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Fine-tuning Serotonin$_{2C}$ Receptor Function in the Brain: Molecular and Functional Implications

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Abstract

The serotonin$_{2C}$ receptor (5-HT$_{2C}$R) is a member of the serotonin$_2$ family of 7-transmembrane-spanning (7-TMS) receptors, which possesses unique molecular and pharmacological properties such as constitutive activity and RNA-editing. The 5-HT$_{2C}$R is widely expressed within the central nervous system, where is thought to play a major role in the regulation of neuronal network excitability. In keeping with its ability to modulate dopamine (DA) neuron function in the brain, the 5-HT$_{2C}$R is currently considered as a major target for improved treatments of neuropsychiatric disorders related to DA neuron dysfunction, such as depression, schizophrenia, Parkinson's disease or drug addiction.

The aim of this review is to provide an update of the functional status of the central 5-HT$_{2C}$R, covering molecular, cellular, anatomical, biochemical and behavioral aspects to highlight its distinctive regulatory properties, the emerging functional significance of constitutive activity and RNA-editing *in vivo*, and the therapeutic potential of inverse agonism.

Keywords

5-HT$_{2C}$ receptor; constitutive activity; RNA-editing; dopamine; microdialysis

1. Introduction

Serotonin$_{2C}$ receptors (5-HT$_{2C}$Rs) are members of the 7-transmembrane spanning (7-TMS or heptahelical) receptor superfamily, frequently referred to as G protein coupled receptors (GPCRs). The 5-HT$_{2C}$Rs couple to multiple cellular signaling pathways and are involved in the regulation of a variety of physiological functions and behaviors. Increasingly, 5-HT$_{2C}$Rs are therapeutic targets for conditions such as schizophrenia, anxiety, depression, Parkinson's disease, drug addiction and obesity. The 5-HT$_{2C}$R is the only 7-TMS receptor whose mRNA undergoes adenosine-inosine editing events which change the coding for amino acids

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located within the putative second intracellular domain (i2) of the receptor. In *in vitro* studies, RNA editing has a dramatic impact on the signaling characteristics of the 5-HT_{2C}R. *In vivo*, RNA editing efficiency differs in different brain regions and in response to various drugs. Further, it has been suggested that alterations in RNA editing of the 5-HT_{2C}R may be involved in the etiology of different disease states such as schizophrenia and affective disorders.

In this review, we discuss some of the various mechanisms that regulate the function of the 5-HT_{2C}R and the implications for this regulation on various physiological functions and behaviors controlled by this receptor.

2. Molecular pharmacological aspects *in vitro*

Like most, if not all, 7-TMS receptors, 5-HT_{2C}Rs couple to multiple cellular effector systems. Perhaps the best studied effector coupled to 5-HT_{2C}Rs is the phospholipase C (PLC) pathway. Albeit somewhat less well studied, other major effectors that are coupled directly to 5-HT_{2C}Rs are the phospholipase A2 (PLA_2) signaling cascade, the phospholipase D (PLD) pathways and extracellular signal-regulated kinase (ERK) (for recent reviews see Leysen, 2004; Raymond et al., 2006; Werry et al., 2006). 5-HT_{2C}Rs couple to PLC via Gq/11 proteins (Chang et al., 2000) and can couple to PLD via Gα13 proteins (McGrew et al., 2002), however, the signaling mediator for PLA_2 activation is unknown. In addition to phospholipid signaling pathways, 5-HT_{2C}Rs also activate desensitization mechanisms, such as G protein coupled receptor kinase (GRK) (Berg et al., 2001b) and arrestin (Marion et al., 2004). The receptor is also known to couple to pertussis toxin-sensitive G proteins (e.g. Gαi/o; Alberts et al., 1999; Cussac et al., 2002; Lucaites et al., 1996) as well as to PDZ domain containing proteins (Backstrom et al., 2000; Becamel et al., 2004). Consequently, the net cellular effect of activation of 5-HT_{2C}Rs is a coalescence brought about by the concurrent activation of several effector pathways within cells.

The second intracellular (i2) domain of 7-TMS receptors plays an important role in receptor function. The highly conserved E/DRY motif is located in i2 at the cytosolic end of transmembrane helix 3 and has been linked strongly to mechanisms of receptor activation and G protein coupling (Flanagan, 2005). Many studies have provided evidence that E/DRY motif and other residues within i2 are involved with direct coupling to G proteins (Burstein et al., 1998; Moro et al., 1993; Sugimoto et al., 2004) or other signaling molecules (Laghmani et al., 2005). In addition, i2 may participate in desensitization mechanisms such as ß-arrestin binding, receptor internalization and downregulation (Marion et al., 2006). Evidence also suggests that residues within i2 regulate the capacity of receptors to isomerize thereby controlling the formation of active receptor conformations and constitutive receptor activity (Alewijnse et al., 2000; Burstein et al., 1998; Flanagan, 2005; Rasmussen et al., 1999; Scheer et al., 2000).

Editing of the 5-HT_{2C}R mRNA leads to changes in amino acids in i2 starting just 2 residues downstream from the E/DRY motif. mRNA transcripts of the human 5-HT_{2C}R undergo adenosine-to-inosine editing events at five sites which encompass amino acids 156-160 within the putative second intracellular loop of the encoded human receptor. In human brain, the non-edited receptor contains the amino acids isoleucine, asparagine, and isoleucine (i.e., INI) at positions 156, 158 and 160, respectively. Consequences of RNA editing can produce potentially 24 different isoforms of the human receptor; although RNA actually encoding for about 14 different receptor isoforms has been detected (Fitzgerald et al., 1999). In rat, adenosine-to-inosine editing events occur with the potential for generating 11 different mRNA transcripts predicted to encode 7 different receptor isoforms (Burns et al., 1997).

Importantly, differences in function of some edited receptor isoforms have been reported (for review see Werry et al., 2008). Pharmacological characterization of two fully edited isoforms (5-$HT_{2C\text{-}VGV}$ and 5-$HT_{2C\text{-}VSV}$) has revealed decreases in agonist affinity (Fitzgerald et al., 1999; Herrick-Davis et al., 1999; Niswender et al., 1999; Quirk et al., 2001), potency (Berg et al., 2001a; Burns et al., 1997; Fitzgerald et al., 1999; Herrick-Davis et al., 1999; McGrew et al., 2004; Price et al., 2001; Wang et al., 2000), ligand-independent (constitutive) receptor activity (Berg et al., 2008; Herrick-Davis et al., 1999; Niswender et al., 1999; Wang et al., 2000) and receptor-arrestin binding and internalization (Marion et al., 2004) as compared with the non-edited receptor (5-$HT_{2C\text{-}INI}$).

In addition to selectivity for receptor subtypes, agonists have selectivity for different signaling pathways coupled to a single receptor subtype; a process known as 'functional selectivity' (Berg and Clarke, 2006; Urban et al., 2007). It has been well-established that 5-HT_{2C} ligands can selectively regulate signaling pathways at the non-edited 5-$HT_{2C\text{-}INI}$ isoforms (Berg et al., 1998, 2001a; Moya et al., 2007; Werry et al., 2005). We have found that RNA editing results in a significant change in agonist functional selectivity for PLC versus PLA_2 signaling via 5-HT_{2C} receptors (Berg et al., 2001a; 2008). For the fully-edited isoforms, 5-$HT_{2C\text{-}VSV}$ and 5-$HT_{2C\text{-}VGV}$, functional selectivity is lost (Berg et al., 2001a). However, at the partially edited receptor, 5-$HT_{2C\text{-}VNI}$, where a single amino acid substitution of valine for isoleucine occurs at position 156 (I156V), the agonist functional selectivity profile is substantially altered but not abolished (Berg et al., 2008). Interestingly, agonists with greater relative efficacy for PLA_2 vs. PLC at the non-edited receptor lost preferential efficacy toward PLA_2 in cells expressing the 5-$HT_{2C\text{-}VNI}$ receptor (i.e., agonist relative efficacy for PLC and PLA_2 were not different), whereas, agonists that were non-selective for PLC vs. PLA_2 at the 5-$HT_{2C\text{-}INI}$ receptor became selective for PLC at the 5-$HT_{2C\text{-}VNI}$ isoform, due to reduced relative efficacy for PLA_2. However, agonists with preferential activity toward PLC retained their PLC signaling preference. Therefore, agonist functional selectivity toward the PLA_2 signaling cascade appears to be sensitive to effects of RNA editing. Overall, these data suggest that the i2 domain of the 5-HT_{2C}R plays a major role in providing agonist-specific information to the signal transduction machinery of the cell.

It has been well documented that 5-HT_{2C}Rs exhibit a great deal of ligand-independent activity toward PLC. RNA editing appears to reduce the ability of 5-HT_{2C}Rs to signal constitutively to PLC (Berg et al., 2008; Herrick-Davis et al., 1999; Niswender et al., 1999; Wang et al., 2000). Reduction in constitutive receptor activity could arise from reduced capacity of the edited receptors to isomerize to an active conformation(s) capable of coupling to the Gq-PLC-IP pathway or to reduced G protein coupling of the active receptor conformation(s). Several reports suggest a role for the E/DRY motif (especially the arginine residue) in i2 of 7-TMS receptors in regulating the capacity of receptors to isomerize between inactive and active conformations (Alewijnse et al., 2000; Burstein et al., 1998; Flanagan, 2005; Rasmussen et al., 1999; Scheer et al., 2000). However, amino acids in the i2 region of 7-TMS receptors close to the conserved DRY sequence are also known to be involved in G protein coupling (Burstein et al., 1998; Moro et al., 1993; Sugimoto et al., 2004). Given the proximity of the amino acids altered by RNA editing to the E/DRY region, either mechanism could be responsible for the reduced constitutive activity of the edited 5-HT_{2C}Rs toward PLC.

To address the mechanism responsible for reduced constitutive activity of 5-HT_{2C}R edited isoforms, we compared the Gq-PLC-IP coupling efficiency of the RNA edited isoforms 5-$HT_{2C\text{-}VNI}$, 5-$HT_{2C\text{-}VSV}$, and 5-$HT_{2C\text{-}VGV}$ occupied by either the full agonist, 5-HT, or the partial agonists, LSD and DOI, to that of the non-edited 5-$HT_{2C\text{-}INI}$ receptor over a range of receptor densities. The slope of the regression line of the receptor density- response curve is

a good measure of the receptor-G protein coupling efficiency (Kang and Leeb-Lundberg, 2002). We found that the slopes of the regression line between receptor density and response to maximal occupancy by agonist (full or partial) for RNA-edited receptors (5-$HT_{2C\text{-}VSV}$, 5-$HT_{2C\text{-}VGV}$, 5-$HT_{2C\text{-}VNI}$) were equal to, or greater than, that for the non-edited 5-$HT_{2C\text{-}INI}$ isoform (Berg et al., 2008). One notable exception was LSD which is known not to activate the 5-$HT_{2C\text{-}VGV}$ isoform (Backstrom et al., 1999; Berg et al., 2001a; Fitzgerald et al., 1999). These results are consistent with the idea that the coupling efficiency of the agonist-occupied receptors to Gq-PLC signaling is not reduced by RNA editing.

To examine the possibility that reduced constitutive activity of RNA edited 5-HT_{2C} isoforms is due to reduced ability to isomerize, we measured thermal stability of the receptors. Constitutively active receptors with a high capacity to isomerize are structurally unstable as a consequence of reduced stabilizing intramolecular constraints and therefore denature more readily at elevated temperature (Alewijnse et al., 2000; Gether et al., 1997; Samama et al., 1997). Receptor instability can be measured by the kinetics of the loss of ligand binding at elevated temperature (Claeysen et al., 2001). Surprisingly, we found that there was no difference in the thermal stability of the 5-$HT_{2C\text{-}VNI}$, 5-$HT_{2C\text{-}VSV}$ or 5-$HT_{2C\text{-}VGV}$ isoforms from the highly constitutively active non-edited 5-$HT_{2C\text{-}INI}$ receptor (Berg et al., 2008). This suggests that RNA editing does not alter the isomerization capacity of 5-HT_{2C} receptors.

If there is no difference between the edited and non-edited 5-HT_{2C} isoforms for either G protein coupling efficiency or isomerization capacity, how can the difference in constitutive receptor activity be explained? We suggest that the reduced constitutive activity toward PLC of the edited 5-HT_{2C} isoforms is due to reduced efficiency of G protein coupling of the unoccupied receptor and that agonist occupancy promotes active receptor conformations that differ from that of unoccupied receptors such that edited and non-edited 5-HT_{2C}Rs have an equal ability to activate Gq-PLC-IP signaling in the presence of agonist.

In summary, RNA editing can produce different 5-HT_{2C}R isoforms with different signaling profiles of agonist-stimulated activity and reduced levels of ligand-independent receptor activity. The dynamic nature of the expression profiles of RNA edited isoforms along with their differential distribution throughout the brain may allow for an exquisite level of fine-tuning of serotonergic neurotransmission via the 5-HT_{2C}R. Moreover, the exciting possibility of development of drugs which have selectivity for 5-HT_{2C}R isoforms may provide for enhanced therapeutic benefit with reduced adverse effects.

3. Functional neuroanatomy of the 5-HT_{2C}R

Localization of the mRNA which encodes for the 5-HT_{2C}R is restricted almost exclusively to the central nervous system, with levels undetectable in liver, kidney, intestine, heart, and lung (Julius et al. 1988). The distribution of the 5-HT_{2C}R protein tracks closely with that of the transcript in regions that receive innervation from 5-HT neurons arising from the midbrain raphe nuclei (Pompeiano et al. 1994). The concordance of mRNA and protein expression (Burnet et al. 1995; Lopez-Gimenez et al. 2001a, 2001b; Mengod et al. 1990a, 1990b; Pompeiano et al. 1994; Wright et al. 1995) suggests predominant postsynaptic localization of these receptors, although the 5-HT_{2C}R may be presynaptically localized in some brain areas (Lopez-Gimenez et al. 2001a; Mengod et al. 1990a, 1990b; Pompeiano et al. 1994). The functional significance of the 5-HT_{2C}R within the limbic-corticostriatal circuits is of current interest given the importance of this circuit in psychiatric and neurological disorders including anxiety, depression, drug addiction, obesity, Parkinson's disease, and schizophrenia. The limbic-corticostriatal circuitry is an integrated collection of nuclei and pathways which functionally connects the prefrontal cortex (PFC), ventral

striatum, amygdala, and hippocampus, among other nuclei. This circuit is integral in coordinating reward-related associative learning and motivated behaviors that contribute to multiple aspects of psychiatric disorders (for reviews, see Hyman et al. 2006; Kalivas and Volkow 2005; Kauer and Malenka 2007).

The distinct patterns of expression of the 5-HT_{2C}R, which is prominently expressed in neurons throughout the limbic-corticostriatal circuit, enables differential modulation of neurotransmission by the 5-HT_{2C}R. For example, microinjection studies indicate that the 5-HT_{2C}R in the PFC and the nucleus accumbens (NAc) do not play an active, tonic role in motor control (Filip and Cunningham 2003; Ramos et al. 2005). However, separate populations of the 5-HT_{2C}R within the PFC and NAc differentially influence the stimulated output of the mesocorticoaccumbens pathway, which is seen as both altered efflux of DA and γ-aminobutyric acid (GABA) neurotransmitters (see below). Microinjection studies employing selective 5-HT_{2C}R ligands have described an opposing stimulatory and inhibitory influence of 5-HT_{2C}R in the NAc and PFC, respectively, over several behavioral effects of the psychostimulant cocaine (Filip and Cunningham 2003; Liu et al. 2007) or 3,4-methylenedioxymethamphetamine (MDMA) (Ramos et al. 2005). Thus, a functional equilibrium within the limbic-corticostriatal circuit *in vivo* is exerted by the 5-HT_{2C}R.

Both the NAc and the PFC are indeed enriched in 5-HT_{2C}R expression. The mRNA for the 5-HT_{2C}R was detected in what appeared to be GABA medium spiny projection neurons in both the core and shell of the NAc, along a rostrocaudal gradient (Eberle-Wang et al. 1997). The 5-HT_{2C}R mRNA was detected in medium-sized GABA interneurons in layer V of the PFC (Pasqualetti et al. 1999) but only low levels of 5-HT_{2C}R mRNA was expressed in cortical pyramidal neurons (Lopez-Gimenez et al. 2001a). In keeping with this finding, expression of the 5-HT_{2C}R protein was recently shown within parvalbumin-containing GABA interneurons localized to the deep layers (V/VI) of the PFC (Liu et al. 2007). While the neurochemistry of the 5-HT_{2C}R in the NAc and the PFC has been of prominent interest (see section 4), the receptor is also enriched in other nodes of the limbic-corticostriatal circuit, notably the amygdala and hippocampus. Functional 5-HT_{2C}R protein within the basolateral amygdala is localized to GABA inhibitory interneurons and glutamate neurons (Stein et al. 2000). The 5-HT_{2C}R mRNA is also expressed in a subset of pyramidal hippocampal cells restricted to the CA3 field of Ammon's horn (Pasqualetti et al. 1999), while 5-HT_{2C}R protein was detected in the pyramidal cell layer in both CA1 and CA3 (Clemett et al. 2000). Since the CA3 pyramidal cells project to the CA1, these data suggest that 5-HT_{2C}R protein in CA1 may be localized to presynaptic axon terminals from CA3 neurons (Pasqualetti et al. 1999). The amygdala 5-HT_{2C}R appears to be critical in the regulation of stress, fear and anxiety (Campbell and Merchant 2003; Harada et al. 2008; Li et al. 2003) while a population of hippocampal 5-HT_{2C}R may be involved in regulation of motility and anxiogenesis (Hackler et al. 2007; Stiedl et al. 2007; Whitton and Curzon 1990). Recent observations that activation of the 5-HT_{2C}R increased hippocampal neurogenesis suggests that this action may underlie learning and memory processes which involve 5-HT (Meneses and Hong 1997; Nic Dhonnchadha and Cunningham 2008) as well as the beneficial effects of serotonergic antidepressants (Banasr et al. 2004).

The ventral tegmental area (VTA) provides an interesting brain nucleus in which to consider the *in vivo* significance of unique regulatory mechanisms afforded to the 5-HT_{2C}R. Intra-VTA microinfusion of 5-HT_{2C}R antagonists did not alter basal nor cocaine-evoked hyperactivity (Filip and Cunningham 2002; McMahon and Cunningham 2001). However, upon activation via intra-VTA 5-HT_{2C}R agonist microinfusion, engagement of 5-HT_{2C}R-controlled pathways originating in the VTA suppressed basal motility and limited the extent of hyperactivity evoked by cocaine (Fletcher et al. 2004). As discussed in section 4, these results are supported by a recent study demonstrating that intra-VTA injection of the 5-

$HT_{2C}R$ agonist RO 60-0175 reduced the enhancement of DA outflow in the NAc induced by a systemic injection of cocaine, while intra-VTA administration of the 5-$HT_{2C}R$ antagonist SB 242084 had no effect (Navailles et al., 2008). These multifaceted effects could potentially be related to the level of constitutive activity of the 5-$HT_{2C}R$, the preponderance of partially to fully edited 5-$HT_{2C}R$ isoforms expressed and/or the dynamic expression patterns of 5-$HT_{2C}R$ protein within the DA vs. GABA neurons in VTA (Bubar et al. 2005; Bubar and Cunningham 2007). Thus, the VTA provides an interesting locus for analyses of the unique biology of the 5-$HT_{2C}R$.

The VTA is localized in the ventral portion of the mesencephalon and is comprised of five subnuclei with distinct afferent and efferent projections (Phillipson 1979; Swanson 1982). The VTA is well known for supplying the major DA innervation for the limbic-corticostriatal circuit and a population of GABA neurons in the VTA send collaterals that synapse locally on DA neurons within the VTA as well as projections that terminate in both the NAc (Van Bockstaele and Pickel 1995) and/or PFC (Carr and Sesack 2000; Steffensen et al. 1998). Protein expression for the 5-$HT_{2C}R$ was found to be relatively uniform in VTA GABA neurons across the rostrocaudal gradient and among the VTA subnuclei (Bubar and Cunningham 2007; Eberle-Wang et al. 1997). Conversely, this protein was shown to be differentially expressed in VTA DA neurons along distinct rostrocaudal and subnuclear patterns (Bubar and Cunningham 2007). The differences in the 5-$HT_{2C}R$ distribution within DA and GABA neurons as well as the variations in the proportion of colocalization across subnuclei and rostral-caudal level suggest that discrete populations of the 5-$HT_{2C}R$ in the VTA may tightly regulate the influence of 5-$HT_{2C}R$ upon DA (and GABA) neurotransmission. Modulatory neurobehavioral effects could result from a functional balance between both populations of 5-$HT_{2C}R$ located on GABA and DA neurons in the VTA (Navailles et al., 2008). Although the distribution of the 5-$HT_{2C}R$ on these two neuronal subtypes appears to vary slightly among the rostral-caudal levels of the various subnuclei, the incidence of co-localization of 5-$HT_{2C}R$ with DA neurons appears to predominate in several subnuclei, particularly in the middle VTA (Bubar and Cunningham 2007). Further examination into the impact of these different 5-$HT_{2C}R$ subpopulations through systematic microinfusion studies is necessary to fully understand how the 5-$HT_{2C}R$ in the VTA regulates activation of the DA mesocorticoaccumbens pathways.

The characteristics of the 5-$HT_{2C}R$ as a constitutively active receptor has been extensively clarified *in vitro* (see section 2) and identified as relevant in the functional neurochemistry of the limbic- corticostriatal circuit *in vivo* (see section 4)(Berg et al. 2005). The VTA 5-$HT_{2C}R$ that controls accumbal DA release appears to lack constitutive activity (Navailles et al., 2006), which may be driven by region-dependent RNA editing of the 5-$HT_{2C}R$ (Burns et al. 1997). Recent studies have also found evidence for 5-$HT_{2C}R$ homodimerization on the plasma membrane (Herrick-Davis et al. 2004, 2005), and endoplasmic reticulum (Herrick-Davis et al. 2006) of living cells suggesting that dimerization may also play an important role in the function of 5-$HT_{2C}R$. Although 5-$HT_{2C}R$ homodimerization is difficult to explore *ex vivo* or *in vivo*, functional studies implicate the involvement of 5-$HT_{2C}R$ homodimerization in ligand binding, signal transduction, and receptor trafficking processes (Herrick-Davis et al. 2004, 2005). The significance of constitutive activity and receptor dimerization *in vivo* has only recently come under investigation; thus, future investigations will provide a greater understanding of the importance of these processes in the regulation of 5-$HT_{2C}R$ function in the VTA and elsewhere in the limbic-corticostriatal circuit and the potential relevance for consideration in the development of pharmacotherapeutics for psychiatric diseases. Drug discovery initiatives have identified a number of new 5-$HT_{2C}R$ binding chemicals and active initiatives are underway to uncover the pharmacology of selective 5-$HT_{2C}R$ agonists, antagonist, partial agonists and inverse agonists. Allosteric modulators of the 5-$HT_{2C}R$ (Im et al. 2003) and small molecule inhibitors that disrupt the

association of the 5-HT_{2C}R with its key binding partners (Ji et al. 2006; Sharma et al. 2007) also provide unique approaches to drug discovery in this system.

4. Neurochemical and functional aspects *in vivo*: focus on the 5-HT/DA interaction

Since the discovery and identification of 5-HT_{2C}Rs in the mammalian brain (for review see Barnes and Sharp, 1999), along with their dense localization in brain dopaminergic regions (Clemett et al., 2000; Eberle-Wang et al., 1997; Pompeiano et al., 1994), much attention has been devoted at studying their functional role in the modulation of DA ascending pathway activity, i.e. the nigrostriatal and the mesocorticolimbic systems. Indeed, the 5-HT_{2C}R is actually considered as a pivotal pharmacological target for improved treatments of neuropsychiatric disorders related to DA neuron dysfunction, such as schizophrenia, depression, Parkinson's disease or drug addiction (Bubar and Cunningham, 2006; Giorgetti and Tecott, 2004; Meltzer et al., 2003; Millan, 2005; Schapira et al., 2006; Wood et al., 2001).

It is now clearly established that central 5-HT_{2C}Rs exert tonic and phasic inhibitory controls on DA neuron function *in vivo*. This was first suggested by electrophysiological studies with non selective 5-HT_{2C}R compounds (Prisco et al., 1994), and then confirmed by several electrophysiological and biochemical studies using more selective 5-HT_{2C}R antagonists and agonists (for review see Alex and Pehek, 2007). Thus, the basal firing rate of DA neurons in the substantia nigra pars compacta (SNc) and the VTA as well as the release of DA at terminals within the striatum, the NAc and the PFC, is increased and decreased by the peripheral administration of 5-HT_{2C}R antagonists and agonists, respectively (De Deurwaerdère and Spampinato, 1999, 2001; Di Giovanni et al., 1999; Di Giovanni et al., 2002; Gobert et al., 2000).

The 5-HT_{2C}R has been also shown to control activated DA neurons by modulating DA neuronal firing (Pierucci *el al*., 2004; Porras et al., 2002) and DA release (Di Matteo et al. 2004; Hutson et al., 2000; Lucas et al., 2000; Navailles et al., 2004; Porras et al., 2002). Specifically, studies with drugs which stimulate the release of DA through different cellular mechanisms (morphine, haloperidol, cocaine phencyclidine and amphetamine) led to the proposal that 5-HT_{2C}R exerts preferential control of DA exocytosis (Navailles et al., 2004; Willins and Meltzer, 1998) likely by regulating DA neuronal firing (Navailles et al., 2004). Indeed, the degree of DA neuronal activity appears as a permissive factor for the modulatory action of 5-HT_{2C}R agonists and antagonists on DA release (Lucas et al., 2000; Navailles et al., 2004; Pozzi et al., 2002).

Mesencephalic regions containing DA cell bodies (VTA and SN) has been first proposed as a primary site of action for the inhibitory control of the mesocorticolimbic and nigrostriatal DA pathways by 5-HT_{2C}Rs (Di Matteo et al., 2001; Navailles et al., 2004; 2006b). Control of DA neuron activity is classically thought to be indirect and to involve a GABA-DA interface (Di Matteo et al., 2001; Navailles et al., 2004) in accord with the presence of 5-HT_{2C}R transcript and protein in VTA and SN GABA neurons (Bubar and Cunningham, 2007; Eberle-Wang *et al*, 1997) and with their ability to modulate GABA function within these brain regions (Bankson and Yamamoto, 2004; Di Giovanni et al., 2001; Invernizzi et al., 2007). However, the absence of effect of intra-VTA administered 5-HT_{2C}R agonists and antagonists on basal DA release in the NAc (Navailles et al., 2006b, 2008), although indirectly, does not support this view, and further microiontophoretic studies assessing the influence of 5-HT_{2C} agents on DA neuron firing are warranted to address this issue. Furthermore, the recent finding that DA neurons in the VTA co-express the protein for the 5-HT_{2C}R (Bubar and Cunningham, 2007; Ji et al., 2006) raises the possibility of direct

excitatory control of DA neuron function. Modulatory effects of VTA DA neuron firing and accumbal DA release could result from a functional balance between both populations of 5-HT_{2C}Rs located on GABA and DA neurons in the VTA (Navailles et al., 2008).

Most intracranial microinjection studies, but not all, have provided evidence that 5-HT_{2C}Rs present within DA terminal regions are capable of modulating DA neuron activity, by exerting not only inhibitory but also excitatory influences on DA release. That striatal 5-HT_{2C}Rs exert a facilitatory control of DA release in the rat striatum was first reported by Lucas and Spampinato (2000), but not confirmed by subsequent studies (Alex et al., 2005). NAc 5-HT_{2C}Rs have been shown to inhibit (Dremencov et al., 2005), facilitate (Yan, 2000) or not affect (Navailles et al., 2006b, 2008) basal DA release in the NAc. Also, they have been shown to exert concentration-dependent excitatory and inhibitory effects on activated accumbal DA release (Navailles et al., 2008). At variance with the NAc and the striatum, compelling evidence indicates that 5-HT_{2C}Rs localized in the PFC do not modulate basal or activated DA release in this region, either tonically or phasically (Alex et al., 2005; Pehek et al., 2006; Pozzi et al., 2002). However, as in the case of the NAC DA (Navailles et al., 2006b), PFC DA release is sensitive to VTA 5-HT_{2C} receptor inhibitory modulation (Pozzi et al., 2002). Furthermore, as previously suggested by behavioral investigations (Filip and Cunningham, 2003), recent neurochemical studies from our laboratory have shown that PFC 5-HT_{2C}Rs are able to modulate activated DA release in the NAc. Intra-PFC administration of 5-HT_{2C}R agonists and antagonists has been shown to facilitate the release of DA induced by cocaine or morphine in the NAc (unpublished observations). Although the neuronal circuits underlying the above reported effects remains to be determined, as discussed elsewhere (Filip and Cunningham 2003; Navailles et al., 2008), 5-HT_{2C}R-dependent controls of DA release in DA terminal-regions, in keeping with the expression of 5-HT_{2C}Rs on GABA cells (Eberle-Wang et al., 1997; Liu et al., 2007), may involve local GABA circuits and/or negative feedback loops to the VTA and the SN, as well as polysynaptic circuits including glutamate pathways relaying the PFC to the VTA and the NAc (Sesack et al., 2003). Hence, studies with peripheral and intracranial administration of 5-HT_{2C} agents altogether indicate that the overall inhibitory control of central 5-HT_{2C}Rs on nigrostriatal and mesocorticolimbic DA pathways may be considered as a composite response involving functional balances between excitatory and inhibitory inputs to DA neurons related to different 5-HT_{2C}R populations located within multiple brain DA areas. Specifically, it appears that, in contrast to striatal and accumbal DA release, PFC DA release is insensitive to local control by 5-HT_{2C}R.

A main step in the advance of the knowledge of the functional role of the 5-HT_{2C}R comes from recent microdialysis studies showing that 5-HT_{2C} constitutive receptor activity participates in the tonic inhibitory control of DA ascending pathways *in vivo* (De Deurwaerdère et al., 2004). In agreement with the pharmacological characteristics of inverse agonist activity (Berg et al., 2005) and consistent with *in vitro* studies in Chinese Hamster ovary (CHO) cells expressing 5-HT_{2C}Rs (Berg et al., 2006; De Deurwaerdère et al., 2004), it has been shown that the purported 5-HT_{2C}R antagonist SB206553 behaves *in vivo* as an inverse agonist at 5-HT_{2C}R. Indeed, SB 206553-stimulated DA release is insensitive to the decrease in 5-HT terminal activity induced by either intra-raphe injections of 5,7-dihydroxytryptamine neurotoxin, or by peripheral administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (De Deurwaerdère et al., 2004). Also, the 5-HT_{2C}R antagonists SB242084 and SB 243213 prevent the increase in striatal and accumbal DA release induced by SB 206553 and reverse the decrease in DA release produced by the 5-HT_{2C}R agonist Ro 60-0175 in both brain regions (Berg et al., 2006; De Deurwaerdère et al., 2004). Thus, these findings altogether indicate that the effect of SB 206553 on *in vivo* DA release is independent of the changes in extracellular levels of 5-HT, and is likely related to its inverse agonist properties at 5-HT_{2C}Rs to silence their level of constitutive activity *in vivo*.

Interestingly, inverse agonist action of SB 206553 at native 5-HT_{2C}Rs has been recently shown in primary culture of mouse cortical neurons (Chanrion et al., 2008).

Further support for the role of 5-HT_{2C} constitutive receptor activity in the control of midbrain DA neuron excitability *in vivo* comes from recent studies with the antipsychotic drugs haloperidol and clozapine which show that 5-HT_{2C}R inverse agonists can have different effects than 5-HT_{2C}R antagonists *in vivo* (Navailles et al., 2006a). Indeed, the increase in accumbal and striatal DA release induced by haloperidol is potentiated by the 5-HT_{2C}R inverse agonist SB 206553 but unaltered by the 5-HT_{2C}R antagonists SB 242084 and SB 243213. Conversely, the effect of clozapine, which is known to be a strong 5-HT_{2C}R inverse agonist *in vitro* (Berg et al., 1999; 2006; Herrick-Davis et al., 2000), is unaffected by SB 206553 but blocked by SB 242084 and SB 243213. These findings, indicating that 5-HT_{2C} constitutive receptor activity participates in the dopaminergic effects of the antipsychotic drugs clozapine and haloperidol, suggest besides that clozapine modulates subcortical DA release by acting as a 5-HT_{2C}R inverse agonist *in vivo*.

Interestingly, intracranial microinjection studies have also shown that the control exerted by 5-HT_{2C} constitutive receptor activity on DA neurons occurs in a brain region-dependent manner and that the NAc may represent a primary site of action for the regulatory effects of constitutive receptor activity on the mesoaccumbens DA pathway (Navailles et al., 2006b). Intra-VTA injections of the 5-HT_{2C}R antagonists SB 242084 and/or SB 243213 prevent the decrease in accumbal DA outflow induced by peripheral administration of the 5-HT_{2C}R agonist Ro 60-0175, but do not affect the increase in DA outflow induced by the peripheral administration of the 5-HT_{2C}R inverse agonist SB 206553. Intra-NAc infusions of SB 242084, as in the case of its peripheral administration (De Deurwaerdère et al., 2004), block both Ro 60-0175- and SB 206553-induced changes of DA outflow. Thus, whereas VTA and NAc 5-HT_{2C}Rs both participate in the inhibitory control exerted by 5-HT_{2C}R agonist on accumbal DA release, 5-HT_{2C}Rs in the NAc are primarily involved in the tonic inhibitory control exerted by the constitutive activity of central 5-HT_{2C}R. In accord with this conclusion, intra-NAc, but not intra-VTA, administration of SB 206553 increases basal DA release in the NAc (Navailles et al., 2006b). The observed region-dependent effect of the inverse agonist SB 206553 could be related to different levels of 5-HT_{2C}R constitutive activity in the VTA and the NAc which may be related to the pre-mRNA editing of the 5-HT_{2C}R. Indeed, as discussed elsewhere (Navailles et al., 2006b), region-dependent RNA editing of 5-HT_{2C}R (Burns et al., 1997) may represent a mechanism generating receptor populations with different levels of constitutive activity (Niswender et al., 1999).

In summary, the findings reported above provide updated insights into the dominant role of the 5-HT_{2C}R in the regulatory neurochemistry of central DA neuron function. The 5-HT_{2C}R appears to possess a unique ability to tonically regulate DA release by combined actions involving the effects of endogenous 5-HT and constitutive receptor activity at different 5-HT_{2C}R populations present in multiple brain regions and thus may provide an exclusive functional basis for the fine-tuning of midbrain DA neuron excitability by the 5-HT_{2C}R in the living brain.

5. Concluding remarks

Twenty-five years after its initial identification in the central nervous system, a sizeable body of evidence has clearly demonstrated the pivotal role of the 5-HT_{2C}R in the regulation of neuronal network excitability. The malleability of signaling processes relative to expression of edited 5-HT_{2C}R isoforms throughout the brain (Schmauss, 2005), together with the relevance of the 5-HT_{2C} constitutive receptor activity in regulating physiological

systems *in vivo* may provide an exceptional molecular basis for fine-tuning of 5-HT neurotransmission via the 5-HT_{2C}R in the living brain.

The 5-HT_{2C}R is thought to be implicated in the pathophysiology of several neuropsychiatric disorders (schizophrenia, depression, anxiety, sleep disorders, drug addiction, obesity), and it is actually considered as a major pharmacological target for the development of improved treatments of these diseases. In this context, it is noteworthy that the discovery of drugs with inverse agonist properties at 5-HT_{2C}R allows for an additional dimension for control of 5-HT_{2C} receptor activity, and has greatly increased the richness of our pharmacological tools. However, the benefits of inverse agonist rather than antagonist properties at the 5-HT_{2C}R remains to be established, and, in the coming years, further experimental and clinical evaluations are needed for a better understanding of the functional significance of constitutive receptor activity and the therapeutic potential of inverse agonism *in vivo*.

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References

Alberts GL, Pregenzer JF, Im WB, Zaworski PG, Gill GS. Agonist-induced GTPgamma35S binding mediated by human 5-HT(2C) receptors expressed in human embryonic kidney 293 cells. Eur. J. Pharmacol. 1999; 383:311–319. [PubMed: 10594325]

Alewijnse AE, Timmerman H, Jacobs EH, Smit MJ, Roovers E, Cotecchia S, Leurs R. The effect of mutations in the DRY motif on the constitutive activity and structural instability of the histamine H(2) receptor. Mol. Pharmacol. 2000; 57:890–898. [PubMed: 10779371]

Alex KD, Pehek EA. Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacol. Ther. 2007; 113:296–320. [PubMed: 17049611]

Alex KD, Yavanian GJ, McFarlane HG, Pluto CP, Pehek EA. Modulation of dopamine release by striatal 5-HT_{2C} receptors. Synapse. 2005; 55:242–251. [PubMed: 15668911]

Backstrom JR, Chang MS, Chu H, Niswender CM, Sanders-Bush E. Agonist-directed signaling of serotonin 5-HT2C receptors: Differences between serotonin and lysergic acid diethylamide (LSD). Neuropsychopharmacology. 1999; 21:S77–S81.

Backstrom JR, Price RD, Reasoner DT, Sanders-Bush E. Deletion of the serotonin 5-HT2C receptor PDZ recognition motif prevents receptor phosphorylation and delays resensitization of receptor responses. J. Biol. Chem. 2000; 275:23620–23626. [PubMed: 10816555]

Banasr M, Hery M, Printemps R, Daszuta A. Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. Neuropsychopharmacol. 2004; 29:450–460.

Bankson MG, Yamamoto BK. Serotonin-GABA interactions modulate MDMA-induced mesolimbic dopamine release. J. Neurochem. 2004; 91:852–859. [PubMed: 15525339]

Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology. 1999; 38:1083–1152. [PubMed: 10462127]

Becamel C, Gavarini S, Chanrion B, Alonso G, Galeotti N, Dumuis A, Bockaert J, Marin P. The serotonin 5-HT2A and 5-HT2C receptors interact with specific sets of PDZ proteins. Journal of Biological Chemistry. 2004; 279:20257–20266. [PubMed: 14988405]

Berg KA, Clarke WP. Development of functionally selective agonists as novel therapeutic agents. Drug Discov. Today Ther. Strat. 2006; 4:421–428.

Berg KA, Cropper JD, Niswender CM, Sanders-Bush E, Emeson RB, Clarke WP. RNA-editing of the 5-HT(2C) receptor alters agonist-receptor-effector coupling specificity. Br J Pharmacol. 2001a; 134:386–392. [PubMed: 11564657]

Berg KA, Dunlop J, Sanchez T, Silva M, Clarke WP. A conservative, single-amino acid substitution in the second cytoplasmic domain of the human serotonin2C receptor alters ligand-dependent and -independent signaling. J. Pharmacol. Exp. Ther. 2008; 324:1084–1092. [PubMed: 18065501]

Berg KA, Maayani S, Goldfarb J, Scaramellini C, Leff P, Clarke WP. Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: Evidence for agonist-directed trafficking of receptor stimulus. Mol. Pharmacol. 1998; 54:94–104. [PubMed: 9658194]

Berg KA, Stout BD, Cropper JD, Maayani S, Clarke WP. Novel actions of inverse agonists on 5-HT2C receptor systems. Mol. Pharmacol. 1999; 55:863–872. [PubMed: 10220565]

Berg KA, Stout BD, Maayani S, Clarke WP. Differences in rapid desensitization of 5-hydroxytryptamine2A and 5-hydroxytryptamine2C receptor-mediated phospholipase C activation. J. Pharmacol. Exp. Ther. 2001b; 299:593–602. [PubMed: 11602671]

Berg KA, Harvey JA, Spampinato U, Clarke WP. Physiological relevance of constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptors. Trends Pharmacol. Sci. 2005; 26:625–630. [PubMed: 16269190]

Berg KA, Navailles S, Sanchez TA, Silva YM, Wood MD, Spampinato U, Clarke WP. Differential effects of 5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxyl]-5-pyridyl]carbamoyl]-6-trifluoromethylindone (SB243213) on 5-hydroxytryptamine(2C) receptor-mediated responses. J. Pharmacol. Exp. Ther. 2006; 319:260–268. [PubMed: 16807362]

Bubar MJ, Cunningham KA. Serotonin 5-HT_{2A} and 5-HT_{2C} receptors as potential targets for modulation of psychostimulant use and dependence. Curr. Top. Med. Chem. 2006; 6:1971–1985. [PubMed: 17017968]

Bubar MJ, Cunningham KA. Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. Neuroscience. 2007; 146:286–297. [PubMed: 17367945]

Bubar MJ, Seitz PK, Thomas ML, Cunningham KA. Validation of a selective serotonin 5-HT(2C) receptor antibody for utilization in fluorescence immunohistochemistry studies. Brain Res. 2005; 1063:105–113. [PubMed: 16274677]

Burnet PWJ, Eastwood SL, Lacey K, Harrison PJ. The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. Brain Res. 1995; 676:157–168. [PubMed: 7796165]

Burns CM, Chu H, Reuter SM, Hutchinson LK, Canton H, Sanders-Bush E, Emeson RB. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. Nature. 1997; 387:303–308. [PubMed: 9153397]

Burstein ES, Spalding TA, Brann MR. The second intracellular loop of the m5 muscarinic receptor is the switch which enables G-protein coupling. J. Biol. Chem. 1998; 273:24322–24327. [PubMed: 9733718]

Campbell BM, Merchant KM. Serotonin 2C receptors within the basolateral amygdala induce acute fear-like responses in an open-field environment. Brain Res. 2003; 993:1–9. [PubMed: 14642825]

Carr DB, Sesack SR. Dopamine terminals synapse on callosal projection neurons in the rat prefrontal cortex. J. Comp. Neurol. 2000; 425:275–283. [PubMed: 10954845]

Chang M, Zhang LS, Tam JP, Sanders-Bush E. Dissecting G protein-coupled receptor signaling pathways with membrane-permeable blocking peptides - Endogenous 5-HT2C receptors in choroid plexus epithelial cells. Journal of Biological Chemistry. 2000; 275:7021–7029. [PubMed: 10702266]

Chanrion B, La Cour C. Mannoury, Gavarini S, Seimandi M, Vincent L, Pujol JF, Bockaert J, Marin P, Millan MJ. Inverse Agonist and Neutral Antagonist Actions of Antidepressants at Recombinant and Native 5-HT_{2C} Receptors: Differential Modulation of Cell Surface Expression and Signal Transduction. Mol. Pharmacol. 2008; 73:748–757. [PubMed: 18083778]

Claeysen S, Sebben M, Becamel C, Parmentier ML, Dumuis A, Bockaert J. Constitutively active mutants of 5-HT4 receptors are they in unique active states? EMBO Rep. 2001; 2:61–67. [PubMed: 11252726]

Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KC. Immunohistochemical localisation of the 5-HT_{2C} receptor protein in the rat CNS. Neuropharmacology. 2000; 39:123–132. [PubMed: 10665825]

Cussac D, Newman-Tancredi A, Duqueyroix D, Pasteau V, Millan MJ. Differential activation of Gq/1 and Gi(3) proteins at 5-hydroxytryptamine(2C) receptors revealed by antibody capture assays:

Influence of receptor reserve and relationship to agonist-directed trafficking. Mol. Pharmacol. 2002; 62:578–589. [PubMed: 12181434]

De Deurwaerdère P, Navailles S, Berg KA, Clarke WP, Spampinato U. Constitutive activity of the serotonin2C receptor inhibits *in vivo* dopamine release in the rat striatum and nucleus accumbens. J. Neurosci. 2004; 24:3235–3241. [PubMed: 15056702]

De Deurwaerdère P, Spampinato U. Role of serotonin(2A) and serotonin(2B/2C) receptor subtypes in the control of accumbal and striatal dopamine release elicited *in vivo* by dorsal raphe nucleus electrical stimulation. J. Neurochem. 1999; 73:1033–1042. [PubMed: 10461892]

De Deurwaerdère P, Spampinato U. The nigrostriatal dopamine system: a neglected target for 5-HT_{2C} receptors. Trends Pharmacol. Sci. 2001; 22:502–504. [PubMed: 11583806]

Di Giovanni G, De Deurwaerdère P, Di Mascio M, Di Matteo V, Esposito E, Spampinato U. Selective blockade of serotonin-2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined *in vivo* electrophysiological and microdialysis study. Neuroscience. 1999; 91:587–597. [PubMed: 10366016]

Di Giovanni G, Di Matteo V, Esposito E. Serotonin/dopamine interaction--focus on 5-HT_{2C} receptor, a new target of psychotropic drugs. Indian J. Exp. Biol. 2002; 40:1344–1352. [PubMed: 12974395]

Di Giovanni G, Di Matteo V, La Grutta V, Esposito E. m-chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin2C receptors. Neuroscience. 2001; 103:111–116. [PubMed: 11311791]

Di Matteo V, De Blasi A, Di Giulio C, Esposito E. Role of 5-HT_{2C} receptors in the control of central dopamine function. Trends Pharmacol. Sci. 2001; 22:229–232. [PubMed: 11339973]

Di Matteo V, Pierucci M, Esposito E. Selective stimulation of serotonin2c receptors blocks the enhancement of striatal and accumbal dopamine release induced by nicotine administration. J. Neurochem. 2004; 89:418–429. [PubMed: 15056285]

Dremencov E, Newman ME, Kinor N, Blatman-Jan G, Schindler CJ, Overstreet DH, Yadid G. Hyperfunctionality of serotonin-2C receptor-mediated inhibition of accumbal dopamine release in an animal model of depression is reversed by antidepressant treatment. Neuropharmacology. 2005; 48:34–42. [PubMed: 15617725]

Eberle-Wang K, Mikeladze Z, Uryu K, Chesselet MF. Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. J. Comp. Neurol. 1997; 384:233–247. [PubMed: 9215720]

Filip M, Cunningham KA. Serotonin 5-HT(2C) receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. Pharmacol. Biochem. Behav. 2002; 71:745–756. [PubMed: 11888566]

Filip M, Cunninghan KA. Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin(2C) (5-HT(2C)) receptors in rat prefrontal cortex. J. Pharmacol. Exp. Ther. 2003; 306:734–743. [PubMed: 12721337]

Fitzgerald LW, Conklin DS, Krause CM, Marshall AP, Patterson JP, Tran DP, Iyer G, Kostich WA, Largent BL, Hartig PR. High-affinity agonist binding correlates with efficacy (intrinsic activity) at the human serotonin 5-HT2A and 5-HT2C receptors: Evidence favoring the ternary complex and two-state models of agonist action. J. Neurochem. 1999; 72:2127–2134. [PubMed: 10217294]

Flanagan CA. A GPCR that is not “DRY”. Mol. Pharmacol. 2005; 68:1–3. [PubMed: 15855406]

Fletcher PJ, Chintoh AF, Sinyard J, Higgins GA. Injection of the 5-HT2C receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. Neuropsychopharmacol. 2004; 29:308–318.

Gether U, Ballesteros JA, Seifert R, Sanders-Bush E, Weinstein H, Kobilka BK. Structural instability of a constitutively active G protein-coupled receptor. Agonist-independent activation due to conformational flexibility. J. Biol. Chem. 1997; 272:2587–2590. [PubMed: 9006889]

Giorgetti M, Tecott LH. Contributions of 5-HT(2C) receptors to multiple actions of central serotonin systems. Eur. J. Pharmacol. 2004; 488:1–9. [PubMed: 15044029]

Gobert A, Rivet JM, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas JP, Cistarelli L, Melon C, Millan MJ. Serotonin(2C) receptors tonically suppress the activity of mesocortical

dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. Synapse. 2000; 36:205–221. [PubMed: 10819900]

Hackler EA, Turner GH, Gresch PJ, Sengupta S, Deutch AY, Avison MJ, Gore JC, Sanders-Bush E. 5-Hydroxytryptamine2C receptor contribution to m-chlorophenylpiperazine and N-methyl-beta-carboline-3-carboxamide-induced anxiety-like behavior and limbic brain activation. J. Pharmacol. Exp. Ther. 2007; 320:1023–1029. [PubMed: 17138863]

Harada K, Yamaji T, Matsuoka N. Activation of the serotonin 5-HT2C receptor is involved in the enhanced anxiety in rats after single-prolonged stress. Pharmacol. Biochem. Behav. 2008; 89:11–16. [PubMed: 18067955]

Herrick-Davis K, Grinde E, Harrigan TJ, Mazurkiewicz JE. Inhibition of serotonin 5-hydroxytryptamine2c receptor function through heterodimerization: receptor dimers bind two molecules of ligand and one G-protein. J. Biol. Chem. 2005; 280:40144–40151. [PubMed: 16195233]

Herrick-Davis K, Grinde E, Mazurkiewicz JE. Biochemical and biophysical characterization of serotonin 5-HT2C receptor homodimers on the plasma membrane of living cells. Biochemistry. 2004; 43:13963–13971. [PubMed: 15518545]

Herrick-Davis K, Grinde E, Niswender CM. Serotonin 5-HT2C receptor RNA editing alters receptor basal activity: implications for serotonergic signal transduction. J. Neurochem. 1999; 73:1711–1717. [PubMed: 10501219]

Herrick-Davis K, Grinde E, Teitler M. Inverse agonist activity of atypical antipsychotic drugs at human 5-hydroxytryptamine2C receptors. J Pharmacol. Exp. Ther. 2000; 295:226–232. [PubMed: 10991983]

Herrick-Davis K, Weaver BA, Grinde E, Mazurkiewicz JE. Serotonin 5-HT2C receptor homodimer biogenesis in the endoplasmic reticulum: real-time visualization with confocal fluorescence resonance energy transfer. J. Biol. Chem. 2006; 281:27109–27116. [PubMed: 16857671]

Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. Annu. Rev. Neurosci. 2006; 29:565–598. [PubMed: 16776597]

Hutson PH, Barton CL, Jay M, Blurton P, Burkamp F, Clarkson R, Bristow LJ. Activation of mesolimbic dopamine function by phencyclidine is enhanced by 5-$HT_{2C/2B}$ receptor antagonists: neurochemical and behavioural studies. Neuropharmacology. 2000; 39:2318–2328. [PubMed: 10974315]

Im WB, Chio CL, Alberts GL, Dinh DM. Positive Allosteric Modulator of the Human 5-HT2C Receptor. Mol. Pharmacol. 2003; 64:78–84. [PubMed: 12815163]

Invernizzi RW, Pierucci M, Calcagno E, Di Giovanni G, Di Matteo V, Benigno A, Esposito E. Selective activation of 5-HT(2C) receptors stimulates GABA-ergic function in the rat substantia nigra pars reticulata: a combined *in vivo* electrophysiological and neurochemical study. Neuroscience. 2007; 144:1523–1535. [PubMed: 17161544]

Ji S, Zhang Y, Van Cleemput J, Jiang W, Liao M, Li L, Wan Q, Backstrom JR, Zhang X. Distruption of PTEN coupling with 5-HT2C receptors suppresses behavioural responses induced by drug of abuse. Nat. Med. 2006; 12:324–329. [PubMed: 16474401]

Julius D, MacDermott AB, Jessel TM, Huang K, Molineaux S, Schieren I, Axel R. Functional expression of the 5-HT1c receptor in neuronal and nonneuronal cells. Cold Spring Harb. Symp. Quant. Biol. 1988; 53(Pt 1):385–393. [PubMed: 3254776]

Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am. J. Psychiatry. 2005; 162:1403–1413. [PubMed: 16055761]

Kang DS, Leeb-Lundberg LM. Negative and positive regulatory epitopes in the C-terminal domains of the human B1 and B2 bradykinin receptor subtypes determine receptor coupling efficacy to G(q/11)-mediated [correction of G(9/11)-mediated] phospholipase Cbeta activity. Mol. Pharmacol. 2002; 62:281–288. [PubMed: 12130679]

Kauer JA, Malenka RC. Synaptic plasticity and addiction. Nat. Rev. Neurosci. 2007; 8:844–858. [PubMed: 17948030]

Laghmani K, Sakamoto A, Yanagisawa M, Preisig PA, Alpern RJ. A consensus sequence in the endothelin-B receptor second intracellular loop is required for NHE3 activation by endothelin-1. Am. J. Physiol. 2005; 288:F732–739.

Leysen JE. 5-HT2 receptors. Curr. Drug Targets CNS Neurol. Disord. 2004; 3:11–26. [PubMed: 14965241]

Li Q, Wichems CH, Ma L, Van De Kar LD, Garcia F, Murphy DL. Brain region-specific alterations of 5-HT2A and 5-HT2C receptors in serotonin transporter knockout mice. J. Neurochem. 2003; 84:1256–1265. [PubMed: 12614326]

Liu S, Bubar MJ, Lanfranco MF, Hillman GR, Cunningham KA. Serotonin2C receptor localization in GABA neurons of the rat medial prefrontal cortex: implications for understanding the neurobiology of addiction. Neuroscience. 2007; 146:1677–1688. [PubMed: 17467185]

Lopez-Gimenez JF, Mengod G, Palacios JM, Vilaro MT. Regional distribution and cellular localization of 5-HT2C receptor mRNA in monkey brain: comparison with [3H]mesulergine binding sites and choline acetyltransferase mRNA. Synapse. 2001a; 42:12–26. [PubMed: 11668587]

Lopez-Gimenez JF, Vilaro MT, Palacios JM, Mengod G. Mapping of 5-HT2A receptors and their mRNA in monkey brain: [3H]MDL100,907 autoradiography and in situ hybridization studies. J. Comp. Neurol. 2001b; 429:571–589. [PubMed: 11135236]

Lucaites VL, Nelson DL, Wainscott DB, Baez M. Receptor subtype and density determine the coupling repertoire of the 5-HT2 receptor subfamily. Life Sci. 1996; 59:1081–1095. [PubMed: 8809227]

Lucas G, De Deurwaerdère P, Caccia S, Spampinato U. The effect of serotonergic agents on haloperidol-induced striatal dopamine release in vivo: opposite role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes and significance of the haloperidol dose used. Neuropharmacology. 2000; 39:1053–1063. [PubMed: 10727716]

Lucas G, Spampinato U. Role of striatal serotonin2A and serotonin2C receptor subtypes in the control of in vivo dopamine outflow in the rat striatum. J. Neurochem. 2000; 74:693–701. [PubMed: 10646521]

Marion S, Oakley RH, Kim KM, Caron MG, Barak LS. A beta-arrestin binding determinant common to the second intracellular loops of rhodopsin family G protein-coupled receptors. J. Biol. Chem. 2006; 281:2932–2938. [PubMed: 16319069]

Marion S, Weiner DM, Caron MG. RNA editing induces variation in desensitization and trafficking of 5-hydroxytryptamine 2c receptor isoforms. J. Biol. Chem. 2004; 279:2945–2954. [PubMed: 14602721]

McGrew L, Chang MS, Sanders-Bush E. Phospholipase D activation by endogenous 5-hydroxytryptamine 2C receptors is mediated by Galpha13 and pertussis toxin-insensitive Gbetagamma subunits. Mol. Pharmacol. 2002; 62:1339–1343. [PubMed: 12435801]

McGrew L, Price RD, Hackler E, Chang MS, Sanders-Bush E. RNA editing of the human serotonin 5-HT2C receptor disrupts transactivation of the small G-protein RhoA. Mol. Pharmacol. 2004; 65:252–256. [PubMed: 14722258]

McMahon LR, Cunningham KA. Role of 5-HT(2a) and 5-HT(2B/2C) receptors in the behavioral interactions between serotonin and catecholamine reuptake inhibitors. Neuropsychopharmacology. 2001; 24:319–329. [PubMed: 11166521]

Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors: their key role in drugs to treat schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2003; 27:1159–1172. [PubMed: 14642974]

Meneses A, Hong E. Role of 5-HT1B, 5-HT2A and 5-HT2C receptors in learning. Behav. Brain Res. 1997; 87:105–110. [PubMed: 9331478]

Mengod G, Nguyen H, Le H, Waeber C, Lübbert H, Palacios JM. The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by in situ hybridization histochemistry. Comparison with receptor binding distribution. Neuroscience. 1990a; 35:577–591. [PubMed: 2381516]

Mengod G, Pompeiano M, Martínez-Mir MI, Palacios JM. Localization of the mRNA for the 5-HT2 receptor by in situ hybridization histochemistry. Correlation with the distribution of receptor sites. Brain Res. 1990b; 524:139–143. [PubMed: 2400925]

Millan MJ. Serotonin 5-HT_{2C} receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. Therapie. 2005; 60:441–460. [PubMed: 16433010]

Moro O, Lameh J, Hogger P, Sadee W. Hydrophobic amino acid in the i2 loop plays a key role in receptor-G protein coupling. J. Biol. Chem. 1993; 268:22273–22276. [PubMed: 8226735]

Moya PR, Berg KA, Gutierrez-Hernandez MA, Saez-Briones P, Reyes-Parada M, Cassels BK, Clarke WP. Functional Selectivity of Hallucinogenic Phenethylamine and Phenylisopropylamine Derivatives at Human 5-Hydroxytryptamine (5-HT)2A and 5-HT2C Receptors. J. Pharmacol. Exp. Ther. 2007; 321:1054–1061. [PubMed: 17337633]

Navailles S, De Deurwaerdère P, Porras G, Spampinato U. In vivo evidence that 5-HT_{2C} receptor antagonist but not agonist modulates cocaine-induced dopamine outflow in the rat nucleus accumbens and striatum. Neuropsychopharmacology. 2004; 29:319–326. [PubMed: 14560323]

Navailles S, De Deurwaerdère P, Spampinato U. Clozapine and haloperidol differentially alter the constitutive activity of central serotonin2C receptors in vivo. Biol. Psychiatry. 2006a; 59:568–575. [PubMed: 16182256]

Navailles S, Moison D, Cunningham KA, Spampinato U. Differential regulation of the mesoaccumbens dopamine circuit by serotonin2C receptors in the ventral tegmental area and the nucleus accumbens: an *in vivo* microdialysis study with cocaine. Neuropsychopharmacology. 2008; 33:237–246. [PubMed: 17429406]

Navailles S, Moison D, Ryczko D, Spampinato U. Region-dependent regulation of mesoaccumbens dopamine neurons *in vivo* by the constitutive activity of central serotonin2C receptors. J. Neurochem. 2006b; 99:1311–1319. [PubMed: 17018023]

Nic Dhonnchadha BÁ, Cunningham KA. Serotonergic Mechanisms in Addiction-Related Memories. Behavioral Brain Res. 2008 in press.

Niswender CM, Copeland SC, Herrick-Davis K, Emeson RB, Sanders-Bush E. RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. J. Biol. Chem. 1999; 274:9472–9478. [PubMed: 10092629]

Pasqualetti M, Ori M, Castagna M, Marazziti D, Cassano GB, Nardi I. Distribution and cellular localization of the serotonin type 2C receptor messenger RNA in human brain. Neuroscience. 1999; 92:601–611. [PubMed: 10408609]

Pehek EA, Nocjar C, Roth BL, Byrd TA, Mabrouk OS. Evidence for the preferential involvement of 5-HT_{2A} serotonin receptors in stress- and drug-induced dopamine release in the rat medial prefrontal cortex. Neuropsychopharmacology. 2006; 31:265–277. [PubMed: 15999145]

Phillipson OT. The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of Tsai in the rat. J. Comp. Neurol. 1979; 187:85–98. [PubMed: 489779]

Pierucci M, Di Matteo V, Esposito E. Stimulation of serotonin2C receptors blocks the hyperactivation of midbrain dopamine neurons induced by nicotine administration. J. Pharmacol. Exp. Ther. 2004; 309:109–18. [PubMed: 14722316]

Pompeiano M, Palacios JM, Mengod G. Distribution of the serotonin 5-HT_2 receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. Brain Res. Mol. Brain Res. 1994; 23:163–178. [PubMed: 8028479]

Porras G, Di Matteo V, Fracasso C, Lucas G, De Deurwaerdère P, Caccia S, Esposito E, Spampinato U. 5-HT_{2A} and 5-$HT_{2C/2B}$ receptor subtypes modulate dopamine release induced *in vivo* by amphetamine and morphine in both the rat nucleus accumbens and striatum. Neuropsychopharmacology. 2002; 26:311–324. [PubMed: 11850146]

Pozzi L, Acconcia S, Ceglia I, Invernizzi RW, Samanin R. Stimulation of 5-hydroxytryptamine (5-HT(2C)) receptors in the ventrotegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. J. Neurochem. 2002; 82:93–100. [PubMed: 12091469]

Price RD, Weiner DM, Chang MS, Sanders-Bush E. RNA editing of the human serotonin 5-HT2C receptor alters receptor-mediated activation of G13 protein. J. Biol. Chem. 2001; 276:44663–44668. [PubMed: 11572865]

Prisco S, Pagannone S, Esposito E. Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. Pharmacol. Exp. Ther. 1994; 271:83–90.

Quirk K, Lawrence A, Jones J, Misra A, Harvey V, Lamb H, Revell D, Porter RH, Knight AR. Characterisation of agonist binding on human 5-HT2C receptor isoforms. Eur. J. Pharmacol. 2001; 419:107–112. [PubMed: 11426831]

Ramos M, Goni-Allo B, Aguirre N. Administration of SCH 23390 into the medial prefrontal cortex blocks the expression of MDMA-induced behavioral sensitization in rats: an effect mediated by 5-HT2C receptor stimulation and not by D1 receptor blockade. Neuropsychopharmacology. 2005; 30:2180–2191. [PubMed: 15841107]

Rasmussen SG, Jensen AD, Liapakis G, Ghanouni P, Javitch JA, Gether U. Mutation of a highly conserved aspartic acid in the beta2 adrenergic receptor: constitutive activation, structural instability, and conformational rearrangement of transmembrane segment 6. Mol. Pharmacol. 1999; 56:175–184. [PubMed: 10385699]

Raymond, J.; Turner, J.; Gelasco, A.; Ayiku, H.; Coaxum, S.; Arthur, J.; Garnovskaya, M. 5-HT receptor signal transduction pathways. In: Roth, BL., editor. The Receptors: The Serotonin Receptors: From Molecular Pharmacology to Human Therapeutics. Human Press; Totowa, New Jersey: 2006. p. 143-206.

Samama P, Bond RA, Rockman HA, Milano CA, Lefkowitz RJ. Ligand-induced overexpression of a constitutively active beta2-adrenergic receptor: pharmacological creation of a phenotype in transgenic mice. Proc. Natl. Acad. Sci. USA. 1997; 94:137–141. [PubMed: 8990174]

Schapira AH, Bezard E, Brotchie J, Calon F, Collingridge GL, Ferger B, Hengerer B, Hirsch E, Jenner P, Novere NL, Obeso JA, Schwarzschild MA, Spampinato U, Davidai G. Novel pharmacological targets for the treatment of Parkinson's disease. Nat. Rev. Drug Discov. 2006; 5:845–854. [PubMed: 17016425]

Scheer A, Costa T, Fanelli F, De Benedetti PG, Mhaouty-Kodja S, Abuin L, Nenniger-Tosato M, Cotecchia S. Mutational analysis of the highly conserved arginine within the Glu/Asp-Arg-Tyr motif of the alpha(1b)-adrenergic receptor: effects on receptor isomerization and activation. Mol. Pharmacol. 2000; 57:219–231. [PubMed: 10648631]

Schmauss C. Regulation of serotonin2C receptor pre-mRNA editing by serotonin. Int Rev. Neurobiol. 2005; 63:83–100. [PubMed: 15797466]

Sesack SR, Carr DB, Omelchenko N, Pinto A. Anatomical substrates for glutamate-dopamine interactions: evidence for specificity of connections and extrasynaptic actions. Ann. N. Y. Acad. Sci. 2003; 1003:36–52. [PubMed: 14684434]

Sharma SC, Rupasinghe CN, Parisien RB, Spaller MR. Design, synthesis, and evaluation of linear and cyclic peptide ligands for PDZ10 of the multi-PDZ domain protein MUPP1. Biochemistry. 2007; 46:12709–12720. [PubMed: 17939682]

Steffensen SC, Svingos AL, Pickel VM, Henriksen SJ. Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. J. Neurosci. 1998; 18:8003–8015. [PubMed: 9742167]

Stein C, Davidowa H, Albrecht D. 5-HT(1A) receptor-mediated inhibition and 5-HT(2) as well as 5-HT(3) receptor-mediated excitation in different subdivisions of the rat amygdala. Synapse. 2000; 38:328–337. [PubMed: 11020236]

Stiedl O, Misane I, Koch M, Pattij T, Meyer M, Ogren SO. Activation of the brain 5-HT2C receptors causes hypolocomotion without anxiogenic-like cardiovascular adjustments in mice. Neuropharmacology. 2007; 52:949–957. [PubMed: 17141810]

Sugimoto Y, Nakato T, Kita A, Takahashi Y, Hatae N, Tabata H, Tanaka S, Ichikawa A. A cluster of aromatic amino acids in the i2 loop plays a key role for Gs coupling in prostaglandin EP2 and EP3 receptors. J. Biol. Chem. 2004; 279:11016–11026. [PubMed: 14699136]

Swanson LW. The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res. Bull. 1982; 9:321–353. [PubMed: 6816390]

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka BK, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton P, Miller K, Spedding M, Mailman RB. Functional selectivity and classical concepts of quantitative pharmacology. J. Pharmacol. Exp. Ther. 2007; 320:1–13. [PubMed: 16803859]

Van Bockstaele EJ, Pickel VM. GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain Res. 1995; 682:215–221. [PubMed: 7552315]

Wang Q, O'Brien PJ, Chen CX, Cho DS, Murray JM, Nishikura K. Altered G protein-coupling functions of RNA editing isoform and splicing variant serotonin2C receptors. J. Neurochem. 2000; 74:1290–1300. [PubMed: 10693963]

Werry TD, Christopoulos A, Sexton PM. Mechanisms of ERK1/2 regulation by seven-transmembrane-domain receptors. Curr. Pharm. Des. 2006; 12:1683–1702. [PubMed: 16712482]

Werry TD, Gregory KJ, Sexton PM, Christopoulos A. Characterization of serotonin 5-HT2C receptor signaling to extracellular signal-regulated kinases 1 and 2. J. Neurochem. 2005; 93:1603–1615. [PubMed: 15935077]

Werry TD, Loiacono R, Sexton PM, Christopoulos A. RNA editing of the serotonin 5-HT-2C receptor and its effects on cell signalling, pharmacology and brain function. Pharmacol. Ther. 2008 in press.

Whitton P, Curzon G. Anxiogenic-like effect of infusing 1-(3-chlorophenyl) piperazine (mCPP) into the hippocampus. Psychopharmacology (Berl). 1990; 100:138–140. [PubMed: 2296623]

Willins DL, Meltzer HY. Serotonin 5-HT_{2C} agonists selectively inhibit morphine-induced dopamine efflux in the nucleus accumbens. Brain Res. 1998; 781:291–299. [PubMed: 9507167]

Wood MD, Heidbreder C, Reavill C, Ashby CR Jr. Middlemiss DN. 5-HT_{2C} receptor antagonists: potential in schizophrenia. Drug Dev. Res. 2001; 54:88–94.

Wright DE, Seroogy KB, Lundgren KH, Davis BM, Jennes L. Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. J. Comp. Neurol. 1995; 351:357–373. [PubMed: 7706547]

Yan QS. Activation of 5-HT2A/2C receptors within the nucleus accumbens increases local dopaminergic transmission. Brain Res. Bull. 2000; 51:75–81. [PubMed: 10654584]