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Distribution of 5-HT and DA receptors in primate prefrontal cortex: Implications for pathophysiology and treatment

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Abstract

The prefrontal cortex (PFC) has attracted great research interest because of its involvement in the control of executive functions in both health and disease, and particularly in cognitive functions such as working memory. In schizophrenia, alterations in the PFC are documented at many different levels: molecular, cellular and functional. Furthermore, deficits in cognitive abilities are considered a core feature of schizophrenia and remain a major unmet medical need with respect to this disorder. In order to understand the sites of action of currently-used drugs, as well as of the new experimental treatments being developed and acting in this brain region, it is important to have detailed knowledge of the corresponding chemical neuroanatomy. Here we review current knowledge regarding the cellular localization of 5-HT_{1A}, 5-HT_{2A}, dopamine D1, D5, and D2, D4 receptors in primate PFC and their possible functions in the neuronal circuits of the prefrontal cortex.

Key words: serotonin receptors, dopamine receptors, GABAergic interneurons, glutamatergic cells

List of abbreviations

5-HT, 5-hydroxytryptamine or serotonin; BA, Brodman's area; CB, calbindin D-28k; DA, dopamine; DLPFC dorsolateral prefrontal cortex; GABA, gamma aminobutyric acid; GAD, glutamic acid decarboxylase; PFC, prefrontal cortex; PV, parvalbumin; vGluT1, vesicular glutamate transporter 1; vGluT1, vesicular glutamate transporter 1; VTA, ventral tegmental area;

Prefrontal cortex: location, connectivity and cytoarchitecture

In mammals the PFC is located in the anterior part of the frontal lobe, anterior to the motor and premotor cortical areas. It is traditionally divided into three major regions: orbitofrontal and ventromedial areas, dorsolateral prefrontal cortex, and the anterior and ventral cingulate cortex. The PFC comprises areas 8-13, 24, 25, 32, and 44-47 according to Brodmann (Brodmann and Garey 2006). The dorsal PFC refers to Brodmann's area (BA) 8, 9 and 10; the dorsolateral PFC to BA 46 and ventral to BA9; the lateral PFC to BA 44, 45, 47; the orbitofrontal PFC to areas BA 11, 12; the anterior cingulate region to BA 24 and 25, and the cortex of the medial surface to BA 32 (Fallon et al. 2003). The PFC is described as a six-layered structure, as other neocortical regions, and it is distinguished by a granular layer IV (see Figure 1).

In primates the PFC orchestrates thoughts and actions, as well as the planning of complex cognitive and affective functions, and it is therefore implicated in mood disorders (Ebert and Ebmeier 1996). The orbital and medial regions are involved in the control of emotional behavior, whereas the lateral regions, which are highly developed in humans, provide cognitive support to the temporal organization of behavior, speech, reasoning and the execution of complex behaviors that require working memory, as already mentioned; for reviews see (Cavada et al. 2000; Elston 2003; Fuster 1997; Goldman-Rakic et al. 1984).

The PFC receives projections from the amygdala (Barbas and de Olmos 1990), the ventral striatum (Kunishio and Haber 1994) and thalamus (Barbas et al. 1991). Specifically, it receives dopaminergic efferents from the ventral tegmental area (VTA) (Berger et al. 1988; Conde et al. 1995; Lindvall et al. 1978) and serotonergic innervation from the median and dorsal raphe nuclei (Berger et al. 1988; Smiley and Goldman-Rakic 1996b), while it sends glutamatergic projections to both the VTA and the nucleus accumbens (Sesack and Pickel 1992; Taber et al. 1995), as well as to the raphe nuclei (Sesack et al. 1989). The PFC is connected with other association cortices but not with primary sensory or motor cortices. There is an important internal network of connectivity between the different divisions of the PFC; indeed, each of the three major prefrontal regions (orbital, medial and lateral) is connected with itself and with the other two. Some of the cortico-cortical connectivity of the PFC is interhemispheric and the majority is organized in a reciprocal and topographical

manner (Cavada and Goldman-Rakic 1989a; 1989b). These cortico-cortical connections originate and terminate in upper cortical layers II and III (Andersen et al. 1985).

As in other cortical regions, there are two main cell types in the PFC. One is the pyramidal and spiny stellate cells characterized by the excitatory asymmetric synapses which they form (glutamatergic). The majority of cortical neurons are pyramidal output neurons, found mainly in layers II-VI. The others are smooth or sparsely spiny neurons, which are interneurons that form inhibitory symmetric synapses (GABAergic) and are found in layers I-VI. In the cerebral cortex several different subclasses of GABA-containing neurons can be distinguished by their content of calcium-binding proteins and electrophysiological characteristics: parvalbumin (PV) cells with physiological properties of fast-spiking interneurons and the morphology of chandelier and large basket cells; and calbindin (CB) cells with physiological properties of non-fast-spiking interneurons, the majority of which show the morphology of double-bouquet cells (Conde et al. 1994; DeFelipe 1997; Zaitsev et al. 2005).

Specific populations of PFC pyramidal neurons have been proposed to be responsible for maintaining “on-line” the information required for working memory (Goldman-Rakic 1995). Furthermore, inhibitory neurons in the PFC seem to play an important role in regulating the spatially-tuned activity of pyramidal neurons during working memory. As mentioned above, there is also evidence that PFC dysfunction is a pathophysiological feature of schizophrenia, and a role has been demonstrated for GABAergic interneurons in the regulation of PFC function (Goldman-Rakic and Selemon 1997; Lewis et al. 1999). Reduced expression for GAD67 (67 kDa isoform of glutamate decarboxylase), a synthesizing enzyme for GABA, has been found in the dorsolateral prefrontal cortex (DLPFC) of individuals with schizophrenia (Volk et al. 2000) but only in a subset of these GABAergic neurons, the parvalbumin-positive neurons (Beasley et al. 2002; Daviss and Lewis 1995; Hashimoto et al. 2003), which also had reduced but detectable levels of parvalbumin mRNA. Thus, the number of PV-expressing GABA neurons in the DLPFC of subjects with schizophrenia is unchanged, but they do have decreased expression of several genes, thus impairing cell function.

The major treatment for schizophrenia is antipsychotic or neuroleptic drugs, all of which are characterized by their ability to interact with dopaminergic and serotonergic

neurons. They are usually divided into two classes, called typical and atypical neuroleptics, differentiated by their profiles of unwanted side-effects. Additionally, the effects of these drugs on cognitive parameters in the disease are limited. In this regard, the need to develop new and more efficacious neuroleptics highlights the need to understand the cellular location of the sites of action of these drugs.

An important element in the chemical machinery of the brain is neurotransmitter receptors. Receptors for certain neurotransmitters, such as dopamine and serotonin, are relevant because they are the sites of action for drugs used in the treatment of psychiatric disorders. In this paper we review recent data on the cellular localization of specific subtypes of these receptors in the primate prefrontal cortex with the goal of integrating this information on the molecular circuitry of this brain area and its relevance, thus laying the basis for the development of drugs to improve psychiatric disorders. The review covers recent data from our laboratory and others on the cellular localization of 5-HT_{1A}, 5-HT_{2A}, dopamine D1, D5, and D2, D4 in primate PFC. In these studies we have used double *in situ* hybridization approaches, which enable the expression of receptors to be correlated with the expression of molecules that define the cellular phenotype. These results are analyzed together with data on radioligand binding autoradiography and immunohistochemistry using receptor antibodies in order to propose the localization of receptors and their possible functions in the neuronal circuits of the PFC. Table 1 summarizes current knowledge on the laminar and cellular localization of these receptors.

5-HT receptors in the monkey PFC

The PFC receives serotonergic innervation from the medial and dorsal raphe nuclei (Mamounas et al. 1991; Steinbusch 1981; Wilson and Molliver 1991a; 1991b). Serotonergic axons are present in all PFC layers, presenting a slight reduction in their density in layer III (Wilson and Molliver 1991a), with beaded axons predominating in layer I and fine axons in layers II-VI. The serotonergic neurons synapse primarily on GABAergic interneurons (Smiley and Goldman-Rakic 1996a). The raphe nuclei receive reciprocal glutamatergic innervation from the PFC (Sesack et al. 1989).

The action of 5-HT in these cells can be mediated by serotonin receptors. There are seven subtypes of 5-HT receptors, which, together with their different subtypes

result in at least fourteen different receptors, plus the many subtypes that result from alternative splicing (Hoyer et al. 2002). The involvement of serotonin in schizophrenia has been a subject of much interest (Breier 1995) due to the particular pharmacological profile exhibited by atypical antipsychotic drugs such as clozapine, olanzapine and quetiapine, which are potent 5-HT_{2A} receptor antagonists and relatively weaker dopamine D2 receptor antagonists (Meltzer 1999). Of the remaining 5-HT receptors with which these drugs also interact, 5-HT_{1A} receptors have been postulated as additional good receptor candidates for their contribution to the antipsychotic action of these drugs. However, in contrast with 5-HT_{2A} receptors, where blockade is required for therapeutic activity, the stimulation of 5-HT_{1A} receptors appears to produce similar effects to the antagonism of 5-HT_{2A} receptors (Meltzer 1999).

The involvement of serotonin in the pathophysiology of schizophrenia is supported by the clinical efficacy of drugs acting on 5-HT receptors. Additionally, in attempts to pinpoint specific brain regions and cells involved in the clinical activity of these drugs a number of studies have examined the changes in postmortem brain tissue from schizophrenic patients. Changes in 5-HT receptor densities have been described in different brain areas. For example, 5-HT_{1A} receptors have been found to have increased densities in the prefrontal cortex of schizophrenic patients (Hashimoto et al. 1993; Sumiyoshi et al. 1996), whereas for 5-HT_{2A} receptors the findings are less consistent since both increments (Joyce et al. 1993) and reduction (Dean et al. 1998) have been described in this brain area. The levels of mRNA coding for 5-HT_{2A} receptors are lower in PFC of schizophrenic patients (Burnet et al. 1996). They remained unchanged in patients treated with neuroleptics and dropped when patients had been free of neuroleptics for more than six months (Hernandez and Sokolov 2000).

We will now review the recent data from our laboratory and others on the cellular localization of 5-HT_{1A} and 5-HT_{2A} receptors in PFC.

Cellular localization of 5-HT_{1A} in PFC

The 5-HT_{1A} receptor is negatively coupled to adenylate cyclase through the G protein $G_{i/o}$. This receptor can be considered as having the opposite functional effect to the 5-HT_{2A} receptor. Activation of the inhibitory 5-HT_{1A} autoreceptor on the raphe nucleus cells attenuates the firing of these neurons.

5-HT_{1A} receptors are found at high densities in the PFC of many species (Marazziti et al. 1994; Pazos et al. 1987a; Pazos and Palacios 1985; Pompeiano et al. 1992), preferentially in external cortical layers (Mengod et al. 1996). Immunohistochemical and *in situ* hybridization studies have revealed the presence of 5-HT_{1A} receptor protein and mRNA in external PFC layers in rat (Abbas et al. 2007; Kia et al. 1996; Pompeiano et al. 1992; Santana et al. 2004) and in monkey and human brain (Burnet et al. 1995; Cruz et al. 2004; DeFelipe et al. 2001; Marazziti et al. 1994; Mengod et al. 1996; Pasqualetti et al. 1996).

There is controversy as to the subcellular location of 5-HT_{1A} receptors. Riad and coworkers (Riad et al. 2000) found a somatodendritic location of the receptor protein in rat brain. However, by using a different receptor antibody (Azmitia et al. 1996), 5-HT_{1A} receptor protein has also been localized in axons of pyramidal cells in rat, monkey and human (Cruz et al. 2004; DeFelipe et al. 2001). In the rat PFC 60% of the glutamatergic cells express 5-HT_{1A} receptors and 25% of the GABAergic interneurons contain this receptor mRNA (Santana et al. 2004). Using dual *in situ* hybridization we found that in monkey PFC the percentage of glutamatergic cells containing 5-HT_{1A} receptor mRNA is higher than in rat, with 5-HT_{1A} receptor mRNA being expressed in about 80% of glutamatergic neurons in external layers II and upper III, and in around 50% in layer VI; they are also present in approximately 20% of GABAergic neurons (de Almeida and Mengod, submitted). An example of this distribution is shown in Figure 2. 5-HT_{1A} receptor transcripts are abundantly expressed mainly in layer II and upper III, whereas they are less abundant in the cells of layers VI and show very low expression in layers III-V in both species. No 5-HT_{1A} receptor mRNA hybridization signal was seen in layer I. A notable co-localization of 5-HT_{1A} receptor mRNA with the glutamatergic marker vGluT1 mRNA, as determined by double *in situ* hybridization histochemistry, can be appreciated in layer II-upper III in monkey PFC (see Figure 2B), which contrasts with the lower co-localization of 5-HT_{1A} receptor mRNA and GAD65/67 mRNA (Figure 2C).

These results suggest that the binding sites localized by autoradiography correspond to those receptors visualized by both immunohistochemistry and *in situ* hybridization, due to the well known somatodendritic localization of these receptors. These data point to the pyramidal cells of layer II-III as being one of the main cells in PFC that expresses 5-HT_{1A} receptors. Since these cells are known to be involved in cortico-cortical projections, both contralateral and ipsilateral, it would seem that these receptors play a role in these connections. 5-HT_{1A} receptors are also found in

GABAergic cells, although in a lower number of cells, thus providing a second way of influencing the role of 5HT in pyramidal and GABAergic cells.

Cellular localization of 5-HT$_{2A}$ in PFC

The 5-HT$_{2A}$ receptor activates phospholipase C by coupling to G proteins. As mentioned above, this receptor can be considered as having the opposite functional effect to the 5-HT$_{1A}$ receptor.

5-HT$_{2A}$ receptors are found at high densities in the PFC of many species (López-Giménez et al. 2001; Pazos et al. 1985; Pazos et al. 1987b; Pompeiano et al. 1994). In monkey PFC, layers I and III-IV presented the highest densities of 5-HT$_{2A}$ labeled receptors (López-Giménez et al. 2001). Immunohistochemical and *in situ* hybridization studies have revealed the presence of 5-HT$_{2A}$ receptors in both rat (Cornea-Hebert et al. 1999; Martin-Ruiz et al. 2001; Pompeiano et al. 1994; Willins et al. 1997; Xu and Pandey 2000) and monkey and human (de Almeida and Mengod 2007; Jakab and Goldman-Rakic 1998; 2000; López-Giménez et al. 2001) PFC. Layers III and IV showed the highest hybridization levels in monkey PFC (de Almeida and Mengod 2007; López-Giménez et al. 2001), although Burnet and coworkers observed that 5-HT$_{2A}$ mRNA was concentrated in two bands, probably corresponding to lamina III and V (Burnet et al. 1995) in the orbitofrontal cortex.

Whereas the pyramidal neuron is the major cortical cell type expressing 5-HT$_{2A}$ receptors, some cortical GABAergic interneurons have also been found to express these receptors in the rat (Santana et al. 2004; Willins et al. 1997) and primate brain (Burnet et al. 1995; de Almeida and Mengod 2007; Jakab and Goldman-Rakic 1998). Several groups have shown that the PFC 5-HT$_{2A}$ receptor protein is localized in the apical dendrites of pyramidal cells (Jakab and Goldman-Rakic 1998; Willins et al. 1997). In PFC interneurons 5-HT$_{2A}$ receptor expression is found in calbindin positive cells (Jakab and Goldman-Rakic 1998). Figure 2D shows that 5-HT$_{2A}$ receptor transcripts are abundantly expressed in the monkey prefrontal cortex in a large number of cells that are distributed preferentially between layers III and V, whereas they are less abundant in the cells of layers II and VI and absent in layer I. It is worth mentioning that the largest amount of 5-HT$_{2A}$ receptor mRNA is found in layer V. Glutamatergic neurons labeled by a dark precipitate are found in all layers, except layer I (Fig. 2E). PFC cells expressing both GABAergic cell markers, GAD65 and GAD67, are found scattered throughout the prefrontal cortex, including layer I (Fig. 2F). The great majority of glutamatergic cells in layers II-V expressed 5-HT$_{2A}$

receptors in the nine prefrontal areas examined (86-100%), with a maximum (almost 100%) observed in layers III and V. In GABAergic cells in layers II-V, this percentage was lower (13-31%). This difference in the percentage of the two cellular populations was, however, much lower when layer VI was analyzed. The proportion of glutamatergic cells expressing 5-HT_{2A} receptors decreased to 52-72%, whereas GABAergic interneurons expressing this receptor increased to 28-46%. This receptor is expressed in 45-69% of parvalbumin and in 61-87% of calbindin positive cells (de Almeida and Mengod 2007).

This cellular location, in pyramidal cells of layers III and V, suggests that these receptors are involved in cortico-cortical contralateral and ipsilateral connections.

Dopamine receptors

Dopamine has been associated with functions such as motivation, affect, reward, movement, and performance on cognitive tasks. Cognitive symptoms have been linked with dopamine dysregulation in several diseases, including schizophrenia (Knable and Weinberger 1997).

The dopaminergic system arises from cells located in the midbrain. Anatomical studies in rodents demonstrate that afferents from the PFC innervate the VTA GABAergic cells which, in turn, project to the nucleus accumbens (Hurley et al. 1991; Sesack et al. 1989; Sesack and Pickel 1992) and the VTA DA cells that project back to the PFC (Carr and Sesack 2000) (mesocortical projections). Dopaminergic neurons synapse on at least two cellular populations: pyramidal excitatory neurons (glutamate) and non-pyramidal GABA interneurons (Cowan et al. 1994; Goldman-Rakic et al. 1989; Sesack et al. 1995).

The central actions of dopamine are mediated by dopamine receptors, which are classified into D1-like or D2-like receptors based on their pharmacological or functional profile (Kebabian and Calne 1979; Missale et al. 1998; Vallone et al. 2000). Receptors belonging to the D1 family (D1 and D5) are positively linked to adenylyl cyclase, whereas those belonging to the D2 family (D2, D3 and D4) are negatively coupled to adenylyl cyclase or to other transduction pathways.

The pharmacological differences between D1-like and D2-like receptors described almost thirty years ago (Kebabian and Calne 1979) remain valid today. The number of purely selective ligands for each receptor is very limited (Alexander et al. 2004). Most of the work done on dopamine receptors has been centred on subcortical

regions such as the striatum, where the density of these receptors is very high (Camps et al. 1989; Cortés et al. 1989; Palacios et al. 1988). Since it was first discovered in the 1970s that the effects of many antipsychotic drugs correlated with their affinities to D2 receptors (Creese et al. 1976) most of the research on the aetiology and treatment of schizophrenia has been centred on these receptors. Subsequently, and given the involvement of cortex and particularly the PFC in schizophrenia (Weinberger 1988), the study of the expression of dopamine receptors in this brain area has become an important issue.

Cellular localization of D1-like receptors in the PFC

Dopamine binds to D5 receptors with a 5-10 fold higher affinity than for D1 receptors (Sunahara et al. 1991). As there are no pharmacological tools capable of differentiating between D1 and D5 receptors the pharmacological effects of D1 agonists are likely to be mediated by both D1 and D5 receptors. The only way to functionally dissect these two receptor subtypes is by knock out mice and antisense strategies (Sibley 1999). The D1 family of dopamine receptors are very abundant in the neocortex (Cortés et al. 1989). Receptor autoradiography studies with D1 specific ligands have demonstrated that D1-like receptors are present in both human (Cortés et al. 1989) and monkey neocortex at high densities in superficial layers I-IIIa and in deep layers V-VI, as well as at lower densities in layers IIIb-IV (Lidow et al. 1991).

High hybridization levels for D1 and D5 receptor mRNAs have been described in the primate neocortex (Huntley et al. 1992; Meador-Woodruff et al. 1996). In human prefrontal cortex Meador-Woodruff et al. (Meador-Woodruff et al. 1996) described the presence of D1 mRNA receptors in the deeper layers, with faint labeling in more superficial layers. *In situ* hybridization studies to examine the expression of D1 and D5 receptor mRNAs in PFC showed their presence primarily in cell layer V in both human and non-human primates (Lidow et al. 1998). In monkey PFC, D1 mRNA can also be found in layer II and part of layer VI in addition to layer V, whereas D5 mRNA is found exclusively in layer V (de Almeida and Mengod, in preparation) (see Fig. 3).

D1-like immunoreactivity is present in cortical interneurons of monkey, it being prevalent in parvalbumin-containing neurons and less common in calretinin-containing interneurons (Muly et al. 1998). However, by using another antibody Paspalas and coworkers were unable to detect any D1-immunoreaction in parvalbumin-positive cells in monkey PFC (Paspalas and Goldman-Rakic 2005). In

monkey PFC, D1-like immunoreactivity is found preferentially in the distal dendrites and spines of pyramidal cells, as well as on the dendrites and axon terminals of putative GABAergic interneurons (Bergson et al. 1995b; Muly et al. 1998; Smiley et al. 1994), whereas D5 receptors are located on perikarya and proximal dendrites of many pyramidal and some non-pyramidal neurons (Bergson et al. 1995a; Bergson et al. 1995b; Ciliax et al. 2000; Khan et al. 2000) in layers IV-VI. D1 immunoreactivity for the heteroreceptor is distinctly localized on perisynaptic and extrasynaptic membranes of excitatory-like varicosities (Paspalas and Goldman-Rakic 2005).

Cellular localization of D2-like receptors in the PFC

The distribution of D2-like receptors in the monkey and human brain has been analyzed by receptor radioligand autoradiography (Camps et al. 1989; Goldman-Rakic et al. 1989; Lidow et al. 1989; Mengod et al. 1992).

In human prefrontal cortex Meador-Woodruff et al. (1996) described the faint presence of D2 mRNA receptors in both superficial and deep layers of the PFC. The D4 mRNA receptor is more abundant and was found to be expressed in the deeper layers with faint labeling in more superficial layers, there being an apparent enrichment in the deep cortex. The same authors comment that the expression of D3 and D5 receptor mRNAs in PFC is particularly rare and they appear to be expressed in the deeper layers. In monkey PFC the mRNA coding for D2, D3 and D4 receptor subtypes is present in all the cellular layers but is especially abundant in layer V (Lidow et al. 1998). Work in progress in our laboratory shows that in monkey PFC D2 mRNA is found exclusively in layer V, whereas D4 mRNA can also be found in almost all layers (except in layer I) and at high levels in layer V (de Almeida and Mengod, in preparation) (see Fig. 3).

D4 immunoreactivity has been detected in both pyramidal and non-pyramidal cells of human cortical areas including prefrontal cortex (Khan et al. 1998a), whereas D2 and D3 immunostaining was found to be mostly associated with non-pyramidal neurons (Khan et al. 1998a; 1998b). About 60% of cortical interneurons showed labeling in soma and dendritic shafts (Khan et al. 2001), while a subset (40%) of these showed immunolabeling in astrocytic processes enwrapping the cell bodies. These authors estimate that approximately 35% of the total D2 receptor binding activity in the cortex may be associated with astrocytes. In primate brain the D4 receptor antibody labeled GABAergic neurons in cerebral cortex, some of which were

parvalbumin (Mrzljak et al. 1996). D2 receptor IR is localized in distal dendritic and axonal processes (Negyessy and Goldman-Rakic 2005).

Interaction of 5-HT, dopamine and their receptors: involvement in schizophrenia

The successful therapeutic application of antypsychotics, such as clozapine, olanzapine, seroquel and sertindole, has focussed much research attention on understanding the interaction between the serotonergic and dopaminergic systems. As one of the characteristics of these compounds is their ability to block both dopamine and serotonin receptors, the initial focus has been on dopamine D2 and $5HT_2$ receptors (Meltzer et al. 1989). More recently, the efficacy and tolerability of the "atypical" antipsychotic drugs is attributed in part to their interaction with specific serotonin receptors such as $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$.

At the neuroanatomical level the interaction between 5-HT and DA mechanisms has been analyzed in different pathways. Serotonin from the dorsal raphe inhibits the firing of dopaminergic neurons in the substantia nigra and antagonizes dopamine-mediated behaviors. This action is modulated by $5\text{-}HT_2$ receptors located in the dopaminergic neurons. The raphe also projects to the striatum and the release of 5-HT is also associated with an inhibition of striatal neuronal firing. In addition, serotonergic influence on striatal cholinergic and GABAergic systems is also well documented (see (Alex and Pehek 2007; Kapur and Remington 1996; Werkman et al. 2006). Again, the role of $5\text{-}HT_2$ receptors in mediating the inhibitory effects of serotonin on dopaminergic activity is well documented. Additionally, $5HT_{1A}$ agonists have also been found to reverse catalepsy in rodents and extrapyramidal symptoms in primate models by acting on the firing of serotonergic neurons, suggesting that the combination of $5\text{-}HT_{1A}$ agonist and D2 antagonist could result in an improved antipsychotic profile (Meltzer et al. 2003; Newman-Tancredi et al. 2005). While most studies have focused on dopaminergic transmission, understanding the serotonin-dopaminergic interactions involved in the mechanism of action of neuroleptics will require a different viewpoint as regards the situation in the cortex.

The PFC is the most appropriate region in which to look for interactions between dopamine and serotonin through their cortical receptors, located either in pyramidal cells or GABAergic interneurons. For example, it has been shown that the increase in prefrontal cortex dopamine release produced by atypical antipsychotics such as

clozapine, olanzapine and ziprasidone, but not by haloperidol, seems to involve 5-HT_{1A} receptor activation (Diaz-Mataix et al. 2005; Ichikawa et al. 2001). 5-HT_{1A} receptor agonists increase dopamine release in PFC, suggesting that this is a potential basis for the action of at least some of the atypical antypsychotics (Rollema et al. 1997; Sakaue et al. 2000).

The role of the cortical glutamatergic pyramidal neuron is to integrate thousands of afferent inputs from GABAergic interneurons, as well as from serotonergic and dopaminergic fibres, and also to control movement and affect through its efferent projection (Goldman-Rakic et al. 2000). Dopamine axons represent a significant source of afferentation of PFC in primates (Williams and Goldman-Rakic 1993; 1998), with area 9 being the one having the greatest density of dopaminergic fibres. Dopaminergic innervation in PFC reveals, in general, a bilaminar pattern of distribution that is especially dense in layer I and less so in layer II, with intermediate density being observed in layers V and VI (Lewis 1992). The PFC in primates also receives serotonergic innervation from the medial and dorsal raphe nuclei (Wilson and Molliver 1991a; 1991b). 5-HT axon fibres are found in a moderate density in all layers in monkey DLPFC (Wilson and Molliver 1991a). DeFelipe and coworkers (2001) found a distinct density of 5-HT fibres in supragranular layers (I-III) and a much lower one in infragranular layers. The serotonergic neurons from the raphe nuclei synapse primarily on GABAergic interneurons (Smiley and Goldman-Rakic 1996a). The raphe nuclei receive reciprocal glutamatergic innervation from the pyramidal cells of PFC (Sesack et al. 1989).

The laminar distribution of cell bodies expressing 5-HT_{1A} and 5-HT_{2A} receptors shows a “complementary” pattern, since 5-HT_{1A} receptors are mainly found in superficial layers II and part of III and at lower levels in deep layer VI, whereas 5-HT_{2A} receptors are located in intermediate layers III-V. This distribution could be related in part with the presence of different types of 5-HT axons (Raghanti et al. 2008; Wilson and Molliver 1991a), as discussed above. The these two 5-HT receptors are present in most PFC pyramidal neurons, where they could probably be co-expressed in some cells, while only a low proportion of cortical GABAergic cells express either receptor. Pyramidal 5-HT_{2A} receptors can modulate excitatory glutamate inputs (Puig et al. 2003), whereas 5-HT_{1A} receptors could act by reducing glutamate release in the thalamus.

All dopamine receptors are found to be expressed in PFC layer V (Lidow et al. 1998); additionally, D1 and D4 are found in other PFC layers in monkey (de Almeida and Mengod, in preparation), except in layer I. The observation that schizophrenia is associated with an altered dopamine innervation of PFC area 9, which is lamina- and neurotransmitter specific (Akil et al. 1999), indicates the importance of understanding the laminar (and subsequently, cell type) distribution of both 5-HT and DA receptors.

In nonhuman primates it has been established that members of the D1-like receptor subfamily modulate excitatory transmission in prefrontal microcircuits, generating stimulus-independent activity that is essential for working memory (Williams and Goldman-Rakic 1995); furthermore, although the cellular basis is unknown it is thought to involve D1-like receptor modulation of pyramidal neuron excitability in a layer- and input-specific manner (see Seamans and Yang, 2004) (Seamans and Yang 2004).

The coexpression of dopamine and serotonin receptors in the same PFC cells has not yet been analyzed in any species. However, the only cortical layer where this could occur is layer V (see Table 1), where most of these receptors, with the exception of 5-HT_{1A}, are present. Greater knowledge about this aspect could therefore help in understanding the array of electrophysiological data concerning the actions of antipsychotic drugs in PFC.

Conclusions

In conclusion, we have reviewed the current neuroanatomical data regarding a role for dopamine and serotonin in the functions of primate PFC through different receptor subtypes, namely 5-HT_{1A}, 5-HT_{2A}, D1, D2, D4 and D5, which are expressed in this brain area. The specific cellular populations expressing these receptors have been identified using *in situ* hybridization techniques.

One of the major findings of these studies is the non-overlapping localization of 5-HT_{1A}, 5-HT_{2A} receptors in the primate PFC with regards to their laminar localization and the involvement of cortical circuitry and projections. This suggests complementary rather than redundant roles for these receptors in regulating functions of the PFC, in addition to their excitatory or inhibitory nature.

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Figure legends

Fig 1. The prefrontal cortex is a group of cortical areas located in the anterior part of the frontal lobe (upper panel) of the monkey brain. A coronal section of monkey PFC stained with cresyl violet shows the main divisions: orbital, ventromedial and dorsolateral, as well as some of the Brodmann areas. The small rectangle of area 46 is shown in the right at higher magnification, where the six laminae are numbered from the pial surface to the white matter, based on the density and the size of the cresyl violet stained cells.

Figure 2. Autoradiographic localization of mRNAs coding for 5-HT_{1A} (a) and 5-HT_{2A} (b) receptor mRNAs in monkey prefrontal cortex. Cellular localization of 5-HT_{1A} (c, e) and 5-HT_{2A} (d, f) receptor mRNAs in glutamatergic (c, d) and GABAergic (e, f) cell populations in monkey PFC. High-magnification bright-field microphotographs of emulsion-dipped sections of layer II-upper III of the dorsolateral prefrontal cortex, simultaneously showing the different mRNAs visualized by double *in situ* hybridization using ^{33}P-labeled oligonucleotides complementary to the mRNA coding for serotonin receptors 5-HT_{1A} and 5-HT_{2A} (clusters of dark silver grains), with DIG-labeled oligonucleotides (dark precipitate) for vGluT1 mRNA (glutamatergic cells), panels **c** and **d**, or for GAD65/67 mRNA (GABAergic cells), panels **e** and **f**. The white arrow head indicates a radioactively-labeled cell, the grey arrow head points to DIG-labeled cells and the red arrow head to a double-labeled cell. Bars=3 mm (a, b), 30 µm (c-f).

Figure 3. Autoradiographic localization of mRNAs coding for D1, D5, D2, and D4 receptor mRNAs in monkey prefrontal cortex. D1 mRNA is found in layers II, III, V and VI. D5 and D2 mRNAs are found in layer V. D4 mRNA is present in layers II-VI, and is especially abundant in layer V. Bar=5 mm.

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Table 1. Layer and Cellular localization of dopamine and serotonin receptors in primate prefrontal cortex

				Receptor				
		D1	D5	D2	D3	D4	5-HT_{1A}	5-HT_{2A}
Layers								
	Receptor Autoradiography	I,II, V[1] I-IIIa, V-VI[2]	nd	I,II, upper III[3] V[2]	nd	nd	Superficial layers[4]	I, III-IV[5,6]
	Receptor Immunoreaction	II, III, V[7]	II, III, V[7] IV-VI[8]	IV-V[9]	IV-V[9]	IV-V[9]	II-upper III[10]	II, III, V, VI[11]
	Receptor mRNA	Deep layers[12] V[13] II,III,V,VI[14]	Deep layers*[12] V[13,14]	Superficial and deep layers*[12] V[13,14]	Deep layers*[12] V[13]	Superficial and deep layers[12] V[13] III-V[15] II-VI[14]	Superficial layers[4,16,17]	III, V[16] III-IV[5,6]
Cell type	**Pyramidal cells**	+[7]	+[7,8]	+[9]	nd	+[9,15]	+[16,17]	+[6,11,16]
	GABAergic cells	+[7]	+[7]	+[9]	+[9]	+[9,15]	+[17]	+[6,11,16]
	parvalbumin	+[19]	nd	nd	nd	nd	nd	+[6]
	calbindin	nd	nd	nd	nd	nd	nd	+[6,11]
	calretinin	+/-[18]	nd	nd	nd	nd	nd	nd

* faint expression

[1] Cortés et al. (1989). [2] Lidow et al. (1991). [3] Camps et al. (1989). [4] Mengod et al. (1996). [5] López-Giménez et al. (2001). [6] de Almeida and Mengod (2007). [7] Bergson et al. (1995). [8] Khan et al. (2000). [9] Khan et al. (1998). [10] DeFelipe et al. (2001). [11] Jakab and Goldman-Rakic (1998). [12] Meador-Woodruff et al. (1996). [13] Lidow et al. (1998). [14] de Almeida and Mengod (in preparation). [15] Mrzljak et al. (1996). [16] Burnet et al. (1995). [17]de Almeida and Mengod G. (submitted). [18] Muly et al. (1998).

Figure 1

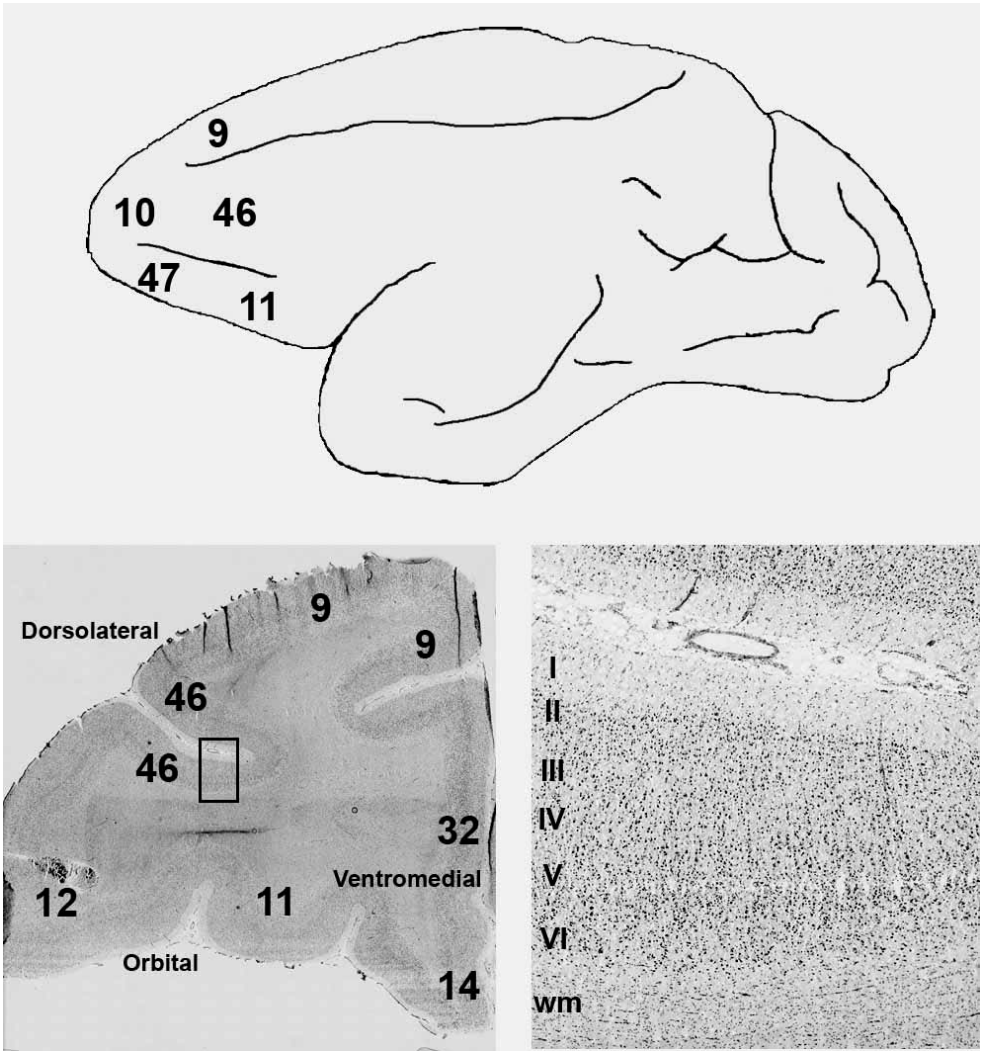
9
10
46
47
11
Dorsolateral
9
9
46
46
32
Ventromedial
12
11
Orbital
14
I
II
III
IV
V
VI
wm

Figure 2

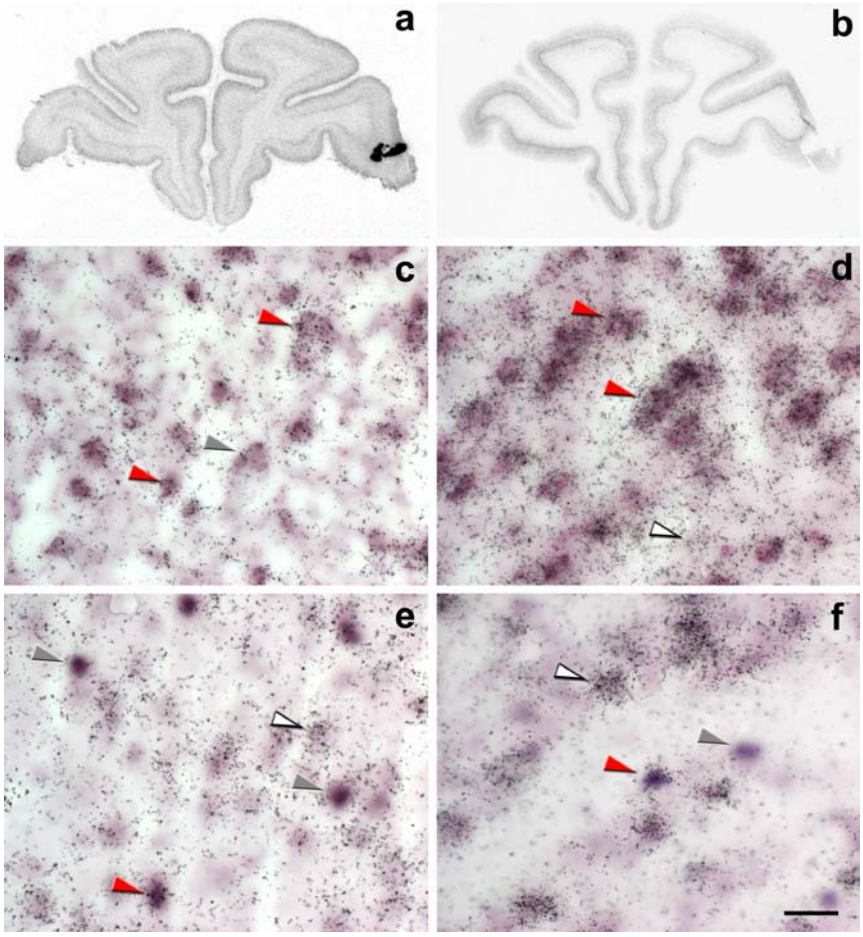

Figure 3