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Role of central 5-HT$_{2C}$ receptor in the control of basal ganglia functions

Ennio Esposito[1], Vincenzo Di Matteo[1], Massimo Pierucci[1]
Arcangelo Benigno[2] and Giuseppe Di Giovanni[2]
[1]Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri
Sud, 66030 Santa Maria, Imbaro (Chieti), Italy; [2]Dipartimento di Medicina
Sperimentale, Sezione di Fisiologia Umana "G. Pagano", Università
di Palermo, 90134 Palermo, Italy

Abstract

The basal ganglia are a highly interconnected group of subcortical nuclei in the vertebrate brain that play a critical role not only in the control of movements but also in some cognitive and behavioural functions. Several recent studies have emphasized that serotonergic pathways in the central nervous system (CNS) are intimately involved in the modulation of the basal ganglia and in the pathophysiology of human involuntary movement disorders. These observations

Correspondence/Reprint request: Dr. Giuseppe Di Giovanni, Dipartimento di Medicina Sperimentale, Sezione
di Fisiologia Umana, "G. Pagano", Università degli Studi di Palermo, Corso Tuköry 129, 90134 Palermo, Italy
E-mail: g.digiovanni@unipa.it

are supported by anatomical evidence demonstrating large serotonergic innervation of the basal ganglia. In fact, serotonergic terminals have been reported to make synaptic contacts with both dopamine (DA)-containing neurons and γ-aminobutyric acid (GABA)-containing neurons in the striatum, globus pallidus, subthalamus and substantia nigra. These brain areas contain the highest concentration of serotonin (5-HT), with the substantia nigra pars reticulata receiving the greatest input. Furthermore, in these structures a high expression of 5-HT different receptor subtypes has been revealed. In this paper, evidence demonstrating the serotonergic control of basal ganglia functions will be reviewed, focusing on the role of the 5-HT$_{2C}$ receptor subtype. Moreover, the involvement of 5-HT$_{2C}$ receptors in neurological disorders such as Parkinson's diseases and other related motor disorders, and their management with drugs acting on 5-HT$_{2C}$ receptor will be discussed.

Introduction

Since the 1950s, when serotonin (5-HT) was discovered in the mammalian central nervous system (CNS), an enormous amount of experimental evidence has revealed the pivotal role of this biogenic amine in a bewildering diversity of behavioural and physiological processes. This is not surprising, considering the almost ubiquitous distribution of 5-HT-containing axon terminals throughout the CNS, although 5-HT is synthesized by a small group of neurons within the raphe nuclei of the brain stem. Despite this broad axon-terminal domain of 5-HT neurons, a closer examination reveals a preferential targeting of motor areas in the CNS [1]. For example, in the rat there is a very dense innervation of the ventral horn of the spinal cord, the motor nucleus of the trigeminal, the facial motor nucleus and all components of the basal ganglia circuitry [2]. It is thus likely that 5-HT plays a role in regulating the appropriate selection of voluntary movements by the basal ganglia and abnormalities in 5-HT transmission might contribute to the neural mechanisms underlying disorders of basal ganglia origin, such as Parkinson's disease (PD), and the treatment complications of such disorders, for example levodopa-induced dyskinesia. Indeed, biochemical evidence suggests that 5-HT transmission is abnormal in the basal ganglia of patients with PD [3]. Moreover atypical antipsychotic drugs (APDs) with affinity for 5-HT$_2$ receptors have less motor side-effects [4].

During the last decades, advances in the understanding of receptors mediating the effect of 5-HT have represented one of the success stories of neuropharmacology. Many of the 5-HT receptors are found within the basal ganglia and are most likely involved in the modulation of basal ganglia circuitry and in the pathology of their correlated disorders. Of particular interest with respect to the development of new treatments for PD and other motor disorders is the 5-HT$_{2C}$ receptor subtype. This will be the subject of further

discussion in the remainder of this review. First, the anatomical and physiological organization of basal ganglia and the 5-HT system will be briefly summarized. Thereafter, several aspects of 5-HT$_{2C}$ control of pathophysiology of basal ganglia nuclei will be discussed.

Overview of basal ganglia anatomy and functions

The basal ganglia are the largest subcortical nuclei of vertebrate brain including human forebrain, and they are placed in a key position to influence motor behaviour, emotions, and cognition [5]. Even the understanding of the basal ganglia circuits still must be considered as highly incomplete, although knowledge has grown rapidly during the last decades. The model presented here is simplified and mainly limited to the aspects most relevant to the discussion [6,7]. The basal ganglia in the vertebrate brain consist of several different nuclei (Fig. **1**), the striatum (ST), the globus pallidus (GP) and its equivalent in primates, the external segment of the globus pallidus (GPe), the entopeduncular nucleus (EP) and its equivalent in primates, the internal segment of the globus pallidus (GPi), the subthalamic nucleus (STN) and the substantia nigra (SN) and each of these is profoundly important clinically [8].

Recently, it has been suggested that the pedunculopontine nucleus (PPN) of the brainstem should be considered as part of the basal ganglia as well [9,10]. Indeed, it is anatomically and physiologically associated with them and affects the function of several nuclei in the basal ganglia circuits [9,10].

The striatum (or caudate-putamen) is the main input nucleus, which receives topographical excitatory projections from almost the entire cerebral cortex, especially from the sensorimotor and frontal cortex [11]. The striatum and the downstream structures in the basal ganglia are organised in topographically and functionally segregated pathways. The cortical inputs to the striatum are convergent, in such a way, for example, that sensory and motor cortex areas converge into single striatal zones [12]. Close to the striatum is located the GPi and the substantia nigra pars reticulata (SNr), the main output nuclei of the basal ganglia [13]. They project, via various thalamic nuclei, to most cortical areas of the frontal lobe [14]. This architecture means that the basal ganglia are part of extensive loops, basal ganglia-thalamocortical circuits, which link almost the entire cerebral cortex to the frontal lobe. The GPi and the SNr also have descending output to the brain stem, especially with the PPT nucleus. Through this pathway the basal ganglia can influence brain stem functions like the inhibition of auditory input [15]. The striatum can be divided into three main parts: the putamen, the caudate nucleus, and the ventral striatum. This division roughly corresponds to a functional division of basal ganglia-thalamocortical circuits: (sensori)motor circuits of the putamen, with output
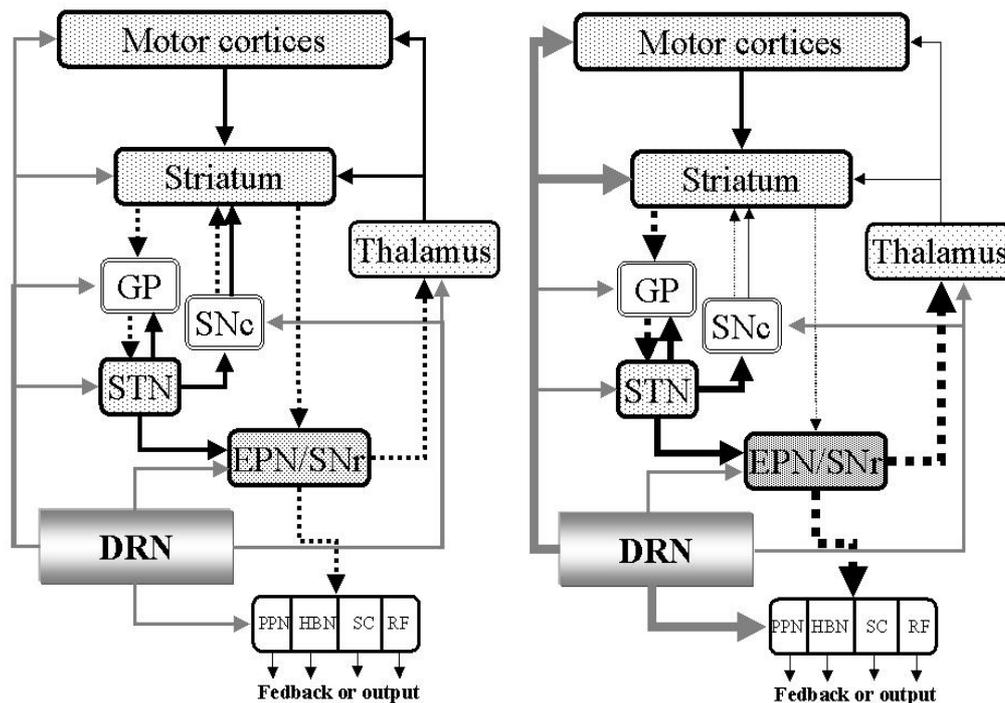
Figure 1. Schematic diagrams of the circuitry of basal ganglia in rodents under normal (left) and hypothetical Parkinsonian conditions (right). The classical model is completed with the presence of the dorsal raphe nucleus (DRN) and its projections to the different basal ganglia nuclei (in grey). Inhibitory projections are shown as black dashed arrows, excitatory as black normal arrows. The presence of the 5-HT$_{2C}$ receptors is indicated by dots in the relative boxes of the diagram, the concentration of dots indicates the amount of expression. The absence of 5-HT$_{2C}$ receptors is shown by empty boxes enclosed with a double line. In the pathological condition, thin lines are pathways with reduced activity; medium lines are physiologically normal pathways; thicker lines are over-active pathways implicated in Parkinson's disease. For further details see the main text. Abbreviations: GP, globus pallidus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; EP, entopeduncular nucleus; PPN, pedunculopontine nucleus; HBN, lateral habenular nucleus; SC, superior colliculus; PR, parvicellular reticular formation.

to primary motor cortex, the supplementary motor cortex (SMA), and the premotor cortex; associative circuits of the caudate nucleus, with output to the prefrontal cortex; and limbic circuits of the ventral striatum, with output to the anterior cingulate cortex and medial prefrontal cortex [11,13]. The ventral (limbic) striatum also receives input from limbic structures, such as the amygdala and hippocampus [16]. The striatum projects to the output structures (GPi and SNr) by two pathways, the so-called direct and indirect pathways. The indirect pathway also includes the STN. All the projections from the striatum, the GPe, the GPi and SNr release GABA and are inhibitory, while the projections from the cortex, the STN and the thalamus are excitatory, and use

glutamate as their neurotransmitter. The GABA-conteining neurons in the GPi and the SNr are tonically active, they project to the ventral tier of thalamus (ventrolateral, ventromedial, ventral anterior nuclei) and form inhibitory synaptic contacts with thalamocortical neurons that project to the motor and premotor cortex. Activation of the direct pathway inhibits GPi/SNr neurons, which in turn disinhibits thalamic neurons, finally resulting in excitation of the cortical neurons. Activation of the indirect pathway has an opposite effect, activating the GPi/SNr and thereby inhibiting the cortex [13]. In this way, the two pathways balance each other, modulating cortical activity. Alexander and Crutcher [17] suggested a model where the indirect pathway provides a diffuse background inhibition of behavioural impulses, while the direct pathway gives a focused activation of the desired behavioural program. In this model, the basal ganglia play an important role in inhibiting potentially competing motor programs. This may be a general mechanism for action selection where "the winner takes all", by facilitation of the strongest cortical signal and suppression of the rest [18]. Recently, it has been proposed by Wilkström and co-workers that the basal ganglia can also elicit a behaviourally meaningful and varied motor pattern without the involvement of the cerebral cortex [19]. Among the basal ganglia nuclei, the striatum seems to have a prominent role in determining when a given motor program should be selected and called into action. For the hypothesised function of the striatum in selection of motor programs, a certain level of tonic dopamine (DA) activity is required. Dopamine projections from the substantia nigra pars compacta (SNc) to the striatum modulate the activity of striatal neurons in a complex way. According to a simplified model, the striatal neurons forming the direct pathway mainly express excitatory D1-receptors, while the striatal neurons in the indirect pathway mainly have inhibitory D2-receptors. This means that DA would facilitate motor behaviours through the activation of the direct pathway and conversely through the inhibition of the indirect one. Reduced DA innervation of the striatum results, indeed, in hypokinesia and difficulty in initiating different motor patterns, including facial expression [20], enhanced striatal DA activity will instead give rise to hyperkinesia (i.e. premature or unintended activation of motor programs). Dopamine also seems to be involved in basal ganglia learning processes, by strengthening or weakening the efficacy of corticostriatal synapses [6,21,22]. In this way the striatum may learn to respond to certain patterns of cortical activation.

Serotonin innervation of basal ganglia

More than fifty years have passed since Twarog and Page [23] isolated an indole, identified as serotonin (5-HT), in the mammalian brain. Subsequently, Brodie and colleagues [24] suggested that 5-HT might serve as a neuro-transmitter in the central nervous system (CNS).

In vertebrates, the majority of the neurons containing 5-HT are grouped in 9 nuclei named B1 to B9, located in the medial part of the brainstem, generically called the raphe nuclei [25] (Fig. **2**). These midline clusters can be divided into two major groups. The caudal or inferior group, localized in the medulla, contains the three nuclei projecting essentially to the grey matter of the spinal cord: the nucleus raphe magnus (NMR, cell group B5), nucleus raphe obscurus (NRO, cell groups B1-B2-B3), and nucleus raphe pallidus (NRP, cell group B4). The rostral or superior group, located in the pons/mesenchepalon, contains the dorsal raphe nucleus (DRN, cell groups B6 and B7) and the medial raphe nucleus (MRN, cell group B8).

These nuclei supply about 80% of the serotonergic innervation to the forebrain. Even if in many brain areas, the innervation coming from the two nuclei overlaps, in certain regions the innervation comes exclusively or pre-valently from one nucleus only. For example, the dorsal hippocampus receives a serotonergic innervation only from MRN, other areas innervated preferentially
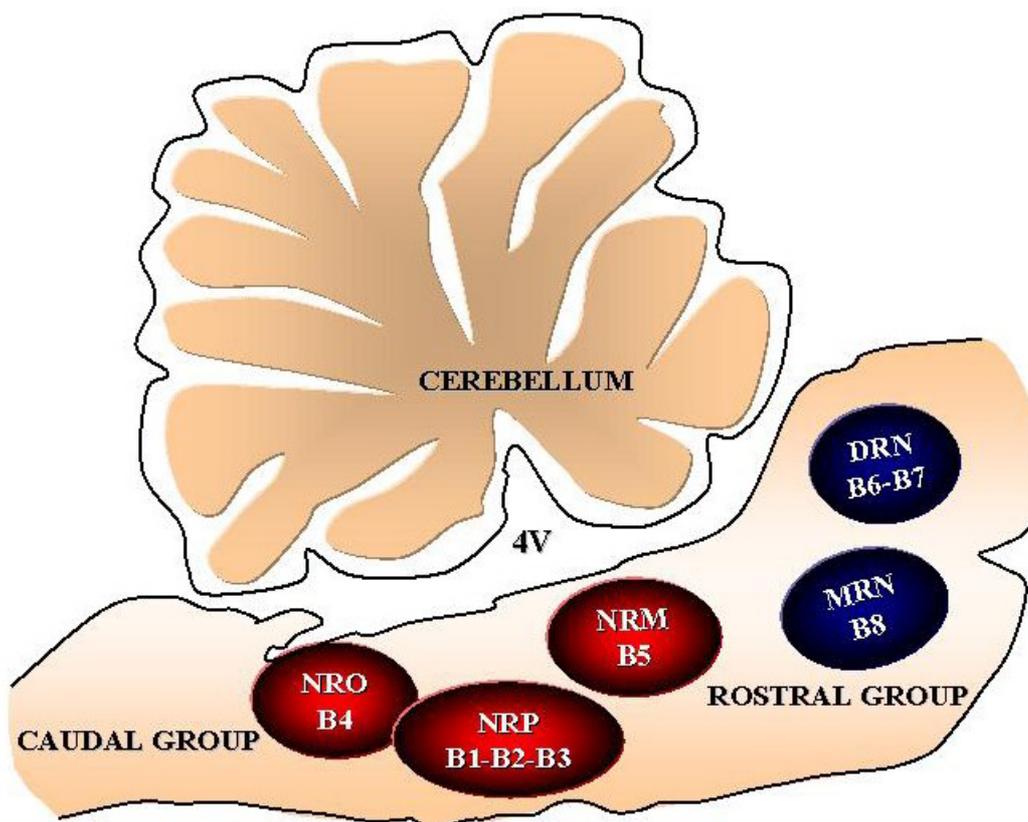

Figure 2. Midsaggital view of the rat brainstem with serotonin-immunoreactive cell body groups. The blue and red ovals encompass the two major subdivisions of the brain serotonergic system. Abbreviations: DRN, dorsal raphe nucleus; MRN, medial raphe nucleus; NRM; nucleus raphe magnus; NRO, nucleus raphe obscurus. Cell groups B1 to B9 according to the terminology of Dahlström and Fuxe [25].

from this nucleus are: the medial preoptic area, the suprachiasmatic nucleus, the olfactory bulb and the medial septum nucleus. The dorsal raphe nucleus innervates all the basal ganglia circuitry (Fig. **1**), sending projections to the corpus striatum, the GP, the STN, SN and the PPN, and provides most of the innervation of the prefrontal cortex, including the motor cortices. Serotonin-containing cell bodies of the raphe send projections to both dopaminergic and GABAergic cells in the substantia nigra (SN), and to their terminal fields [26-29]. Moreover, electron microscopy demonstrates the presence of synaptic contacts of [^{3}H]5-HT labeled terminals with both dopaminergic and non-dopaminergic dendrites in the SN pars compacta and reticulata [9,26,29]. The DRN innervates, together with the MRN the ventral part of the hippocampus, the nucleus accumbens and various nuclei of the thalamus among them the ventral lateral nuclear group that processes motor information and the hippocampus [31-33]. Moreover, extensive serotonergic connections between the DRN and the MRN also exist [34].

Serotonin$_{2C}$ receptor and its distribution within the basal ganglia nuclei

Serotonin$_{2C}$ receptors

The first evidence for the existence of multiple subtypes of 5-HT receptors was provided by Gaddum and Picarelli who discovered the so-called M and D receptors [35]. Definitive evidence for two distinct recognition sites was reported by Peroutka and Snyder [36] who classified 5-HT in two subtypes 5-HT$_1$ or 5-HT$_2$ depending on their affinity to [^{3}H]-5-HT and [^{3}H]-spiperone, respectively. A vast amount of research has led to the discovery and characterisation of a plethora of 5-HT receptor subtypes. At present, seven classes of 5-HT receptors (5-HT$_{1-7}$ receptors) have been identified which comprise at least a total of 15 subtypes [37] (Table **1**). Not surprisingly, with so many potential targets, distributed throughout all the CNS, 5-HT is a major neurotransmitter involved in a such large number of physiological and pathological processes. 5-HT receptors are cell surface receptors that have been classified on the basis of their pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organisation, and second messenger coupling pathways [37,38]. Except for the 5-HT$_3$ receptor, which is a ligand gated channel, all 5-HT receptors belong to the seven transmembrane domain G-protein-coupled receptor (GPCR) superfamily [37,39,40].

Among the multiple classes of 5-HT receptors described in the central nervous system [37,38,41], much attention has been devoted to the 5-HT$_2$ receptor family since it has been shown by experimental and clinical observation that it may represent a possible therapeutic target for the development of drugs

Table 1

Nomenclature and classification of the serotonin receptors subtypes
Receptor
Seven-transmembrane-spanning, G-protein-coupled receptors
Negatively coupled to adenylate cyclase ($G_{i/o}$)
5-HT_{1A}
5-HT_{1B}
5-HT_{1D}
5-HT_{1E}
5-HT_{1F}
Positively coupled to adenylate cyclase (G_s)
5-HT_4
5-HT_6
5-HT_7
Coupled to phospholipase C (G_q)
5-HT_{2A}
5-HT_{2B}
5-HT_{2C}
Unknowing coupling
5-HT_{5A}
5-HT_{5B}
Ligand-gated ion channel/receptor
5-HT_{3A}
5-HT_{3B}
5-HT_{3C}

for a range of CNS disorders such as schizophrenia, depression, drug abuse, eating disorders, Parkinson's disease and epilepsy [42-45]. In fact, 5-HT_2 receptors are major targets for a wide array of psychoactive drugs, ranging from atypical APDs, anxiolytics and antidepressants, which have a 5-HT_2 antagonistic action, to hallucinogens, which are agonists of the 5-HT_2 receptors [46-49]. The 5-HT_2 receptors form a closely related subgroup of G-protein-coupled receptors and show the typical heptahelical structure of an integral membrane protein monomer. They are currently classified as 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} subtypes [37,38,41,50], based on their close structural homology, pharmacology and signal transduction pathways and they are differently localized in the brain [37,38,41,50]. The amino acid sequence of the 5-HT_2 receptors shares a high degree (>70%) of identity within the transmembrane segments [37,38,41,50], consequently, it is not surprising that many compounds bind with high affinity to all these three receptor subtypes.

The 5-HT_{2C} receptor has received less attention in psychopharmacology compared to the 5-HT_{2A} sites. This may relate to the inadequacy of techniques

to determine the receptor in tissue samples. However, evidence from a variety of sources has demonstrated the involvement of this receptor in several important physiological and psychological processes including motor function, anxiety, ingestive behaviour and brain development [45,51-54]. 5-HT$_{2C}$ receptor-deficient mice are overweight as a result of abnormal control of feeding behaviour [55,56], thus this receptor could play a role in the serotonergic control of appetite. Mutant animals are also prone to spontaneous death from seizures, suggesting that receptors of this type mediate tonic inhibition of neuronal network excitability. Direct activation of the 5-HT$_{2C}$ receptors by agonists in normal rats decreases their food intake, and central 5-HT systems probably directly activate pro-opiomelanocortin (POMC) neurons via the 5-HT$_{2C}$ receptors [57-60]. The 5-HT$_{2C}$ receptor knockout mice are also more wakeful, and show several abnormalities in rapid eye movement (REM) sleep expression, and enhanced response to sleep deprivation compared with wild-type control mice. These findings suggest that the 5-HT$_{2C}$ receptors may mediate several effects on sleep that have been ascribed to 5-HT [61]. Furthermore, some interesting pharmacogenetic observations have focused on the potential importance of this receptor for the development of novel antipsychotic, antidepressant, antiparkinsonian and antiepileptic drugs [43,44,52].

The 5-HT$_{2C}$ receptor gene is unique among the members of the 5-HT receptors family by virtue of its genomic organization. The human 5-HT$_{2C}$ receptor gene has been mapped to X-chromosome band q24 [62] and shown to contain six exons and five introns. The gene product is a protein of 460 amino acids, heavily glycosylated and migrating as a 60-kDa protein [63]. The 5-HT$_{2C}$ receptor is a member of the GPCR superfamily with 7-transmembrane spanning domains and three intracellular loops connecting them. The 5-HT$_{2C}$ receptor is the only known GPCRs whose mRNA undergoes post-trascriptional editing to yield different isoforms [64]. Although RNA editing produces at least 14 functional isoforms (and potentially many more) of the receptor, these isoforms are associated with a variation in the efficacy of their interaction with G protein [65-67]. In this regard, it was recently reported that the depletion of 5-HT increases expression of 5-HT$_{2C}$ mRNA isoforms encoding receptors with higher sensitivity to 5-HT. This evidence suggests that this editing acts as a fine-tuning mechanism that adjusts receptor function to changes in synaptic activity, to maintain their normal response properties to agonist stimulation [68]. Interestingly, alteration of the 5-HT$_{2C}$ pre-mRNA editing has been implied in several psychiatric disorders [69] but, to date, no evidence exists for its involvement in neurological disorders, even though Fox and colleagues have suggested that this could occur in PD [70]. Decreased agonist binding affinity has also been linked to RNA editing, however antagonist binding remained unaltered [71].

As regards the signalling pathway, agonist binding to the 5-HT$_{2C}$ receptor activates phospholipase C (PLC) via G protein (G$_{q/11}$) [72-75]. However, other

different signalling pathways for the 5-HT_{2C} receptor subtype have been shown. Indeed, some agonists binding to the 5-HT_{2C} receptor can instead activate phospholipase A_2 (PLA_2) leading to the release of arachidonic acid (AA) [76]. Furthermore, 5-HT_{2C} receptors can also activate phospholipase D (PLD), involving $G_{\alpha 13}$ and free $G_{\beta-\gamma}$ subunits [77], the mitogen-activated protein kinase, and the extracellular signal-regulated kinase (ERK) 1 and 2 [78]. Interestingly, it was shown that coupling of 5-HT_{2C} receptors to $G_{\alpha 13}$ and $G_{q/11}$ can be differentially stimulated by certain agonists; ergots preferentially stimulates $G_{q/11}$, whereas 5-HT and RO 600175 are equally efficacious towards both pathways. Differential G-protein coupling appears to depend on receptor reserve [79] and is regulated by RNA editing [65,80]. These observations suggested that it may be possible to develop agonists which preferentially act in specific tissues via a specific signalling pathway. Amongst the 5-HT_2 receptor family, 5-HT_{2C} appears more prone to exhibit intrinsic constitutive activity. In this regard, studies have demonstrated the considerable ability of the native 5-HT_{2C} receptor to spontaneously activate intracellular signalling pathways, including PLC and PLA_2, in the absence of agonist stimulation [81-84]. This quite peculiar 5-HT_{2C} receptor characteristic occurs not only in recombinant cells but also in natural tissues and it seems to play an important role in the tonic control of the central dopaminergic function [85,86]. The occurrence of constitutive receptor activity allows the detection of compounds that can block this activity, the so-called inverse agonists [81]. Inverse agonists may show differential pharmacological and clinical effects as compared to mere antagonists and bring up important therapeutic perspectives [87]. For instance, several atypical antipsychotic drugs display inverse agonist activity at constitutive 5-HT_{2C} receptors [86,88].

In contrast to most other receptors 5-HT_{2C} is not classically regulated. Indeed, 5-HT_{2C} receptors appear not only to decrease their responsiveness upon chronic agonist stimulation, but also and paradoxically after chronic treatment with antagonists [89,90]. This mechanism appears to be related to an internalisation process that removes activated cell surface receptors from the plasma membrane involving a phosphorylation step and possible degradation in lysosomes [49]. Inasmuch as a large number of psychotropic drugs, including atypical antipsychotics, antidepressants, and anxiolytics, can all induce down-regulation of 5-HT_{2C} receptors, it has been suggested that this alteration plays a role in the therapeutic action of these drugs [47,49].

Serotonin$_{2C}$ receptor in the basal ganglia nuclei

Pazos and colleagues first identified the 5-HT_{2C} receptors by radioligand binding of $^3\text{H-5-HT}$ and $^3\text{H-mesulergine}$ in the pig choroid plexus, and initially called it 5-HT_{1C} because of its high affinity for tritiated 5-HT [91]. However, once the receptor was cloned and more information about its characteristics

became available, a shift to the 5-HT$_2$ receptor family and reclassification as 5-HT$_{2C}$ became accepted. Later studies using autoriadiography with ^{3}H-mesulergine have confirmed a conspicuously high binding site density in the choroid plexus in all mammalian species, including subhuman and human primates. High density is also found in the SNr, globus pallidus and ventromedial thalamus with low amounts detected in many other brain areas [92-94] (Fig. **1**). A widespread distribution of 5-HT$_{2C}$ receptor mRNA has been demonstrated in rat, monkey and human brains using in situ hybridization techniques [95-98]. 5-HT$_{2C}$ receptors are more abundant and more widely expressed in the brain than 5-HT$_{2A}$ receptors, although there are several areas where both receptors coexist [99]. As was found for 5-HT$_{2C}$ binding sites, the choroid plexus also contained the highest amounts of 5-HT$_{2C}$ receptor mRNA and protein, mainly associated with choroid epithelial cells [100]. High levels of both 5-HT$_{2C}$ protein and mRNA have been found in several cortical areas, in the hippocampus, striatum, septal nuclei, thalamic nuclei, midbrain nuclei, brain stem nuclei and the spinal cord [95,99,101]. A number of studies have concentrated on the localization and potential role of 5-HT$_{2C}$ receptors in the basal ganglia and have also revealed marked differences in the distribution of this mRNA among subregions of the basal ganglia [96,102,103,104], also its level of expression varied markedly within labeled nuclei [104] (Fig. **1**). In the striatum most labeled neurons were medium-sized, probably, efferent neurons. Labeling was not detected in large cholinergic interneurons or in glial cells. Neurons that express mRNA for the 5-HT$_{2C}$ receptors were found in discrete areas of the caudate-putamen, mostly in the ventral and ventrolateral parts of the nucleus. 5-HT$_{2C}$ receptor mRNA did not show a preferential colocalization with neuropeptide markers for either the striatonigral pathway (substance P and dynorphin) or the striato-pallidal projections (enkephalin). This indicates that 5-HT$_{2C}$ expression is not differentially influenced by the two main targets to which the striatum projects, and the signal mediated via the 5-HT$_{2C}$ receptor is likely to have downstream consequences in both striatal output locations showing a substantial difference from the dopaminergic system. Furthermore, 5-HT$_{2C}$ receptor mRNA is differentially localized with regard to patch/matrix striatal structure. In fact, 5-HT$_{2C}$ receptor mRNA occurs almost exclusively in the patch compartment areas, [105] revealing a preferential modulation of the projection to the SNc and thus the striatal dopaminergic input [106,107]. In the striatal target areas, dense labeling is found in the entopeduncular nucleus (internal pallidum) while no mRNA is found in the globus pallidus (external pallidum), an area where 5-HT$_{2C}$ receptor binding sites have been detected (Fig. **1**). Therefore, the differential distribution of 5-HT$_{2C}$ receptor mRNA may be indicative of differential regulation of the direct and indirect pathways of striatal output by 5-HT. The subthalamic nucleus contained large numbers of

cells that expressed a high level of 5-HT$_{2C}$ receptor mRNA, where the receptors could be involved in the oral dyskinesia that is induced by drugs with 5-HT$_{2C}$ agonist action [104]. In the SN, neurons labeled for 5-HT$_{2C}$ receptors were present in both the SNc and the SNr from rostrocaudal and mediolateral levels. Despite an homogenous 5-HT innervation of the SN, the 5-HT$_{2C}$ receptors were express in about half of the neuronal population, however, the density was markedly higher caudally than rostrally, in both nuclear subdivisions. Receptor expression is confined to GABAergic neurons and not to dopaminergic cell bodies. Other immunoistochemical and electrophysiological studies [28,108-110] have corroborated this piece of evidence suggesting an indirect control of dopaminergic transmission *via* 5-HT$_{2C}$ receptors and a preferential effect on SNr GABAergic projecting neurons as compared to SNr GABAergic interneurons (Fig. **3**).

The distribution of 5-HT$_{2C}$ receptor mRNA-expressing cells does not present any obvious similarity with the lamellar distribution of neurons projecting to different sets of target structures [111]. However, the much larger number of neurons expressing the 5-HT$_{2C}$ receptor mRNA in the caudal part of the SNr raises the possibility that this receptor may be preferentially associated

Figure 3. Schematic drawing showing the distribution of 5-HT$_{2C}$ receptors throughout the rat substantia nigra according to Di Giovanni et al. [110]. Dopaminergic neurons of the dorsal pars compacta do not express the 5-HT$_{2C}$ receptor; in the par reticulata the 5-HT$_{2C}$ receptors are present essentially on GABAergic projecting neurons and probably not on presumed GABAergic interneurons. Abbreviations: GABA, γ-aminobutyric acid; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata.

with neurons projecting to the tegmentopontine nucleus, which are more abundant in the caudal than in the rostral pars reticulata [111]. However, this hypothesis is too simplistic if we take in to consideration the complexity of collateral projections of SNr neurons that could influence neurons located in other regions within the nucleus [104,110].

Serotonin$_{2C}$ modulation of basal ganglia circuitry
Serototnin$_{2C}$ modulation of dopaminergic nigrostriatal pathway

The precise role of 5-HT in the SNc has not yet been firmly established, although some evidence indicates a possible inhibitory role for 5-HT over the SNc neurons, mainly supported by studies involving electrical stimulation of the DRN [112-115] and microionthophoretic application of 5-HT on SNc DA neurons [116,117]. However, 5-HT itself has been shown to increase the firing rate of SNc DA neurons in vitro [118] and to facilitate a voltage-dependent calcium conductance [119].

The involvement of 5-HT$_{2C}$ receptor subtypes in the control of mesencefalic DA neurons activity is now well established [52,86,110,120-128], and evidence has been provided that it exerts both tonic and phasic modulation of DA function [85,123-131]. Furthermore, some studies carried out in our laboratory have shown a noteworthy preferential control of the mesorticolimbic compared to the nigrostriatal DA pathway by the 5-HT$_{2C}$ receptor subtype, this evidence has also been confirmed by Tecott and colleagues [132]. Indeed, firstly we showed that selective serotonin reuptake inhibitors (SSRIs) reduce the spontaneous activity of midbrain DA neurons, by acting on 5-HT$_{2C}$ receptors, in the VTA but not in the SNc [133,134]. In addition, there is the evidence that 5-HT$_{2C}$ receptor agonists mCPP, MK212 and RO 600175 and the selective antagonist SB 242084 neither significantly affect the activity of SNc DA-containing neurons nor the in vivo DA release in the striatum [38,135-137]. Lastly, the mixed 5-HT$_{2C/2B}$ receptor antagonist SB 206553 caused only a slight increase in the basal activity of DA neurons in the SNc and in the striatal DA release [138]. Consistently, it has been reported that the new selective antagonist SB 243213 does not significantly alter the basal firing rate or pattern of SNc neurons while its repeated administration modifies only the pattern discharge but not the number of spontaneously active SNc DA cells [139]. Nevertheless, another group did not find this decrease in sensitivity of the nigrostriatal DA system following the acute administration of 5-HT$_{2C}$ ligands [140]. It is worth noting, that these authors also showed that SB 206553 elicited a dose related increase in striatal DA release behaving as a 5-HT$_{2C}$ inverse agonist in vivo. Indeed, they found that SB 242084 prevented the SB 206553-induced increase and reversed the RO 600175-induced decrease in

DA release in the striatum [86]. The discrepancy between these data and our evidence is unknown. In spite of a lack of a local effect on DA cell bodies, the 5-HT$_{2C}$ receptors located on the nerve terminal regions seem to play a role in the regulation of DA release. Indeed, local administration of the antagonist SB 206553 increased DA efflux in the striatum and this effect was attenuated by systemic administration of the agonist mCPP [128].

Serotonin$_{2C}$ modulation of striatal activity

In agreement with anatomical data, most of the reports on this subject indicate that striatal cells are predominantly affected by dorsal raphe stimulation, the median raphe stimulation being unable to alter striatal cell activity directly [141,142]. In addition, it has been found that suppression of spontaneous firing activity is the main response of striatal cells following dorsal raphe stimulation, excitation being observed in only about 10% of them. Nevertheless, a rebound excitation was observed in some cells that were initially inhibited. All these changes were rapid in onset and prevented by methesergide, a 5-HT$_2$ antagonist [141,142]. Local application of 5-HT was found to produce similar changes to that caused by raphe stimulation and most of the cells responsive to the raphe stimulation were also affected by nigral stimulation [142]. The data obtained by Blier and colleagues [143] concerning single-unit recordings coupled with microiontophoresis in vivo, in rats, guinea pigs and in 5-HT$_{2C}$ receptor mutant mice, are consonant with the hypothesis of an inhibitory action of the 5-HT system on the neuronal activity of the striatum. These authors reported that in the caudate nucleus the inhibitory effect of 5-HT was mimicked by the 5-HT$_{2C}$ receptor agonist mCPP [143,144]. Furthermore, significantly less quisqualate was required to activate neurons in the caudate nucleus of mutant mice than in the wild-type mice, suggesting that 5-HT$_{2C}$ receptors serve a tonic inhibitory role in membrane excitability [145]. In concordance with these electrophysiological findings, there is the behavioural evidence that caudate injections of 5-HT provoked contraversive turning, conversely intracaudate methysergide induced ipsiversive circling [146]. These data are compatible with 5-HT having a tonic inhibitory function in the striatum. In addition, the facilitation by hyoscine and the attenuation by eserine of the 5-HT induced contraversive circling, together with the converse effects of these drugs on methysergide-evoked ipsiversive rotations, are consistent with raphe-caudate 5-HT fibres synapsing directly with, and inhibiting, striatal cholinergic neurons. Given that motor behaviour recruits multiple striatal neurotransmitter systems, recent attention has focused on the interaction between DA and 5-HT receptors. In the intact striatum, several studies have consistently demonstrated that intrinsic 5-HT$_2$ receptors can modify DA function, and have postulated divergent roles for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes [123,147]. 5-HT$_{2A}$ antagonists reduce hyperlocomotion induced by cocaine, amphetamine and 3,4-methylenedioxymethyamphetamine

(MDMA) [148,149], whereas, 5-HT$_{2C}$ receptor antagonists have been shown to enhance or reduce these effects depending upon which compounds and neuronal sites are studied [150-152]. Recent studies from Walker's group have shown that following DA depletion D1-induced locomotor activity can be reduced by antagonism of striatal 5-HT$_{2A}$, but not 5-HT$_{2C}$ receptors [153]. This preferential involvement of the 5-HT$_{2A}$ subtype is confirmed by the evidence that intrastriatal injections of the selective 5-HT$_{2C}$ antagonist RS 102221 had no effect on motor activity, conversely the 5-HT$_{2A}$ agonist DOI induced motor behaviour in neonate 6-OHDA-lesioned rats which was attenuated by the 5-HT$_{2A}$ receptor antagonism [154].

However, using intracellular recording techniques, Kitai and co-workers [155] found that dorsal raphe stimulation was consistently capable of generating excitatory postsynaptic potentials. These findings were subsequently repeated in the Kitai laboratory, and it was indicated that non-5-HT dorsal raphe-striatal neurons could be involved in striatal responses to 5-HT. They also showed that the increase of the firing frequency in rat neostriatal medium-spiny neurons induced by 5-HT depended on reducing voltage-dependent potassium currents [156,157]. A recent in vivo study showed a consistent excitation of the striatal neurons induced by microintophoretical application of 5-HT while the mixed agonist 5-HT$_{2A/2C}$ DOI caused a preferential inhibitory response, highlighting varying effects of different 5-HT receptor subtypes [158].

Interestingly, recent pharmacological data and lesion studies have established that the biosynthesis of neuropeptides in the striatum is regulated by the 5-HT innervation originating from the dorsal raphe suggesting that 5-HT in the striatum might exert a metabolic regulatory function on the biosynthesis of neuropeptides rather than acting as ion channel modulator [159,160].

Serotonin$_{2C}$ modulation of substantia nigra pars reticulata activity

The SNr neurons receive the largest 5-HT innervation from the DRN of all brain regions [161,162], and express a high level of 5-HT$_{2C}$ receptors which are post-synaptic and locatedon the somato-dendritic region of SNr neurons. It has been calculated that the density of 5-HT-immunoreactive varicosities in the SNr is in the order of $9x10^{6}/mm^{3}$, of which about 74% form synaptic specializations with GABAergic projection neurons [163]. This picture gives an idea of the great influence that 5-HT could exert on the activity of SNr neurons. Early studies have revealed a primarily inhibitory action of serotonin on SNr neurons. Electrical stimulation of the DRN caused mostly inhibitory responses in the SNr, as measured by single-unit extracellular recording in vivo [161]. Similarly, the iontophoresis of 5-HT into the SN produced mixed, although mostly inhibitory, effects in the SNr [164,116]. The inhibition of SNr

neurons by 5-HT was supported by the finding that unilateral injection of 5-HT into the SNr induced controlateral circling, as did muscimol [165]. Bilateral injection of 5-HT into the SNr increased vacuous chewing movements (a model of drug-induced dyskinesia) [166], mimicking another of the behavioural effects of intranigral muscimol. Furthermore, the bilateral intranigral injection of fluoxetine was anticonvulsant in an animal model of epilepsy. In spite of this evidence, Lacey and co-workers have shown, by using in vitro electrophysiological methods, that 5-HT not only excites SNr neurons directly but also disinhibits them by reducing GABA release from striotonigral terminals [167,168]. The 5-HT-induced excitation is observed in the majority of the SNr neurons recorded and most probably mediated by a direct action on 5-HT_{2C} receptors being blocked by ketanserin and ritanserin and mimicked by α–methyl 5-hydroxytriptamine, unselective antagonists and agonist of the 5-HT_{2C} receptor subtype, respectively [167,168]. In addition, Góngora-Alfaro and colleagues' in vitro study revealed that 5-HT can excite about half of SNr neurons tested, this effect was seen in the neurons and blocked by methysergide, thus confirming the involvement of 5-HT_{2C} receptors [169]. These authors also showed a direct 5-HT-inhibitory action on SNr neurons probably mediated by 5-HT_1 receptors [169]. Consistent with these findings, we showed that mCPP excites SNr neurons by activating 5-HT_{2C} receptors in vivo [110]. This effect was evident both after systemic administration and local microiontophoretic application of mCPP. Although mCPP is not a selective 5-HT_{2C} agonist, because it also acts on other 5-HT receptor subtypes, its effects on neurons in the SNr are mediated by the 5-HT_{2C} receptor, inasmuch as SB 242084, a potent and selective 5-HT_{2C} antagonist, completely blocks the excitation elicited by mCPP. An interesting finding of our study was the differential effect exerted by mCPP on subpopulations of SNr neurons. Thus, mCPP caused a marked excitation of the so-called P(0) non-DA neurons in the SNr, whereas it did not affect the P(+) neurons. These neurons are identified on the basis of the presence P(+) or the absence P(0) of an excitatory response to a noxious stimulus (footpinch). There is evidence that P(+) neurons in the SNr are GABAergic interneurons that exert a direct inhibitory influence on DA neurons in the SNc, whereas P(0) cells represent SNr projection neurons. Thus, mCPP caused a marked excitation of presumed SNr projection neurons but did not modify the SNr interneurons firing discharge [110]. It is tempting to speculate that this differential response to mCPP might be the basis of the preferential inhibitory effect of 5-HT_{2C} agonists on the mesolimbic versus the nigrostriatal dopaminergic function. These data have been confirmed by some experiments that are presently under way in our laboratory using the most selective agonist and antagonist currently available for the 5-HT_{2C} receptor to date. In fact, about 50% of SNr neurons are excited by the selective agonist RO 600175 and this effect is counteracted by pre-treatment with the new antagonist SB 243213. Microintophoretic application of RO 600175

clearly showed a direct effect of the 5-HT$_{2C}$ receptors on the SNr neurons, antagonised by SB 243213 applied by pressure ejection (unpublished observations).

Nevertheless, unilateral microinjection of 5-HT, 5-HT$_{1D}$ agonist, and SSRIs, into the SNr of freely moving rats elicited a contraversive circling behaviour blocked by systemic pretreatment with methysergide [146,165,170-172]. Current theories of circling behaviour hypothesise that the animal turns away from the basal ganglia output where activity has been reduced. Taken together this evidence, apparently in contradiction with the latest in vivo and in vitro electrophysiological studies, suggests that endogenously released 5-HT exerts a predominantly inhibitory effect on the SNr output neurons. In order to explain these discrepancies, it is possible to argue that 5-HT released in vivo elicits an excitatory response in a discrete population of SNr neurons, which in turn inhibits a greater number of neighbouring SNr cells through GABA release from their extensive axon collaterals [173]. The fact that methysergide inhibited the contraversive circling induced by SSRIs nicely fits with the ability of the unselective 5-HT$_{2C}$ receptor antagonists to block the excitatory action of 5-HT or 5-HT$_{2C}$ receptor agonists on SNr neurons in vitro and in vivo [110,167-169, our unpublished observations]. Interestingly, it has been shown that 5-HT$_{2C}$ receptor transmission may be a key determinant in the activity of SNr in parkinsonism but not in the "normal" basal ganglia. Indeed, intranigral infusion of SB 206553 into the SNr on the unlesioned side of 6-hydroxydopamine (6-OHDA)-lesioned rat did not elicit a significant rotational response [174]. In keeping with this, there is evidence that a number of selective antagonists, SB 200646A, SB 206553 and SB 242084, do not elicit locomotory activity when given alone [175-177]. Conversely, infusion of SB 206553 into the SNr on the 6-OHDA-lesioned side elicited a marked rotational response, contraversive to the injection [174]. Such behaviour represents a reduction in activity of basal ganglia outputs and can be taken as representing a potential anti-parkinsonian action. Moreover, systemic administration of SB 206553 enhanced the action of D2 agonist quinpirole and D1 agonist SKF 82958 in eliciting a rotational response contraversive to the lesioned side [174,178]. The mechanism whereby 5-HT$_{2C}$ receptor antagonists enhance the anti-parkinsonian action of DA receptor agonists may involve reducing the overactivity of the SNr. When given alone, 5-HT$_{2C}$ receptor antagonists may only be capable of reducing the activity to a certain degree following systemic administration. Therefore, there may not be sufficient reduction in the activity of SNr to restore the normal thalamo-cortical output and have an overt anti-parkinsonian effect.

Serotonin$_{2C}$ modulation of subthalamic nucleus activity

The subthalamic nucleus, is an important mediator of the output circuits subserving basal ganglia motor function. It is interposed in the direct pathway between the GPe and the GPi/SNr. The STN also has projections that interact

with the other primary output pathway from the striatum, the direct pathway, at the level of the GPe [179,180]. This connectivity provides the STN with a unique ability to mediate basal ganglia motor function, and accordingly, the STN has a strong influence on motor behaviour related to basal ganglia DAergic neurotransmission [181]. For instance, the STN has been implicated in the mediation of Parkinsonian movement disorders. An increase in the basal activity of the excitatory glutamatergic afferent neurons of the STN, associated with the loss of DA terminals in the striatum, may play a role in the hypokinetic symptoms of this condition [182]. Indeed, STN lesion as well as its inactivation by deep brain stimulation has shown to have antiparkinsonian effects in primates experimental PD model and in PD patients [183-185]. Under normal DA function, in both humans and primates, unilateral lesions of the STN result in hemiballism and chorea which are characterised by involuntary, hyperkinetic movements of the contralateral limbs [186,187]. Serotonin neurons, mainly from DRN, innervate the STN, and clearly modulate its neuronal activity. The $5-HT_{2C}$ receptors are most likely involved in 5-HT effects, since they are present in a relatively high concentration in this nucleus. The most frequent response to 5-HT seems to be an excitation of STN neurons [188-190]. This effect is mediated by the activation of the $5-HT_{2C}$ and $5-HT_4$ receptors being reversed by the combined use of selective antagonists for $5-HT_4$ and $5-HT_{2C}$ receptors [189,190]. Moreover, an inhibitory action of 5-HT over a small subpopulation (about 20%) of STN neurons has been also shown by Stanford et al. [190], and it seems to be mediated by $5-HT_{1A}$ receptors activation [190]. Thus, this electrophysiological evidence indicates that 5-HT-induced excitation or inhibition in the STN are separate entities and most likely to arise as a consequence of independent, direct post-synaptic effects mediated by $5-HT_{2C}$, $5-HT_4$ and $5-HT_{1A}$ subtypes. However, these electrophysiological findings are not in accordance with a large body of behavioural evidence which shows an inhibitory action of 5-HT over the STN by acting on $5-HT_{2C}$ receptors. As a result, a decrease in the excitatory input from the STN to GPe/SNr occurs, which in turn enhances the activity of the ipsilateral motor thalamus. Indeed, the unilateral injection of 5-HT into the STN induces a contralateral dose-dependent turning behaviour which is blocked by a non selective $5-HT_2$ antagonist mianserin. The contribution of the $5-HT_{2C}$ receptor in 5-HT-induced behaviour was revealed by the intrasubthalamic injection of the $5-HT_{2C}$ receptor agonist MK 212 that, in concordance, increased the net turns [191]. In addition, the blockade of subthalamic $5-HT_{2C}$ receptors suppressed the stereotypic behaviour induced by apomorphine administration [192] while both systemic administration and local unilateral infusion of m-CPP into the STN induced an increase in oral movements in rats [193-195], that resemble the orofacial dyskinesias occurring as a severe side effect of prolonged treatment with antipsychotic drugs in humans [196,197]. Oral dyskinesia observed after peripheral injections of m-CPP was enhanced by

5,7-dihydroxytryptamine (5,7-DHT)-induced lesion of the serotonergic neurons, probably due to an altered sensitivity to 5-HT$_{2C}$ receptor stimulation in the STN [194]. Interestingly, these authors observed m-CPP induced seizure-like behaviours in a subset of lesioned rats that were never observed in sham-lesioned animals, thus demonstrating a pivotal role for the 5-HT$_{2C}$ receptor in the control of the normal neuronal excitability, a phenomenon already noted by others [194]. Despite the fact that the mechanism by which serotonergic inputs to the STN contribute to its normal functioning remains controversial, the behavioural data discussed above clearly suggest that excess stimulation of 5-HT$_{2C}$ receptors in this region may lead to hyperkinetic movement disorders. Thus 5-HT$_{2C}$ antagonists can be useful to treat the side effects of long-term administration of neuroleptics in schizophrenia [198,199].

Serotonin$_{2C}$ receptors in Parkinson's disease and other related motor disorders

Since Hornykiewicz's pioneering work in identifying the SNc as the site of major pathological change in PD, reduced dopaminergic innervation of the striatum has been thought to be central to its pathogenesis [200]. However, relatively little research has been focused on other parts of the dopaminergic system and on the influence of other neurotransmitter systems. Nonetheless, the fact that several other neurotransmitters, and among them 5-HT, are also involved in PD, has long since been known [201-204]. There is no doubt that a gradual degeneration of 5-HT neurons occurs in the raphe nuclei in PD. Decarboxylase activity has been shown to be reduced in these nuclei in PD patients. Further, 5-HT is reduced in ascending pathways as noted by Chase and co-workers [205,206]. Indeed, post-mortem examinations have shown a reduction of up to 50% of 5-HT in some areas of the cortex and the basal ganglia [202,207,208], and antemortem studies have shown reduced cerebrospinal fluid levels of the major 5-HT metabolite, 5-hydroxy-indolacetic acid (5-HIIA) in PD patients. Recently, imaging studies of PD patients have shown decreased activity of 5-HT transporter (5-HTT) in the thalamus, medial frontal areas, caudate nucleus and the putamen, indicating a pathophysiological involvement of 5-HT in the disease process [209-211]. It is, however, very likely that the degree of serotonergic degeneration depends on the stage of the disease [204]. No consistent morphologic change has been identified in parkinsonian brain stem raphe nuclei [210] but, in primates, surgical lesion of upper brainstem producing contralateral resting tremor, and bradykinesia were associated with reduced homolateral striatal 5-HT [212].

However, preclinical research on animal models of PD has provided discrepant results from the above mentioned evidence in humans, highlighting that these experimental models only represent an imperfect replica of human

disorders [204]. Indeed, it has been shown that DRN serotonergic neurons in 6-OHDA-lesioned rats fire at an higher frequency and preferentially display burst firing activity [213]. Since it has been demonstrated that the consequence of the repetitive burst firing pattern for DRN neurons enhances both 5-HT release and its postsynaptic effect [214], it is possible to speculate that in rat 6-OHDA-model of PD, a hyperfunction of the serotonin system occurs. According to this hypothesis, several studies have shown a hyperinnervation of 5-HT nerve terminals in the striatum of rats and mice lesioned with 6-OHDA [215-221]. Consistently, striatal 5-HT levels and 5-HTT binding have been reported to be increased in this animal model of PD [219,220,222-225]. Interestingly, it has been proposed that striatal serotonergic hyperinnervation in lesioned animals occurs to compensate for the lost function of DAergic terminals, indeed raphe-striatal serotonergic neurons produce DA from exogenously administered L-DOPA and release it as "false transmitter" [226]. This suggests that 5-HT neurotransmission is impaired and the clarification of the mechanism of its striatal hyperinnervation can provide unique information regarding the treatment of parkinsonism from a point of view that differs from conventional therapy.

An additional consequence of neonatal DA depletion is the up-regulation of striatal postsynaptic 5-HT receptors. Indeed, it has been shown that the inhibitory response of neostriatal neurons to the iontophoretic application of 5-HT and to the preferential 5-HT_{2C} receptor agonist mCPP is enhanced in neonatal 6-OHDA lesioned rats [144]. However, in situ hybridization and autoradiographic radioligand studies from lesioned rats and human postmortem tissue from patients with PD have revealed that striatal 5-HT_{2C} receptors are not influenced by DA depletion, in contrast to 5-HT_{2A} receptors that appear to be up-regulated [70,227]. Indeed, mRNA expression and the number of 5-HT_{2C} receptors is unchanged in this rodent model of PD and in parkinsonian patients [70,227]. Fox and colleagues [70] also showed that there was no significant difference in 5-HT_{2C} binding level for control versus PD tissue in the GPi and GPm. Conversely, 5-HT_{2C} receptor binding in the SNr of aged-matched control tissue was less than half that in the SNr of patients with PD, strictly in accordance with the 50% increase in 5-HT_{2C} receptor binding observed in 6-OHDA-lesioned rats [70,228].

This evidence highlights a selective change in 5-HT_{2C} receptor activity only in the output regions of the basal ganglia. 5-HT_{2C} receptors up-regulation might be compensatory, being a consequence of a decreased level of 5-HT in this nuclei and thus potentially underlie a role for them in the neuronal mechanisms involved in PD [70]. However, the changes in 5-HT_{2C} receptor binding reported by Fox and colleagues were seen in PD patients with levodopa-induced dyskinesia, it is thus possible that they could be ascribed to

the process underlying dyskinesia rather than parkinsonism [70]. Thus, reduced stimulation of 5-HT$_{2C}$ receptors would lead to decreased activity of the basal ganglia output nuclei and increased levels of abnormal movements. This hypothesis may predict an efficacious use of 5-HT$_{2C}$ agonists for alleviating the side-effects of long-term treatment with levodopa, although clozapine and quetiapine, antagonists for this receptor subtype, have been used successfully for this purpose [229]. On the other hand, systemic administration of the selective 5-HT$_{2C}$ antagonist SB 206553 was shown to enhance the action of the anti-parkinsonian action of the dopamine D1 and D2 agonists in the 6-hydroxydopamine-lesioned rats [230,231], suggesting that the use of a 5-HT$_{2C}$ receptor antagonist in combination with a dopamine receptor agonist may reduce the reliance upon dopamine replacement therapies. Hitherto, no studies have been conducted in either non-human primates or humans to address this issue.

Another interesting application of the data regarding the functional role of 5-HT$_{2C}$ receptors in the basal ganglia is the possible use of 5-HT$_{2C}$ receptor antagonists in the treatment of drug-induced parkinsonian syndromes. Moreover, in addition to the neurodegenarative, idiopathic form, parkinsonian symptoms are also produced by typical APDs therapy such as haloperidol, a mixed DA antagonist. Conversely, clozapine and other atypical APDs, that also have a high affinity to 5-HT$_{2C}$ receptors [232], reduced motor side-effect liability because of the same degree of intrinsic antiparkinsonian characteristics, which act to counteract the pro-parkinsonian effects of DA blockade. This was suggested by early clinical studies indicating that the 5-HT$_{2A/2C}$ receptor antagonist ritanserin [233,234] can ameliorate negative symptoms as well as attenuate exciting extrapiramidal side effects (EPS) in schizophrenics treated with classical APDs [235,236]. The relevance of 5-HT$_{2C}$ receptor blocking in the effect of atypical APDs was shown by Canton and colleagues [237], who revealed the high affinity of clozapine and risperidone for 5-HT$_{2C}$ sites in the rat choroid plexus. These findings were subsequently confirmed [238-242] and extended to brain sections [241,242], and in transiently expressed 5-HT$_{2C}$ receptors [47,243]. Antagonism at 5-HT$_{2C}$ receptors by several atypical antipsychotics was also observed in vivo. Indeed, clozapine produces an increase in extracellular levels of DA in the nucleus accumbens [244,245], reverses the inhibition of accumbal DA release induced by the 5-HT$_{2C}$ agonist RO 60175 [244] and blocks the hypolocomotion induced by the 5-HT$_{2C}$ agonist mCPP [246].

It is worth noting that clozapine, like several atypical APDs, behaves as a 5HT$_{2C}$ inverse agonist in heterologus expression systems in vitro [247,86,88] and in vivo [86]. Thus, the 5-HT$_{2C}$ receptor inverse agonist might underlie the unique clinical properties of atypical APDs such as low EPS profile and good antidyskinetic efficacy [86,247,229].

The involvement of 5-HT$_{2C}$ receptor in the neuroleptics EPS disturbance is underlined also by the behavioural evidence that clozapine inhibits the cataleptic response to loxapine and olanzapine [248] and SB 228357, a selective 5-HT$_{2B/2C}$ receptor antagonist reverses haloperidol-induced catalepsy [249]. This hypothesis is further reinforced by the observation that the 5-HT$_{2C}$ receptor activation by RO 600175 *per se* induces catalepsy [250].

It has been suggested that 5-HT$_{2C}$ receptors are also involved in the resting tremor that occurs in PD. Parkinsonian resting tremor in humans is usually quite focal. The most common tremor seen in patients with PD is a "pill-rolling" movement of the hands [251]. Despite a number of clinical and basic studies, the neural substrate for this motor complication remains unclear. A glimmer of light was recently thrown on this, by Carlson and colleagues using cholinomimetic-induced jaw movements in the rat as a model of parkinsonian tremor [252]. The authors showed that local injections of a mixed 5-HT$_{2A/2C}$ receptor antagonist into the SNr block tremulous jaw movements in rat [252]. This result is consistent with previous studies showing that the jaw movement activity was suppressed potently by clozapine, olanzapine and risperidone [253-255] and with clinical reports demonstrating serotonergic involvement in the generation and treatment of parkinsonian symptoms and other motor dysfunction [256].

In addition to the possible role of 5-HT$_{2C}$ receptors in regulating movement, we have recently suggested that manipulation of 5-HT$_{2C}$ receptor-mediated transmission might prove useful in addressing psychiatric problems such depression which is often associated to PD [52,257]. Indeed, we have showed that 5-HT$_{2C}$ receptor antagonists cause an increase of VTA DA neurons activity and in the accumbal DA release [85,124,128], an effect that might be useful in treating the comorbid depression that often accompanies PD [258,259].

Conclusions and implications

From the large amount of literature here reviewed it is apparent that the serotonergic neurotransmitter system plays a pivotal role in the modulation of basal ganglia circuitry, and its dysfunction is involved in the pathophysiology of PD. Among all the 5-HT receptors present in the basal ganglia nuclei, the 5-HT$_{2C}$ receptor subtype seems to be one of the principal receptors through which 5-HT exerts its function. Twenty five years of 5-HT$_{2C}$ receptors research has generated detailed information on the molecular biology and regional and cellular localization of these receptors. Furthermore, this research has revealed that the 5-HT$_{2C}$ receptor could be a potential target for therapeutic intervention in mood disorders, anxiety, stress, sexual dysfunctions, eating disorders, and drug abuse. From the above discussion it seems clear that drugs acting at the

5-HT$_{2C}$ receptor might be an important feature of the treatment of PD, drug-related motor disturbances, and the depressive symptoms often associated with these neurological disorders.

Nevertheless, further clarification of the role of 5-HT$_{2C}$ transmission in the pathophysiology of basal ganglia disorders is required, since the overall picture is still confusing. Furthermore, most of the data came from animal studies and animal models of PD that yield contrary results compared to clinical studies. This leaves room for speculation about the real value of preclinical research for clinical PD. The enthusiasm generated by the research of Fox and colleagues [70,174,178,230,231] at the end of the 90's has been dampened by the lack of clinical results to date. Although several selective agents for this receptor have been discovered, none have reached the market for the treatment of motor disorders as yet. However, several companies are very active in 5-HT receptors research in PD, though they have concentrated on different receptor subtypes such as 5-HT$_{1A}$, 5-HT$_{1B/D}$ and 5-HT$_{2A}$. Moreover, there are also many avenues that remain unexplored, so there are undoubtedly further advances to be made. In the next few years, we will certainly see compounds selective for 5-HT$_{2C}$ receptors making further significant impacts on the treatment of the major motor disturbances such as PD.

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