

Reciprocal interactions between monoamines as a basis for the antidepressant response potential

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Thesis submitted to the
Faculty of Graduate and Postdoctoral studies
In partial fulfillment of the requirements
For the PhD degree in Neuroscience

Cellular and Molecular Medicine/Neuroscience
Faculty of Graduate and Postdoctoral studies
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ABSTRACT

Despite substantial progress in the area of depression research, the current treatments for Major Depressive Disorder (MDD) remain suboptimal. Therefore, various medications are often used as augmenting agents in pharmacotherapy of treatment-resistant MDD. Despite the relative clinical success, little is known about the precise mechanisms of their antidepressant action.

The present work was focused on describing the effects of three drugs with distinctive pharmacological properties (pramipexole, aripiprazole, and quetiapine) on function of the monoaminergic systems involved in the pathophysiology and treatment of MDD. Reciprocal interactions between the monoamines serotonin, norepinephrine, and dopamine systems allow the drugs targeting one neuronal entity to modify the function of the other two chemospecific entities.

Electrophysiological experiments were carried out in anaesthetized rats after 2 and 14 days of drug administration to determine their immediate and the clinically-relevant long-term effects upon monoaminergic systems.

Pramipexole is a selective D2-like agonist with no affinity for any other types of receptors. It is currently approved for use in Parkinson's disorder and the restless leg syndrome. Long-term pramipexole administration resulted in a net increase in function of both dopamine and serotonin systems.

Aripiprazole is a unique antipsychotic medication. Unlike all other representatives of this pharmacological class that antagonize D2 receptor, this

drug acts as a partial agonist at this site. Chronic administration of aripiprazole elevated the discharge rate of the serotonin neurons, presumably increasing the overall serotonergic neurotransmission.

Like aripiprazole, quetiapine is one of three atypical antipsychotic drugs approved for use in MDD. Prolonged administration of quetiapine led to a significant increase in both noradrenergic and serotonergic neurotransmission. Importantly, the clinically counter-productive decrease in the spontaneous firing of catecholaminergic neurons, induced by SSRIs, was overturned by the concomitant administration of both aripiprazole and quetiapine.

The increase in serotonergic neurotransmission was a consistent finding between all three drugs studied herein. In every case this enhancement was attained in a distinctive manner. Understanding of the precise mechanisms leading to the amplification/normalization of function of monoamines enables potential construction of optimal treatment strategies thereby allowing clinicians greater pharmacological flexibility in the management of depressive symptoms.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor Dr. Pierre Blier for the years of unparalleled mentorship and professionalism. I am forever indebted for giving me the opportunity to join his group and thus opening to me the exciting world of neuropsychopharmacology; I can only hope to be able to repay the debt one day.

I am extremely grateful to Dr. Mostafa El Mansari for providing not only continuous guidance and technical expertise, but also his endless patience and a unique sense of humor. I would like to thank Dr. Bruno Guiard, Dr. Eliyahu Dremencov and Dr. Ramez Ghanbari for donating their time, effort and knowledge to teach me the basics of electrophysiology. Not only none of the present work would be possible without them, but the entire experience of my graduate life would have been bland without our (always fun) talks.

A special thanks (and a place in my heart) belongs to Maria da Silva, administrative assistant to Dr. Blier, and a caring mother to the rest of the unit. The ease in dealing with endless paperwork and administrative hurdles makes her one of my superheroes.

I would like to express my gratitude to each and every member of our unit for creating a great work environment and countless pleasant memories.

Last, but not the least, I would like to thank my husband Alex for his love and support, and to our cat Lucy for generously providing her whiskers and thus enabling the microiontophoretic experiments for the entire lab.

LIST OF ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine (serotonin)
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino) tetralin
AADC	L-amino acid decarboxylase
AAP	atypical antipsychotic
AC	adenylyl cyclase
AMPT	α -methyl-para-tyrosine
ANOVA	analysis of variance
ARI	aripiprazole
BDNF	brain derived neurotrophic factor
c-AMP	cyclic adenosine monophosphate
CNS	central nervous system
COMT	catechol-O-methyltransferase
CSF	cerebrospinal fluid
DA	dopamine
DAG	diacylglycerol
DAT	dopamine transporter
DBS	deep brain stimulation
DNA	deoxyribonucleic acid
DOPAC	3,4-dihydroxyphenylacetic acid
DRN	dorsal raphe nucleus
ECT	electro convulsive therapy

GABA	gamma-aminobutyric acid
GH	growth hormone
GIT	gastro-intestinal tract
hQuet	human quetiapine
HVA	homovanilic acid
i.p.	intraperitoneal
i.v.	intravenous
IP3	inositol triphosphate
LC	locus coeruleus
L-dopa	L-dihydroxyphenylalanine
LSD	lysergic acid diethylamide
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder
MHPG	into 3-methoxy-4-hydroxyphenylglycol
MRN	medial raphe nucleus
NE	norepinephrine
NET	norepinephrine transporter
NQuet	norquetiapine
NRI	norepinephrine reuptake inhibitor
PCPA	parachlorophenylalanine
PD	Parkinson's disease
PET	positron emission tomography
PFC	prefrontal cortex
PKA	protein kinases A

PLC	phospholipase C
PPX	pramipexole
PTSD	posttraumatic stress disorder
REM	rapid eye movement
S.E.M.	standard error of mean
SERT	serotonin transporter
SNRI	serotonin-norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
TH	tyrosine hydroxylase
TPH	tryptophan hydroxylase
VMA	vanillylmandelic acid
VMAT	vesicular monoamine transporter
VNS	vagus nerve stimulation
VTA	ventral tegmental area
WHO	World Health Organization

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1.

1.1. Major depressive disorder

Major depressive disorder (MDD) is one of the most predominant psychiatric illnesses. Indeed, World Health Organization (WHO) determined that more than 120 million people worldwide are affected. Presently MDD is ranked as the third leading cause of disability globally (WHO, 2008). Despite several generations of depression research, the WHO prognosis is grim and the magnitude of the problem is expected to worsen in the future, making the MDD the leading cause of disability worldwide by 2030. The loss of productivity, need for sustained medical care, and an increased susceptibility for the co-morbid illnesses, associated with MDD, result in the largest socio-economic burden of all disorders in developed countries. Moreover, suicidal ideation, often present in MDD, makes 15-20% of depressed individuals to take their own life (Nemeroff et al. 2001). Among others, the lack of objective diagnostic tools, high stigmatization of the mental illness, and insufficient understanding of the disorder pathophysiology and treatment strategies by the health professionals result in MDD being often undiagnosed, untreated or undertreated. The difficulty in understanding of the genesis of depression and its successful treatment is likely related to the high heterogeneity of the illness. In fact, two individuals diagnosed with MDD may not share a single common symptom. Such a diverse clinical presentation is indicative of the complex multifactorial cause(s) of depression. Indeed, similarly to many other psychiatric and some somatic illnesses, MDD is linked to the genetically predisposing factors. The risk of

depression is 2-4 times higher in family members of depressed patients, compared to the general population (Sanders, Detera-Wadleigh et al. 1999). Even though 40-50% of the risk for depression development is believed to be genetically-driven (Fava, Kendler 2000), the role of non-genetic factors is undoubted. Not only stress and adverse life events may predispose for the MDD manifestations, but also endocrine disturbances, traumatic head injuries, random processes during the CNS development and even viral infections were shown to be implicated in depression etiology (Fava, Kendler 2000; Akiskal 2000). The complex nature of MDD is likely causative of the very recent development of the efficient pharmacological treatments. Ironically, first classes of drugs that were shown to possess the antidepressant properties were discovered in 1950s by accident. The tricyclic antidepressants (TCAs) were derived from the antihistamine research, while the monoamine oxidase inhibitors (MAOIs) were initially tested as antitubercular agents. The nature of the biochemical changes evoked in the brain by these drugs led to the development of the monoamine hypothesis of depression. The increase in levels of monoamines, achieved via the blockade of norepinephrine (NE) and/or serotonin (5-HT) reuptake by TCAs, and via inhibited deactivation of NE, 5-HT and dopamine (DA) by MAOIs, was postulated to underlie the antidepressant properties of the above drugs. This hypothesis was further strengthened by the observation that reserpine, depleting the synaptic amounts of NE, 5-HT and DA, produced depressive-like effects in healthy individuals. As the subsequent research documented the involvement of all three monoaminergic systems in MDD pathophysiology (discussed in detail in section 1.3), the present

work will be focused on the description of the physiology and pathology of NE, 5-HT and DA systems (section 1.2), the reciprocal neuronal interactions between them (section 1.4), and the effects of antidepressant treatments on their function (section 1.5).

1.2. Monoaminergic systems involved in pathophysiology and/or treatment of depression

1.2.1.1.1. Serotonin system

1.2.1.1. Neuroanatomy:

Serotonin was initially identified in the blood serum (Rapport et al. 1948) and gastric mucosa (Erspamer, Asero 1952). Its serum origin along with the endogenous vasoconstrictive properties originated the term 'serotonin'. Soon after the discovery of serotonin in peripheral system it was found to be also present in CNS, serving as a neurotransmitter (Bogdanski et al. 1956). Despite the immense role of 5-HT in brain function that will be discussed later in detail, only 2% of the total body serotonin is located in the CNS (90% - mucous membranes of GIT, 8% - blood platelets). In the brain 5-HT neurons were initially discovered in the midline of the brainstem by Ramon y Cajal. Creation of first anatomical map of the 5-HT system became possible with the advancement of histochemical techniques. In 1964 Dahlstrom and Fuxe divided groups of cells along the brainstem midline, deemed to be serotonergic, into 9 units accordingly to their caudal to rostral

orientation: B1-B9 (Dahlström, Fuxe 1964). Together these structures were named raphe nuclei (from latin *raphe* – midline). This nucleus can be further subdivided into rostral (B5-B9) and caudal (B1-B4) nuclei (Tork 1990). Further, dorsal (DRN; B6 and B7) and medial raphe nuclei (MRN) within the rostral cluster, provide 80% of serotonergic forebrain innervation (Azmitia, Segal 1978); whereas the caudal part innervates medulla and spinal cord. In the mammalian CNS the DRN is the largest brainstem 5-HT nucleus and contains 50-60% of all 5-HT neurons (MRN – 5%). Composition of these nuclei, is not homogeneously serotonergic and as much as 50-75% (DRN) and 70-80% (MRN) of cells within them are non-serotonergic in their nature (Moss, Glazer et al. 1981).

The projections from raphe are so extensive that virtually every neuron within

the brain receives 5-HT innervation.

Consequently, the proper functioning of this nucleus is curtail for the maintenance of the overall brain function.

Serotonin is implicated in the processes of mood, sleep, aggression, cognition, memory, emesis, and feeding behavior, as well as the pathophysiology of disorders including major depression,

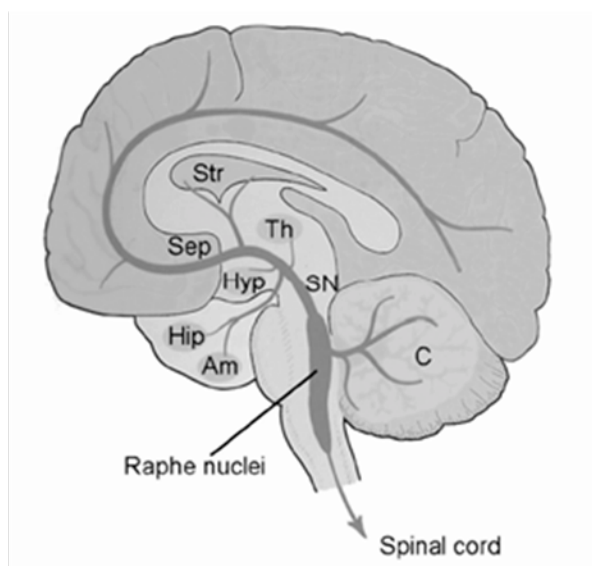


Figure 1. Serotonergic pathways in the human brain. AM: amygdala; C: cerebellum; Hip: hippocampus; Hyp: hypothalamus; Sep: septum; SN: substantia nigra; Str: striatum. Modified from (Rang et al., 1999)

schizophrenia, obsessive–compulsive disorder, and anxiety.

1.2.1.2. Synthesis, storage, release and metabolism

Though over 95% of the body 5-HT is circulating in the periphery, it can not cross the blood-brain barrier. Therefore, neurons synthesize 5-HT from the dietary amino acid tryptophan. The first step of 5-HT synthesis involves hydroxylation of L-tryptophan. This reaction is catalyzed by the enzyme tryptophan hydroxylase (TPH). Availability of this enzyme is rate-limiting for the 5-HT formation. Due to its extreme importance, the TPH is a subject for complex intracellular regulatory processes. Two forms of TPH are known – THP1, present in periphery and CNS, and TPH2, expressed exclusively in CNS (Walther et al., 2003). As well, its pharmacological inhibition by parachlorophenylalanine (PCPA) (Sanders-Bush et al. 1974) is often used to study the effects of central 5-HT depletion (Goodwin, Post 1974; Carlsson 1976). Noteworthy, being localized exclusively to the 5-HT neurons, TPH is a good marker for detection of these cells.

The majority of 5-HT is stored in vesicles located in 5-HT cell bodies and nerve terminals, the residual amount is also found in cytoplasm. Two different pools of 5-HT are believed to exist (Morot-Gaudry et al. 1981; Tracqui et al. 1983). The smaller pool of 5-HT holding about 20% of the transmitter is deemed to be the functional pool. It contains newly synthesized 5-HT and is preferentially released. The larger pool, on the other hand, is considered to be a reserve pool.

The release of 5-HT occurs mainly via exocytosis. It is Ca^{2+} -dependent and

sensitive to the Na^+ blockade by tetrodotoxin. The reverse release through the serotonin transporter (SERT) is also possible.

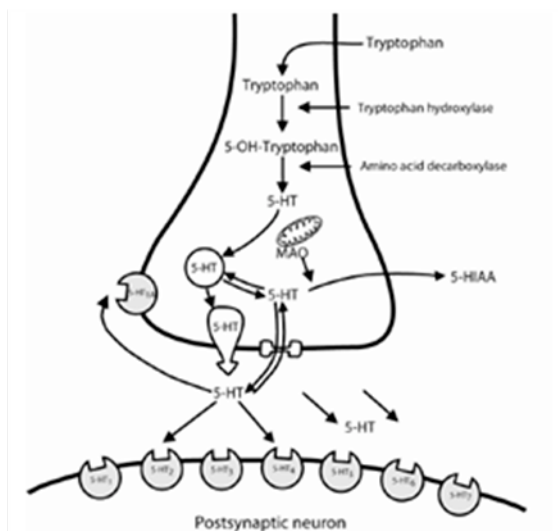


Figure 2. Schematic drawing of a serotonergic synapse. 5-HT serotonin. See text for other abbreviations. Modified from (Cooper *et al.*, 2003)

The degradation of 5-HT is achieved through the oxidative deamination catalyzed by the enzyme monoamine oxydase (MAO). The main metabolite of 5-HT formed through this process is 5-hydroxyindoleacetic acid (5-HIAA). It is eliminated from the neuron into the CSF, thus serving as an indirect measure of the 5-HT brain turnover.

Though 5-HT can be catabolyzed by both

forms of MAO (MAOA and MAOB), the potency of MAOA is almost ten-fold greater for this neurotransmitter (Fowler, Ross 1984; Willoughby *et al.* 1988). Paradoxically, 5-HT neurons predominantly express MAOB (Westlund *et al.* 1985, Konradi *et al.* 1988), suggesting that the vesicular uptake and storage is favored over the degradation.

1.2.1.3. 5-HT transporter

The reuptake of 5-HT limits its duration of action on pre- and postsynaptic receptors and also its diffusion to other synapses within the biophase. Moreover, the uptake process also allows the recycling and reuse of nonmetabolized 5-HT in the neurotransmission process. The reuptake of released 5-HT by neurons is

the major mode of inactivation. The uptake is mediated by the SERT – a membrane-spanning, Na/Cl dependent plasma protein. The uptake process is characterized by saturability, high affinity to 5-HT, Na^+ dependence and requirement for metabolic energy. While SERT mRNA is expressed only in the raphe region, being most concentrated in the DRN and MRN, SERT protein is ubiquitous in the CNS. It is also present in the periphery in platelets (Qian et al. 1995), lung membranes and placenta (Cool et al. 1990; Ramamoorthy et al. 1993).

The transporter gene was cloned from the rat (Blakely et al. 1991; Hoffman et al. 1991), mouse (Chang et al. 1996) and human (Ramamoorthy, et al. 1993; Lesch et al. 1993). The gene sequence was found to have a great degree of interspecies conservation and significant amino acid homology with transporters for DA, NE, GABA and glycine.

Serotonin transporter cellular localization has been determined using immunocytochemistry (Qian et al. 1995; Lawrence et al. 1995). The staining could be detected in both neuronal and glial cells in the terminal and somatic areas (Lawrence et al. 1995).

The regional differences in binding of the pharmacological agents were found using radiolabeling techniques (Langer et al. 1980; Habert et al. 1985; D'Amato et al. 1987). For instance, the TCA imipramine in comparison to SSRIs showed much higher binding in postsynaptic areas like cortex and hippocampus. This phenomenon is likely explained by the ability of imipramine to bind to both high and low affinity SERT, whereas SSRIs only bind to the high affinity SERT.

However, only high affinity sites are believed to be relevant for the 5-HT reuptake (Marcusson et al. 1986; Moret, Briley 1986). Needs to be remembered that the difference in binding may also be related to the NET-binding property of imipramine.

The prominent role of the 5-HT carrier in regulating the amount of 5-HT present in synaptic regions and, consequently, the degree of activation of the postsynaptic areas, made it a therapeutic target of extreme importance. Today the vast majority of first-line antidepressants are targeting this site.

1.2.1.4. 5-HT receptors

Initially, the existence of multiple 5-HT receptors was suggested by Gaddum and Picarelli who found that the neurotransmitter could produce opposing actions on the ileum smooth muscle contraction (Gaddum 1957). The extensive research in 5-HT field supplemented with the significant advancement in microbiological techniques and the increased number of selective pharmacological agents made possible characterization of 14 types of 5-HT receptors belonging to 7 subgroups (5-HT₁₋₇; (Hoyer et al. 1994)). All but one of these receptors belong to the superfamily of G-protein coupled metabotropic receptors. The 5-HT_{1A,B,D,E,F} subtypes are negatively coupled to the adenyl cyclase via Gi proteins; 5-HT_{2A,B,C} subtypes are coupled to Gq proteins and are positively coupled to the phospholipase C activation; 5-HT_{4,6,7} subtypes are positively coupled to the adenyl cyclase via Gs proteins; coupling of 5-HT_{5R} is unknown. The 5-HT_{3R} is

unique among not only 5-HT, but also other monoamines in that it is the only ligand-gated ion channel.

Only the receptors believed to be involved in MDD pathophysiology and/or antidepressant response will be further discussed in details.

1.2.1.4.1. 5-HT₁ receptors

In 1981, Pedigo et al. identified the 5-HT_{1A} receptor-binding site in the rat brain (Pedigo et al. 1981), but the sequence encoding the receptor was not isolated until 1988 (Fargin et al. 1988). The 5-HT_{1A} receptor inhibits adenylyl cyclase activity through coupling to G i/o proteins.

The immunohistochemical studies using selective 5-HT_{1A} receptor antibodies have provided greater resolution of the receptor expression through light and electron microscopy. Within the raphe nuclei, the 5-HT_{1A} receptor appears to be expressed somatodendritically by the serotonergic neurons projecting to the forebrain, with dendritic receptors predominantly located in extrasynaptic regions (Kia et al. 1996; Riad et al. 2000). This receptor is also found in many regions of the forebrain, including the frontal, periform, and entorhinal cortices, the hippocampus, preoptic areas, lateral and medial septum, the diagonal band of Broca, hypothalamus, amygdala, and thalamic regions (Aznar et al. 2003). Within the hippocampus, granule and pyramidal cells are also believed to express the 5-HT_{1A} receptor on both the soma and dendrites (Riad et al. 2000; Aznar et al. 2003). In the cortex this receptor was found to be expressed by both pyramidal

neurons and interneurons (Aznar et al. 2003). Activation of the somatodendritic 5-HT_{1A} autoreceptor in the DRN induces membrane hyperpolarization, leading to the reduced 5-HT neuron excitability, firing, and ultimately a reduction in the 5-HT release in the raphe forebrain projection areas (Sharp et al. 1996). 5-HT_{1A} receptor agonists also inhibit neuronal firing in forebrain regions, including the hippocampus (Sprouse, Aghajanian 1988). The release of other neurotransmitters, including acetylcholine, noradrenaline, and dopamine, is thought to be regulated by the 5-HT_{1A} receptor activation.

Modulation of 5-HT_{1A} receptor is believed to be involved in depression, anxiety and panic disorders, suicidal and aggressive behavior, as well as control of circadian rhythms, sleep, and learning processes.

The 5-HT_{1B} receptor-binding site was initially distinguished from the 5-HT_{1A} receptor due to its low affinity for 8-OH-DPAT (Middlemiss, Fozard 1983) and the rat receptor sequence was identified in 1991 by Voigt et al. (Voigt et al. 1991). As 5-HT_{1B} receptors in rats share 74% sequence homology with human 5-HT_{1D} receptor, which rats do not express and has identical distribution patterns, it is believed to be a rodent analog of human 5-HT_{1D} receptors (Saxena et al. 1998; Bruinvels et al. 1993). The distribution of the 5-HT_{1B} receptor in the brain has been extensively characterized through the receptor autoradiography (Pazos, Palacios 1985) and immunohistochemistry (Sari et al. 1999), whereas the location of 5-HT_{1B} receptor mRNA has been determined by in situ hybridization (Varnäs et al. 2005; Boschert et al. 1994). The 5-HT_{1B} receptor appears to be expressed at highest

levels in the basal ganglia, particularly the globus pallidus and substantia nigra, with lower levels being found in the periaqueductal gray, superficial layer of the superior colliculus, cortex, amygdala, hypothalamus, hippocampus, cerebellum, and dorsal horn of the spinal cord (Sari et al. 1999; Varnäs et al. 2001; Bonaventure et al. 1997). Correspondingly, the distribution of receptor transcripts does not completely match the location of the receptor-binding sites, as 5-HT_{1B} mRNA has been identified in the raphe nuclei, striatum, hippocampus, cortex, and thalamus (Varnäs et al. 2005). The transcripts are notably absent from the substantia nigra and globus pallidus, which display the highest levels of the binding sites. The 5-HT_{1B} receptor is thought to act as an auto- and heteroreceptor on 5-HT and non-5-HT neurons, respectively. The 5-HT_{1B} receptor activation has been shown to mediate the inhibition of 5-HT release in the forebrain, including the frontal cortex and hippocampus (Trillat et al. 1997).

Similarly to 5-HT_{1A} receptors, numerous studies have documented the role of 5-HT_{1B} autoreceptors in modulation of anxiety, depression, circadian rhythms and aggressive behavior. In addition, it is believed to be a key player in migraine physiopathology and treatment.

1.2.1.4.2. 5-HT₂ receptors

The recent advance in the development of selective pharmacological tools have enabled the precise characterization of 3 types of 5-HT₂ receptors (5-HT_{2A}, 2B, 2C).

1.2.1.4.2.1. 5-HT_{2A}

The 5-HT_{2A} receptor was initially identified as a binding site in the rat cortical membranes (Peroutka, Snyder 1979), with subsequent identification of the rat sequence a decade later (Pritchett et al. 1988; Julius et al. 1990). The distribution of the 5-HT_{2A} receptor in the brain has been well characterized. The receptor autoradiography with selective ligands, such as [3H]-MDL 100907, has shown high levels of expression in the human and rodent forebrain, including the neocortex, entorhinal and piriform cortices, hippocampus, caudate nucleus, nucleus accumbens, and olfactory tubercles (López-Giménez et al. 1997). The localization of 5-HT_{2A} mRNA corresponds well to the receptor distribution (Burnet et al. 1995), generally following the distribution of 5-HT neuron innervation, implying that the receptor has a postsynaptic location. The cellular expression of the 5-HT_{2A} receptor protein appears to be predominantly neuronal, both on GABAergic interneurons in the cortex and glutamatergic pyramidal cells within the cortex and hippocampus (Pompeiano et al. 1994; Jakab, Goldman-Rakic 1998; Burnet et al. 1995). A detailed study of the subcellular location of 5-HT_{2A} receptors in rat PFC (Miner et al. 2003) reported expression on both the shafts and spines of proximal and distal pyramidal dendrites, reinforcing the likely postsynaptic location of the receptor. In addition to the 5-HT_{2A} receptor being expressed on the plasma membrane (Willins et al. 1997), it also exhibits a degree of intracellular localization, which may be indicative of a high rate of the receptor turnover (Cornea-Hébert et al. 2002). The precise ultrastructural positioning of the receptor is supported by the

work of Cornea-Hébert et al. (2002), who demonstrated that the 5-HT_{2A} receptor physically interacts with the cytoskeletal protein, MAP_{1A}, suggesting that the receptor may regulate neuronal development or dendritic plasticity (Cornea-Hébert et al. 2002). The 5-HT_{2A} receptor is coupled to the activation of phospholipase C (PLC), inducing the mobilization of intracellular Ca²⁺ stores, in both recombinant systems and native tissue (Pritchett et al. 1988; Conn, Sanders-Bush 1984). Additionally, the 5-HT_{2A} receptor may activate second-messenger cascades responsible for the receptor agonist-induced reduction in the levels of BDNF in the dentate gyrus of the hippocampus, while increasing its levels in the neocortex, which has a potentially profound effects on the neuronal growth (Vaidya et al. 1997). The 5-HT_{2A} receptor regulates the release of many neurotransmitters, including glutamate, dopamine, and GABA. Within the forebrain, for example, the 5-HT_{2A} receptor increases both glutamate release from layer V pyramidal neurons in the PFC (Aghajanian, Marek 1999) and GABA release onto CA1 pyramidal neurons in the hippocampus (Shen, Andrade 1998). The 5-HT_{2A} receptor also appears to have a regulatory effect on dopaminergic neuron firing, supported by the receptor expression being associated with the dopaminergic neurons within the VTA and substantia nigra (Ikemoto et al. 2000). Furthermore, the 5-HT_{2A} receptor antagonism attenuates dopamine release in the VTA (De Deurwaerdère, Spampinato 1999) and striatum (Lucas, Spampinato 2000). It has been suggested that aside from its integral role in schizophrenia treatment, the 5-HT_{2A} receptors may be involved in the pathogenesis of depression and mediate some of the effects of the antidepressant treatment. Moreover, this receptor is believed to play

a modulatory effect upon hormonal secretion in hypothalamus, and to be involved in regulation of feeding and, thus, treatment of eating disorders. The hallucinogenic effect of psychotomimetic drugs is mediated via these receptors. Additionally, 5-HT₂ receptors may be involved in regulation of sleep and memory and learning processes.

The expression of 5-HT_{2B} receptors is prominent in periphery and rather low but still physiologically significant in the brain (Lucas, Spampinato 2000), shown to be linked to severe impulsivity (Doly et al., 2010).

1.2.1.4.2.2. 5-HT_{2C}

The 5-HT_{2C} receptor-binding site was originally identified in the choroid plexus, and displayed high affinity for [3H]5-HT (Pazos et al. 1984), resulting in the initial classification within the 5-HT₁ receptor family high affinity for 5-HT being a key characteristic to guide classification at the time. The 5-HT_{2C} receptor sequence was subsequently identified in the rat (Julius et al. 1988). In contrast to the 5-HT_{2B} receptor, the 5-HT_{2C} receptor has a widespread distribution throughout the brain. The receptor autoradiographical and immunohistochemical studies have complemented each other, identifying putative sites of receptor expression in the choroid plexus, cortex, amygdala, hippocampus, substantia nigra, caudate nucleus, and cerebellum (Abramowski et al. 1995). Generally, in situ hybridization has colocalized 5-HT_{2C} receptor transcripts with the binding sites, suggesting that the receptor is postsynaptic, with the exception of potentially presynaptic receptor localization in the medial habenula. Activation of the 5-HT_{2C} receptor is

thought to induce membrane depolarization, and may mediate some of the excitatory effects of 5-HT, for instance in periform cortical pyramidal neurons (Sheldon, Aghajanian 1991) and nigral neurons (Rick et al. 1995).

1.2.1.4.3. 5-HT₄ receptors

The 5-HT₄ receptor was identified in 1988 (Dumius et al., 1988) in mouse collicular neurons. The 5-HT₄ receptor stimulates adenylyl cyclase activity through coupling to Gs proteins (Bockaert et al., 1992). This receptor was found to be expressed in globus pallidus, olfactory tubercles, substantia nigra and caudate nucleus as well as hippocampus and cortex (Waeber et al., 1993). Activation of central 5-HT₄ receptors was found to exert an excitatory control on rat DRN 5-HT neuronal firing activity in an indirect manner (Lucas and Debonnel, 2002).

Modulation of 5-HT₄ receptor is believed to be involved in depression and anxiety (Costall and Naylor, 1993; Lucas et al. 2007).

1.2.1.5. 5-HT neuron electrophysiology:

The electrophysiological studies in awake behaving rats allowed to determine that the activity of the 5-HT neurons is dependent upon the physiological state of the body – it is highest during the active waking, decreases in quiet waking, further slows during slow wave sleep and virtually absent during the REM stage of sleep (Jacobs, Fornal 1993). Such a dependence is consistent with the role of 5-HT in

facilitation of the motor output and inhibition of sensory input processing. The intrinsic cell activity can be detected as early as 3-4 days before birth, highlighting the importance of the 5-HT system in the overall brain function.

In anaesthetized rats the 5-HT cells of DRN were given precise electrophysiological characterization by Aghajanian and colleagues. The discharge pattern of these neurons is characterized by the regular, slow (0.5-2.5 spikes per second) firing rate and the long duration of bi-triphasic action potential (2-5ms) (Aghajanian, Vandermaelen 1982b). The regular, pacemaker-like firing activity is attributed to the outward Ca-dependent K^+ current. The depolarization of 5-HT neuron is accompanied by entry of Ca^{2+} via voltage-dependent Ca channels. This leads to the activation of outward K^+ conductance leading to the afterhyperpolarization period, which diminishes slowly with Ca^{2+} extrusion. A new action potential is fired as the membrane potential reaches the low threshold Ca^{2+} conductance (Aghajanian, Lakoski 1984; Burlhis, Aghajanian 1987).

1.2.2. Norepinephrine System

1.2.2.1. Neuroanatomy

The term 'Norepinephrine' (NE) is derived from Greek *epi nephros* (upon the kidney), reflects the fact that the substance was initially discovered in adrenal glands that are situated above the kidneys. In the middle 1950s, NE was identified as a neurotransmitter in CNS (Vogt 1954). In mammalian CNS NE cells are

subdivided into 7 clusters: A1-A7. These clusters form two major NE centers – lateral tegmental system (areas A1-A5, A7) and locus ceruleus (LC, A6) (Paxinos, Watson 1986). The lateral tegmental system has rostral and caudal projections. The rostral projections inhibit synaptic connections to the sympathetic preganglionic neurons in the spinal cord, thus acting as a bridge between the central and peripheral sympathetic systems. The caudal projection innervates thalamus and other diencephalic structures, thus regulating physiological homeostasis (Guyenet, Cabot 1981). On the other hand, around 90% of the brain NE projections is coming from the LC (Fuxe, Sedvall 1965; Foote et al. 1983). This nuclei is bilateral and contains only 1500 neurons on each side in rat brain (Swanson 1976) and around 13000 neurons per side in humans (this number represents around 60% of total brain NE cells; Mouton et al. 1994). Despite such a small number of neurons, LC is the most widely projecting nucleus in the CNS (Foote et al. 1983) with one axon branching up to 100,000 times (Moore, Bloom 1979). The efferent pathways extending from the LC play a modulatory role on postsynaptic structures, inhibiting the

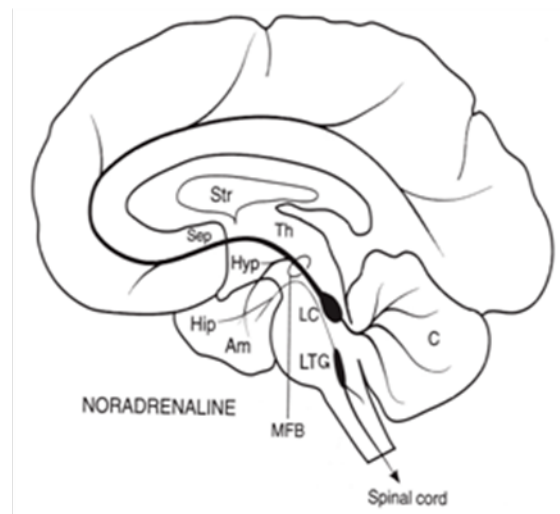


Figure 3. Noradrenergic pathways in the human brain. AM: amygdala; C: cerebellum; Hip: hippocampus; Hyp: hypothalamus; LC: locus coeruleus; LTG: lateral tegmental group; MFB: medial forebrain bundle; Sep: septum; SN: substantia nigra; Str: striatum; Th: thalamus. Modified from (Rang et al., 1999)

spontaneous discharge in these areas.

These neurons are associated with the stress response and with the control of drive and motivation, alertness and sleep patterns, along with stress-related manifestations such as anxiety and fear. Taken together the physiological reactions in the peripheral and central nervous systems mediated by the adrenergic and NE-ergic systems make up the substrate of the response to stress that is best illustrated by the “Fight or Flight” paradigm.

1.2.2.2. NE synthesis, storage, release and metabolism

The sequence of enzymatic steps required for the NE production was first documented by Blashko in 1939. In the brain NE precursor L-tyrosine, derived from the dietary proteins, is hydroxylated at position 3 by the tyrosine hydroxylase (TH) and 3,4-dihydroxy-L-phenylalanine (L-DOPA) is formed. This is a rate limiting step. Thus, by either depleting the L-tyrosine or inhibiting the TH (often achieved with α -methyl-paratyrosine(AMTP)) synthesis of NE can be dramatically decreased, allowing to study the effects or lack of this neurotransmitter. Subsequently, L-DOPA is rapidly decarboxylated to dopamine (DA) by the aromatic L-amino acid decarboxylase (AADC). The last step involves hydroxylation of DA into NE by DA- β -hydroxylase. Synthesis of NE occurs in the nerve terminals.

Once synthesized, NE is accumulated in the vesicles by the vesicular monoamine transporter (VMAT2). The vesicular uptake process has a low substrate specificity and a variety of biogenic amines including tryptamine, tyramine, and amphetamines can be transported. Indeed, the vesicular packaging of DA and 5-HT are regulated by the same protein suggesting a common storage mechanism (Erickson et al. 1992).

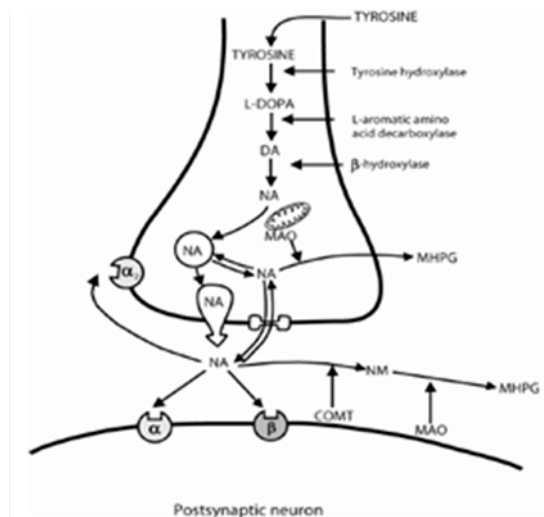


Figure 4. Schematic drawing of a noradrenaline synapse NA: noradrenaline; NM: normethanephine; MHPG: 3-methoxy-4-hydroxy-phenylglycol. See text for other abbreviations. Modified from (Cooper et al., 2003)

The release of NE occurs mainly via stimulus-evoked exocytosis in a Ca^{2+} -dependent manner (Thureson 1983). As well, NE can be pumped out through the membrane transport proteins in a Ca^{2+} -independent way by diffusion from cytoplasm through the channel-like pores (Raiteri et al. 1979). Two distinct vesicular pools of NE are believed to exist within the nerve terminal –

preferentially released (newly synthesized) and the reserve pool.

Norepinephrine can be metabolized intra- and extracellularly. Extracellularly NE is primarily degraded by the catechol-O-methyltransferase (COMT). COMT metabolizes NE into 3-methoxy-4-hydroxyphenylglycol (MHPG) in a Mg^{2+} -dependent manner. On the other hand MAO, mainly expressed intrasynaptically, catalyzes oxidative deamination. As a result NE is transformed into

vanillylmandelic acid (VMA).

1.2.2.3. NE transporters

One of the main mechanisms of inactivation of synaptically released NE is reuptake through the norepinephrine transporter (NET). Cloning of NET DNA revealed the structure of the transporter (Pacholczyk et al. 1991). It is a 12-membrane spanning hydrophobic glycoprotein with a high degree of sequence homology with transporters for 5-HT, DA, GABA, glycine and choline (Amara, Kuhar 1993; Uhl 1992). NET amino acid sequence is highly homologous between species (Pacholczyk, Blakely et al. 1991; Lingen 1994). The uptake process is saturable, energy-dependent, and depends on Na⁺ co-transport (Brüss et al. 1997; Krueger 1990). In addition, extracellular Cl⁻ is required.

Upon uptake the neurotransmitter is either repackaged back into vesicles for future release or degraded by MAO.

The NET mRNA expression is seen primarily in the LC, lateral tegmentum and nucleus tractus solitarius (Lorang et al. 1994; Eymin et al. 1995). After translation, the NET protein is transported from the NE cell bodies to the terminals in all projection areas (Tejani-Butt 1992; Cheetham et al. 1996). The levels of NET are highest in the LC, followed by dentate gyrus, hippocampus and DR (Tejani-Butt 1992).

Interestingly, despite NE and DA neurons expressing only the gene for their own carrier (Amara, Kuhar 1993), transporters do not possess a high degree of

selectivity for their own transmitter. Indeed, NET not only takes up DA, but it was found to have a higher affinity for this neurotransmitter, than for NE itself (Raiteri et al. 1977). For instance, in prefrontal cortex DA is predominantly taken up by NET (Di Chiara et al. 1992).

The NET activity is not constant and is dependent on the neuronal activity, peptide hormones, levels of catabolic proteins and second messengers (Kaye et al. 1997). The level of protein phosphorylation, mediated by the latter, seem to be of the greatest importance. The NET function can be inhibited by either selective NET blockers or by toxins inhibiting Na^+ , K^+ -ATPase (required for maintenance of Na^+ gradient crucial for proper NET activity). Pharmacological agents blocking NET represented by tricyclic antidepressants, selective NE reuptake inhibitors and 5-HT/NE inhibitors, are important players in MDD therapy.

1.2.2.4. NE receptors

Existence of two distinct types of noradrenergic receptors was suggested in 1948 by Ahlquist. Later, based on pharmacological and functional criteria, these two groups were further subdivided into $\alpha_{1A/B/C/D}$, $\alpha_{2A/B/C}$ and β_1 , β_2 , β_3 receptors (Langer 1974). All noradrenergic receptors belong to the superfamily of G-protein coupled receptors. Differential G-protein coupling of these receptors classifies them into three categories: all β adrenoceptors activate G_s to stimulate adenylate cyclase; $\alpha_{2A/B/C}$ adrenoceptors inhibit adenylate cyclase through G_i coupling; $\alpha_{1A/B/C/D}$ adrenoceptors stimulate phospholipase C action through coupling to G_q .

Adrenergic receptors are present throughout the brain and in the periphery. The focus of this document will be directed at adrenoceptors located in DRN and LC brainstem nuclei, hippocampus and frontal cortex, as these structures are believed to be related to the symptomatology of psychiatric disorders.

1.2.2.4.1. α -adrenoceptors

Distribution of α_1 -adrenergic receptors was attained through use of the autoradiography techniques. Moderate levels of binding were detected in LC, RD and hippocampus (Unnerstall et al. 1985; Palacios et al. 1987). The levels and distribution of α_1 -adrenergic receptors mRNA follow a similar trend (Pieribone et al. 1994). These receptors are predominantly expressed on non-NE cells and thus act as heteroreceptors, mediating the excitatory effect of NE. The α_1 -adrenergic receptors (subdividing into 1A, 1B, and 1D subtypes) are coupled to the Gi/Gq proteins. The Gi/Gq proteins activate the phospholipase C-protein kinase (PLC), which subsequently triggers the cascade of events generating second messengers, inositol triphosphate (IP3) and diacylglycerol (DAG). The IP3 promotes Ca^{2+} release from the intracellular stores, thus increasing the concentration of available intracellular Ca^{2+} utilized in regulation of several protein kinases (Berridge 1993). The DAG is a potent activator of protein kinase C (PKC), which is involved in the activation of many substrates including membrane proteins such as channels, pumps, and ion exchange proteins (Fields, Casey 1997). These events lead to the decrease in K^+ conductance, thus depolarizing neurons which renders them more excitable.

Though potential benefits of pharmacological activation of these receptors in the CNS exists, it is largely overshadowed by the increase in blood pressure mediated through the α_1 -adrenergic receptors in the periphery.

The molecular cloning has identified four different subtypes of α_2 -adrenergic receptors (2A,B,C, D) (Bylund et al. 1994). The α_2 -adrenergic receptors were found to be expressed predominantly in LC, DR, cortex and hippocampus (Bruning et al. 1987). The mRNA labelling was detected in the same areas (Nicholas et al. 1993; Scheinin et al. 1994). The α_2 -adrenergic receptors are expressed both pre- and postsynaptically (Boehm, Kubista 2002). The postsynaptic localization is believed to be predominant, as majority of the binding sites are unaffected by the neurotoxin-produced destruction of LC neurons (Heal et al. 1991). The presynaptic α_2 -adrenoceptors, however, regulate the NE system homeostasis via negative feedback autoregulation. The α_2 -adrenergic receptors are coupled to the Gi/o protein family whose activation results in the inhibition of cAMP accumulation (Neer 1995). The latter leads to an increase in the K^+ conductance via activation of the G protein-gated K^+ channels. Thus, α_2 -adrenoceptors mediate a hyperpolarization of the neuronal membrane, making the neuron less excitable.

Drugs with α_2 -blocking properties (like mirtazapine, clozapine, etc.) were shown to increase the level of NE via activation of autoreceptors, and of 5-HT via heteroreceptors on 5-HT terminals, they were found to be efficacious in treatment of depression (Maes et al. 1999).

1.2.2.4.2. β -adrenoceptors

Based on a differential response to pharmacological manipulation, 3 subtypes of β -adrenoceptors were described ($\beta_{1,2,3}$). Both β_1 and β_2 -adrenergic receptors were found to be widely expressed throughout the CNS, whereas β_3 -adrenergic receptors are mainly present in the adipose tissue. There are regional differences in the regional distribution of β_1 - and β_2 -adrenergic receptors – the intense labeling for β_2 receptors is present in cerebellum, thalamus and olfactory bulb, whereas levels of β_1 are high in cortex, hippocampus and caudate-putamen (Nicholas et al. 1993). The β -adrenergic receptor expression is exclusively postsynaptic. Functionally β -adrenergic receptors stimulate adenylyl cyclase (AC) via Gs protein coupling (Bylund et al. 1994).

Many antidepressants are known to downregulate/desensitize β -adrenergic receptors in rats' forebrain structures, the functional importance of this finding is not entirely known (Anand, Charney 2000; Blier, De Montigny 1994). Interestingly, the blockade of β receptors was proposed to have a potential in PTSD treatment, as these receptors are believed to be involved in regulation of the emotional memories.

1.2.2.5. NE neuron electrophysiology:

The firing pattern of NE neurons is greatly dependent on the physiological state of the body – it is highest during the active waking, decreases in quiet waking, further slows during slow wave sleep and virtually absent during the REM

stage of sleep. In anaesthetized rats neurons discharge 0.5-5 spikes per second in a pacemaker-like manner with action potentials of long duration (0.8-1.2 ms) (Aghajanian, Vandermaelen 1982a). Characteristically, the NE neurons are responsive to the noxious stimuli – by pinching the contralateral, but not ipsilateral paw, burst discharge followed by a brief quiescent period and restoration of normal firing occurs (Chiang, Aston-Jones 1993b). The regular, almost clock-like firing activity is dependent on the levels of endogenous cAMP, which induces persistent Ca^{2+} -independent/ TTX-insensitive inward current that depolarizes the cell membrane (Alreja, Aghajanian 1995).

1.2.3. Dopamine System

1.2.3.1. Neuroanatomy and function

After Carlsson and his group had shown that despite presence of both NE and DA in the CNS, their regional distribution varied significantly, the role of DA as a neurotransmitter was established (Carlsson 1976). Up until then DA was only viewed as a NE precursor. In rats the number of DA cells in the mesencephalic tegmentum has been estimated at about 15,000–20,000 on each side of the brain (Hedreen, Chalmers 1972; Swanson 1982): some 9,000 in the ventral tegmental area (VTA) and the remainder in the zona compacta of the substantia nigra and retrorubral field (Swanson 1982). Unlike 5-HT and NE, the general neuronal organization of the DA system is rather compartmentalized with DA neurons

distributed across several nuclei. In the rat CNS, four major DA projection subsystems have been described — mesocortical, mesolimbic, nigrostriatal and tuberoinfundibular. Additionally, several parts of the diencephalon (A11-A15) as well as both the olfactory bulb (A16) and retina (A17), contain DA neurons. The VTA (A8, A10) projections to the cingulate and medial prefrontal cortex constitute the mesocortical pathway, while VTA afferents to the limbic structures like nucleus accumbens, amygdala, hippocampus and olfactory tubercle form the mesolimbic pathway. Together these two pathways mediate regulation of the emotional control, motivation, reward and cognition. Therefore, their malfunction is believed to be involved in etiology of several psychiatric conditions including affective disorders, schizophrenia and addiction. The substantia nigra pars compacta DA neurons innervate the dorsal striatal structures like caudate, putamen and globus pallidus forming the nigrostriatal DA pathway. The proper function of this system is crucial for the sensorimotor coordination; degradation of DA cells within this pathway underlies pathophysiology of Parkinson's Disease (PD). The tuberoinfundibular DA system is comprised of DA projections from the hypothalamus arcuate and periventricular nuclei to the median eminence, and is involved in the hormonal regulation. Disruption of its function may produce (unfavorable) neuroendocrine effects. The descending DA projection to the spinal cord originates from DA neurons (A11) in hypothalamus.

1.2.3.2. Synthesis, storage, release and metabolism of DA

Synthesis of DA takes place in the nerve terminals. There are two enzymatic steps involved in the synthesis process (Von Bohlen Und Halbach et al. 2004). In the brain catecholamine precursor L-tyrosine is hydroxylated to L-DOPA by the enzyme TH. Dopamine synthesis is completed in the next step, when L-aromatic amino acid decarboxylase converts L-DOPA to DA (Deutch, Roth 1987). Tyrosine hydroxylase is the rate-limiting step in synthesis of DA and thus controls the neuronal concentrations of DA. The physiological tyrosine concentrations

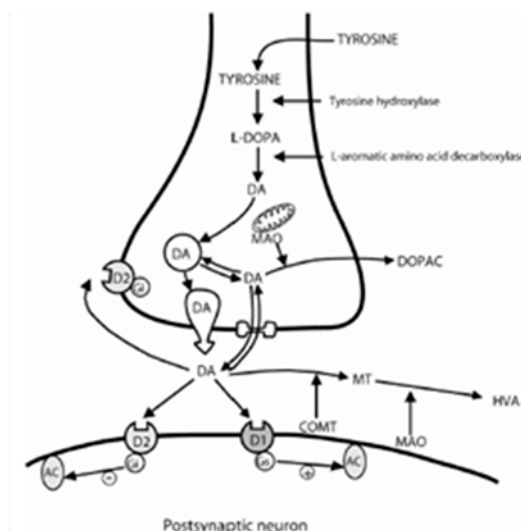


Figure 6. Schematic drawing of a dopamine synapse. DA: dopamine; D1: D1-like receptor; D2: D2-like receptor. See text for other abbreviations. Modified from (Cooper et al., 2003)

saturate TH and its increase usually does not elevate the rate of DA synthesis. The activation of DA neurons leads to the increase of TH activity; its expression can be either upregulated or downregulated by different drugs such as nicotine, caffeine, morphine, or antidepressants via modulation of the transcriptional regulatory elements of TH gene promoter.

Accumulation of DA in the vesicles depends on the operation of the VMAT2 (Weihe, Eiden 2000). The driving force for uptake into the synaptic vesicles is an ATP-dependent proton electrochemical gradient generated in the synaptic vesicle membrane. Thus, VMAT2 decreases cytoplasmic concentration of DA and prevents its metabolism by MAO. Administration of reserpine that competes with 5-HT, NE and DA for the VMAT binding site, thus preventing their effective storage,

produces drastic reduction in the release of monoamines (Henry, Scherman 1989). Two vesicular compartments of DA are believed to exist. One vesicular pool is designated for rapid release of DA. This releasable compartment represents the vesicles located near the presynaptic membrane and contains 5– 20% of the total DA content. The larger pool is designed for the reserve transmitter storage and is inactive during most physiological processes.

The release of DA mainly occurs via exocytosis in a Ca^{2+} -dependent manner, when the action potential invades the terminal. The extent of DA release is dependent on both rate and pattern of DA neuronal firing. Indeed, burst firing, characteristic for DA neurons, is believed to be a more efficient form of the signal propagation, as more transmitter is released per pulse fired in burst, than single-spike mode (Gonon 1988; Garris et al. 1994). The reverse transport of DA across the membrane by DA transporter (DAT) represents another form of DA release (Raiteri et al. 1979). It occurs in Ca^{2+} -independent manner, however its role in release under physiological conditions is not functionally significant.

The metabolism of DA occurs via enzymatic degradation by COMT (extracellularly) and by MAO (both extra- and intracellularly) (Von Bohlen Und Halbach et al. 2004). MAO oxidatively deaminates DA and its O-methylated derivative, 3-methoxytyramine, forming transient derivative 3,4-dihydroxyphenylacetaldehyde. This aldehyde is then rapidly catabolised by the aldehyde dehydrogenases to 3,4-dihydroxyphenylacetic acid (DOPAC). About 40% of DOPAC is eliminated from the brain, while other 60% get further metabolized by

COMT resulting in homovanilic acid (HVA) formation. Accumulation of DOPAC and HVA in the brain or cerebrospinal fluid (CSF) is often used as an index of functional activity of the DA system.

1.2.3.3. DA transporters

The termination of action of synaptically released DA is primarily mediated by the reuptake process carried out by the membrane carrier - DAT. Dopamine transporter is a 12-membrane spanning hydrophobic glycoprotein, member of the family of Na^+/Cl^- -dependent plasma membrane transporters. As follows from the protein family name, DAT is dependent on the Na^+ co-transport and requires extracellular Cl^- (Norregaard, Gether 2001). As reuptake depends on the Na^+ gradient across the neuronal membrane, drugs that inhibit Na^+/K^+ -transporting adenosine triphosphase or open Na^+ channels subsequently decrease the DA reuptake. As Na^+ gradient across the plasma membrane varies by the neuron state, DAT may operate in a reverse-mode pumping out DA from the cell to the synaptic cleft (Gainetdinov et al. 2002). The neuronal reuptake is saturable and energy-dependent.

The molecular characterization of the DAT showed that it exhibits 66% homology with NET and is highly conserved between species.

The DA carrier mRNA occurs in brain areas in which DA is synthesized: it is highest in the substantia nigra and the VTA, but is also detected in arcuate nucleus, olfactory bulb, and the retina. The regional distribution of the carrier follows the expected localization of the distinct DA neurons; however, DAT

expression varies greatly among DA cell groups and is not expressed in all DA neurons. For instance, the hypothalamic tuberoinfundibular DA cells that release DA into the pituitary portal blood stream, lack DAT mRNA and protein. The DAT expression is also low in primate prefrontal cortex where DA reuptake is predominantly carried out by NET (Gresch et al. 1995). Not only neurons, but glia also express DAT. However the functional significance of the glial DA reuptake is not clear.

As DAT maintains transmitter homeostasis its modulation plays an important role in neuropsychiatric disorders. Blockade of this protein by cocaine and other drugs of abuse leads to the drastic increase in synaptic DA concentration and thus underlies their reinforcing properties. However, the binding site of these molecules is distinct from the transmitter binding site. Thus, DAT blockers interacting with the neurotransmitter binding site are devoid of addictive properties and were shown to possess antidepressant potential.

1.2.3.4. DA receptors

The existence of two distinct groups of DA receptors was proposed based upon a combination of biochemical, pharmacological and anatomical criteria (Garau et al. 1978; Keabian, Calne 1979). Thus, DA receptor family is comprised by D1- like (D1) and D2-like (D2) receptors. Subsequent molecular biological studies postulated that based on the sequence homology as well as similarity in function, D1 group contained D₁ and D₅ receptors, and D2 group was made of D₂, D₃ and D₄ receptors (Rashid et al. 2007; Garau et al 1978; Keabian, Calne

1979). The D1 receptors activate AC via coupling to a Gs protein and increase cAMP formation, whereas D2 receptors inhibit AC via Gi coupling decreasing cAMP and/or increasing IP3 production (Kebabian, Calne 1979). Interestingly, despite opposite effects on the signal transduction, D1 and D2 receptors can form hetero-oligomeric complexes (Rashid et al. 2007).

1.2.3.4.1. D1 receptors

The distribution of D1 receptors was determined by the autoradiography. The D₁ receptors are localized throughout the brain regions receiving DA afferents with highest densities in the nucleus accumbens, caudate-putamen, olfactory tubercle and substantia nigra, as well as thalamus, hypothalamus and cortex. The D₅ receptors are limited to thalamus, hypothalamus and hippocampus. Frontocortical localization of D1 receptors implicates their importance in the cognitive function.

1.2.3.4.2. D2 receptors

Similarly to D1 receptors, binding sites for D2 receptors were detected in numerous brain areas receiving DA innervation (Meador-Woodruff, Mansour et al. 1991). However, levels of D2 expression were much greater in projecting loci - VTA and substantia nigra, and in pituitary gland. The somatodendritic localization of these receptors hints their involvement in autoregulation. Indeed, activation of D2 autoreceptors located on the cell body of midbrain DA neurons decreases the firing activity of the latter, while stimulation of terminal D2 autoreceptors inhibits synthesis and release of the neurotransmitter. The D₂ subgroup of the D2-like

receptors is further subdivided into two categories in accordance with the splice variation of the receptor gene D_{2S} , expressed predominantly presynaptically and functioning as an autoreceptor, and D_{2L} acting as a heteroreceptor.

The D2 receptors are believed to be important players in psychopathology and/or treatment of schizophrenia, depression, PD and attention deficit hyperactivity.

1.2.3.5. DA neuron electrophysiology:

In parallel to other monoamines the firing pattern of DA neurons is highest during the active waking, decreases in quiet waking, further slows during the slow wave sleep and is virtually absent during REM stage of sleep. The extracellular electrophysiological recordings of these neurons have elucidated their distinct properties. These neurons exhibit slow spontaneous firing rate (0.5-7 Hz), the action potentials have a long duration (>2.6 msec) and often present a notch on the rising phase (Grace, Bunney 1984). The DA neurons discharge in a single-spike or bursting pattern in which 3-10 spikes of decreasing amplitude are fired (Freeman et al. 1985). The discharge pattern of DA neurons presents a spontaneous firing followed by a quiescent period due to temporary hyperpolarization (Bunney, Grace 1978). Sometimes neighboring DA cells fire in a synchronous mode, indicative of gap-junction mediated electric coupling (Grace, Bunney 1983).

1.3. Evidence of involvement of monoaminergic systems in pathophysiology of depression

1.3.1. Serotonin

The deficiency in serotonergic function is believed to be a factor predisposing for depression (Maes et al. 1990; Deakin et al. 1990; Maes, Meltzer 1995; Mann 1999). Indeed, investigations into the levels of neurotransmitter metabolites, changes in the receptor functioning, and neuroendocrine challenge testing have consistently reported the altered 5-HT neurotransmission in depression. First lines of evidence supporting the importance of 5-HT component in the antidepressant response come from the studies where the 5-HT deficiency was induced by either p-chlorophenylalanine or tryptophan depletion. The remission produced by MAOIs or TCAs was reversed with these manipulations, illuminating the necessity of 5-HT for the treatment response (Shopsin et al. 1976; Smith et al. 1997). Indeed, the levels of 5-HT precursor - tryptophan were inversely correlated with the severity of the depletion-induced depressive symptoms (Booij et al. 2005; Delgado et al. 1990). Furthermore, the plasma levels of tryptophan were found to be significantly lower in depressed individuals, when compared to controls (Maes 1990; Deakin et al. 1990). Several studies have also put into evidence that the levels of 5-hydroxyindoleacetic acid (5-HIAA), the primary 5-HT metabolite, were significantly decreased in MDD patients (Asberg et al. 1976; Roy et al. 1989). Subsequent studies, however, demonstrated that low 5-HIAA is strongly associated with

impulsiveness and aggression rather than depression per se (Faustman et al. 1991).

Additionally, alternations in the 5-HT receptors population are evident in the depressed brain. The binding towards both 5-HT_{1A} and 5-HT_{2A} receptors was found to be increased in cortical areas of the post-mortem tissue of depressed individuals (Arango et al. 1992; Mann et al. 1986; Matsubara et al. 1991). Interestingly, the binding potential of 5-HT_{1A} receptor was not restored after successful SSRI treatment (Bhagwaga et al. 2004; Sargent et al. 2000). The persistence of this alteration in patients who recovered from depression suggests that the impaired 5-HT function is trait rather than state related (Mann 1999). It needs to be mentioned, however, that some investigators report the normalization of 5-HT_{1A} receptor levels following treatment with SSRIs (Miller et al., 2009).. Furthermore, a similar lack of normalization of the 5-HT response after successful treatment was noted with prolactin challenge. The ability of fenfluramine (which increases 5-HT release and reduces its reuptake) to stimulate the prolactin secretion from the pituitary gland can be used to examine the 5-HT function. The response to this neuroendocrine challenge is decreased in both currently depressed and remitted patients, when compared to controls (Maes et al. 1990; Lichtenberg et al. 1992; Flory et al. 1998).

Another piece of evidence pointing at the 5-HT abnormality in MDD is a decrease in the SERT binding in brain and platelets of the depressed individuals (the platelets are considered to be a good model for a state-dependent brain

serotonergic function as they take up 5-HT via the SERT in a manner similar to the CNS neurons) (Kaplan, Mann 1982; Malison et al. 1998; Nemeroff et al. 1988). Indeed, in comparison to controls, the levels of SERT were significantly lower in people who died by suicide (Leake et al. 1991). It needs to be emphasized, however, that around 30% of suicide victims are not depressed at the time of death (Beautrais et al. 1996; Mannet al. 1999). The diminished SERT function is paralleled by the notion that the functional polymorphism of SERT plays an important role in depression vulnerability. The longitudinal study aimed at determining the association between the number of significantly stressful life events and the depression outcomes, determined that individuals carrying one or two copies of short allele of the SERT promoter were significantly more susceptible for development of depression and suicidal ideations, than carriers of two long alleles (Caspi, Sugden et al. 2003). This finding was recently confirmed by the meta-analysis of all 54 studies, assessing this correlation (Karg et al. 2011). Moreover, individuals homozygous for short form allele in comparison to the long allele carriers were found to be less responsive and more prone to side effects when treated with SSRI, but not with NE targeting drug mirtazapine (Murphy Jr. et al. 2004).

1.3.2. Norepinephrine

The reduction in energy levels and cognitive function, as well as commonly comorbid anxiety, are likely related to perturbations of the NE function in

depression. Indeed, several post-mortem studies in depressed suicidal victims elucidated an increase in density of α_2 -adrenoceptors, compared to the matched controls (Ordway 1997; Meana et al. 1992). Interestingly, the same alteration in α_2 -adrenergic receptors count is observed in rats undergoing chronic mild stress (overactivation of LC) or subjected to the pharmacologically-induced decrease of NE levels (Ordway 1997; Willner 1997). As these receptors act as autoreceptors exerting negative feedback modulation of NE release, this finding ultimately suggests a decrease in the overall levels of NE (Leonard 1997). Additionally, this increase in density of α_2 -adrenergic receptors seems to be balanced out by the antidepressants: their chronic administration was found to lead to the decrease in number of α_2 -adrenergic receptors in both animal and clinical studies (Charney et al. 1983; Garcia-Sevilla et al. 1981; Giralt, Garcia-Sevilla 1989). In humans the sensitivity of α_2 -adrenergic receptors can be indirectly measured by using clonidine, a centrally acting α_2 receptor agonist. Activation of the postsynaptic α_2 -adrenergic receptors in the hypothalamus stimulates the release of growth hormone (GH), subsequently causing its secretion from the pituitary gland. Several groups have reported that the GH response was significantly blunted in patients with depression and anxiety (Matussek et al. 1980; Siever et al. 1992).

Furthermore, another post-mortem study documented the decrease in the number of NET in the LC of depressed individuals (Ordway 1997). This alteration is likely taking place as a compensatory downregulation in response to the decreased levels of neurotransmitter. Moreover, as number of NE uptake sites is

believed to be indicative of the NE neuronal viability, the observed reduction suggests the decline in the number of NE neurons in LC (Ordway 1997). In line with this assumption, in patients with PD the severity of depressive symptoms was found to be inversely correlated with the integrity of limbic NE innervation (Remy et al. 2005).

Another line of evidence showing direct involvement of NE system in depression pathology and treatment comes from the α -methyl-para-tyrosine (AMPT) challenge experiments. The AMPT is a competitive tyrosine hydroxylase inhibitor producing acute drop in synthesis of DA and NE (Brodie et al. 1971). Administration of this compound produces a recurrence of depressive symptoms in MDD patients who respond to the NE-specific treatments (like NET inhibitors or mirtazapine), but not in patients stabilized on SSRIs. (Miller et al. 1996; Delgado et al. 1993; Delgado et al. 2002). Thus, depressive patients may present with a decrease in NE neurotransmission.

1.3.3. Dopamine

Anhedonia – one of the core symptoms of depression is associated with the dysfunction of the DA reward system. The amphetamine challenge leading to increased DA release was found to produce greater reward response and altered activation of brain regions mediating it in depressed individuals, than controls (Tremblay et al. 2002; Tremblay et al. 2005). Such effect suggests the diminished

basal function of DA neurotransmission in depression. Furthermore, the increased sensitivity to the psychostimulant-induced DA release was found to be potentiated by the glucocorticoids (Oswald et al. 2005). As glucocorticoid levels are known to be increased in depressed patients, alternations in DA function may be in part influenced by this neuroendocrine pathology.

Further evidence confirming the involvement of the DA system in depression comes from the genetic studies. The metaanalysis combining the data from 12 studies totaling 2071 patients showed a consistent association between the D₄ receptor polymorphism and the vulnerability for depression (López León et al. 2005). Two more studies have documented the increased risk of affective disorders in individuals with a D₃ receptor polymorphism (Chiaroni et al. 2000; Dikeos et al. 1999).

Another line of evidence of DA malfunction in MDD comes from neuroimaging studies. The binding towards D2 receptor was found to be enhanced in patients hospitalized with depression (D'haenen, Bossuyt 1994; Ebert et al. 1996; Shah et al. 1997). It is not clear, however, whether this alternation in D2 neuronal population is secondary to depression or psychomotor retardation often comorbid with it. This raise may indicate sensitization of the receptors and/or increase in their number and/or decrease of the synaptically available endogenous DA which competes with the testing agent for the binding site. In fact, the increase in sensitivity of D2 receptors was linked to treatment resistance (Healy, McKeon 2000). In addition, PET studies revealed a decrease in the DAT density in patients

suffering from depression, in comparison to controls (Martinot et al. 2001; Meyer et al. 2001). Interestingly, the same changes were produced by the induced DA depletion (Kilbourn et al. 1992; Gordon et al. 1996). The increase in binding for D2 receptors, paralleled by the decrease in binding for DAT likely reflecting an adaptive change secondary to the drop in levels of available DA.

Indeed, the level of homovanilic acid (HVA), a DA metabolite, was found to be negatively correlated with the severity of depression (Roy et al. 1985; Hamner, Diamond 1996). Furthermore, HVA levels were found to be elevated in suicide attempters and victims (Engström et al. 1999; Roy et al. 1992). Indeed, the longitudinal study put into evidence an increase in risk of suicide attempts in MDD patients with low CSF HVA levels (Roy et al. 1989).

The high incidence of MDD comorbidity in PD serves as yet another indication of the importance of the DA system in depression. In fact, almost 50% of parkinsonian patients present with depressive symptoms, which improve after treatment with pro-dopaminergic agents (Tandberg, Larsen et al. 1996).

Taking together these changes clearly indicate a decrease in the function of DA system in depression.

In summary, the increase in the level of autoreceptors accompanied by the decrease in the level of reuptake transporters was documented for all three monoaminergic systems. These changes are likely secondary adaptation

compensating for the elucidated decrease in level of the given neurotransmitter in depressed patients. Though it is not clear if these changes are causative of the neurobiological and behavioral changes observed in depression, or are consecutive of them, they reflect a strong link between the deficiency in function of DA, NE and 5-HT systems and the depression pathophysiology.

1.4. Structural and functional interactions between the monoaminergic system

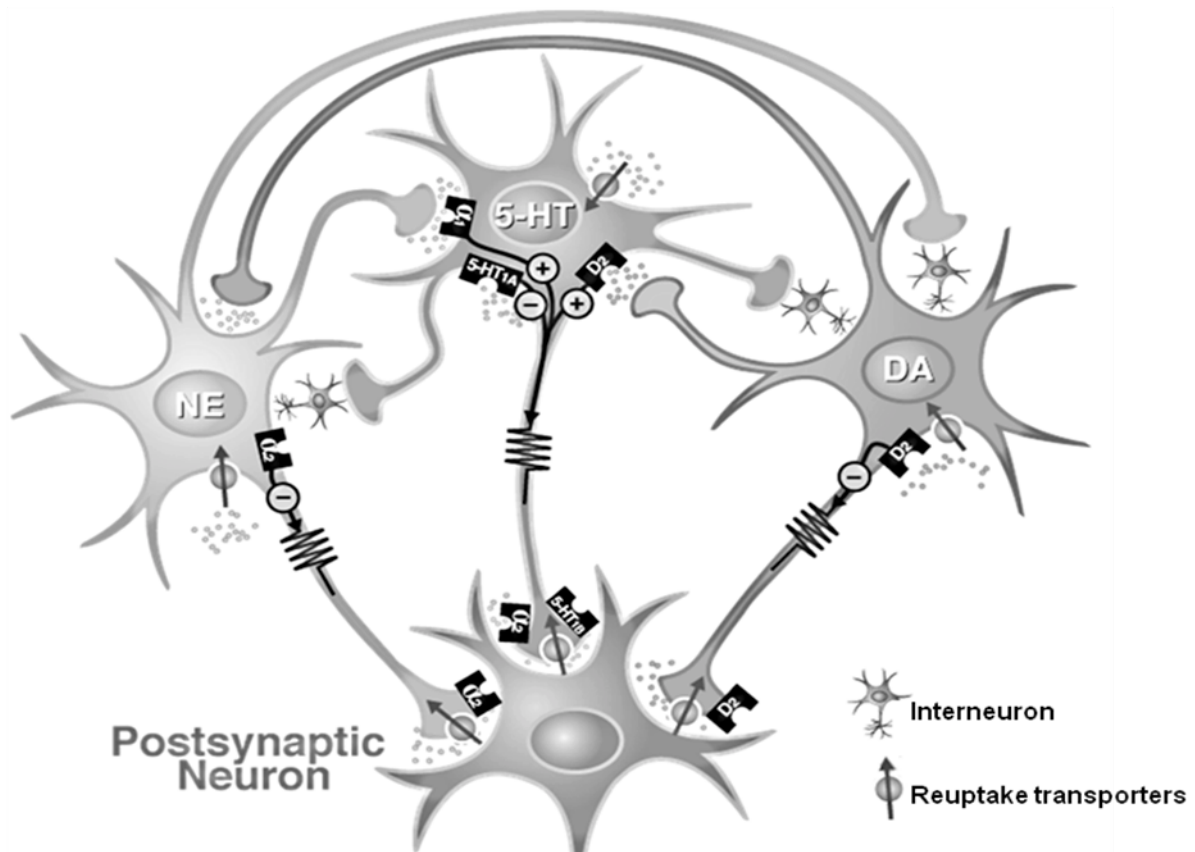


Figure 7. Diagram representing the reciprocal interactions between the cell bodies of dopamine, norepinephrine and serotonin neurons. A (+) sign indicates a stimulatory effect and a (-) sign indicates an inhibitory effect. The interneurons have been identified as GABA neurons mediating an inhibitory action via 5-HT_{2A} receptors for NE neurons and 5-HT_{2C} receptors for DA neurons. Modified from (Trivedi et al., 2008).

1.4.1. Serotonin-norepinephrine interactions

The 5-HT neurons in DRN are innervated by the LC NE neurons (Baraban, Aghajanian 1981; Sakai et al. 1977). The firing activity of DRN 5-HT neurons is significantly decreased and irregular after destruction of LC by the selective lesion

(Svensson et al. 1975). This suggests that NE provides tonic excitation of the 5-HT neurons. The activation of α_1 -adrenoceptors located on the 5-HT neuronal membranes is believed to be the main determinant of this effect (Baraban, Aghajanian 1981). Indeed, when raphe slices are prepared, 5-HT neurons do not discharge spontaneously, unless the α_1 -adrenergic receptor agonist is added to the medium. The decrease in release of 5-HT in hippocampus after the systemic administration of selective α_1 receptor antagonist prazosin serves as another evidence of the facilitatory effect of these receptors. Numerous studies postulated that 5-HT neurons are tonically activated by NE via the α_1 -adrenoceptors located on the 5-HT neurons. In contrast, activation of terminal α_2 adrenergic receptors reduces the 5-HT release in cortex, hypothalamus and hippocampus (Tao, Hjorth 1992), whereas their blockade prevents this effect (De Boer et al. 1996). Indeed, the prolonged administration of mirtazapine - antidepressant drug displaying a potent α_2 -adrenergic receptor antagonism, enhances the 5-HT spontaneous firing (Haddjeri et al. 1995). Taken together these findings suggest that NE exerts stimulatory effect over 5-HT neurons mediated via α_1 - and modulated by α_2 -adrenoceptors.

Conversely, 5-HT is believed to inhibit the NE neuronal function. Indeed, the decrease in available 5-HT levels, produced by either pharmacological lesion of RD or inhibition of 5-HT synthesis by PCPA, leads to a marked activation of NE neuronal firing rate (Reader et al. 1986; Crespi et al. 1980). Furthermore, the above 5-HT-depleting manipulations also prevent the inhibition of NE discharge

induced by the stimulation-evoked 5-HT (Segal 1979). This inhibitory modulation is believed to be conducted via several types of 5-HT receptors. Indeed, the labelling for 5-HT_{1A} as well as 5-HT_{2A} receptors is present in LC (Pompeiano et al. 1992). However, no mRNA hybridization signal for presence of neither of these receptors is detected in LC, suggesting that they are expressed on nerve terminals of other neuronal elements (Pompeiano et al. 1992). As destruction of 5-HT projections by selective DR lesion does not affect the binding profile of the above receptors in LC (Weissmann-Nanopoulos et al. 1985), it is likely that the 5-HT receptors mediating the inhibition of NE function are expressed on projecting Glu and/or GABA neurons. Indeed, the 5-HT_{2A} receptors are expressed on the GABA cells innervating the NE neurons in LC (Chiang, Aston-Jones 1993a). These receptors

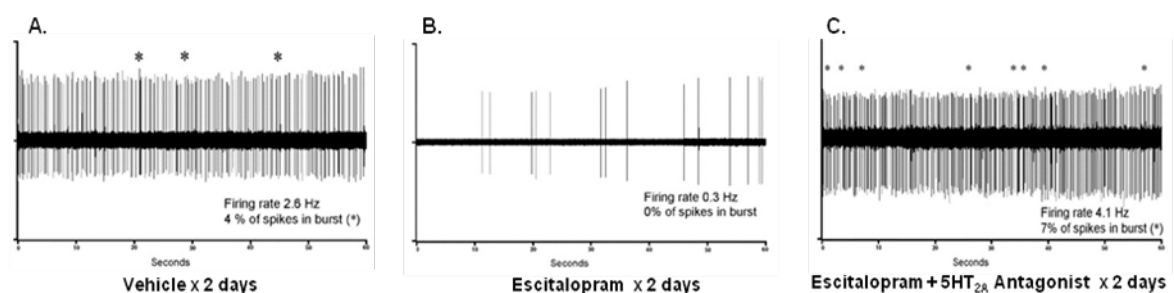


Figure 8. Representative single-unit extracellular recordings from the locus coeruleus norepinephrine neurons. Baseline NE discharge rate in a control rat (A); the inhibitory influence of the SSRi administration on a rat NE neuronal discharge (B), and a reversal of SSRi-induced inhibition by addition of the 5HT_{2A} receptor antagonist (C). Each asterisk indicates a burst. Modified from (Dremencov et al., 2007)

are believed to be chief players in mediation of the 5-HT-mediated inhibition of NE.

The administration of SSRIs, ultimately leading to the increase in 5-HT transmission, is known to decrease the NE spontaneous discharge (Haddjeri et al. 1998b). This effect is, in fact, believed to be mediated via activation of GABA neurons through excitatory 5-HT_{2A} receptors, which then results in the inhibition of NE neuronal activity by elevated GABA (Szabo, Blier 2001a). The inhibition in NE neuronal discharge rate induced by SSRIs as well as hallucinogens can be

reversed by administration of 5-HT_{2A} blockers (Dremencov et al. 2007d). Moreover, administration of 5-HT_{2A} antagonists increases the firing rate and NE metabolite levels in LC (Clement et al. 1992; Rasmussen, Aghajanian 1986).

In addition, 5-HT_{1A} receptor activation by the systemic administration of agonists increases both the firing activity of NE neurons and its metabolite levels in LC (Engberg 1989). Consistently with the latter observations, blockade of these receptors by selective 5-HT_{1A} receptor antagonist WAY 100635 suppresses the spontaneous discharge of NE neurons (Haddjeri et al. 1997). As local application of 5-HT_{1A} agonists does not alter the activity of these neurons (Haddjeri et al. 1997), the effects observed through the systemic administration of 5-HT_{1A} agonists are likely taking place due to the reduction in inhibitory 5-HT tone resulting from the inhibition of 5-HT firing produced by these agents.

The 5-HT system is thus believed to exert inhibitory influence over the activity of NE neurons. This effect is indirect and is primarily mediated via the 5-HT_{2A} receptors.

1.4.2. Serotonin-dopamine interactions

Various studies have shown anatomical similarities and functional interactions between the 5-HT neurons of the DRN and the DA neurons of mesencephalic DA systems (Martín-Ruiz et al. 2001; Aman et al. 2007a). For instance, D2-like receptors are expressed on the cell body of 5-HT neurons (Suzuki et al. 1998;

Mansour et al. 1990), which suggests that DA might be able to modulate 5-HT neuronal firing. Accordingly, recent *in vivo* study confirmed the existence of the excitatory effect of DA upon the DRN 5-HT neuronal firing: the mean firing activity of 5-HT neurons in DA-lesioned rats was decreased by 60% compared to the sham-operated rats (Guiard et al. 2008c). This finding is consistent with previously documented increase in firing rate (Martín-Ruiz et al. 2001) and 5-HT outflow in RD (Martín-Ruiz et al. 2001; Ferre, Artigas 1993b; Ferré et al. 1994) in response to

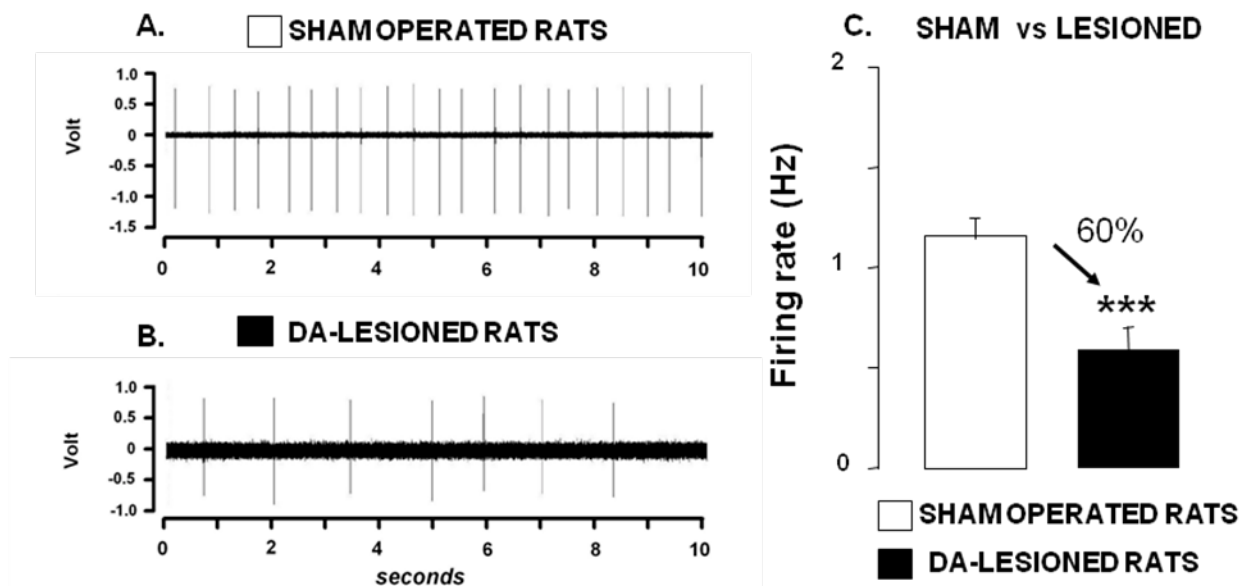


Figure 9. The effect of dopaminergic lesion on the electrophysiologic activity of 5-HT neurons in the dorsal raphe (DR). Examples of typical recordings of DR 5-HT neurons obtained in (A) a sham-operated rat and (B) a DA neuron-lesioned rat. (C) Mean $\pm$ S.E.M. of frequency (Hz) of 5-HT neurons recorded in the DR in sham-operated and DA neuron-lesioned rats. Modified from (Guiard et al., 2008)

the systemic administration of DA receptor agonist apomorphine. Furthermore, the D2 agonist quinpirole depolarized 5-HT neurons in tetrodotoxin-insensitive way, thus confirming that DA exerts direct excitatory effect upon 5-HT neurons via activation of D2 receptors located on the cell body of DR 5-HT neurons (Haj-Dahmane 2001b).

In turn, several receptors were found to mediate 5-HT effects upon the DA

neurotransmission. For example, activation of 5-HT_{1A} receptors by intravenous injection of selective agonists elevated the spontaneous firing of VTA DA neurons (Aborelius et al. 1993; Lejeune, Millan 1998; Lejeune et al. 1997) and consequently, DA release in somatodendritic (Chen, Reith 1995) and terminal areas (Aborelius et al. 1993; Rasmusson et al. 1994; Tanda et al. 1994). However, direct application of the 5-HT_{1A} receptor agonist 8-OH-DPAT onto the cell body failed to produce such effect (Prisco et al. 1994). As well, 5-HT_{1B} receptors were also shown to affect DA neurotransmission. For instance, ethanol-induced increases in VTA DA neuronal activity were suppressed by the selective 5-HT_{1B} receptor antagonist, SB 216641, and enhanced by the 5-HT_{1B} receptor agonist CP 94253 (Yan et al. 2005). Moreover, DA levels in the nucleus accumbens were elevated in the 5-HT_{1B} receptor knockout mouse (Shippenberg et al. 2000). In contrast, it was documented that the 5-HT_{2C} receptor has a tonic inhibitory control on the firing activity of mesolimbic and mesostriatal DA neurons. These receptors are excitatory in nature and are expressed on the GABA cells in VTA. Their activation leads to the increased inhibitory GABAergic tone over DA neuronal activity. For instance, acute administration of the 5-HT_{2B/C} receptor antagonist SB 206553 increased the rate of firing of neurons in the VTA, resulting in elevated dopamine release in the nucleus accumbens and striatum (Di Giovanni et al. 2000; Alex et al. 2005), whereas the 5-HT_{2C} receptor agonist Ro 60-0175, produced an opposing effect (Di Matteo et al. 2000). As selective lesioning of 5-HT neurons was found to enhance the spontaneous discharge of DA neurons in VTA (Guiard et al. 2008c), the overall effect of 5-HT system upon DA function appears to be inhibitory

and thus is predominantly mediated via 5-HT_{2C} receptors.

1.4.3. Dopamine-norepinephrine interactions

Several radioligand binding studies documented the presence of D2 receptors in the LC (Suzuki et al. 1998; Yokoyama et al. 1994). Lesion of VTA was found to significantly decrease the DA concentrations in LC (Ornstein et al. 1987). This decrease is likely a cause of nearly 50% elevation in the firing rate of NE neurons, following the 6-hydroxydopamine selective VTA lesion (Guiard et al. 2008c). This suggests a negative influence of VTA DA on the LC NE neurons. In line with the above observation, pharmacological blockade of DA receptors enhances the LC NE neuronal activity (Guiard et al. 2008a; Nilsson et al. 2005; Piercey et al. 1994), whereas the direct microiontophoretic application of DA to the cell body of NE neurons inhibits their activity (Guiard et al. 2008a; Elam et al. 1986; Cedarbaum, Aghajanian 1977). Interestingly though, despite the presence of D2 neurons in LC, the inhibitory effect of DA appears to be mediated via activation of α_2 -adrenergic receptors. The dampening of NE neuronal activity induced by systemic administration of D2 receptor agonist 3PP was reversed by the α_2 adrenoceptor antagonist yohimbine, but not the D2 receptor antagonist haloperidol (Elam et al. 1986). Similarly, the effect of the microiontophoretically applied DA was overturned by the blockade of α_2 but not D2 receptors (Guiard et al. 2008a). Together these findings point at the inhibitory influence of DA system over the activity of NE

neurons, which is, however, likely mediated via the α_2 -adrenergic receptors.

In turn, the LC NE neurons were found to innervate VTA (Jones et al. 1977; Geisler, Zahm 2005; Simon et al. 1979). Furthermore, the immunoreactivity for α_2 -adrenergic receptors was detected on VTA DA neurons (Lee et al. 1998). Decrease in the brain NE levels, produced by the selective lesion of LC, resulted in a significant increase in the discharge rate of DA neurons, implicating the

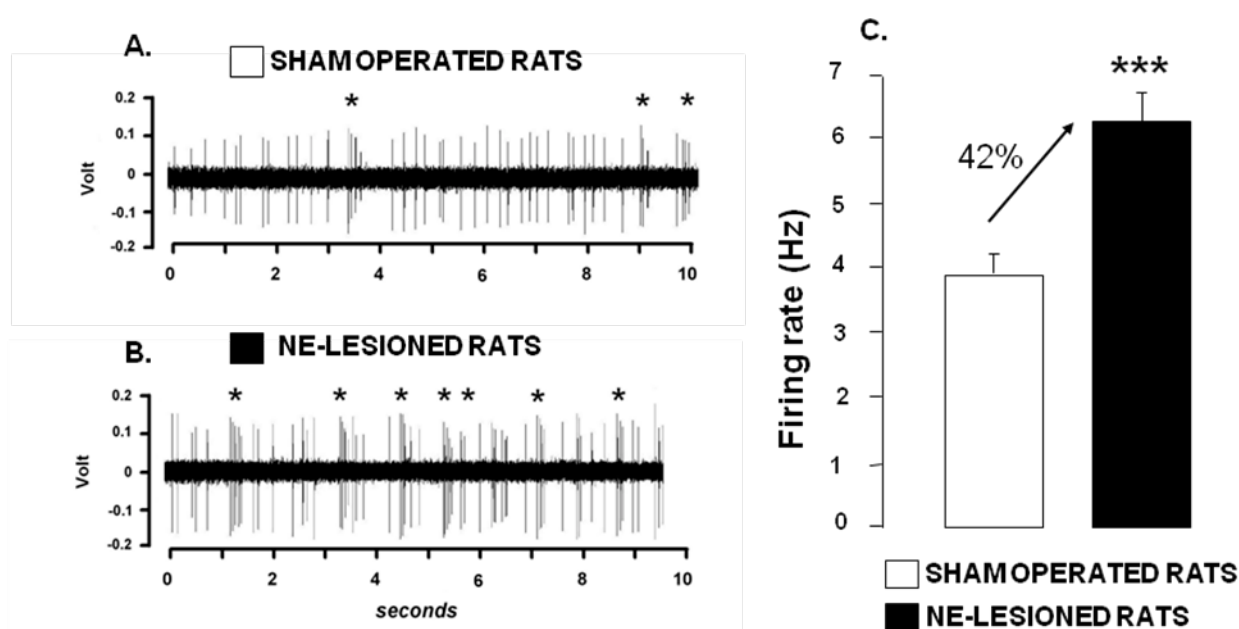


Figure 10. The effect of noradrenergic lesion on the electrophysiological activity of DA neurons in the ventral tegmental area (VTA). Examples of typical recordings of VTA DA neurons obtained in (A) a sham-operated rat and (B) a NE neuron-lesioned rat, each asterisk indicating a burst. (C) Mean $\pm$ S.E.M. of frequency (Hz) of DA neurons. Modified from (Guiard et al., 2008)

inhibitory influence of NE over the DA neuronal activity (Guiard et al. 2008c). In line with this observation, dampening of the NE neuronal tone produced by administration of low dose of α_2 -adrenergic receptor agonist clonidine (Szabo, Blier 2001a; Haddjeri et al. 1998a), also led to the increase in the spontaneous firing of DA neurons (Georges, Aston-Jones 2003; Millan et al. 2000). Additionally, the direct application of NE decreased the VTA DA neuronal discharge rate

(Guiard et al. 2008a). This effect was prevented by the blockade of α_2 -adrenergic receptors (Guiard et al. 2008a; White, Wang 1984) as well as D2 receptors (White, Wang 1984; Aghajanian, Bunney 1977). It needs to be mentioned, however, that contradictory results were also reported: a rise in extracellular NE levels produced by the systemic administration of α_2 -adrenergic antagonist or NET inhibitors resulted in an increase in the burst activity of VTA DA neurons (Shi et al. 2000; Grenhoff, Svensson 1989; Linnér et al. 2001). Moreover, unlike α_2 -, α_1 -adrenergic receptors mediate the direct excitation of VTA DA neurons, but also indirectly inhibit them via stimulation of inhibitory GABA interneurons (Grenhoff et al. 1995; Steffensen et al. 1998). Thus, modulation of DA neuronal activity by NE system appears to be more complex. However, the net effect of NE over function of VTA DA neurons is likely inhibitory and it is mediated via activation of both α_2 -adrenergic and D2 VTA receptors.

1.5. Impact of treatments used in depression on monoaminergic systems

Abundant clinical and fundamental research data documented the effects of the antidepressant treatments upon function of DA, NE and 5-HT systems. As the above monoaminergic systems influence each other in a reciprocal manner (as outlined in detail in section 1.4), even the treatments selectively targeting one of these neuronal entities alternate the state of the other systems through connections at the cell body and terminal levels. This section will thus be directed at outlining the effects of the antidepressant treatments, both

pharmacological and non-pharmacological, on the activity of monoaminergic neurons. Furthermore, as DA, NE and 5-HT neurons innervate the forebrain structures implicated in a genesis of depression, the monoamine-driven effects of therapies upon the activity of hippocampus and prefrontal cortex will be highlighted. As most of the antidepressant therapies require a prolonged administration for the manifestation of clinical efficacy, the description of the effects will reflect the changes observed over the chronic course. Antidepressant treatments may, in part, exert their effect via alteration in hormonal state, neurotrophins expression and intracellular signaling cascades, among others. However, the discussion of the above changes will be omitted and the scope of the present work will be focused on the antidepressant-driven changes in function of monoamines.

1.5.1. Pharmacological treatments

1.5.1.1. TCA

For many years since their discovery in the 1950s, TCAs were the first choice for the pharmacological treatment of depression, and they remain effective for the treatment of a wide range of disorders including depression, panic disorder, generalized anxiety disorder, eating disorders, obsessive–compulsive disorder (OCD), and pain syndromes (Anderson 2000). Although they are effective, owing to their high toxicity, narrow therapeutic window, and a potentially lethal outcome if used in suicide attempts, these drugs are now seldom prescribed (Mir, Taylor

1997).

Though most of the TCAs block both norepinephrine and serotonin, some have higher affinity for SERT (clomipramine, amitriptyline, imipramine, etc.) and some (desipramine, maprotiline, nortriptyline, protriptyline, etc.) for NET (Sánchez, Hyttel 1999). As well they block muscarinic, histamine, 5-HT₂ and α_1 adrenergic receptors. While the blockade of NET and SERT largely accounts for their therapeutic actions, the antagonism at the other receptors and NET inhibiting potential accounts for their side effects.

Though TCAs display 5-HT and/or NE reuptake blocking properties, their effect upon monoaminergic neuronal activity differs significantly from the alterations produced by the drugs selectively targeting the respective transporter complexes. In fact, contrary to SSRIs, prolonged treatment with TCA does not alter 5-HT neuronal discharge (Blier, De Montigny 1980). The sensitivity of 5-HT_{1A} and 5-HT_{1B} autoreceptors, decreased by the SSRIs, remains unchanged with prolonged TCA administration (Blier, De Montigny 1980). Though unlike selective NRIs, the TCAs do not change the firing rate of NE neurons, they do desensitize terminal α_2 auto- and heteroreceptors that regulate the release of NE and 5-HT, respectively, similarly to the former drugs (Mateo et al. 2001; Yoshioka et al. 1995; Mongeau et al. 1997). As a matter of fact, the concentration of 5-HT was found to be increased in striatum of rats subjected to a long-term desipramine regimen (Kreiss, Lucki 1995).

In sharp contrast to all other pharmacological treatments discussed herein, the long-term administration of TCAs sensitizes the postsynaptic 5-HT_{1A} receptors in the hippocampus and likely other 5-HT receptor subtypes in other brain regions (Chaput et al. 1991; De Montigny, Aghajanian 1978; Blier 1987), thus making the neurons in target areas to produce more functional output.

1.5.1.2. MAOIs

The MAOI family of antidepressants was discovered accidentally by the clinical observation of tuberculosis patients taking an MAOI pro-drug, iproniazid. Many of these patients were found to become happy and energetic. Later studies involving psychiatric patients given MAOI revealed mood improvement.

The MAOIs act by inhibiting the monoamine oxidase enzyme that carries out the intracellular breakdown of monoamines. Therefore, the administration of inhibitors of these catabolic enzymes was shown to elevate the levels of monoamines throughout the CNS (Eisenhofer et al. 2004). In the body, there are two major subtypes of MAO— A and B. MAO-A preferentially metabolizes NE and 5-HT, whereas DA gets deactivated by both isoforms (Hall et al. 1969; Yang, Neff 1974). The antidepressant properties of MAOIs are carried out by the MAO-A subtype, as agents selective for A, but not B isoforms were shown to be effective in the clinical management of MDD (Blier, de Montigny 1987). Similarly to other treatments influencing the 5-HT system, the initial decrease in the firing rate 5-HT neurons resulting from the acute activation of 5-HT_{1A} autoreceptors due to the increased synaptic availability of 5-HT, is followed by the normalization of the

serotonergic firing. This normalization of spontaneous discharge is permitted by the desensitization of 5-HT_{1A} autoreceptors. Chronic blockade of MAO also dampens the sensitivity of somatodendritic 5-HT_{1B} autoreceptors, controlling the release of the neurotransmitter (Piñeyro, Blier 1996). Moreover, both the density and sensitivity of 5-HT_{1A} receptors in hippocampus was diminished by prolonged MAO inhibition (Blier et al. 1986; Sleight et al. 1988). These adaptive changes ultimately reflect the increase in overall 5-HT transmission stemming from the MAOI-produced elevation in the releasable pool of 5-HT.

Unlike 5-HT system, NE neuronal firing does not regain the baseline levels even after chronic MAOI administration because of lack of adaptation of somatodendritic α_2 autoreceptors (Blier, De Montigny 1985). Regardless, the synaptic availability of NE increases in the rat cortical areas after prolonged MAOI administration (Greenshaw et al. 1988).

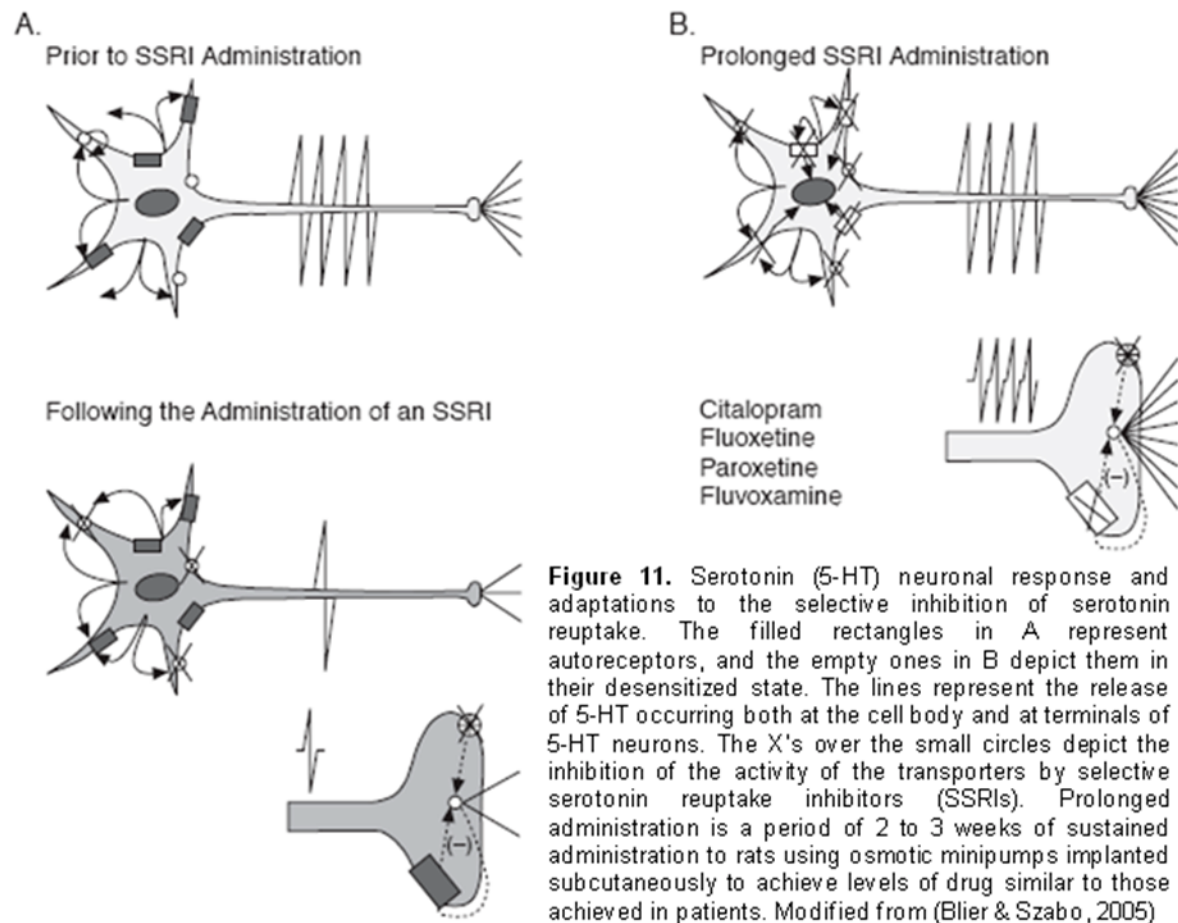
Similarly to NE, DA neuronal firing rate was decreased following the prolonged administration of MAO-A and unselective MAO inhibitors (Chenu et al. 2009). The observed attenuation of the neuronal discharge was found to be indirect and fully dependent on 5-HT system integrity, as 5-HT depletion antagonized the effect of MAOI (Chenu et al. 2009). Interestingly, the sustained administration of MAO-B inhibitor, affecting primarily DA degradation, had no effect on the firing rate of DA neurons. However, the *in vitro* cellular responses to DA, attributable to the activation of somatodendritic D2 autoreceptors, were found to be prolonged by pharmacological blockade of MAO A/B (Mercuri et al. 1997).

1.5.1.3. SSRI

Since approval of the first SSRI fluoxetine for treatment of depression in 1987, this class of drugs has become the most prescribed antidepressant pharmacotherapy. An important reason for their current popularity is that, apart from their low propensity for side effects, SSRIs have virtually no potential for lethality in overdose and are therefore considered very safe medications for use in a population at risk for suicide.

Drugs belonging to this class rapidly cross the blood-brain barrier to inhibit SERT. The blockade of the 5-HT reuptake leads to the amplification of synaptically available neurotransmitter. In fact, even a single administration of SSRI increases the levels of 5-HT in both DRN and projection areas (Bel, Artigas 1992; Fuller 1994). As a consequence of the enhanced activation of somatodendritic 5-HT_{1A} autoreceptors by the augmented levels of 5-HT, the firing rate of DRN 5-HT neurons falls significantly in rats (Chaput et al. 1986; Hajos et al. 1995). When SSRIs are administered over prolonged period of time, the rate of firing, however, returns to the baseline level (Chaput et al. 1986). This recovery is taking place due to the desensitization of the 5-HT_{1A} receptors which was demonstrated both *in vivo* and *in vitro* (Blier et al. 1987; Schechter et al. 1990). Indeed, not only somatodendritic 5-HT_{1A} receptors are subjected to the SSRI-induced decrease in sensitivity, but the terminal 5-HT_{1B} autoreceptors that control the release of 5-HT are also desensitized by the long-term SSRI administration. The electrically evoked neurotransmitter overflow is enhanced and the inhibitory effect of 5-HT_{1B} receptor

agonists is blunted (Piñeyro, Blier 1999). This adaptation allows the greater



release of 5-HT. Unlike their presynaptic counterparts however, the postsynaptic 5-HT_{1A} receptors, mediating the 5-HT signal transduction in the target areas, preserve their level of responsiveness following the sustained blockade of 5-HT reuptake (Béïque et al. 2000). Thus the attenuated responsiveness of 5-HT_{1A} and 5-HT_{1B} autoreceptors along with the normal sensitivity of postsynaptic 5-HT_{1A} receptors, and SSRI-induced synaptic 5-HT accumulation leads to the net increase in 5-HT neurotransmission.

The SSRI-driven enhancement of the 5-HT transmission also affects the activity of catecholamines. As 5-HT exerts tonic inhibitory influence over activity of both NE and DA neurons, their spontaneous firing decreases after SSRIs are administered in a sustained fashion. It needs to be mentioned that the lag phase and the degree of inhibition of catecholamine neurons is dependent upon the potency of the SSRI. The effect of SSRIs on the firing activity of NE and DA neurons is indirect and is mediated by the enhanced activation of 5-HT_{2A} and 5-HT_{2C} receptors, respectively (Dremencov et al. 2009b). Pharmacological blockade of these receptors prevents the SSRI-induced decrease in the catecholaminergic firing. Thus the increase in levels of 5-HT, resulting from the blockade of SERT, leads to an increased tonic inhibition of activity of NE and DA neurons. This dampening may be responsible for some side effects and the suboptimal response to SSRIs in a subset of patients.

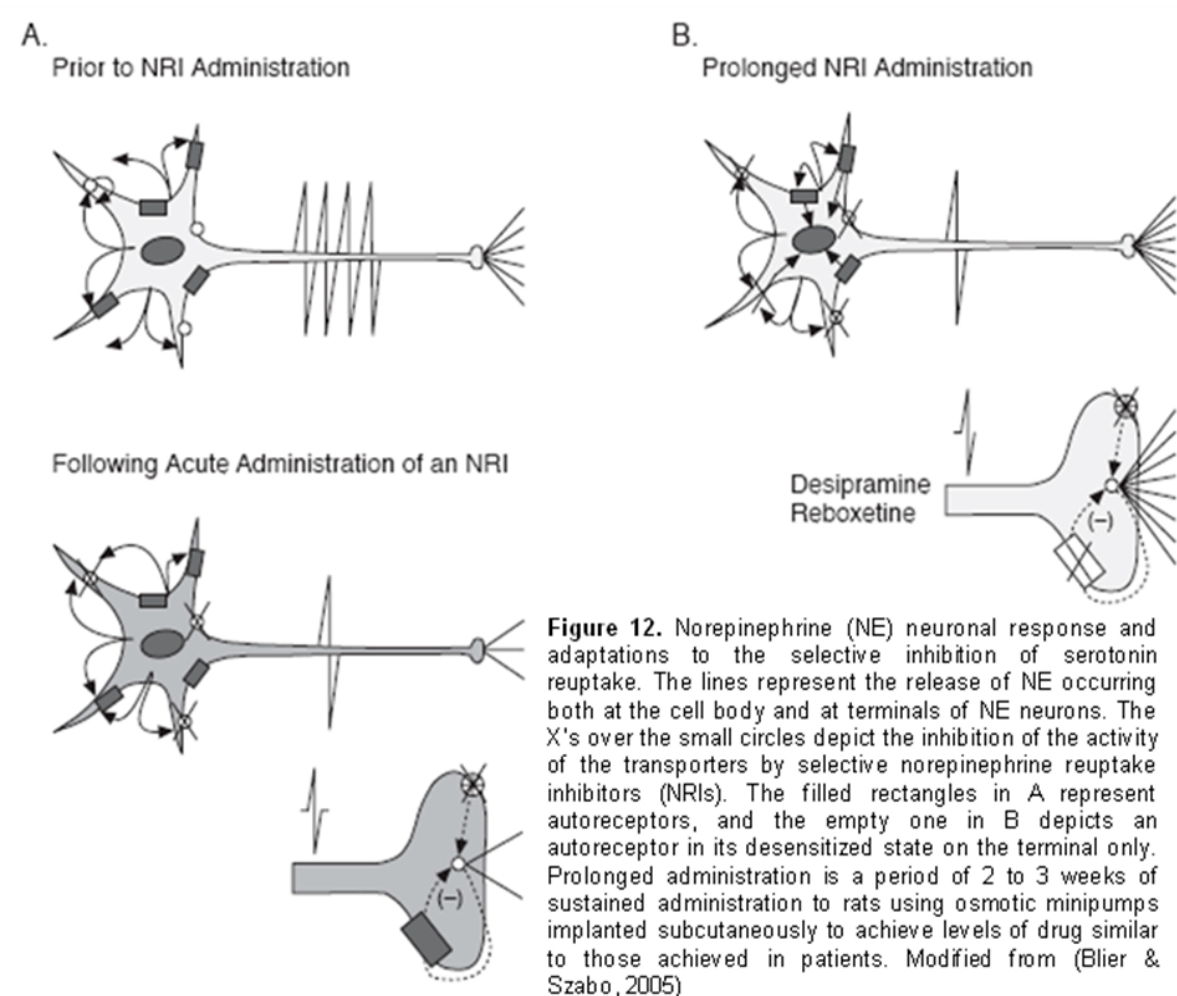
1.5.1.4. Norepinephrine reuptake inhibitors (NRI)

The blockers of NET desipramine and reboxetine were shown to be effective in clinical management of the depressive disorder (Olivier et al. 2000; Brunello et al. 2003).

Administration of these drugs leads to the elevation of synaptic levels of NE in LC and projection areas (Olivier, Soudijn et al. 2000). The increase in available NE overactivates the α_2 -adrenergic autoreceptor resulting in a decrease in the firing rate of NE neurons. Unlike 5-HT_{1A} autoreceptors desensitizing with the sustained blockade of 5-HT transporter, α_2 -adrenergic autoreceptors maintain their

sensitivity after NRIs are administered on a long-term basis (Szabo, Blier 2001a). In contrast, α_2 -adrenergic terminal autoreceptors, controlling the release of NE, do desensitize after NRI treatment (Szabo, Blier 2001a). The differential response of somatodendritic and terminal α_2 -adrenoceptors may potentially be due to different subtypes of these receptors to be present in respective areas (α_{2A} and α_{2C} , respectively). This allows the increase in release of NE. Taken together, the blockade of reuptake along with the augmented release leads to the elevation of synaptic levels of NE.

Despite their selective action at NET sites, blockers of NE reuptake also



impact the 5-HT neuronal transmission. Similarly to the terminal α_2 -adrenergic receptors located on NE terminals and controlling the release of NE, α_2 -adrenergic receptors are also present on the 5-HT terminals where they regulate the release of the latter transmitter. The desensitization of these terminal α_2 -adrenergic heteroreceptors by the sustained blockade of NET results in an increased synaptic availability of 5-HT in hippocampus (Szabo, Blier 2001a).

Furthermore, NRIs elicit a robust elevation of the DA in prefrontal cortex (Yamamoto, Novotney 1998). This phenomenon is likely explained by the ability of NET to uptake not only NE, but DA as well. As DA transporters are sparse in this brain area, the uptake of DA in cortical areas is predominantly mediated by the NET (Yamamoto, Novotney 1998).

Therefore, despite their selective action at NET, NRIs not only enhance the NE transmission, but also increase the levels of both DA and 5-HT, at least in some brain areas.

1.5.1.5. Serotonin-norepinephrine reuptake inhibitors (SNRI)

Venlafaxine and duloxetine are representatives of the class of drugs known as the SNRIs. At low doses, both agents has an action of serotonin reuptake inhibitor, and at higher doses it also possesses norepinephrine reuptake inhibition properties. The mechanism of action of these agents is similar to that of the TCA imipramine, without anticholinergic, sedative, or hypotensive side effects of the

latter.

Similarly to SSRIs, both duloxetine and venlafaxine initially decrease the firing activity of 5-HT neurons. With time, the discharge activity normalizes, as the sensitivity of somatodendritic 5-HT_{1A} autoreceptor decreases (Béïque et al. 2000; Rueter et al. 1998).

In accord with its potency at 5-HT and NE reuptake sites, higher doses of SNRIs is required to alter the function of NE neurons (Béïque, De Montigny et al. 2000, Rueter, De Montigny et al. 1998). Analogously to selective NRIs, SNRIs administered on a long-term basis also decrease the firing of NE neurons and desensitize terminal α_2 -adrenergic receptors on both NE and 5-HT neurons (Mongeau et al. 1998).

Thus SNRIs enhance both 5-HT and NE neurotransmission via elevation of the neurotransmitter levels achieved through the blockade of reuptake in combination with the increased release of the respective neurotransmitters, stemming from the desensitization of the release-regulating receptors.

1.5.1.6. Atypical antipsychotics

The antidepressant potential of atypical antipsychotics (AAPs) was discovered when clinical outcomes in treatment-resistant depressed patients improved with addition of olanzapine to an SSRI treatment regimen (Shelton et al. 2001). This finding resulted in an approval of olanzapine, aripiprazole and quetiapine alone and/or in combination with antidepressants for the treatment of

depressive states.

All agents in the class (with the exception of aripiprazole) share an antagonism at 5-HT₂ and D2 receptors, with much higher potency at the former site. Aripiprazole is a sole drug within the group that acts as a partial agonist, rather than antagonist, at D2 receptors, while preserving the 5-HT₂ blocking property. Since the first generation antipsychotics, acting primarily at the D2 receptors, do not possess antidepressant properties, blockade of the D2 receptors does not appear to be a valid mechanism explaining the AAPs antidepressant action. Furthermore, as the dose of AAPs used in depression treatment is much lower than that prescribed in psychotic states and thus provides clinically insignificant occupancy of D2 receptors, the importance of latter in the antidepressant potential is unlikely. It is, therefore, believed that the 5-HT₂ receptors are the main determinants of the beneficial clinical action of the AAPs in SSRI-resistant depression (Celada et al. 2004). As SSRIs attenuate the NE neuronal activity via activation of 5-HT_{2A} receptor, its blockade by AAPs reverses this effect, which potentially contributes to additive efficacy of such augmentation treatment (Szabo, Blier 2002). Similarly, the SSRI-induced inhibition of spontaneous firing rate of DA neurons, occurring due to the activation of 5-HT_{2C} receptors, is prevented with co-administration of AAPs (Dremencov et al. 2009b). Importantly, both NE and DA output in cortical areas is enhanced by AAPs administration (Dean, Scarr 2004). While the efficacy of AAPs as SSRI augmenting agents is largely explained by the reversal of tonic inhibition of catecholamines,

the effects at other receptors are also making an important input into the observed clinical benefit. However, the changes in the monoaminergic function vary from one AAP to another. This is due to the differential affinity of the agents within the pharmacological group for the various receptors that regulate the activity of the discussed neurotransmitters. For example, risperidone, quetiapine and clozapine effectively block α_2 -adrenoceptors, whereas ziprasidone and aripiprazole act at 5-HT_{1A} receptors (Schotte et al. 1996; Stark et al. 2007). The functional significance of mechanism of multireceptorial effect of AAPs is discussed in details in papers 3 and 4.

1.5.1.7. Bupropion

Bupropion is widely prescribed for the depression treatment (Zisook, Rush et al. 2006). Mechanism of action of this agent is distinct from the groups described above. Though bupropion was initially marketed as a blocker of DA and NE transporters, further pharmacological tests proved that the affinity for these neuronal elements was clinically insignificant (Tatsumi et al. 1997; Meyer et al. 2002; Gobbi et al. 2003).

The main site of action of this drug is believed to be the NE system. As bupropion does not possess a significant affinity for adrenergic receptors or transporters, it is believed to exert its action via stimulation of NE release (Dwoskin et al. 2006; Ferris et al. 1981; Li et al. 2002). In fact, several *in vivo* microdialysis studies documented an increase in the extracellular levels of NE in hippocampus, hypothalamus, nucleus accumbens and prefrontal cortex of rats (Li et al.

2002; Piacentini et al. 2003). The increase in the amount of synaptically available NE leading to enhanced activation of regulatory somatodendritic α_2 -adrenergic autoreceptors is believed to be responsible for initial drop in the frequency of NE neuronal firing (Dong, Blier 2001). The long-term administration of bupropion, however, desensitizes the above autoreceptor allowing the gradual recovery in the rate of discharge (El Mansari et al. 2008b; Ghanbari et al. 2010a). While the overall firing rate equalized with that of control rats, the burst activity of NE neurons was significantly augmented (Ghanbari et al. 2010a). The latter finding fits well the NE-releasing property of bupropion, as the release of the neurotransmitter is known to be higher when the action potentials are discharged in a burst-, rather than single-spike mode. Furthermore, α_2 -adrenergic autoreceptors that are located on the terminals of NE neurons and regulate the release of the neurotransmitter onto the postsynaptic neurons, were also desensitized by the prolonged bupropion administration. As the function of postsynaptic α_1 - and α_2 -adrenergic receptors mediating the transduction of NE neuronal signal remained unchanged, the increase in release of NE led to an overall increase in the NE neurotransmission (Ghanbari et al. 2011).

Aside from altering the function of NE system, bupropion also facilitates the 5-HT transmission. Bupropion increases the firing rate of 5-HT neurons above the baseline (Dong, Blier 2001; El Mansari et al. 2008b; Ghanbari et al. 2010a). This effect is mediated by the increased activation of the stimulatory α_1 -adrenergic heteroreceptors located on the 5-HT neurons (Ferris et al. 1981). Thus the

bupropion-induced increase in the spontaneous discharge rate combined with the desensitization of release-controlling α_2 -adrenergic heteroreceptors located on the 5-HT terminals leads to the increase in the 5-HT neurotransmission.

Though the DA neuronal firing rate is not altered, the extracellular levels of this neurotransmitter were shown to be increased by bupropion in some but not all brain structures (Li et al. 2002; Piacentini et al. 2003; Nomikos et al. 1992).

1.5.1.8. Mirtazapine

Mirtazapine is another antidepressant drug with a unique pharmacological profile. It appears particularly useful in depressed patients with prominent insomnia, anxiety, agitation and eating disorders. Mirtazapine is a potent antagonist of α_2 -adrenergic as well as 5HT₂ receptors (De Boer et al. 1988).

Mirtazapine administration elevates both NE and 5-HT neuronal firing rates (Haddjeri et al. 1998a; Haddjeri et al. 1996). The effect upon NE spontaneous discharge is direct and takes place due to the blockade of somatodendritic α_2 adrenoceptors (Haddjeri et al. 1998a). On the other hand, the increase in the 5-HT firing is indirect and takes place due to additional activation of the stimulatory α_1 receptor located on the cell body of 5-HT neuron by the mirtazapine-increased endogenous NE. Aside from blockade of LC somatodendritic α_2 -adrenergic autoreceptors, mirtazapine also antagonises terminal α_2 -adrenergic autoreceptors, likely increasing NE release, and desensitizes terminal α_2 -adrenergic heteroreceptors that regulate the release of 5-HT (Haddjeri et al. 1998a). The latter

adaptation, common with NE-enhancing drugs, allows 5-HT neuron to escape the dampening effect of α_2 -adrenergic heteroreceptor stimulation by NE, thus elevating the release of 5-HT into synapse (Mongeau et al. 1993). In fact, mirtazapine was shown to augment levels of 5-HT, as well as DOPAC – one of the main NE metabolites, in the ventral hippocampus (De Boer et al. 1994). Conclusively, both NE and 5-HT neuronal transmission is facilitated by mirtazapine in a NE-dependent manner (Haddjeri et al. 1998a; De Boer, Ruigt 1995).

The release of both NE and DA is enhanced by mirtazapine, likely due to the blockade of 5-HT₂ receptors that mediate the 5-HT inhibitory tone (Devoto et al. 2004). Additionally, the blockade of 5-HT_{2A} receptor subtype by mirtazapine decreases the stress-induced hypersecretion of glucocorticoids and restores sleep cycles, often perturbed in depression (Davis, Wilde 1996). The latter properties might be of additional benefit in treatment of depressive states. (Haddjeri et al. 1998a)

1.5.1.9. Trazodone

Trazodone is an approved antidepressant medication structurally and pharmacologically divergent from other described drugs (Al-Yassiri et al. 1981). The low affinity for the H1 receptor is only partially responsible for the significant sedation induced by trazodone. The combined antagonism of 5-HT₂ and α_1 -adrenoceptors also contributes to its beneficial action in restoration of sleep architecture. The presence of significant somnolence in most patients prevents physicians from increasing the trazodone dosing to an antidepressant level.

Thus presently it is mostly used as a non habit-forming sleeping aid agent in both depressed and non- depressed individuals. The new extended-release formulation may minimize the sedative properties of trazodone and provide clinicians with another MDD treatment option.

Trazodone likely exerts its antidepressant effects in a multireceptorial way. Similarly to SSRIs, it blocks the reuptake of 5-HT, thus enhancing the synaptic availability of the transmitter (Owens et al. 1997). Furthermore, like SSRIs, trazodone administered on a long-term basis was shown to desensitize the terminal 5-HT_{1B} receptors that modulate the 5-HT release (Ghanbari et al. 2010b). In addition, trazodone acts as an agonist at 5-HT_{1A} receptors (Odagaki et al. 2005). Thus the documented increase in the 5-HT tone, following sustained trazodone administration, is likely attributable to several components: the enhancement of the synaptic levels of 5-HT, resulting from the inhibited reuptake, the elevated release, summated with the direct activation of postsynaptic 5-HT_{1A} receptors that ultimately mediate the 5-HT signal transduction (Ghanbari, in press). The increase in levels of 5-HT, produced by SSRIs, overactivates the inhibitory 5-HT₂ receptors thus leading to the potentially clinically counterproductive decrease in the firing rate of NE and DA neurons. Trazodone, however, is an antagonist of 5-HT₂ receptors (Millan 2006), and therefore the elevation in 5-HT neuronal transmission produced by this drug does not dampen the discharge of the catecholaminergic neurons.

It thus can be concluded that trazodone increases the 5-HT neuronal

transmission while leaving the function of NE and DA systems largely unaltered.

1.5.1.10. 5-HT_{1A} agonists

The 5-HT_{1A} agonists were shown to possess antidepressant (-like) properties both in rodent models of depression and in humans (Lucki 1991; Blier, Ward 2003).

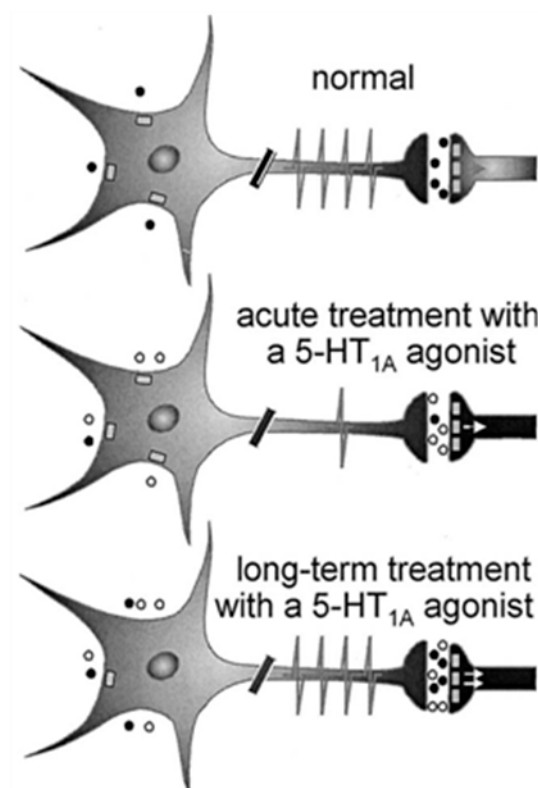


Figure 13. The effect of serotonin 1A (5-HT_{1A}) agonists on serotonergic neurotransmission. The rectangles represent the 5-HT_{1A} autoreceptors on the cell body of 5-HT neurons on the left and on postsynaptic neuronal elements on the right. The filled circles represent the 5-HT molecules and the open circles the 5-HT_{1A} agonist. The numbers of action potentials on the axons represent the firing rates of 5-HT neurons. The small arrows within the postsynaptic element represent and are proportioned to 5-HT_{1A} neurotransmission. Note the disappearance of the rectangles in the cell body of 5-HT neurons but not on the postsynaptic neurons. This corresponds to the desensitization of the presynaptic but not postsynaptic 5-HT_{1A} receptors (in the hippocampus) after long-term administration of 5-HT_{1A} agonists.

Indeed, the 5-HT_{1A} receptor has also been linked to depression through the ability of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, following 5-HT depletion, to induce granule cell proliferation within the dentate gyrus of the hippocampus (Huang, Herbert 2005), a process thought to facilitate the treatment of depression (Santarelli et al. 2003). Buspirone, the sole representative of its class to be marketed, is indicated for anxiety treatment. Augmentation of SSRIs with buspirone was found to accelerate and enhance the antidepressant effect of the former (Dimitriou, Dimitriou 1998; Bouwer, Stein 1997)

. This clinical synergy might be, in part, explained by the 5-HT_{1A} receptor-mediated increase in the frontocortical levels of DA and NE (Hughes et al. 2005). With regards to the 5-HT system, 5-HT_{1A} agonists initially decrease the firing rate of the DR 5-HT neurons (Blier et al. 1987; Sprouse, Aghajanian 1987). With chronic administration, however, the spontaneous firing normalizes due to desensitization of somatodendritic 5-HT_{1A} autoreceptor, which controls the firing activity (Blier et al. 1987). Unlike autoreceptors, postsynaptic 5-HT_{1A} receptors controlling the firing rate of pyramidal neurons in hippocampus are resistant to desensitization (Blier et al. 1987). Thus, normal firing of 5-HT neurons, achieved through desensitization of autoreceptors, combined with the direct activation of normosensitive postsynaptic 5-HT_{1A} receptors by the exogenous agonist (i.e. buspirone) results in an increase of the overall 5-HT neurotransmission, evidenced by the increase in tonic activation of postsynaptic 5-HT_{1A} receptors (Haddjeri et al. 1998a; Rueter, Blier 1999).

1.5.2. Non-pharmacological interventions

1.5.2.1. DBS

The deep brain stimulation (DBS), which premiered as a treatment of severe movement disorders (Benabid et al. 1987), was shown to also possess prominent antidepressant properties (Blomstedt et al. 2011). Despite its efficacy and minimal incidence of adverse effects, the invasive nature of the electrode and stimulator

implantation procedure reserves this experimental therapeutic option to the extremely treatment-resistant cases. To date, fewer than 100 patients, suffering from the resistant MDD, were operated worldwide.

The behavioral animal studies, aiming at deciphering the mechanism of action of DBS, were conducted (Hamani et al. 2010). Based on anatomical connections and cytoarchitectural parameters, the ventromedial prefrontal cortex (vmPFC) in rats, in particular its infralimbic portion, was determined to be a good anatomical correlate of human Brodmann area 25 (Takagishi, Chiba 1991). The latter brain region is a part of the subcallosal cingulate gyrus - one of the most widely used sites for electrode implantation in treatment of MDD (Hamani et al. 2009). In rodents, in turn, the infralimbic cortex is implicated in the stress mechanisms (Diorio et al. 1993). Stimulation parameters of vmPFC were closely correlated to those used in clinic (Hamani et al. 2010).

Similarly to other tested antidepressant treatments, both pharmacological and non-pharmacological, DBS of vmPFC decreased the immobility time in forced swim test - a standard behavioral measure of an antidepressant-like response in rodents (Hamani et al. 2010). Interestingly, this response was dependent on the integrity of 5-HT, but not NE neuronal system, as indicated by the complete loss of DBS antidepressant-like effect in animals with lesioned 5-HT neurons (Hamani et al. 2010). Selective NE denervation, in turn, had no effect on the DBS-induced behavioral changes. In addition, the increase in the 5-HT efflux in hippocampus was documented following DBS (Hamani et al. 2010). This effect is in accord with

the previous data, reporting a significant increase in 5-HT levels in various brain regions in rodents and primates, following the stimulation of infralimbic cortex (Juckel et al. 1999). The behavioral measures assessing the hedonic status did not change following the DBS, thus suggesting that DA neurotransmission, implicated in the reward and pleasure responses, is likely unaltered by this treatment (Hamani et al. 2010).

It can thus be concluded, that DBS-induced neurobiological changes underlying antidepressant (-like) response are dependent on the function of 5-HT system.

1.5.2.2. ECT

Despite the emergence of numerous pharmacological antidepressant classes, the electro-convulsive therapy (ECT) remains the golden standard of the MDD treatment, providing the greatest response and the lowest relapse rates among all therapeutic interventions. Though the ECT was first implemented in clinical practice in 1930s, precise mechanisms underlying its efficacy in treatment of MDD and other disorders are not fully understood.

It is known, nonetheless, that repeated ECT increases the overall 5-HT neurotransmission, as the sensitivity of postsynaptic 5-HT_{1A} receptors increases, thus facilitating the 5-HT responses (De Montigny 1984). Interestingly, this finding mirrors the changes produced by the tricyclic antidepressants (Blier et al. 1987). The observed sensitization of the 5-HT_{1A} receptor in hippocampus stands in

contrast to normosensitive presynaptic 5-HT_{1A}, 5-HT_{1B} autoreceptors and normal firing rate of DRN 5-HT neurons (Blier, Bouchard 1992). The mRNA expression of SERT, however, was found to be reduced following the ECS course (Shen et al. 2003), thus suggesting that the levels of synaptic 5-HT might in fact be elevated by the repeated electric stimulations. Indeed, the increase in levels of 5-HT following the repeated ECT stimulation was documented in several brain regions (Shen et al. 2003; Zis et al. 1992). Additionally, sprouting of the 5-HT neurons projecting to hippocampus is promoted by the ECT (Madhav et al. 2000).

The firing rate of LC NE neurons is not affected by the ECT (Tsen, in press). Postsynaptically responses to the exogenously applied NE were found to differ by region – the sensitivity of postsynaptic α -adrenergic receptors in hippocampus remained unchanged (De Montigny 1984). The latter effect, again, follows the trend of tricyclic antidepressants that also sensitize the facial motor nucleus neurons to both NE and 5-HT (Menkes et al. 1980). Moreover, both TCAs and ECT were shown to increase mRNA density for α_1 adrenergic receptors in cortical areas (Nalepa et al. 2002).

Repeated ECT does not alter the spontaneous discharge rate of the DA neurons (Tsen, in press). However, levels of DA were found to be decreased in striatum of animals subjected to ECT (Brannan et al. 1993). This finding is somewhat counterintuitive, as similarly to SSRIs and MAOIs the ECT downregulates the functional activity of 5-HT_{2C} receptors, inhibitory to the DA neuronal function (Butler et al. 1993).

It is thus evident, that in animals receiving ECT in a regiment following the parameters used in clinic, the NE, and, to a greater extent, the 5-HT neuronal transmission increase due to the sensitization of the postsynaptic receptors that mediate the effects of respective transmitters.

1.5.2.3. Sleep deprivation

More than 50% of depressed individuals experience a significant improvement of the depressive symptoms, following a single night of sleep deprivation (Gillin et al. 2001; Boivin 2000; Adrien 2002). Interestingly, an antidepressant-like effects were also documented in animals subjected to the forced wakefulness (Lopez-Rodriguez et al. 2004).

The effects of this intervention are mediated, in part, by alterations in the NE system function – depletion of NE in rodents prevents the antidepressant-like effect of sleep deprivation (Asakura et al. 1994). This observation is in line with the proposed role of α_1 - and β -adrenoceptors in control of sleep patterns (Mallick et al. 2005). In fact, β adrenergic receptors were shown to be downregulated in hippocampus and frontal cortex in animals following sleep deprivation (Pedrazzoli, Benedito 2004). The analogous modulation is also produced by several classes of antidepressants. Furthermore, the density of both NET and SERT, as well as the activity of MAO-A, catabolizing both NE and 5-HT, was found to be decreased by the sleep deprivation (Thakkar, Mallick 1993; Hipólido et al. 2005). These findings thus underscore that not only NE, but also 5-HT system is likely playing a part in an antidepressant (-like) activity of the discussed intervention. In fact, the

levels of 5-HT following sleep deprivation were found to be increased in hippocampus, frontal cortex and suprachiasmatic nucleus – the main regulator of the circadian rhythms (Adrien 2002). The rebound sleep, ameliorating the beneficial effects, was found to normalize the 5-HT levels (Lopez-Rodriguez et al. 2004). Intriguingly, the decrease in levels of 5-HT by tryptophan depletion not only didn't reverse the antidepressant effects of sleep deprivation, but prevented the depressive relapse after the recovery night in most of the patients (Neumeister et al. 1998).

The positive effects of sleep deprivation were found to resemble the psychostimulant-induced behavioral changes (Ebert, Berger 1998), suggesting that an increase in DA transmission within corticolimbic structures may be taking place. Indeed, the release of DA was found to be increased in humans subjected to the sleep deprivation (Wu et al. 2001). The enhancement of DA tone might be related to the downregulation of 5-HT_{2C} receptors that tonically inhibit DA neuronal function, by the sleep deprivation (Moreau et al. 1993).

Though the effects of the discussed intervention are transient and only last until the next period of normal sleep, the prompt changes in the functional state of monoaminergic systems, often resembling those induced by the antidepressant treatments, emphasize the role of NE, 5-HT and DA in the elimination of depressive symptoms.

1.5.2.4. Vagus nerve stimulation

The electric stimulation of the vagus nerve afferents is the most common non-pharmacological treatment of epilepsy (Buoni, Mariottini et al. 2004). The mood improvement observed in epileptic patients treated with vagus nerve stimulation (VNS), prompted the investigation of its effects in mood disorders (Schachter 2004, Elger, Hoppe et al. 2000). The bulk of data confirming its antidepressant(-like) effects in animals and humans led to the approval of VNS for the treatment resistant depression in Canada, US and Europe (Krahl et al. 2004; Daban et al. 2008).

The primary brain target of the vagal nerve is the nucleus tractus solitarius, which in turn innervates several brain regions (Chae et al. 2003). In fact, studies in both humans and animals documented the VNS-driven changes in activity of hippocampus, amygdala, LC and several cortical regions (Naritoku et al. 1995; Groves, Brown 2005). The effects of VNS are believed to be taking place due to activation of the main NE brain nucleus – LC (Groves, Brown 2005). Indeed, the anticonvulsant properties of the VNS were lost after the LC was destroyed by the neurotoxic lesion (Krahl et al. 1998). Furthermore, the increase in the firing rate of 5-HT neurons, following the prolonged VNS, was no longer present when the NE input was ablated (Manta et al. 2009b). In fact, the increase in the discharge rate of NE neurons was evident earlier and was also more pronounced than that of 5-HT, further suggesting that the changes in NE function are primary, whereas the effects on 5-HT are indirect and occur due to the NE-driven neuronal adaptations (Dorr, Debonnel 2006). Not only regular-, but also burst-mode firing of NE neurons

increased (Manta, Dong et al. 2009b). The latter type of discharge is functionally more efficient, as more neurotransmitter is released per action potential. Thus the increase in the 5-HT is driven by the superior tonic activation of stimulatory α_1 -adrenoceptors, located on the 5-HT neurons, by the VNS-induced increase in the NE rate of firing combined with the greater release potential. Interestingly, the sensitivity of the 5-HT_{1A} autoreceptors, controlling the rate of discharge of 5-HT neurons in a negative-feedback manner, did not change following VNS (Dorr, Debonnel 2006). This allows a conclusion that the NE tone, stimulating the 5-HT activity, is indeed so pronounced, that the negative effect of 5-HT_{1A} receptor activation becomes overridden by the α_1 -adrenergic stimulatory input (Manta et al. 2009b). The firing rate of DA neurons was decreased by the prolonged VNS (Manta, in press). Despite it, the levels of DA were found to be elevated in nucleus accumbens and cortical regions of the rat brain (Manta, in press).

Taken together these lines of evidence suggest that the VNS induces a profound increase in the NE tone, which, indirectly, stimulates the 5-HT neuronal transmission. Considering the common vector of neuronal activity modulation by the VNS and pharmacological antidepressants, the described changes are likely underlying the antidepressant effects of the VNS.

1.6. Study rationale

The conducted series of studies were aimed at several goals. First, as previous research focused greatly on the interaction between the NE and 5-HT systems it was deemed important to determine the previously overlooked role

of the DA system in the network of neuronal interactions potentially responsible for the antidepressant response. To test the effects of DA system manipulation upon the function of the other two monoamines the drug selective for the D2-like receptors, pramipexole, was chosen. Pramipexole is a pharmacological agent approved for treatment of the Parkinson's disorder and restless legs syndrome. Aside from its efficacy in the above conditions, it was shown to possess antidepressant properties (Lattanziet al. 2002; Corrigan et al. 2000). As dopaminergic afferents innervate both NE and 5-HT neurons, it was hypothesized that pramipexole administration would alter the function of not only DA, but also, indirectly, NE and 5-HT neurons.

Secondly, the characterization of the effects of two atypical antipsychotics with distinct pharmacological profiles alone and in combination with SSRIs was performed. As discussed in previous section, all atypical antipsychotics were shown to possess antidepressant properties, however only aripiprazole, olanzapine, and quetiapine were approved for use in depression either in combination with antidepressants or alone. Aripiprazole is a unique antipsychotic medication. Unlike all other representatives of this pharmacological class that antagonize D2 receptors, this drug acts as a partial agonist at this site (Burris et al., 2002; Hirose et al., 2005). This distinctive property of aripiprazole, along with its effect at number of other receptors implicated in an antidepressant response, made important the characterization of its effects on the firing rates of monoaminergic neurons. Augmentation of SSRI and SNRI treatments with

aripiprazole was shown to result in an increase in response rate and, sometimes, faster clinical benefit (Marcus et al. 2008; Berman et al.2007; Berman et al. 2008). Numerous studies documenting this phenomenon led to the approval of aripiprazole for use in MDD as an adjunct to the standard antidepressants. Considering the above, examining the effects of not only the sole administration of aripiprazole, but also its concomitant use with the SSRI escitalopram were deemed important and relevant. An increase in the 5-HT tone, produced by the SSRIs, is known to dampen the firing rate of NE and DA neurons via excessive activation of inhibitory 5-HT_{2A} and 5-HT_{2C} receptors, respectively (Dremencov et al. 2007; Dremencov et al. 2009). This decrease in catecholaminergic tone may be responsible for the suboptimal response rate as well as some adverse effects of SSRIs. Since aripiprazole blocks both 5-HT_{2A} and 5-HT_{2C} receptors (Shapiro et al. 2003), it was hypothesized that its addition to an SSRI regimen will reverse the inhibition of NE and DA firing. In addition, since aripiprazole activates multiple monoaminergic receptors (Shapiro et al. 2003), it was hypothesized that it may alter the activity of DA and/or NE and/or 5-HT system even when administered on its own.

Quetiapine is another member of the atypical antipsychotic family. Aside from the blockade of 5-HT₂ and D₂ receptors, the pharmacological profile varies greatly between different agents within this class. Thus the generalization about the mechanism of action of atypicals can not be made, and each agent needs to be studied separately. Like aripiprazole, quetiapine is one of the three atypical

antipsychotic drugs approved for use in MDD either alone (Canada & EU), or as antidepressants augmenting agent (USA). Considering the above, the effects of use of quetiapine administered both alone and in combination with an SSRI were important to assess. The following study was aimed at characterization of the effects produced by mono- and combination use of quetiapine on the spontaneous firing rate of NE and 5-HT neurons and the overall neurotransmission within the above systems, and at determination of the neuronal elements conveying these changes. The assessment of effects of quetiapine on DA neurotransmission was omitted, since the potential alterations produced at the presynaptic level are likely functionally insignificant, as the D2 receptors are systemically blocked by the drug itself only at doses higher than those used to treat depression than psychosis.

Quetiapine is actively degraded in the human body, resulting in a formation of over 20 metabolites. One of the principal metabolites – norquetiapine is structurally similar to the tricyclic antidepressants and shares some pharmacological properties of these drugs. For instance, norquetiapine not only largely follows the pharmacological profile of quetiapine, but it is also a potent inhibitor of NET, like many TCAs, whereas the parent compound is totally devoid of this property. As norquetiapine is believed to be partially responsible for the antidepressant properties of quetiapine, modeling of the kinetic balance between these two compounds was of great importance for proper understanding of its mode of action. Unlike humans, in rats quetiapine is not metabolized to norquetiapine. The norquetiapine was thus added to the quetiapine, at the concentration mimicking

that seen in humans.

The effects of the above drugs upon function of 5-HT and/or NE and/or DA systems were determined by utilizing the *in vivo* electrophysiological recordings in anaesthetized male rats. The electrophysiological experiments were carried out after 2 and 14 days of drug administration to determine the immediate and the clinically-relevant long-term effects.

The results of the above studies are presented in the following sections.

2. Collection of manuscripts

2.1. Manuscript I

For several decades the antidepressant research was focused on the 5-HT and NE systems. As the role of the DA in depression pathophysiology and treatment becomes more and more recognised, the effects of pure dopaminergic agents upon monoaminergic systems, mediating the antidepressant response, was of interest. Pramipexole (PPX) is a selective D2-like agonist with no affinity for any other types of receptors. This drug is currently approved for use in treatment of the Parkinson's disorder and the restless legs syndrome (Guttman et al.. 2001; Piercey et al. 1994; Reichmann et al.. 2006). Aside from its efficacy in the above conditions, it was shown to possess antidepressant properties (Lattanziet al. 2002; Corrigan et al. 2000). As dopaminergic afferents innervate both NE and 5-HT neurons, it was hypothesized that pramipexole administration would alter the function of not only DA, but also, indirectly, NE and 5-HT neurons. The electrophysiological experiments were carried out after 2 and 14 days of drug PPX administration to determine the immediate and the clinically-relevant long-term effects. Series of pharmacological experiments aimed at the determination of the receptor(s) responsible for the observed effects were performed.

The experimental design was drafted by Dr. Pierre Blier, Dr. Mostafa El Mansari and Olga Chernoloz. The experiments were carried out and analyzed by

Olga Chernoloz. All authors assisted in drafting the article, and approved the final manuscript. The manuscript was published at the *Neuropsychopharmacology*, 2009, 34 (3), pp. 651-66.

The study was carried out as a part of the CIHR grant entitled 'Role of the dopamine system in the antidepressant response', awarded to Dr. Pierre Blier.

Sustained administration of Pramipexole modifies the spontaneous firing of dopamine, norepinephrine and serotonin neurons in the rat brain

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Journal: Neuropsychopharmacology

Figures: 8

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ABSTRACT

Pramipexole (PPX) is a D₂/D₃ receptor agonist which has been shown to be effective in the treatment of depression. Serotonin (5-HT), norepinephrine (NE) and dopamine (DA) systems are known to be involved in the pathophysiology and treatment of depression. Due to reciprocal interactions between these neuronal systems, drugs selectively targeting one system-specific receptor can indirectly modify the firing activity of neurons that contribute to firing patterns in systems which operate via different neurotransmitters. It was thus hypothesized that PPX would alter the firing rate of DA, NE and 5-HT neurons. To test this hypothesis, electrophysiological experiments were carried out in anaesthetized rats. Subcutaneously implanted osmotic minipumps delivered PPX at a dose 1 mg/kg/d for two or fourteen days. After a two-day treatment with PPX the spontaneous neuronal firing of DA neurons was decreased by 40%, NE neuronal firing by 33% and the firing rate of 5-HT neurons remained unaltered. After 14 days of PPX treatment, the firing rate of DA had recovered as well as that of NE, whereas the firing rate of 5-HT neurons was increased by 38%. It was also observed that sustained PPX administration produced desensitization of D₂/D₃ and 5-HT_{1A} cell body autoreceptors, as well as a decrease in sensitivity of α ₂-adrenergic cell body autoreceptors. These adaptive changes are implied in long-term firing rate adaptations of DA, NE and 5-HT neurons after prolonged PPX administration. In conclusion, the therapeutic action of PPX in depression might be attributed to increased DA and 5-HT neurotransmission.

Keywords: pramipexole, electrophysiology, dopamine, norepinephrine, serotonin, depression

INTRODUCTION

Dopamine (DA) agonists, such as quinpirole, pergolide, pramipexole and bromocriptine have been shown to possess antidepressant-like properties in animal studies and therapeutic action in depressed patients (Anisman et al. 1979; Izumi et al. 2000; Muscat et al. 1992; Waehrens and Gerlach 1981; Brocco et al. 2006). Pramipexole (PPX) is a D₂/D₃ receptor agonist customarily used in treatment of Parkinson's disease and restless legs syndrome (Guttman and Jaskolka 2001; Piercey 1998; Reichmann et al. 2006). This drug was also shown to be efficacious in treatment of major depressive disorder (MDD) as monotherapy (Corrigan et al. 2000; Lattanzi et al. 2002), and to be a useful augmentation strategy in treatment-resistant depressed patients (Cassano et al. 2004; Goldberg et al. 2004; Sporn et al. 2000).

Even though pathophysiological mechanisms of depression have yet to be fully elucidated, a consensus exists for a central involvement of serotonergic (5-HT) and noradrenergic (NE) systems in this disease and in its effective treatment. Furthermore, reciprocal interactions between these two neuronal entities is now well established (Blier 2001; Szabo and Blier 2001; Guiard et al. 2008a). However, during the last decade substantial data suggesting participation of dopaminergic system in this neuronal network of interactions have emerged (Aman et al. 2007; Esposito 2006; Haj Dahmane 2001). Consequently, in the light of an apparent involvement of the DA system in pathophysiology of depression (Dunlop and Nemeroff 2007), it is important to ascertain the effects of DA on the above-

mentioned monoaminergic systems.

Various studies have shown anatomical similarities and functional interactions between the 5-HT neurons of the raphe dorsalis (RD) and the DA neurons of mesencephalic DA systems (Aman et al. 2007; Martin-Ruiz 2001) which can help to guide research regarding the pharmacological action of antidepressant drugs (Aman et al. 2007; Dremencov et al. 2004). For instance, D2-like receptors are expressed on the cell body of 5-HT neurons (Mansour et al. 1990; Suzuki et al. 1998). This anatomical commonality first suggested that DA might be able to modulate 5-HT neuronal firing. Accordingly, this led to a recent *in vivo* study, which confirmed the existence of the excitatory effect of DA upon RD 5-HT neuronal firing: the mean firing activity of RD 5-HT neurons in DA-lesioned rats was decreased by 60% compared to sham-operated rats (Guiard et al. 2008a).

In the locus coeruleus (LC), dopamine is, however, thought to exert an inhibitory effect on NE cells. Several radioligand binding studies documented presence of D₂ as well as D₃ receptors in the LC (Suzuki et al. 1998; Yokoyama 1994). As predicted, pharmacological blockade of these receptors or selective lesioning of VTA DA neurons enhances LC NE neuronal activity (Guiard et al. 2008a; Piercey et al. 1994). This suggests a negative influence of ventral tegmental area (VTA) DA neurons on LC NE neurons.

Clinical attenuation of depressive symptoms correlates in time with desensitization of autoreceptors achieved after long-term treatment with pharmacological agents acting on the respective neuronal systems Waning of

the responsiveness of somatodendritic 5-HT_{1A} autoreceptor following chronic administration of SSRI was previously described (Blier and De Montigny 1983; Pineyro and Blier 1999). It was observed that attenuated autoreceptor regulation leads to an overall increase in 5-HT transmission in the presence of 5-HT reuptake inhibitor (Chaput et al. 1986; Haddjeri et al. 1998a). Analogously, desensitization of terminal α_2 -adrenergic autoreceptor as a result of sustained NE reuptake inhibition has been described using electrophysiology and microdialysis (Lacroix et al. 1991; Parini et al. 2005). Similar biochemical and electrophysiological aspects of dopaminergic autoreceptor desensitization have also been described in a large body of literature (Jeziorski and White 1989; Pitts et al. 1995; Subramaniam et al. 1992).

The in vivo electrophysiological studies which we present here were designed to test the hypothesis that acute and sustained administration of the D₂/D₃ receptor agonist PPX will alter not just DA neuronal activity, but that of 5-HT and NE neurons as well. This endeavor was prompted by reports of the clinical effectiveness of PPX in the MDD treatment (Cassano et al. 2004, Goldberg et al. 2004; Lattanzi et al. 2002; Maj et al. 1997; Sporn et al. 2000), the presence of D₂ as well as D₃ receptors in VTA, LC and RD (Levant et al. 1993; Suzuki et al. 1998; Yokoyama 1994), as well as the existence of reciprocal interactions between DA, NE and 5-HT systems involved in the pathophysiology of depression (Millan et al. 2000a; Tremblay and Blier 2006).

MATERIAL AND METHODS

Animals

Male Sprague Dawley rats (Charles River, St. Constant, QC) weighing 270 to 320 g at the time of recording, were used for the experiments. They were kept under standard laboratory conditions (12:12 hour light/dark cycle with access to food and water ad librum). All animal handling and procedures were carried out according to the guidelines of the Canadian Council on Animal Care and protocols of this study were approved by the local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, ON, Canada).

Treatments

Rats were anesthetized with isoflurane for the subcutaneous implantation of osmotic minipumps, delivering PPX at a daily dose of 1 mg/kg for 2 or 14 days. Control rats were implanted with minipumps delivering physiologic saline.

***In vivo* electrophysiological recordings**

Rats were anesthetized with chloral hydrate (400 mg/kg; i.p.) and placed in a stereotaxic frame. To maintain a full anesthetic state, chloral hydrate supplements of 100 mg/kg, i.p., were given as needed to prevent any nociceptive reaction to paw pinching. Extracellular recordings of the 5-HT, DA and NE neurons in the RD, the VTA and the LC respectively, were obtained using single-barreled glass micropipettes. Their tips were of 1-3 μm in diameter and impedance ranged

between 4-7 MΩ. All glass micropipettes were filled with a 2 M NaCl solution. Using this approach, during all recordings signal to noise ratio was between 2 and 10, therefore making spike amplitude discrimination extremely reliable. In cases when more than one neuron was recorded simultaneously, neurons were discriminated automatically by the Spike 2 software based on the spike shape and amplitude. Prior to electrophysiological experiments, a catheter was inserted in the lateral tail vein for systemic i.v. injection of appropriate pharmacological agents when applicable.

Recording of the VTA DA neurons

Single-barreled glass micropipettes were positioned using the following coordinates (in mm from Lambda): AP, +3.0 to +3.8; L, 1 to 0.6; V, 6.5 to 9. The presumed DA neurons were identified according to the well established electrophysiological properties in vivo: a typical triphasic action potential with

a marked negative deflection; a characteristic long duration (> 2.5 ms) often with an inflection or “notch” on the rising phase; a slow spontaneous firing rate (0.5 – 5 Hz) with an irregular single spiking pattern with slow bursting activity (characterized by spike amplitude decrement) (Grace and Bunney 1983). Additionally, as previously described, a criterion of duration (> 1.1 msec from the start of the action potential to the negative trough) was used. (Ungless et al. 2004)

Recording of the LC NE neurons

Single-barreled glass micropipettes were positioned using the following coordinates (in mm from Lambda): AP, - 1.0 to - 1.2; L, 1.0 to 1.3; V, 5 to 7. Spontaneously active NE neurons were identified using the following criteria: regular firing rate (0.5–5.0 Hz) and positive action potential of long duration (0.8–1.2 ms) exhibiting a brisk excitation followed by period of silence in response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen 1982 b).

Recording of the RD 5-HT neurons

Single-barreled glass micropipettes were positioned using the following coordinates (in mm from Lambda): AP, +1.0 to 1.2; L, 0 ± 0.1 ; V, 5 to 7. The presumed 5-HT neurons were then identified by applying the following criteria: a slow (0.5 - 2.5 Hz) and regular firing rate and long-duration (2 - 5 ms) bi- or triphasic extracellular waveform (Aghajanian and Vandermaelen 1982a).

Dose response curves

Dose-response curves were generated to determine response of DA, NA and 5-HT neurons to acute i.v. administration of PPX. Dose-response curves were also constructed for systemic i.v. administration of the DA agonist apomorphine, the 5-HT autoreceptor agonist LSD and the α_2 -adrenergic agonist clonidine to assess the effect of sustained administration of PPX on the sensitivity of D_2/D_3 , 5-HT_{1A} and α_2 -adrenergic autoreceptors. Dose-response curves were obtained using

only the initial response to the first dose injected to a single neuron of each rat. Dose-response curves were plotted using GraphPad software.

Firing rate and burst analysis

The firing patterns of DA and NE neurons were analyzed by interspike interval burst analysis following the criteria set by Grace and Bunney (Grace and Bunney 1984). The onset of a burst was defined as the occurrence of two spikes with an interspike interval shorter than 0.08 s. The termination of burst was defined as an interspike interval of 0.16 s or longer. Burst activity of 5-HT neurons mostly occurs in doublets. Furthermore, firing was analyzed using the following parameter: the onset of a burst, defined as the occurrence of two spikes with an interspike interval of 0.01 s or shorter (Hajos and Sharp 1996).

Statistical analysis

All results are expressed as mean $\pm$ S.E.M., unless otherwise specified. Statistical comparisons between differences in spontaneous firing rate and burst activity DR, VTA and LC of control and PPX-treated rats were carried out by using one-way analysis of variance and multiple comparison procedures using Fisher's PLSD post hoc test. Data were obtained from 3 to 5 rats per experimental group. Statistical significance was taken as $p < 0.05$.

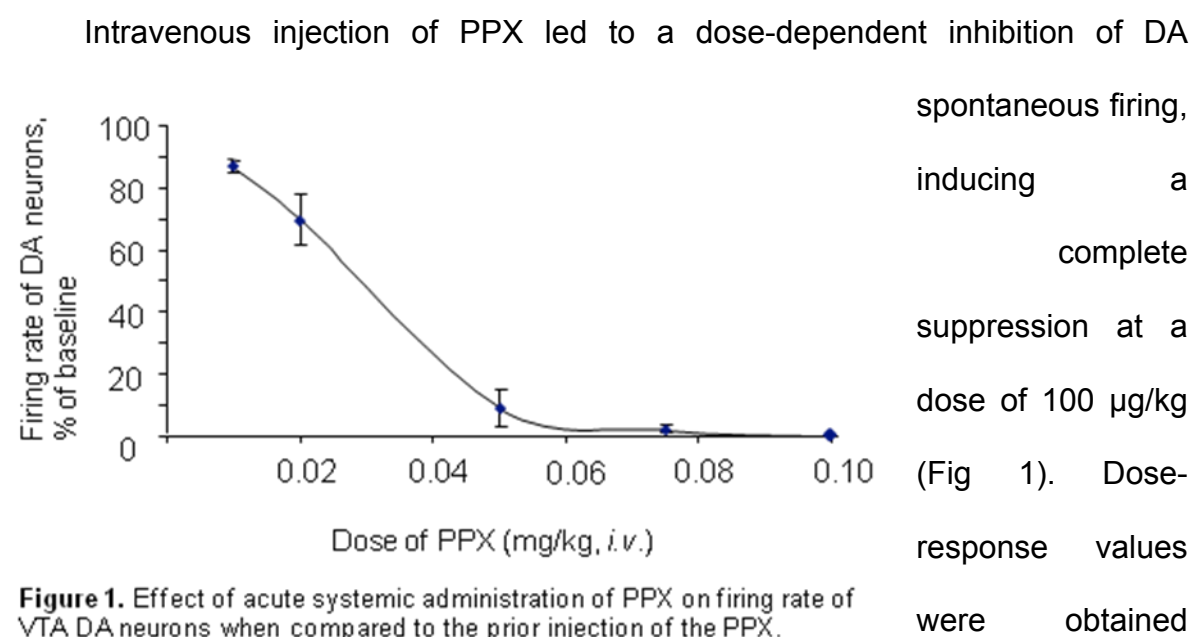
Drugs

Pramipexole was generously provided by Boehringer Ingelheim

Pharmaceuticals (Ingelheim, Germany); S 33084 was generously provided by Servier Research Institute (Paris, France); L-741,626 was purchased from Tocris Biopharmaceuticals (Bristol, UK); Apomorphine, Haloperidol, Clonidine, Idazoxan, WAY 100635 were purchased from Sigma (St. Louis, USA); Lysergic Acid Diethylamide (LSD) was obtained through Health Canada. All drugs except haloperidol and S 33084 were dissolved in distilled water. Haloperidol and S 33084 were dissolved in distilled water acidified with lactic acid (followed by pH control and normalization, as needed).

RESULTS

Effects of acute systemic administration of PPX on the mean firing rate of VTA DA, LC NE and RD 5-HT neurons



using only the initial response to the first dose injected to a single neuron of each rat (n = 14 in 14 rats). In contrast, administration of PPX in doses of up to 3-6 mg/kg did not produce any significant effect on 5-HT and NE discharge rate (data not shown).

Effects of PPX administration for 2 and 14 days on the mean firing rate and burst activity of VTA DA neurons

The mean firing rate of recorded DA neurons in vehicle treated rats was 4.2 ± 0.33 Hz (n=41 in 8 rats). A two-day treatment with PPX at a dose of 1 mg/kg/d resulted in a 40% attenuation of the spontaneous

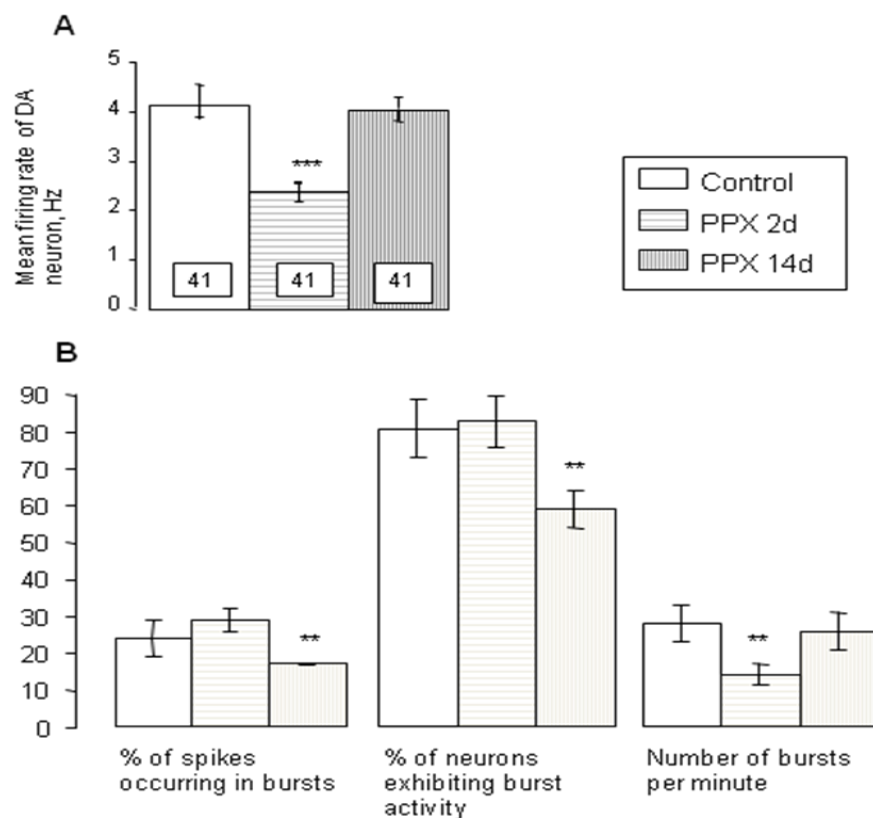


Figure 2. Effect of acute and sustained administration of PPX on VTA DA spontaneous firing rate (A) and burst activity (B). (A) The number of neurons recorded in each group is displayed in respective histogram. (B). Percentage of spikes occurring in bursts as well as % of neurons exhibiting burst activity was assessed in all the neurons recorded (n=41 in control, n=41 in PPX 2d treated rats and n=41 in PPX 14d treated rats). Number of bursts discharged in a minute was analyzed only in neurons exhibiting burst-mode activity (n=33 in control, n=34 in PPX 2d treated rats and n=24 in PPX 14d treated rats). The data expressed as mean firing rate $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$; n - number of neurons, SEM - standard error mean.

firing of DA neurons (n=41 in 8 rats) when compared to the vehicle treated rats. However, following 14 days of treatment with the same dose of PPX, the firing activity of DA neurons had fully recovered (n=41 in 7 rats) (Fig 2A).

Burst firing activity, characteristic of most DA neurons, was significantly altered by PPX. In controls, 24% of all spikes were occurring in bursts, 81 % of neurons exhibited burst firing, with an average of 29 bursts per minute (assessed only in neurons exhibiting burst firing) (Fig 2B). After 2 days of Pramipexole administration at dose of 1 mg/kg, there was no alteration of the percentage of neurons displaying burst-mode activity. However, the number of bursts per minute was decreased by 50% and the percentage of spikes occurring in bursts was not changed when compared to the control level. Interestingly, after 14 days of PPX administration the number of bursts per minute returned to the baseline level (Fig 2B). Despite the recovery of this parameter, the percentage of spikes occurring in bursts significantly decreased to 70% of control level (Fig 2B), possibly due to significantly decreased number of neurons exhibiting burst activity (Fig 2B).

Assessment of long-term administration of PPX on the function of the D2-like autoreceptors

In order to explain the recovery of firing of DA neurons following the 14-day administration of PPX, the responsiveness of D₂/D₃ autoreceptors was assessed using the i.v. administration of DA agonist apomorphine. Injection of apomorphine in doses of 10 µg/kg to 40 µg/kg led to a dose-dependent inhibition of DA firing activity in control rats. Injection of 30 µg/kg of apomorphine resulted in a

complete and lasting inhibition of spontaneous firing in the control group ($ED_{50}=13\pm1.1$ $\mu\text{g/kg}$; $n=8$ in 8 rats). In contrast, rats subjected to 14 days of PPX administration responded to apomorphine only to a minor extent. Apomorphine administered in doses of up to 1000 $\mu\text{g/kg}$ induced an inhibition of only up to 33% (Fig 3) ($n=7$ in 7 rats). In order to determine if the attenuated response to apomorphine was due to a true desensitization of DA autoreceptors, or to a competition of apomorphine with PPX at the autoreceptor sites, the effect of apomorphine was examined after a wash-out period (minipumps delivering PPX

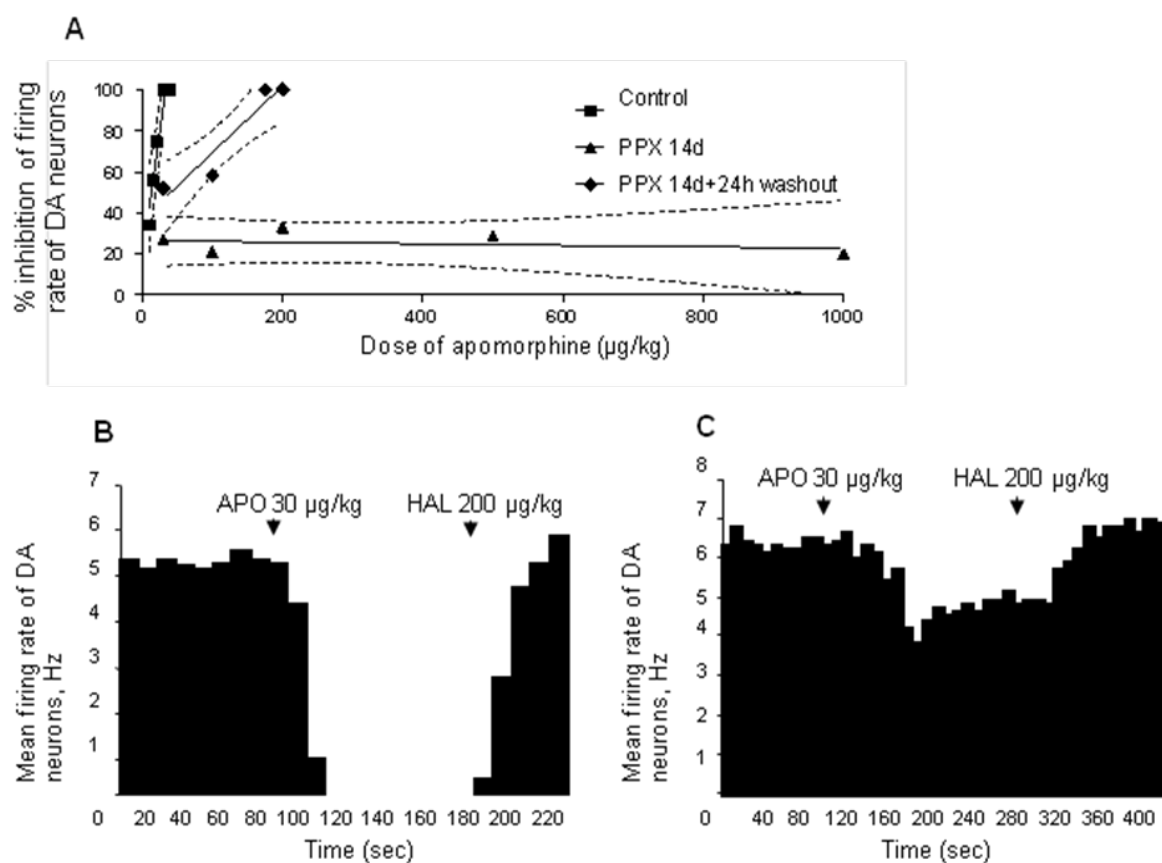


Figure 3. Assessment of the sensitivity of the VTA DA D2 autoreceptor:

(A) Relationship between the degree of suppression of VTA DA firing activity and doses of D2 receptor agonist apomorphine administered intravenously in control, 14-day PPX treated rats and 14-day PPX treated rats after 24-hour PPX washout. Outer lines represent the standard error of the regression line. Representative integrated firing rate histogram illustrating the effect of apomorphine administration in control (B) and 14-day PPX treated rats (C). Effect of the D2 agonist apomorphine is reversed by administration of the D2 antagonist haloperidol in both control, PPX14-day treated rats and 14-day PPX treated rats after 24-hour PPX washout.

were taken out under isoflurane anesthesia and electrophysiological recordings were carried out 24 hours later). After PPX was washed-out, a complete inhibition of DA neuronal firing was achievable with 175 µg/kg of apomorphine ($ED_{50}=32.7\pm1.5$ µg/kg; $n=7$ in 7 rats), thus indicating that despite apparent competition between the two agonists, a desensitization of the D₂-like receptor had occurred (Fig 3). Besides the sensitivity of the autoreceptor, other parameters such as spontaneous and burst-mode firing of the DA neurons were not affected after the 24 h PPX wash-out, when compared to the PPX 14-day treated group tested with the minipump delivering the drug present in the rats (data not shown).

Assessment of the role of D₂ and D₃ receptors in the suppressant effect of PPX administration on VTA DA firing

Since PPX is an agonist on both D₂ and D₃ receptors, pharmacological dissection of observed inhibitory action of PPX on DA neuronal firing was attempted using highly selective antagonists of D₂ and D₃ receptors (data not shown). Dopamine neuronal firing in control rats was suppressed by an i.v. bolus injection of PPX (100 µg/kg). In order to determine whether PPX was acting on the firing activity of the DA neuron via D₂ and/or D₃ receptors, the selective D₂ receptor antagonist L-741,626 or the selective D₃ receptor antagonist S 33084 were injected thereafter in doses of 250-500 µg/kg. These doses were based on previous studies (Millan et al. 2000b). Several experiments yielded inconsistent results possibly due to heterologous distribution of D₂ and D₃ receptors on VTA DA neurons and their similar effects on DA spontaneous firing.

Effects of PPX administration for 2 and 14 days on the mean firing rate and burst activity of LC NE neurons

The mean firing rate of NE neurons in vehicle-treated rats was 1.7 ± 0.11 Hz ($n=61$ in 5 rats). Similarly to DA neurons, a two-day regimen of PPX led to a significant 33 % decrease in firing activity of NE neurons ($n=53$ in 4 rats) compared to controls. Norepinephrine neuronal firing returned to the baseline levels after 14 days of PPX administration (Fig 4A) ($n=41$ in 4 rats).

Overall burst activity, represented by the percentage of spikes occurring in bursts, was drastically decreased by both short- and long-term treatment with PPX (Fig 4B). This change is potentially attributed to the significant decrease in the number of bursts per minute (assessed only in neurons exhibiting burst firing) in response to both 2- and 14 days of PPX treatment compared to baseline control

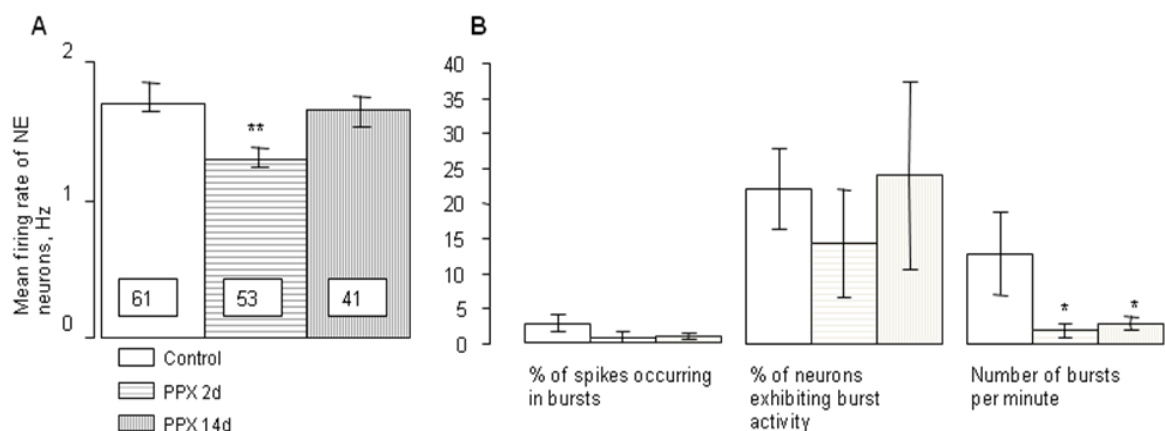


Figure 4. Effect of acute and sustained administration of PPX on LC NE spontaneous firing rate (A) and burst activity (B). (A) The number of neurons recorded in each group is displayed in respective histogram. (B). Percentage of spikes occurring in bursts as well as % of neurons exhibiting burst activity was assessed in all the neurons recorded ($n=61$ in control, $n=53$ in PPX 2d treated rats and $n=41$ in PPX 14d treated rats). Number of bursts discharged in a minute was analyzed only in neurons exhibiting burst-mode activity ($n=14$ in control, $n=8$ in PPX 2d treated rats and $n=6$ in PPX 14d treated rats). The data expressed as mean firing rate $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$; n - number of neurons, SEM - standard error mean.

level (Fig 4B). The percentage of NE neurons exhibiting burst activity, however,

was not changed by PPX treatment (Fig 4B).

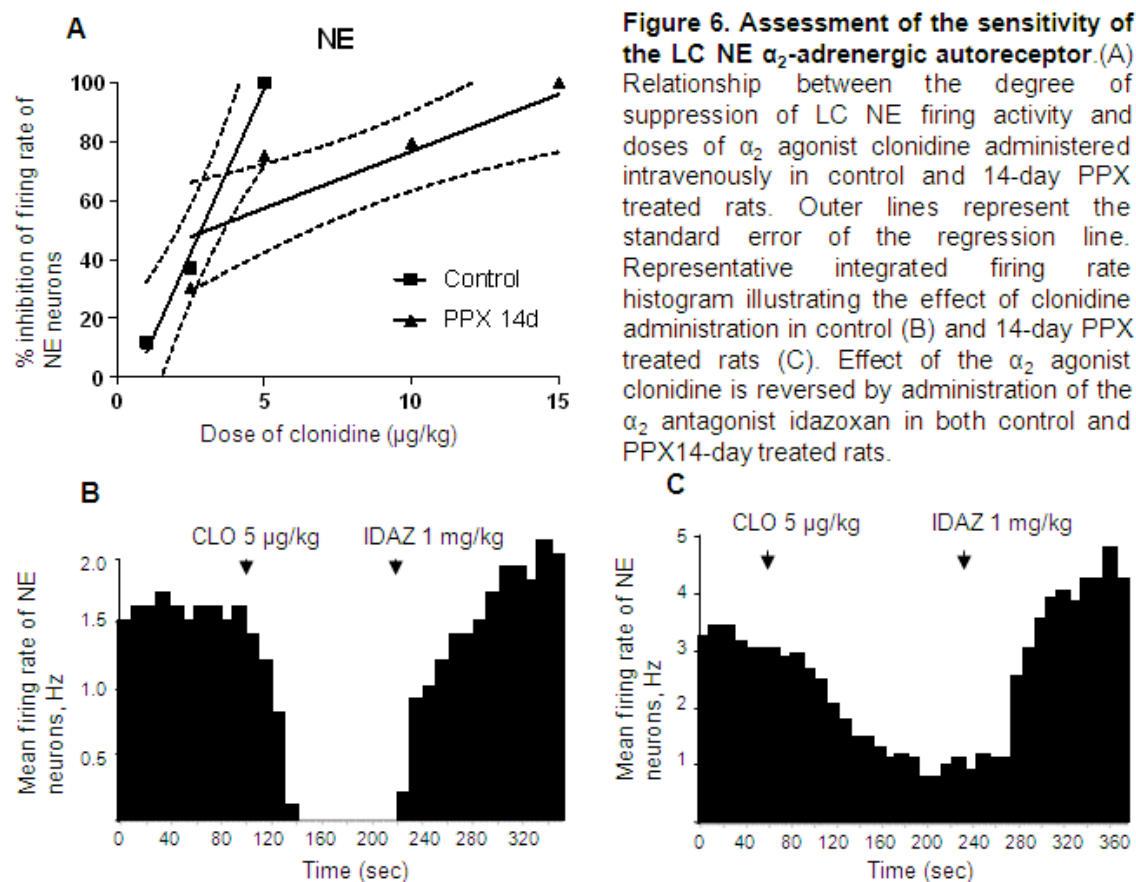
Assessment of short-term treatment with PPX on the function of the α_2 -adrenergic autoreceptor

Dopamine-induced decrease of the NE firing rate has recently been attributed to the stimulation of α_2 -adrenergic autoreceptors (Guiard et al 2008,b). If this receptor is solely responsible for the inhibition of spontaneous firing of NE neurons by PPX, then its blockade would lead to the same increase of firing rate in vehicle and PPX treated rats (See Szabo and Blier 2002). To address this possibility, the selective α_2 -adrenoreceptor antagonist idazoxan was administered at a dose 1 mg/kg in the control group (6 rats) and in rats given PPX for 2 days (6 rats). Firing rates of the NE neurons were recorded prior to and following administration of the antagonist in both groups. Despite significantly lower initial rates of discharge in PPX treated group, they were equalized in both groups after idazoxan administration (Fig 5), thus indicating that no other receptor than α_2 -adrenoceptors contributed to the inhibition of firing of NE neurons by PPX.

Effect of long-term PPX administration on the function of the α_2 -adrenergic autoreceptor

In an attempt to explain the recovery of the mean firing of NE neurons following the 14-day administration of PPX, the sensitivity of the cell body α_2 -adrenergic autoreceptor was assessed using the α_2 -adrenoreceptor agonist clonidine.

Although the ED50 values for clonidine in the PPX-treated rats (ED50=3.4±1.1 µg/kg; n=6 in 6 rats) did not significantly differ from the controls



(ED50=2.7±1.2 µg/kg; n=10 in 10 rats), there was some evidence for an attenuated responsiveness of the α_2 -adrenergic autoreceptor based on the differential doses required to completely inhibit firing between PPX-treated and control rats. The dose of clonidine required for silencing of NE neurons in control rats was determined to be 5 µg/kg; however, chronic treatment with PPX 1 mg/kg/d resulted in a marked attenuation of the inhibitory effect of clonidine, with a required dose of 15 µg/kg for complete inhibition of firing (Fig 6).

Effects of PPX administration for 2 and 14 days on the mean firing rate and burst activity of RD 5-HT neurons

The baseline firing rate of 5-HT neurons (controls: 1.0 ± 0.1 Hz; $n=51$ in 5 rats) remained unchanged after 2-day treatment with PPX (1 mg/kg/d; $n=65$ in 6 rats). However, after 14 days of the same regimen, the spontaneous firing of 5-HT neurons was increased by 38 % ($n=66$ in 6 rats) (Fig 7A). This increase was observed in both single-spike and burst-firing neurons (data not shown).

As for the mean firing rate of 5-HT neurons, the percentage of spikes occurring in bursts was not changed by the 2-day PPX administration. It was, however, significantly elevated after the drug was administered for 14 days (Fig 7B). This change was not due to the increase in the number of neurons exhibiting burst activity, since this parameter was not altered by either 2 or 14-day PPX

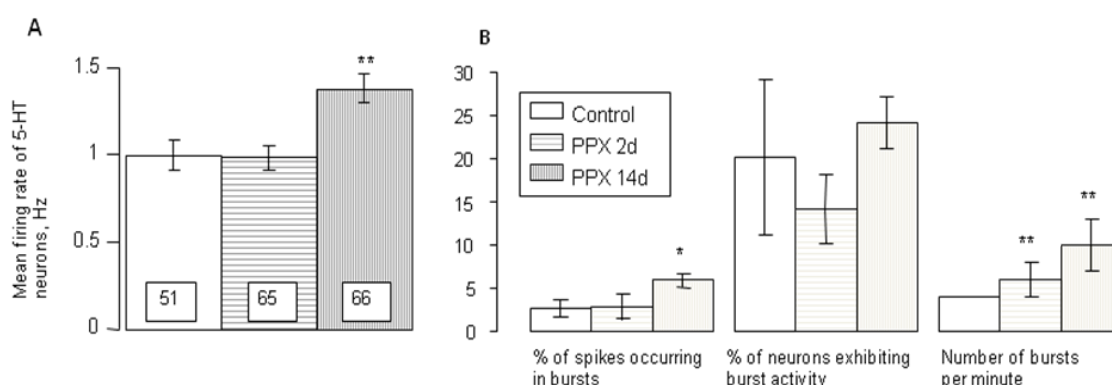


Figure 7. Effect of acute and sustained administration of PPX on RD 5-HT spontaneous firing rate (A) and burst activity (B). (A) The number of neurons recorded in each group is displayed in respective histogram. (B). Percentage of spikes occurring in bursts as well as % of neurons exhibiting burst activity was assessed in all the neurons recorded ($n=51$ in control, $n=65$ in PPX 2d treated rats and $n=66$ in PPX 14d treated rats). Number of bursts discharged in a minute was analyzed only in neurons exhibiting burst-mode activity ($n=17$ in control, $n=9$ in PPX 2d treated rats and $n=16$ in PPX 14d treated rats). The data expressed as mean firing rate $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$; n - number of neurons, SEM - standard error mean.

administration, when compared to saline treated rats (Fig 7B). Thus, the observed increase in percentage of spikes occurring in bursts is likely due to the substantial

difference in the number of bursts per minute at different stages of the treatment (assessed only in neurons exhibiting burst firing). On average, 5-HT neurons in vehicle-treated rats exhibited 4 bursts per minute. After two days of PPX administration, this parameter was amplified by 50%, and after 14 days of PPX administration it increased even more, reaching a level of 150% of control (Fig 7B).

Effect of long-term PPX administration on the function of the 5-HT_{1A} autoreceptor

Given the potent inhibitory role of the 5-HT_{1A} autoreceptor on 5-HT neuronal firing, its sensitivity had to be determined in order to explain the elevated firing activity of 5-HT neurons in 14-day PPX treated rats. The degree of 5-HT neuron firing rate suppression due to LSD, a 5-HT autoreceptor agonist, was assessed. LSD is considered to be a more reliable tool for testing 5-HT_{1A} autoreceptor sensitivity and was chosen over the other widely used 5-HT_{1A} agonist 8-OH-DPAT because, unlike the latter, it does not have an effect on postsynaptic cortical 5-HT_{1A} receptors and therefore does not activate a feedback loop (Blier et al. 1987).

A dose-dependent suppression of the 5-HT firing was observed with the administration of LSD in the range of 1-10 µg/kg. In control rats, LSD completely suppressed the firing activity of 5-HT neurons with a dose of 10 µg/kg (ED₅₀=5.6±1.1 µg/kg; n=7 in 7 rats), whereas in rats treated with PPX 1 mg/kg/d for 14 days, the dose required for complete inhibition was 40 µg/kg

(ED₅₀=15.1±1.2 µg/kg; n=6 in 6 rats) (Fig 8).

DISCUSSION

The present electrophysiological study documented the effects of acute and prolonged administration of the D₂-like receptor agonist PPX on VTA DA, LC NE and RD 5-HT neuronal firing. The decrease in the mean spontaneous firing of DA and NE neurons observed after 2-day PPX treatment was no longer present after prolonged administration, although their burst activity remained attenuated. Serotonergic neurons, which did not show any response to acute or subacute administration of PPX, significantly increased their firing rate and burst activity after prolonged treatment.

As expected from the negative feedback action of DA D₂-like autoreceptors (localized on the VTA DA neurons) on DA firing (Piercey et al. 1996), their acute as well as short-term activation by PPX resulted in a reduction of DA spontaneous discharge rate. As is the case with other DA agonists (Pitts et al. 1995), sustained treatment with PPX (which overstimulates somatodendritic D₂/D₃ receptors), led to a decrease in their responsiveness and a subsequent restoration of the mean firing rate of DA neurons. This adaptive change was found to be due to the desensitization of the D₂-like autoreceptors. Pramipexole and apomorphine have a similar affinity for the D₂-like receptors (Piercey 1998). To rule out the possibility of competition between these two pharmacological agents at the autoreceptor sites, a

24-hour washout period was carried out. This procedure allowed to determine that, even in rats subjected to sustained PPX administration, a complete inhibition of the DA firing activity with DA autoreceptor agonist apomorphine was still possible. Therefore, there was some competition between PPX and apomorphine for the autoreceptor site when the minipumps delivering the drug were present in the rats. Indeed, the dose of apomorphine needed to fully suppress DA firing was nevertheless six times greater than that in control rats, thus clearly indicating desensitization of the autoreceptor. This finding is consistent with previously reported decreased sensitivity and density of D2-like receptors following chronic administration of the D2-like agonist quinpirole (Pitts et al. 1995; Subramaniam et al. 1992)

Interestingly, the burst activity of DA neurons was also modulated by PPX administration. The physiological role of the latter mode of firing needs to be emphasized. It has been shown to lead to increased transmitter release for the same number of impulses delivered at regular intervals during the same time period (Gonon 1988). Chronic PPX treatment resulted in a decrease in the percentage of neurons discharging in bursts. On the other hand, the number of bursts per minute returned to baseline levels, and the overall DA firing rate was accordingly markedly attenuated. The recovery of the DA tonic activity in the presence of the decreased burst-type activity after chronic stimulation of D₂/D₃ receptors by PPX implies a compensation from the single spiking activity of DA neurons. This difference on the two types of DA firing mode might be explained by

PPX agonism on both D₂ and D₃ cell body receptors because it was proposed they contribute to tonic and phasic suppression of DA tone, respectively (Millan et al. 2000a). Pramipexole, being slightly more potent at D₃ than D₂ receptors, might affect them in different ways, when administered chronically. However, PPX acting on both subtypes of DA autoreceptors makes it difficult to fully differentiate their effects on DA firing

Acute PPX administration is known to inhibit neuronal firing in the nucleus accumbens, a postsynaptic target of VTA DA neurons (Piercey 1998). However, the long-term outcome of chronic PPX treatment on postsynaptic neurons has not been examined. It is anticipated that the recovery of firing of DA neurons, despite a 29% reduction of spikes occurring in bursts, may lead to a net enhancement of the DA transmission in these structures because of the presence of PPX, which directly activates postsynaptic neurons. A definite answer will have to come from the assessment of the tonic activation of D₂/D₃ receptors in postsynaptic areas.

The presence of D₂-like receptors in the LC was previously put into evidence in a number of studies (Suzuki et al. 1998; Yokoyama 1994). It has been presumed that DA exerts an inhibitory influence on NE neurons through these receptors. For example, systemic administration of the selective D₂-like receptor antagonist haloperidol enhances the spontaneous firing activity of NE neurons in the LC (Piercey 1994). Moreover, a recent study showed a 47 % increase in firing of NE neurons in VTA-lesioned rats (Guiard et al. 2008a). Accordingly, in the current study it was found that despite the absence of any effect due to an i.v. bolus, a 2-

day PPX regimen did reduce NE firing activity by 30% (Fig 4A).

It is noteworthy that PPX has some affinity for α_2 -adrenoceptors (K_i = 188 nM)

(Piercey et al. 1996). It is

thus possible, though

unlikely, that

accumulation of PPX

after 2 days of sustained

infusion can directly

activate α_2 -adrenergic

autoreceptors. On the

other hand, availability of

NE is known to be

inversely proportional to

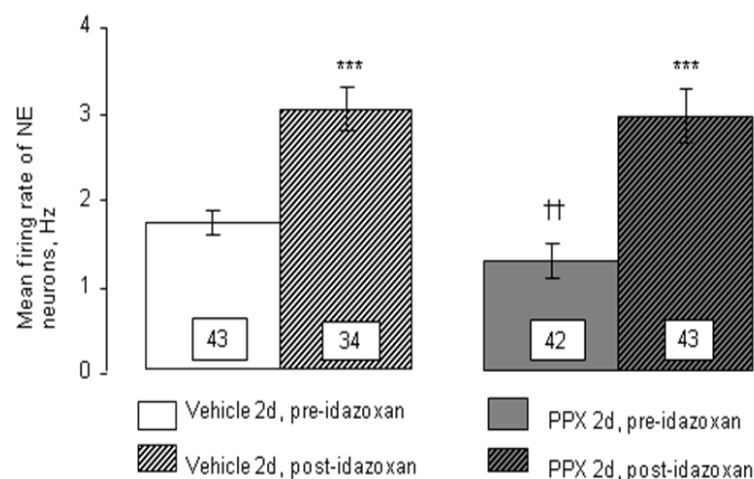


Figure 5. Effect of the α_2 -adrenoreceptor antagonist idazoxan on LC NE neuronal firing in control and 2-day PPX treated rats. †† indicates decreased NE neuronal firing in 2-day PPX treated rats when compared to control. The number of neurons recorded in two electrode descents before and after i.v. idazoxan administration recorded displayed in each column. The data expressed as mean firing rate $\pm$ S.E.M. ** $p < 0.01$

the levels of endogenous DA (Misu et al. 1985). Thus, a short-term PPX treatment

resulting in a decreased DA tone causes disinhibition of NE release in the LC.

Increased synaptic availability of NE would, therefore, stimulate inhibitory α_2 -

adrenergic autoreceptors. Consequently it was hypothesized that the initial

decrease in firing activity of NE neurons in 2-day PPX treated rats is due to α_2 -

adrenoreceptor stimulation. This possibility was supported by the ability of the α_2 -

adrenergic antagonist idazoxan to increase spontaneous firing to equal levels in

controls and rats sub-acutely given PPX (Fig 5), thus indicating that observed NE

inhibition in 2-day treated rats is mediated solely by α_2 -adrenergic receptors.

Interestingly, after chronic PPX treatment, NE neurons regained their normal firing rate. This adaptive change likely occurred due to a decreased responsiveness of the cell body α_2 -adrenergic autoreceptor (Fig 6). This modification can be attributed either to the direct effect of PPX on the α_2 -adrenoceptors or, more likely, to activation of the α_2 -adrenoceptors by endogenous NE, levels of which are likely to be increased due to dampened inhibitory influence of the DA neuronal system.

Unlike for the spontaneous firing of NE neurons, their burst-mode discharge did not recover after chronic treatment with PPX, thus suggesting an involvement of modulating factors different from those affecting single-spike firing activity. Even though mechanisms affecting NE burst firing are not fully understood, it may be speculated that an increase in 5-HT neurotransmission, exerting a suppressant action on NE neurons, may prevent burst mode activity from recovery. For instance, burst firing of NE neurons completely disappears during sustained administration of the potent selective serotonin reuptake inhibitor escitalopram (Dremencov et al. 2007). Another possibility would be that PPX dampens the glutamatergic activation of LC neurons coming from the nucleus paragigantocellularis, which has been shown to drive the activity of NE neurons (Ennis and Aston-Jones 1988).

Previous reports documented that the firing activity of 5-HT neurons is positively influenced by administration of dopaminergic agonists without direct 5-HT effects (Haj Dahmane 2001). Based on these data, an increase in the

discharge rate of 5-HT neurons in rats acutely treated with PPX was expected. Surprisingly, both an i.v. bolus injection and a 2-day regimen of PPX failed to alter 5-HT neuronal firing. Nonetheless, after prolonged stimulation of the D2-like receptors by systemic PPX administration, the frequency of 5-HT neuron firing was significantly increased. Considering that other types of drugs that directly or indirectly lead to an enhancement of serotonergic neurotransmission cause a desensitization of somatodendritic 5-HT_{1A} autoreceptor (Haddjeri et al. 1998b; Haddjeri and Blier 2000; Kreiss and Lucki 1995), it was assumed that similar adaptation could occur in response to chronic PPX administration. Indeed, the observed shift of the LSD dose-response curve (Fig 8A) implies a desensitization of somatodendritic 5-HT_{1A} autoreceptors following prolonged administration of PPX. Taking into consideration that PPX has no affinity for 5-HT_{1A} receptors, such a desensitization probably resulted from an indirect action evoked by PPX. The latter observation is in line with previous in vivo and in vitro studies suggesting enhancement of the 5-HT tone in response to the stimulation of RD D2-like receptors by various pro-dopaminergic agents (Ferre and Artigas 1993; Haj Dahmane 2001).

Even though mechanisms involved in burst-mode firing of 5-HT neurons are not well established, it is hypothesized that observed elevation of this parameter in response to sustained PPX administration likely results from the activation of D2-like receptors located on 5-HT neurons. This assumption is supported by the fact that bath application of DA as well as DA agonists produces a train of action

potentials in 5-HT neurons in vitro (Aman et al. 2007). On the other hand, pharmacological blockade of the 5-HT_{1A} autoreceptors was reported to produce an increase in the number of bursts in the RD 5-HT neurons exhibiting burst-mode activity (Hajós et al. 1995). Since similar changes in burst firing were observed in rats chronically treated with PPX, desensitization of the 5-HT_{1A} autoreceptors might serve as another possible mechanism responsible for increased 5-HT bursting.

Indeed, the latter mode of discharge activity functionally correlates with

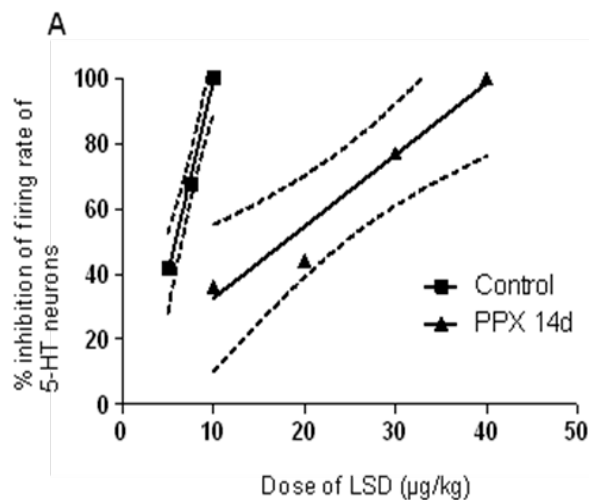
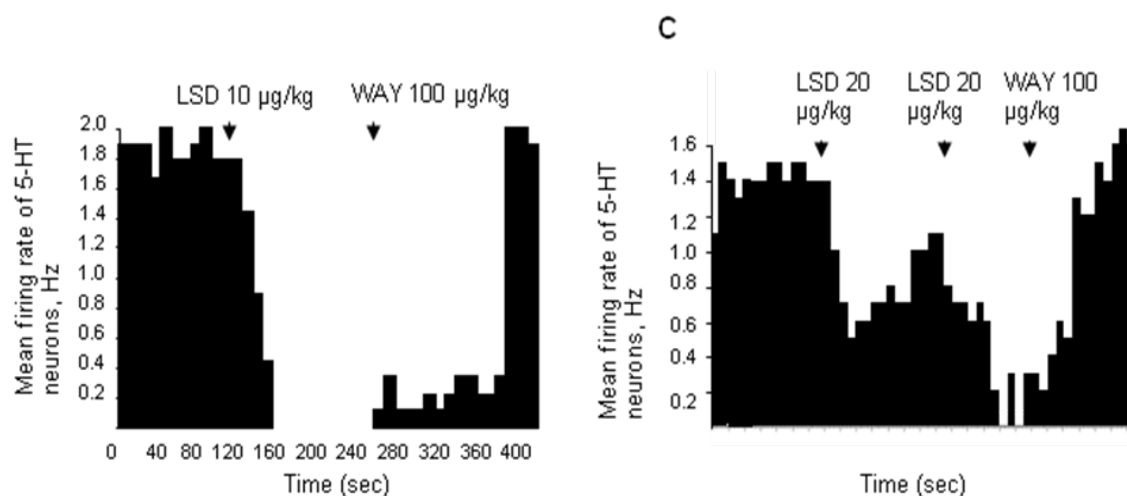


Figure 8. Assessment of the sensitivity of the RD 5-HT 5-HT_{1A} autoreceptor. (A) Relationship between the degree of suppression of RD 5-HT firing activity and doses of 5-HT_{1A} autoreceptor agonist LSD administered intravenously in control and 14-day PPX treated rats. Outer lines represent the standard error of the regression line. Representative integrated firing rate histogram illustrating the effect of LSD administration in control (B) and 14-day PPX treated rats (C). Effect of the 5-HT_{1A} autoreceptor agonist LSD is reversed by administration of the 5-HT_{1A} antagonist WAY in both control and PPX 14-day treated rats.



increased release of 5-HT (Gartside et al. 2000). These observations, in addition to the 38% increase in the mean firing rate of 5-HT neurons, suggest that the long-term administration of PPX should enhance tonic activation of the postsynaptic 5-HT receptors. These alterations of 5-HT neuronal function may be an important contributor to the antidepressant effect of PPX.

Considering the facilitatory effect of chronic PPX administration on DA neurotransmission resulting from a normalized mean firing rate of DA neurons in the presence of sustained activation of postsynaptic D2-like receptors by PPX, it can be hypothesized that drugs possessing pro-dopaminergic properties act through both the DA and the 5-HT systems. In conclusion, the present study provided possible mechanism(s) of action of PPX, which likely underlies its clinical effectiveness in the treatment of depression. These results also serve as yet another line of evidence for the central involvement of reciprocal interactions between the monoaminergic systems involved in the pathophysiology and/or therapeutics of depression.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare that the present study was fully funded by the CIHR grant to PB. Aside from the above PB has financial involvements with these companies:

Biovail

C

Cyberonics	C, G
Eli Lilly & Company	C, SB
Forest Laboratories	CE
Janssen Pharmaceuticals	C, SB, CE, G
Lundbeck	G, C, SB
Organon Pharmaceuticals	C, SB, G
Sepracor	C, G
Wyeth Ayerst	C, SB, G, CE
Sanofi-Aventis	C
Astra-Zeneca	G, SB
Takeda	C
Novartis	C
Shire	C

C = Consultant

SB = Speaker's Bureau

G = Grant Funding

CE = Contract employee

The above companies have no relevance to the work covered in the submission.

OC and ME have no financial involvements to disclose.

ACNOWLEDGEMENTS

This study was supported by the Canadian Institutes of Health Research grant to PB. The authors would like to thank Boehringer Ingelheim Pharmaceuticals and Servier Research Institute for kindly providing pramipexole and S 33084 respectively.

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2.2 Paper II

The results of previous study have shown that the prolonged administration of PPX led to an increase in the firing rate of 5-HT neurons and a normalization of DA neuronal discharge, initially dampened by PPX (Chernoloz et al. 2009). As firing rate is not the only determinant of the overall neuronal transmission, further experiments allowing documentation of the net effect of sustained PPX administration were deemed necessary. The measurement of the levels of the neurotransmitter in the synapse does not offer a reliable evidence of the overall transmission (i.e. even if the amount of neurotransmitter is increased, the overall effect mediated by it will not be enhanced, should the sensitivity/number of postsynaptic receptors be decreased as a result of some adaptive changes). In turn, the electrophysiological experiments allow to determine not only the change in the firing rate and the neurotransmitter release, but also the state of the postsynaptic receptors that ultimately convey the mediated signal. The following study was thus aimed at the determining the overall neuronal 5-HT and DA neurotransmission. It was hypothesized that the normalized firing (/release capacity) of DA neurons, summated with the direct activation of D2 receptors by PPX, will result in the enhancement of the overall DA neurotransmission. The sustained PPX administration was also hypothesized to augment the 5-HT tone, as the firing rate of 5-HT neurons was increased in indirect manner by the PPX-driven DA activation. The increase in the 5-HT spontaneous discharge rate, attained via different mechanisms was, indeed, previously shown to result in an elevation of the

5-HT neuronal transmission (Ghanbari et al.2010; Manta et al. 2009). Only the effects of the prolonged PPX administration were assessed as this treatment regimen 1) produced functionally significant changes at the presynaptic level and 2) is consistent with the delayed onset of action of antidepressants on clinic. Considering a documented role of hippocampus and prefrontal cortex in depression (Drevets et al. 2008), and a substantial innervation of the above brain regions by 5-HT and DA, respectively, these areas were picked for the assessment of postsynaptic PPX effects.

The experimental design was drafted by Dr. Pierre Blier, Dr. Mostafa El Mansari and myself. The experiments were carried out and analyzed by me. All authors assisted in drafting the article, and approved the final manuscript. The manuscript was submitted to the *Journal of Psychiatry and Neuroscience* and was accepted for publication on August 22nd, 2011.

The study was carried out as a part of the CIHR grant entitled 'Role of the dopamine system in the antidepressant response', awarded to Dr. Pierre Blier.

Long-term administration of the dopamine D_{3/2} receptor agonist pramipexole increases dopamine and serotonin neurotransmission in the male rat forebrain

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Journal: ***Journal of Psychiatry & Neuroscience***

Abstract : 237

Introduction : 453

Discussion : 1077

Figures : 4

References : 73

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Olga Chernoloz

Abstract

Background: Long-term administration of the dopamine (DA) D2-like ($D_{3/2}$) receptor agonist pramipexole (PPX), was previously found to desensitize D2 autoreceptors, thereby allowing a normalization of the firing of DA neurons, and serotonin (5-HT_{1A}) autoreceptors permitting an enhancement of the spontaneous firing of 5-HT neurons. It was therefore hypothesized that PPX would increase overall DA and 5-HT neurotransmission in the forebrain as a result of these changes at the presynaptic level.

Methods: Osmotic minipumps were implanted subcutaneously in male Sprague-Dawley rats delivering PPX at a dose of 1 mg/kg/d for fourteen days. The *in vivo* electrophysiological microiontophoretic experiments were carried out in anesthetized rats.

Results: The sensitivity postsynaptic D2 receptors in PFC remained unaltered following PPX, as indicated by the unchanged responsiveness to the microiontophoretic application of DA. Their tonic activation was, however, significantly increased by 104% compared to the control level. The sensitivity postsynaptic 5-HT_{1A} receptors was not altered, as indicated by the unchanged responsiveness to the microiontophoretic application of 5-HT. Similarly to other antidepressant treatments, long-term PPX administration enhanced by 142% the tonic activation of 5-HT_{1A} receptors on CA3 pyramidal neurons, when compared to the control level.

Limitations: The assessment of DA and 5-HT neuronal tone was restricted to

the prefrontal cortex and the hippocampus, respectively.

Conclusion: Chronic PPX administration led to a net enhancement in DA and 5-HT neurotransmission as indicated by the increased tonic activation of postsynaptic D2 and 5-HT_{1A} receptors in forebrain structures.

Introduction

Pramipexole (PPX) is a selective D2-like ($D_{3/2}$) receptor agonist approved for the treatment of Parkinson's disease and restless legs syndrome ¹⁻³. Aside from its use in the above neurological conditions, PPX was also shown to be efficacious in the treatment of major depressive disorder (MDD), both as a monotherapy ^{4, 5} and as an augmenting agent in treatment-resistant depressed patients ⁶⁻⁸. The efficacy of PPX against depressive symptoms was first noted in patients with Parkinson's disorder ^{1, 9}. This illness, characterized by a critical loss of the dopamine (DA) neurons, has a high incidence of comorbidity with MDD – up to 50% ¹⁰. These observations fall in line with a bulk of research suggesting an important role of the DA system in both pathophysiology and treatment of depression ¹¹. Furthermore, not only PPX, but other D2 receptor agonists with unrelated chemical structures, such as pergolide, priribedil and bromocriptine have also been shown to possess antidepressant-like properties in animal studies and a therapeutic action in depressed patients ¹²⁻¹⁵. Imaging studies provided evidence that in depressed patients who achieve remission using PPX, the metabolic activity in brain areas affected by MDD was normalized ¹⁶. Moreover, prolonged PPX treatment not only brought brain metabolism to the control level, it was also found to restore cortical plasticity in patients suffering from restless leg syndrome ¹⁷.

Interestingly, chronic but not short-term stimulation of D2 receptors was found to promote neuronal proliferation in rat hippocampus ^{18, 19}. This finding is of crucial importance as enhanced neurogenesis appears to be one of the common changes occurring with drugs endowed with antidepressant properties. Despite

their proven efficacy, the mechanisms responsible for the therapeutic actions of DA D2 agonists have not been fully elucidated.

Hippocampus and prefrontal cortex (PFC), structures manifesting volume decreases in depressed individuals, are also affected in rodents undergoing chronic stress ²⁰⁻²⁴. In light of the above facts, it is not surprising that one of the common pathways for antidepressant response is an increase in the gene expression of neurotrophic/neuroprotective factors in the PFC and hippocampus ²⁵, ²⁶. Previous work documented that prolonged administration of PPX to rats induced a desensitization of somatodendritic D2 autoreceptors in ventral tegmental area (VTA), allowing the firing of DA neurons to normalize, and of 5-HT_{1A} receptors in dorsal raphe (DR) that enabled spontaneous firing rate of 5-HT neurons to elevate above control levels. Considering the effectiveness of PPX in treatment of MDD, the importance of both DA and 5-HT systems in depression pathophysiology, and the DA innervation of the PFC and the 5-HT innervation of hippocampus, the assessment of the net effect of chronic PPX administration on DA and 5-HT neuronal tone in PFC and hippocampus, respectively, was deemed relevant to understand its antidepressant action.

Materials and methods

Animals

Male Sprague-Dawley rats (Charles River, St Constant, QC), weighing 270–

320 g at the time of recording, were used for the experiments. They were kept under standard laboratory conditions (12:12 h light/dark cycle with access to food and water *ad libitum*). All animal handling and procedures were carried out according to the guidelines of the Canadian Council on Animal Care and protocols of this study were approved by the local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, ON, Canada).

Treatments

Rats were anesthetized with isoflurane for the subcutaneous implantation of osmotic minipumps (Alza, Palo Alto, CA), delivering PPX at a daily dose of 1 mg/kg for 14 days. Control rats were implanted with minipumps delivering physiologic saline. The electrophysiological experiments were carried out with the minipumps in place.

In Vivo Electrophysiological Recordings

Rats were anesthetized with chloral hydrate (400 mg/kg, *i.p.*) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). To maintain a full anesthetic state, chloral hydrate supplements of 100 mg/kg, *i.p.*, were given as needed to prevent any nociceptive reaction to paw pinching. Throughout the experiments, body temperature was maintained at 37°C using thermistor-controlled heating pad. Extracellular recordings of pyramidal neurons in hippocampal CA1

region and in PFC were obtained using five-barreled glass micropipettes. Their tips were of 3-5 μm in diameter and impedance ranged between 4 and 7 M Ω . Using this approach, during all recordings signal-to-noise ratio was between 2 and 10, therefore making spike amplitude discrimination reliable. Prior to electrophysiological experiments, a catheter was inserted in the lateral tail vein for systemic *i.v.* injection of appropriate pharmacological agents, when necessary.

Extracellular recordings and microiontophoresis of pyramidal neurons in PFC

The central barrel or the recording electrode was filled with a 2 M NaCl solution, the four side barrels were filled with the following solutions: DA hydrochloride (5 mM in 200 mM NaCl, pH 4), and 2 M NaCl solution used for automatic current balancing. The micropipettes were descended into the PFC using the following coordinates: 2.5 mm anterior and 1 mm lateral to bregma ²⁷. Pyramidal neurons were found at a depth of 2 to 4 mm below the surface of the brain. Pyramidal neurons were characterized by firing at a range of 0.5-20 spikes/sec, biphasic waveform with initial negative phase deflection and long-duration (0.8–1.2 ms) simple action potentials, alternating with complex spike discharges ²⁸. The duration of microiontophoretic application of DA was 50 seconds. The 50-second duration of microiontophoretic application of the pharmacological agents and the ejection currents (nA) were kept constant before and after each *i.v.* injection throughout the experiments. Neuronal responsiveness

to the microiontophoretic application of DA, prior to and following *i.v.* injections, was assessed by determining the number of spikes suppressed per nA. To calculate the number of spikes suppressed per nA, the difference between the average number of spikes 50 s prior to the start of ejection and the average number of spikes during 50 s of ejection, was divided by the current of the ejected DA in nA.

Extracellular recordings and microiontophoresis of pyramidal neurons in CA3 dorsal hippocampus

Extracellular recording and microiontophoresis of CA3 pyramidal neurons were carried out with five-barreled glass micropipettes. The central barrel used for the unitary recording was filled with a 2 M NaCl solution, the four side barrels were filled with the following solutions: 5-HT creatinine sulfate (10 mM in 200 mM NaCl, pH 4), quisqualic acid (1.5 mM in 200 mM NaCl, pH 8), and the last barrel was filled with a 2 M NaCl solution used for automatic current balancing. The micropipettes were descended into the dorsal CA3 region of the hippocampus using the following coordinates: 4 mm anterior and 4.2 mm lateral to lambda ²⁷. Pyramidal neurons were found at a depth of 4.0 ± 0.5 mm below the surface of the brain. Since the pyramidal neurons do not discharge spontaneously in chloral hydrate anesthetized rats, a small current of quisqualate +1 to -6 nanoampere (nA) was used to activate them to fire at their physiological rate (10 to 15 Hz) ²⁹. Pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-

duration (0.8–1.2 ms) simple action potentials, alternating with complex spike discharges³⁰. The duration of microiontophoretic application of 5-HT was 50 seconds. The 50-second duration of microiontophoretic application of the pharmacological agents and the ejection currents (nA) were kept constant before and after each *i.v.* injection throughout the experiments. Neuronal responsiveness to the microiontophoretic application of 5-HT, prior to and following *i.v.* injections, was assessed by determining the number of spikes suppressed per nA. To calculate the number of spikes suppressed per nA, the difference between the average number of spikes 50 s prior to the start of ejection and the average number of spikes during 50 s of ejection, was divided by the current of the ejected 5-HT in nA.

Assessment of the tonic activation of postsynaptic D2 receptors

The degree of tonic activation of postsynaptic D2 receptor was assessed following 14-day PPX administration. After stable firing baseline was obtained, the D2-like antagonist haloperidol was administered systemically at a dose of 200 µg/kg. The change in the discharge rate of pyramidal neurons was expressed as percentage of baseline firing. This value was compared to that of the control group. Control rats were subjected to the same testing paradigm. In order to avoid drug residual effects, only one neuron in each rat was tested.

Assessment of the tonic activation of postsynaptic 5-HT_{1A} receptors

The degree of tonic activation of postsynaptic 5-HT_{1A} receptors was assessed following 14-day PPX administration. The assessment of the tonic activation of postsynaptic 5-HT_{1A} receptor is more accurate when the firing rate of the recorded neuron is low. Therefore, the firing rate of pyramidal neurons was reduced by lowering the ejection current of quisqualate. After stable firing baseline is obtained, the selective 5-HT_{1A} antagonist WAY 100,635 was administered systemically in 4 incremental doses of 25 µg/kg each, at time intervals of 2 minutes. Neuronal response at each dose-point was obtained for construction of the dose-response curve. Such curves represent stable changes in the firing rate of pyramidal neurons as percentages of baseline firing following each systemic drug administration. In order to avoid drug residual effects, only one neuron in each rat was tested.

Stimulation of the ascending DA pathway

The VTA was electrically stimulated using a bipolar electrode (NE-100, David Kopf, Tujunga, CA, USA). The electrode was implanted 5.2 ± 0.6 mm anterior and 1.0 ± 0.5 mm lateral to bregma 7.4 ± 1 mm from the surface of the brain. VTA was stimulated in a burst mode (train rate=0.5 Hz, train duration=30 ms, 6 pulses per train) via stimulator (S48, Grass Instruments, West Warwick, RI, USA) at an intensity of 500 µA. These stimulation parameters led to durations of suppression of firing of PFC neurons similar to those obtained in previous reports^{31, 32}. The

inhibition of the spontaneous activity of the PFC pyramidal neuron takes place due activation of postsynaptic inhibitory D2 receptors by the DA, endogenously released as a result of stimulation of the DA afferents ⁷³. Different frequencies of stimulation were not used, as DA terminals in this brain region are devoid of D2 autoreceptors ³³. The firing activity in relation to stimulation trains were analyzed by computer using Spike 2 (Cambridge Electronic Design Limited, UK). Peristimulus time histograms of PFC pyramidal neurons were generated to determine the suppression of firing measured in absolute silence (SIL) value in milliseconds.

Stimulation of the ascending 5-HT pathway

The ascending 5-HT pathway was electrically stimulated using a bipolar electrode. The electrode was implanted 1 mm anterior to lambda on the midline with a 10° backward angle in the ventromedial tegmentum and 8.0 ± 0.2 mm below the surface of the brain. Two hundred square pulses of 0.5 msec in duration were delivered by a stimulator at an intensity of 300 μ A and frequencies of 1 and 5 Hz. The inhibition of the spontaneous activity of the hippocampus pyramidal neuron takes place, at least in part, due to activation of the postsynaptic inhibitory 5-HT_{1A} receptors by the 5-HT, endogenously released as a result of stimulation of the 5-HT afferents ⁷². The different frequencies were used to determine the function of terminal 5-HT_{1B} autoreceptors ³⁴. This approach is based on the evidence that when the frequency is increased to 5 Hz, more 5-HT is released in the extracellular

cleft, which consequently exerts a greater negative feedback on the 5-HT release via the terminal 5-HT_{1B} autoreceptors³⁴. Therefore, the release of 5-HT is inhibited quickly during the 5 Hz stimulation leading to a smaller release of transmitter in the synapse for each action potential reaching the terminals. The stimulation pulses and the firing activity were analyzed by computer using Spike 2. Peristimulus time histograms of hippocampus pyramidal neurons were generated to determine the suppression of firing measured in absolute silence (SIL) value in milliseconds. The SIL represents the duration of a total suppression of the hippocampal neuron by endogenously released 5-HT.

Drugs

Pramipexole was generously provided by Boehringer Ingelheim Pharmaceuticals (Ingelheim, Germany); haloperidol, 5-HT creatinine sulfate, DA hydrochloride, quisqualic acid and WAY 100635 were purchased from Sigma (St Louis, MO, USA); All drugs except haloperidol were dissolved in distilled water. Haloperidol was dissolved in distilled water acidified with lactic acid (followed by pH control and normalization, as needed).

Statistical analyses

All results are expressed as mean $\pm$ SEM. The n values represent the number of neurons tested. In the experiments where pharmacological agents were

systemically administered, only the last neuron in each rat was used in order to avoid residual drug effects. Data were obtained from 5 to 7 rats per experimental group. Statistical comparisons were carried out using the two-tailed Student's *t* test when a parameter was studied in control and treated rats. The paired Student's *t* test was used to assess the statistical significance of the variation of the measured parameter from the same neurons under two conditions such as the SIL value at 1 and 5 Hz (for 5-HT). Analysis of covariance was used to assess statistical significance of the difference in the degree of reduction in the response of hippocampus neurons when the frequency of stimulation was increased from 1 to 5 Hz in control and PPX-treated rats. Statistical significance was taken as $p < 0.05$.

Results

Effect of 14-day PPX administration on the responsiveness of prefrontocortical pyramidal neurons to exogenous DA.

In line with previous data, DA applied microiontophoretically to the cell body of the neuron resulted in suppression of 31 out of 36 recorded PFC pyramidal neurons. Such variability is normal for the given type of neurons in the PFC and was documented by previous studies³⁵⁻³⁷. Dopamine-induced inhibition of spontaneous firing in PFC pyramidal neurons is believed to be mediated by the D2 receptors^{28, 38}. Therefore, to determine the responsiveness of postsynaptic D2 receptors only the neurons responding with inhibition were analyzed. Chronic PPX

treatment left the responsiveness of these receptors at the control level, as indicated by the unchanged number of spikes suppressed/nA (control: 14 ± 5 , $n=31$, baseline firing rate = 0.7 ± 0.4 Hz; PPX 14 days: 18 ± 8 , $n=38$, baseline firing rate = 0.9 ± 0.5 Hz; non-significant; see Fig 1 A/B for examples).

Effect of 14-day PPX administration on the degree of tonic activation of D2 receptors in PFC.

In the control group the blockade of inhibitory D2 receptors located on the cell body of PFC pyramidal neurons, achieved with the systemic administration of selective D2 antagonist haloperidol, led to the decrease of their firing rate (Fig 1A). However, after sustained PPX administration this blockade led to a significant 104% disinhibition in

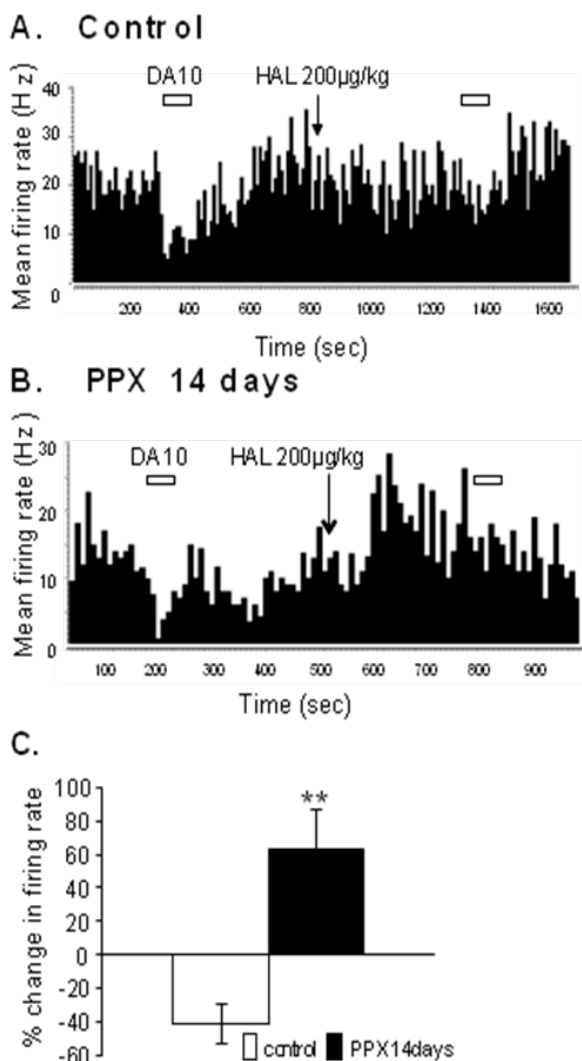


Figure 1. Assessment of tonic activation of D2 receptors in prefrontal cortex.

A and B, integrated firing rate histograms of prefrontal cortex pyramidal neurons illustrating systemic administration of D2 receptor antagonist haloperidol (HAL, 0.2 mg/kg) in rats subjected to vehicle (A) and 14-day PPX (1 mg/kg/day; B) administration. Each bar corresponds to 50-s application of DA, and the number above each bar corresponds to the ejection current in nA. Arrow indicates a point of injection of 200 µg/kg of haloperidol. C, the overall effect of systemic administration of haloperidol on baseline firing of PFC pyramidal neuron in vehicle and PPX-administered rats (expressed as % of change in basal firing). **, $p < 0.01$

the firing rate of pyramidal neurons, when compared to the control value (control $n=6$, PPX 14d $n=7$; $t=4.01$, $df=12$, $p=0.002$; Fig. 1B, 1C). The increase in firing following haloperidol administration indicates that the overall DA tone is increased by the prolonged PPX administration.

Effect of 14-day PPX administration on the PFC DA release potential.

To assess the ability of PPX to modify the endogenous release of DA, the VTA bundle sending afferents to PFC via the mesocortical pathway was electrically stimulated at a time DA neurons have recovered their normal firing rate following sustained administration of PPX ³⁹. Dopamine release, as a result of stimulation produced a suppressant effect on prefrontocortical neuronal firing, was quantified as the absolute silence value (SIL). In rats treated with PPX for 14 days SIL remained at level of the control group under both stimulation conditions (control:

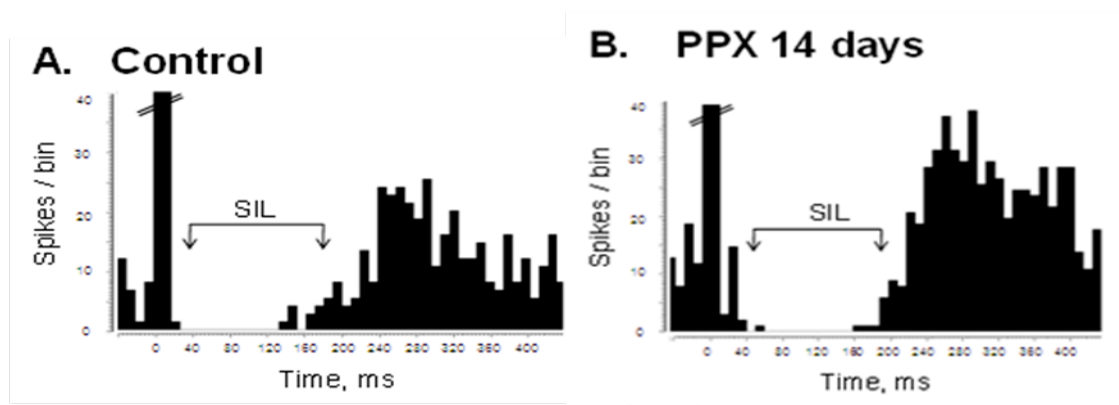


Figure 2. Assessment of DA release in prefrontal cortex

Peristimulus time histograms illustrating effects of stimulation of the ascending DA pathway on the firing activity of PFC pyramidal neurons in controls (A) and rats administered PPX for 14 days (B). The two 10 msec bins at time 0 represent the stimulation artifacts.

SIL= 130 ± 9 , $n=20$; PPX 14d: SIL= 115 ± 6 , $n=34$; non-significant; Fig 2), indicating that the release of DA per impulse was not altered by prolonged administration of

PPX.

Effect of 14-day PPX administration on the responsiveness of dorsal hippocampus pyramidal neurons to exogenous 5-HT.

The firing rate of hippocampus pyramidal neurons in control rats was decreased by 5-HT applied microiontophoretically in a current-dependent fashion. The sensitivity of the postsynaptic 5-HT_{1A} receptors located on the cell body of CA3 pyramidal neurons was found to be unaltered by PPX, as indicated by the lack of change in the number of spikes suppressed/nA in comparison to the control group (control: 18±1, n=19; PPX 14 d: 18±1, n=24, non-significant; see figure 3A/B for examples).

Effect of 14-day PPX administration on the degree of tonic activation of hippocampal 5-HT_{1A} receptors. In the control group, blockade of inhibitory 5-HT_{1A} receptors located on CA3 pyramidal neurons, achieved with the systemic administration of the selective 5-HT_{1A} antagonist WAY 100635, did not modify their firing rate (Fig. 3A). Following 14 days of PPX administration this blockade led to the significant 142% disinhibition in the firing rate of pyramidal neurons in dorsal hippocampus when compared to the control value (control n=6, PPX 14d n=7, t=3.57, df=11, p=0.044; Fig. 3B, 3C). This increase, also observed with all effective antidepressant treatments⁴⁰, indicates that the overall 5-HT tone is enhanced by the long-term PPX administration.

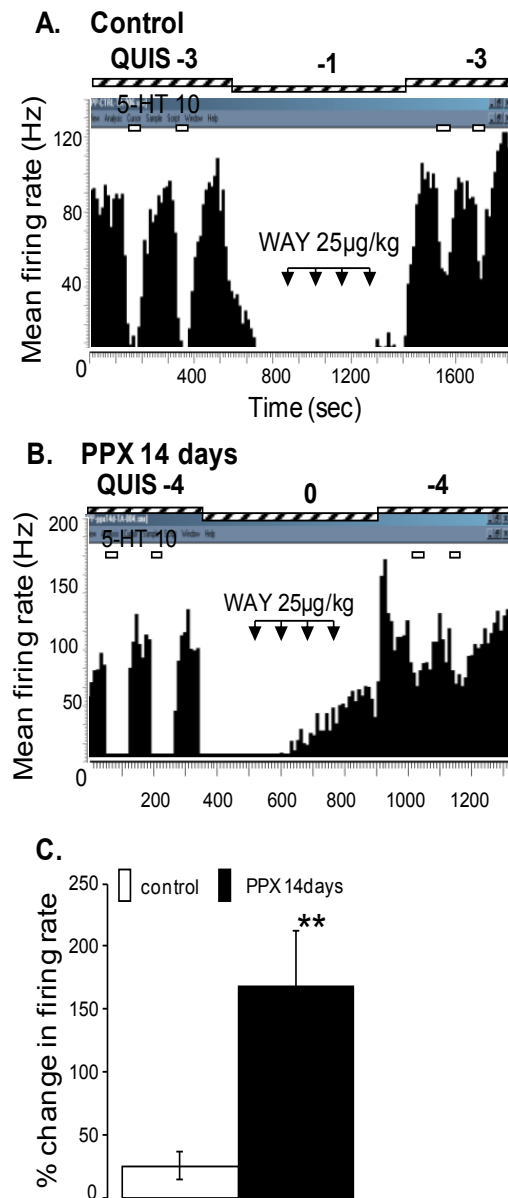


Figure 3. Assessment of tonic activation of 5-HT_{1A} receptors in dorsal hippocampus.

A and B, integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons illustrating systemic administration of 5-HT_{1A} receptor antagonist WAY-100635 in 4 incremental doses of 25 µg/kg in vehicle (A) and 14-day PPX (1 mg/kg/day; B) treated rats. Each bar corresponds to 50-s application of 5-HT, and the number above each bar corresponds to the ejection current in nA. Each arrow indicates a single injection of 25 µg/kg of WAY-100635. C, the overall effect of cumulative systemic administration of WAY-100635 on baseline firing of CA3 pyramidal neuron in vehicle and PPX-treated rats (expressed as % of change in basal firing). *, $p < 0.05$; **, $p < 0.01$

Effect of 14-day PPX administration on the responsiveness of dorsal hippocampal pyramidal neurons to endogenous 5-HT.

To assess the ability of PPX to modify the endogenous release of 5-HT, the 5-HT bundle containing most of the brain 5-HT afferents was electrically stimulated at a physiological (1 Hz) and a maximal (5 Hz) rate³⁴. Serotonin released as a result of stimulation produced a suppressant effect on hippocampal neuronal firing which was quantified as the absolute silence value (SIL). In rats exposed to PPX for 14 days, SIL remained at level of the control group (Fig. 4), indicating that the sensitivity of terminal 5-HT_{1B} receptors controlling the release of 5-HT remained unchanged.

Discussion

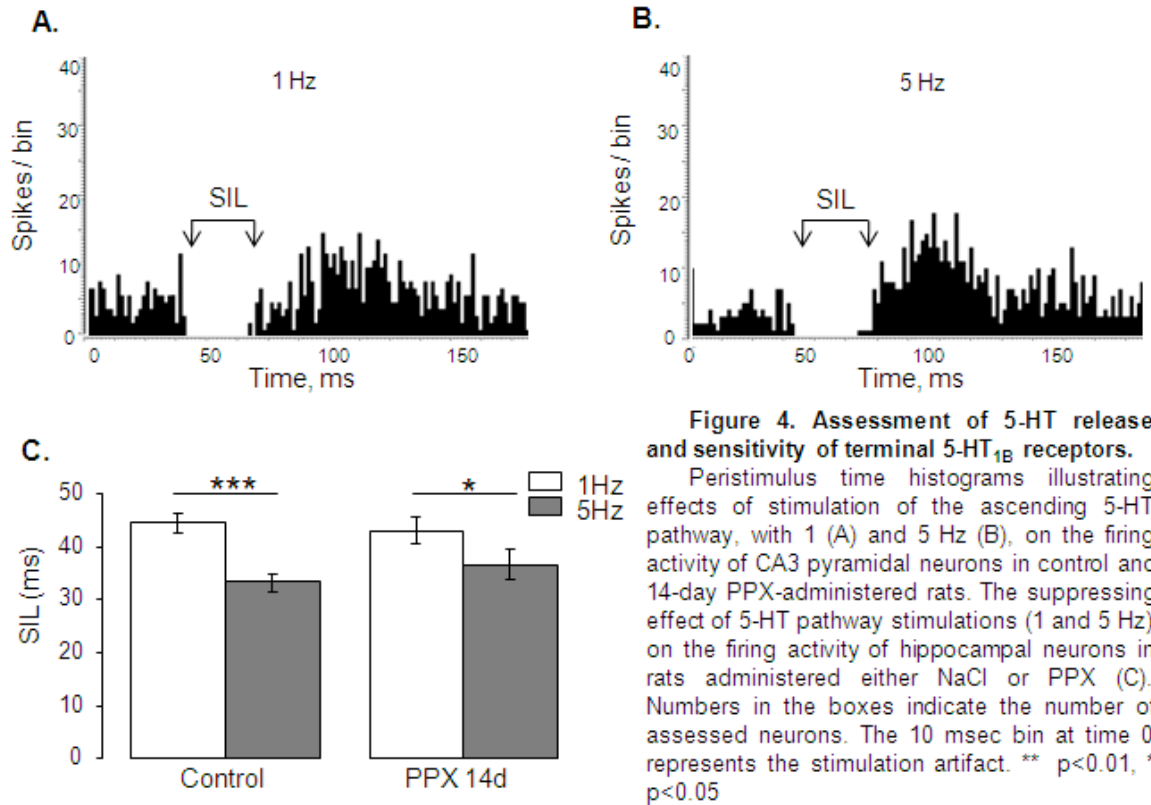
The present electrophysiological study showed that long-term administration of the D2-like agonist PPX increased overall DA neurotransmission, as indicated by the disinhibition of spontaneous neuronal firing of PFC pyramidal neurons by systemic administration of the D2-like antagonist haloperidol. This enhancement of DA tone was not attributable to alterations of the release of DA or to an enhanced responsiveness of postsynaptic D2 receptors. It is therefore concluded that it resulted from a summation of the normalized DA firing, presumably restoring DA release, and the presence of PPX in the synapse. The present study also showed that prolonged PPX administration increased the overall 5-HT tone without changing the release of 5-HT per action potential reaching hippocampus terminals, or the sensitivity of terminal 5-HT_{1B} autoreceptors. It can thus be concluded that the increase in 5-HT neuronal transmission resulted from the enhanced firing of 5-HT neurons³⁹.

The PFC is believed to be under tonic inhibitory influence from endogenous DA²⁸. Microiontophoretic DA administration has an inhibitory effect on spontaneous firing rate of PFC pyramidal neurons³⁵. The same effect can be produced by the endogenous DA, as evidenced by the suppression of PFC discharge in response to the VTA stimulation^{36, 37}. Though both D1-like and D2-like receptors are present in PFC^{41, 42}, the suppressant effect of DA was shown to be mediated by the latter, as selective blockade of D2-like, but not D1-like receptors reversed this action^{28, 38}.

It was previously documented that the PPX-induced activation of somatodendritic D2 autoreceptors in the VTA leads to the decrease in the firing rate of DA neurons, driven by the negative feedback mechanism exerted by the cell body D2 autoreceptors ³⁹. With ongoing administration of PPX over 14 days, these receptors desensitize, allowing the firing to return to baseline. Conversely, the degree of inhibition of PFC pyramidal neurons by both exogenous (iontophoretically-applied) and endogenous (stimulation-induced) DA was equal in control and PPX-treated rats. This lack of change indicated an unaltered responsiveness of PFC postsynaptic D2 receptor after 14-days of PPX sustained exposure. Nevertheless, in rats receiving PPX on a long-term basis, but not in the control group, blockade of the inhibitory D2 receptors by *i.v.* administration of selective antagonist haloperidol led to significant disinhibition of the spontaneous firing of pyramidal neurons (Fig. 1). As the sensitivity of the receptors mediating this response was found to be unchanged, the observed PPX-induced increase in the tonic activation of D2 receptors in PFC was most likely attributable to the direct effect of this D2 agonist, present on board at the time of experiment, on the target receptor summing with a normalized DA release resulting from a recovered DA firing activity ³⁹. Yet, it needs to be mentioned that both DA modulation and its effects in PFC are complex and multisided and no unified view is established ⁴³. For instance, depending on the dose, the state of the system, predominance of direct vs. indirect activation, the same drug may produce opposing effects upon the elicited responses ⁴³⁻⁴⁵. Terminal D2 receptors, playing a prominent role in the control of DA release in limbic structures, are fewer in number in PFC ^{33, 46}. Thus,

under physiological conditions, their stimulation by endogenous DA and/or exogenous D2 receptor agonists plays a negligible role in the amount of the transmitter released ⁴⁷.

The maintenance of proper mesocortical DA levels known play an important role in different aspects of attention and learning, as well as behavioral and physiological mechanisms of the stress response ⁴⁸⁻⁵⁰. These functions are often perturbed in depression and may be related to the decrease in the levels of DA. The decrease in function of the frontal lobe is one of the most constant findings in the depressive state ⁵¹⁻⁵³. The normalization of the fronto-cortical metabolism is consistently seen in patients who achieve the remission following the pharmacological antidepressant treatment ⁵⁴⁻⁵⁶. The VTA provides a dense DA projection to the PFC. The PPX-induced increase in the DA function, known to be dampened in depression, potentially leads to the normalization of the modulatory DA tone in PFC and a consequent restoration of the functions controlled by this brain region. As the 5-HT tone in the hippocampus was significantly increased after prolonged PPX administration, it was important to determine the mechanism of such an enhancement. The 5-HT ascending bundle was electrically stimulated first to assess the amount of 5-HT released per electrical pulse reaching the terminals, and second to assess the sensitivity of terminal 5-HT_{1B} autoreceptors that control 5-HT release. The stimulation at a physiological rate of 1 Hz led to the same degree of suppression of the firing activity of CA3 pyramidal neurons in rats that received PPX for 14 days when compared to controls. When the frequency of stimulation was increased from 1 Hz to 5 Hz, the suppression of the firing was



reduced to a similar extent in treated and control rats, indicating an unaltered responsiveness of terminal 5-HT_{1B} autoreceptors. This result stands in contrast with the decreased responsiveness of the terminal 5-HT_{1B} autoreceptors occurring with long-term administration of SSRIs such as citalopram, paroxetine, fluvoxamine, and fluoxetine⁵⁷. The sensitivity of postsynaptic 5-HT_{1A} receptors was also unaltered, as shown by the unchanged inhibitory potency of 5-HT applied on the CA3 pyramidal neurons by iontophoresis. Since 5-HT activating the postsynaptic 5-HT_{1A} receptor equally suppressed the firing in both PPX and saline exposed rats, it can be concluded that in rats receiving PPX the disinhibition in response to 5-HT_{1A} receptor blockade (Fig. 3) is due to an overall increase of the 5-HT tone, and not the modified sensitivity of the receptor mediating this response.

This elevation of the 5-HT neuronal tone likely stemmed from the PPX-

induced amplification in the firing rate of DR 5-HT neurons that occurred after the same 14-day, but not 2-day, PPX regimen ³⁹. Enhancement of the tonic activation of postsynaptic 5-HT_{1A} receptors resulting from the increase in the firing rate of 5-HT neurons is not unique to PPX. The catecholamine releasing agent bupropion and prolonged vagus nerve stimulation produce an analogous change ^{58,59}. Similarly to PPX, the increase in the spontaneous discharge of DR 5-HT neurons produced by subchronic administration of the atypical antipsychotic aripiprazole was found to be due to activation of the D2-like receptors and desensitization of 5-HT_{1A} autoreceptors ⁶⁰. Such a phenomenon is in line with previous *in vivo* and *in vitro* studies documenting the enhancement of the 5-HT tone in response to the stimulation of DR D2-like receptors by pro-dopaminergic agents ⁶¹⁻⁶³.

Limitations

The sensitivity of postsynaptic DA in the frontal cortex was not assessed using a range of ejection currents of DA, unlike for the 5-HT sensitivity in the hippocampus. Therefore, it is possible that we may have missed a subtle difference in sensitivity. Nevertheless, the 10 nA current did not produce a maximal inhibition of firing which would not place that value at the extremes of a current-effect curve. The neuronal tone was assessed within the mesocortical system, but not within the mesolimbic system. Nevertheless, similar changes combining activation of postsynaptic D2 receptors with both endogenous DA as well as with the exogenous agonist PPX are likely taking place within the

mesolimbic system as well because VTA gives rise to the DA innervation in both circuits. Stress response and cognitive functions, regulated by the mesocortical DA, as well as hedonia, regulated by the mesolimbic DA, are impaired in depression ⁶⁴⁻⁶⁶. Major depression is characterized by abnormalities in activity and/or functional connectivity within both these systems ^{67,68}, thus changes in their function produced by prolonged PPX administration likely contribute to the clinical benefits of this drug in MDD. Indeed, a recent clinical study documented that depressed patients responding to the long-term PPX treatment showed normalization of the regional blood flow in orbitofrontal cortex, anteromedial and ventrolateral PFC, posterior cingulate, hippocampus and accumbens ¹⁶. Importantly, activity of these brain areas is known to be altered in the depressed state ^{23, 24}. It is noteworthy that the metabolic changes produced by sustained PPX closely follow those of antidepressants and deep-brain stimulation ⁶⁹⁻⁷¹.

Conclusion

It can thus be concluded that despite the lack of affinity towards any component of 5-HT system, PPX produces a significant increase in the 5-HT neurotransmission in an indirect manner. These observations with PPX therefore add to large body of data showing the commonality of this change by all effective antidepressants thus far tested ⁴⁰.

The current study thus put into evidence that chronic treatment with the D2

agonist PPX increased DA neurotransmission in rat PFC and 5-HT neurotransmission in hippocampus. Considering the documented normalization of the brain function within the same regions in depressed patients treated with this drug ¹⁶, it is likely that the observed changes in the function of the abovementioned modulatory monoaminergic systems may underlie to some degree the clinical effectiveness of PPX in treatment of depression.

Acknowledgments

This study was supported by the Canadian Institutes of Health Research grant to PB. The authors would like to thank Boehringer Ingelheim Pharmaceuticals for kindly providing pramipexole. P.B. received support for investigator initiated grants, and/or honoraria for advisory boards and/or speaking engagements from Astra-Zeneca, Biovail, Bristol Myers Squibbs, Eli Lilly & Company, Janssen Pharmaceuticals, Labopharm, Lundbeck, Pfizer, Schering-Plough/Merck, Servier, Takeda and Wyeth.

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2.3. Paper III

All atypical antipsychotics were shown to possess an antidepressant properties, however only aripiprazole, olanzapine and quetiapine were approved for use in depression either in combination with antidepressants or alone. Aripiprazole is a unique antipsychotic medication. Unlike all other representatives of this pharmacological class that antagonize D2 receptor, this drug acts as a partial agonist at this site (Burris et al., 2002; Hirose et al., 2005). This distinctive property of aripiprazole, along with its effect at number of other receptors implicated in an antidepressant response, made important the characterization of its effects on the firing rates of monoaminergic neurons. Augmentation of SSRI and SNRI treatments with aripiprazole was shown to result in an increase in response rate and, sometimes, faster clinical benefit (Marcus et al. 2008; Berman et al. 2007; Berman et al. 2008). Numerous studies documenting this phenomenon led to the approval of aripiprazole for use in MDD as an adjunct to the standard antidepressants. Considering the above, examining the effects of not only the sole administration of aripiprazole, but also its concomitant use with an SSRI escitalopram were deemed important and relevant. Increase in the 5-HT tone, produced by the SSRIs, is known to dampen the firing rate of NE and DA neurons via excessive activation of inhibitory 5-HT_{2A} and 5-HT_{2C} receptors, respectively (Dremencov et al. 2007; Dremencov et al. 2009). This decrease in catecholaminergic tone may be responsible for the suboptimal response rate as well as some adverse effects of SSRs. Since aripiprazole blocks both 5-HT_{2A} and 5-HT_{2C} receptors (Shapiro et al. 2003), it was hypothesized that its addition to an

SSRI regimen will reverse the inhibition of NE and DA firing. In addition, since aripiprazole activates multiple monoaminergic receptors (Shapiro et al. 2003), it was hypothesized that it may alter the activity of DA and/or NE and/or 5-HT system even when administered on its own. Experiments were carried out after 2 and 14 days of drug(s) administration to determine the immediate and the clinically-relevant long-term effects.

The experimental design was drafted by Dr. Pierre Blier, Dr. Mostafa El Mansari and Olga Chernoloz and approved by Bristol Myers Squibb, supporting the study. The experiments were carried out and analyzed by Olga Chernoloz. All authors assisted in drafting the article, and approved the final manuscript. The manuscript was published at the *Psychopharmacology*, 2009, 206 (2), pp. 335-344.

Electrophysiological studies in the rat brain on the basis for aripiprazole augmentation of antidepressants in major depressive disorder

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Journal: Psychopharmacology

Figures: 4

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ABSTRACT

Rationale: Aripiprazole is an atypical antipsychotic approved by the FDA for use in major depressive disorder as an adjunct to antidepressants. However, the precise mechanisms responsible for the effectiveness of aripiprazole augmentation are not fully understood.

Objectives: The current study was aimed at examining the effects of aripiprazole administration alone and in combination with the SSRI escitalopram, on the firing of serotonin (5-HT), norepinephrine (NE) and dopamine (DA) neurons.

Methods: Electrophysiological experiments were carried out in anaesthetized Sprague-Dawley rats. Escitalopram was delivered via subcutaneously implanted osmotic minipumps at a dose 10 mg/kg/d. Aripiprazole was injected s.c. daily at a dose 2 mg/kg/d. Both drugs were administered for 2 and 14 days alone and in combination. Control rats received physiological saline in analogous regimens.

Results: Two-day escitalopram administration resulted in a significant decrease in the firing rate of 5-HT, NE and DA neurons. Following 14 days of escitalopram administration, 5-HT firing returned to the baseline. Firing rate of NE and DA neurons remained significantly decreased.

Aripiprazole administered for 2 or 14 days significantly increased the firing rate of 5-HT neurons by 36 and 48%, respectively, but not those of DA and NE neurons. Desensitization of 5-HT neurons was observed after 2 days of aripiprazole administration.

The combination of the two drugs reversed the inhibitory action of escitalopram on the firing rate of 5-HT, NE and DA neurons.

Conclusion: The present study showed that addition of aripiprazole to an SSRI regimen reverses the inhibitory action of the SSRI on monoaminergic neuronal firing.

Keywords: Antidepressant – Antipsychotic – Electrophysiology – Depression
– Dopamine – Norepinephrine – Serotonin

INTRODUCTION

Despite substantial progress in the area of depression research, existing therapies of the Major Depressive Disorder (MDD) remain far from optimal. The majority of patients diagnosed with MDD do not achieve an optimal response to the selective serotonin reuptake inhibitors (SSRIs) – the current first-line depression treatment. Therefore, various augmentation strategies are often used to optimize treatment, especially in treatment-resistant depression. One such approach is an addition of an atypical antipsychotic to an SSRI regimen (Ostroff and Nelson. 1999; Shelton et al. 2001; Thase et al. 2007; Garakani et al. 2008; Keitner et al. 2009).

The group of drugs composed of atypical antipsychotics is very heterogeneous in terms of the targeted receptors. The only common denominator of the above drugs is the antagonism at serotonin (5-HT) 5-HT_{2A} (with the exception of amisulpride which has no significant affinity for 5-HT_{2A} receptors) and D2 receptors with a high 5-HT_{2A}:D2 affinity ratio (Creese et al. 1976; Meltzer. 1999). In contrast, aripiprazole (ARI) presents higher affinity for D2 than to 5-HT_{2A} receptors and was shown to have partial agonistic rather than antagonistic activity at D2 receptors (Burris et al. 2002; Shapiro et al. 2003; Hirose and Kikuchi. 2005; Uzun et al. 2005). The partial agonism of ARI at D2 receptors likely contributes not only to the low level of extrapyramidal symptoms, but also underlies its ability to normalize dopamine (DA) transmission accordingly to the levels of endogenous DA (Kikuchi et al. 1995; Inoue et al. 1996; Lawler et al. 1999; Matsubayashi et al.

1999; Burris et al. 2002). In addition, ARI acts as a partial agonist at D3, D4, 5-HT_{1A}, 5-HT_{2C} and 5-HT₇ receptors, as an antagonist at 5-HT_{2A} and 5-HT₆ receptors (Burris et al. 2002; Shapiro et al. 2003). It also has moderate affinity at α_1 -adrenergic and H1-histamine receptors (Shapiro et al. 2003). The pharmacological profile of ARI appears to be favorable for its use in the treatment of depression. First, increased activation of postsynaptic 5-HT_{1A} receptors is believed to underlie antidepressant effects of various drugs used in depression treatment (Blier and Ward. 2003). ARI is a 5-HT_{1A} agonist like buspirone and gepirone – agents shown to be effective in the treatment of depression (Feiger et al. 2003; Rush et al. 2006). Furthermore, activation of 5-HT_{1A} receptors by ARI was shown to increase DA release in the prefrontal cortex and hippocampus (Ichikawa et al. 2001; Li et al. 2004). The latter effect is believed to play a positive role on cognitive symptoms in depressed patients. Secondly, agents possessing D2 receptor agonism were reported to be effective adjuncts in treatment-resistant depression (Waehrens and Gerlach. 1981; Cassano et al. 2004; Goldberg et al. 2004). For instance, the selective D2 receptor agonist pramipexole has been reported to be an effective antidepressant in large studies (Corrigan et al. 2000; Lemke et al. 2006).

Even though SSRIs are considered to be a first-line treatment in the current therapy of MDD, many treatment failures and adverse effects are also associated with their use. Such unfavorable outcomes could be due to, at least in part, an inhibitory action of SSRIs on both DA and norepinephrine (NE) neurotransmission. SSRIs administered either acutely or chronically were shown to decrease the firing

rate and pattern of the DA and NE neurons via activation of 5-HT_{2C} and 5-HT_{2A} receptors, respectively (Gobert et al. 2000; Szabo and Blier. 2001; Dremencov et al. 2009). By blocking these receptors, ARI could possibly counterbalance the inhibitory action of SSRIs, as in the case of other atypicals (Dawe et al. 2001; Dremencov et al. 2007b). The resulting preservation of DA and NE firing could be of crucial importance in the treatment of MDD.

Most atypical antipsychotics were reported to be an effective addition to the therapeutic regimen of treatment-resistant depressed patients (Ostroff and Nelson. 1999; Shelton et al. 2001; Thase et al. 2007; Garakani et al. 2008; Keitner et al. 2009). ARI became the first antipsychotic to be approved by the FDA as an augmenting agent in the treatment of depression. Three large placebo-controlled studies documented increased remission rates in depressed patients treated with combination of antidepressant and ARI (Berman et al. 2007; Berman et al. 2009; Marcus et al. 2008).

Despite proven clinical efficacy of ARI as an augmenting agent in MDD treatment, the neurobiological mechanism explaining its mode of action has not been fully elucidated. The unique pharmacologic profile of ARI sets it apart from all other agents in its class and necessitates closer scrutiny. The present study was thus aimed at examining the effects of ARI on its own and in addition to the SSRI escitalopram (ESC) on the spontaneous firing of locus coeruleus (LC) NE, ventral tegmental area (VTA) DA and raphe dorsalis (RD) 5-HT neurons.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Charles River, St. Constant, QC) weighing 270 to 320 g at the time of recording, were used for the experiments. They were kept under standard laboratory conditions (12:12 hour light/dark cycle with access to food and water *ad librum*). All animal handling and procedures were carried out according to the guidelines of the Canadian Council on Animal Care and protocols of this study were approved by the local Animal Care Committee (University of Ottawa, Institute of Mental Health Research, Ottawa, ON, Canada).

Treatments

Escitalopram was delivered via subcutaneously implanted osmotic minipumps at a daily dose of 10 mg/kg/d. Aripiprazole at a dose of 2 mg/kg/d was injected *s.c.* daily with a last dose administered 1 hour prior to the experiment. Both drugs were administered for 2 or 14 days alone and in combination. Control rats received physiological saline in analogous regimens.

To test the input of DA on 5-HT neuronal function, paliperidone at a dose of 1 mg/kg was administered *i.v.* to rats treated with ARI for 2 days. To test the input of 5-HT on DA neuronal function, WAY 100635 was administered *i.v.* at a dose of 0.1 mg/kg to rats treated with ARI for 2 days.

In vivo electrophysiological recordings

Rats were anesthetized with chloral hydrate (400 mg/kg; *i.p.*) and placed in a stereotaxic frame. To maintain a full anesthetic state chloral hydrate supplements of 100 mg/kg, *i.p.*, were given as needed. Extracellular recordings of NE, DA and 5-HT neurons in the LC, the VTA and the RD respectively, were obtained using single-barreled glass micropipettes. Three to six electrode descents per nucleus were made. Their tips were of 1-3 μm in diameter and impedance ranged between 4-7 M Ω . All glass micropipettes were filled with a 2 M NaCl solution. Prior to the electrophysiological experiments, a catheter was inserted in the lateral tail vein for systemic *i.v.* injection of pharmacological agents.

Recording of the LC NE neurons

Single-barreled glass micropipettes were positioned using the following coordinates (in mm from Lambda): AP, - 1.0 to - 1.2; L, 1.0 to 1.3; V, 5 to 7. Spontaneously active NE neurons were identified using the following criteria: regular firing rate (0.5–5.0 Hz) and positive action potentials of long duration (0.8–1.2 ms) exhibiting a brisk excitation followed by period of silence in response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen. 1982a).

Recording of the VTA DA neurons

Single-barreled glass micropipettes were positioned using the following coordinates (in mm from Lambda): AP, +3.0 to +3.8; L, 1 to 0.6; V, 6.5 to 9. The presumed DA neurons were identified according to these *in vivo* electrophysiological properties: a triphasic action potential with a marked negative deflection; a characteristic long duration (> 2.5 ms) often with an inflection or “notch” on the rising phase, a slow spontaneous firing rate (0.5 – 5 Hz) with an irregular single spiking pattern with slow bursting activity (characterized by spike amplitude decrement; Grace and Bunney. 1983). In addition, a criterion of duration (> 1.1 msec from the start of the action potential to the negative trough) was used (Ungless et al. 2004).

Recording of the RD 5-HT neurons

Single-barreled glass micropipettes were positioned using the following coordinates (in mm from Lambda): AP, +1.0 to 1.2; L, 0 ± 0.1 ; V, 5 to 7. The presumed 5-HT neurons were then identified using the following criteria: a slow (0.5 - 2.5 Hz) and regular firing rate and long-duration (2 - 5 ms) bi- or triphasic extracellular waveform (Aghajanian and Vandermaelen. 1982b).

Dose-response curves

Dose-response curves assessing the effect of 2-day administration of ARI on the sensitivity of 5-HT_{1A} autoreceptors were constructed for systemic *i.v.* administration of the 5-HT autoreceptor agonist LSD. Dose-response curves were obtained using only the initial response to the first dose injected to a single neuron

of each rat. Dose-response curves were plotted using GraphPad software (Smallville, USA).

Statistical analysis

All results are expressed as means $\pm$ S.E.M. Statistical comparisons between differences in spontaneous firing rate of LC NE, VTA DA and DR 5-HT neurons in rats treated with saline, ESC, ARI and ESC+ARI combination were carried out by using one-way analysis of variance and multiple comparison procedures using Fisher's PLSD *post hoc* test. Statistical comparisons between differences in spontaneous firing rate of 5-HT neurons in rats treated with aripiprazole for 2 days prior to and following the administration of paliperidone were carried out using the Student's t-test. Statistical data assessing the effect of 2-day administration of ARI on the sensitivity of 5-HT_{1A} autoreceptors was obtained using one-way analysis of variance followed by Tukey's Multiple Comparison Test. Firing rate data were obtained from 3 to 5 rats per experimental group. Statistical significance was taken as $p < 0.05$.

Drugs

Aripiprazole was provided by *Bristol-Myers Squibb* (Ingelheim, Germany); ESC was provided by *Lundbeck* (Copenhagen, DK); paliperidone was provided by *Janssen* (Titusville, NJ); WAY 100635 was purchased from *Sigma* (St. Louis, USA); lyserginic acid diethylamide (LSD) was obtained through *Health Canada*. WAY 100635 and ESC were dissolved in distilled water. Aripiprazole and

paliperidone were dissolved in distilled water acidified with lactic acid (followed by pH control and normalization, as needed).

RESULTS

Effects of 2- and 14-day administration of ESC, ARI and their combination on the mean firing rate of LC NE neurons

In line with previous data (Dremencov et al. 2007a; Ghanbari et al. 2008), both short and long-term ESC administration led to significant decrease in NE spontaneous firing by 45 and 49%, respectively, when compared to controls (control 2 days vs. ESC 2 days $p < 0.001$, $F = 10.56$; control 14 days vs. ESC 14 days $p < 0.001$, $F = 12.84$) (Fig. 1). The ARI regimen left NE firing rate unaltered. When the two drugs were administered in combination for 2 days, NE firing was partially restored, compared to that of ESC-treated rats, reaching 74% of saline-

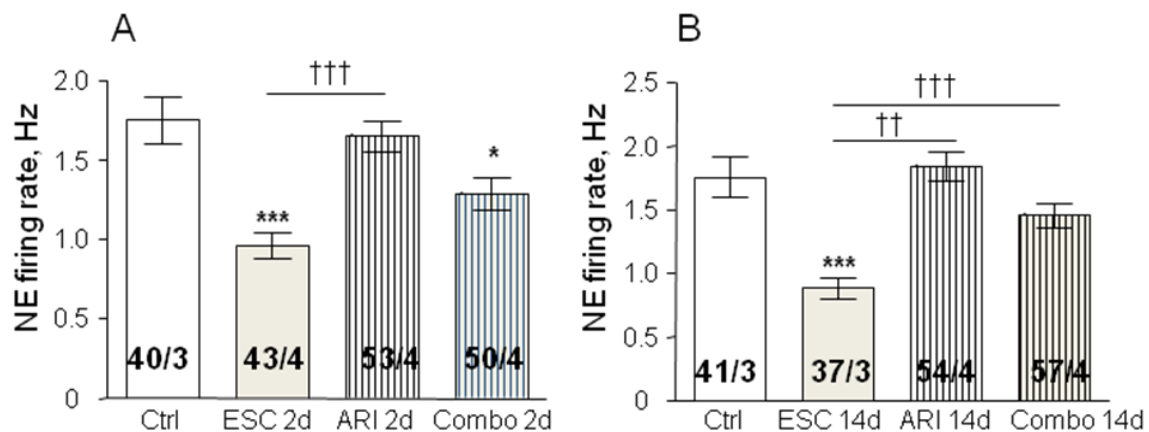


Figure 1. Effect of acute and sustained administration of ESC, ARI and their combination on NE spontaneous firing rate

The firing rate of NE neurons after 2 (A) and 14 days (B) of treatment. The number of neurons recorded / rats used in each group is displayed in respective histograms. The data expressed as mean firing rate $\pm$ SEM. * represents comparison to the control group; † represents comparison to the ESC group. *(†) $p < 0.05$, ** (††) $p < 0.01$, *** (†††) $p < 0.001$

treated rats (control vs. ESC+ARI 2 days $p<0.05$, $F=10.56$) (Fig. 1). This inhibitory action of the ESC was no longer significant after two drugs were co-administered for 14 days.

Effects of 2- and 14-day administration of ESC, ARI and their combination on the firing rate of VTA DA neurons

Dopaminergic neuronal firing was significantly decreased by 41% in response to both 2- and 14-day administration of ESC (control 2 days vs. ESC 2 days $p<0.001$, $F=5.81$; control 14 days vs. ESC 14 days $p<0.001$, $F=7.15$) (Fig. 2). Mean firing rates remained unchanged in response to ARI administration on its

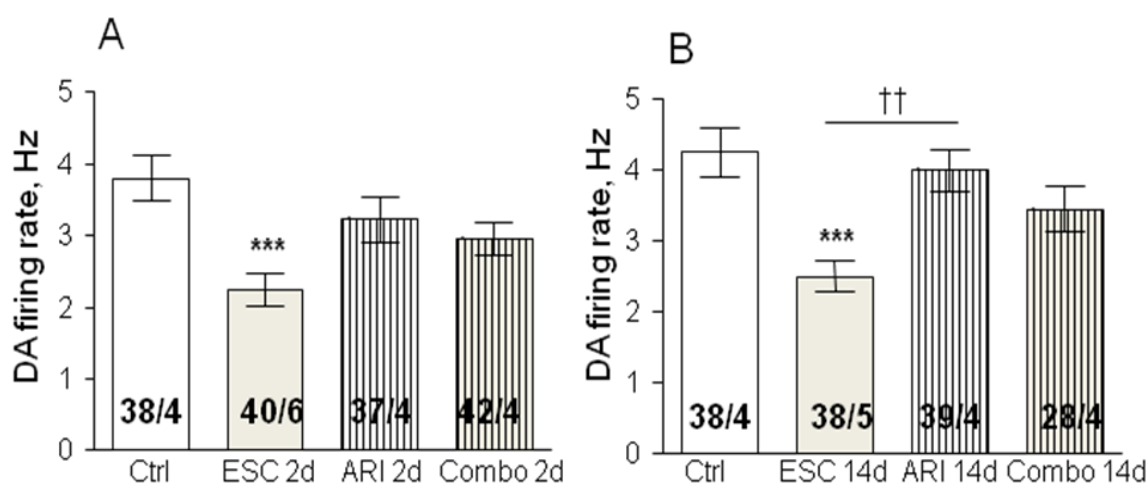


Figure 2. Effect of acute and sustained administration of ESC, ARI and their combination on DA spontaneous firing rate

The firing rate of DA neurons after 2 (A) and 14 days (B) of treatment. The number of neurons recorded / rats used in each group is displayed in respective histogram. The data expressed as mean firing rate $\pm$ SEM. * represents comparison to the control group; † represents comparison to the ESC group. ** (††) $p<0.01$, *** (†††) $p<0.001$

own. The combination of the two drugs administered concomitantly, reversed the inhibitory action of ESC, resulting in an equalization of the firing rate with that of

controls (Fig. 2).

Assessment of the 5-HT_{1A} input on DA firing in rats treated with ARI for 2 days

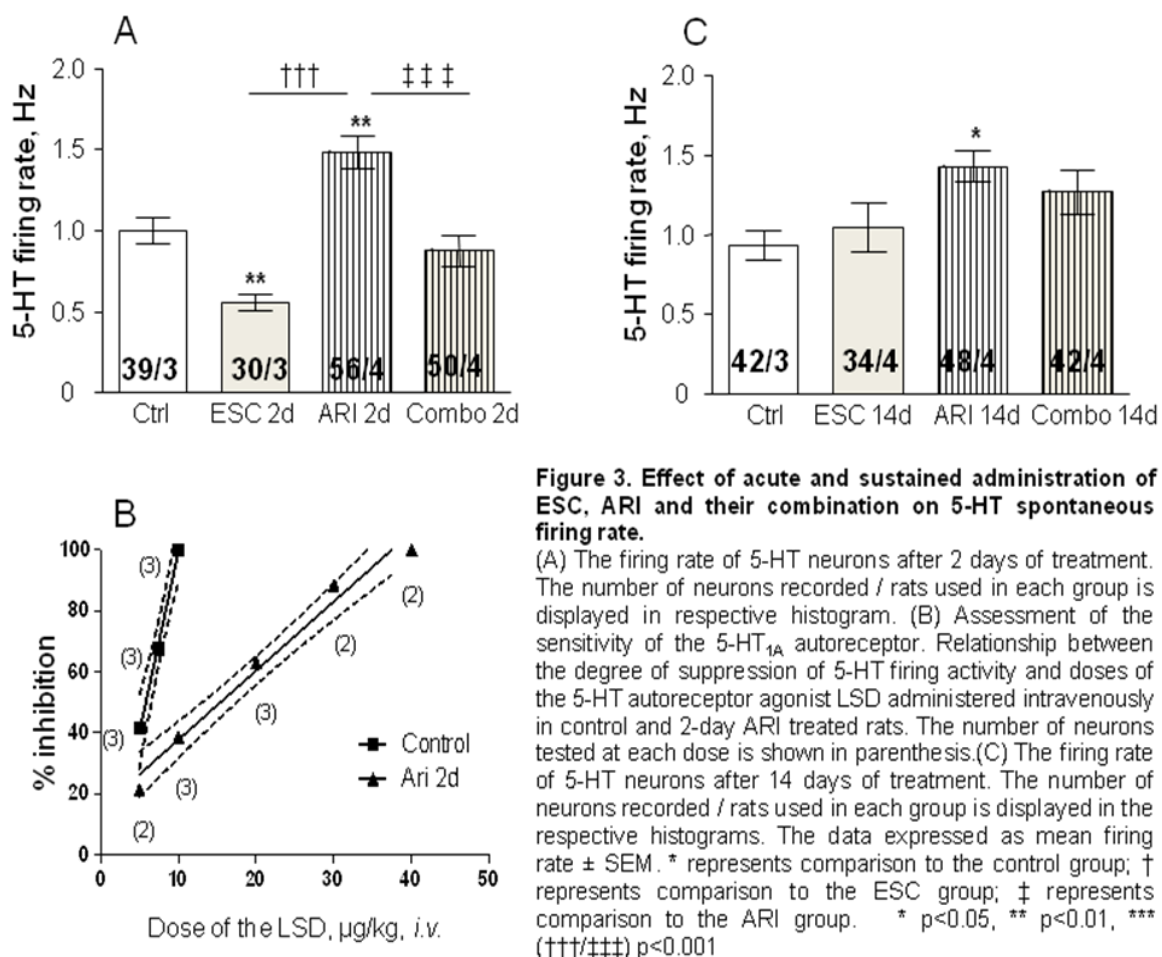
Aripiprazole was previously shown to decrease DA firing rate when administered acutely (Bortolozzi et al. 2007; Dahan et al. 2008). In an attempt to possibly account for the lack of effect of 2-day ARI administration on DA spontaneous firing, the effect of blockade of 5-HT_{1A} receptor, which is known to positively influence the DA firing, was tested (Prisco et al. 1994; Díaz-Mataix et al. 2005). The spontaneous firing of VTA DA neurons did not differ prior to and following the injection of 5-HT_{1A} selective antagonist WAY 100635 at a dose of 100 µg/kg (Firing rate: before 3.26 ± 0.41 Hz (n=23) and 2.98 ± 0.39 Hz (n=20) after *i.v.* administration of WAY 100635 100 µg/kg), thus indicating that the 5-HT_{1A} receptor agonism of ARI did not have a substantial role in the preservation of DA firing (data not shown).

Effects of 2- and 14-day administration of ESC, ARI and their combination on the firing rate of 5-HT neurons

Short-term ESC administration resulted in a 44% decrease in the spontaneous firing rate of 5-HT (control vs. ESC 2 days $p < 0.01$, $F = 20.67$). ARI administered for 2 days increased the spontaneous firing rate of 5-HT neurons by 48% (control vs. ARI 2 days $p < 0.01$, $F = 20.67$) (Fig. 3A). Aripiprazole combined with ESC reversed the inhibitory action of the SSRI, restoring the spontaneous

firing of 5-HT neurons to that of controls (Fig. 3A).

In accordance with previous studies, 5-HT neuronal firing returned to the control level after ESC was administered for 14 days (El Mansari et al. 2005). Chronic ARI administration yielded a significantly elevated firing, when compared



to controls (control vs. ARI 14 days $p < 0.05$, $F = 3.75$). Aripiprazole given in combination with ESC did not increase 5-HT firing above the control level (Fig. 3C).

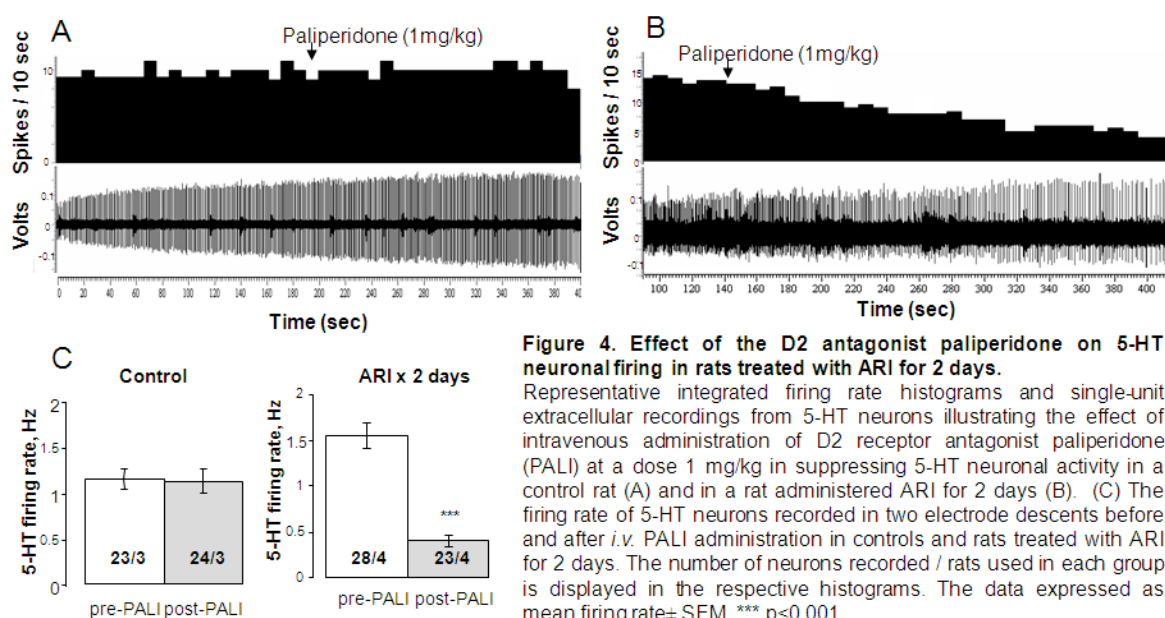
Effect of 2-day ARI administration on the function of the 5-HT_{1A} autoreceptor

The enhanced firing of 5-HT neurons, after 2 and 14 days of sustained ARI administration, stood in sharp contrast to its suppressant effect upon acute *i.v.* injection. In order to explain such a discrepancy, given the potent inhibitory role of the 5-HT_{1A} autoreceptor on 5-HT neuronal firing, the sensitivity of the 5-HT_{1A} autoreceptors was examined after a 2-day ARI regimen. The degree of 5-HT neuronal firing rate suppression produced by the 5-HT autoreceptor agonist LSD was determined. LSD is a more reliable tool for testing 5-HT_{1A} autoreceptor sensitivity and was chosen over other 5-HT_{1A} agonists because it does not act on postsynaptic cortical 5-HT_{1A} receptors and therefore does not activate a feedback loop interfering with the detection of alternations of 5-HT_{1A} autoreceptor sensitivity (Blier et al. 1987). In control rats, LSD completely suppressed the firing activity of 5-HT neurons with a dose of 10 µg/kg (ED_{50} 5.6±1.1 µg/kg), whereas in rats treated with ARI for 2 days, 40 µg/kg was required (ED_{50} 12.6±1.1 µg/kg) (Fig. 3B).

Assessment of D2 receptor activation on the enhancement of 5-HT firing in rats treated with ARI for 2 days

Serotonin_{1A} receptor desensitization achieved through sustained administration of 5-HT_{1A} agonists or SSRI results only in a recovery of firing, not an elevation above normal. Therefore, other mechanisms potentially enhancing firing rate of 5-HT neurons had to be present to allow the increase of 5-HT neuronal firing above the baseline. Activation of D2 receptors is known to exert a

stimulatory effect on 5-HT firing rate (Haj-Dahmane. 2001; Aman et al. 2007). Therefore, the D2 agonism produced by ARI might be a contributing factor to the increase of the spontaneous firing of 5-HT neurons. To address this possibility paliperidone (PALI), a drug with D2 antagonistic properties, was used. Despite its affinity for several other receptors, PALI was chosen over other D2 antagonists because it does not alter the spontaneous 5-HT firing (Dremencov et al. 2007b).



Firing rates of 5-HT neurons were recorded prior to and following *i.v.* administration of PALI in rats treated with ARI for 2 days (i.e. 7 to 10 neurons were recorded in rats treated with ARI for 2 days, then PALI was administered *i.v.* and another 7-10 neurons were recorded in the same rat). PALI administration resulted in a significant decrease in 5-HT firing, thus indicating that D2 receptor agonism contributed to the increase in 5-HT firing after 2 days of ARI administration (Fig. 4).

DISCUSSION

The results of the present study showed that sustained administration of ARI increased the firing rate of DR 5-HT neurons while leaving DA and NE spontaneous discharge unchanged. The inhibitory drive of ESC on monoaminergic firing rate was overridden by the addition of ARI: the spontaneous firing rate of DA and 5-HT neurons was reversed after two days and that of NE after 14 days of treatment.

Neither 14-, nor 2-day ARI administration produced any change of LC NE spontaneous firing. In line with previous studies, 2-day ESC administration resulted in a significant decrease of the NE firing (Dremencov et al. 2007a; Ghanbari et al. 2008). Increased extracellular 5-HT concentration, produced by the blockade of reuptake, leads to activation of the excitatory 5-HT_{2A} receptors expressed on the GABA cells innervating LC NE neurons (Szabo and Blier. 2001). By antagonizing these receptors, the negative influence of SSRIs on NE firing can be blocked (Szabo and Blier. 2002; Dremencov et al. 2007a). The partial recovery of NE firing observed in rats treated with combination of ESC and ARI for 2 days likely took place due to the antagonistic properties of ARI on 5-HT_{2A} receptors. Therefore, ARI shares this property with the atypical antipsychotics olanzapine, risperidone and paliperidone (Dawe et al. 2001; Dremencov et al. 2007a,b).

The current findings confirmed the notion that unlike 5-HT neurons, NE

neurons do not regain their normal firing after long-term SSRI administration. This effect was, however, reversed by addition of ARI to the 14-day ESC regimen, thus allowing NE neurons to maintain their firing rate at the control level.

Dopamine neuronal firing was shown to be moderately decreased by *i.v.* ARI administration (Bortolozzi et al. 2007; Dahan et al. 2008). However, following 2 days of ARI administration, there was no change in the firing rate of DA neurons. 5-HT_{1A} agonists were previously shown to stimulate the electrical activity of the VTA DA neurons and to enhance the DA release in mPFC (Prisco et al. 1994; Díaz-Mataix et al. 2005). Therefore, in an attempt to explain the lack of inhibition of DA firing activity in rats treated with ARI for 2 days, a possible contribution of such an excitatory influence of 5-HT_{1A} receptors was examined. Dopamine neuronal firing rate was recorded in rats subjected to 2-day ARI administration prior to and following the administration of the selective 5-HT_{1A} antagonist WAY 100635. It was concluded that 5-HT_{1A} receptor activation by subacute ARI administration was not responsible for the lack of DA inhibition, since there was no change in the DA firing after 5-HT_{1A} receptor blockade.

Despite the lack of effect of 2-day ARI administration on the DA neuronal electrical activity, its addition to the ESC treatment was sufficient to reverse the inhibitory action of the latter on DA spontaneous firing. Serotonin exerts a negative effect on DA neuronal firing since in rats with their 5-HT neurons lesioned, DA firing is significantly increased (Guiard et al. 2008). SSRIs were shown to decrease the DA spontaneous firing (Dremencov et al. 2009). This effect is believed to be

mediated by activation of the 5-HT_{2C} receptors located on the cell body of the VTA GABA interneurons (Dremencov et al. 2009). For instance, 5-HT_{2C} selective agonists were shown to decrease the VTA DA firing, while selective 5-HT_{2C} antagonist increased it and reversed the SSRI-induced inhibition of the DA firing (Millan et al. 1998; Gobert et al. 2000; Lucas et al. 2000). Based on these observations, the reversal of the inhibitory effect exerted by ESC on the firing of DA neurons can be explained by the 5-HT_{2C} receptor functional antagonism of ARI.

Prolonged administration of ARI did not produce any changes on DA firing activity, compared to the 2-day treatment, thus leaving it at the control level. Conflicting data exists as for the effect of chronically administered SSRIs on DA firing. Di Matteo et al. (2002) reported an initial decrease in the DA firing followed by full restoration after chronic administration of the SSRI. The authors speculated that the observed recovery of the firing rate could be attributed to the desensitization of 5-HT_{2C} receptors, although the R enantiomer of fluoxetine is a potent 5-HT_{2C} antagonist (Koch et al. 2002). In contrast, the inhibition of firing of DA neurons by ESC was identical after both 2 and 14 days of sustained administration (Dremencov et al. 2009). This effect was reversed by administration of selective 5-HT_{2C} antagonist SB 242084 (Dremencov et al. 2009). The present data replicated the findings of the latter study with ESC. This inhibitory effect of ESC was reversed by addition of ARI, probably due to its 5-HT_{2C} receptor functional antagonism.

Previous electrophysiological studies documented a complete inhibition of 5-HT firing in response to acute *i.v.* administration of ARI (Stark et al. 2007; Dahan et al. 2008). This effect was reversed using the selective 5-HT_{1A} antagonist WAY 100635, thus confirming that the inhibitory effect of ARI on 5-HT neurons is mediated via activation of 5-HT_{1A} autoreceptors. A decrease in 5-HT firing was thus expected in rats treated with ARI for 2 days. Unexpectedly, a significant increase of 5-HT spontaneous firing activity was observed. Since a desensitization of 5-HT_{1A} autoreceptors by bupropion and mirtazapine was previously shown to allow the firing rate of 5-HT neurons above baseline (Haddjeri et al. 1998; Ghanbari et al. 2008), it was hypothesized that its prompt desensitization took place with ARI administration. Indeed, the responsiveness of 5-HT_{1A} autoreceptors in rats treated with ARI for 2 days was attenuated. Due to the fact that selective 5-HT_{1A} agonists require a longer lag of time to allow a recovery of 5-HT neuronal firing, another factor had to be at play in the enhancement of firing taking place as early as after 2 days of ARI administration. Considering that activation of D2 receptors located on the cell body of 5-HT neurons within the DR was shown to stimulate 5-HT firing (Haj-Dahmane 2001; Martin-Ruiz et al. 2001; Aman et al. 2007; Chernoloz et al. 2009), it was assumed that ARI as a D2 agonist might produce such an effect. To test this possibility, the 5-HT firing rate was examined in rats treated with ARI for 2 days prior to and following the blockade of D2 receptors by PALI. Even though this drug is not a selective D2 antagonist, it does effectively block this receptor while leaving 5-HT firing unchanged, unlike other D2 antagonists (Dremencov et al. 2007b). As shown in Fig. 4, 5-HT firing was

markedly decreased after PALI administration in rats treated with ARI for 2 days, but not in control rats. Therefore, it was concluded that activation of D2 receptors by ARI exerted a significant effect in the observed increase of 5-HT firing. The resulting direct activation of 5-HT_{1A} autoreceptors and D2 heteroreceptors by ARI could potentially explain the early desensitization of the 5-HT_{1A} autoreceptor after only 2 days.

Even though 5-HT neuronal firing rate was normalized and significantly increased in rats chronically treated with ESC and ARI, respectively, combined administration of the two drugs for 14 days did not bring the 5-HT spontaneous firing above the control level. This result stands in contrast with the effect of ESC co-administered with bupropion. The latter drug, similarly to ARI, increases the 5-HT neuronal firing above the baseline and desensitizes the 5-HT_{1A} autoreceptor when administered alone for 2 and 14 days (Ghanbari et al. 2008). However, in contrast to the ARI+ESC combination, bupropion added to the chronic ESC regimen enhances the 5-HT firing to a greater extent than that achieved with bupropion alone (Ghanbari et al. 2008). Such a difference observed between two different SSRI augmentation strategies might be attributed to the ability of bupropion to promote NE release and thus activate 5-HT neuronal activity via stimulatory α_1 -adrenergic receptors (Millan et al. 1994). The exact role of α_1 -adrenergic and D2 heteroreceptors located on 5-HT neurons in enhancing firing activity is currently under further investigation.

The *in vivo* electrophysiological results of the current study provide a possible

rationale for the clinical efficacy of ARI augmentation in treatment-resistant MDD. As for other atypical antipsychotics, ARI reversed the inhibitory action of SSRI on the firing of NE neurons. In addition, the direct 5-HT_{1A} and D2 agonistic activity of ARI may contribute to enhancing overall 5-HT and DA transmission because the firing rates of 5-HT and DA neurons would not be diminished by the combination of a SSRI and ARI.

ACNOWLEDGEMENTS

This research was supported by *Bristol-Myers Squibb*. PB received a Canada Research Chair in Psychopharmacology and an Endowed Chair from the University of Ottawa, Institute of Mental Health Research. We thank Lundbeck and Janssen for supplying escitalopram and paliperidone, respectively.

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2.4. Paper IV

Quetiapine is another member of the atypical antipsychotic family. Aside from the blockade of 5-HT₂ and D2 receptors, the pharmacological profile varies greatly between different agents within this class. Thus the generalization about the mechanism of action of atypicals can not be made, and each agent needs to be studied separately. Like aripiprazole, quetiapine is one of the three atypical antipsychotic drugs approved for use in MDD either alone (Canada & EU), or as antidepressant augmenting agent (USA). Considering the above, the effects of use of quetiapine administered both alone and in combination with SSRI were important to assess. The following study was aimed at characterization of the effects produced by mono- and combination use of quetiapine on the spontaneous firing rate of NE and 5-HT neurons and the overall neurotransmission within the above systems, and at determination of the neuronal elements conveying these changes. The assessment of effects of quetiapine on DA neurotransmission was omitted, since the potential alterations produced at the presynaptic level are likely functionally insignificant, as the D2 receptors are systemically blocked by the drug itself only at doses higher than those used to treat depression than psychosis.

Quetiapine is actively degraded in the human body, resulting in a formation of over 20 metabolites. One of the principal metabolites – norquetiapine is structurally similar to the tricyclic antidepressants and shares some pharmacological properties of these drugs. For instance, norquetiapine not only largely follows the pharmacological profile of quetiapine, but it is also a potent inhibitor of NET, like

many TCAs, whereas the parent compound is totally devoted of this property. As norquetiapine is believed to be partially responsible for the antidepressant properties of quetiapine, modeling of the kinetic balance between these two compounds was of great importance for proper understanding of its mode of action. Unlike humans, in rats quetiapine is not metabolized to norquetiapine. The norquetiapine was thus added to the quetiapine, at the concentration mimicking that seen in humans. Therefore in the manuscript term 'quetiapine' pertains to the quetiapine+norquetiapine mixture, unless specified otherwise.

The experimental design was drafted by Dr. Pierre Blier, Dr. Mostafa El Mansari and myself and approved by Astra Zeneca, supporting the study. The experiments were carried out and analyzed by me. All authors assisted in drafting the article, and approved the final manuscript. The manuscript was submitted to the *Neuropsychopharmacology*.

Effects of sustained administration of quetiapine alone and in combination with a serotonin reuptake inhibitor on norepinephrine and serotonin transmission.

***Running title:** Quetiapine: norepinephrine and serotonin neurotransmission*

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Journal: Neuropsychopharmacology

Abstract : 250

Introduction : 784

Methods : 1564

Figures : 9

References : 69

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Abstract

Quetiapine is now used in the treatment of unipolar and bipolar disorders, both alone and in combination with other medications. In the current study, the sustained administration of quetiapine and N-desalkylquetiapine (NQuet) in rats in a 3:1 mixture (hQuet) was used to mimic quetiapine exposure in patients because rats do not produce the latter important metabolite of quetiapine. Sustained administration of hQuet for 2 and 14 days significantly enhanced the firing rate of norepinephrine (NE) neurons by blocking the cell body α_2 -adrenergic autoreceptors on NE neurons, whether it was given alone or with a serotonin (5-HT) reuptake inhibitor. The 14-day regimen of hQuet enhanced the tonic activation of postsynaptic α_2 - but not α_1 -adrenergic receptors in the hippocampus. This increase in NE transmission was attributable to increased firing of NE neurons, the inhibition of NE reuptake by NQuet, and the attenuated function of terminal α_2 -adrenergic receptors on NE terminals. Sustained administration of hQuet for 2 and 14 days significantly inhibited the firing rate of 5-HT, whether it was given alone or with a 5-HT reuptake inhibitor, because of the blockade of excitatory α_1 -adrenergic receptors on 5-HT neurons. Nevertheless, the 14-day regimen of hQuet enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus. This increase in 5-HT transmission was attributable to the attenuated inhibitory function of the α_2 -adrenergic receptors on 5-HT terminals, and possibly to direct 5-HT_{1A} receptor agonism by NQuet. The enhancement of NE and 5-HT transmission by hQuet may contribute to its antidepressant action in mood disorders.

Key words: quetiapine, norquetiapine, SSRI, depression, serotonin, norepinephrine,

Introduction

Major depressive disorder (MDD) is the most predominant illness among mental, neurological, and substance-use disorders (Collins et al., 2011). Indeed, the World Health Organization (WHO) determined that more than 120 million people worldwide are affected. Presently MDD is ranked as the leading cause of disability globally in middle to high-income countries (WHO, 2008, Ayuso-Mateos, 2000). Despite significant progress in development of antidepressant treatments, the response and remission rates in depressed patients remain suboptimal (Shelton et al., 2010). Novel therapeutic approaches yielding better clinical outcomes are eagerly awaited. Lately combination strategies in treatment of MDD, and especially its treatment-resistant form, find more and more empiric support (Papakostas, 2009; Stahl, 2010). The effectiveness of augmentation of antidepressants with low doses of atypical antipsychotics (AAPs) is now well documented (Shelton et al., 2010; Nelson and Papakostas, 2009; DeBattista and Hawkins, 2009). Moreover, extensive clinical studies resulted in an official approval of some of these drugs for use in MDD.

The group of AAPs comprises agents with a wide variety of pharmacological profiles, with the antagonism at serotonin (5-HT)_{2A} and dopamine D₂ receptors serving as a common denominator. Since the first generation antipsychotics, acting primarily at the D₂ receptors, do not possess antidepressant properties, the blockade of the latter receptors therefore does not appear to be the mechanism explaining the antidepressant action of AAPs. Indeed, the doses of AAPs used in

depression treatment are much lower than those prescribed in psychotic states and generally provide clinically insignificant occupancy of D2 receptors. It is thus likely that the 5-HT₂ receptors may be the main determinants of the beneficial clinical action of the AAPs in depression treatment (Celada et al., 2004; Szabo and Blier, 2002; Blier and Szabo, 2005). As selective serotonin reuptake inhibitors (SSRIs) attenuate NE neuronal activity via activation of 5-HT_{2A} receptors, their blockade by AAPs reverses this effect (Dremencov et al., 2007a; Seager et al., 2005). This mechanism potentially contributes to the additive efficacy of such augmentation treatment. While the efficacy of AAPs as SSRI augmenting agents may largely be explained by the reversal of tonic inhibition of catecholamines by 5-HT, their action at other receptors may also contribute to their clinical benefits. The monoaminergic properties vary from one AAP to another. This is due to their differential affinity for various receptors that regulate the activity of monoamine neurotransmitters. For example, risperidone, paliperidone, quetiapine and clozapine effectively block α_2 -adrenoceptors (Schotte et al., 1996). Ziprasidone blocks 5-HT_{1D} receptors that normally inhibit 5-HT release, aripiprazole acts as a partial agonist at D2 receptors, while both latter agents are potent 5-HT_{1A} receptors (Ballas et al., 2004; Stark et al., 2007).

AAPs, just like other effective antidepressant strategies, were shown to positively influence the expression of brain derived neurotrophic factor (BDNF) as well as neuroplasticity (Molteni et al., 2009). For instance, long-term quetiapine administration in rats was shown to reverse the stress-induced suppression of

hippocampal neurogenesis and to increase the levels of BDNF in hippocampus and cortex of both stressed and control groups (Luo et al., 2005; Bai et al. 2003). Clinically, AAP augmentation resulted in an increase of plasma BDNF levels in patients with MDD that responded to treatment (Yoshimura et al., 2010).

To date, the effectiveness of extended-release quetiapine in unipolar and bipolar depression has been assessed in twelve controlled, randomized, double blind clinical studies totaling 4485 patients (McElroy et al., 2010). It was shown to be effective in the treatment of treatment-resistant MDD when used alone, combined with antidepressants or cognitive behavior therapy (McIntyre et al., 2007; El-Khalili et al., 2010; Bauer et al., 2009; Bortnick et al., 2011; Chaput et al., 2008; Cutler et al., 2009; Katila et al., 2008; Weisler et al., 2009). Not only the remission rate was increased, but also the relapse was found to be less likely when patients were maintained on quetiapine when compared to placebo (Liebowitz et al., 2010). This data set resulted in approval of the drug for use in MDD as an augmenting agent in the USA and EU, and as a second-line monotherapy in Canada.

Despite the established efficacy of quetiapine in the treatment of MDD, its mechanism of action is not entirely understood. Though the extended release quetiapine formulation is approved for monotherapy use in depression, in many cases it is used in combination with SSRIs. Thus the current study was aimed at investigating the effects of short- and long-term use of quetiapine alone, and in combination with the SSRI escitalopram (ESC) on neurotransmission in the 5-HT

and norepinephrine (NE) system, which are known to play an important role in pathophysiology and treatment of MDD.

It is important to mention that in humans quetiapine is extensively metabolized leading to over 20 metabolites (Goldstein and Arvanitis, 1995; Lindsay DeVane, 2001). N-desalkylquetiapine (NQuet) is one of the main active metabolites. It largely shares the pharmacological profile of quetiapine but has additional pharmacological targets, potentially important in the treatment of MDD (Jensen et al., 2008). Having significant structural similarity with tricyclic antidepressants, NQuet has one of their prominent properties, a moderate affinity to the NE transporter (NET) (Jensen et al., 2008). Unlike humans, rodents do not metabolize quetiapine to NQuet. In order to mimic the therapeutic conditions, NQuet was thus added to quetiapine in a ratio present in humans. The mixture used for experiments was thus termed hQuetiapine (for human quetiapine; hQuet).

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Charles River, St. Constant, QC) weighing 270 to 320 g at the time of recording, were used for the experiments. They were kept under standard laboratory conditions (12:12 hour light/dark cycle with free access to food and water). All animal handling and procedures were approved by our local Animal Care Committee (University of Ottawa, Institute of Mental Health Research,

Ottawa, ON, Canada).

Treatments

Quetiapine and ESC were delivered via subcutaneously implanted osmotic minipumps at a daily dose of 10 mg/kg and NQuet at a dose of 3.3 mg/kg. These drugs were administered for 2 or 14 days alone and in combination. Control rats received physiological saline through an osmotic minipump as well.

***In vivo* electrophysiological recordings**

Rats were anesthetized with chloral hydrate (400 mg/kg; *i.p.*) and placed in a stereotaxic frame. To maintain a full anesthetic state, chloral hydrate supplements of 100 mg/kg, *i.p.*, were given as needed. Extracellular recordings of the 5-HT and NE neurons in the RD and the LC, respectively, were obtained using single-barreled glass micropipettes. Their tips were of 1-3 μm in diameter and impedance ranged between 4-7 M Ω . All glass micropipettes were filled with a 2 M NaCl solution. Prior to electrophysiological experiments, a catheter was inserted in a lateral tail vein for systemic *i.v.* injection of appropriate pharmacological agents when applicable.

Recording of the LC NE neurons

Micropipettes were positioned in mm from lambda at: AP, - 1.0 to - 1.2; L, 1.0 to 1.3; V, 5 to 7. Spontaneously active NE neurons were identified using the following criteria: regular firing rate (0.5–5.0 Hz) and positive action potentials of

long duration (0.8–1.2 ms) exhibiting a brisk excitation followed by period of silence in response to a nociceptive pinch of the contralateral hind paw (Aghajanian and Vandermaelen, 1982a). Dose-response curves were obtained using only the initial response to the first dose injected to a single neuron of each rat.

Recording of the RD 5-HT neurons

Single-barreled glass micropipettes were positioned in mm from lambda at: AP, +1.0 to 1.2; L, 0 ± 0.1 ; V, 5 to 7. The presumed 5-HT neurons were then identified using the following criteria: a slow (0.5 - 2.5 Hz) and regular firing rate and long-duration (2 - 5 ms) bi- or triphasic extracellular waveform (Aghajanian and Vandermaelen, 1982b).

Dose response curves

Dose-response curves assessing the effect of 2-day administration of hQuetiapine on the responsiveness of 5-HT_{2A} receptors and α_2 -adrenergic autoreceptors were constructed for systemic *i.v.* injections of the 5-HT_{2A} agonist DOI and the α_2 -adrenergic agonist clonidine. Dose-response curves were plotted using GraphPad software.

Extracellular recordings and microiontophoresis of pyramidal neurons in CA3 dorsal hippocampus

Extracellular recordings and microiontophoresis of CA3 pyramidal neurons were carried out with five-barreled glass micropipettes. The central barrel used for

the unitary recording was filled with a 2 M NaCl solution, the four side barrels were filled with the following solutions: 5-HT creatinine sulfate (10 mM in 200 mM NaCl, pH 4), ($\pm$)-NE bitartrate (10 mM in 200 mM NaCl, pH 4), quisqualic acid (1.5 mM in 200 mM NaCl, pH 8), and the last barrel was filled with a 2 M NaCl solution used for automatic current balancing. The micropipettes were descended into the dorsal CA3 region of the hippocampus using the following coordinates: 4 mm anterior and 4.2 mm lateral to lambda (Paxinos and Watson, 1986). Pyramidal neurons were found at a depth of 4.0 ± 0.5 mm below the surface of the brain. Since the pyramidal neurons do not discharge spontaneously in chloral hydrate anesthetized rats, a small current of quisqualate +1 to -6 nanoampere (nA) was used to activate them to fire at their physiological rate (10 to 15 Hz; Ranck, 1975). Pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple action potentials, alternating with complex spike discharges (Kandel and Spencer, 1961). The duration of microiontophoretic application of the agonists, 5-HT and NE, was 50 seconds. The 50-second duration of microiontophoretic application of the pharmacological agents and the ejection currents (nA) were kept constant before and after each i.v. injection throughout the experiments. Neuronal responsiveness to the microiontophoretic application of 5-HT and NE, prior to and following i.v. injections, was assessed by determining the number of spikes suppressed per nA.

Assessment of the tonic activation of postsynaptic α_2 - and α_1 -adrenoceptors

The degree of tonic activation of postsynaptic α -adrenergic receptors was assessed following 14-day hQuet administration. The assessment of the tonic activation of postsynaptic receptors is more accurate when the firing rate of the recorded neuron is low. Therefore, the firing rate of pyramidal neurons was reduced by lowering the ejection current of quisqualate. The degree of tonic activation of postsynaptic α_2 - and α_1 -adrenoceptors was assessed using the selective antagonists idazoxan and prazosin, respectively. Upon obtaining a low steady firing baseline, idazoxan (1 mg/kg) and prazosin (100 μ g/kg) were systemically administered to assess the changes in the firing activity in rats administered saline or hQuet for 14 days. In order to avoid drug residual effects, only one neuron in each rat was tested.

Assessment of the tonic activation of postsynaptic 5-HT_{1A} receptors

The degree of tonic activation of postsynaptic 5-HT_{1A} receptors was assessed following 14-day hQuet administration. The assessment of the tonic activation of postsynaptic 5-HT_{1A} receptor is more accurate when the firing rate of the recorded neuron is low. Therefore, the firing rate of pyramidal neurons was reduced by lowering the ejection current of quisqualate. After stable firing baseline is obtained, the selective 5-HT_{1A} antagonist WAY 100,635 (100 μ g/kg) was administered systemically in 4 incremental doses of 25 μ g/kg each, at time intervals of 2 minutes. Neuronal response at each dose-point was obtained for construction of the dose-response curve. Such curves represent stable changes in the firing rate of pyramidal neurons as percentages of baseline firing following each

systemic drug administration. In order to avoid drug residual effects, only one neuron in each rat was tested.

Assessment of NE reuptake *in vivo*

To evaluate the effectiveness of hQuet on the blockade of NE transporter reuptake, the recovery of the firing activity of pyramidal neurons following the microiontophoretic application of NE was assessed using the recovery time 50 (RT₅₀) value. Norepinephrine exerts an inhibitory action upon firing of pyramidal neurons. The time necessary for their firing to recover (RT₅₀) is entirely dependent on the activity of the NE transporter (De Montigny et al., 1980; Piñeyro et al., 1994). The RT₅₀ value was obtained by calculating the time in seconds required for the neuron to recover 50% of its initial firing rate at the end of the microiontophoretic application of NE onto CA₃ pyramidal neurons (De Montigny et al., 1980).

Stimulation of the ascending 5-HT pathway

The ascending 5-HT pathway was electrically stimulated using a bipolar electrode (NE-100, David Kopf, Tujunga, CA, USA). The electrode was implanted 1 mm anterior to lambda on the midline with a 10° backward angle in the ventromedial tegmentum and 8.0 ± 0.2 mm below the surface of the brain. Two hundred square pulses of 0.5 ms in duration were delivered by a stimulator (S48, Grass Instruments, West Warwick, RI, USA) at an intensity of 300 µA, and a frequency of 1 Hz. The effects of 1 Hz stimulations of the ascending 5-HT fibers

were assessed prior to and following *i.v.* injections of the α_2 -adrenoceptor agonist clonidine (10 and 400 $\mu\text{g/kg}$), while recording from the same neuron. The low and high doses of clonidine were used to assess the responsiveness of the α_2 -adrenergic auto- and heteroreceptors, respectively. Previous studies showed that clonidine is 10-fold more potent at α_2 -adrenergic autoreceptors than the α_2 -adrenergic heteroreceptors on 5-HT terminals (Frankhuyzen and Mulder, 1982; Maura et al., 1985). The low dose of clonidine (10 $\mu\text{g/kg}$) potentiates the effect of stimulation of 5-HT pathway by stimulating the α_2 -adrenergic autoreceptors that are present on NE terminals, leading to inhibition of NE firing and disinhibition of 5-HT terminals (Lacroix et al., 1991). Indeed, the effect of the low, but not the high, dose of clonidine was abolished when the NE neurons were lesioned (Mongeau et al., 1993). On the other hand, the high dose of clonidine (400 $\mu\text{g/kg}$) inhibits the effect of 5-HT stimulation by acting on α_2 -adrenergic heteroreceptors, located on the 5-HT terminals, leading to inhibition of 5-HT release. Therefore, 1 Hz stimulations of 5-HT bundle result in a greater 5-HT release and increased SIL value after the *i.v.* injection of the low clonidine dose, and a smaller 5-HT release resulting in a shorter inhibition of pyramidal firing (smaller SIL) following a high dose of clonidine.

The stimulation pulses and the firing activity were analyzed by computer using Spike 2 (Cambridge Electronic Design Limited, UK). Peristimulus time histograms of hippocampal pyramidal neurons were generated to determine the suppression of firing measured in absolute silence (SIL) value in msec. The SIL represents the duration of a total suppression of the hippocampal neuron.

Statistical analysis

All results are expressed as means $\pm$ S.E.M. Statistical comparisons between differences in spontaneous firing of DR 5-HT and LC NE neurons in rats treated with saline, ESC, hQuet and ESC + hQuetiapine combination were carried out by one-way analysis of variance and multiple comparison procedures using Fisher's PLSD *post hoc* test. Data were obtained from 3 to 5 rats per experimental group. Statistical significance was taken as $p < 0.05$.

Drugs

Quetiapine and NQuet were provided by Astra Zeneca; ESC was provided by Lundbeck (Copenhagen, DK); WAY 100635, clonidine hydrochloride, idazoxan hydrochloride, DOI, 5-HT creatinine sulfate, ($\pm$)-NE bitartrate, quisqualic acid, MDL100907, and desipramine were purchased from Sigma (St. Louis, USA); WAY 100635 and ESC were dissolved in distilled water.

Results

Assessment of the effects of 2- and 14-day administration of ESC, hQuet and their combination on the mean firing rate of NE neurons

In line with previous data (El Mansari et al., 2005), both short and long-term ESC administration led to significant decreases in NE spontaneous firing when compared to controls (2 days: -47%, $p < 0.001$; 14 days: -35%, $p < 0.01$; Fig 1 A, B).

Administration of hQuet led to a significant increase in the NE neuronal firing after

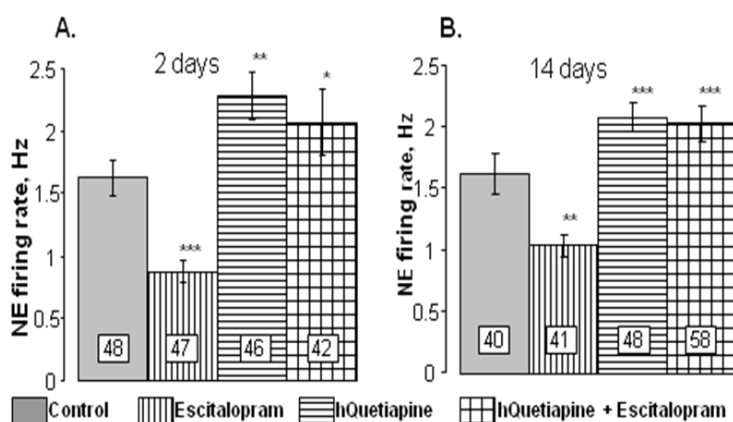


Figure 1. Effect of acute and sustained administration of hQuet, ESC, and their combination on NE spontaneous firing rate
Firing rate of LC NE neurons after 2- (A) and 14-days (B) of drugs administration. The number of neurons recorded in each group is displayed in respective histograms. The data are expressed as mean firing rates $\pm$ standard error of the means (SEM). * p<0.05, ** p<0.01, *** p<0.001.

both 2 and 14 days (2 days: -

40%, p<0.01 ; 14 days: -

28%, p<0.001; Fig 1 A, B).

When the two drugs were

co-administered for either 2

or 14 days, NE neuronal

firing was not only fully

restored compared to that of

ESC-treated rats, but

increased significantly

compared to the control level (2 days: +27%, p<0.05; 14 days: +25%, p<0.001; Fig

1 A, B).

Assessment of the effects of 14-day administration of hQuet of the tonic activation of postsynaptic α_2 - and α_1 -adrenoceptors on the dorsal hippocampus CA3 pyramidal neurons

Pyramidal neurons in the CA3 layer of the dorsal hippocampus experience constant (tonic) activation by NE released from terminals. The effect of NE on pyramidal neurons is inhibitory and mediated by α_1 - and α_2 -adrenoceptors. Systemic application of the selective α_2 - and α_1 -adrenoceptor antagonists idazoxan and prazosin, respectively, did not modify the firing activity of pyramidal neurons in control rats (Fig. 2 A). However, in rats administered hQuet for 14 days

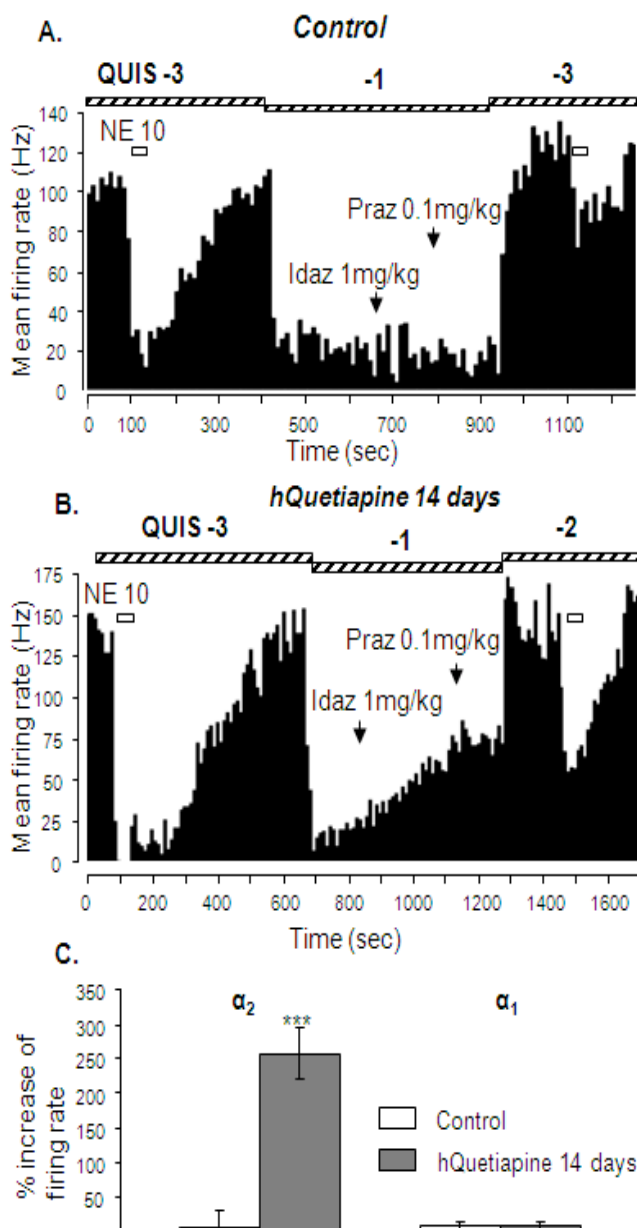


Figure 2. Assessment of tonic activation of α -adrenoceptors in dorsal hippocampus.

Integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons illustrating the effects of systemic administration of idazoxan (1000 μ g/kg), prazosin (100 μ g/kg) in control (A) and 14-day hQuet (B) treated rats. The arrows represent the consecutive injections of idazoxan and prazosin. The overall changes of the firing activity of pyramidal neurons after systemic injections of idazoxan and prazosin in controls and rats that received hQuet for 14 days (C). The numbers in the columns correspond to the number of recorded neurons. The number above each bar corresponds to the ejection current of NE in nA applied for 50 seconds. *** $p < 0.001$

consecutive *i.v.* injections of idazoxan significantly enhanced the firing activity of CA3 pyramidal neurons by $260 \pm 38\%$, $p < 0.001$ (Fig. 2 B). The blockade of α_1 -adrenergic receptors with prazosin did not alter the firing of pyramidal neurons in rats receiving hQuet for 14 days.

Assessment of NE reuptake potential of NQuet

NQuet, an active metabolite of quetiapine produced in humans but not in rats, appears to be a moderate blocker of NET ($K_i = 58$ nM; Jensen et al., 2008). To assess the potential of NQuet to inhibit the reuptake of NE *in vivo*, the effect of direct microiontophoretic application

of NE onto pyramidal neurons of the hippocampus was studied in anesthetized rats. It was found that NQuet administered *i.v.* at a dose of 0.5-1 mg/kg significantly increased the RT₅₀ value, compared to control rats ($p < 0.01$; Figs 2 and 3). Furthermore, when rats were given hQuet (given as a 3:1 mixture of quetiapine and NQuet) for 14 days, RT₅₀ values were increased more than twofold ($p < 0.001$; Fig. 3). These observations indicate that hQuet exerts significant NE reuptake blockade *in vivo*.

Assessment of the effects of hQuet on locus coeruleus NE neurons: role of 5-HT_{2A} receptors

The 5-HT system can inhibit NE neuronal activity *via* the activation of 5-HT_{2A} receptors (Szabo and Blier, 2001). hQuet is known to have affinity for these receptors (Jensen et al., 2008). As expected, the dose of the selective 5-HT_{2A} receptor agonist DOI required for the complete inhibition of NE neuronal firing rate was significantly higher in rats administered hQuet for 2 days, compared to controls (DOI ED₅₀: control = 20 ± 8 $\mu\text{g/kg}$ versus hQuet = 55 ± 16 $\mu\text{g/kg}$; Fig. 4 A, B). The blockade of 5-HT_{2A} receptors by hQuet, documented by the present experiments, would thus prevent a potential 5-HT-mediated attenuation of the NE neuronal activity.

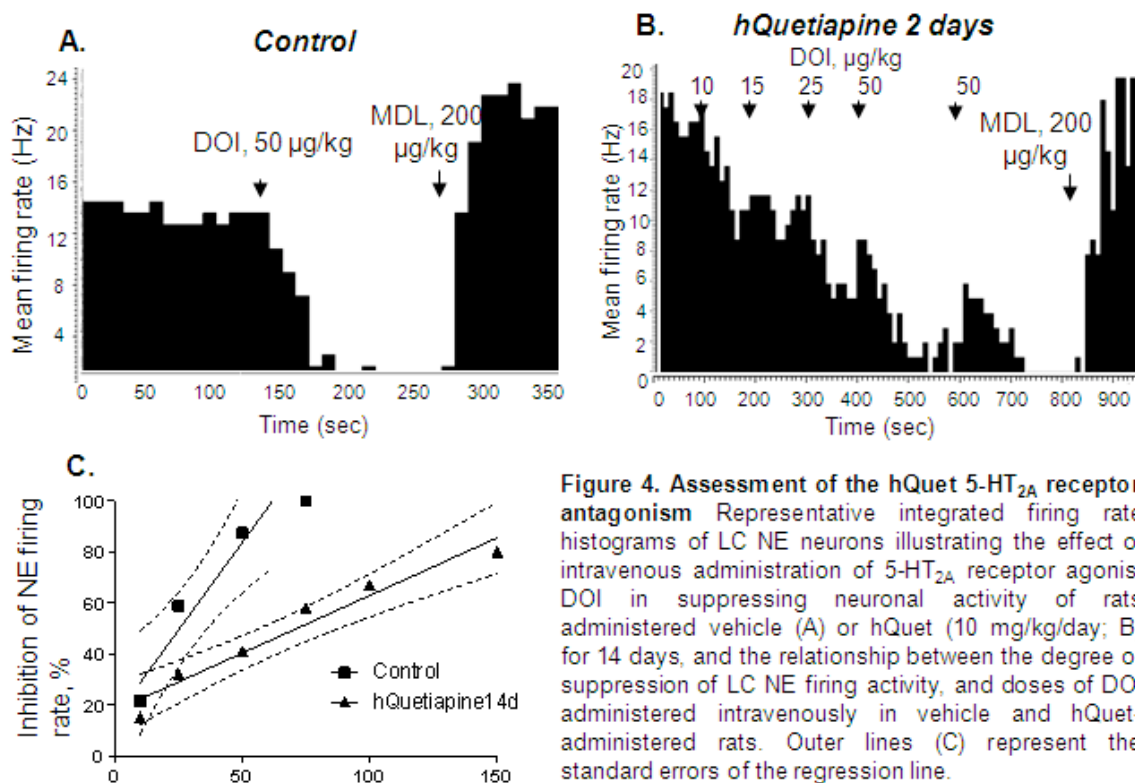


Figure 4. Assessment of the hQuet 5-HT_{2A} receptor antagonism Representative integrated firing rate histograms of LC NE neurons illustrating the effect of intravenous administration of 5-HT_{2A} receptor agonist DOI in suppressing neuronal activity of rats administered vehicle (A) or hQuet (10 mg/kg/day; B) for 14 days, and the relationship between the degree of suppression of LC NE firing activity, and doses of DOI administered intravenously in vehicle and hQuet-administered rats. Outer lines (C) represent the standard errors of the regression line.

Assessment of the effects of hQuet on locus coeruleus NE neurons: role of α_2 -adrenoceptors

Adrenergic α_2 -autoreceptors regulate the firing rate and the release capacity of NE neurons in a negative feedback manner. Thus in control rats activation of these receptors by systemic administration of the selective α_2 -adrenergic agonist clonidine led to the complete cessation of the spontaneous discharge ($ED_{50} = 2.1 \pm 0.5$ µg/kg; Fig. 5A). In rats exposed to hQuet for 2 days, the dose of clonidine required for the complete inhibition of neuronal charging was significantly greater ($ED_{50} = 5.4 \pm 1$ µg/kg; Fig. 5B). In line with its documented pharmacological properties (Jensen et al., 2008), this increase indicates that hQuet effectively

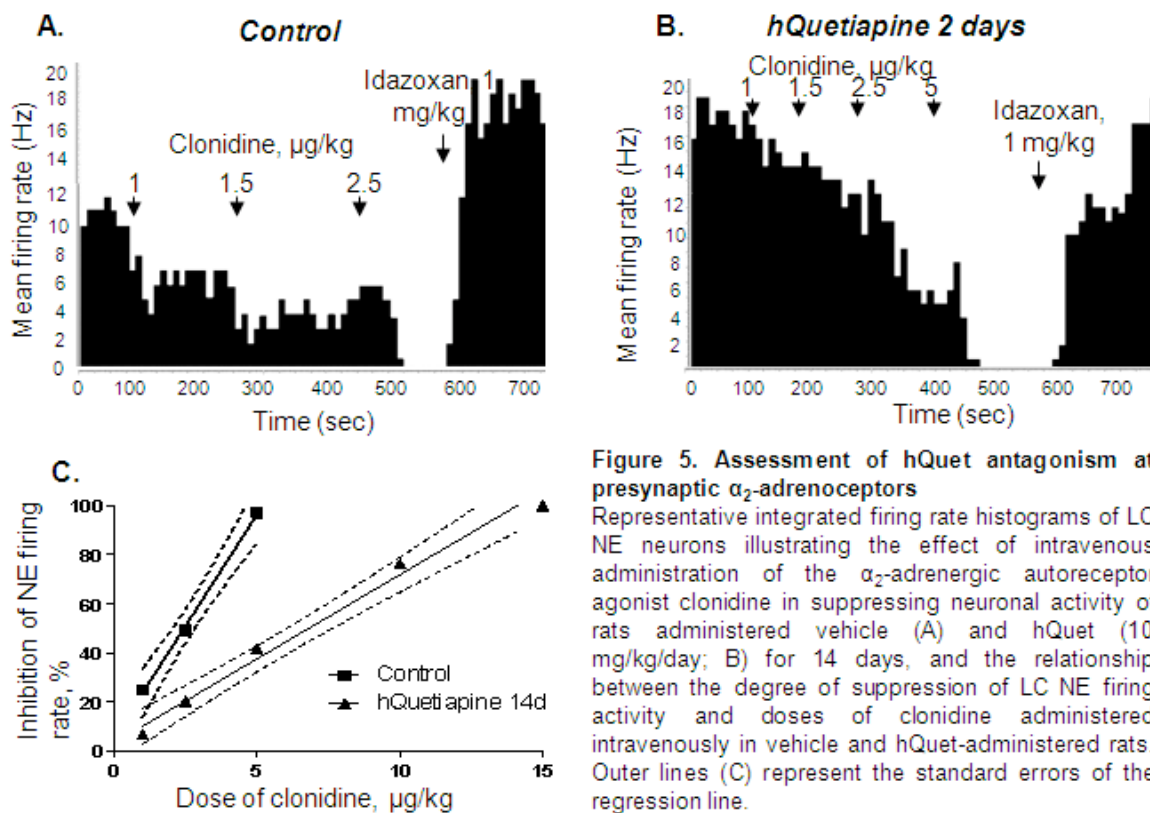


Figure 5. Assessment of hQuet antagonism at presynaptic α_2 -adrenoceptors
Representative integrated firing rate histograms of LC NE neurons illustrating the effect of intravenous administration of the α_2 -adrenergic autoreceptor agonist clonidine in suppressing neuronal activity of rats administered vehicle (A) and hQuet (10 mg/kg/day; B) for 14 days, and the relationship between the degree of suppression of LC NE firing activity and doses of clonidine administered intravenously in vehicle and hQuet-administered rats. Outer lines (C) represent the standard errors of the regression line.

blocks somatodendritic α_2 -adrenergic autoreceptors. This property is likely responsible for the increase in the discharge rate of NE neurons, following both 2 and 14 days of hQuet administration.

Effects of 14-day hQuet administration on the responsiveness of terminal α_2 -adrenoceptors

The ascending 5-HT pathway was stimulated to determine whether 14-day administration of hQuet had the ability to antagonize terminal α_2 -adrenoceptors

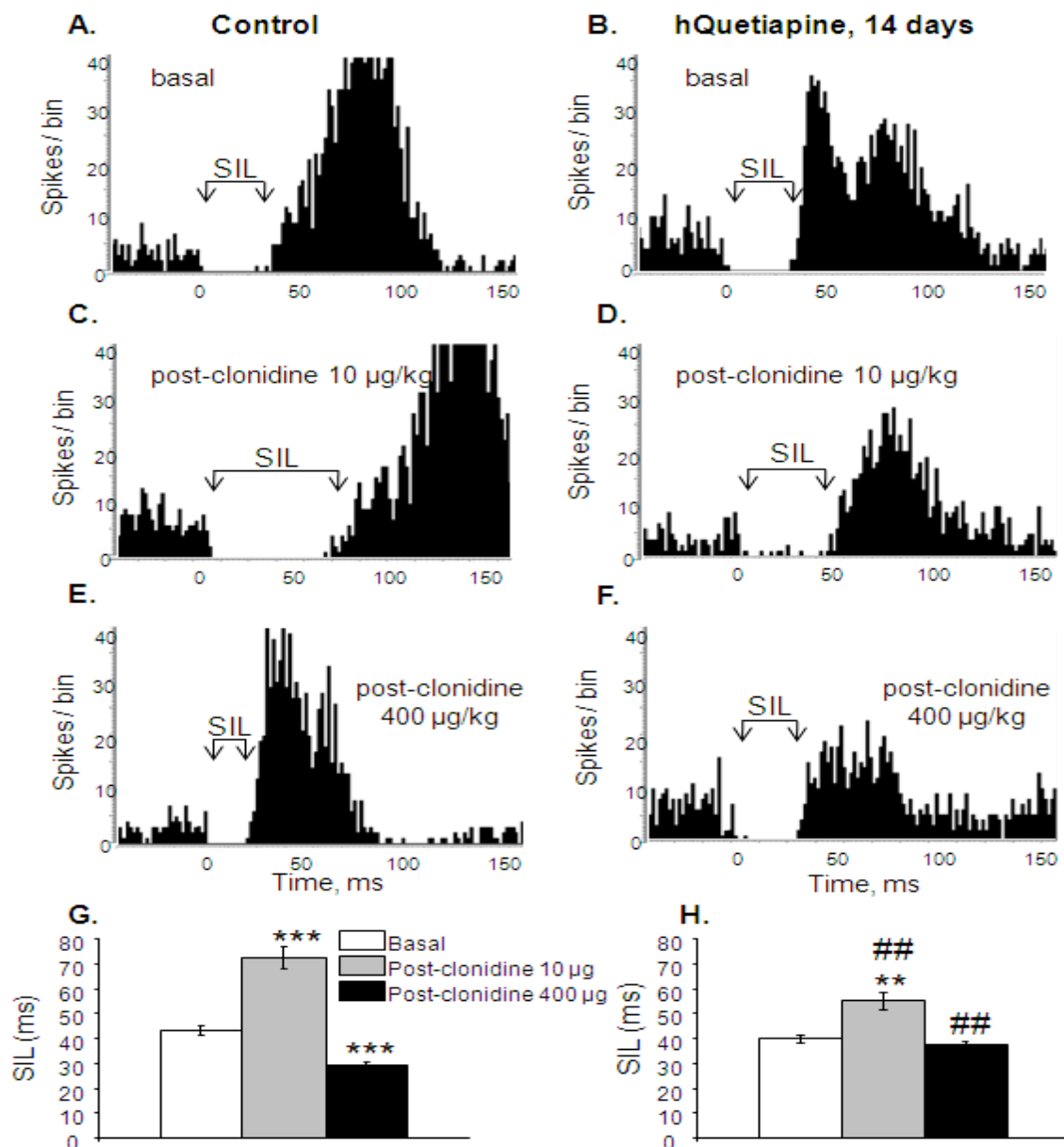


Figure 6. Assessment of hQuet antagonism at postsynaptic α_2 -adrenoceptors

Peristimulus time histograms illustrating effects of stimulation of the ascending 5-HT pathway on the firing activity of CA3 pyramidal neurons in control (A) and 14-day hQuet exposed rats (B). The effect of 5-HT pathway stimulation prior to and following the systemic administration of clonidine at doses of 10 and 400 $\mu\text{g/kg}$ (control: C and D, hQuet: E and F, respectively). The overall effect of clonidine administration in controls (G) and hQuet treated rats (H). The numbers in the columns correspond to the number of recorded neurons. ** indicates $p < 0.01$, *** indicates $p < 0.001$ comparing the SIL value of the respective group to the basal level. ## Indicates $p < 0.01$, comparing the SIL value following clonidine in hQuet group to the respective values in controls. SIL: absolute silence

and thus modulate the endogenous release of 5-HT and NE in the synaptic cleft. Systemic administration of the low dose of the α_2 adrenoceptor agonist clonidine (10 μ g/kg) significantly enhanced the suppression of the firing rate of hippocampus pyramidal neurons in the control rats, whereas high dose of clonidine (400 μ g/kg) reversed this effect bringing the SIL below the pre-injection value (control, pre-clonidine: 43 ± 2 ms; post-clonidine 10: 73 ± 5 ms, $p < 0.001$; post-clonidine 400: 29 ± 1 ms; $p < 0.001$; Fig 6 A, C, E). The low dose of clonidine still significantly increased the suppression of CA3 pyramidal neurons in rats administered hQuet for 14 days (hQuet 14 days: pre-clonidine 40 ± 2 ms; post-clonidine 10: 55 ± 3 ms, $p < 0.01$; Fig. 6 B, C), although to a lesser extent than in the control rats ($p < 0.01$, compared to post-clonidine 10 in controls), thus suggesting a diminished function of α_2 -adrenergic autoreceptors on NE terminals. Following the 14-day administration of hQuet, the high dose of clonidine reversed the SIL-prolonging action of 10 μ g/kg clonidine injection. The magnitude of the effect was blunted, and the post-clonidine 400 value in rats receiving hQuet for 14 days was significantly higher than that in controls (hQuet 14 days: post-clonidine 400: 38 ± 2 ms, control: post-clonidine 400: 29 ± 1 ms; Fig 6 E, F), indicating diminished functioning of α_2 -adrenergic receptors on 5-HT terminals.

Assessment of the effects of 2- and 14-day administration of ESC, hQuet and their combination on the firing rate of 5-HT neurons

Short-term ESC administration resulted in a 65% decrease in the spontaneous firing rate of 5-HT neurons ($p < 0.001$). hQuet administered for 2 days

decreased the spontaneous firing rate of 5-HT neurons by 43% ($p<0.001$; Fig. 2A). hQuet combined with ESC for 2 days led to the same decrease of the

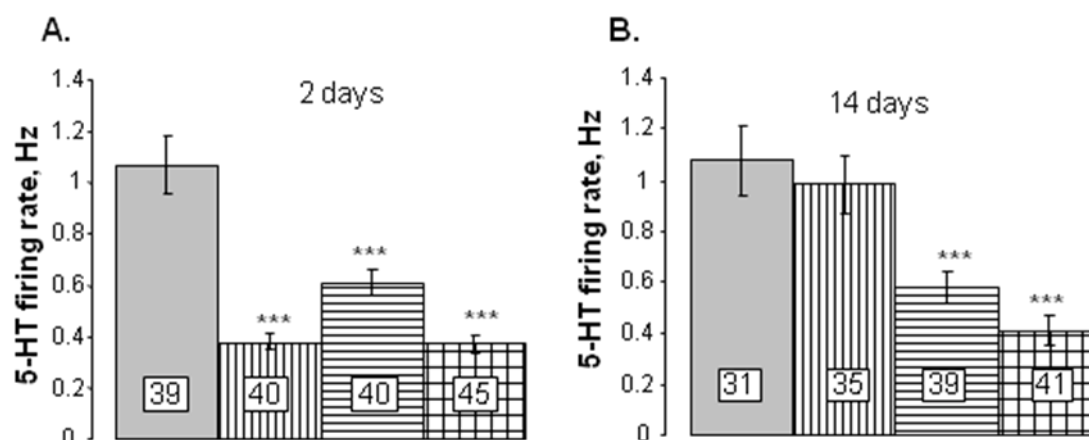


Figure 7. Effect of acute and sustained administration of hQuet, ESC, and their combination on 5-HT spontaneous firing rate

The firing rate of RD 5-HT neurons after 2- (A) and 14-days (B) of drugs administration. The number of neurons recorded in each group is displayed in each histograms. The data expressed as mean firing rate $\pm$ standard error of the mean (SEM). *** $p<0.001$.

spontaneous firing of 5-HT neurons as that of rats treated with ESC alone (65% decrease, $p<0.001$; Fig. 7 A).

As previously reported, 5-HT neuronal firing returned to the control level after ESC was administered for 14 days (El Mansari et al., 2005) (Fig. 7 B). Sustained hQuet administration yielded a significantly dampened firing, when compared to controls (46% decrease, $p<0.001$). hQuet given in combination with ESC also led to significant inhibition of spontaneous firing activity of 5-HT neurons (62% decrease, $p<0.001$; Fig. 7 B).

Assessment of the effect of 14-day administration of ESC, hQuet and their combination on the tonic activation of postsynaptic 5-HT_{1A} receptors on the

dorsal hippocampus CA3 pyramidal neurons

Pyramidal neurons in CA3 layer of the dorsal hippocampus receive its serotonin innervation from the dorsal and median raphe nuclei. The effect of 5-HT on pyramidal neurons is inhibitory and mainly mediated by 5-HT_{1A} receptors. All antidepressant medications thus far tested, as well as electro-convulsive shocks and stimulation of the vagus nerve (undertaken to achieve antidepressant action) produce an increase in tonic activation of pyramidal neurons (Manta et al., 2009; Haddjeri et al., 1998) (indicated by the disinhibition of firing rate in response to the blockade of 5-HT_{1A} receptors by highly potent and selective antagonist WAY 100635). Importantly, no disinhibition occurs in control rats (Fig. 8A).

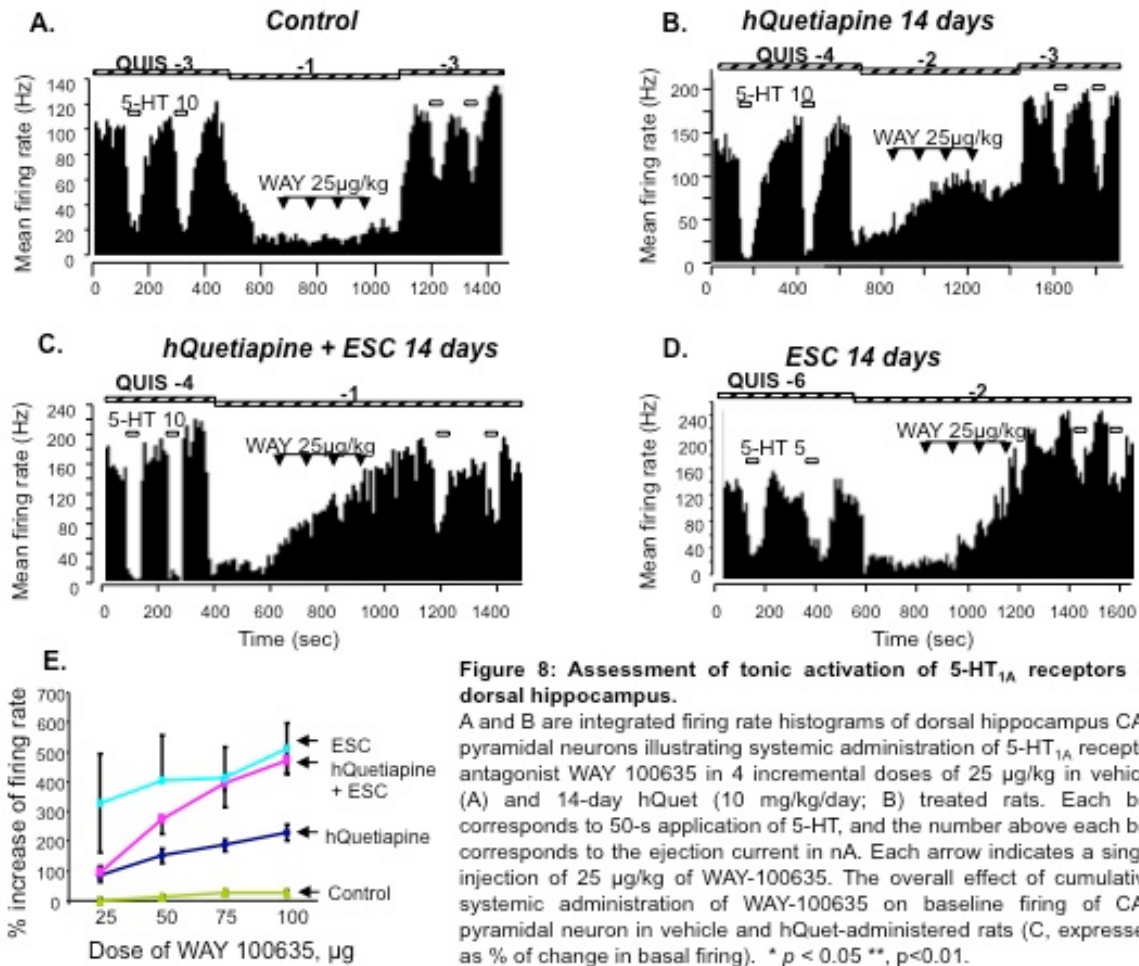


Figure 8: Assessment of tonic activation of 5-HT_{1A} receptors in dorsal hippocampus.

A and B are integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons illustrating systemic administration of 5-HT_{1A} receptor antagonist WAY 100635 in 4 incremental doses of 25 µg/kg in vehicle (A) and 14-day hQuet (10 mg/kg/day; B) treated rats. Each bar corresponds to 50-s application of 5-HT, and the number above each bar corresponds to the ejection current in nA. Each arrow indicates a single injection of 25 µg/kg of WAY-100635. The overall effect of cumulative systemic administration of WAY-100635 on baseline firing of CA3 pyramidal neuron in vehicle and hQuet-administered rats (C, expressed as % of change in basal firing). * $p < 0.05$ **, $p < 0.01$.

It was found that chronic administration of hQuet produced a significant increase in tonic activation of postsynaptic 5-HT_{1A} receptors located on the dorsal hippocampus CA₃ pyramidal neurons ($230 \pm 28\%$; Fig. 8 B). Escitalopram administered on its own for 14 days also produced a marked increase ($511 \pm 87\%$; Fig. 8 D). When hQuet was co-administered with ESC, the increase in tonic activation was in the same range as that obtained with ESC alone ($471 \pm 46\%$; Fig. 8 C).

Assessment of the effects of hQuet on dorsal raphe nucleus 5-HT neurons:

role of α_1 -adrenoceptors

Stimulation of α_1 -adrenoceptors located on the cell bodies of 5-HT neurons leads to the decrease of their spontaneous firing rate. hQuet has moderate affinity for α_1 -adrenoceptors (K_i for quetiapine = 22 nM and for NQuet = 144 nM) (Jensen

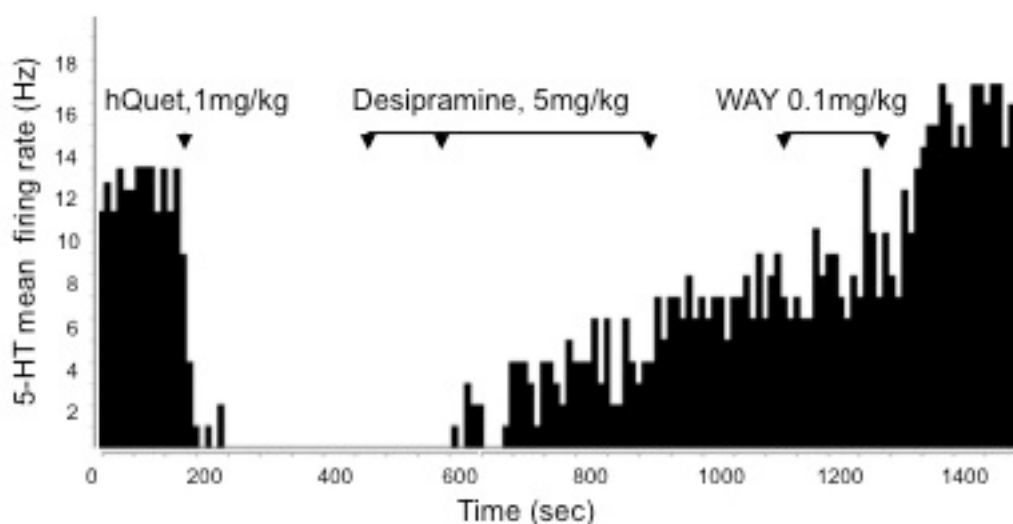


Figure 9: Effect of acute hQuet administration on 5-HT neuronal firing

Representative single-unit extracellular recording from the 5-HT neuron during the consecutive acute injections of hQuet, followed by the NET inhibitor desipramine and the selective 5-HT_{1A} receptor agonist WAY 100635. hQuet was given as a 3:1 mixture of quetiapine and NQuet.

et al., 2008). It was found that acute *i.v.* injection of hQuet completely inhibited the firing of 5-HT neurons ($ED_{50}=0.5 \pm 0.2$ mg/kg; Fig. 9). This inhibition could be partially reversed by the administration of the potent NE reuptake blocker desipramine by displacing hQuet from α_1 -adrenoceptors through an additional enhancement of endogenous NE. As NQuet also has moderate affinity for 5-HT_{1A} receptors (K_i = 45 nM), the desipramine injection was expectedly followed by administration of potent and the selective 5-HT_{1A} receptor antagonist WAY100635 which led to the complete restoration of 5-HT neuronal firing. It is worth mentioning

that the blockade of 5-HT_{1A} receptors by WAY100635 without desipramine administration could not reverse the inhibitory effect of hQuet at all, emphasizing the principal role of α_1 -adrenoceptors. These results provide a possible explanation for the decrease of the 5-HT neuronal firing observed with both the 2- and 14-day regimens of hQuet.

Discussion

The present study put into evidence that hQuet, administered for both 2 and 14 days, increased the NE neuronal discharge rate and overall NE neurotransmission. hQuet was found to block cell body and terminal α_2 -adrenergic receptors, but not the α_2 -adrenergic receptors located postsynaptically. In contrast, both pre- and postsynaptic α_1 receptors were blocked by the hQuet. The documented antagonism of 5-HT_{2A} receptors by hQuet was demonstrated *in vivo*. NQuet was shown to possess the significant NET blocking property, both when acutely administered on its own and when given on a long-term basis as a part of hQuet. The inhibitory influence of SSRI ESC on NE spontaneous neuronal discharge was reversed by hQuet, both after 2 and 14 days of concomitant drug administration. The firing rate of 5-HT neurons, however, was significantly decreased in rats receiving hQuet alone or in combination with ESC after both 2- and 14 days. Despite this dampening of firing, the overall 5-HT neuronal transmission was enhanced following long-term hQuet administration.

hQuet was found to produce very profound noradrenergic effects: both the spontaneous firing and the overall NE neuronal transmission were increased by sustained administration of hQuet. This effect is likely due to action of hQuet at several NE neuronal elements. First, antagonism of α_2 -adrenergic cell-body autoreceptors that exert a negative feedback control over NE neuronal firing, is known to increase the NE neuronal discharge. Both the optimal blockade of this receptor by the selective antagonist idazoxan, and its sustained antagonism by mirtazapine- an effective antidepressant with prominent α_2 -adrenergic blocking properties, were previously documented to elevate the NE neuronal firing rate above the control level (Dremencov et al., 2007a; Freedman and Aghajanian 1984; Haddjeri et al., 1998). The α_2 antagonistic potential of hQuet was assessed after 2 days of administration. The observed right shift of the α_2 agonist clonidine dose-response curve clearly confirms that hQuet effectively blocks this receptor. The potency of hQuet was nevertheless lower than that of idazoxan since the latter was still able to reverse the suppression action of clonidine (Fig. 5). This is consistent with the greater affinity of idazoxan for α_2 -adrenergic receptors than NQuet (11nM vs. 240 nM, respectively; Hudson et al., 1992; Jensen et al., 2008).

SSRIs administered for short-term or chronically are known to inhibit the spontaneous firing of NE neurons (Dremencov et al., 2007a; Szabo and Blier, 2001a). This phenomenon was reproduced in our study (Fig. 1). The above effect takes place due to the SSRI-induced increased endogenous stimulation of excitatory 5-HT_{2A} receptors, located on the GABA neurons that inhibit the firing

rate of the NE neurons (Aston-Jones et al., 1991; Szabo and Blier, 2001c). The observed drop in the discharge rate of NE neurons is likely counterproductive in treatment of MDD, and may underlie the fatigue and asthenia observed in some patients chronically treated with SSRIs (Montgomery et al., 1993). Our results demonstrate that the addition of hQuet (exhibiting 5-HT_{2A} receptor antagonism confirmed by the right shift of the 5-HT_{2A} agonist DOI dose-response curve in rats subjected to 2-day hQuet administration) to the ESC regimen not only reversed the inhibitory influence of an SSRI upon NE neuronal firing, but even increased it above the baseline level. This observation is in line with previous electrophysiological data, as well as notion that concomitant administration of SSRI with 5-HT_{2A}-receptor blockers produces a significant increase in levels of the extracellular NE in rat frontal cortex (Szabo and Blier, 2002; Seager et al., 2005; Hatanaka et al., 2000). It is noteworthy that the addition of 5-HT_{2A} receptor antagonist to the SSRI has been shown to result in an increased antidepressant effect in numerous animal and clinical studies (Nemeroff, 2005; Tohen et al., 2003; Papakostas, 2005\). The potency of hQuet was nevertheless lower than that of MDL100,907 which completely prevents the inhibitory effect of DOI on NE neuronal firing (Szabo and Blier, 2001b)

The present study also put into evidence the NET-inhibiting properties of NQuet. This compound, when administered both acutely alone and over the long term as a part of hQuet preparation, was found to prolong the recovery time of pyramidal neuronal firing, following the topical application of NE to the neuronal

cell body. The recovery time is a validated and reliable method of *in vivo* characterization of the reuptake blocking potential. NQuet thus contributes to the NE-activating profile of the parent compound by preventing recycling and thus increasing the levels of synaptically available NE. Interestingly, the tricyclic antidepressant desipramine, structurally related to NQuet, provides similar degree of NE inhibition in rats, when administered acutely (Lacroix et al., 1991; Curet et al., 1992). Considering that in humans long-term administration of desipramine leads to the effective 85% blockade of the NET (Gilmor et al., 2002), it can be speculated that NQuet, forming as a result of Qeut metabolism, also blocks NE reuptake to a clinically-significant degree. The NET-inhibiting potential of NQuet remains, however, to be determined in humans.

When terminal α_2 auto- and heteroreceptors that control the release of NE and 5-HT, respectively, are overstimulated by the reuptake-produced increased synaptic levels of NE, they gradually desensitize (Szabo and Blier, 2001a). This decrease in sensitivity of terminal inhibitory α_2 receptors leads to the increased release in NE and 5-HT. A similar functional change is produced by the α_2 -adrenergic antagonist mirtazapine: though the decreased function of terminal α_2 -adrenergic receptors stems from their blockade, and not the desensitization as in the case with NET inhibitors. The outcome is therefore identical – both NE and 5-HT release are enhanced (Haddjeri et al., 1998).

The overall increase in the NE neuronal transmission can be attributed to the increased firing of NE neurons, the inhibition of NE reuptake by NQuet, and the

attenuated function of terminal α_2 -adrenergic receptors on NE terminals. This was put into evidence by the observed enhancement in tonic activation of the postsynaptic adrenoceptors. The degree of activation of postsynaptic α_2 - adrenoceptors was enhanced in rats receiving hQuet on a long-term basis. No such increase could be detected at postsynaptic α_1 -adrenergic receptors because their effective blockade by the hQuet. The variability of the α_2 -antagonistic potential of hQuet between different receptor sites (i.e. ability to block auto- and terminal receptors, but not the α_2 -adrenoceptors located on the cell body of pyramidal neurons in hippocampus) is not unusual. Similar changes were previously documented with the α_2 -adrenoceptor antagonist mirtazapine (Haddjeri et al., 1998; Mongeau et al., 1994).

hQuet administered *i.v.* at a dose of 1 mg/kg abolished the discharge of 5-HT neurons. Indeed, all AAPs, but paliperidone, decrease the spontaneous firing rate of 5-HT neurons, when administered acutely (Dremencov et al., 2007b; Gartside et al., 1997; Hertel et al., 1997; Sprouse et al., 1999; Stark et al., 2007). Two actions on the cell body of DR 5-HT neurons can mediate this decrease: the blockade of α_1 -adrenoceptors and the activation of 5-HT_{1A} autoreceptors. The inhibition of 5-HT neuronal discharge induced by aripiprazole and ziprasidone can be reversed by the blockade of 5-HT_{1A} autoreceptor with the selective antagonist WAY100635 (Stark et al., 2007; Sprouse et al., 1999). On the other hand, the suppression of firing of 5-HT neurons produced by clozapine and olanzapine is overturned by the increase of endogenous NE, obtained with the administration of

NE reuptake blocker desipramine (Gartside et al., 1997). The latter process is mediated by activation of α_1 adrenoceptors, as desipramine reverses the decrease in 5-HT neuronal discharge produced by α_1 -adrenoceptor antagonist prazosin (Gartside et al., 1997). In turn, quetiapine and NQuet have significant affinities for both 5-HT_{1A} and α_1 -adrenergic receptors (Jensen et al. , 2008; Schotte et al., 1996), and thus likely suppress the 5-HT firing by acting on both receptors. This was confirmed by the observation that the quetiapine-induced suppression of 5-HT spontaneous firing could be completely reversed only when both these receptors were blocked (Fig. 9). The same phenomenon was true for risperidone (Dremencov et al., 2007a,b). The decrease in the firing rate of 5-HT neurons observed in rats treated with hQuet for both 2 and 14 days, likely took place due to the same inhibitory mechanisms. The firing rate of 5-HT neurons decreased with short-term ESC administration, returns to control levels when the SSRI is given chronically (El Mansari et al., 2005). When hQuet and ESC are co-administered, however, this recovery did not take place (Fig. 7). The latter is likely explained by the sustained blockade of the α_1 -adrenoreceptors.

Despite the observed decrease in 5-HT spontaneous firing in both the hQuet and hQuet + ESC groups, the overall 5-HT neurotransmission was found to be enhanced, as indicated by the increase in tonic activation of postsynaptic 5-HT_{1A} receptors located on the CA3 hippocampal pyramidal neurons. The 5-HT neuronal tone thus appears to increase independently of the firing rate of 5-HT neurons in DR. The same is likely the case for other AAPs: long-term

administration of risperidone, for instance, dampens the spontaneous activity of 5-HT neurons (Dremencov et al., 2007b), however the concentration of 5-HT increases in both DR and prefrontal cortex (Hertel et al., 1999) . The observed increase in 5-HT neuronal tone in rats administered hQuet on a long-term basis, likely stems from the direct activation of 5-HT_{1A} receptors (Quetiapine Ki=717 nM, NQuet Ki= 45 nM) combined with the augmented 5-HT release capacity, stemming from the blockade of release-inhibiting terminal α_2 heteroreceptors. Interestingly, even though the firing rate of 5-HT neurons was the same in rats receiving hQuet alone and those administered hQuet in combination with ESC (Fig. 7), the degree of tonic activation of postsynaptic 5-HT_{1A} receptors was significantly higher in the latter group (Fig. 8). This finding advocates for the additive benefit of combined administration of hQuet and SSRIs.

Conclusion: Both short- and long-term administration of hQuetiapine enhanced the firing rate of NE neurons. Addition of hQuetiapine to the SSRI regimen reversed the inhibitory action of the latter upon NE spontaneous firing (which is likely contributing to the limited benefit of SSRIs in some patients, as well as to some of their side-effects). The overall NE neuronal transmission was enhanced by long-term hQuetiapine administration. Despite the inhibited spontaneous firing of 5-HT neurons after 2 and 14 days of treatment with both hQuetiapine and its combination with ESC, the overall 5-HT neurotransmission likely increased, as indicated by the enhancement of tonic activation of

hippocampal 5-HT_{1A} receptors. The effectiveness of hQuetiapine and its combination with SSRIs in depression treatment can possibly be explained by its positive effect on NE and 5-HT neuronal tone.

Disclosure:

The study was sponsored by Astra Zeneca.

P.B. received support for investigator initiated grants, and/or honoraria for advisory boards and/or speaking engagements from Astra-Zeneca, Biovail, Bristol Myers Squibbs, Eli Lilly & Company, Janssen Pharmaceuticals, Labopharm, Lundbeck, Pfizer, Schering-Plough/Merck, Servier, Takeda and Wyeth.

O.C. and M.E.M have no conflicting interests.

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General discussion

Though in depression DA, NE and 5-HT systems are known to be malfunctioning in one way or another, considering the heterogeneity of clinical presentations of MDD, the specific balance of functional state of the above systems likely varies from patient to patient. This point is confirmed by the depletion studies: patients responding to serotonergic treatments relapse after the synthesis of 5-HT, but not catecholamines is disrupted (Shopsin, Friedman et al. 1976, Smith, Fairburn et al. 1997); whereas those achieving a remission on medications targeting the NE system, display depressive symptoms after NE (but not 5-HT) precursor is depleted (Brodie, Murphy et al. 1971). Moreover, though few at the moment, the techniques allowing to determine the state of the given monoamine system may shed the light at the possible pathophysiological mechanisms predisposing individuals to depression and predicting the response to one class of the drugs or another. For instance, individuals carrying the s allele in the promoter region of 5-HTT gene are known to respond to SSRIs in suboptimal way (Caspi, Sugden et al. 2003). Thus, profound deficiency in function of one of the monoaminergic systems may not be sufficiently compensated by the drug selectively targeting this site, however the ability to augment the neuronal transmission in indirect manner via other monoamines may lead to the desired clinical outcome. Considering the above, the precise understanding of reciprocal influence of monoamines upon function of each other has utmost therapeutic significance.

The present work provided new data documenting in vivo reciprocal interactions between NE, DA and 5-HT systems. A major part of the studies was dedicated to the description of DA effects of drugs under investigation as well as to previously overlooked dopaminergic modulation of NE and 5-HT signaling.

For instance, studies I and II showed that long-term administration of the D2-like agonist pramipexole increased the overall DA neurotransmission. This enhancement of DA tone was not attributable to alterations of the release of DA or to an enhanced responsiveness of postsynaptic D2 receptors. It is therefore concluded that it resulted from a summation of the normalized DA firing, presumably restoring DA release, and the presence of PPX in the synapse.

The maintenance of proper mesocortical DA levels known play an important role in different aspects of attention and learning, as well as behavioral and physiological mechanisms of the stress response (Berridge, Espana et al. 2003, Deutch, Roth 1990). These functions are often perturbed in depression and may be related to the decrease in the levels of DA. The decrease in function of the frontal lobe is one of the most constant findings in the depressive state (Mayberg, Liotti et al. 1999, Drevets 1999, Drevets, Price et al. 2008). The normalization of fronto-cortical metabolism is consistently seen in patients who achieve the remission following pharmacological antidepressant treatment (Kennedy, Evans et al. 2001, Mayberg, Brannan et al. 2000, Stefurak, Mahurin et al. 2001). The PPX-induced increase in the DA function, known to be dampened in depression, potentially leads to the normalization of the modulatory DA tone in PFC and a

consequent restoration of the functions controlled by this brain region.

Moreover, studies I and II, for instance unveiled an important finding that despite the lack of affinity towards any component of 5-HT system, selective DA agonist pramipexole produced a significant increase in the 5-HT neurotransmission in an indirect manner. This elevation of the 5-HT neuronal tone likely stemmed from the pramipexole-induced amplification in the firing rate of DRN 5-HT neurons that occurred after the same 14-day, but not 2-day, pramipexole regimen. Enhancement of the tonic activation of postsynaptic 5-HT_{1A} receptors resulting from the increase in the firing rate of 5-HT neurons is not unique to PPX. The catecholamine releasing agent bupropion and prolonged vagus nerve stimulation produce an analogous change (Manta, Dong et al. 2009a, El Mansari, Ghanbari et al. 2008a). Such a phenomenon is in line with previous in vivo and in vitro studies documenting the enhancement of the 5-HT tone in response to the stimulation of DR D2-like receptors by pro-dopaminergic agents (Ferre, Artigas 1993a, Haj-Dahmane 2001a, Aman, Shen et al. 2007b). The above studies have thus put into evidence that chronic treatment with the D2 agonist pramipexole increased DA neurotransmission in rat PFC and 5-HT neurotransmission in hippocampus. Considering the documented normalization of the brain function within the same regions in depressed patients treated with this drug (Mah, Zarate et al. 2010), it is likely that the observed changes in the function of the abovementioned modulatory monoaminergic systems may underlie to some degree the clinical effectiveness of PPX in treatment of depression.

Similarly to pramipexole, the increase in the spontaneous discharge of DR 5-HT neurons produced by subchronic administration of the atypical antipsychotic aripiprazole was found to be due to activation of the D2-like receptors and prompt desensitization of 5-HT_{1A} autoreceptors (study III). The increase in the firing rate of 5-HT neurons above the baseline level in rats receiving aripiprazole hints to the overall increase in the 5-HT neurotransmission.

While agonism at D2-like receptors by aripiprazole played a role in the effect of the drug upon 5-HT neuronal tone, paradoxically it failed to alter the electrical activity of DA neurons. Despite the lack of effect aripiprazole administration on the DA neuronal electrical activity, its addition to the SSRI treatment was sufficient to reverse the inhibitory action of the latter on DA spontaneous firing. Serotonin exerts a negative effect on DA neuronal firing since in rats with their 5-HT neurons lesioned, DA firing is significantly increased (Guiard, El Mansari et al. 2008b). SSRIs were shown to decrease the DA spontaneous firing (Dremencov, El Mansari et al. 2009a). This effect is believed to be mediated by activation of the 5-HT_{2C} receptors located on the cell body of the VTA GABA interneurons (Dremencov, El Mansari et al. 2009a). For instance, 5-HT_{2C} selective agonists were shown to decrease the VTA DA firing, while selective 5-HT_{2C} antagonist increased it and reversed the SSRI-induced inhibition of the DA firing (Gobert, Rivet et al. 2000, Lucas, De Deurwaerdère et al. 2000, Millan, Dekeyne et al. 1998). Based on these observations, the reversal of the inhibitory effect exerted by SSRI escitalopram on the firing of DA neurons can be explained by the 5-HT_{2C} receptor functional

antagonism of aripiprazole.

SSRIs administered for short-term or chronically are known to inhibit the spontaneous firing of not only DA, but also NE neurons (Dremencov, El Mansari et al. 2007c, Szabo, Blier 2001c). The latter effect takes place due to the SSRI-induced increased endogenous stimulation of excitatory 5-HT_{2A} receptors, located on the GABA neurons that inhibit the firing rate of the NE neurons (Aston-Jones, Akaoka et al. 1991, Szabo, Blier 2001d). The observed drop in the discharge rate of NE neurons is likely counterproductive in treatment of MDD, and may underlie the fatigue and asthenia observed in some patients chronically treated with SSRIs (Montgomery, Rasmussen et al. 1993). By antagonizing these receptors, the negative influence of SSRIs on NE firing can be blocked (Szabo, Blier 2002, Dremencov, El Mansari et al. 2007b). It is noteworthy that the addition of 5-HT_{2A} receptor antagonist to the SSRI has been shown to result in an increased antidepressant effect in numerous animal and clinical studies (Nemeroff 2005, Tohen, Vieta et al. 2003, Papakostas 2005).

Addition of aripiprazole to the escitalopram regimen reversed the negative effect of the latter upon NE neuronal discharge (study III) and likely took place due to the antagonistic properties of aripiprazole at the 5-HT_{2A} receptors. Of note, the administration of aripiprazole on its own had no effect over the firing activity of NE neurons (study III).

Similarly to aripiprazole, another atypical antipsychotic studied within the present work – quetiapine, also blocks 5-HT_{2A} receptors, thus reversing the

inhibitory SSRI input (study IV). However, quetiapine not only reversed the impeding influence of an SSRI upon NE neuronal firing, but even increased it above the baseline level. This amplification is likely taking place because quetiapine was found to produce very profound noradrenergic effects: both the spontaneous firing and the overall NE neuronal transmission were increased by its sustained administration (study IV). This effect is likely due to action of quetiapine at several NE neuronal elements (among other properties, it antagonizes α_2 -adrenergic receptors and blocks the reuptake of NE). Antagonism of α_2 -adrenergic cell-body autoreceptors that exert a negative feedback control over NE neuronal firing, is known to increase the NE neuronal discharge. Both the optimal blockade of this receptor by the selective antagonist idazoxan, and its sustained antagonism by mirtazapine- an effective antidepressant with prominent α_2 -adrenergic blocking properties, were previously documented to elevate the NE neuronal firing rate above the control level (Dremencov, El Mansari et al. 2007c, Freedman, Aghajanian 1984, Haddjeri, Blier et al. 1998c).

When terminal α_2 -adrenergic auto- and heteroreceptors that control the release of NE and 5-HT, respectively, are overstimulated by the reuptake-produced increased synaptic levels of NE, they gradually desensitize (Szabo, Blier 2001b)a). This decrease in sensitivity of terminal inhibitory α_2 -adrenergic receptors leads to the increased release in NE and 5-HT. A similar functional change is produced by the α_2 -adrenergic antagonist mirtazapine: though the decreased function of terminal α_2 -adrenergic receptors stems from their blockade, and not the

desensitization as in the case with NET inhibitors. The outcome is therefore identical – both NE and 5-HT release is enhanced (Haddjeri, Blier et al. 1998c).

The overall increase in the NE neuronal transmission by quetiapine can be attributed to the increased firing of NE neurons, the inhibition of NE reuptake, and the attenuated function of terminal α_2 -adrenergic receptors on NE terminals.

Unlike pramipexole and aripiprazole, the administration of quetiapine decreased the spontaneous firing of 5-HT neurons (study IV). Despite the observed decrease in 5-HT discharge rate, the overall 5-HT neurotransmission was found to be enhanced. Thus in case of quetiapine the 5-HT neuronal tone appears to increase independently of the firing rate of 5-HT neurons in DR. The same is likely the case for some other AAPs: long-term administration of risperidone, for instance, dampens the spontaneous activity of 5-HT neurons (Dremencov, El Mansari et al. 2007a), however the concentration of 5-HT increases in both DR and prefrontal cortex (Hertel, Nomikos et al. 1999) . The observed increase in 5-HT neuronal tone in rats administered quetiapine on a long-term basis, likely stems from the direct activation of 5-HT_{1A} receptors by the drug, combined with the augmented 5-HT release capacity, stemming from the blockade of release-inhibiting terminal α_2 heteroreceptors.

It is noteworthy that the net increase in the 5-HT neuronal tone, was found to be the common denominator not only between the three drugs studied herein, but also between all treatments (both pharmacological and non-pharmacological)

endowed with the antidepressant properties.

As follows from the results of studies described in the present work, the same functional outcome may be achieved via different mechanisms. For instance, while both SSRIs and quetiapine increase the overall 5-HT neuronal transmission (albeit in a different fashion), the former dampen, while the latter increase the NE tone (see paper IV). Knowing that the excessive NE activation is undesired in patients suffering from profound agitation, but it is much needed in patients complaining of the diminished energy levels, the clinician obtains additional benefits allowing the customization of treatment. Therefore understanding of the principles underlying the antidepressant action would enable the clinician to better tailor therapeutic strategies minimizing the time needed for patient recovery.

Limitations

An argument against the relevance of findings obtained in anaesthetized animals towards the clinical effects of the studied drugs can be made. However, several factors suggest that the used method does provide accurate and reliable data.

While the data from awake behaving animals would provide a closer alternative to the conditions observed in clinic, several drawbacks make the use of such model highly impractical. As many experimental techniques require invasive procedures (like electrode descent or canulae implantation, etc.) significant peri- and post-surgical manipulations (use of analgesics, wound healing, handling

required for habituation of animals to the testing environment, acute stress associated with experimental procedure, etc.) not only alter the neuronal activity on their own but allow a great number of variants to impact the experimental outcome. Electrophysiological experiments, in particular, present with number of challenges: the isolation of monoaminergic neurons and obtaining of the stable recordings is extremely difficult in awake animals. Thus acquisition of data necessary for the reliable interpretation of results not only becomes tremendously labor-intensive, but also requires use of much greater number of animals making this experimental approach ethically questionable. These factors, along with the natural deviation of neuronal activity related to the environmental variations (circadian rhythms, temperature, etc.) make it difficult to compare data from different studies with even slightly different experimental conditions. Use of anesthesia, on the other hand, allows standardized measurements, minimizing the effects of uncontrollable external factors.

Most importantly, several studies conducted in different species have consistently shown that while the neuronal firing rates of monoaminergic neurons vary throughout the sleep-wake cycle (Jacobs, Fornal 1993) being fastest in awake state and slowest during deep sleep, the direction of change of spontaneous discharge produced by the tested pharmacological agents persists regardless of biological rhythm (Levine, Jacobs, 1992; Bjorvatn et al., 1998). Thus use of anesthesia, providing controlled stable experimental conditions without hindering the physiological responses is deemed justified and optimal.

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