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# Neuroplasticity of Neocortical Circuits in Schizophrenia

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The core features of schizophrenia include deficits in cognitive processes mediated by the circuitry of the dorsolateral prefrontal cortex (DLPFC). These deficits are associated with a range of molecular and morphological alterations in the DLPFC, each of which could be a cause, consequence, or compensation in relation to other changes, and thus reflect the neuroplasticity of the brain in response to the underlying disease process. In this review, we consider disturbances in excitatory, inhibitory, and modulatory connections of DLPFC circuitry from the perspective of disease- and development-related neuroplasticity and discuss their implications for the identification of novel therapeutic targets.
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## INTRODUCTION

In a broad sense of the term, neuroplasticity refers to the capacity of the brain to change the molecular and structural features that dictate its function. In the context of a disease process such as schizophrenia, these changes, as observed directly in postmortem tissue or reflected in biomarkers detectable by neuroimaging, electroencephalography or other noninvasive assessments of brain structure and function, could represent any of the following four 'Cs': (1) Cause, an upstream factor related to the disease pathogenesis; (2) Consequence, a deleterious effect of a cause; (3) Compensation, a response to either cause or consequence that helps restore homeostasis; or (4) Confound, a product of factors frequently associated with, but not a part of, the disease process, or an artifact of the approach used to obtain the measure of interest. Thus, understanding neuroplasticity (as defined above) in schizophrenia requires the identification of the brain alterations that are reliably associated with the clinical syndrome that we term schizophrenia, and then the determination of the 'C' category to which each alteration belongs.

This process is, of course, complicated by the apparent heterogeneity associated with the diagnosis of schizophrenia. From the classic view of a disease process, the etiology of schizophrenia unleashes pathogenetic mechanisms that produce a pathological entity, a conserved set of molecular and cellular disturbances in the brain. This pathological entity so alters the brain's normal function that the resulting pathophysiology gives rise to the emergent properties recognized as the clinical features of the illness (Lewis *et al*, 2005). Etiologically, most cases of schizophrenia are thought to be the consequence of a complex interplay of genetic liabilities and environmental risk factors that alter the developmental trajectories of neural circuits (Harrison and Weinberger, 2005; Lewis and Levitt, 2002). At the other end of the disease process, the broad range of clinical features found in individuals who meet diagnostic criteria for schizophrenia suggests that multiple brain systems are affected in this disorder. Thus, a critical challenge in exploring neuroplasticity in schizophrenia is to understand this heterogeneity from the perspectives of diversity (the existence of different disease entities within the population meeting diagnostic criteria) and variability (variance of particular parameters within a disease entity).

One approach to addressing this problem is to focus on a well-defined clinical component of the illness. In this regard, it has been argued that among the clinical manifestations of schizophrenia, the deficits in cognitive abilities are the core features of the illness (Elvevag and Goldberg, 2000). For example, a characteristic pattern of cognitive deficits occurs with high frequency in individuals with schizophrenia, is relatively stable over the course of the illness, is independent of the psychotic symptoms of the disorder, and is present in a milder form in individuals at genetic risk who do not become clinically ill (Gold, 2004). Furthermore, the degree of cognitive impairment is the strongest determinant of functional outcome for individuals with schizophrenia (Green, 1996).

Of the different domains of cognitive dysfunction in schizophrenia, working memory, the ability to transiently maintain and manipulate a limited amount of information

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to guide thought or behavior, has been studied extensively. Working memory involves several component processes including storage buffers for different types of information (eg visuo-spatial scratch pad (short-term storage of visual information) and phonological loop (articulatory rehearsal and phonological coding of verbal information) and a central executive component that controls the manipulation of information within the storage buffers (Baddeley, 1992). In subjects with schizophrenia, tasks that depend primarily on the phonological loop show relatively little impairment. In addition, activation of the brain regions (eg ventral lateral prefrontal cortex and posterior parietal cortex) that mediate the components of the phonological loop appear to be intact (Barch, 2006). In contrast, central executive function, especially the manipulation of transiently stored information, is clearly disturbed in subjects with schizophrenia (Cannon *et al*, 2005; Tan *et al*, 2005), and this dysfunction is accompanied by altered activation of the dorsolateral prefrontal cortex (DLPFC) (Box 1), a brain region known to be associated with executive function (Callicott *et al*, 2003). The altered activation of the DLPFC under such conditions might be specific to the disease process of schizophrenia because these disturbances are present in medication-naive individuals with schizophrenia, but not in subjects with other psychotic disorders or major depression (Barch *et al*, 2003; MacDonald *et al*, 2005).

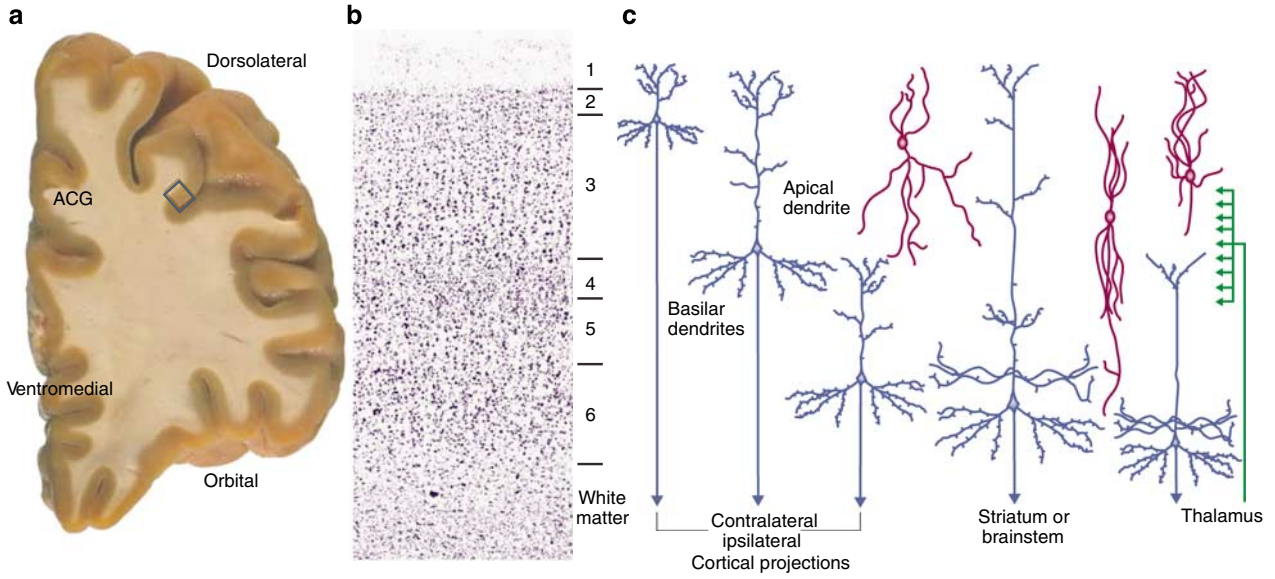
Consequently, in this paper, we examine the alterations in excitatory, inhibitory, and modulatory components of DLPFC circuitry in schizophrenia that might contribute to the impairments in working memory. Each component is examined in relation to the four 'Cs' and considered from the perspectives of disease and developmental neuroplasticity. It is important to note that while we focus on the DLPFC as a prototypic cortical region affected in the illness, the alterations described below are likely to be present in multiple other cortical regions as well.

## NEUROPLASTICITY OF EXCITATORY CORTICAL CONNECTIONS IN SCHIZOPHRENIA

### Evidence of Altered Excitatory Connections in the DLPFC in Schizophrenia

The disease process of schizophrenia appears to involve deficient glutamate-mediated excitatory neurotransmission through the NMDA receptor (Moghaddam, 2003; Olney and

**Box 1**




In the primate brain, the prefrontal cortex is generally considered to encompass a set of structurally and functionally heterogenous cortical areas located anterior to the motor and premotor regions of the frontal lobe. These regions share a granular cytoarchitecture (that is, a discernible layer 4) and they are the recipients of axonal projections from the mediodorsal thalamus, although each of these features has exceptions. The prefrontal cortex can be divided into dorsolateral, orbital, and ventromedial regions as shown in (a), an unstained coronal block cut immediately anterior to the corpus callosum through the left hemisphere of a postmortem human brain. This block also includes the adjacent anterior cingulate gyrus (ACG) of the limbic lobe. The portion of the dorsolateral prefrontal cortex (DLPFC) delineated by the small rectangle is shown at higher magnification after staining for Nissl substance in (b). This section shows the typical appearance of six layers or lamina, numbered from the pial surface of the cortex to the underlying white matter, based on the size and packing density of neurons. Distributed across these layers are different types of pyramidal neurons (red cells in (c)), which represent about 75% of cortical neurons. These neurons typically have triangularly shaped cell bodies, a single apical dendrite directed towards the pial surface and an array of basilar dendrites. Depending on their laminar location, the axons of pyramidal neurons provide excitatory projections to different brain regions, with those located in layers 2 and 3 projecting principally to other cortical regions in the same (ipsilateral) or opposite (contralateral) hemisphere, those located in layer 5 projecting principally to the striatum or brainstem and those in layer 6 projecting principally to the thalamus. Axons that project to the DLPFC from other brain regions also tend to innervate different subsets of cortical layers. For example, axonal projections (green) from the thalamus terminate in layers deep 3 and 4. The remaining ~25% of DLPFC neurons are local circuit or interneurons (blue cells). These neurons use the inhibitory neurotransmitter GABA, and have axons that arborize locally and innervate other neurons in the same area of the prefrontal cortex. Figure adapted from Lewis (2004).

Farber, 1995). NMDA receptor antagonists such as phencyclidine (PCP) or ketamine increase both positive and negative symptoms in patients with schizophrenia (Coyle, 2004), and the administration of subanesthetic doses of ketamine to healthy individuals produces thought disorder and other features similar to those seen in schizophrenia (Adler *et al*, 1999; Krystal *et al*, 1994). In addition, systemic administration of NMDA receptor antagonists disrupts working memory in rats (Verma and Moghaddam, 1996), and application of an NMDA receptor antagonist to the DLPFC (but not to the primary visual cortex) impairs working memory performance in monkeys (Dudkin *et al*, 2001). Furthermore, computational modeling studies of neuronal network activity in the DLPFC suggest that an NMDA receptor activation supports slow reverberating excitation (Durstewitz and Seamans, 2006; Wang, 2006). This pattern of excitation is critical for the sustained firing of DLPFC neurons that is considered to be the cellular basis of working memory (Goldman-Rakic, 1995).

Hypoactivation of only NMDA receptors in schizophrenia is unlikely to result from deficits in glutamate release because, in the adult monkey DLPFC, both AMPA and NMDA receptors mediate fast synaptic transmission at glutamate synapses (Gonzalez-Burgos *et al*, 2007b); thus, decreased glutamate release would also result in deficits in AMPA receptor-mediated transmission. Consequently, selective NMDA receptor hypofunction would appear more likely to result from postsynaptic deficits in NMDA receptor-mediated signaling. However, although postmortem studies have reported alterations in measures of glutamate receptor binding, transcription and subunit protein expression in several brain regions in subjects with schizophrenia (Konradi and Heckers, 2003), such findings for mRNA and protein levels of NMDA receptor subunits in the DLPFC have been limited in magnitude and not always replicated (Akbarian *et al*, 1996; Dracheva *et al*, 2001; Kristiansen *et al*, 2006; Mirnics *et al*, 2000). Indeed, as noted elsewhere (Kristiansen *et al*, 2007), the modest and inconsistent findings in postmortem studies of NMDA receptor transcripts and protein expression levels in schizophrenia suggest that other components of NMDA receptor signaling might be affected in the illness (Kristiansen *et al*, 2007). It is possible, however, that NMDA hypoactivity in schizophrenia is associated with decreased levels of synaptic NMDA receptor proteins in particular classes of inputs that are difficult to detect.

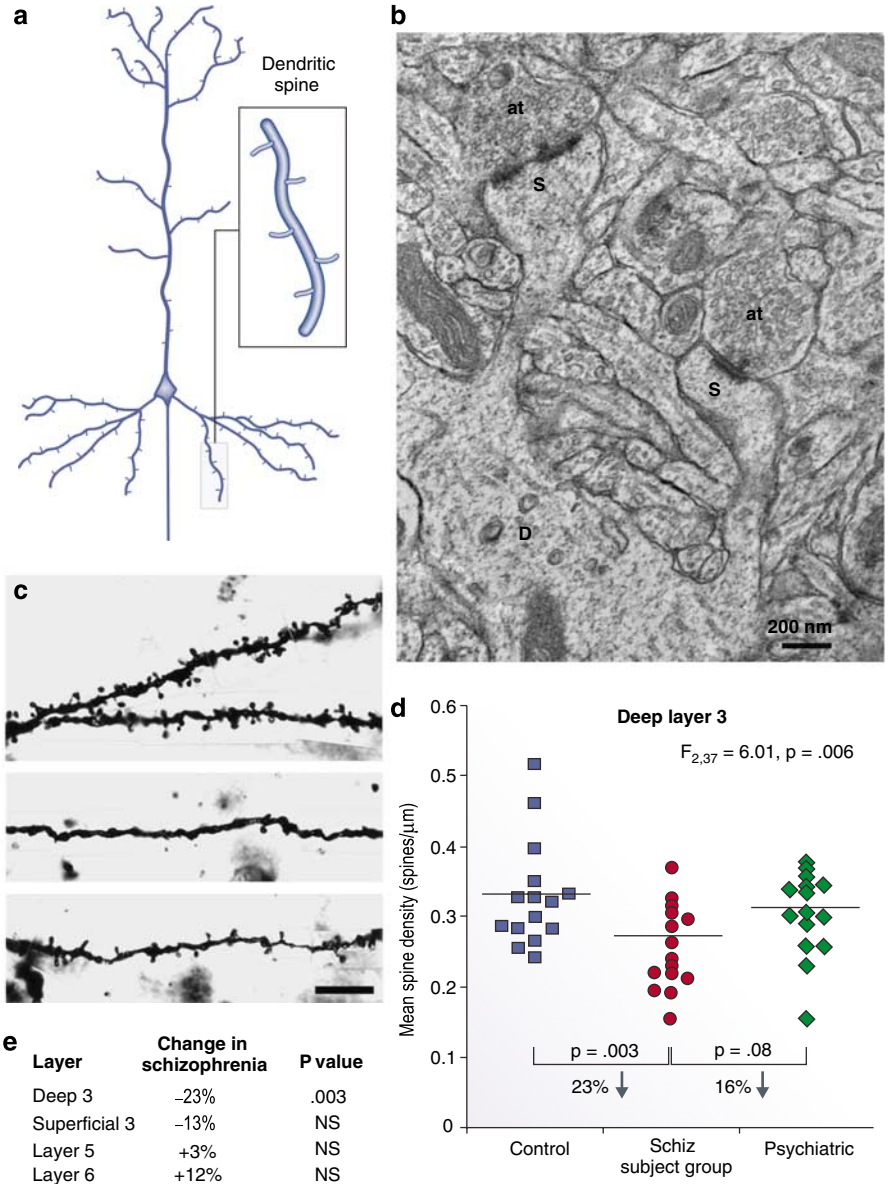
Anatomical studies do support the presence of input-specific alterations of excitatory connections in the DLPFC in schizophrenia. In the DLPFC, pyramidal neurons are the principal source of glutamate neurotransmission, as well as the targets of the majority of glutamate-containing axon terminals (White, 1989). Although the number of these neurons does not appear to be altered in schizophrenia (Akbarian *et al*, 1995; Thune *et al*, 2001), neuronal density in the DLPFC and certain other cortical regions, such as the primary visual cortex, has been reported to be increased in schizophrenia (Selemon *et al*, 1995). (However, more recent studies have failed to support this finding, at least in the visual cortex; Dorph-Petersen *et al*, 2007.) Increased cell packing density has been interpreted as evidence of a reduction in the amount of cortical neuropil, the axon terminals, dendritic spines, and glia processes that occupy the space between neurons (Selemon and Goldman-Rakic, 1999). Consistent with this interpretation, decreased synaptophysin protein, a marker of axon terminals, has been reported to be decreased in the DLPFC of subjects with schizophrenia (Glantz and Lewis, 1997; Karson *et al*, 1999; Perrone-Bizzozero *et al*, 1996). Furthermore, gene expression profiling studies have found reduced tissue levels of gene transcripts that encode proteins involved in the presynaptic regulation of neurotransmission (Mirnics *et al*, 2000).

Dendritic spines are the principal targets of excitatory synapses to pyramidal neurons. Approximately 75% of the dendritic spines present in the somatosensory cortex of young adult mice are numerically stable over adulthood (Zuo *et al*, 2005), but they are subject to a number of neuroplastic changes, such as a loss of their presynaptic excitatory input. In schizophrenia, dendritic spine density in pyramidal neurons has been reported to be lower in both the DLPFC and other cortical regions by multiple research groups (Black *et al*, 2004; Broadbelt *et al*, 2002; Garey *et al*, 1998; Glantz and Lewis, 2000; Kalus *et al*, 2000). These findings have raised a series of questions regarding the neuroplastic nature of the apparent changes in spine density in schizophrenia.

## Is the Lower Spine Density in Schizophrenia Specific to Certain Subpopulations of DLPFC Pyramidal Neurons?

Pyramidal neurons can be divided into subgroups based on the brain region targeted by their principal axonal projection and the sources of their excitatory inputs; both of these characteristics are associated with the laminar location of pyramidal cell bodies (Box 1). For example, many pyramidal cells in layers 2 and 3 send axonal projections to other cortical regions, pyramidal neurons in layer 5 tend to project to the striatum and other subcortical structures, and pyramidal neurons in layer 6 furnish projections primarily to the thalamus (Jones, 1984). Studies of basilar dendritic spine density on Golgi-impregnated pyramidal neurons in each cortical layer of the DLPFC in the same cohort of subjects found a significant effect of diagnosis on spine density only for pyramidal neurons in deep layer 3 (Figure 1; Glantz and Lewis, 2000; Kolluri *et al*, 2005). In subjects with schizophrenia, spine density on these neurons was significantly decreased by 23 and 16%, respectively, compared to normal and psychiatrically ill comparison subjects. In contrast, spine density on pyramidal neurons in superficial layer 3 was reduced to a lesser degree, whereas the density of those on the basilar dendrites of pyramidal neurons in layer 5 or 6 did not differ across subject groups.

The functional integrity of the pyramidal neurons with lower dendritic spine densities may be reflected in changes in their somal volume. For example, shifts in somal size may indicate disturbances in neuronal connectivity, given that somal size has been shown to be correlated with measures of a neuron's dendritic tree (Hayes and Lewis, 1996; Jacobs *et al*, 1997) and axonal arbor (Gilbert and Kelly, 1975; Lund *et al*, 1975). Indeed, the mean cross-sectional somal area of the Golgi-impregnated, deep layer 3 pyramidal neurons was 9.1% smaller in the subjects with schizophrenia relative to

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| Layer | Change in schizophrenia | P value |
|---|---|---|
| Deep 3 | –23% | .003 |
| Superficial 3 | –13% | NS |
| Layer 5 | +3% | NS |
| Layer 6 | +12% | NS |

**Figure 1** Pyramidal neuron dendritic spines in the human dorsolateral prefrontal cortex (DLPFC). (a) Schematic diagram illustrating the dendritic tree and dendritic spines on a prototypic pyramidal neuron. (b) Electron micrograph showing a dendrite (D) with two spines (S). Each spine receives an asymmetric (presumably excitatory) synapse from an axon terminal (at). (c) Golgi-impregnated basilar dendrites and spines on deep layer 3 pyramidal neurons from a normal comparison (top) and two subjects with schizophrenia (bottom). Note the reduced density of spines in the subjects with schizophrenia in these extreme examples. (d) Scatter plot demonstrating the lower density of spines on the basilar dendrites of deep layer 3 pyramidal neurons in the DLPFC of subjects with schizophrenia relative to both normal and psychiatrically ill comparison subjects. (e) Laminar-specificity of the spine density differences in the same subjects. (c–e) Adapted from Glantz and Lewis (2000); Kolluri *et al* (2005).

normal control subjects, although this difference did not achieve statistical significance (Glantz and Lewis, 2000). Consistent with this observation, the mean somal volume of Nissl-stained pyramidal neurons in DLPFC deep layer 3 was 9.2% smaller in a different cohort of subjects with schizophrenia, relative to matched normal comparison subjects, a decrease that was not explained by either antipsychotic medication history or duration of illness (Pierri *et al*, 2001). Similarly, in another study, the mean somal size of all layer 3 neurons in DLPFC area 9 was smaller in subjects with schizophrenia, and was accompanied by a decrease in the density of the largest neurons in deep layer 3, without a change in somal volume in layer 5 (Rajkowska *et al*, 1998). Furthermore, in both primary and association auditory cortices, somal volumes of deep layer 3, but not of layer 5, pyramidal neurons were smaller in schizophrenia (Sweet *et al*, 2003, 2004). Together, these findings suggest that in schizophrenia, (1) basilar dendritic spine density is lower and somal volume is smaller in deep layer 3 pyramidal neurons, (2) these alterations are specific to or at least most prominent in deep layer 3, (3) this pattern of alterations is not restricted to the DLPFC, and (4) these differences reflect the underlying disease process and not confounding factors. However, these findings do not reveal the biochemical or connectional identity of the affected pyramidal neurons.

Even within the same cortical layer, different subpopulations of pyramidal neurons exhibit quantitative differences in dendritic morphology and qualitative differences in the gene products that they express, and these subpopulations of pyramidal neurons tend to differ in the targets of their principal axon projections. For example, pyramidal neurons in the supragranular layers of the monkey DLPFC with axons that project callosally have larger dendritic arbors and a greater density of dendritic spines than do neighboring pyramidal cells that furnish axons to the adjacent regions of the ipsilateral DLPFC (Duan *et al*, 2002; Soloway *et al*, 2002). Pyramidal neurons that furnish axonal projections to distant cortical regions also tend to have larger cell bodies and to express high levels of non-phosphorylated epitopes of neurofilament proteins (NNFPs) compared to pyramidal cells in the same location that provide shorter corticocortical projections (Hof *et al*, 1996). Attempts to identify the affected pyramidal neurons based on such markers have not yet yielded fruit. For example, the somal volumes of NNFP-containing layer 3 neurons were reported to be unaltered in schizophrenia (Law and Harrison, 2003; Pierri *et al*, 2003), but these studies appear to have been subject to a methodological confound that resulted in an overestimation of somal volumes in schizophrenia (Maldonado-Aviles *et al*, 2006). Furthermore, the ability to distinguish other subpopulations of pyramidal neurons based on their molecular phenotype still awaits the types of gene expression profiling studies that have been successfully utilized for characterizing subclasses of cortical interneurons (Sugino *et al*, 2006).

## Are the Reductions in Spine Density Associated with a Deficit in a Specific Source of Excitatory Inputs?

Excitatory projections from the mediodorsal thalamus, the principal source of thalamic inputs to the DLPFC (Giguere and Goldman-Rakic, 1988), synapse primarily on dendritic spines (Melchitzky *et al*, 1999). These axons densely arborize in DLPFC layers deep 3 and 4, but do not innervate the deep cortical layers (Erickson and Lewis, 2004). Since the laminar distributions of these afferents matches that of the basilar dendrites of DLPFC deep layer 3 pyramidal neurons, a reduction in the number or activity of these afferents could contribute to the observed decrement in spine density in schizophrenia. Initial reports indicated that the total number of neurons in this nucleus was lower in schizophrenia (Byne *et al*, 2002; Pakkenberg, 1990; Popken *et al*, 2000; Young *et al*, 2000), suggesting that a reduced number of these afferents might contribute to lower spine density in the DLPFC in schizophrenia. However, more recent studies, including some from the same research groups that reported the initial positive findings, have failed to find a decrement in thalamic neuron number (Cullen *et al*, 2003; Dorph-Petersen *et al*, 2004; Kreczmanski *et al*, 2007; Young *et al*, 2004). Some neuroimaging studies have reported smaller thalamic volumes, altered shape and decreased activity (Manoach *et al*, 1999) in subjects with schizophrenia, including studies in first-episode subjects in whom the potential confounds of medication effects and illness chronicity are mitigated (Gilbert *et al*, 2001). Thus, even in the face of a normal number of thalamic neurons, these observations might suggest that the nature, if not the number, of thalamic projections is altered in schizophrenia. However, a reduction in thalamic inputs to the DLPFC cannot completely account for the decrease in dendritic spine density since thalamocortical terminals appear to comprise a small proportion ($<10\%$) of the total excitatory inputs to the targeted cortical neurons in animals (Guillery and Sherman, 2002). If this holds true in the human DLPFC, then (in the absence of some synergistic factor—see below) even a total absence of thalamocortical afferents would not be sufficient to account for the apparent magnitude of the reduction in basilar dendritic spine density on deep layer 3 pyramidal cells observed in schizophrenia.

Two other major sources provide excitatory inputs to deep layer 3 pyramidal neurons in the primate DLPFC, although these inputs are less laminarly restricted than those from the mediodorsal thalamus. First, the axons of pyramidal neurons in layers 2 and 3, which project to other cortical regions, also give rise to both local (within 300 μm of the cell body) and long-range axon collaterals that travel through the gray matter for up to several millimeters before arborizing in discrete stripe-like clusters in the same cortical region (Levitt *et al*, 1993; Pucak *et al*, 1996). Both the extrinsic and long-range intrinsic collaterals of these neurons target almost exclusively the dendritic spines of other pyramidal cells, whereas the synaptic targets of the local axon collaterals are equally divided between dendritic spines and the dendritic shafts of the parvalbumin-containing class of GABA neurons (Melchitzky *et al*, 1998, 2001; Melchitzky and Lewis, 2003). Second, associational or callosal projections from other cortical regions also terminate in these layers (Barbas, 1992; Pucak *et al*, 1996). Thus, the smaller decrease in spine density on superficial layer 3 (relative to deep layer 3) pyramidal cells raises the possibility that abnormalities in thalamocortical afferents to deep layer 3 have an additive effect to a disturbance in the cortical axon terminals that are distributed across layer 3. However, even if these interpretations are correct, they do not reveal the direction of the pathogenetic mechanisms. For example, the reduced number of excitatory inputs to DLPFC layer 3 pyramidal cells might not be the consequence of a more primary disturbance in the source of these afferents, but an abnormality intrinsic to these pyramidal cells could render them unable to support a normal complement of excitatory inputs.

## What Molecular Mechanisms Mediate the Lower Spine Density in Schizophrenia?

Attempts to explore the direction of these pathogenetic mechanisms have looked at both extracellular and intracellular molecular mediators of spine number in schizophrenia. One potential extracellular mediator is the secreted neurotrophin, brain-derived neurotrophic factor (BDNF). BDNF stimulates the growth of dendrites and increases the spine density of cortical pyramidal neurons in organotypic brain slices in a lamina-specific fashion (McAllister *et al*, 1995, 1996). The expression of BDNF mRNA is lower in the

DLPFC of subjects with schizophrenia (Hashimoto *et al*, 2005; Weickert *et al*, 2003), and BDNF mRNA levels and spine density on the basilar dendrites of deep layer 3 pyramidal neurons were positively correlated ($r = 0.91$; $p = 0.004$) in the same subjects with schizophrenia (Hill *et al*, 2005). However, large ($\sim$80%) genetically engineered reductions in BDNF expression, induced either prenatally or in early adulthood, were not sufficient to alter basilar dendritic morphology in the prefrontal cortex of adult mice (Hill *et al*, 2005). Similarly, in heterozygous *bdnf* knockout mice, a 50% reduction in BDNF protein levels did not affect the amount of cortical neuropil (Genoud *et al*, 2004), of which excitatory axon terminals and dendritic spines are a major component. Therefore, these findings suggest that reduced levels of BDNF mRNA expression in subjects with schizophrenia are not sufficient to account for the observed deficit in spine density. However, it must be kept in mind that potential compensatory mechanisms, such as increased expression of the neurotrophin NT-4 or of the receptor for BDNF, tyrosine kinase B (trkB), might occur in *bdnf* knockout mice but not in schizophrenia. For example, in subjects with schizophrenia, reduced BDNF levels are accompanied by a significant decrease in trkB mRNA levels (Hashimoto *et al*, 2005; Weickert *et al*, 2005). Thus, reduced levels of both BDNF and trkB may be required to produce the decrease in spine density observed in schizophrenia. Alternatively, the murine models may fail to reproduce a cell type-specific decrement in BDNF mRNA expression in schizophrenia that is required for alterations in spine density.

An increasing number of intracellular signaling pathways have been shown to regulate the actin cytoskeleton that is critical for spine structure (Calabrese *et al*, 2006). In neurons, different members of the RhoGTPase family regulate specific aspects of dendritic morphology and spine number (Luo, 2000; Negishi and Katoh, 2005). For example, the RhoGTPase Cdc42 (cell division cycle 42), which regulates the outgrowth of the actin cytoskeleton, is involved in the formation of thin protrusions (filopodia), which may develop into mature spines. In neuronal cultures, Cdc42 is essential for the establishment of the full complement of dendritic spines (Ramakers, 2002; Scott *et al*, 2003). Rac1 (Ras-related C3 botulinum toxin substrate 1) helps to stabilize the actin cytoskeleton, and thus appears to contribute to the outgrowth of spine-like structures (Nobes and Hall, 1995; Ramakers, 2002) and to be a critical regulator of dendritic spine density (Nakayama *et al*, 2000). In contrast, RhoA (Ras homologous member A) regulates the destabilization of the actin cytoskeleton, such that the activation of RhoA results in a reduction in the density of dendritic spines (Nakayama *et al*, 2000; Nobes and Hall, 1995). Other proteins that are selectively localized to spines also play roles in spine structure. Duo, the human ortholog of the murine Kalirin-7, is a GDP–GTP exchange factor that activates Rac1 (Penzes *et al*, 2000) and is essential for spine maintenance and for coordinating the apposition of presynaptic terminals to the sites of postsynaptic densities (Ma *et al*, 2003; Penzes *et al*, 2001). Drebrin, an actin-binding protein (Hayashi *et al*, 1996), regulates the maturation of filopodia into mature spines by promoting the formation of the postsynaptic density and appears to be involved in spine morphogenesis by promoting the conversion of filopodia into mature spines (Takahashi *et al*, 2003).

Studies of the transcripts for these five proteins in the DLPFC of subjects with schizophrenia revealed that only the mRNAs for Cdc42 and Duo showed significantly decreased expression levels that were (1) restricted to the cortical gray matter; (2) significantly correlated with spine density in the same subjects; and (3) not due to chronic treatment with antipsychotic medications or other potential confounds (Hill *et al*, 2006). Because Cdc42 regulates the polymerization of the actin cytoskeleton into filopodia (Nobes and Hall, 1995), many of which are precursors for mature spines (Dailey and Smith, 1996), reduced levels of Cdc42 might impair the ability of pyramidal neurons to generate new filopodia in the adult neocortex. Furthermore, the formation of spines is closely tied to the generation of stable constructs of the actin cytoskeleton (Nobes and Hall, 1995). This stability is regulated by Rac1, whose upstream regulator is Duo (Carlisle and Kennedy, 2005). Therefore, a decrease in Duo mRNA expression would result in reduced activation of Rac1, and consequently impaired stability of dendritic spines. In combination, a decrease in the expression of both Cdc42 and Duo mRNAs would be expected to result in a reduction in the formation of new spines and impaired maintenance of existing, mature spines.

However, expression levels of both Cdc42 and Duo mRNAs were comparable in both deep layer 3 and 6 in the subjects with schizophrenia. Although this observation suggests that the decreased expression of Cdc42 and Duo mRNAs in schizophrenia is not a consequence of a reduced number of pyramidal neuron dendritic spines, since the expression of these mRNAs was similarly reduced in pyramidal neurons with or without a reduction in spine density, it also indicates that reduced expression of Cdc42 and Duo mRNAs is not sufficient to cause reduced spine density. Because excitatory synaptic activity (via NMDA receptors) can stimulate the formation of new spines, in addition to changing the shape of existing spines (Carlisle and Kennedy, 2005; Jourdain *et al*, 2003), a laminar-specific reduction in excitatory inputs, such as those discussed above, might interact with alterations in the molecular cascades involving Cdc42 and Duo to produce a reduction in spine density in schizophrenia. Although speculative, this interpretation suggests the testable hypothesis that spine density is decreased on the portions of the apical dendrites of pyramidal neurons in layer 5 or 6 that pass through layer deep 3, while being unaltered on the basilar dendrites of the same neurons (Figure 2).

It must also be kept in mind that other molecular cascades contribute to the regulation of the actin cytoskeleton (Carlisle and Kennedy, 2005), and their potential role in the reduced density of dendritic spines in schizophrenia remains to be determined. For example, inhibitors of spine formation, such as Nogo, which has been reported to be increased in the frontal cortex in schizophrenia (Novak and Tallerico, 2006), could contribute to the observed reductions in spine density. In addition, it could be that molecules selectively expressed by layer 3 pyramidal neurons (Arion *et al*, 2007) provide the essential factor that determines the apparent heightened vulnerability of these neurons in schizophrenia.

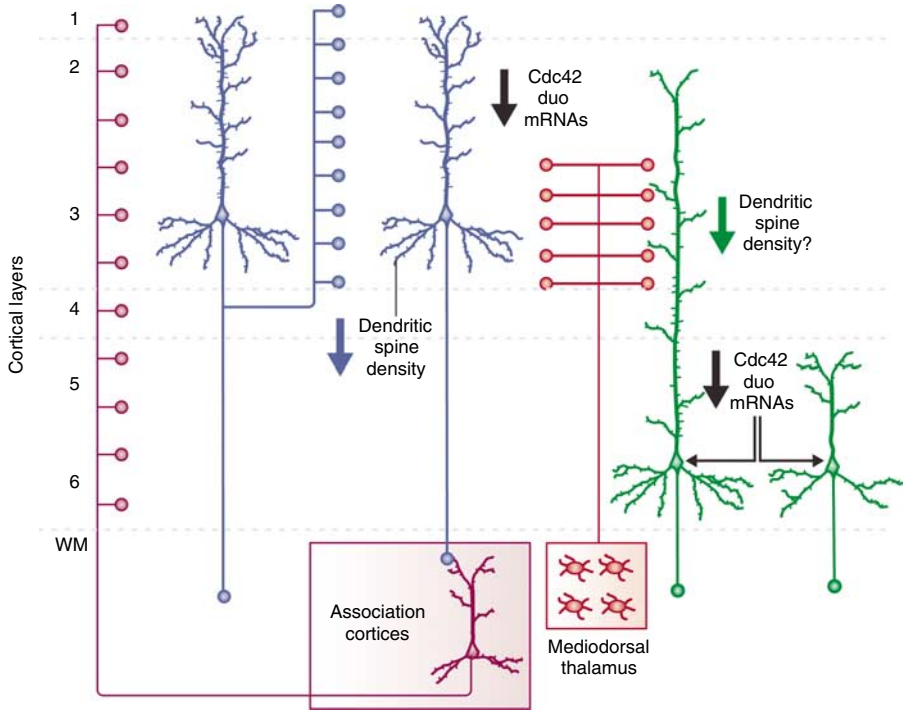



**Figure 2** Schematic diagram summarizing the idea that reduced dendritic spine density in schizophrenia might require alterations both in regulators of the actin cytoskeleton that are intrinsic to pyramidal neurons and in the extrinsic excitatory inputs to pyramidal neurons. In this example, reduced expression of Cdc42 and Duo mRNAs and reduced inputs from the mediodorsal (MD) nucleus of the thalamus or the intrinsic axon collaterals of layer 3 pyramidal neurons are hypothesized to produce the apparent laminar-specific reduction in dendritic spine density on the basilar dendrites of deep layer 3 pyramidal neurons in subjects with schizophrenia. As illustrated in the figure, the apical dendrites of pyramidal neurons in layer 6 that extend through the thalamic termination zone are predicted to show a reduction in spine density that is similar to that observed on the basilar dendrites of pyramidal neurons in deep layer 3. In contrast to the restricted termination of excitatory inputs from the thalamus and intrinsic axon collaterals to specific cortical layers, those originating in other cortical association regions tend to terminate across all cortical layers. Thus, changes in these inputs would be expected to produce alterations in spine density across all cortical layers, unless critical regulators of the actin cytoskeleton that determine spine development, stability or retraction are selectively altered in a subset of pyramidal neurons. Adapted from Hill *et al* (2006).

## Does Developmental Plasticity Contribute to Dendritic Spine Alterations in Schizophrenia?

Dendritic spine density on DLPFC layer 3 pyramidal neurons undergoes a substantial decline during adolescence in primates (Anderson *et al*, 1995). Consistent with the findings that dendritic spines are the main site of excitatory synaptic input onto pyramidal cells and that all mature dendritic spines contain an excitatory synapse (Arellano *et al*, 2007), the number of asymmetric (presumably excitatory) synapses declines in a similar age-related fashion in both monkey and human DLPFC (Bourgeois *et al*, 1994; Huttenlocher and Dabholkar, 1997). In humans, this synaptic pruning is thought to underlie the decrease in cortical gray matter thickness that occurs during adolescence (Giedd, 1999; Gogtay *et al*, 2004). Interestingly, the late developmental refinements in excitatory connectivity are more marked in layer 3 than in the deeper cortical layers (Bourgeois *et al*, 1994), suggesting that they may be associated with the apparent lamina-specific alterations in spine density in schizophrenia. The observation of alterations in the expression of certain synaptic proteins in schizophrenia suggested the possibility that the exuberant synapses present before adolescence somehow compensated for a dysfunction in excitatory transmission in individuals with schizophrenia (Mirnics *et al*, 2001). Alternatively, such alterations in synaptic protein expression might disturb the mechanisms of adolescence-related synapse elimination leading, for instance, to excessive synapse pruning and decreased spine number in the illness (Feinberg, 1982; Keshavan *et al*, 1994).

Understanding how reductions in excitatory synaptic density during adolescence could contribute to disease-related changes in DLPFC function depends, in part, on knowledge of the functional properties of the synapses that are pruned. Current views on the mechanisms by which synapses are eliminated during early brain development suggest that the pruned synapses are functionally immature. Immature glutamate synapses are relatively weak and their maturation involves an activity-dependent increase in strength. Such activity-dependent strengthening might underlie synapse stabilization, and thus mark for elimination of the immature synapses that are not strengthened (Katz and Shatz, 1996; Le Be and Markram, 2006; Waites *et al*, 2005). Furthermore, spine morphology correlates with synaptic strength, that is, spines with smaller spine heads have a smaller postsynaptic density, fewer AMPA receptors and a weaker response to glutamate (Matsuzaki *et al*, 2001; Matsuzaki, 2007). Moreover, patterns of activity consistent with those that induce long-term potentiation of synaptic strength may increase spine head size concomitantly with

the synapse-specific increase in strength (Matsuzaki, 2007). Conversely, activity patterns that produce long-term depression may lead to a reduction in synaptic strength together with spine head shrinkage and eventually spine disappearance (Matsuzaki, 2007; Zhou *et al*, 2004). Activity-dependent changes in dendritic spine shape and size, as well as spine formation and retraction, are dependent on regulation of actin dynamics and clathrin-dependent membrane endocytosis (Blanpied and Ehlers, 2004; Tada and Sheng, 2006).

Determining if the synapses that are eliminated during adolescence in the primate DLPFC are functionally immature is essential for understanding how synaptic pruning might inform neurodevelopmental models of schizophrenia. For example, if the eliminated synapses are functionally immature, then they may not be able to provide the hypothesized compensation for synaptic dysfunction prior to adolescence in individuals with schizophrenia (Mirnics *et al*, 2001). This lack of compensatory capacity is due to the facts that neurotransmission at immature synapses has (1) very low AMPA receptor contribution, rendering these synapses silent at the resting membrane potential; and (2) relatively high probability of glutamate release, such that these synapses are not able to be repetitively activated without quickly exhausting glutamate vesicle pools (Schneggenburger *et al*, 2002; Zucker and Regehr, 2002).

In a living slice preparation of the monkey DLPFC (Gonzalez-Burgos *et al*, 2007b), excitatory inputs to layer 3 pyramidal neurons in 3-month-old monkeys (early postnatal), had immature functional properties, including a higher probability of glutamate release, lower AMPA/NMDA receptor ratio and a longer duration of NMDA receptor-mediated excitatory synaptic currents. In contrast, the excitatory synaptic inputs to layer 3 pyramidal neurons from 15-month-old monkeys (pre-adolescence and pre-pruning) had mature functional properties that were similar to those observed in neurons from 42-month-old (post-adolescence and post-pruning) and 84-month-old (adult) animals. Therefore, the contribution of functionally immature synapses decreases significantly before synapse elimination begins, and remains essentially constant thereafter. These data suggest that the substantial remodeling of excitatory connectivity of the primate DLPFC during adolescence primarily involves the elimination of mature synapses, and that some other factor, such as the neuronal source of input, somehow tags mature synapses for pruning (Woo *et al*, 1997b).

Recent imaging studies of the dynamics of neocortical dendritic spines and synaptic boutons *in vivo* suggest that synaptogenesis is an active process throughout life (Holtmaat *et al*, 2006; Knott *et al*, 2006; Stettler *et al*, 2006). Thus, synapse density at a given stage of development depends on the rates of active synapse formation and elimination (reviewed in Alvarez and Sabatini, 2007). For instance, in the adult monkey visual cortex *in vivo*, about 7% of the synapses are lost per week, but the rate of synapse formation is very similar, resulting in no net change in synapse density (Stettler *et al*, 2006). If these findings are true for the human DLPFC, then lower synapse density in schizophrenia could result from decreased synapse formation or increased synapse elimination at any developmental stage, including adulthood.

## NEUROPLASTICITY OF INHIBITORY CORTICAL CONNECTIONS IN SCHIZOPHRENIA

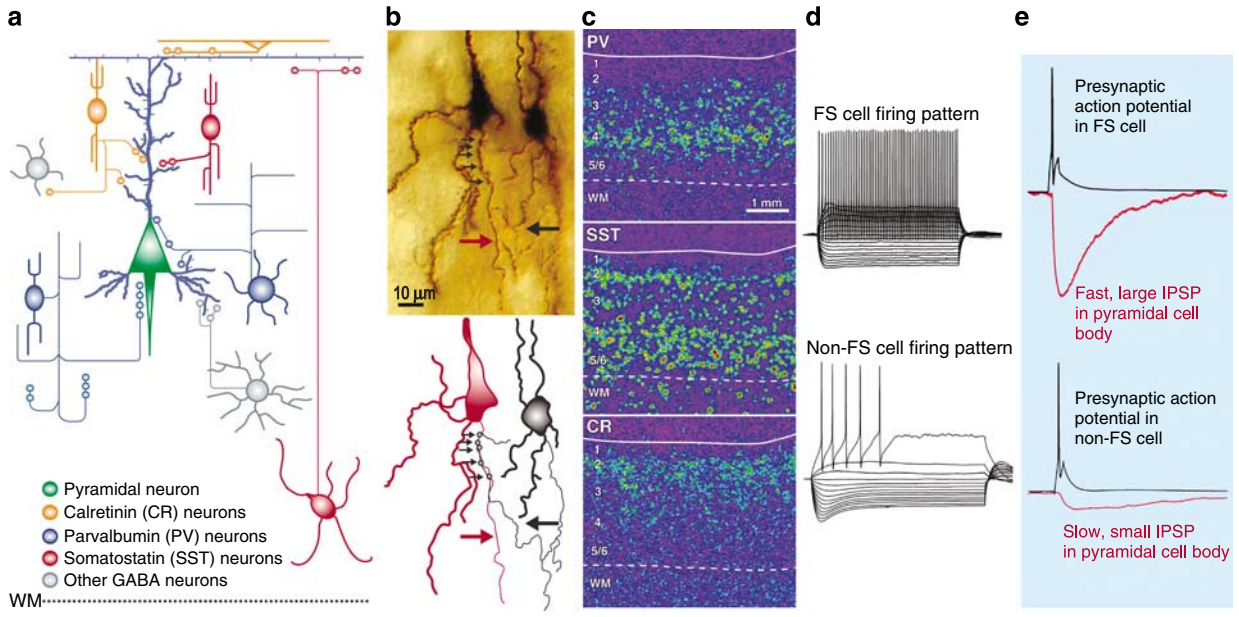
### Evidence of Altered Inhibitory Connections in the DLPFC in Schizophrenia

Initial postmortem studies found evidence of decreased glutamic acid decarboxylase (GAD) activity (Bird *et al*, 1979), decreased GABA reuptake (Simpson *et al*, 1989) and increased binding to $GABA_A$ receptors (Benes *et al*, 1996; Hanada *et al*, 1987) in the neocortex of subjects with schizophrenia. Studies conducted over the past decade, using DNA microarray, real-time quantitative PCR or *in situ* hybridization, have consistently found reduced levels of the transcript for the 67-kDa isoform of glutamic acid decarboxylase ($GAD_{67}$), the principal synthesizing enzyme for GABA, in the DLPFC of subjects with schizophrenia (Akbarian *et al*, 1995; Guidotti *et al*, 2000; Hashimoto *et al*, 2005, 2007a; Mirnics *et al*, 2000; Straub *et al*, 2007; Vawter *et al*, 2002; Volk *et al*, 2000). Similar findings have also been reported in other neocortical regions (Akbarian and Huang, 2006; Woo *et al*, 2004). Indeed, an analysis of all postmortem studies of schizophrenia conducted in specimens from the Stanley Neuropathology Consortium revealed that three genes expressed in GABA neurons (reelin, parvalbumin, and $GAD_{67}$) had the most abnormal transcript and protein levels in schizophrenia (Torrey *et al*, 2005). At the cellular level, the expression of $GAD_{67}$ mRNA was not detectable in ~25–35% of GABA neurons in layers 1–5 of the DLPFC, but the remaining GABA neurons exhibited normal levels of $GAD_{67}$ mRNA (Akbarian *et al*, 1995; Volk *et al*, 2000). Furthermore, levels of the mRNA for the GABA membrane transporter (GAT1), a protein responsible for reuptake of released GABA into nerve terminals, was also decreased (Ohnuma *et al*, 1999), and this decrease was restricted to a similar minority of GABA neurons (Volk *et al*, 2001). These findings suggest that both the synthesis and reuptake of GABA are lower in a subset of DLPFC neurons in schizophrenia.

### Which Subclasses of DLPFC GABA Neurons are Affected in Schizophrenia?

As in other cortical regions, the inhibitory GABA neurons in the primate DLPFC appear to form subclasses that can be distinguished on the basis of a number of molecular, electrophysiological, and anatomical properties. For example, as illustrated in Box 2, the $Ca^{2+}$-binding proteins, parvalbumin (PV) and calretinin (CR), and the neuropeptide somatostatin (SST) are, with a few exceptions, expressed in separate populations of cortical GABA neurons (Condé *et al*, 1994; DeFelipe, 1997; Gabbott and Bacon, 1996). These subtypes tend to exhibit different membrane-firing properties (Kawaguchi and Kubota, 1993; Krimer *et al*, 2005; Zaitsev *et al*, 2005) and to have axons with different arborization patterns and synaptic targets (DeFelipe, 1997). For example, the axon terminals of PV-containing chandelier and basket neurons principally target the axon initial segments and cell body/proximal dendrites, respectively, of pyramidal neurons (Melchitzky *et al*, 1999;

**Box 2**




As illustrated in Box 1, excitatory pyramidal cells convey to other brain regions the neural signals generated by the local circuits in each cortical area. The activity of pyramidal neurons is strongly regulated by interneurons (or local circuit neurons) that utilize the inhibitory neurotransmitter GABA. Many subclasses of cortical interneurons can be identified on the basis of difference in several parameters. At the level of gene expression (a), some interneurons express the $Ca^{2+}$-binding proteins parvalbumin (PV) or calretinin (CR), whereas others contain the neuropeptide somatostatin (SST). Furthermore, these biochemically defined subclasses of interneurons also exhibit morphological differences; the axons of SST-containing interneurons predominantly target the dendrites of pyramidal cells, many of the axons of CR-containing interneurons innervate other GABA cells, and the GABA synapses formed by PV-containing neurons are found at or close to pyramidal cell bodies. For example, as shown in (b), the axons of the chandelier class of PV-containing interneurons (black arrow) make multiple synaptic contacts (small arrows) at the axon initial segment (red arrow) of a pyramidal cell. Anatomically, these three subclasses of interneurons have differential distributions across the six layers of the neocortex, as illustrated by the distinctive expression patterns of their messenger RNAs. As shown in (c), cells that express PV mRNA are found mostly in layers 3 and 4, those that express SST mRNA have a bilaminar pattern in layers 2, 5, and 6, and those that express CR are located predominately in layers 1 and 2. Electrophysiologically, PV-containing neurons are called fast-spiking (FS) because during sustained stimulation they fire fast action potentials at a constant interval (d). In contrast, non-FS neurons, such as the SST neurons, fire slower action potentials at progressively longer intervals.

In interneurons, the discharge of an action potential (black traces in (e)) triggers synaptic GABA release which activates $GABA_A$ receptors in the targeted pyramidal cell and produces an inhibitory postsynaptic potential (IPSP). The IPSPs generated by FS/PV interneurons have a large amplitude and fast time course ((e), top red trace), reflecting the perisomatic location of the synaptic contacts. In contrast, IPSPs generated by dendrite-targeting non-FS/SST neurons are small and slow at the pyramidal cell body ((e), bottom red trace), indicating that their physiological inhibitory effect is strongest in the dendrites.

Interneuron-mediated inhibition does not simply constrain pyramidal cell excitation, but precisely controls the timing of pyramidal cell firing, causing large groups of nearby pyramidal cells to oscillate between active and silent states. GABA neuron activity induces rhythms of different frequencies, with each oscillation frequency apparently involving different interneuron subclasses. The synaptic signaling by cell body-targeting PV neurons and by dendritic-targeting SST neurons appears to be impaired in schizophrenia, suggesting that the alterations in circuit oscillations and neuronal synchrony observed in the illness are due, at least in part, to selective disturbances in GABA transmission mediated by these interneuron subtypes. Because neuronal synchrony increases the efficacy of synaptic communication within and between cortical areas, interneuron alterations in schizophrenia are likely to have substantial effects on cognitive processes that are dependent on the integration of neural signals across the neocortex. Adapted from Gonzalez-Burgos *et al* (2007a).

Williams *et al*, 1992), the SST-containing Martinotti cells innervate the distal dendrites of pyramidal neurons (DeLima and Morrison, 1989; Kawaguchi and Kubota, 1996; Ma *et al*, 2006), and the CR-containing double-bouquet cells tend to synapse on the dendrites of other GABA cells (Melchitzky *et al*, 2005).

The affected GABA neurons in schizophrenia include the PV-positive neurons, which comprise ~25% of GABA neurons in the primate DLPFC. In individuals with schizophrenia the expression level of PV mRNA is reduced, although the number of PV neurons appears to be unchanged (Woo *et al*, 1997a); in addition, approximately half of PV mRNA-containing neurons lack detectable levels of $GAD_{67}$ mRNA (Hashimoto *et al*, 2003). In contrast, the ~50% of GABA neurons that express CR appear to be unaffected (Hashimoto *et al*, 2003).

In the DLPFC of subjects with schizophrenia, GAT1 immunoreactivity is selectively reduced in the characteristic axon terminals (cartridges) of the chandelier class of PV-containing neurons (Woo *et al*, 1998). In the postsynaptic targets of these axon cartridges, the axon initial segments of pyramidal neurons, immunoreactivity for the $GABA_A$ receptor $\alpha_2$-subunit (which is present in most $GABA_A$ receptors in this location; Nusser *et al*, 1996) is markedly increased in schizophrenia (Volk *et al*, 2002). These changes appear to be specific to the disease process of schizophrenia because they are not found in subjects with other psychiatric disorders or in monkeys exposed chronically to antipsychotic medications (Hashimoto *et al*, 2003; Volk *et al*, 2000, 2001, 2002).

Several lines of evidence suggest that the reductions in presynaptic GABA markers (GAT1 and PV) and increased

postsynaptic $GABA_A$ receptors are compensatory responses to a deficit in GABA release from chandelier neurons. For example, PV is a slow $Ca^{2+}$ buffer that does not affect the amplitude, but accelerates the decay, of $Ca^{2+}$ transients in GABA nerve terminals (Collin *et al*, 2005; Muller *et al*, 2007). Thus, PV decreases the residual $Ca^{2+}$ levels that normally accumulate in nerve terminals and facilitate GABA release during repetitive firing (Collin *et al*, 2005). Studies in PV-deficient mice have demonstrated that a decrease in PV increases residual $Ca^{2+}$ and favors synaptic facilitation (Collin *et al*, 2005; Vreugdenhil *et al*, 2003). Furthermore, the enhanced facilitation of GABA release from fast-spiking neurons with reductions in PV is associated with increased power of $\gamma$ oscillations (Vreugdenhil *et al*, 2003) (see below). Similarly, the blockade of GABA reuptake via GAT1 prolongs the duration of IPSCs when synapses located close to each other are activated synchronously (Overstreet and Westbrook, 2003); the resulting prolongation of IPSCs increases the probability of IPSC summation and enhances the total efficacy of IPSC trains. The upregulation of the postsynaptic $GABA_A$ receptors that contain $\alpha_2$-subunits would be expected to increase the efficacy of the GABA that is released from chandelier neurons. Thus, the combined reduction of PV and GAT1 proteins in chandelier cell axon cartridges, and the upregulation of postsynaptic $GABA_A$ receptors, would act synergistically to increase the efficacy of GABA neurotransmission at pyramidal neuron axon initial segments during the types of repetitive neuronal activity associated with working memory. However, the persistence of cognitive impairments in individuals with schizophrenia suggests that these neuroplastic changes in GABA neurotransmission from chandelier neurons are insufficient as compensatory responses. Alternatively, it is possible that compensation at chandelier cell synapses is not effective because additional interneuron subclasses are also functionally deficient in schizophrenia (Lewis *et al*, 2005).

## Are PV-containing Basket Cells also Altered in Schizophrenia?

The other major subclass of PV-containing GABA neurons are basket (or wide arbor) cells whose axons target the cell body and proximal dendritic spines and shafts of pyramidal neurons. Although more difficult to directly assess, similar presynaptic and postsynaptic alterations may also be present in the inputs of PV-containing, basket neurons to the perisomatic region of pyramidal neurons. For example, the density of PV-immunoreactive puncta, possibly the axon terminals of wide arbor neurons (Erickson and Lewis, 2002), is reduced in the middle layers, and not in the superficial layers, of the DLPFC of subjects with schizophrenia (Lewis *et al*, 2001a), paralleling the laminar pattern of decreased PV mRNA expression in schizophrenia (Hashimoto *et al*, 2003). Furthermore, the increased density of $GABA_A$ receptors in the DLPFC of subjects with schizophrenia found in ligand-binding studies (Benes *et al*, 1996; Hanada *et al*, 1987) was most prominent at pyramidal neuron cell bodies (Benes *et al*, 1996). Together, these data suggest that the perisomatic inhibitory input from both PV-containing chandelier and basket neurons is reduced in schizophrenia.

## What are the Consequences of Altered GABA Neurotransmission in DLPFC PV-containing Neurons in Schizophrenia?

How could reduced GABA signaling from PV-containing GABA neurons to the perisomatic region of pyramidal neurons in the DLPFC contribute to the pathophysiology of working memory dysfunction? First, it is well established that the activity of DLPFC GABA neurons is essential for normal working memory function in monkeys (Rao *et al*, 2000; Sawaguchi *et al*, 1989). Second, PV-positive GABA neurons and pyramidal neurons share common sources (eg thalamic afferents) of excitatory input (Melchitzky *et al*, 1999). The resulting feedforward, disynaptic inhibition limits the time window for the summation of excitatory inputs required to evoke pyramidal neuron firing (Pouille and Scanziani, 2001). Third, both chandelier and basket neurons target multiple pyramidal neurons (Peters *et al*, 1982), enabling them to use this timing mechanism to synchronize the activity of local populations of pyramidal neurons (Klausberger *et al*, 2003). Finally, networks of PV-positive GABA neurons, formed by both chemical and electrical synapses, give rise to oscillatory activity in the $\gamma$ band range, the synchronized firing of a neuronal population at 30–80 Hz (Whittington and Traub, 2003). Interestingly, $\gamma$ band oscillations in the human DLPFC increase in proportion to working memory load (Howard *et al*, 2003), and in subjects with schizophrenia, prefrontal $\gamma$ band oscillations are reduced bilaterally during a working memory task (Cho *et al*, 2006). Thus, a deficit in the synchronization of pyramidal cell firing, resulting from impaired regulation of pyramidal cell networks by PV-positive GABA neurons, may contribute to reduced levels of induced $\gamma$ band oscillations, and consequently to impairments in cognitive tasks that involve working memory in subjects with schizophrenia (Lewis *et al*, 2005).

The decreased $GAD_{67}$ mRNA expression in schizophrenia is not restricted to the DLPFC, with similar observations reported in studies of the anterior cingulate and temporal cortices (Akbarian and Huang, 2006). In addition, disturbances in $\gamma$ oscillations evoked with auditory and visual stimuli have been reported in other cortical regions (Spencer *et al*, 2004) in subjects with schizophrenia. Thus, a conserved alteration in GABA neurotransmission across cortical regions could underlie a common abnormality in $\gamma$ oscillations that is associated with different clinical features of schizophrenia. Consistent with this interpretation, a recent study found the same pattern of altered GABA-related transcripts across four different cortical regions in subjects with schizophrenia (Hashimoto *et al*, 2007b).

## Is the Output of Chandelier Neurons Inhibitory or Excitatory?

Chandelier neurons have been considered to be powerful inhibitors of pyramidal cell output, exercising 'veto power' by virtue of the close proximity of their synaptic inputs to the site of action potential generation in pyramidal neurons. Like other cortical GABA neurons, the effect of GABA released from chandelier neuron axon terminals is mediated by binding to postsynaptic $GABA_A$ receptors, which results in the opening of chloride ion channels. In the adult brain,

high expression of the potassium–chloride co-transporter (KCC2) results in the extrusion of chloride from the cell (Stein and Nicol, 2003). Thus, when $GABA_A$ receptors are activated, chloride ions flow into the cell along a concentration gradient, resulting in hyperpolarization of the membrane, and a reduced probability of cell firing. However, a recent study found that KCC2, while readily detectable in the cell body of adult pyramidal neurons, was apparently absent in the axon initial segment. Consistent with this observation, Szabadics *et al* (2006) found that the release of GABA from chandelier neuron axon terminals resulted in depolarization of pyramidal cells in an *in vitro* slice preparation. In fact, the chandelier cell-mediated depolarization was so powerful that in ~50% of the cases in which a single chandelier cell was stimulated, the postsynaptic pyramidal cell was depolarized to the point of firing an action potential. In contrast, excitatory input from a single pyramidal neuron can rarely, if ever, cause a postsynaptic neuron to fire.

The findings of this study are quite striking, and suggest that the scenario described above for the physiological consequences of altered GABA neurotransmission in chandelier neurons in schizophrenia might need to be reconsidered. For example, if chandelier cell inputs to pyramidal neurons are usually depolarizing, then reduced synthesis of GABA in these neurons could result in a marked decrease in the excitatory output of pyramidal neurons that project to other cortical regions or subcortical sites. Alternatively, it is possible that instead of providing a substantial fraction of background excitatory synaptic input over time, chandelier neurons excite pyramidal cells only during specific patterns of network activity. For instance, by virtue of the divergent connections of a given chandelier cell to several hundred pyramidal cells (and assuming that every pyramidal cell receives chandelier cell inputs), then excitatory input from chandelier cells could provide a strong synchronization signal during network oscillations. However, the findings of the Szabadics *et al*'s (2006) study, while awaiting replication, also need to be integrated with observations in the hippocampus of living animals showing that during $\theta$ oscillations the maximal activity of chandelier neurons is perfectly out of phase with that of pyramidal neurons (Klausberger *et al*, 2003), indicative of a powerful inhibitory timing effect of chandelier cells.

## Are Other Populations of GABA Neurons Altered in Schizophrenia?

Although the findings reviewed above provide convergent evidence for alterations in PV-containing GABA neurons in schizophrenia, they also suggest that these neurons cannot account for all of the observed findings in postmortem studies. For example, the levels of $GAD_{67}$ and GAT1 mRNAs are reduced to comparable degrees in layers 2–5 (Volk *et al*, 2000, 2001), even though the density of PV neurons is much greater in layers 3 and 4 than in layers 2 and 5 (Condé *et al*, 1994). In addition, PV mRNA expression was reduced in layers 3 and 4, but not in layers 2 and 5, in subjects with schizophrenia (Hashimoto *et al*, 2003). Together, these findings suggest that one or more populations of GABA neurons in layers 2 and 5, which express neither PV nor CR, is altered in schizophrenia.

A recent study using a customized cDNA microarray with enhanced sensitivity and specificity for 85 GABA-related transcripts found that, in addition to decreased expression of $GAD_{67}$ and GAT1 mRNAs, the tissue concentrations of the mRNAs for three neuropeptides (SST, neuropeptide Y (NPY) and cholecystokinin (CCK)) were also reduced in the DLPFC of subjects with schizophrenia (Hashimoto *et al*, 2007a; Figure 3). These findings were confirmed by quantitative real-time PCR and/or *in situ* hybridization, and they are consistent with previous reports of decreased CCK peptide (Gabriel *et al*, 1996) and mRNA levels (Virgo *et al*, 1995) in other frontal areas. In the cortex, SST is expressed by GABA neurons that do not express PV or CR (González-Albo *et al*, 2001), and a subset of SST-containing neurons largely overlaps with the majority of NPY-containing neurons (Hendry *et al*, 1984; Kubota *et al*, 1994). Because SST- and NPY-containing neurons are predominantly located in layers 2 and 5 (Hendry *et al*, 1984; Kubota *et al*, 1994), alterations in these neurons could contribute to the deficits in $GAD_{67}$ and GAT1 mRNA expression in these layers (Volk *et al*, 2000). Consistent with this interpretation, the gene expression differences between subjects with schizophrenia and control subjects were highly positively correlated for $GAD_{67}$, SST, and NPY, suggesting that $GAD_{67}$ mRNA expression is also decreased in the subset of GABA neurons that express both SST and NPY. Because SST- and NPY-containing neurons selectively target distal dendrites of pyramidal neurons (DeLima and Morrison, 1989; Hendry *et al*, 1984; Kawaguchi and Kubota, 1996), these coordinated gene expression changes suggest that GABA neurotransmission is altered at the dendritic domain of pyramidal neurons in the DLPFC of subjects with schizophrenia.

CCK is heavily expressed in GABA neurons that do not contain either PV or SST (Kawaguchi and Kondo, 2002; Lund and Lewis, 1993). The expression changes of CCK and $GAD_{67}$ in the DLPFC of subjects with schizophrenia were highly correlated, suggesting a deficit of GABA synthesis in CCK-containing GABA neurons. Interestingly, the axon terminals of CCK-containing large basket neurons, which target selectively pyramidal neuron cell bodies, contain type I cannabinoid receptors (CB1R) (Eggan and Lewis, 2007). The activation of these receptors by exogenous cannabinoids suppresses GABA release (Bodor *et al*, 2005; Katona *et al*, 1999). Thus, exposure to cannabis could exacerbate an intrinsic deficit in GABA synthesis in these neurons, providing a mechanism by which cannabis use increases the risk for, and the severity of, schizophrenia (Fergusson *et al*, 2006). In addition, preliminary evidence of reduced mRNA and protein levels of CB1R in the DLPFC of subjects with schizophrenia suggests that downregulation of this receptor may represent a compensatory response to reduce the ability of endogenous cannabinoids to decrease GABA release from CCK/CB1R-containing axon terminals (Eggan *et al*, 2007). Interestingly, CCK/CB1R- and PV-containing basket cells provide convergent sources of perisomatic inhibition to pyramidal neurons that play specific roles in shaping network activity, including complementary roles in regulating $\gamma$ band oscillations (Hajos *et al*, 2000). Thus, alterations in CCK-containing basket cells could also contribute to impaired $\gamma$ oscillations in schizophrenia.

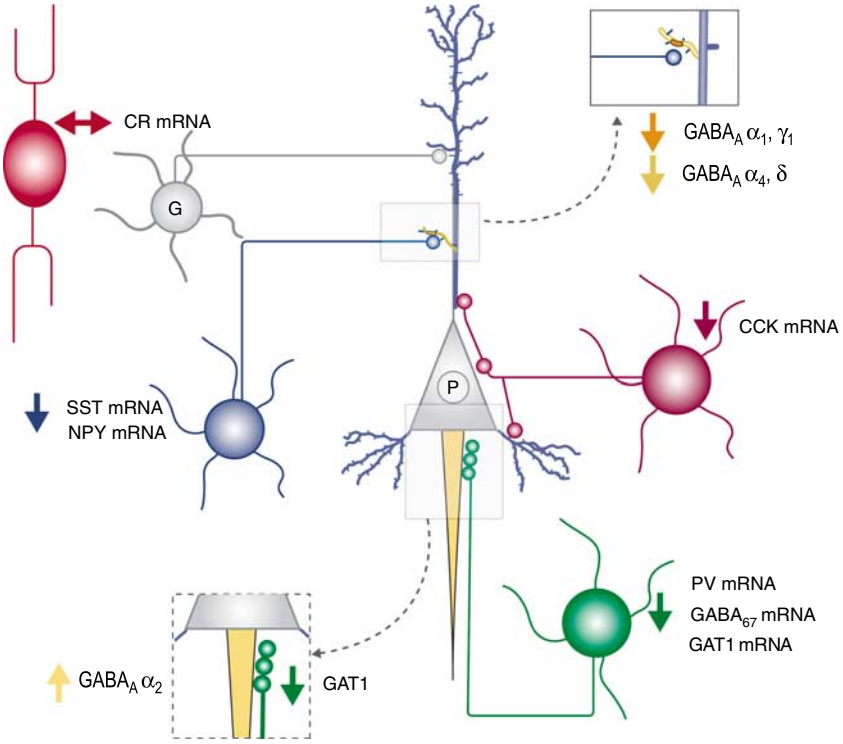



**Figure 3** Schematic summary of alterations in GABA-mediated circuitry in the dorsolateral prefrontal cortex (DLPFC) of subjects with schizophrenia. Altered GABA neurotransmission by parvalbumin (PV)-containing neurons (green) is indicated by gene expression deficits in these neurons and associated changes in their synapses, a decrease in GAT1 expression in their terminals and an upregulation of $GABA_A$ receptor $\alpha_2$-subunit at the axon initial segments of pyramidal neurons (lower enlarged square). Decreased gene expression for both somatostatin (SST) and neuropeptide Y (NPY) indicates alterations in SST and/or NPY-containing neurons (blue) that target the distal dendrites of pyramidal neurons. Decreased cholecystokinin (CCK) mRNA levels indicate an alteration of CCK-containing large basket neurons (purple) that represent a separate source of perisomatic inhibition from PV-containing neurons. Gene expression in CR-containing GABA neurons (red) does not seem to be altered. Other neurons, such as PV-containing basket neurons, are not shown because the nature of their involvement in schizophrenia is unclear. G, generic GABA neuron; P, pyramidal neuron. Adapted from Hashimoto *et al* (2007a).

## Could the Alterations in the Affected PV-containing GABA Neurons Reflect Disturbances in the Postnatal Maturation of their Synapses?

Of the subpopulations of GABA neurons that are affected in schizophrenia, the development of PV-containing neurons has been best studied in the primate DLPFC. For example, the density of the PV-positive axon terminals of basket neurons changes markedly during postnatal development (Erickson and Lewis, 2002), increasing steadily in an almost linear fashion from 1 month of age to adulthood (Figure 4). These developmental changes in the number of PV-positive boutons most likely reflect a shift in the detectability of basket axon terminals with immunocytochemical techniques, secondary to a change in the concentration of PV protein within the terminals, since the total number of inhibitory synapses, and the axonal arbors of basket cells specifically, appear to remain relatively constant over this same period of development (Bourgeois *et al*, 1994; Lund and Lewis, 1993).

The chandelier class of PV-containing GABA neurons also exhibits developmental changes in the expression of biochemical markers. During postnatal development, the density of chandelier neuron axon cartridges immunoreactive for either PV or GAT1 changes markedly in monkey DLPFC (Cruz *et al*, 2003). Although the precise time course differs for the two markers, the density of labeled cartridges is low in the newborn, increases to reach a peak prior to the onset of puberty, and then declines markedly to adult levels (Figure 4). Because cartridges are readily visualized with the Golgi technique over this same time period (Lund and Lewis, 1993), the changes in PV- and GAT-immunoreactive cartridges likely reflect developmental shifts in the concentration of these proteins. Interestingly, the peak and subsequent decline in the density of labeled cartridges occur prior to the age when the peak density of labeled basket terminals is achieved, but appears concomitant with the pruning of intracortical excitatory connections (Woo *et al*, 1997b). Although the basis for this temporal coincidence remains to be investigated, it may be that the synchronous firing of a group of pyramidal cells, facilitated by inputs from chandelier neurons, is essential for promoting the maintenance of a subset of excitatory connections.

Postsynaptically, the influence of released GABA on the pyramidal cells targeted by chandelier neurons is determined by the particular type of $GABA_A$ receptor to which it binds. Interestingly, $GABA_A$ receptors undergo changes in subunit composition during cortical development. For example, the $\alpha_2$-subunit is most prevalent prenatally and is replaced in the majority of, but not all, $GABA_A$ receptors postnatally by the $\alpha_1$-subunit (Fritschy *et al*, 1994). In the adult cortex, the majority of $GABA_A$ receptors containing the $\alpha_2$-subunit are found in pyramidal cell axon initial segments (Loup *et al*, 1998). The detectability of $GABA_A$ $\alpha_2$-subunits at the axon initial segment undergoes substantial

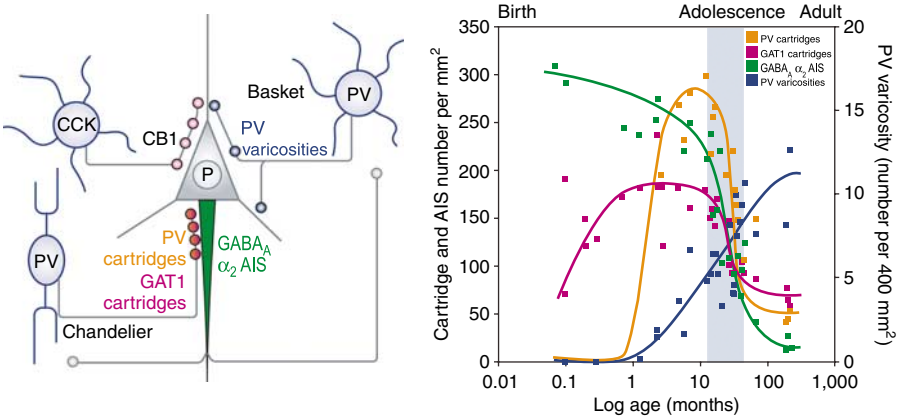



**Figure 4** Postnatal development of chandelier neuron inputs to pyramidal neurons in monkey dorsolateral prefrontal cortex (DLPFC). The axon terminals of chandelier neurons are vertical arrays of boutons (cartridges) that are immunoreactive for parvalbumin (PV) or the GABA membrane transporter (GAT1) and synapse on the axon initial segment of pyramidal neurons (a). As shown in (b), the density of cartridges immunoreactive for either PV or GAT1 is low in the DLPFC of newborn monkeys, increases to reach a peak before the onset of puberty, and then declines markedly during adolescence (shaded area between 15 and 42 months of age) to adult levels (Cruz *et al*, 2003). These density changes in PV- and GAT1-immunoreactive cartridges seem to reflect developmental shifts in the concentration of these proteins (and thus in the detectability of cartridges) because cartridges are readily visualized with the Golgi technique over this same time period (Lund and Lewis, 1993). Postsynaptically, the detectability of the $\alpha_2$-subunit of the $GABA_A$ receptor in pyramidal neuron axon initial segment is high at birth, and then markedly declines during adolescence before stable adult levels are achieved. In contrast, the density of PV-immunoreactive varicosities, putative axon terminals from the basket class of PV-expressing interneurons that innervate the cell body and proximal dendrites of pyramidal neurons, rises during adolescence (Erickson and Lewis, 2002). Together, these developmental changes in markers of inhibition at the perisomatic region of pyramidal neurons suggest that the capacity to synchronize pyramidal neuron output in the primate DLPFC is in substantial flux until adulthood. Consequently, the protracted developmental time course of improvements in performance on working memory tasks (Crone *et al*, 2006; Diamond, 2002; Luna *et al*, 2004) might depend on both refinements in the number of excitatory connections among pyramidal neurons and changes in their proximal inhibitory inputs. These developmental changes during adolescence might contribute to unmasking the consequences of inherited abnormalities in the regulation of GABAergic neurotransmission and might help explain why certain life experiences during adolescence (eg stress or cannabis exposure) seem to increase the risk for schizophrenia (Lewis and Levitt, 2002). Adapted from Lewis *et al* (2005).

changes during postnatal development (Cruz *et al*, 2003). As shown in Figure 4, the density of pyramidal cell axon initial segments immunoreactive for the $\alpha_2$-subunit is very high in the postnatal period, and then steadily declines through adolescence into adulthood. Because GABA receptors including the $\alpha_2$-subunit have a higher affinity for GABA, faster activation times, and slower deactivation times than receptors containing the $\alpha_1$-subunit (Lavoie *et al*, 1997), this decrease in the density of $\alpha_2$-labeled axon initial segments may be interpreted as a change in the speed and efficacy of GABAergic transmission at the axon initial segment during postnatal maturation, rather than a reduction in the number of GABAergic synapses onto the axon initial segment, because the total number of inhibitory synapses remains constant over this same time period (Bourgeois *et al*, 1994).

This complex and protracted postnatal maturation of the inputs from PV-containing GABA neurons in the primate DLPFC provides a number of opportunities for any disturbances, even subtle ones, to have their effects amplified as they alter the trajectories of the developmental events that follow. In particular, the marked developmental changes in the axon terminals of PV-containing basket and chandelier neurons, and their postsynaptic receptors, during the perinatal period and adolescence raises the possibility that the alterations in schizophrenia of these markers reflect a disturbance in these patterns of development. These temporal correlations may explain how a range of environmental factors (eg labor-delivery complications, urban place of rearing, and marijuana use during adolescence) are all associated with increased risk for the appearance of schizophrenia later in life. Although it seems unlikely that the GABA-related disturbances in schizophrenia represent an arrest of development, they may reflect an alteration of DLPFC circuitry that makes it unable to support higher levels of working memory load, rendering the impaired performance in schizophrenia analogous to the immature levels of working memory function seen in children (Crone *et al*, 2006; Diamond, 2002; Luna *et al*, 2004).

## NEUROPLASTICITY OF MODULATORY CONNECTIONS IN SCHIZOPHRENIA

### Evidence of Altered Dopamine Neurotransmission in the DLPFC in Schizophrenia

In addition to disturbances in excitatory and inhibitory connectivity in the DLPFC, alterations in modulatory systems, such as dopamine (DA), have also been found in schizophrenia. The positive or psychotic features of the illness were originally related to a functional excess of DA neurotransmission because (1) DA agonists, such as amphetamine, can induce a schizophrenia-like psychosis, and (2) antipsychotic medications have antagonistic activity at DA receptors of the D2 subtype (Carlsson, 2006). Indeed, the efficacy of classical antipsychotics against the positive symptoms of schizophrenia is strongly correlated with their potency at D2 receptors in subcortical structures (Carlsson, 2006). In contrast, impairments of cognitive function in

schizophrenia might be related to a deficit in DA signaling in the DLPFC (Davis *et al*, 1991; Weinberger, 1987). For example, working memory performance is markedly impaired by depletion of DA (Brozoski *et al*, 1979), and by blockade of D1 receptor-mediated DA signaling (Sawaguchi and Goldman-Rakic, 1991, 1994), in the monkey DLPFC. Potential mechanisms underlying both an increase in DA input to subcortical sites and a simultaneous decrease in the DA signal in the DLPFC in schizophrenia have been reviewed elsewhere (Carlsson, 2006; Lewis and Gonzalez-Burgos, 2006). Briefly, it is thought that prefrontal pyramidal neurons may directly excite mesocortical DA neurons in the ventral mesencephalon and indirectly inhibit mesostriatal DA cells through activation of GABAergic neurons in mesencephalic cell nuclei (Lewis and Gonzalez-Burgos, 2006). Such opposing effects of the prefrontal projections on DA cells are in line with the idea that reduction in PFC cell activity leads to an excess of DA receptor activation at subcortical nuclei that is prevented by antipsychotics that antagonize D2 receptors. However, decreased DA input to cortex by reduced PFC cell activity could be at least partially counteracted by D1 receptor agonist administration.

In the primate DLPFC, D1 receptors are the most abundant DA receptor subtype (Goldman-Rakic *et al*, 1990) and mediate most of the cellular effects of DA in this region (González-Burgos *et al*, 2002; Gonzalez-Burgos *et al*, 2005; Henze *et al*, 2000; Kroner *et al*, 2007). Moreover, D1 receptors regulate sustained firing of DLPFC neurons during the delay phase of delayed-response tasks that require working memory (Sawaguchi, 2001; Williams and Goldman-Rakic, 1995). Sustained firing of DLPFC neurons is thought to be the cellular basis of information storage in the short-term buffers associated with working memory (Goldman-Rakic, 1995). DA input to the DLPFC may be necessary, in addition, for proper DLPFC neuron activity during other stages of the working memory process. For instance, certain effects of DA on neurons from the rat medial PFC suggest an important role of DA in gating the information loaded into working memory buffers (Rotaru *et al*, 2007; Seamans and Yang, 2004); Yang *et al*, 1999).

Postmortem studies indicate that the total length of tyrosine hydroxylase (TH)- and DA transporter-containing axons is significantly reduced in the DLPFC of subjects with schizophrenia (Akil *et al*, 1999). These findings suggest that the cortical DA signal might be diminished in schizophrenia, due to a reduced content of TH per axon, to a reduced density of DA axons or to both. However, schizophrenia does not seem to be associated with a substantial loss of midbrain DA neurons (Bogerts *et al*, 1983), although the DA cells in subjects with schizophrenia have reduced soma volume (Bogerts *et al*, 1983). Because reductions in soma volume are usually correlated with restricted axonal projections (Gilbert and Kelly, 1975; Lund *et al*, 1975), decreased DA signaling in the DLPFC in schizophrenia might thus result, at least in part, from a reduced DA innervation of these cortical areas. Consistent with the idea of altered midbrain DA neuron function in schizophrenia, a recent study reported reduced levels of tyrosine hydroxylase protein in midbrain DA cells in schizophrenia (Perez-Costas *et al*, 2007).

## What Factors might Account for a Deficit in DA Signaling in the DLPFC?

The morphological alterations of DA neurons in schizophrenia, including reduced innervation of the DLPFC, might be the consequence of sustained hypoactivity of these neurons. DA cell activity is under control of multiple synaptic input pathways including a strong inhibitory input originating in subcortical structures (Grace *et al*, 2007; White, 1996). Glutamatergic inputs from subcortical structures, from the prefrontal cortex and, indirectly, from the hippocampus, provide excitation to DA cells (Grace *et al*, 2007; White, 1996). In rodents, excitatory projections from the PFC are thought to be an essential source of glutamate-mediated excitation to the PFC-projecting DA neurons (Abi-Dargham and Moore, 2003; Sesack and Carr, 2002). Sustained hypoactivity of PFC pyramidal neurons in schizophrenia may result from reduced NMDA-mediated signaling or from other changes in the excitatory inputs to these cells (see above). If such alterations ultimately affect the output of cortical pyramidal cells projecting to midbrain DA cell nuclei, then DLPFC cell hypoactivity may lead to persistently decreased activation of DA cells. However, a recent study suggested that in primates, unlike in rodents, prefrontal projections to midbrain DA cells are sparse (Frankle *et al*, 2006).

Altered DA input to the DLPFC in schizophrenia may also result from disturbances in the development of DA innervation. In the monkey DLPFC, the density of TH-containing DA fibers (Rosenberg and Lewis, 1995) and the number of TH-positive appositions onto pyramidal cell dendrites (Lambe *et al*, 2000) increase gradually during postnatal development, reaching a maximum during adolescence. In contrast, tissue levels of DA and TH protein are high in the primate DLPFC shortly after birth, but decrease markedly during the early postnatal period, prior to adolescence (Goldman-Rakic and Brown, 1982; Weickert *et al*, 2007). Thus, between childhood and adolescence, the DA fibers innervating the DLPFC are in a highly dynamic state, adjusting the levels of TH expression and increasing the density of axon branches and varicosities in target cortical areas. The mechanisms regulating the innervation of cortical areas by DA axons during development are not well understood, but neurotrophic factors such as the glia-derived neurotrophic factor (GDNF) and BDNF seemed to be involved. For instance, GDNF expression by cortical tissue promotes its innervation by DA axons, regulating the density of DA fibers in the adult state (Kholodilov *et al*, 2004). In addition, experiments with BDNF knockout mice suggest that BDNF stimulates TH expression by midbrain DA neurons (Baquet *et al*, 2005). Alterations of BDNF-mediated signaling in schizophrenia are well documented (Hashimoto *et al*, 2005; Weickert *et al*, 2005), but no similar changes have been reported for GDNF.

In contrast to subcortical structures, the DA transporter in cortical axons is located distant from release sites, limiting the ability of these transporters to regulate extracellular DA levels (Lewis *et al*, 2001b). In contrast, inhibition of the DA-degrading enzyme catechol-*O*-methyl transferase (COMT) significantly increases prefrontal DA levels (Tunbridge *et al*, 2004b), suggesting that alterations in COMT levels could contribute to altered DA signaling in

schizophrenia. The levels of COMT mRNA and protein do not appear to be altered in schizophrenia (Matsumoto *et al*, 2003; Tunbridge *et al*, 2004a), although one study found significantly increased levels of a membrane-bound form of COMT in both schizophrenia and bipolar disorder (Tunbridge *et al*, 2006). The COMT gene is located in a chromosomal region associated with increased risk for schizophrenia, and its allelic variants code for enzymes with marked differences in catalytic activity (Chen *et al*, 2004). In particular, the Val158Met COMT allele was hypothesized to confer susceptibility for schizophrenia, because its presence is associated with lower DA levels and less robust cognitive performance in normal subjects (Egan *et al*, 2001). Consistent with this prediction, the presence of the Val allele, which codes for an isoform of COMT protein with higher enzymatic activity, is associated with higher levels of TH mRNA in mesencephalic DA neurons in healthy human subjects (Akil *et al*, 2003). However, studies of the association between COMT gene variants and schizophrenia have been inconclusive (Barnett *et al*, 2007; Craddock *et al*, 2006; Meyer-Lindenberg *et al*, 2006; Williams *et al*, 2007). Interestingly, COMT is mostly an intracellular cytosolic soluble protein, and although membrane-bound forms can be isolated (Tunbridge *et al*, 2006), these are probably associated with the intracellular side of the plasma membrane or with internal cell membranes (Mannisto and Kaakkola, 1999). Therefore, extracellular DA must be transported through the plasma membrane before becoming a substrate of COMT enzymatic activity, raising the question of potential interactions between genetic variants of COMT and the DA transporter. Indeed, allelic variants of the DA transporter gene are associated with different levels of transporter protein expression and may have significant effects on cortical physiology (Demiralp *et al*, 2007). Thus, certain genotypic combinations of COMT and DA transporter alleles might be associated with greater DA metabolism, decreased DA levels, deficits in cognitive functions and possibly an increased risk of developing schizophrenia. Although in some studies, the effects of both COMT and DA transporter gene polymorphisms on cognitive function have been assessed (Rybakowski *et al*, 2006), to our knowledge no studies have reported significant interactions between COMT and DA transporter gene variants. One possibility is that, because COMT expression levels in DA neurons are relatively low (Kastner *et al*, 1994; Lundstrom *et al*, 1995), the availability of DA to be inactivated by COMT is dependent on DA transport through the membrane of cortical cells that express higher levels of COMT (eg postsynaptic neurons or glia), and that may express non-specific membrane transporters with the ability to uptake DA. It is also important to note that COMT metabolizes norepinephrine which heavily innervates the primate DLPFC (Lewis and Morrison, 1989) and influences working memory function (Arnsten and Goldman-Rakic, 1985).

## What are the Functional Consequences of Decreased DA Signaling in the DLPFC?

A combination of DA cell hypoactivity, decreased cortical DA innervation, and increased DA turnover in the DLPFC would lead to markedly reduced extracellular DA levels in the DLPFC in schizophrenia, which in turn may produce DA receptor hypostimulation. A potential consequence of long-term deficits in DA receptor stimulation, as indicated by some animal studies (Butkerait and Friedman, 1993; Davidoff *et al*, 2000; Guo *et al*, 2003; Tassin *et al*, 1986), is a compensatory increase in the DA receptor levels. Because antipsychotics decrease the levels of DA receptors in monkey and human DLPFC (Hirvonen *et al*, 2006; Lidow and Goldman-Rakic, 1994, 1997), assessment of effect of disease on DA receptor binding in the DLPFC of schizophrenic subjects should be performed, ideally, in drug naive patients. A positron emission tomography study revealed an increase in the binding of NNC112, a DA D1 receptor ligand, in drug-free and drug-naive patients, consistent with a compensatory D1 receptor upregulation in response to reduced DA levels (Abi-Dargham *et al*, 2002). Using SCH23390 instead of NNC112 to label D1 receptors *in vivo*, a separate study found decreased levels of D1 receptors in the DLPFC of drug naive and drug-free schizophrenia patients (Okubo *et al*, 1997). A third study found no differences between the *in vivo* binding of SCH23390 to the DLPFC of schizophrenics and control subjects (Karlsson *et al*, 2002). Further research is necessary to determine the factors underlying these discrepant findings. Interestingly, sustained DA depletion differentially affects NNC112 and SCH23390 binding in the rat brain, elevating the *in vivo* binding of the first but not the second ligand (Guo *et al*, 2003).

The degree of D1 receptor upregulation in schizophrenia was found to be inversely related to working memory performance (Abi-Dargham *et al*, 2002). However, D1 upregulation does not contribute to working memory dysfunction since D1 receptor antagonists worsen cognitive deficits in schizophrenia (Abi-Dargham and Moore, 2003). These results are consistent with the idea that D1 upregulation is a compensatory, but insufficient, response to the DA deficit in the DLPFC. In other studies, however, the decrease in cognitive function was associated with a lower D1 receptor binding (Okubo *et al*, 1997) or the magnitude of negative symptoms was not correlated to D1 receptor levels (Karlsson *et al*, 2002). Further research is necessary to determine the relationship between D1 receptor levels and cognitive/negative symptoms in schizophrenia, to establish the possibility of targeting D1 for treatment with novel drugs as well as to determine potential side effects of classic antipsychotics on cognitive function.

The cellular mechanisms of DA modulation of DLPFC neuron activity are important to understand the functional consequences of decreased DA signaling in the DLPFC in schizophrenia as well as the compensatory responses triggered by the DA deficit. DA modulatory connections differ substantially from glutamate and GABA synaptic connections because DA acts exclusively via G-protein-coupled receptors (Missale *et al*, 1998; Neve *et al*, 2004) unlike glutamate and GABA, which also act via fast ligand-gated channels. Thus, DA does not act via fast, point-to-point synaptic transmission, but in a slow and diffuse manner consistent with volume transmission mechanisms (Paspalas and Goldman-Rakic, 2004). Indeed, D1 receptors are found in dendritic shafts and spines (Bergson *et al*, 1995; Paspalas and Goldman-Rakic, 2004, 2005; Smiley *et al*, 1994), and in glutamate axonal boutons (Paspalas and

Goldman-Rakic, 2005), most of which are not associated with DA terminals. DA signaling via volume transmission suggests that changes in DA degradation by COMT or in the uptake of DA by DA transporters could lead to significant changes in the amount of DA available to activate D1 receptors. As noted above, robust changes in the levels of COMT, consistent with either deficiency or compensation, have not been found in schizophrenia. In contrast, the decrease in detectable DA transporter-containing fibers in the DLPFC in schizophrenia (Akil *et al*, 1999), may reflect to some extent a compensatory decrease in DA uptake that favors volume transmission.

Physiological studies have focused on the DA modulation of intrinsic membrane properties and cellular excitability and of glutamate- and GABA-mediated transmission. Application of DA or D1 agonists to monkey DLPFC slices does not affect the membrane potential of pyramidal neurons (González-Burgos *et al*, 2002; Henze *et al*, 2000). In contrast, DA consistently depolarizes interneurons of the fast-spiking subclass, but not interneurons of other subtypes (Kroner *et al*, 2007). Because most fast-spiking cells contain PV, and most PV cells are fast-spiking, selective regulation of fast-spiking interneuron activity by DA is consistent with studies showing that PV neurons, but not calretinin-positive cells, receive synaptic inputs from DA axons (Sesack *et al*, 1998) and preferentially express D1 receptors (Muly *et al*, 1998). Probably as a consequence of increased FS cell excitability, D1 receptor activation also increases the frequency of action potential-dependent inhibitory synaptic currents in pyramidal cells (Kroner *et al*, 2007). Because the expression of $GAD_{67}$ mRNA in interneurons is activity-dependent (Benson *et al*, 1994) and because DA upregulates the firing of fast-spiking cells (Kroner *et al*, 2007), a deficient DA signal in DLPFC may contribute to a decrease in fast-spiking cell activity and thus decreased $GAD_{67}$ expression in these neurons (see Figure 5).

Although the interneuron depolarization and the increase in inhibitory currents produced by DA suggests net inhibitory effects in the primate DLPFC network, DA also changes the response of pyramidal neurons to excitatory currents, although in complex ways. *In vitro*, DA increases the excitability of primate DLPFC pyramidal cells with a delayed time course (González-Burgos *et al*, 2002; Henze *et al*, 2000) that is consistent with other delayed D1 receptor-mediated effects (Seamans and Yang, 2004). However, more detailed studies of the effects of DA receptor activation on rat medial PFC pyramidal neurons revealed effects consistent with an early decrease in excitability, followed by a delayed enhancement (Seamans and Yang, 2004). The mechanisms underlying short-term and long-lasting effects of DA receptor activation, as well as their relative importance *in vivo*, remain to be determined. In addition to such temporally biphasic effects *in vitro*, DA seems to have effects *in vivo* that are biphasic as a function of its extracellular concentration or occupancy of DA receptors. More specifically, DA elicits effects on monkey DLPFC cell firing that follow an 'inverted-U' dose–response relationship (Vijayraghavan *et al*, 2007; Williams and Goldman-Rakic, 1995). However, such 'inverted-U' effects *in vivo* seem to result from complex modulatory effects and not simply from modulation of monkey DLPFC pyramidal cell excitability such as that found *in vitro* (González-Burgos *et al*, 2002; Henze *et al*, 2000). For instance, low levels of DA receptor stimulation do not generally enhance the firing of DLPFC cells *in vivo*, but selectively increase the firing of neurons that are tuned to preferred stimuli during tasks that require working memory (Vijayraghavan *et al*, 2007; Williams and Goldman-Rakic, 1995). Therefore, the effects of DA receptor activation on the output of DLPFC pyramidal cells most likely result from complex network effects.

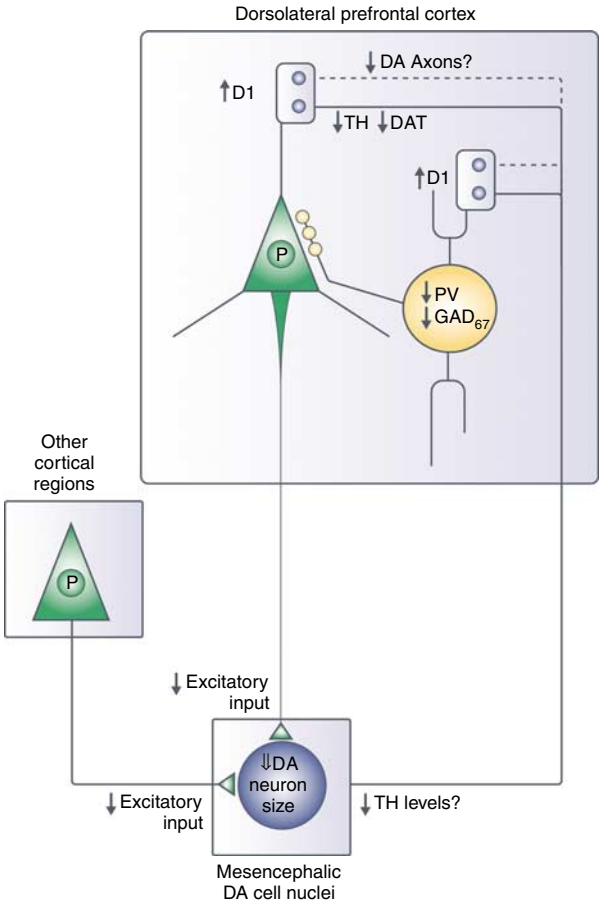



**Figure 5** Summary of the putative causes and consequences of altered dopamine (DA) signaling in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia. A decrease in glutamatergic excitatory input onto mesencephalic DA cells may result from hypoactivity in pyramidal cells (P) located in the DLPFC or in other cortical areas that are a source of excitatory input to DA cells. Reduced excitatory input may produce sustained hypoactivity of DA cells providing DA input to the DLPFC, which may result in both morphological and biochemical changes in the DA cells. For instance, in schizophrenia the size of DA neuron cell bodies has been reported to be reduced and might be associated with a lower number of DA fibers innervating the DLPFC. In addition, the amount of tyrosine hydroxylase (TH) in the mesencephalic DA cell nuclei has been reported to be reduced in schizophrenia as is the detectability of TH and of dopamine transporter (DAT)-immunoreactive DA axons in the DLPFC. Decreased DA innervation and reduced DA levels in the DLPFC may lead to compensatory, but functionally insufficient, upregulation of D1 receptors in pyramidal cells or GABA neurons or both. Because D1 receptor activation increases the activity of parvalbumin (PV)-positive, fast-spiking GABA neurons, reduced D1-mediated signaling may reduce the activity of these neurons and lead to activity-dependent downregulation of the expression of $GAD_{67}$ and PV.

Enhancement of tuned firing of DLPFC neurons by low levels of DA receptor stimulation during cognitive tasks is

consistent with the idea that cognitive deficits in schizophrenia result, at least in part, from decreased DA input to the DLPFC. Moreover, D1 receptor upregulation in schizophrenia might partially compensate for such deficits in working memory-related DLPFC activity. Because high levels of DA receptor stimulation, via this 'inverted-U' model, disrupt tuned firing of DLPFC neurons, D1 receptor-based treatment of cognitive deficits in schizophrenia should consider the changes in D1 receptor levels produced by compensatory brain mechanisms and also the changes in D1 receptors induced by antipsychotic medications. For instance, PET-based assessments of the degree of D1 receptor upregulation in individual patients may help in guiding therapy to maximize the possibility that therapeutic compounds are combined with the compensatory plasticity in D1 receptor levels in a manner that restores optimal levels of D1 receptor stimulation.

## FUTURE RESEARCH DIRECTIONS

The findings reviewed above indicate that the dysfunction of the DLPFC and associated working memory impairments in schizophrenia are likely the result of a complex set of alterations in glutamate, GABA, and DA neurotransmission. The existing data suggest that some of these alterations are deleterious causes or consequences of disturbances in the functional architecture of the DLPFC and interconnected brain regions, whereas others are best explained as compensatory responses. In each case, they reflect the neuroplasticity of DLPFC circuitry in a disease state.

How do these changes arise in the context of neuroplastic responses? Clearly, the nature of the relationships of these alterations within and across excitatory, inhibitory, and modulatory circuits requires further investigation. For example, it remains unclear whether these changes define diversity and/or variance in the disease process(es) that underlie the clinical syndrome we call schizophrenia. That is, do some individuals have predominately disturbances in one of these systems, but not in another, yet with the frequency of alterations in each system common enough to be detected in cohorts of subjects identified by the presence of the clinical syndrome and not specific phenotypic features? It is also critical to refine the cellular and circuitry specificity of the molecular alterations in the illness. For example, as summarized in the section on Excitatory Cortical Connections, morphological evidence of alterations in excitatory circuits in the DLPFC appear to be most pronounced in deep layer 3 pyramidal neurons. However, further explication of these findings requires the availability of a panel of molecular markers that define distinct subsets of pyramidal neurons based on their functional and connectional properties. In the case of the disturbances in GABA neurotransmission, a fuller understanding of whether changes in other $GABA_A$ receptor subunits (Hashimoto *et al*, 2007a) or $GABA_B$ receptor subunits (Zhao *et al*, 2007) consistently occur in the illness, and if so, whether they occur in a cell type and circuit-specific manner may inform their potential utility as therapeutic targets.

Understanding these issues is necessary for a pathophysiologically based rationale for the development of novel and selective pharmacotherapic interventions for the illness, that is, any molecule identified as a drug target must be understood in the cellular and circuitry context of the DLPFC, as well as other brain regions that express the molecule of interest. In addition, understanding whether the disease-related change in the targeted molecule is a consequence or compensation in the disease process has important implications both for the nature of activity of the drugs designed against that target, and perhaps for the potential therapeutic value of the target. For example, is the neuroplastic capacity of cortical circuitry sufficiently limited that pharmacological augmentation of a compensatory response is feasible? If so, is such an approach more likely to be effective than attempts to reverse detrimental consequences when the pathological cause is still present? Furthermore, given the marked developmental changes that occur in each of these systems during adolescence, this type of pharmacological intervention may have particular value as an intervention strategy for high-risk adolescents in the prodromal phase of the illness. However, the effectiveness and safety of such interventions requires a complete understanding of the maturation of these neural circuits, of the functional consequences of these circuitry changes and of the vulnerability of these developmental processes to pharmacological agents.

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