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Catecholamine/Serotonin Interactions: Systems Thinking for Brain Function and Disease

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Abstract

This chapter brings together the work of several leading laboratories, each an outstanding example of integrative approaches to complex diseases of the central nervous system. Cognitive dysfunction and negative symptoms associated with schizophrenia are believed to result from hypofunction of the mesocortical dopaminergic projections to prefrontal cortex (PFC). Noradrenergic targets for the augmentation of dopaminergic function in PFC show promise to improve cognitive deficits as well as negative symptoms. Serotonergic targets for the modulation of mesocortical dopaminergic neurotransmission include 5-HT_{2A} and 5-HT_{1A} receptors. The hallmark of Parkinson's disease is the destruction of nigrostriatal dopaminergic neurons. L-DOPA, a metabolic precursor of dopamine, is the standard of treatment. However, the ectopic release of dopamine (DA) from serotonin neurons and the clearance of extracellular DA by the norepinephrine transporter in areas enriched with noradrenergic terminals contribute to extracellular DA produced by L-DOPA and offer opportunities to improve L-DOPA therapy. The high-affinity transporters for monoamines are the primary targets for antidepressant drugs. However, many patients experience suboptimal therapeutic benefit or fail to respond to treatment. Organic cation transporters and plasma membrane monoamine transporter serve an important function in regulating monoamine neurotransmission and hold potential utility as targets for the development of therapeutic drugs. Improved therapeutic approaches will arise from not only understanding how monoamines influence one another within the central nervous system as an integrated whole but also addressing the pathophysiology of specific core symptoms or distinct

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CONFLICT OF INTEREST

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syndromal dimensions (cognitive impairment, motor slowing, and negative affect) regardless of disease classification, for example, psychotic, affective, and neurodegenerative.

1. INTRODUCTION

The monoaminergic neurotransmitter systems, dopamine (DA), norepinephrine (NE), and serotonin (5-HT), are often viewed in isolation, as separate and distinct entities. Yet the neuroanatomy of these individual systems in brain is consistent with robust anatomical interconnections and functional interactions between monoaminergic neurons. Although the cell bodies for dopaminergic, noradrenergic, and serotonergic neurons are localized to discrete nuclei, their projections to the forebrain create a complex network of overlapping innervation. In addition, there is reciprocal innervation of monoaminergic nuclei from forebrain structures, as reciprocal innervation between these cell body groups, providing the opportunity for the elegant coordination of neuronal activity. The organization of the central monoamine transmitter systems is consistent with intricate and complex interactions between systems in the regulation of key brain functions, for example, executive function and cognition, motor function, and the coordination of voluntary movement, emotion, and mood.

For disorders and degenerative diseases of the central nervous system, current treatments are inadequate, providing less than satisfactory remission or resulting in significant unwanted side effects that reduce compliance. The inadequate response rate to current medications, and the chronic nature of these diseases, underscores the critical need for the development of new treatment strategies. For this common goal, “systems thinking,” the process of understanding how monoamines influence one another within central nervous system as an integrated whole, will be most productive.

This chapter brings together the work of several leading laboratories in the field of neuropsychopharmacology, each an example of truly integrative approaches to complex diseases, each with an established track record of examining and characterizing monoaminergic interactions as they relate to disorders of the central nervous system, that is, schizophrenia, Parkinson’s disease, and major depression. Each addresses the limitations of current pharmacotherapies and offers compelling evidence for the success of this integrative approach to monoaminergic systems in the development of novel approaches or strategies to improve drug design and clinical outcomes.

In the pages that follow, Analia Bortolozzi and Francesc Artigas provide a comprehensive overview of their significant work delineating mechanisms of antipsychotic drug (APD) action and novel strategies to specifically target negative symptoms and/or cognitive deficits associated with schizophrenia. Philippe De Deurwaerdère, Sylvia Navailles, and Léa Milan offer compelling evidence for the involvement of serotonergic and noradrenergic fibers in mechanisms of action of L-DOPA, currently the standard of treatment in the management of Parkinson’s disease. Lynette Daws and Wouter Koek provide a very timely review of studies of organic cation transporter (OCT) and plasma membrane monoamine transporter (PMAT) function in brain. Significant early findings encourage continued investigation of OCT and PMAT function and, importantly, their potential as novel therapeutic targets for antidepressant and neuroprotective drugs.

2. CONTROL OF DA NEUROTRANSMISSION IN THE PREFRONTAL CORTEX: FOCUS ON NORADRENERGIC AND SEROTONERGIC SYSTEMS

2.1. Monoaminergic innervation of the prefrontal cortex

Many psychiatric and neurological disorders present cognitive and emotional alterations that are related to an abnormal function of the prefrontal cortex (PFC) and anatomically related brain areas. The PFC exerts top-down control of brain activity and is involved in many higher executive tasks such as learning, working memory, and behavioral flexibility. In order to accomplish these tasks, the PFC is densely interconnected with numerous cortical and subcortical structures. The reciprocal connectivity between the PFC and the brainstem monoamine nuclei may be particularly important for the pathophysiology and treatment of several psychiatric disorders. The cingulate, prelimbic, and infralimbic cortices are innervated by DA axons from ventral tegmental area (VTA), NE axons from locus coeruleus (LC), and 5-HT axons from raphe nuclei (RN). Anatomical and functional studies reveal that the PFC sends projections back to these brainstem nuclei, providing the substrate for feedback control of cortical DA, NE, and 5-HT release (Carr & Sesack, 2000; Celada & Artigas, 2007; Jodo, Chiang, & Aston-Jones, 1998) (Fig. 9.1).

The robust anatomical and functional interconnections between the PFC and monoaminergic nuclei further suggest that monoamines play an important role in regulating executive function. Extensive research has associated cognitive dysfunction with DA dysregulation in the PFC. Pioneering studies of Patricia Goldman-Rakic and others in nonhuman primates have shown an inverted-U relationship between the cognitive performance in spatial working memory tasks and the occupancy of DA D₁ receptors (Goldman-Rakic, Muly, & Williams, 2000; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007; Williams & Castner, 2006). Thus, very low (for instance, in Parkinson's disease and schizophrenia) or excessive (such as during stress) D₁-mediated signaling results in poor cognitive function. Likewise, the overstimulation of DA D₂ receptors may reduce PFC pyramidal excitability, a detrimental effect for maintenance of persistent activity patterns in PFC during short-term memory processes.

However, despite the greater proportion of PFC neurons expressing receptors for NE and 5-HT *versus* DA (Table 9.1), the exact role of NE and 5-HT neurotransmission in PFC is less well understood than that of cortical DA transmission (see Artigas, 2010; Masana, Santana, Artigas, & Bortolozzi, 2012 for review). Electrophysiological studies have shown that alpha₁-adrenergic receptors mediate the excitatory actions of NE on PFC pyramidal neurons. Rats and monkeys exposed to uncontrollable stress exhibit prominent deficits in working memory related to excessive NE stimulation of alpha₁-adrenergic receptors. Conversely, moderately elevated NE levels, acting on alpha_{2A}-adrenergic receptors, improve cognitive performance, helping to protect memory from irrelevant stimulation. Noradrenergic targets are actively being explored as potential therapeutic agents to modulate cortical DA neurotransmission for treating the cognitive deficits in schizophrenia, bipolar disorder, and major depression (see succeeding text).

Serotonin 5-HT_{1A} and 5-HT_{2A} receptors are localized on pyramidal and GABAergic neurons in the medial PFC with a particularly high density (Table 9.1). These receptors mediate, respectively, the direct hyperpolarizing and depolarizing actions of 5-HT and selective agonists on PFC pyramidal neurons (see Celada & Artigas, 2007 for review). However, selective 5-HT_{1A} receptor-mediated signaling in GABAergic neurons paradoxically increases the *c-fos* mRNA expression and induces excitation in PFC pyramidal neurons (see Masana et al., 2012 and references therein). Hence, 5-HT may influence the descending excitatory input into subcortical areas, including the

monoaminergic nuclei, through the direct and indirect modulation of pyramidal neuron activity in PFC. Specifically, the activation of 5-HT_{1A} and 5-HT_{2A} receptors in medial PFC is deeply involved in the positive control of mesocortical DA neurotransmission (Bortolozzi, Díaz-Mataix, Scorza, Celada, & Artigas, 2005; Díaz-Mataix et al., 2005).

2.2. Cognitive deficits in schizophrenia and APDs

Schizophrenia is associated with many anatomical, molecular, cellular, and neurochemical alterations as well as dysfunctions in several brain circuits. Deficits in PFC cognitive function are considered as a fundamental feature of the illness. Cognitive derangements frequently precede the onset of psychotic symptoms and often persist after successful treatment of the latter symptoms. Considerable research has been carried out to examine the role of PFC DA function in cognitive deficits in schizophrenia, as previously described.

Current views on the role of DA transmission in schizophrenia suggest an imbalance between subcortical and cortical DA systems. Subcortical DA projections to the striatum and nucleus accumbens (mesolimbic pathway) are hyperactive, causing excessive stimulation of DA D₂ receptors in these regions. This is believed to produce psychotic symptoms. In contrast, the mesocortical dopaminergic projections to PFC are hypoactive, producing suboptimal stimulation of cortical D₁ receptors, and consequently negative and cognitive symptoms. An abundant literature suggests that a deficiency in the mesocortical DA function might produce the disinhibition of mesolimbic DA activity (Karreman & Moghaddam, 1996; Wilkinson, 1997).

Classical APDs, such as haloperidol, are required to occupy >70% of DA D₂ receptors to be clinically effective to relieve psychotic symptoms. The modulation of information processing in the “affective” circuit of the basal ganglia (the mesolimbic pathway) is thought to produce the therapeutic properties of classical APDs (reviewed in Artigas, 2010). However, the blockade of DA D₂ receptors in other brain areas causes a series of unwanted and severe side effects. Blockade of DA D₂ receptors in the dorsal striatum (caudate–putamen) results in motor disturbances analogous to parkinsonian symptoms. Antagonism of DA D₂ receptors (likely in PFC) evokes negative symptoms. Hence, DA D₂ receptor blockade in the nigrostriatal and mesocortical pathways results in significant side effects that limit the effectiveness of classical APDs and reduce compliance.

By contrast, the action of atypical APDs (with clozapine as a prototype) takes place predominantly through the blockade of cortical 5-HT_{2A} receptors for which they display a higher *in vitro* affinity. In addition, 5-HT_{1A} receptor agonism plays a key role in their mechanism of action. Although it remains unclear how atypical APDs exert their therapeutic action with subthreshold occupancy of DA D₂ receptor (<70%, except for risperidone), the involvement of PFC 5-HT receptors has been suggested (Artigas, 2010). In addition, there is some evidence for the superiority of atypical APDs over classical APDs in treating negative symptoms and improving cognitive performance, although the latter effect size is relatively small and has not been consistently reproduced.

The primary molecular targets identified for treating cognitive dysfunction include nicotinic and muscarinic acetylcholine receptors, the glutamatergic and GABAergic systems, and monoaminergic receptors, mainly in the PFC. The recent withdrawal of a potential APD targeting mGluR2/3 due to lack of efficacy in phase III trials has reduced the hopes of glutamatergic drugs, although compounds acting at the NMDA receptor are being developed for treating negative symptoms. In absence of new agents, the modulation of cortical DA and NE function remains a potential way to improve the negative symptomatology and cognitive deficits. Given the presence of DA receptors in the mesocortical and mesolimbic DA pathways, and the presumed opposite changes of these DA systems in schizophrenia, the

modulation of cortical DA transmission must be carried out in such a way to take advantage of connectivity (Carr & Sesack, 2000) and functional differences (Masana, Bortolozzi, & Artigas, 2011) between mesolimbic and mesocortical DA neurons.

2.3. Noradrenergic targets for augmentation of dopaminergic function in PFC: add-on strategies

Preclinical studies show that NE terminals in PFC largely contribute to the control of DA neurotransmission in the mesocortical, but not in mesolimbic pathway by coreleasing DA and taking up DA via NE transporter (NET) (see Masana et al., 2011 and references therein). A marked and selective enhancement of DA function in medial PFC of rats is feasible through the combined administration of NET blockers such as reboxetine and α_2 -adrenergic receptor antagonists (e.g., RX-821002, mirtazapine) (Masana et al., 2011, 2012). Recently, we showed that this latter effect occurs when these drugs are administered alone or in combination with APDs such as haloperidol or clozapine. This suggests that this approach may be useful to improve cognitive deficits as well as to treat negative symptoms in patients with schizophrenia (Masana et al., 2011) (Fig. 9.2). Likewise, an improvement of antipsychotic-like effects and increases in cortical DA neurotransmission are found after adjunctive treatment of a NET inhibitor with D2/3 antagonist raclopride or atypical ADPs such as olanzapine and quetiapine (Björkholm et al., 2013; Masana et al., 2012).

Clinically, initial attempts to use reboxetine as add-on therapy to haloperidol in the treatment of schizophrenia failed to show significant benefit. However, this combination therapy was effective in patients with prominent depressive and negative symptoms. In addition, adjunctive treatment of the NET inhibitor atomoxetine with atypical APDs was found to activate certain brain areas related to working memory. Similarly, norquetiapine, the major metabolite of quetiapine in humans, acts as a potent NET inhibitor and differs from other atypical APDs in terms of its antidepressant activity (Björkholm et al., 2013). Consequently, this opens the way to perform more comprehensive clinical trials in which reboxetine or other NET blockers, which are used as antidepressants, can be combined with α_2 -adrenergic antagonists in order to test their clinical efficacy on emotional and cognitive dysfunction in schizophrenia and other psychiatric disorders (Masana et al., 2011).

2.4. Serotonergic targets for the modulation of mesocortical dopaminergic neurotransmission: 5-HT_{2A} and 5-HT_{1A} receptors

5-HT_{2A} receptors are particularly abundant on the pyramidal neurons of cortical layer V, where they have been shown to colocalize with NMDA glutamate receptors, suggesting a role in modulating cognitive functions (Celada & Artigas, 2007). A prominent role of 5-HT_{2A} receptors in antipsychotic action is to stabilize dopaminergic tone in the mesocortical and mesolimbic pathways (Masana et al., 2012). Preclinical studies indicate that stimulation of 5-HT_{2A} receptors in the PFC increases the activity of VTA dopaminergic neurons as well as the DA release in medial PFC and VTA; the selective 5-HT_{2A} antagonist M-100907 reverses these effects (Bortolozzi et al., 2005). These results suggest that blockade of excitatory 5-HT_{2A} receptors on pyramidal neurons of the PFC may reduce excitatory drive to subcortical regions potentially involved in schizophrenia symptoms and normalize the function of ascending monoaminergic systems in schizophrenia. The observation that PFC pyramidal neurons projecting to the VTA express 5-HT_{2A} receptors provides an anatomical support to the observations in the preceding text (Artigas, 2010). More recently, it has been shown that many atypical APDs are inverse agonist at the 5-HT_{2A} receptor rather than antagonists. In contrast to antagonists, inverse agonists can attenuate basal constitutive receptor signaling activity and block agonist-induced responses. Pimavanserin tartrate (ACP-103) is the first 5-HT_{2A} inverse agonist to enter clinical trials as a treatment for L-DOPA-induced psychosis in Parkinson's disease and for augmentation of low-dose

risperidone treatment in schizophrenia. In preclinical studies, ACP-103 potentiates haloperidol-induced DA neurotransmission in mesocortical pathway (see Masana et al., 2012 and references therein).

Among the various G protein-coupled serotonergic receptors, there is growing interest in the cortical 5-HT_{1A} receptors as potential targets for APD action. 5-HT_{1A} receptors are expressed postsynaptically to 5-HT axon terminals in forebrain regions including frontal and cingulate cortices. Their regional distribution is similar in rodent and human brains (Artigas, 2010). This receptor seems to contribute to the ability of atypical (but not classical) APDs to increase cortical DA neurotransmission, an effect involved in the improvement of negative symptoms and cognitive dysfunction in schizophrenia. Of the marketed atypical ADPs, only two, aripiprazole and ziprasidone, are partial 5-HT_{1A} receptor agonists. Clozapine, however, occupies 5-HT_{1A} receptors in primate brain *in vivo* at clinically relevant doses despite its negligible *in vitro* affinity for such receptors. A similar study in healthy subjects failed to find a significant occupancy. Hence, it is still controversial whether clozapine behaves as a 5-HT_{1A} agonist *in vivo* in human brain (Masana et al., 2012).

Preclinical studies in rodents have shown that several atypical APDs including clozapine, olanzapine, risperidone, ziprasidone, and aripiprazole (with markedly different *in vitro* affinities for 5-HT_{1A} receptors) require the functional activation of cortical 5-HT_{1A} receptor to increase the DA neuronal activity and DA release in the mesocortical pathway (Díaz-Mataix et al., 2005) (Fig. 9.3). In addition, some clinical reports indicate that the partial 5-HT_{1A} agonists, tandospirone and buspirone, can enhance certain domains of cognition such as verbal memory in patients treated with classical or atypical APDs. In agreement with these views, different compounds that possess 5-HT_{1A} receptor agonism combined with D₂ receptor antagonism, including SLV-313, SSR-181507, F-15063, S-16924, BSF 190555 (BTS 79018), and RGH-188, are being developed as potential APDs. It is suggested that the balance between DA D₂ receptor blockade and 5-HT_{1A} receptor activation may be critical in determining the superior efficacy of these compounds. Of particular interest is the development of drugs showing preferential activity at postsynaptic 5-HT_{1A} receptors, such as F15599, which are effective when given alone in animal models of schizophrenia and cognition (Masana et al., 2012).

2.5. Conclusion

Schizophrenia has been historically characterized by the presence of positive symptoms. However, negative symptoms and cognitive deficits are also key features of the illness and are poorly treated by current medications. Given (1) the DA deficiency in PFC as putative mediator of negative symptoms and cognitive deficits and (2) the key role of DA neurotransmission in motivation and cognition, a selective regional increase of DA neurotransmission in PFC appears as a possible way to treat nonpsychotic disturbances in schizophrenia. However, it is unlikely that such selective action on PFC DA neurotransmission can be evoked by DA drugs, because of the similar neurochemistry/pharmacology of the mesolimbic and mesocortical DA pathways. Hence, indirect approaches such as those reviewed herein appear more effective. On the one hand, the presence of 5-HT receptors on PFC pyramidal or GABAergic cells controlling DA neuronal activity offers the possibility to indirectly control mesocortical DA activity. This appears to be the way atypical APDs—and in particular, clozapine—increase cortical dopaminergic neurotransmission. The knowledge of the circuitry and of the neurochemical elements involved offers the possibility to develop new therapeutic strategies that specifically target negative symptoms and/or cognitive deficits. On the other hand, the observation that a substantial part of PFC DA arises from noradrenergic axon terminals and is sensitive to noradrenergic drugs is a second way to modulate cortical DA neurotransmission.

3. L-DOPA AND THE BRAIN PATTERN OF EXTRACELLULAR DA LEVELS: A NEW DEAL IMPOSED BY SEROTONERGIC AND NORADRENERGIC FIBERS

The interaction between catecholamine and 5-HT neurons has been evidenced in the mechanism of action of L-DOPA. L-DOPA is the gold standard medication to treat Parkinson's disease. It was introduced in the mid-1960s soon after the demonstration that DA content in the brain is drastically reduced in parkinsonian patients. The hallmark of the disease is the destruction of DA neurons from the substantia nigra pars compacta innervating the striatum. As L-DOPA is a metabolic precursor of DA, it has been assumed for several years that the conversion of L-DOPA to DA *via* its decarboxylation inside remaining DA terminals may help to maintain adequate levels of extracellular DA (Fahn, 2008). However, the widespread nature of DA released by L-DOPA in a situation in which DA neurons are destroyed limits the sole involvement of spared DA neurons if any. The purpose of this section is to highlight the involvement of 5-HT and NE fibers in the mechanism of action of L-DOPA.

3.1. L-DOPA and DA release: from DA neurons to 5-HT neurons

DA from exogenous L-DOPA appears to be responsible for the motor benefits in parkinsonian patients, but the role of spared DA neurons for supplying extracellular DA is questionable. Although the decarboxylation of L-DOPA into DA can occur in every system (endothelial, glial, and neuronal) that expresses the enzyme aromatic amino acid L-decarboxylase (AADC), the newly synthesized DA must be released to exert its effects on DA receptors. In the 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease, L-DOPA-induced striatal or nigral DA release is sensitive to tetrodotoxin, which blocks fast-sodium voltage-dependent channels, implying a neuronal origin (Lindgren, Andersson, Lagerkvist, Nissbrandt, & Cenci, 2010). The monoamine vesicular transporter inhibitor reserpine suppresses L-DOPA-induced striatal DA release, implying that the release of DA occurs *via* an exocytotic process. The remaining DA neurons are not involved in this model because numerous experiments have shown that L-DOPA-induced DA release is higher in the DA-depleted striatum than in the intact striatum and is no longer sensitive to DA autoregulatory processes (DA-D₂ autoreceptor stimulation/blockade and DA transporter blockade).

5-HT neurons are able to convert L-DOPA into DA and store newly synthesized DA into exocytotic vesicles via the vesicular monoamine transporter VMAT2. The distribution of 5-HT neurons in the brain is very different from normal DA innervation (Björklund & Lindvall, 1986; Steinbusch, 1984). According to the widespread distribution of 5-HT neurons, we found using multiple-site, intracerebral microdialysis that L-DOPA dose-dependently enhances DA release in various rat brain regions including the PFC, the striatum, the substantia nigra pars reticulata (SNr), or the hippocampus (HIPP) of 6-OHDA-treated rats. Our data indicate that 5-HT neurons are exclusively involved in this mechanism over a large range of doses. Selective lesion of 5-HT neurons using 5,7-dihydroxytryptamine abolishes L-DOPA-induced DA release (Navailles, Bioulac, Gross, & Deurwaerdère, 2010; Fig. 9.4). Furthermore, L-DOPA-induced DA release is sensitive to various 5-HT autoregulatory processes (5-HT_{1A/1B} autoreceptor stimulation and 5-HT transporter blockade) or to other strategies reducing 5-HT nerve activity (high-frequency stimulation of subthalamic nucleus, HFS-STN) (Navailles, Bioulac, Gross, & Deurwaerdère, 2011).

The increase in extracellular DA levels induced by moderate (6 mg/kg) to very high doses (100 mg/kg) L-DOPA is slightly higher in the striatum (two- to threefold) compared to the other regions (Fig. 9.4). Nevertheless, the magnitude of the effect in those extrastriatal regions is tremendous compared to physiological situations, including at lower doses

(Navailles et al., 2010). It is likely that these extrastriatal effects induced by L-DOPA also occur in normal brain because 5-HT neurons are unable to regulate the release of newly synthesized DA. Consequently, L-DOPA is not an appropriate tool to probe the physiological role of DA neurons in sensorimotor, motivational, or cognitive processes.

L-DOPA-induced dyskinesias (LIDs) are thought to emerge as a consequence of the dysregulated release of DA as a 'false neurotransmitter' from 5-HT neurons (Muñoz et al., 2008). According to this presynaptic model of LIDs, the efficacy of 5-HT_{1A} and/or 5-HT_{1B} agonists and HFS-STN to reduce LIDs may depend on their ability to control the excessive rise in L-DOPA-induced DA levels in brain regions innervated by 5-HT neurons. Selective serotonin reuptake inhibitors (SSRIs) citalopram or fluoxetine, which are known to reduce the activity of 5-HT neurons, have been also shown to reduce L-DOPA-induced DA release and attenuate LIDs in 6-OHDA-treated rats (Bishop et al., 2012; Navailles, Bioulac, et al., 2011). The reduction of LIDs occurs without compromising the motor benefit of L-DOPA. The exception to this is in the case of compounds (e.g., 5-HT_{1A} agonists) that have detrimental effects on their own (Muñoz et al., 2008). Consequently, reduction in inappropriate DA release may be essential for the antidyskinetic properties of these treatments (Navailles, Bioulac, et al., 2011). Because SSRIs and these other treatments mentioned in the preceding text decrease 5-HT release, their effect on 5-HT release would not be expected to be involved in alleviating LIDs (Navailles, Lagière, Contini, & De Deurwaerdère, 2013). Additional data are warranted to further these ideas in clinical practice.

3.2. L-DOPA and noradrenergic fibres: a region-dependent clearance of extracellular DA

The exclusive involvement of 5-HT neurons in the release of DA induced by L-DOPA indirectly rules out the contribution of NE neurons in the process of release. More directly, we have found that lesion of NE terminals coming from the LC using the NE neurotoxin DSP-4 (50 mg/kg 7 days before the dialysis experiments), which leads to 85% reduction in cortical NE tissue content, does not modify L-DOPA-induced DA release in the striatum and even enhances it in the SNr or the HIPP of 6-OHDA-treated rats (Navailles et al., unpublished observation).

In addition to the release process, one must consider the mechanisms of clearance of extracellular DA released after L-DOPA administration in the 6-OHDA rat model of Parkinson's disease. These include catechol-*O*-methyltransferase, OCTs including OCT3 (see succeeding text), or the NET, which has almost the same affinity for DA and NE. At variance with the other monoaminergic systems, NE fibers and the NET are poorly represented in the striatum compared to other brain regions (Aston-Jones, 2004). However, the NET plays a major role in the reuptake of extracellular DA in brain areas poorly innervated by DA fibers, for example, the PFC and HIPP. This mechanism, as postulated from the data obtained with the lesion of NE terminals, could participate to the lower magnitude of the effect of L-DOPA on DA levels in extrastriatal regions.

The administration of the NET blockers desipramine (10 mg/kg) or reboxetine (3 mg/kg i.p.) enhances L-DOPA (12 mg/kg)-induced DA release in the PFC, the SNr, and the HIPP, but not in the striatum (Fig. 9.5). The magnitude of the effect of L-DOPA on DA extracellular levels becomes similar between all brain regions in the presence of a NET blocker (Navailles et al., 2013: Fig. 9.5). Therefore, the higher magnitude of response of L-DOPA on DA extracellular levels in the striatum would not be related to 5-HT terminals themselves, but to the low presence of NE fibers limiting the clearance of extracellular DA in this brain region (Figs. 9.5 and 9.6).

3.3. The brain pattern of L-DOPA-induced DA release evolves after chronic treatment

The brain pattern of extracellular levels of DA in 6-OHDA-treated rats after L-DOPA administration evolves after chronic treatment, but no clear consensus emerges from the different studies available (Lindgren et al., 2010; Navailles, Carta, Guthrie, & De Deurwaerdere, 2011). In our laboratory, a 10-day treatment with L-DOPA (12 mg/kg) reduces the ability of acute L-DOPA (12 mg/kg) challenge to enhance extracellular levels of DA in all brain regions including the striatum (Navailles, Carta, et al., 2011). This effect is associated with a reduction in both basal levels of extracellular 5-HT and its metabolite and tissue content of 5-HT and its metabolite (Navailles, Carta, et al., 2011). This would be consistent with the idea that L-DOPA alters the integrity of 5-HT cells after chronic exposure via oxidative metabolism (Stansley & Yamamoto, 2012), possibly leading to the loss of L-DOPA's ability to enhance DA release in the 6-OHDA rat model of Parkinson's disease.

As 5-HT and NE fibers may be severely altered in the disease process (Eskow, Angoa-Perez, Kuhn, & Bishop, 2011 for review), the efficacy of L-DOPA to enhance DA extracellular levels should be affected accordingly and vary among patients.

3.4. Conclusion

The ectopic release of DA induced by L-DOPA creates a new biochemistry in the parkinsonian brain (Fig. 9.6). The brain pattern of extracellular DA levels produced by L-DOPA in the brain involves 5-HT neurons to release DA in an impulse-dependent manner and NE fibers for the clearance of extracellular DA in areas enriched with NE terminals. Such high extrastriatal DA levels have functional consequences in the parkinsonian brain and may contribute to the motor benefits and undesirable side effects of L-DOPA. Based on the mechanism of action of L-DOPA, numerous strategies are available to control extracellular levels of DA produced by L-DOPA, opening therapeutic opportunities to improve L-DOPA therapy (Eskow et al., 2011; Navailles, Bioulac, et al., 2011).

4. UNFAITHFUL NEUROTRANSMITTER TRANSPORTERS: FOCUS ON BIOGENIC AMINE REUPTAKE AND IMPLICATIONS FOR ANTIDEPRESSANT EFFICACY

Dysfunction of biogenic amine neurotransmission is implicated in many psychiatric disorders, including depression. The high-affinity transporters for these amines (i.e., serotonin transporter (SERT), NET, and dopamine transporter (DAT)) are the primary targets for drugs used to treat depression, which include SSRIs, selective norepinephrine reuptake inhibitors (SNRIs), DAT blockers, and drugs that block both SERT and NET or that block SERT, NET, and DAT. It is the increase in extracellular levels of one or more of these biogenic amines that is thought to be critical to initiate downstream events that ultimately lead to therapeutic benefit. However, many patients experience suboptimal therapeutic benefit or fail to respond to treatment with these antidepressants. The reason for this remains unclear, but it underscores an urgent need to discover new targets to develop novel drugs with improved therapeutic efficacy.

To this end, the discovery of additional transporters for biogenic amines in brain has opened new avenues for antidepressant research. These transporters include OCTs, first cloned and characterized in the 1990s (for reviews, see Eisenhofer, 2001; Koepsell, Lips, & Volk, 2007), and the PMAT cloned by Wang and coworkers in 2004 (Engel & Wang, 2005; Engel, Zhou, & Wang, 2004). These transporters move a wide range of organic cations across membranes, including cationic drugs and toxins, as well as biogenic amines (Koepsell et al., 2007; Koepsell, Schmitt, & Gorboulev, 2003). However, their relatively recent discovery in

brain leaves us with much yet to understand about their function in the central nervous system, as well as their potential utility as targets for the development of therapeutic drugs.

4.1. OCTs and PMAT: functional significance in regulation of central biogenic amine neurotransmission

OCTs and PMAT are low-affinity transporters for biogenic amines but have a high capacity to transport these neurotransmitters, including 5-HT, DA, and NE. This raises the question, how significant is the contribution of OCTs and PMAT to biogenic amine uptake in brain, if any? In this regard, it is important to consider the mode of biogenic amine neurotransmission. It is now well established that following release of biogenic amines into the synaptic cleft, these neurotransmitters spill out of the synapse and, via volume (or paracrine) neurotransmission, can travel quite some distance from the synapse (~20 microns) (see Daws, 2009 for review; Bunin & Wightman, 1999; Cragg & Rice, 2004). This means that released neurotransmitter can interact with many extrasynaptic elements, including not only their “native” high-affinity transporters (which are located perisynaptically) but also with less specific low-affinity transporters, including OCTs and PMAT. When high-affinity transporters (e.g., SERT, NET, and DAT) are functioning normally, they are likely responsible for the majority of uptake of their “native” substrate (i.e., 5-HT, NE, and DA). However, when their function is reduced, as may occur following administration of therapeutic drugs, or because of genetic mutation, uptake of biogenic amines by OCTs and PMAT may become crucial to control extracellular levels of these amines.

4.2. A role for OCTs and PMAT in antidepressant response?

This concept becomes especially intriguing when we consider the mechanism of action of existing antidepressant drugs. These drugs block SERT, NET, and/or DAT, the high-affinity transporters for 5-HT, NE, and DA, respectively, thereby increasing extracellular levels of one or more of these biogenic amines. As we have described elsewhere (Daws, 2009; Daws, Koek, & Mitchell, 2013), activity of OCTs and/or PMAT could serve to limit this increase, thereby reducing or abolishing the ability of current antidepressant medications to trigger the downstream events thought to be necessary for ultimate therapeutic benefit.

We and others have provided support for this notion. For example, we found that when SERT expression in mice is genetically reduced, expression and activity of the OCT3 subtype increases (Baganz et al., 2008). In these mice, which either lack SERT (SERT^{-/-}) or express half as many SERT (SERT^{+/-}) as wild-type mice (SERT^{+/+}), decynium-22 (D-22), a blocker of OCTs and PMAT, produced antidepressant-like effects. D-22 also inhibited clearance of 5-HT from extracellular fluid in HIPP of SERT^{-/-} and SERT^{+/-} mice. In contrast, in SERT^{+/+} mice, D-22 did not inhibit 5-HT clearance, nor did it produce antidepressant-like effects (Baganz et al., 2008). These findings are consistent with a prominent role for OCTs, putatively OCT3 (see Baganz et al., 2008), in 5-HT uptake when SERT expression is reduced genetically.

What if SERT function is reduced pharmacologically? Based on our findings in SERT^{+/-} and SERT^{-/-} mice, we reasoned that uptake of 5-HT by OCTs and/or PMAT might likewise become more pronounced when SERT is blocked by an SSRI. If so, D-22 given together with an SSRI should increase the effectiveness of the SSRI to inhibit 5-HT uptake and produce antidepressant-like effects. To test this hypothesis, we gave wild-type mice the SSRI fluvoxamine together with D-22. We selected a dose of fluvoxamine that by itself did not produce significant antidepressant-like activity as measured by a reduction in time spent immobile in the tail suspension test. D-22 had no effect on immobility time in the tail suspension test in wild-type mice at any dose tested. In contrast, and consistent with our hypothesis, D-22 given together with fluvoxamine produced marked antidepressant-like

effects, with mice spending essentially no time immobile during the tail suspension test. Of note, this drug combination had no effect on general locomotor activity, nor did it produce any signs of the serotonin syndrome (Horton et al., 2013), which in mice include hind limb abduction, low body posture, Straub tail, tremor, backward movement, head weaving, and forepaw treading (Fox, Jensen, Gallagher, & Murphy, 2007). These data suggest that the anti-immobility effect produced by the combination of D-22 and fluvoxamine in the tail suspension test was not due to increases in general activity. Consistent with the enhanced antidepressant-like effect of D-22 and fluvoxamine in the tail suspension test, this drug combination also inhibited 5-HT clearance to a greater extent than fluvoxamine alone (Horton et al., 2013). This finding provides a mechanistic basis, at least in part, for the suboptimal therapeutic effects of SSRIs. Conceivably, uptake of 5-HT by OCTs and/or PMAT prevents extracellular levels rising to those needed to trigger downstream events that lead to therapeutic effects.

Our data are consistent with earlier reports that also support a role for these low-affinity, high-capacity transporters in antidepressant activity. For example, Kitaichi et al. (2005) showed that the antidepressant-like effect of the tricyclic, imipramine, was augmented in mice treated with antisense to decrease OCT3 expression. Low-affinity, high-capacity transporters for biogenic amines have also been coined “uptake-2,” whereas the high-affinity, low capacity transporters are known as “uptake-1.” Normetanephrine, the *O*-methylated metabolite of NE, inhibits “uptake-2.” Rahman et al. (2008) found that normetanephrine enhanced the ability of venlafaxine to increase extracellular NE in mouse cortex and enhanced the ability of desipramine to produce antidepressant-like activity in the tail suspension test. According to Rahman and colleagues, these effects of normetanephrine most likely resulted from its ability to block OCT3. More recently, Bacq et al. (2012) showed that genetic inactivation of OCT2 potentiated the antidepressant-like effects of three different classes of antidepressant: the NET blocker, reboxetine; the dual SERT and NET blocker, venlafaxine; and, to a lesser extent the SERT blocker, citalopram. Thus, there is converging evidence implicating OCT2 and OCT3 in dampening the antidepressant activity of SERT and NET blockers.

OCT1 has received less attention, likely because it is not as highly expressed in brain. In contrast, PMAT is highly expressed in brain. D-22 also inhibits uptake of biogenic amines via PMAT, so it is possible that some of the antidepressant actions of D-22 reported in the preceding text may also be mediated via this transporter. Recently, Duan and Wang generated a global PMAT knockout (KO) mouse (Duan & Wang, 2012). Using these mice, they showed that PMAT is the major transporter for uptake of bio-active amines in the choroid plexus, which forms the blood–cerebrospinal fluid barrier. Through uptake of substrates from cerebral spinal fluid, PMAT may be important in protecting the brain from cationic neurotoxins. These mice will be a useful tool in future studies investigating a role for PMAT in psychiatric disorders and as a potential target for development of novel therapeutic drugs.

In sum, several lines of evidence converge on the idea that low-affinity, high-capacity transporters for biogenic amines, such as OCT2, OCT3, and potentially other D-22-sensitive transporters such as PMAT, limit the ability of SERT and NET blockers to produce antidepressant-like effects. Figure 9.7 illustrates this idea. Uptake of biogenic amines by low-affinity, high-capacity transporters limits the increase in extracellular 5-HT, NE, and/or DA that can be produced by blockade of the high-affinity SERT, NET, and/or DAT. When the low-affinity, high-capacity transporters are also blocked, for example, by D-22, levels of extracellular 5-HT, NE, and/or DA can now increase further and presumably, therefore, increase antidepressant efficacy (see also Daws, 2009; Daws et al., 2013).

Although this an exciting area for future research, with the potential to uncover new targets for the development of drugs with improved antidepressant efficacy, there are limitations to keep in mind. Currently D-22 is the only commercially available compound with selectivity for OCTs and PMAT. Thus, the pharmacological tool belt in hand does not allow ready dissection of which OCT subtype(s) and/or PMAT may be most important in mediating the antidepressant-like effects of D-22. Using KO strategies is another approach, and recent studies suggest involvement of both OCT3 and PMAT in the antidepressant-like activity of D-22 (Horton et al., 2013). However, KO approaches have their own set of limitations, particularly constitutive KO where compensation of other transporters likely occurs, thereby creating a new set of potential confounds. Another caveat could lie in the remarkable promiscuity among both high- and low-affinity transporters for each of the biogenic amines. With this in mind, one prediction might be that a blanket inhibition of all high- and low-affinity transporters would be required to achieve improved antidepressant action. Consistent with this view, clinical data suggest that antidepressants that block SERT, NET, and DAT have greater antidepressant efficacy than drugs that block only one or two of these transporters (Skolnick et al., 2006). However, given that there is regional variability in the expression of high- and low-affinity biogenic amine transporters in limbic regions of brain thought to be important for antidepressant efficacy, it is likely that only a subset of transporters may be pivotal targets for improved therapeutic outcome, encouraging further study of OCTs and PMAT as such targets.

4.3. OCT3 and stress

So far, we have discussed the potential of OCTs and PMAT as targets for the development of novel antidepressants. In addition, there is a growing literature supporting an important role for OCT3 in the regulation of stress-induced enhancement of DA neurotransmission. The stress hormone, corticosterone, blocks OCT3, but not other OCT subtypes or PMAT. Gasser and coworkers have shown that concurrent blockade of DAT with GBR12909 and of OCT3 with corticosterone inhibits DA clearance in the nucleus accumbens to a greater extent than GBR12909 alone (Gasser et al., 2012, *XICS*). Since many abused drugs, including cocaine and amphetamine, act at DAT to inhibit DA uptake, OCT3 could play an important role in stress-induced reinstatement of drug taking. Others have reported similar results for 5-HT: when corticosterone levels are elevated, extracellular 5-HT levels are increased (Feng et al., 2010), and 5-HT clearance from extracellular fluid is prolonged (Baganz, Horton, Martin, Holmes, & Daws, 2010). Interestingly, short-term treatment with low levels of corticosterone produces antidepressant-like effects, consistent with corticosterone's ability to inhibit biogenic amine clearance via OCT3 (Stone & Lin, 2008).

4.4. OCT3 as a modulator of neurotoxicity

A role for OCT3 in neuroprotection has also been demonstrated. Studies from the Tieu lab reveal that the neurotoxic effect of the herbicide paraquat on DA neurons is increased in mice lacking OCT3 (Rappold et al., 2011). Neither DAT nor OCT3 can transport paraquat in its divalent cation state. However, when it is converted to a monovalent cation, it becomes a substrate for both transporters. Once taken into DA neurons by DAT, monovalent paraquat causes oxidative stress and cytotoxicity. OCT3 is richly expressed in non-DA cells, including astrocytes (Cui et al., 2009). Astrocytes are the most abundant cell type in brain and are located adjacent to the soma and terminals of midbrain DA neurons. Rappold and coworkers found that paraquat-induced striatal damage to DA neurons was increased in OCT3-deficient mice, suggesting that OCT3 serves as a critical buffer against paraquat-induced neurotoxicity. By reducing the amount of extra-cellular monovalent paraquat available for uptake by DAT, OCT3 protects DA neurons (Rappold et al., 2011). Similarly, Cui et al. (2009) showed that genetic deletion or pharmacological blockade of OCT3 protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced

nigrostriatal DA neuron degeneration. MPTP can be converted into its neurotoxic form, 1-methyl-4-phenylpyridinium (MPP⁺), by astrocytes. These investigators showed that MPP⁺ escapes from astrocytes via OCT3, where it can then be taken up by DAT, and leads to degeneration of DA neurons. Thus, preventing OCT3-dependent escape of MPP⁺ from astrocytes protects DA neurons (Cui et al., 2009).

4.5. Location

OCT3 is located on neurons and glia, including astrocytes (Cui et al., 2009; Vialou, Amphoux, Zwart, Giros, & Gautron, 2004), whereas PMAT is found on neurons, but not on astrocytes (Dahlin, Xia, Kong, Hevner, & Wang, 2007). The distribution of OCT3 and its cellular partition between astrocytes and neurons is region dependent. For example, OCT3 was found in nigrostriatal astrocytes, but not in astrocytes from cerebellum, HIPP, and cortex (Cui et al., 2009). While there is still much to be learned regarding precisely which neurons OCTs and PMAT occupy, and their regional distribution in brain, their presence on neurons raises the possibility that ectopic uptake of neurotransmitters into their nonnative neurons may occur more often than previously thought. Clearly, such activity could have important implications for monoaminergic interactions (see also Section 2). For example, uptake of 5-HT into DA neurons may induce intracellular changes in enzymatic activity and/or receptor and transporter trafficking to the plasma membrane, which differ from those induced by uptake of DA, the native substrate, by DAT. There is much to be learned about the physiological significance of “transporter promiscuity” and the fate of ectopically transported biogenic amines, but the preceding section suggests that this is an area ripe for further study.

4.6. Conclusion

From this brief overview, it is clear that OCTs and PMAT serve an important function in regulating biogenic amine neurotransmission. While these low-affinity, high-capacity transporters have been considered a “backup” system for the high-affinity SERT, NET, and DAT, there is growing evidence that they play a more prominent role than previously expected. For example, preclinical studies suggest that these transporters are important determinants of the therapeutic efficacy of existing antidepressant drugs. Thus, OCTs and PMAT are emerging as new targets for the development of novel antidepressant drugs with improved therapeutic effects. Moreover, OCT3 in particular appears to be an important modulator of neurodegeneration and might also be a novel target for neuroprotective drugs. Studies of OCT and PMAT function in brain are in their infancy, but these significant early findings encourage continued investigation of their fundamental biology and, importantly, their potential as therapeutic targets.

5. CONCLUSION

Neuroanatomical, electrophysiological, and neurochemical data are consistent with robust anatomical interconnections and functional interactions between DA, NE, and 5-HT systems in brain. In our review of catecholamine/5-HT interactions in brain function and disease, it is clear that successful therapeutic advances for improved clinical outcomes require an integrative approach. The development of promising novel treatment strategies takes advantage of knowledge of complex circuitry, the localization of receptor populations, and the role transporters and receptors play determining monoamine neurotransmission.

These intricate functional interactions between monoaminergic systems are vital in the regulation of brain function, for example, executive function and cognition, motor function and the coordination of voluntary movement, emotion and mood. The dysregulation of cognition, motor function, and emotion is present across disease states as core symptoms of

psychotic, neurodegenerative, and affective disorders. In this chapter, we have discussed three complex disorders of the central nervous system: schizophrenia, Parkinson's disease, and major depression. Although commonly viewed as very distinct disorders, they share common core symptoms. Motor symptoms include motor slowing, reduced movement, involuntary movements, and rigidity. Cognitive impairments include problems with making sense of information, attentional and memory deficits, and reduced ability to plan or carry out activities. Negative symptoms include negative mood, loss of interest in everyday activities, and social withdrawal.

Improved therapeutic approaches may also arise from addressing specific core symptoms or distinct syndromal dimensions (cognitive impairment, motor slowing/retardation, and negative affect/mood) regardless of disease classification, for example, psychotic, affective, and neurodegenerative. An example of this is the use of NET blockers to modulate DA levels in cortical areas (where DA innervation is relatively sparse), a strategy effective in treating cognitive deficits and negative symptoms associated not only with major depression but in schizophrenia and Parkinson's disease as well. Consideration of anatomical interconnections and functional interactions between DA, NE, and 5-HT systems, to address distinct syndromal dimensions, each with its distinct pathophysiology, will further the development of more effective treatment strategies for complex disorders of the central nervous system.

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ABBREVIATIONS

1A-KO	5-HT _{1A} receptor knockout mice
5-HT	serotonin
5-HT_{1A}	serotonin _{1A} receptor
5-HT_{2A}	serotonin _{2A} receptor
6-OHDA	6-hydroxydopamine
AADC	aromatic amino acid L-decarboxylase
APDs	antipsychotic drugs
D-22	decynium-22
DA	dopamine
DAT	dopamine transporter
DR	dorsal raphe nucleus
HFS-STN	high-frequency stimulation of subthalamic nucleus
HIPP	hippocampus
LC	locus coeruleus

LIDs	l-DOPA-induced dyskinesias
MPP⁺	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NE	norepinephrine
NET	norepinephrine transporter
OCT	organic cation transporter
PFC	prefrontal cortex
PMAT	plasma membrane monoamine transporter
RN	raphe nuclei
SERT	serotonin transporter
SNr	substantia nigra pars reticulata
SNRI	selective norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
VTA	ventral tegmental area

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Figure 9.1.

Schematic representation of the anatomical and functional relationships between the medial prefrontal cortex (PFC) and the brainstem monoaminergic nuclei. The PFC receives innervation from brainstem monoaminergic nuclei such as the ventral tegmental area (VTA, dopamine (DA)), locus coeruleus (LC, norepinephrine (NE)), and raphe nuclei (RN, serotonin (5-HT)), which are reciprocally connected with the PFC (Carr & Sesack, 2000; Celada & Artigas, 2007; Jodo et al., 1998). DA, NE, and 5-HT neurons modulate the activity of pyramidal cells in medial PFC through various receptors such as D₁, D₂, α₁-adrenergic, 5-HT_{1A}, and 5-HT_{2A} (Artigas, 2010; Masana et al., 2012). These receptors are identified as potential molecular targets for treating cognitive dysfunction in schizophrenia. Not shown: The PFC also innervates subcortical structures of the basal ganglia (nucleus accumbens and ventral pallidum), which feedback to PFC through the thalamus. The hippocampus (HIPP) and the amygdala provide contextual and emotional inputs to the PFC and nucleus accumbens.

Figure 9.2.

Bars show the effect of the combination treatment of NET inhibitor reboxetine (REB, 3 mg/kg, i.p.) plus selective α_2 -adrenergic receptor antagonist RX-821002 (RX, 1 mg/kg, s.c.) on DA release in medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) of freely moving adult rats assessed by *in vivo* microdialysis. Rats were implanted with two probes in mPFC and NAc. After initial 30 min stabilization period, four baseline fractions were collected (20 min per fraction) before systemic drug administration. Rats were pretreated with (i) vehicle, (ii) atypical APD clozapine (CLZ, 3 mg/kg, s.c.), or (iii) classical APD haloperidol (HAL, 0.1 mg/kg, s.c.). Saline was injected in fraction 5, and combined treatment was administered in fraction 11. Note that a marked and selective enhancement of DA output in mPFC is produced by the combined treatment of the NET inhibitor and α_2 -adrenergic receptor antagonists. Data are mean $\pm$ SEM of AUC of fractions 12–16, expressed as percentage of baseline. * $P < 0.01$ versus control treatment (veh/veh/veh) and ^ $P < 0.01$ between mPFC and NAc. Adapted from Masana et al. (2011).

Figure 9.3.

Local perfusion of atypical APDs: clozapine (300 μ M, $n = 9$), olanzapine (100 μ M, $n = 7$), and risperidone (100 μ M, $n = 5$) significantly increase the extracellular DA concentrations in medial PFC of wild-type (WT) mice. This effect was absent when APDs were infused in the medial PFC of 5-HT_{1A} receptor knockout (1A-KO) mice ($n = 6$ for each treatment). Data are mean $\pm$ SEM. Adapted from Bortolozzi *et al.* (2010).

Figure 9.4.

Effect of full 5-HT lesion on L-DOPA-induced extracellular DA levels in the STR, substantia nigra pars reticulata (SNr), HIPP, and PFC of hemiparkinsonian rats. Data represent the mean $\pm$ SEM of DA extracellular levels ($n=5-7$ animal/group) in each sample. Dialysis experiments were performed 3-4 weeks after the unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle combined with intraraphe injections of 5,7-DHT or its vehicle (sham). L-DOPA was administered intraperitoneally successively at 6 and 12 mg/kg at 2 h intervals as indicated by the vertical arrows, and each injection was preceded 20 min before by the administration of benserazide (15 mg/kg, i.p.; not shown in the figure). Asterisks refer to the overall statistical effect of each dose of L-DOPA. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus the sham group (Fisher's PLSD test). Adapted from Navailles *et al.* (2010).

Figure 9.5.

(A) Effect the nonselective NET inhibitor desipramine (DMI) on L-DOPA-induced extracellular DA levels in the striatum (STR), HIPP, and SNr (results in the prefrontal cortex are similar to SNr and HIPP and are not shown). Data represent the mean $\pm$ SEM of extracellular DA levels ($n=5-7$ animal/group) in each sample. Dialysis experiments were performed 3–4 weeks after the injection of 6-OHDA into the medial forebrain bundle. L-DOPA was administered intraperitoneally at 12 mg/kg as indicated by the vertical arrows, and each injection was preceded 20 min before by the administration of both benserazide (15 mg/kg, i.p.; not shown in the figure) and DMI (10 mg/kg, i.p.) or its vehicle. Asterisks refer to the overall statistical effect of L-DOPA. $**P < 0.01$ versus the L-DOPA group (Fisher's PLSD test; for experimental procedures, see Navailles et al., 2010). (B) Drawing representing the mechanism of action of L-DOPA. Briefly, L-DOPA enters 5-HT neurons. It is converted into DA via the L-aromatic amino acid decarboxylase (AADC). 5-HT and DA are costored in exocytotic vesicles through the vesicular monoamines transporter (VMAT2) inside 5-HT neurons and coreleased. Then, NE neurons take up extracellular DA and/or NE via the NE transporter (NET). DMI blocks the clearance of extracellular DA by the NET. Adapted from Navailles et al. (2013).

Relative innervation of each monoamine system in regions of interest

DA tone in 6-OHDA rats: response to L-DOPA

Figure 9.6.

The brain pattern of extracellular DA induced by L-DOPA involves 5-HT and NA fibers. The upper drawings correspond to an approximate illustration of the density of innervation of each monoaminergic system in some brain areas. It depicts that the normal DA innervation is extremely high in the striatum compared to the other brain regions. By contrast, 5-HT innervation is comparatively quite homogenous. NE innervation is extremely low in the striatum. The lower panels depict the DA tone in 6-OHDA rats. DA tone is dramatically increased in all brain regions after L-DOPA (corresponding to doses over 6 mg/kg) with a preferential response in the striatum. In rats receiving L-DOPA and bearing an additional lesion of 5-HT neurons, DA tone in all brain regions is almost absent. In rats receiving NET inhibitors, the magnitude of the effect induced by L-DOPA on DA tone is similar in all brain regions.

Figure 9.7.

Schematic representation of how volume (paracrine) transmission may be an important role in determining the contribution of high- and low-affinity transporters for biogenic amines to their clearance from extracellular fluid. The *left panel* depicts the hypothetical amount of biogenic amines in extracellular fluids when neither high- nor low-affinity transporters are blocked. The *middle panel* shows that when high-affinity transporters are blocked, biogenic amine uptake continues via D-22-sensitive low-affinity, high-capacity transporters such as organic cation transporters or plasma membrane monoamine transporter located on neighboring neurons or glia. The *right panel* shows that when these D-22 sensitive transporters are also blocked, levels of extracellular biogenic amines increase dramatically.

Table 9.1

Expression of some monoaminergic receptor mRNAs in prelimbic cortex of rat

Receptor	Prelimbic cortex		
	Layers II–III	Layer V	Layer VI
D₁ positive			
Pyramidal neurons	19.0 ± 3.0	21.0 ± 2.0	38.0 ± 3.0
GABAergic neurons	28.0 ± 1.0	30.0 ± 2.0	38.0 ± 4.0
D₂ positive			
Pyramidal neurons	5.0 ± 1.0	25.0 ± 2.0	13.0 ± 1.0
GABAergic neurons	5.0 ± 1.0	8.0 ± 2.0	17.0 ± 2.0
5-HT_{1A} positive			
Pyramidal neurons	n.e.	61.0 ± 2.0	n.e.
GABAergic neurons	n.e.	20.0 ± 1.0	n.e.
5-HT_{2A} positive			
Pyramidal neurons	n.e.	52.0 ± 3.0	n.e.
GABAergic neurons	n.e.	34.0 ± 1.0	n.e.
Alpha₁ positive			
Pyramidal neurons	80.0 ± 3.0	79.0 ± 8.0	59.0 ± 2.0
GABAergic neurons	79.0 ± 5.0	72.0 ± 5.0	57.0 ± 8.0

Data are means ± SEM of three rats (four adjacent sections per rat) and show the percentage of neurons of each type (pyramidal or GABAergic) expressing several monoaminergic receptors in superficial, intermediate, or deep layers of the prelimbic area in the medial PFC of rat. n.e. not examined.

Adapted from Masana et al. (2012)