on Ranks; Dunn's method pairwise comparisons, p > .05; Figure 6B). The administration of LY266097 alone, for two days, had no significant effect on the firing and bursting activity of 5-HT neurons, despite increasing the firing activity of 5-HT neurons by 40%. The co-administration of LY266097 did not rescue escitalopram-induced inhibition of 5-HT neuron activity (p < .05; Figure 6C/D).

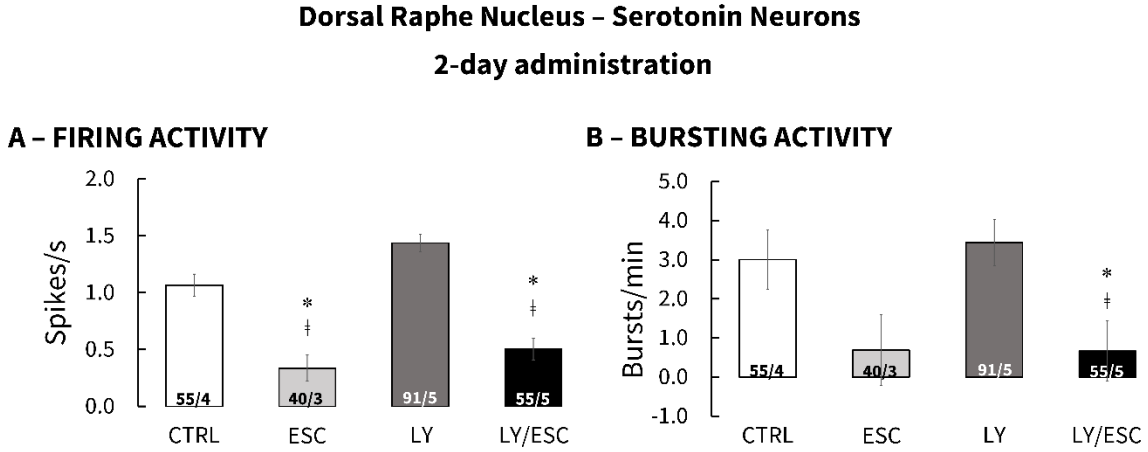

Figure 6. (A) Firing rate of dorsal raphe nucleus (DRN) 5-HT neurons in rats administered vehicle, escitalopram (10 mg/kg/day, s.c.), the 5-HT_{2B} receptor antagonist LY266097 (0.6 mg/kg/day, i.p.) and their combination for two days **(B)** Bursting rate of 5-HT neurons in the same rats administered these same regimens. Note that the inhibitory effects of escitalopram on firing rate were prevented in DA neurons. Data and are presented as mean ± S.E.M. *p < .05 relative to control group; ǂp > .05 relative to LY266097 group. Pairwise comparisons using Dunn's method. Numerators within the bars represent the total number of neurons recorded, and denominators within the same bars represent the total number of rats used.

Chapter 3.3: Acute and long-term aripiprazole alone and in combination with escitalopram increases mPFC pyramidal neuron activity, and may be mediated by 5-HT_{2B} receptor blockade

Previous experiments from our laboratory had demonstrated the effects of aripiprazole on DA and 5-HT neuron activity (Chernoloz et al., 2009a), however, there have been no studies to date investigating the effects of aripiprazole administration on mPFC pyramidal neuron activity. Thus, we sought to determine the effects of aripiprazole in acute and long-term administration regimens. Acute aripiprazole administration (0.6 mg/kg, i.v.) increased the firing activity of mPFC pyramidal neurons by 30%, but it was not statistically significant ($t [7] = -1.79$, $p > .05$; paired samples t-test; Figure 7A). However, it significantly increased their bursting activity ($t [7] = -3.05$, $p < .05$; paired samples t-test; Figure 7B).

Long-term administration of escitalopram had no effect on the firing and bursting activity of mPFC pyramidal neurons. Aripiprazole and its combination with escitalopram significantly increased the firing activity of mPFC pyramidal neurons ($H [3] = 15.76$, $p < .01$; Kruskal-Wallis One-Way ANOVA on Ranks; Dunn's method pairwise comparisons, $p > .05$; Figure 7C) and the bursting activity of mPFC pyramidal neurons after a 14-day regimen ($H [3] = 12.05$, $p < .01$; Dunn's method pairwise comparisons, $p > .05$; Figure 7D). Further analysis showed a lack of a differential effect of these regimens on the two cortical subregions, namely the prelimbic and infralimbic cortices.

To assess 5-HT_{2B} receptor involvement in the increase of mPFC pyramidal neuron firing and bursting activity, the 5-HT_{2B} receptor antagonist LY266097 was administered concomitantly with escitalopram. Indeed, the longer-term administration of LY266097 alone and when combined with escitalopram, significantly enhanced the firing activity of mPFC pyramidal neurons ($H [3] = 27.32$, $p < .001$; Kruskal-Wallis One-Way ANOVA on Ranks; Dunn's method pairwise comparisons, $p > .05$; Figure 7E) and their bursting activity ($H [3] = 15.16$, $p < .01$; Dunn's method pairwise comparisons, $p > .05$; Figure 7F) as did the combination of aripiprazole and escitalopram.

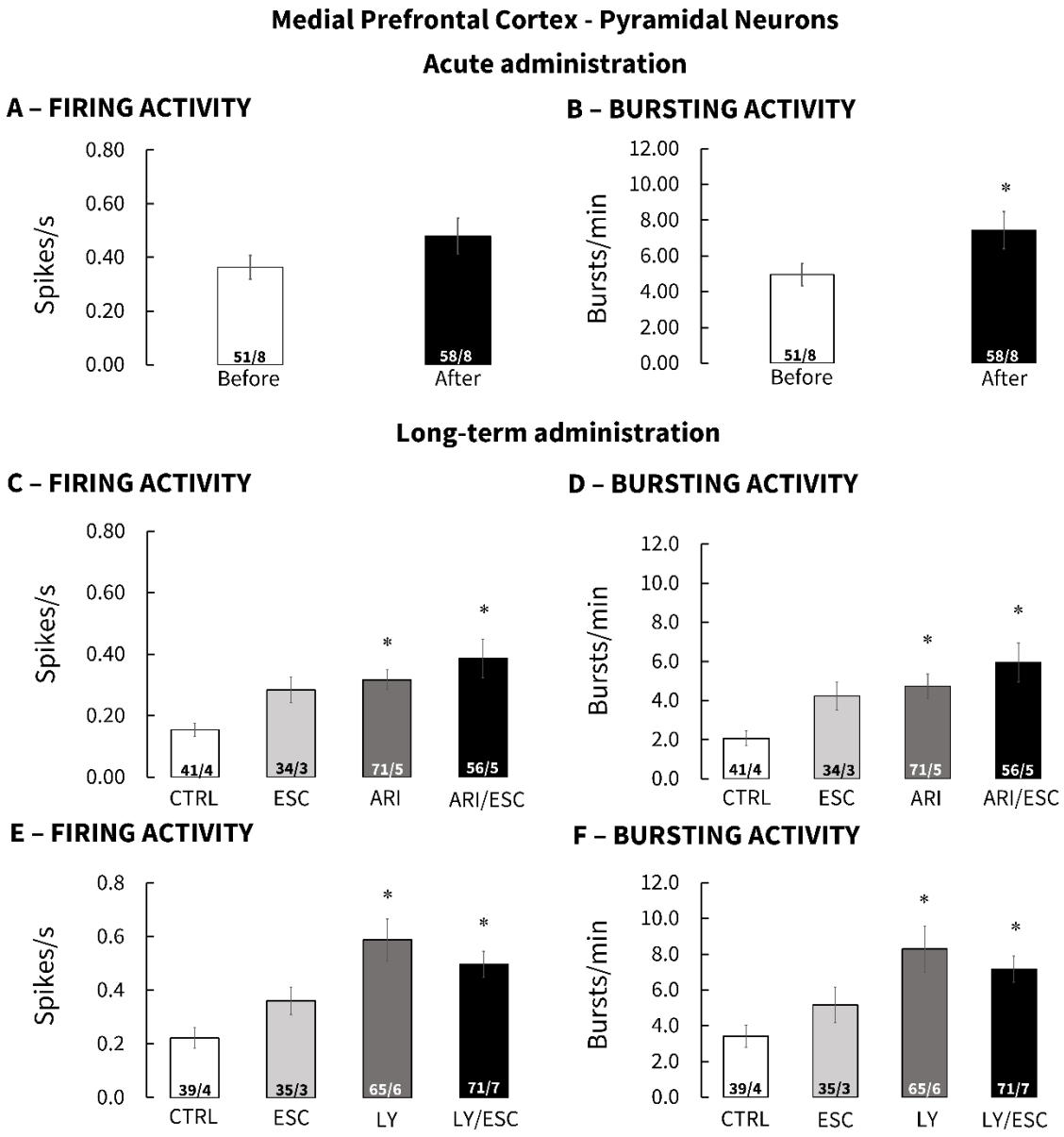

Figure 7. (A) Firing rate of medial prefrontal cortex (mPFC) pyramidal neurons before the administration of an acute dose of aripiprazole (0.6 mg/kg, i.v.; white bars) and after (black bars) **(B)** Bursting rate of pyramidal neurons before the administration of an acute dose of aripiprazole (0.6 mg/kg, i.v.; white bars) and after (black bars) **(C)** Firing rate of pyramidal neurons in rats administered vehicle, escitalopram (10 mg/kg/day, s.c.), aripiprazole (2 mg/kg s.c.) and their combination for 14 days **(D)** Bursting rate of pyramidal neurons in the same rats administered the same regimens **(E)** Firing rate of pyramidal neurons in rats administered vehicle, escitalopram for 14 days (10 mg/kg/day, s.c.), the 5-HT_{2B} receptor antagonist LY266097 (0.6 mg/kg/day, i.p.) for the last three days of a 14-day sham-regimen and their combination. **(F)** Bursting rate of pyramidal neurons in the same rats administered the same regimens. Data and are presented as mean ± S.E.M. *p < .05, relative to before or control group. Pairwise comparisons using Dunn's method. Numerators within the bars represent the total number of neurons recorded, and denominators within the same bars represent the total number of rats used.

Chapter 3.4: 5-HT_{2B} receptor agonism may impair SSRI binding to 5-HTT in vivo

Several studies have demonstrated the effects of various medications, including aripiprazole and escitalopram, on 5-HTT reuptake capacity in the CA3 region of the dorsal hippocampus (Béïque et al., 1998; El Mansari el., 2015; Ebhrahimzadeh et al., 2019). For consistency and comparability, this region was chosen to assess the effects of 5-HT_{2B} receptor agonism on 5-HTT reuptake capacity. CA3 dorsal hippocampus pyramidal neuron activity was supressed by microiontophoretic application of 5-HT and displayed a recovery to 50% of baseline firing (RT-50) after an average of 70 seconds (Figure 8A/B). The acute administration of escitalopram 0.2 mg/kg significantly increased the RT-50 value ($F[2,6] = 9.53$, $p < .05$; One-way repeated-measures ANOVA; Holm-Sidak pairwise comparisons, $p < .05$; Figure 8B). Afterwards, CA3 dorsal pyramidal neuron activity was partially supressed by BW723c86 ejection (Figure 8A). However, the co-ejection of 5-HT during BW723c86 ejection returned the RT-50 to baseline values ($p > .05$; Figure 8B).

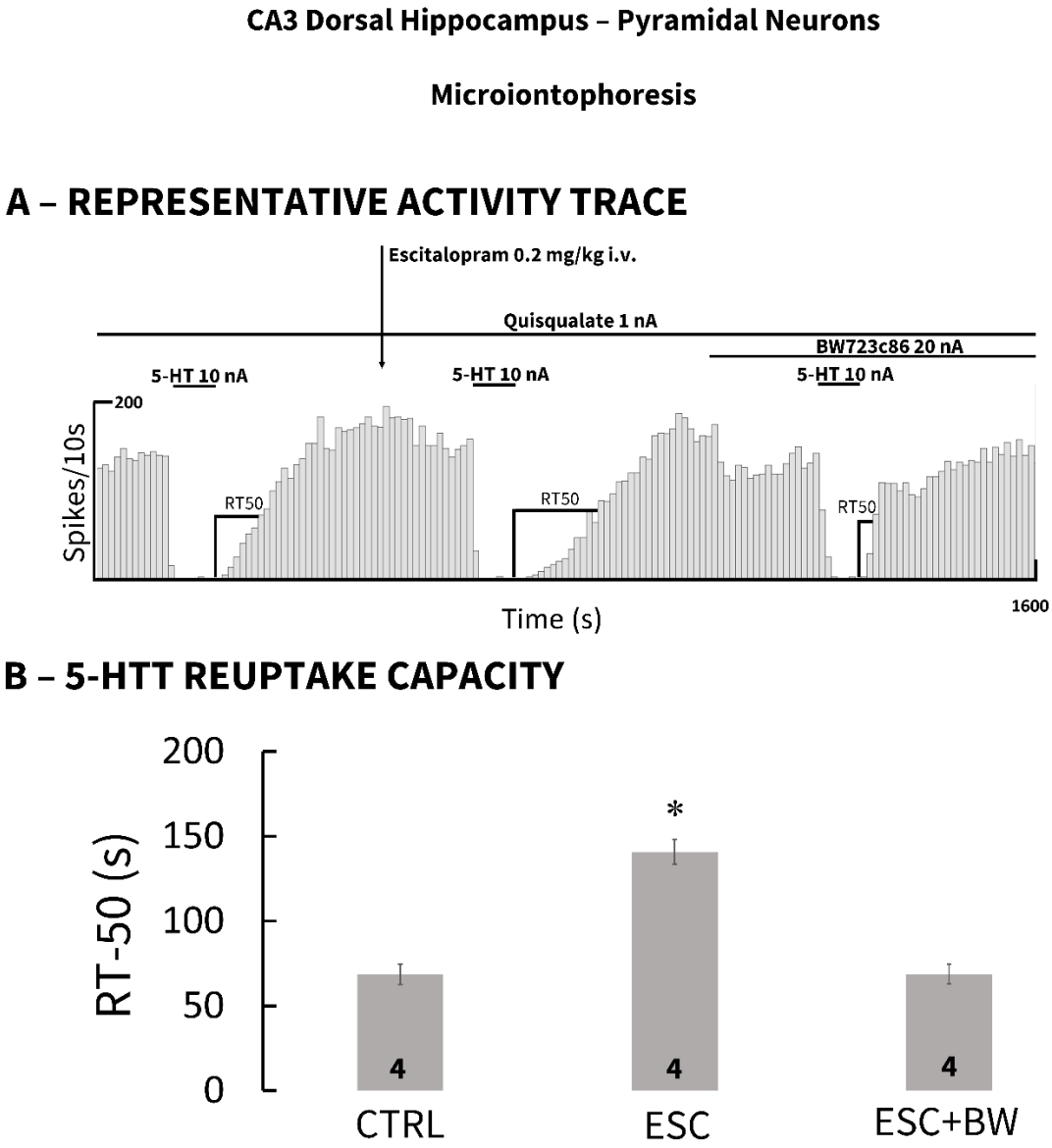

Figure 8. (A) Integrated firing rate histogram of a representative CA3 dorsal hippocampal pyramidal neuron illustrating microiontophoretic application of 5-HT three times; before any treatment (baseline), after escitalopram 0.2 mg/kg i.v., and during co-ejection of the 5-HT_{2B} receptor agonist BW723c86. **(B)** RT-50 values in seconds as an index of 5-HTT activity following the microiontophoretic application of 5-HT within the same rat, administered the aforementioned treatments. Data and are presented as mean ± S.E.M. *p < .05, relative to control group. Pairwise comparisons using the Holm-Sidak method. Numbers within the bars represent the total number of neurons recorded, and each neuron was recorded from a separate rat.

Chapter 4: Discussion

In summary, acute modulation of the 5-HT_{2B} receptor altered the activity of DA neurons. Furthermore, the 5-HT_{2B} receptor antagonist LY266097, which has no effect by itself, reversed an inhibition of DA neuron firing activity induced by escitalopram. However, similar inhibition by escitalopram on firing activity of 5-HT neurons was not reversed by LY266097. In the mPFC, acute administration of aripiprazole increased bursting but not firing activity of pyramidal neurons, although it did enhance firing and bursting activities when chronically administered. On the other hand, although escitalopram by itself has no effect of firing and bursting activities of mPFC pyramidal neurons, blockade of 5-HT_{2B} receptor with LY266097 resulted in an augmentation in the effect on firing and bursting activities. Finally, activation of the 5-HT_{2B} receptor impaired the ability of escitalopram to bind to the 5-HTT in dorsal hippocampal CA3 pyramidal neurons.

The present study demonstrated that the 5-HT_{2B} receptor agonist BW723c86 significantly decreased firing and bursting activity of DA neurons. Previous studies showed no significant effect of BW723c86 on DA neurons or DA dialysate levels in the mPFC (Di Matteo et al, 2000; Gobert et al., 2000) probably because a low dose was administered and since many of the acute anxiolytic effects of BW723c86 were only observed after the administration of a relatively higher dose (Kennett et al., 1996; 1998). The same study showed that Ro 60-0175, another agonist with equivalent affinity for 5-HT_{2B} receptor and 5-HT_{2C} receptor, decreased firing activity of DA neurons (Di Matteo et al., 2000). Taking in account the fact that BW723c86 has similar affinity for 5-HT_{2B} receptor and 5-HT_{2C} receptor (see review - Devroye et al., 2018), it is possible that the dose used in the present experiments (6 mg/kg) acted on both receptors. On the other hand, a mixture of antagonism at both 5-HT_{2B} receptor and 5-HT_{2C} receptor by SB206553 has been shown to result in an increase in firing and bursting activity (Di Giovanni et al., 1999). In the present study, inhibition of firing and bursting activity induced by BW723c86 was prevented by pretreatment with the 5-HT_{2B} receptor antagonist RS127445, indicating that the 5-HT_{2B} receptor mediated the inhibition of dopamine neuron firing and bursting activity. However, the RS127445 administration on its own has been previously reported to decrease the firing of DA neurons (Devroye et al., 2016). It was reported that blockade of 5-HT_{2B} receptor receptors by RS127445 decreased DA levels in the shell of the nucleus accumbens (NAc) and increased DA levels in the mPFC (Devroye et al., 2016; 2017), suggesting a heterogenous response of dopamine neurons to this antagonist. It is hence

possible that the electrophysiological effects of RS127445 on DA neurons depends on the type (mesoaccumbal versus mesocortical) of neuron recoded.

In the present study, 5-HT_{2B} receptor blockade did not change DA bursting and firing activity after a two-day LY266097 administration. However, after fourteen days, the firing activity was shown to be increased by LY266097 (Chenu et al., 2014). Hence, eliciting an increase in DA activity may require a longer pharmacological inactivation of the 5-HT_{2B} receptor receptor, as is the case with long-term administration of the agomelatine, which possesses a strong affinity for 5-HT_{2B} receptors (Milan et al., 2003). In line with previous results, two-day administration of escitalopram induced a decrease in DA neuron firing activity (Chernoloz et al., 2009a; Dremencov et al., 2009). Since 5-HT_{2B} receptor mRNA was found in the VTA (Doly et al., 2017), and the fact that 5-HT_{2B} receptor mediated inhibition of DA neuron firing and bursting activity in the present study, it is possible that blockade of these receptors rescues an inhibition of DA neuron activity induced by escitalopram. Indeed, co-administration of escitalopram and LY266097 for two days blocked an escitalopram-induced dampening of DA activity. Similar rescue of DA neuron firing activity was also demonstrated when escitalopram was co-administered with aripiprazole (Chernoloz et al., 2009a). As the role of 5-HT_{2C} receptors in this rescue was already established (Chernoloz et al., 2009a; Dremencov et al., 2009), the present study indicates that the 5-HT_{2B} receptor is implicated as well. Altogether, these results indicate that aripiprazole rescue of escitalopram-induced inhibition of DA neurons is mediated, at least in part, by 5-HT_{2B} receptors expressed on DA and GABAergic interneurons and 5-HT_{2B} receptors possibly expressed on DA neurons (Bubar et al., 2011; Doly et al., 2017).

Despite a 40% increase in 5-HT neuron firing activity following 5-HT_{2B} receptor blockade by LY266097 for two days, this did not reach significance in our study. Similar blockade of these receptors by RS127445, however, resulted in an increase in 5-HT firing activity (Devroye et al., 2016). In the latter study, RS127445 was given acutely while LY266097 was administered for two days herein. Altogether, these results suggest a role for the 5-HT_{2B} receptor in controlling activity of 5-HT neurons. Indeed, a recent study has reported that 5-HT_{2B} receptors are located on GABA interneurons in the rat DRN (Cathala et al., 2019), demonstrating the existence of a disinhibitory mechanism and confirms the importance of the DRN in mediating the effects of 5-HT_{2B} receptor antagonists. The addition of LY266097 did not rescue inhibition of 5-HT neuron firing activity and

bursting induced by escitalopram in the present study. Since the inhibition induced by escitalopram is also mediated by 5-HT_{1A} autoreceptors (El Mansari et al., 2005), it is possible that inhibition through this autoreceptor predominates over the 5-HT_{2B} receptor in the control of 5-HT neuron activity and that it's desensitization could unmask the effects mediated by 5-HT_{2B} receptor blockade. A previous study from our laboratory has shown that following the desensitization of the 5-HT_{1A} receptor, subacute aripiprazole administration resulted in an increase 5-HT neuron firing activity (Chernoloz et al., 2009a). It is possible that in addition to this desensitization, 5-HT_{2B} receptor blockade may have been involved in this enhancement of activity.

A low dose of aripiprazole previously shown to increase DA dialysate levels in the mPFC (Li et al., 2004; Tanahashi et al., 2012) was administered once to observe the acute effects of mPFC pyramidal activity. Acute aripiprazole administration elicited an increase in the bursting rate but not the firing rate of mPFC pyramidal neurons relative to pre-administration recordings in the same rodents. These data are in line with a previous report showing that acute olanzapine administration had no effect on mPFC pyramidal firing activity (Gronier and Rasmussen, 2003). In contrast, the acute administration of clozapine elicited an increase in mPFC pyramidal neuron activity (Kim et al., 2001). However, long-term drug administration regimens are more relevant to the therapeutic effects of drugs since their effects take place after sustained treatment in the clinic.

LY266097 administered on its own induced an increase in the firing and bursting activity of pyramidal neurons in the mPFC. These results suggest that 5-HT_{2B} receptor activation must be inhibitory on pyramidal neurons. Indeed, these effects may be due to a direct action on 5-HT_{2B} receptors, as they have been shown to be expressed on pyramidal neurons of the cortex (Niebert et al., 2011). In addition, the ejection of the preferential 5-HT_{2B} receptor partial agonist mCPP (Cussac et al., 2002) inhibited the activity of pyramidal neurons in the mPFC, which was reversed by clozapine, the latter also possessing a strong affinity for the 5-HT_{2B} receptor (Wainscott et al., 1996; Bergqvist et al., 1999). However, mCPP is also a weaker, yet, full 5-HT_{2C} receptor agonist (Cussac et al.,, 2002) and inhibition of activity mediated through this receptor cannot be discounted.

Long-term administration of escitalopram did not induce any change in the firing and bursting activity of pyramidal neurons in the mPFC, which is line with previous results (Riga et al., 2017). However, the addition of LY266097 to escitalopram resulted in a similar increase of

comparable magnitude to that induced by LY266097 administration alone. This increase in firing and bursting activity of mPFC pyramidal neurons was also present following the long-term administration of aripiprazole and its combination with escitalopram. This suggests that 5-HT_{2B} receptor blockade by aripiprazole may be involved, at least in part, in this enhancement of activity. Similarly, 3-week administration of olanzapine, which has moderate affinity for the 5-HT_{2B} receptor (Wainscott et al., 1996), increased basal firing rate of mPFC pyramidal neurons and reversed fluoxetine-induced inhibition of these neurons (Gronier and Rasmussen, 2003). Interestingly, aripiprazole was reported to enhance dopamine levels in the mPFC (Li et al., 2004; Zocchi et al., 2005; Tanahashi et al., 2012), while the effects on 5-HT were inconclusive (Zocchi et al., 2005; Bortolozzi et al., 2007; Carli et al., 2011). This increase in DA concentration is partly due to blockade of 5-HT_{2B} receptors since blockade of 5-HT_{2B} receptors by the selective antagonists LY266097 and RS127445 elicited a comparable effect (Devroye et al., 2016; 2017), although SB204741 did not (Gobert et al., 2000). Eliciting an increase in mesocortical DA neurotransmission via 5-HT_{2B} receptor blockade may contribute to the antidepressant response. Preclinical studies have demonstrated that stress induces a hypo-dopaminergic state in the mPFC that is partly characterized by DA-D1 receptor dysfunction (Mizoguchi et al., 2000; Goldwater et al., 2009). Furthermore, selectively supressing mesocortical DA output increases susceptibility to stress (Chaudhury et al., 2012). Thus, facilitating an increase in mesocortical DA release via pharmacological inactivation of 5-HT_{2B} receptors may be beneficial for an individual with MDD. This is especially relevant for individual taking SSRIs, as it is well documented that this class of medication may decrease DA activity (Chernoloz et al., 2009a; Dremencov et al., 2009) thereby possibly diminishing the antidepressant response in certain patients (El Mansari and Blier, 2013).

To our knowledge, this is the first study to demonstrate the long-term effects of a DA-$D_{2/3}$ partial receptor agonist medication on mPFC activity, which may be due to a combination of direct 5-HT_{2B} receptor antagonism on mPFC pyramidal neurons and/or an enhancement of DA efflux into the mPFC. These data are congruent with recent evidence demonstrating that vortioxetine, but not escitalopram, increased mPFC pyramidal neuron activity recorded under identical conditions (Riga et al., 2017). First, this comparison proposes that increased mPFC pyramidal neuron activity is a correlate of the antidepressant response despite these medications having distinct mechanisms of action. Second, this suggests SRI alone may not be sufficient to increase cortical activity, and that blockade of certain serotonin receptor sub-types such as 5-HT_3 receptors (Leiser et al., 2014; Riga

et al., 2016) and now 5-HT_{2B} receptors may facilitate this effect. Increasing the activity of mPFC pyramidal neurons may lead to a pro-cognitive effect that is seen with vortioxetine treatment in human (McIntyre et al., 2016). Although the pro-cognitive effects of aripiprazole alone have been reported in preclinical studies (Burda et al., 2011; Russo et al., 2013), the effects of aripiprazole on cognition in humans with MDD is currently being examined in the CAN BIND-I study and will be reported soon (Kennedy et al., 2019).

A previous study showed that activation of the 5-HT_{2B} receptor by BW723c86 impairs 5-HT uptake in vitro (Launay et al., 2006). In vivo as in the present study, however, the iontophoretic application of BW723c86 did impair the escitalopram-induced blockade of 5-HTT resulting in greater 5-HT uptake. This unusual phenomenon may have arisen due to hyperphosphorylation of the 5-HTT via activation of the 5-HT_{2B} receptor, which may impair antidepressant recognition on 5-HTT binding pockets (Zhang et al., 2005; Launay et al., 2006). This finding becomes especially relevant for individuals who carry mutations in the 5-HT_{2B} receptor leading to a five-fold increased affinity of 5-HT for this receptor, enhanced constitutive signalling and slower desensitization kinetics (Belmer et al., 2013). Or, for those who use 5-HT_{2B} agonist agents such as LSD (Porter et al., 1999) and MDMA (Setola et al., 2003). However, when aripiprazole, which possesses 5-HT_{2B} receptor antagonist activity, was administered concomitantly with escitalopram for two weeks, the inhibition of reuptake induced by escitalopram was similar to when aripiprazole was added (Ebrahimzadeh et al., 2019). This suggests that the addition of 5-HT_{2B} receptor antagonism along with serotonin reuptake inhibition may occur without any negative interactions in the hippocampus.

Conclusion

Until recently, understanding the capacity of the 5-HT_{2B} receptor to alter neuronal activity has been largely ignored. 5-HT_{2B} receptor blockade may prevent an SSRI-induced decrease in DA activity. Aripiprazole may increase mPFC pyramidal neuron activity by acting on 5-HT_{2B} receptors expressed within the mPFC and/or by facilitating mesocortical dopaminergic neurotransmission mediated by these same receptors. These long-term effects of aripiprazole on the mPFC may explain, at least in part, the contribution of 5-HT_{2B} receptor blockade to the antidepressant response and is strengthened by data showing a late increase in dlPFC activity is a marker of treatment-response in MDD (Mayberg et al., 2000). Lastly, 5-HT_{2B} receptor activation

may impair SSRI binding, which may be prevented by the addition of a 5-HT_{2B} receptor antagonist medication. Ultimately, aripiprazole adjunctively and other psychopharmacologic agents may exert their therapeutic effects partly by acting as 5-HT_{2B} receptor antagonists in the CNS.

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